Reactivating memories during sleep by odors: odor specificity and associated changes in sleep oscillations

Rihm, Julia S; Diekelmann, Susanne; Born, Jan; Rasch, Björn

Abstract: Memories are reactivated during sleep. Reexposure to olfactory cues during sleep triggers this reactivation and improves later recall performance. Here, we tested if the effects of odor-induced memory reactivations are odor specific, that is, requiring the same odor during learning and subsequent sleep. We also tested whether odor-induced memory reactivation affects oscillatory EEG activity during sleep, as a putative mechanism underlying memory processing during sleep. Participants learned a visuospatial memory task under the presence of an odor. During subsequent SWS, the same odor, a different odor, or an odorless vehicle was presented. We found that odor reexposure during sleep significantly improves memory only when the same odor was presented again, whereas exposure to a new odor or the odorless vehicle had no effect. The memory-enhancing effect of the congruent odor was accompanied by significant increases in frontal delta (1.5-4.5 Hz) and parietal fast spindle (13.0-15.0 Hz) power as well as by an increased negative-to-positive slope of the frontal slow oscillation. Our results indicate that odor-induced memory reactivations are odor specific and trigger changes in slow-wave and spindle power possibly reflecting a bottom-up influence of hippocampal memory replay on cortical slow oscillations as well as thalamo-cortical sleep spindles.

DOI: https://doi.org/10.1162/jocn_a_00579

Reactivating Memories during Sleep by Odors: Odor Specificity and Associated Changes in Sleep Oscillations

Julia S. Rihm[1*], Susanne Diekelmann[2*], Jan Born[2], and Björn Rasch[3,4]

Abstract

■ Memories are reactivated during sleep. Reexposure to olfactory cues during sleep triggers this reactivation and improves later recall performance. Here, we tested if the effects of odor-induced memory reactivations are odor specific, that is, requiring the same odor during learning and subsequent sleep. We also tested whether odor-induced memory reactivation affects oscillatory EEG activity during sleep, as a putative mechanism underlying memory processing during sleep. Participants learned a visuospatial memory task under the presence of an odor. During subsequent SWS, the same odor, a different odor, or an odorless vehicle was presented. We found that odor reexposure during sleep significantly improves memory only when the same odor was presented again, whereas exposure to a new odor or the odorless vehicle had no effect. The memory-enhancing effect of the congruent odor was accompanied by significant increases in frontal delta (1.5–4.5 Hz) and parietal fast spindle (13.0–15.0 Hz) power as well as by an increased negative-to-positive slope of the frontal slow oscillation. Our results indicate that odor-induced memory reactivations are odor specific and trigger changes in slow-wave and spindle power possibly reflecting a bottom–up influence of hippocampal memory replay on cortical slow oscillations as well as thalamo-cortical sleep spindles. ■

INTRODUCTION

Olfactory stimuli are potent cues for memories. In his oeuvre "En recherche du temps perdu," Marcel Proust elegantly describes how the smell and taste of a tea-dipped cake instantly reactivate a highly detailed scene of the protagonist's childhood, which he had not recalled for a long time. This efficacy of odors as memory cues has been confirmed in experimental studies. When stimuli have been learned in the presence of a contextual odor, retrieval of the stimuli was improved when the olfactory stimulus was also present during retrieval testing (Smith, 1992; Schab, 1990, 1991). Importantly, olfactory context effects are odor specific: After learning under the presence of a positive or negative odor, retrieval performance only increased when the same odor was presented again during retrieval testing (Schab, 1990; Cann & Ross, 1989).

In recent studies, we applied the approach of odor cueing to reactivate memories during sleep (Diekelmann, Büchel, Born, & Rasch, 2011; Rasch, Büchel, Gais, & Born, 2007). Sleep promotes memory consolidation, and it is widely assumed that the beneficial effect of sleep on memory relies on memory reactivations during SWS (Oudiette & Paller, 2013; Rasch & Born, 2013; Diekelmann & Born, 2010). According to this concept, hippocampal memory reactivations facilitate the gradual integration of memories from hippocampal into neocortical networks for long-term storage. This process occurs in close coordination with slow oscillatory and fast spindle activity during SWS (Mölle & Born, 2011). Reactivations embedded in spindles during the excitable up state of slow oscillations have been proposed as a mechanism supporting the hippocampo-to-neocortical transfer of reactivated memory information (Ngo, Martinetz, Born, & Mölle, 2013; Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012). Indeed, experimentally inducing memory reactivation by reexposure to a contextual olfactory memory cue during SWS activated hippocampal areas during sleep and resulted in improved memory recall the next day (Diekelmann et al., 2011; Rasch et al., 2007). This concept has received further support by recent findings indicating that reactivating memories during sleep by auditory cueing leads to a strengthening of individual memory traces, suggesting a high degree of specificity of the effects of reactivation on memory consolidation during sleep (Oudiette, Antony, Creery, & Paller, 2013; Antony, Gobel, O'Hare, Reber, & Paller, 2012; Rudoy, Voss, Westerberg, & Paller, 2009). Although a first study on creativity suggests that odor effects on reactivation are absent when different odors are used before and during sleep (Ritter, Strick, Bos, van Baaren, & Dijksterhuis, 2012), the specificity of olfactory cueing for memory consolidation processes during sleep has not yet been examined in previous studies.

[1]University of Zurich, Switzerland, [2]University of Tübingen, Germany, [3]University of Fribourg, Switzerland, [4]Zurich Center for Interdisciplinary Sleep Research, Switzerland
*Julia S. Rihm and Susanne Diekelmann contributed equally to this article.

Journal of Cognitive Neuroscience X:Y, pp. 1–13
doi:10.1162/jocn_a_00579

Here, we tested the specificity of olfactory cueing during sleep on memory consolidation. Participants learned the position of card pairs in a two-dimensional object-location task under the presence of either a positive or negative odor. During subsequent SWS, the same odor or the other odor was presented. We hypothesized that only reexposure to the same odor effectively increases sleep-related memory consolidation, resulting in improved memory performance the next day. In addition, we predicted that these changes in memory because of reactivation are associated with changes in sleep parameters implicated in sleep-dependent memory consolidation processes, according to the active system consolidation hypothesis (Rasch & Born, 2013), namely, slow delta, delta, and fast spindle power. For further fine-grained exploratory analysis, we calculated the slope, amplitude, and number of the slow oscillations.

METHODS

Participants

Thirty-six nonsmoking healthy participants naive to the experimental protocol participated in the study (12 men, mean age = 23.4, *SD* = 3.2 years, range = 19–32 years). They were divided into three groups (n = 12 per group) depending on the congruency of the odors presented during learning and during sleep: "congruent group" (same odor during learning and sleep), "incongruent group" (different odors during learning and sleep), and "vehicle group" (odor during learning and odorless vehicle during sleep). Data from three participants (two men, one woman; one in the congruent group and two in the incongruent group) had to be excluded from the EEG analysis because of technical problems with the EEG recordings resulting in 33 participants for EEG data analysis. Age ($F(2, 33) = 0.08$, $p > .90$) and gender ($F(2, 33) = 0.35$, $p > .70$) distributions were highly comparable between groups. Participants were in good physical and mental health condition according to a routine examination, did not take any medication at the time of the experiments, and reported a normal sleep–wake cycle with habitual bedtimes starting between 11:00 p.m. and 1:00 a.m. and ending between 6:00 and 8:30 a.m. They had not been on night shift and did not have any major sleep disturbances during 6 weeks before the experiment. Any nasal infections were excluded on the days of the experiments. Participants were habituated to the experimental setting by spending an adaptation night in the sleep laboratory under the conditions of the experiment including the placement of electrodes and of the nasal mask used for delivery of odors during sleep. On experimental days, participants were instructed to get up at 7:00 a.m., not to take any naps, and not to ingest alcohol- or (after 3:00 p.m.) caffeine-containing drinks. Written informed consent was obtained from all participants before participation. The experiment was approved by the ethics committee of the University of Lübeck.

Memory Task

The two-dimensional object-location memory task resembles the game "concentration" and consists of 15 card pairs showing colored pictures of different animals and everyday objects. Performance on this type of task relies on temporal lobe structures including the hippocampus (Sommer, Rose, Gläscher, Wolbers, & Büchel, 2005; Kessels, de Haan, Kappelle, & Postma, 2001). Throughout the task, all possible spatial locations are shown as gray squares on a 15-in. flat screen ("the back of the cards"). The locations are geometrically ordered in a checkerboard-like fashion.

At learning, the first card of each card pair was presented alone for 1 sec followed by the presentation of both cards for 3 sec. After an ISI of 3 sec, the next card pair was presented in the same way. The whole set of card pairs was presented twice. Immediately after these two runs, recall of the spatial locations was tested using a cued recall procedure, that is, the first card of each pair was presented and the participant had to indicate the location of the second card with a computer mouse. Visual feedback was given in each case by presenting the second card at the correct location for 2 sec independent of whether the response was correct or not, to enable reencoding of the correct location of the card pair. The cued recall procedure was repeated until the participant reached a criterion of 60% correct responses. After presenting a card pair, both cards were replaced by gray squares again, so that guessing probability remained the same throughout each run. The odor was delivered in a stimulus-locked way, starting with the onset of the presentation of the first card of each pair and stopping when presentation of both cards ended.

At retrieval testing the next morning, the same cued recall procedure was used during the learning phase, but without odor presentation. To indicate overnight memory consolidation, we used the percentage of correctly recalled card locations at retrieval, with performance on the last run during learning set to the baseline value of 100%. Note that this measure yields values of >100% if more card locations are recalled at retrieval testing than during learning. (Values of >100%, however, do not reflect "true gains" in memory because feedback was given during the last learning trial.) In Table 1, overnight changes are additionally indicated as absolute difference between the number of recalled card locations at retrieval minus performance at learning.

Odor Delivery and Substance

We used two highly distinct olfactory stimuli in the experiment: Odor A was isobutyraldehyde (IBA, unpleasant; Sigma-Aldrich, Munich, Germany; similarly used in Diekelmann et al., 2011), and Odor B was citral (pleasant; Sigma-Aldrich,

Table 1. Performance on the Two-dimensional Object-location Task

	Congruent Odor	*Incongruent Odor*	*Vehicle*	*F(2, 33)*	*p*
Number of trials before sleep	3.1 ± 0.4	3.5 ± 0.5	2.3 ± 0.5	1.8	.19
Recalled card pairs before sleep	9.5 ± 0.3	10.3 ± 0.3	10.0 ± 0.3	2.0	.16
Change in recalled card pairs (before/after sleep)	0.9 ± 0.6	−0.6 ± 0.4	−0.7 ± 0.3	4.5	.02*

The task included 15 card pair locations. Learning trials were repeated until participants reached a learning criterion of 60% correct responses. Number of trials to reach the criterion and number of card locations recalled at learning are indicated. Change denotes the difference between retrieval performance after sleep and performance at the criterion trial at learning. Data are means ± *SEM*. Right columns indicate *F* and *p* values for one-way ANOVA.

*$p < .05$.

Munich, Germany; tested in pilot studies). Both odors were diluted in odorless mineral oil (1, 2-propanediol; Sigma-Aldrich, Munich, Germany) at a concentration of 1:100 (citral) and 1:200 (IBA). The odorless mineral oil served as stimulus in the control condition. The experimental odors were delivered via a 12-channel computer-controlled olfactometer designed after Lorig (2000). Room air was filtered before entering the system, and airflow was held constant at 3 L/min. To avoid tactile or thermal shifts associated with odor onset, half of the air stream was presented continuously to the participant, and only the other half was switched between room air and vehicle or odor presentation by computer-controlled valves. The olfactometer was placed in a separate room (adjacent to the participant's room) and was connected to the participant's mask via teflon tubes, which allowed regulating the odor stimulation without disturbing the participant. The participant received the odor via a small nasal mask, which assured constant stimulation but permitted normal breathing. A 1-m tube with a 12.6-ml volume connected the glass bottles containing the stimulus fluids with the mask, thus allowing rapid odor onset and offset times of 300–500 msec.

Design and Procedure

Half of the participants learned the two-dimensional object-location task under the presence of Odor A. The other half of the participants received Odor B during learning. In a balance between participant design, either the same odor ("congruent group") or the other odor ("incongruent group") was delivered during subsequent SWS. A third group received the odorless mineral oil ("vehicle group") during SWS. No odor was presented during retrieval testing the next morning (see Figure 1A).

Sessions started at 8:30 p.m. with the application of electrodes for standard polysomnography and of the nasal mask. Next, participants performed an odor detection test with the odor applied during learning, to ensure normal olfactory sensitivity. The learning phase of the visuospatial two-dimensional object-location memory task started at 9:30 p.m. The experimental odor was presented time-locked to the presentation of the stimuli to be learned via a nasal mask. The odor detection test was repeated after the learning phase. At 11:00 p.m., participants went to bed and were allowed to sleep for 7.5 hr. The olfactory stimuli were presented during SWS in the first 3 hr after sleep onset. Presentations started as soon as online polysomnographic recordings indicated more than

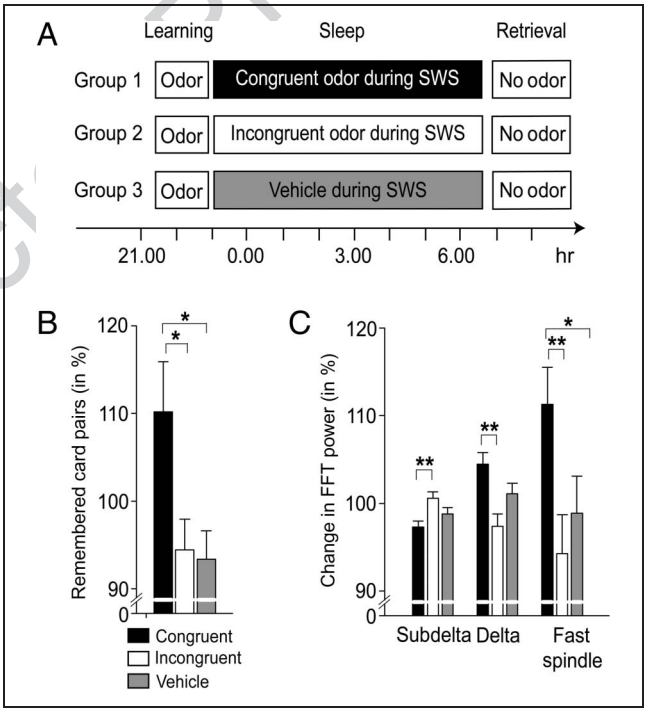

Figure 1. (A) Procedure. Participants learned the two-dimensional object-location task under the presence of a specific odor. According to the group, the same odor, a novel odor, or an odorless vehicle was presented during the first two periods of subsequent SWS. Retrieval took place the morning after sleep without odor. (B) Percentage of remembered card pairs after sleep relative to the number of correctly identified card pairs during learning before sleep. Values greater than 100% indicate more remembered card pairs during retrieval than during learning. (C) Changes in relative EEG power during the first 10 sec of odor-on intervals compared with the last 10 sec of odor-off intervals. Data for slow delta (0.5–1.5 Hz) and delta (1.5–4.5 Hz) power are retrieved from frontal electrodes. Data for fast spindle power (13.0–15.0 Hz) are retrieved from parietal electrodes. For B and C, displayed values are mean values ± *SEM*. *p* Values from planned pairwise post hoc comparisons are indicated (*$p < .05$, **$p < .01$).

20% delta waves (i.e., the presence of SWS) during a 30-sec period. The stimulation was interrupted whenever polysomnographic signs of arousal, awakening, or changes in sleep stage appeared. The experimenter was entirely unaware whether odor or vehicle was applied on a given night. In each experimental session, the olfactometer contained Odor A, Odor B, and vehicle, and the selection was performed automatically by a preprogramed algorithm unknown to the experimenter. Stimulation followed an alternating pattern of 30-sec on-phases/30-sec off-phases to reduce habituation. Participants were awakened at approximately 6:30 a.m. from nonrapid eye movement (NREM) sleep stage 2 or 1, and the nasal mask and electrodes were removed. If these sleep stages were not present at 6:30 a.m., we waited until the next appearance of sleep stage 2 or 1 to awaken participants. About 30 min later, recall was tested on the memory task, without any odor presentation.

As a control measure of vigilance, the participants' RTs were measured before learning and before retrieval. RTs were assessed by a standardized test that required pressing a button as fast as possible whenever a large red disk appeared on a computer screen (as described in Little, Johnson, Minichiello, Weingartner, & Sunderland, 1998). In 40 trials, the participants fixated their gaze on a cross, displayed for 50–000 msec on a white screen. Then, in 35 trials, a red disk appeared, and in five random no-go trials, the screen remained white.

Sleep and EEG Recordings

Sleep was recorded by standard polysomnography (Rechtschaffen & Kales, 1968). EEG was recorded from six scalp (Ag–AgCl) electrodes (F3, F4, C3, C4, P3, and P4; according to the International 10–20 System) and a nose reference. EEG signals were filtered between 0.15 and 35.0 Hz and sampled at 200 Hz. Additionally to the online identification of sleep stages, polysomnographic recordings were scored offline by two experienced technicians. The sleep stages scored were wake after sleep onset (WASO); NREM sleep stages 1, 2, 3, and 4, with sleep stages 3 and 4 defining SWS; REM sleep; and movement.

For a more fine-grained exploratory analysis of immediate effects of odor cueing during SWS, EEG recordings were subjected to power spectral analysis. Data of the 30-sec on-and-off phases of odor and vehicle stimulation were separated each into three blocks of artifact-free EEG including 2,048 data points each (≈10.2 sec) with an overlap of 205 data points between blocks. A Hanning window was applied on each 2,048-point block before calculating power spectra using fast Fourier transformation (FFT) with a resolution of 0.2 Hz. Individual mean power in the following EEG bands was determined for the odor-on and odor-off periods: frontal slow delta (0.5–1.5 Hz), frontal delta (1.5–4.5 Hz), frontal slow spindle (11.0–13.0 Hz), and parietal fast spindle (13.0–15.0 Hz) bands.

Because recordings from each pair of electrodes (F3 and F4; P3 and P4) revealed the same results, data were collapsed across both hemispheres. The blocks of odor-on and odor-off periods were used to calculate the percent change of spectral power such that power during the first 10-sec interval of the odor-on period was expressed as percentage of the power during the last 10-sec interval of the preceding odor-off period (set to 100%).

Identification of Slow Oscillations and Slope Analysis

In addition to spectral EEG power, we identified discrete slow oscillations and calculated their slopes during odor-on and odor-off intervals. The slopes of the slow oscillation are considered a sensitive measure of synchronization of cortical activity and are possibly related to network synaptic connectivity and its changes over time (Vyazovskiy, Cirelli, & Tononi, 2011). Slow oscillation detection and slope calculation were performed in frontal recording sites, as described previously (Bölsterli et al., 2011; Riedner et al., 2007). In brief, artifact-free EEG data were low-pass filtered at 30.0 Hz and band-pass filtered between 0.5 and 4.0 Hz (stopband of 0.1 and 10.0 Hz) using a Chebyshev Type II filter (MATLAB, The Math Works, Inc., Natick, MA). The chosen filter parameters provided minimal amplitude and wave shape distortion. For each frontal channel (F3, F4), individual negative half-waves were identified. A half-wave was defined as the negative deflection of the EEG between two consecutive zero crossings. We considered only those half-waves whose consecutive zero crossings were separated by 0.25–1.0 sec (i.e., a frequency between 0.5 and 2.0 Hz), which had a corresponding quarter-wave from the negative peak to the next zero crossing lasting more than 0.11 sec (<2.25 Hz) and a minimal amplitude of −75 μV. For these individually identified slow oscillations, we calculated slopes from the negative peak to the next zero crossing (negative-to-positive slopes) and slopes from the previous zero crossing to the negative peak (positive-to-negative slopes). Whereas positive-to-negative transitions of the surface EEG have been associated with the onset and synchronicity of neuronal down states, negative-to-positive transitions might be more related to the onset of the subsequent up state (Vyazovskiy et al., 2009). As in the power analysis, the relative change in numbers, slopes, and amplitudes in slow oscillations was calculated for the first 10-sec interval of the odor-on period with reference to the last 10-sec interval of the preceding odor-off period (set to 100%).

Spindle Analysis

Spindle counts and density during odor-on and odor-off intervals were analyzed because of their well-known relationship with overnight retention of memories (Saletin, Goldstein, & Walker, 2011; Fogel, Nader, Cote, & Smith, 2007; Nishida & Walker, 2007; Gais, Mölle, Helms, &

Born, 2002). Discrete spindles are a characteristic feature of sleep stage 2 and occur also in SWS but are virtually absent during REM sleep. Slow (11.0–13.0 Hz) and fast (13.0–15.0 Hz) spindles were separately identified at the six EEG recording sites based on an algorithm adopted from previous studies (Gais et al., 2002; Schimicek, Zeitlhofer, Anderer, & Saletu, 1994). In brief, power was extracted in the frequency bands of interest (11.0–13.0 Hz; 13.0–15.0 Hz), and the events were counted as spindles for which the power signal exceeded a fixed threshold (±10 μV) for an interval lasting 0.5–3 sec. Spindles were counted separately in each channel during 30-sec NREM EEG segments free of movement artifacts (maximal difference in EMG activity of <150 μV). Mean spindle counts were calculated by averaging spindle counts of all six channels. To calculate mean spindle density, mean spindle counts were divided by the number of analyzed NREM 30-sec epochs. One participant of the odorless vehicle group had to be excluded because the algorithm did not detect any discrete sleep spindles during the odor-on and odor-off periods. Thus, 32 participants were included in the spindle analysis. The two separate spindle bands were chosen based on previous studies, which demonstrated the presence of two kinds of spindles in humans possibly linked to different aspects of cognitive function, that is, slow spindles that prevail over frontal cortex and show greater topographical variability than the fast spindles that concentrate over parietal cortex (Mölle, Bergmann, Marshall, & Born, 2011; Schabus et al., 2007; Zeitlhofer et al., 1997).

Arousal Analyses

We performed analyses on EMG power, EMG arousal counts, and EEG arousal counts to control for arousal-induced changes in sleep parameters. Three additional participants had to be excluded from the EMG analyses because of loss of the EMG signal at the beginning of the night, resulting in 30 participants (congruent: $n = 10$, incongruent: $n = 9$, vehicle: $n = 11$).

EMG arousal counts were analyzed using the EMG channel. Data were rectified, a moving average of 125 msec was applied, and a baseline of 500 msec before each segment was subtracted. Signal peaks above 40 μV were counted as EMG arousal. The number of EMG arousals during the 10-sec odor-off periods was subtracted from the number of EMG arousals during the 10-sec odor-on periods and relativized on the overall number of stimulations. We used the same method over the total sleep period to detect the number of EMG arousals for the entire night and relativized the number of EMG arousals on the total sleep time.

For the power analysis of the EMG signal, we applied a notch filter (50 Hz) and calculated the FFT in the 10- to 100-Hz range (Fridlund & Cacioppo, 1986). We set the upper restriction to 100 Hz because of our sampling rate of 200 Hz. We performed this FFT similar to the FFT for EEG analysis and calculated the percent change in power during the first 10 sec of the odor-on interval with power in the preceding last 10 sec of the odor-off interval set to 100%.

In addition to the EMG analysis, we also counted EEG arousals during 10-sec and 30-sec odor-on and odor-off intervals by visual inspection of the six EEG channels. We used the EEG arousal scoring rules of the American Sleep Disorders Association and Sleep Disorders Society (ASDA, 1992). An EEG arousal was counted if there was a change to alpha, theta, or frequencies greater than 16 Hz, which lasted for at least 1.5 sec (De Gennaro, Ferrara, & Bertini, 2001). EMG was not considered because we only analyzed NREM sleep. To demonstrate that odors do not cause differences in arousals of higher frequencies (above 16 Hz), we additionally applied a high-pass filter with 16 Hz to our data and counted the remaining arousals (an arousal was counted if it lasted longer than 1.5 sec). We calculated the absolute difference in the number of both types of EEG arousals between odor-on and odor-off periods (rather than percentages to avoid division by zero, as several participants had no EEG arousal at all during these periods).

Data Analyses

Data were analyzed using 2 × 3 ANOVA with the factors "odor during learning" (Odor A vs. Odor B) and "group" (congruent odor vs. incongruent odor vs. vehicle). For significant main effects or interactions, post hoc pairwise comparisons were performed using the least significant difference method. If Mauchly sphericity test reached significance, we displayed degrees of freedom and p values that were Greenhouse–Geisser corrected. Correlation analyses were conducted using Pearson correlation. A p value $< .05$ was considered significant.

RESULTS

Memory Performance

As expected, administration of the same odor was critical for the memory-improving effect of odor reexposure during sleep. When the same odor was presented during learning and subsequent SWS ("congruent group"), participants recalled 110.2 ± 5.8% of the card pairs they had learned before sleep. Participants who received a different odor during learning than during sleep ("incongruent group") recalled only 94.5 ± 3.5% of the card pairs, which was comparable with those who received the odorless vehicle during SWS ("vehicle group," 93.4 ± 3.3%). Recall performance between the three groups differed significantly (main effect group: $F(2, 33) = 4.7$, $p = .02$; results for absolute differences are indicated in Table 1). Post hoc pairwise comparisons revealed that performance in the congruent group was significantly higher as compared with both the incongruent ($p = .02$) and vehicle ($p = .01$)

groups, whereas the incongruent and vehicle groups did not differ ($p > .80$; see Figure 1B). The results were not affected by the valence of the odor presented during learning ($p > .80$ for main and interaction effects of Odors A and B). Memory performance in the incongruent and vehicle groups was roughly comparable with performance after a night of sleep without any intervention as reported previously (90.9 ± 4.5%; see Rasch et al., 2007, supporting material, p. 6).

The three experimental groups did not differ in their learning performance before sleep, neither in the number of card pairs recalled in the last learning trial nor in the number of trials to achieve the learning criterion of 60% (both ps ≥ .16, Table 1). The number of trials to reach the learning criterion was not correlated with changes in memory improvement ($r = .26$, $p > .15$), frontal delta power ($r = -0.26$, $p > .15$), frontal slow delta power ($r = -0.16$, $p > .30$), parietal fast spindle power ($r = .11$, $p > .50$), and slow oscillation slopes ($r = -0.07$, $p > .60$).

Furthermore, participants were asked after the experiment whether they had received the same odor during sleep as during learning, a different odor or no odor. Only 1 of 36 participants gave the correct answer, 16 gave incorrect answers, and 15 participants indicated "I don't know."

Sleep Architecture

The three groups did not differ in total sleep time ($p > .70$), and there were also no differences in the percentage of time they spent in sleep stages S1, S2, SWS, REM sleep, or awake (all ps ≥ .17, Table 2). The number of movements during the whole night revealed a trend for significance between groups ($F(2, 30) = 3.27$, $p = .052$), with highest values in the incongruent odor group (1.4 ± 0.35%; congruent group: 0.77 ± 0.14%; vehicle group: 0.62 ± 0.12%). Post hoc pairwise comparisons revealed a significant difference between the incongruent and vehicle conditions ($p = .02$) and marginal significance between the congruent and incongruent conditions ($p = .06$), whereas the congruent and vehicle conditions did not differ ($p > .60$; see Table 2). When including the number of arousals as covariate for the difference of memory improvement between groups, the difference between groups was still significant ($F(2, 29) = 6.51$, $p = .005$) with a similar pattern of performance (congruent: 112.76 ± 4.45%, incongruent: 94.47 ± 4.72%, vehicle: 92.03 ± 4.55%).

Effects of Odor-induced Reactivation on Oscillatory Activity during Sleep

In accordance with our hypothesis, exposure to the same odor during learning and subsequent SWS increased oscillatory activity in the delta and fast spindle bands shortly after odor onset, whereas slow delta power was surprisingly reduced (see Table 3 and Figure 1C; for all other frequency bands at all electrode sites, see Supplementary Tables 1–4).

During the first 10-sec interval of the odor-on period, power in the 1.5- to 4.5-Hz delta band over frontal electrodes significantly increased to 104.5 ± 1.2% in the congruent group, with the preceding 10 sec of the odor-off interval set to 100% ($t(10) = 3.68$, $p = .004$, one-sample t test). In contrast, no changes in delta power were observed during administration of an odor different from that during learning (incongruent group, 97.4 ± 1.6%; $t(9) = -1.61$, $p = .14$) or an odorless vehicle (vehicle group, 101.1 ± 1.1%; $t(11) = 1.0$, $p > .30$) during sleep.

Table 2. Sleep Parameters

	Congruent Odor	*Incongruent Odor*	*Vehicle*	*F(2, 33)*	*p*
WASO %	1.2 ± 0.5	6.2 ± 3.0	3.5 ± 0.9	1.8	.17
S1 %	5.0 ± 0.6	5.4 ± 1.0	7.0 ± 0.6	1.9	.17
S2 %	55.9 ± 2.4	53.1 ± 2.6	51.9 ± 1.8	0.8	>.40
SWS %	16.2 ± 2.0	17.4 ± 1.6	17.2 ± 1.8	0.1	>.80
REM %	20.1 ± 1.6	17.3 ± 1.8	19.5 ± 1.5	1.3	>.20
Movement %	0.8 ± 0.14	1.4 ± 0.35	0.6 ± 0.12	3.27	.052
Sleep time (min)	445 ± 8.2	447 ± 13.8	456 ± 13.6	0.3	>.70
SOL (min)	25.0 ± 2.4	35.1 ± 9.2	31.0 ± 4.6	0.7	>.40
Sleep efficiency %	93.6 ± 0.9	86.8 ± 3.5	90.2 ± 1.4	2.3	.12
Number of stimulations	65.8 ± 6.0	56.7 ± 4.9	59.4 ± 5.4	0.7	>.40

WASO, S1, S2 (NREM sleep stages 1 and 2), SWS (combined sleep stages 3 and 4), and REM sleep in percent of total sleep time (sleep time). Number of arousals in percent of the total time scored as sleep. SOL = sleep onset latency. Data are means ± *SEM*. Right columns indicate *F* and *p* values for one-way ANOVA.

Table 3. Percentage of Changes in FFT Power

	Congruent Odor	*Incongruent Odor*	*Vehicle*	*F(2, 30)*	*p*
Slow delta	97.3 ± 0.7	100.6 ± 0.7	98.8 ± 0.7	5.5	.009**
Delta	104.5 ± 1.3	97.4 ± 1.4	101.1 ± 1.2	7.1	.003**
Fast spindle	111.3 ± 4.2	94.3 ± 4.4	98.9 ± 4.0	4.2	.03*

Data are retrieved from frontal (slow delta and delta band) and parietal (fast spindle band) electrodes during the first 10 sec of the odor-on interval compared with the last 10 sec of the preceding odor-off interval set to 100%. Data are means ± *SEM*. Right columns indicate *F* and *p* values for one-way ANOVA.

The change in delta power differed significantly between the three experimental groups (ANOVA: $F(2, 30) = 7.13$, $p = .003$). Post hoc group-wise comparison revealed a stronger increase in delta activity in participants of the congruent group compared with the incongruent ($p = .001$) and a trend for a stronger increase compared with the vehicle group ($p = .07$). The difference between the incongruent and vehicle groups also almost reached significance ($p = .054$; Figure 1C). An additional analysis of the delta band using the 1.0- to 4.5-Hz range revealed similar results (overall: $F(2, 30) = 3.43$, $p = .046$). The reported differences in changes in delta power between the groups were not related to differences in baseline power: Values of the last 10 sec of the odor-off interval did not differ between the three groups (all *p*s > .50 for all frequency bands of interest). In addition, including these odor-off values as a covariate did not alter any of the reported results.

The increase in delta activity during odor-on periods in the congruent group was accompanied by a parallel reduction of 0.5- to 1.5-Hz slow delta power over frontal electrodes in the congruent group for odor-on intervals (97.3 ± 0.7%, $t(10) = -4.07$, $p = .002$, one-sample *t* test). Similarly, the vehicle group showed a decrease in slow delta power (98.7 ± 0.6%, $t(11) = -2.17$, $p = .052$), whereas no changes in slow delta power were observed during administration of a novel odor (100.6 ± 0.8%, $t(9) = 0.71$, $p > .40$). The overall difference in slow delta power between the three conditions was also significant (ANOVA: $F(2, 30) = 5.50$, $p = .009$). Post hoc tests indicated a significant difference between the congruent and incongruent groups ($p = .009$). The congruent and vehicle groups did not differ ($p = .13$), whereas marginal significance resulted for the difference between the vehicle and incongruent groups ($p = .07$; Figure 1C). An additional analysis of the slow delta band using the 0.5- to 1.0-Hz range revealed similar results (overall: $F(2, 30) = 7.26$, $p = .003$).

In addition, we observed a significant increase in 13.0- to 15.0-Hz fast spindle power over parietal electrodes during odor-on periods in the congruent group (111.2 ± 3.8%; $t(10) = 2.96$, $p = .014$, one-sample *t* test), whereas no changes occurred in the two control groups (incongruent group: 94.2 ± 4.1%, $t(9) = -1.39$, $p = .20$; vehicle group: 98.9 ± 4.6%, $t(11) = -0.24$, $p > .80$). The overall difference between the three experimental conditions was significant (ANOVA: $F(2, 30) = 4.21$, $p = .03$). Post hoc pairwise comparisons revealed significant differences between the congruent compared with the incongruent ($p = .009$) and the vehicle ($p = .043$) groups, whereas the incongruent and vehicle groups did not differ ($p > .40$; Figure 1C). Using a spindle algorithm to detect spindle density averaged over all channels, no significant group differences were found for fast spindle counts ($F(2, 29) = 0.78$, $p > .40$) or density ($F(2, 29) = 0.13$, $p > .80$; for fast spindle density values of the single channels, see Supplementary Table 5).

In contrast to parietal fast spindle power, changes in frontal slow spindle power over frontal electrodes did not differ between the three groups ($F(2, 30) = 0.21$, $p > .90$). Furthermore, there were no significant correlations between memory performance and changes in delta ($r = .28$, $p = .12$), slow delta ($r = -0.25$, $p = .17$), or fast spindle ($r = .04$, $p > .80$) power.

The relative increase in delta power together with a relative reduction in slow delta power during odor-on periods in the congruent group might reflect a shift in the number and/or morphology of slow oscillations. To test this, we detected and analyzed individual slow oscillations (>75 μV) in frontal electrodes during the odor-on and odor-off intervals. For negative-to-positive slopes, relative changes in slopes of the frontal EEG slow oscillations were significantly higher in the congruent group (102.8 ± 2.1%) as compared with the incongruent (95.2 ± 3.5%) and the vehicle (90.4 ± 3.7%; $F(2, 30) = 4.03$, $p = .03$) groups. Post hoc tests indicated only a significant difference between the congruent and vehicle groups ($p = .008$), whereas there was no difference between the congruent and incongruent groups ($p = .11$) or between the incongruent and vehicle groups ($p > .30$; Figure 2A). Neither relative changes in amplitude (99.8 ± 1.9% vs. 98.5 ± 1.7% vs. 98.8 ± 1.4%, respectively; $F(2, 30) = 0.15$, $p > .80$; Figure 2A) nor numbers (107.5 ± 7.2% vs. 96.7 ± 4.3% vs. 96.1 ± 3.2%; $F(2, 30) = 1.57$, $p > .20$) of slow oscillations significantly differed between groups. Also, no differences in slope changes between the three experimental groups were observed for positive-to-negative slopes (99.5 ± 3.5% vs. 93.2 ± 3.4% vs. 97.1 ± 4.9%; $p > .50$). Altogether, this pattern suggests that the shift in power from the slow delta to the delta band primarily

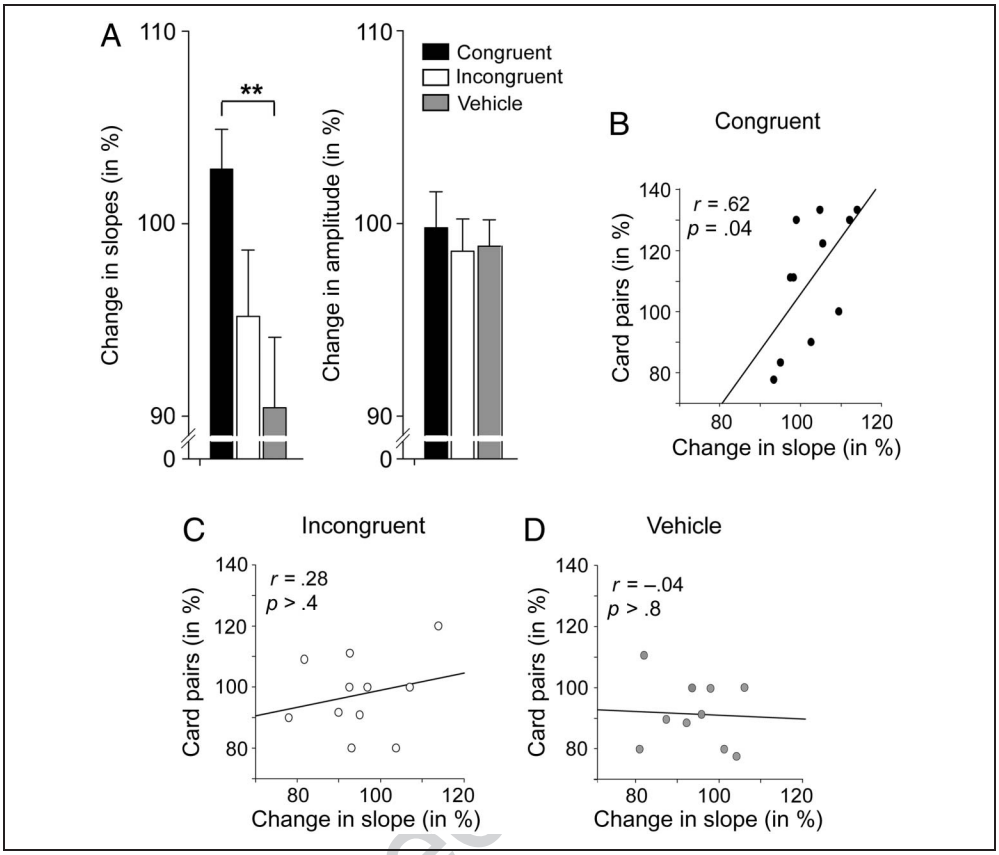

Figure 2. (A) Changes of slopes and amplitudes of slow oscillations between groups in the frontal electrodes during the first 10 sec of odor-on intervals compared with the last 10 sec of odor-off intervals. Displayed values are retrieved from one-way ANOVA, and p values from planned pairwise post hoc comparisons are indicated (**$p < .01$). Differences between groups were significant only for slopes ($p = .03$). (B–D) Correlations between memory performance during retrieval (% retrieval) and relative changes in slopes in frontal electrodes for the congruent group (B), the incongruent group (C), and the vehicle group (D). Overnight memory consolidation is indicated as percentage of correctly recalled card locations at retrieval, with performance on the last run during learning set to 100%. Note that this measure yields values of >100% if more card locations are recalled at retrieval testing than during learning.

originated from an increase in the negative-to-positive slope of the slow oscillations. Furthermore, changes in negative-to-positive slopes of slow oscillations over frontal cortex correlated significantly with improved memory consolidation across sleep ($r = .40, p = .02$). Remarkably, this correlation was significant only in the congruent group ($r = .62, p = .04$), but not in the incongruent ($r = .28, p > .40$) or vehicle ($r = -.04, p > .80$; Figure 2B–D) group.

When considering the whole 30-sec period of odor stimulation relative to the preceding 30-sec off period, the group difference remained significant for frontal delta power ($F(2, 30) = 4.57, p = .02$), with a significantly higher value for the congruent compared with the incongruent condition ($p = .02$) and also for the vehicle compared with the congruent condition ($p = .01$). Incongruent and vehicle conditions did not differ ($p > .80$). Parietal fast spindle power also differed significantly between conditions ($F(2, 30) = 3.74, p = .04$). Post hoc pairwise comparisons revealed only a significant difference between the congruent and incongruent conditions ($p = .01$), whereas the incongruent and vehicle conditions ($p > .30$) as well as the congruent and vehicle conditions did not differ significantly ($p = .09$). Frontal slow delta power revealed no difference between groups ($F(2, 30) = 1.40, p > .20$; for detailed values, see Supplementary Table 6). Concerning the changes in frontal slopes, the difference for the entire stimulation interval revealed only a trend for significance ($F(2, 30) = 2.60, p = .09$).

Arousal

To exclude that the reported EEG changes during the presentation of the congruent odor reflect increased intrasleep wakefulness instead of increased neural synchrony, we additionally analyzed EMG and EEG arousal responses during the 10-sec odor-on periods with reference to the previous 10-sec odor-off periods. These analyses revealed that arousal responses did not differ between the three experimental conditions, neither in the number of EMG arousals (congruent: -0.43 ± 0.61, incongruent: -0.57 ± 0.42, vehicle: -0.54 ± 0.62; $F(2, 29) = 0.02, p > .90$) nor in changes in EMG power (congruent: $97.49 \pm 2.44\%$, incongruent: $102.10 \pm 4.38\%$, vehicle: $96.14 \pm 2.01\%$; $F(2, 29) = 1.07, p > .30$).

We could also not find any differences in EEG arousals when considering all arousals between groups for the first 10 sec of the odor-on period compared with the last 10 sec of the odor-off period (congruent: -0.36 ± 0.60, incongruent: -0.20 ± 0.36, vehicle: 0.17 ± 0.34; $F(2, 30) = 0.4, p > .60$) or for the full 30-sec odor-on interval compared with the preceding full 30-sec odor-off interval (congruent: -0.09 ± 0.68, incongruent: -0.50 ± 0.72, vehicle: -0.50 ± 0.65; $F(2, 30) = 0.1, p > .80$; for mean

EEG arousal values, see Supplementary Table 7). Similarly, after high-pass filtering the EEG at 16 Hz and considering only frequencies above 16 Hz, arousal counts for the first 10 sec (congruent: −0.36 ± 0.47, incongruent: −0.50 ± 0.40, vehicle: −0.75 ± 0.30; $F(2, 30) = 0.3$, $p > .70$) and for the full 30-sec interval did not differ significantly between groups (congruent: −0.36 ± 0.61, incongruent: −1.40 ± 0.76, vehicle: −1.58 ± 0.51; $F(2, 30) = 1.1$, $p > .30$; for mean EEG arousal values greater than 16 Hz, see Supplementary Table 8; for examples of raw EEG data, see Supplementary Figure 1).

Odor Stimulation, Odor Sensitivity, and Vigilance

Participants in all conditions underwent on average of 60.6 ± 3.1 odor stimulations during the experimental night. The number of stimulations did not differ between groups ($F(2, 33) = 0.73$, $p > .40$). In all three conditions, the number of stimulations was not correlated with changes in retrieval performance (all ps $> .70$).

The odor detection test performed before the experiment proper required participants to indicate the presence or absence of the experimental odor stimulus on 10 trials. The number of correct responses was, on average, 91.1 ± 1.7% and did not differ between odor and vehicle conditions ($F(2, 33) = 1.10$, $p > .30$). Participants rated on 10-point scales the familiarity, arousal, intensity, valence, and penetrance of the odors. One participant was excluded from this analysis because of data loss. Judgments did not differ between the three experimental groups (all Fs$(2, 32) < 1.81$, all ps $\geq .18$). When divided into groups according to the odor received during learning, appraisals between groups differed. Participants rated the odor citral as more familiar ($t(33) = -4.34$, $p < .001$), more positively valenced ($t(33) = -2.14$, $p = .04$), less arousing ($t(33) = -2.45$, $p = .02$), and more intense ($t(33) = -2.27$, $p = .03$) compared with the odor IBA. The odors did not differ concerning penetrance ($t(33) = 1.02$, $p > .30$).

Valence ratings of the odors did not correlate with memory performance or relative changes of frontal delta power, frontal slow delta power, parietal fast spindle power, or slow oscillation slopes (all ps $> .3$).

RT on the vigilance task during learning was, on average, 277.9 ± 5.10 msec and did not differ between groups ($F(2, 33) = 2.31$, $p = .12$). RT during retrieval was, on average, 270.22 ± 5.55 msec and also did not differ ($F(2, 33) = 1.40$, $p > .20$).

DISCUSSION

Our results show that reexposure during SWS to the same odor presented during learning enhances memory performance and triggers an increase in EEG delta and fast spindle power, in comparison with two control groups receiving either another odor or an odorless vehicle during SWS. Our findings indicate that (a) the same odor during learning and sleep is required for reactivating memories during SWS and for improving retrieval of these memories the next day and that (b) successful reactivation of odor-associated memories during SWS is associated with a specific response of the slow oscillation and fast spindles.

Olfactory stimuli are powerful cues for memories (Willander & Larsson, 2006; Chu & Downes, 2000, 2002; Herz & Schooler, 2002; Herz & Engen, 1996; Herz & Cupchik, 1995; Laird, 1934). The great efficacy of odors to reactivate memories might be a consequence of the close connections of the olfactory cortex to memory-related brain regions like the hippocampus and amygdala. Importantly, there are direct projections from the olfactory cortex to the hippocampus that bypass the thalamus (Gottfried, 2010; Zelano & Sobel, 2005) and might be particularly involved in mediating olfactory stimulation during sleep. Because of these connections, thalamic gating during sleep is expected to affect olfactory processing during sleep to a lesser extent than other sensory modalities. This could result in an increased effectiveness of olfactory cueing during sleep in comparison with memory reactivation induced by other sensory stimuli, for example, auditory cues. Additionally, olfactory stimuli do not disturb ongoing sleep when presented during deeper sleep stages (Carskadon & Herz, 2004).

The memory-improving effect of odor reexposure during SWS is quite robust and has now been replicated in three independent studies including the current one (Diekelmann et al., 2011; Rasch et al., 2007). These studies have also specified that odor reexposure during sleep activates the left hippocampus (Rasch et al., 2007) and results in an immediate stabilization of memory traces, even in the absence of REM sleep (Diekelmann et al., 2011). Another research group found that odor reexposure during sleep after a learning phase also improves creativity (Ritter et al., 2012).

EEG slow-wave activity (SWA) including the 0.5- to 1.5-Hz slow delta and the 1.5- to 4.5-Hz delta band is the hallmark of SWS and has been implicated in declarative memory consolidation during sleep (Diekelmann & Born, 2010). Declarative memory consolidation mainly profits from early, SWS-rich sleep (Drosopoulos, Wagner, & Born, 2005; Plihal & Born, 1999; Yaroush, Sullivan, & Ekstrand, 1971), and learning-dependent increases in SWA have been observed after encoding of declarative and procedural memories (Wilhelm et al., 2011; Huber et al., 2006). Most of the theoretical work on declarative memory consolidation during sleep assumed a crucial role of slow oscillations in this process. Slow oscillations show a prominent spectral peak in the <1-Hz frequency range, whereas the power spectrum particularly of the falling and rising flanks of the slow oscillations includes also faster frequencies in the delta range (>1 Hz). It is proposed that the slow oscillation up state synchronizes hippocampal sharp-wave ripples (which are associated with memory reactivations) with thalamic spindle activity to optimize strengthening of memories on the cortical level (Mölle & Born, 2011; Diekelmann & Born, 2010). This

was confirmed by studies showing that experimentally increasing slow waves by electrical stimulation or tones improves declarative memory consolidation (Ngo et al., 2013; Marshall, Helgadóttir, Mölle, & Born, 2006). Here, we found an increase in delta activity during reactivation of declarative memories, which was accompanied by reduced power in the slow delta band. Further analyses suggested that this shift in power toward higher frequencies primarily reflects an increase in the negative-to-positive slope of slow oscillations largely corresponding to down-to-up-state transition, whereas amplitude of the slow oscillations remained largely unchanged. Thus, this shift in the slow oscillation slopes changes the EEG power from slow delta to delta frequencies while leaving the amplitude and number of the slow oscillations unchanged. Interestingly, the congruent odor changes in delta and spindle power are not accompanied by changes in the number of slow oscillations or discrete sleep spindles. In the case of delta, we believe that the increase in delta activity is induced by a change in slope of the slow oscillations rather than by a change in number or amplitude, which is supported by our slope analysis. For sleep spindles, we can only speculate that the congruent odor increases in spindle power are either only transient or not sufficiently large to induce distinct sleep spindles detected by the spindle detection algorithm. Alternatively, higher spindle power could be indicative of a stronger functional efficacy of sleep spindles, for example, in the temporal grouping of hippocampal sharp wave/ripples, which does not necessarily need to express in a higher number of spindles. The change in slow oscillation slopes significantly predicted memory retrieval after sleep, however, only in the group with congruent odor stimulation, that is, the group with effective reactivation of memories. Slopes of slow oscillations have been associated with processes of synchronization on the neural level in studies using simultaneous recordings of EEG and multiunit activity (Vyazovskiy et al., 2009, 2011). Thus, both the reactivation-induced shift of power toward higher frequencies in the delta band and the associated increase in slow oscillation slopes together with an increase in parietal fast spindle power might reflect an increase in neural synchrony in cortical neurons triggered by induced memory reactivations during sleep. This increased neural synchrony might well favor synaptic plastic processes mediating the observed enhancement in memory. As a speculation, the degree of cortical synchronization induced by reactivation during sleep could be relevant for improvements in offline memory consolidation. Alternatively and/or simultaneously, induced memory reactivation could directly facilitate or trigger other processes supporting the consolidation of the newly learned information during sleep. Interestingly, the changes that we observed in sleep oscillations on congruent odor reactivation were most pronounced during the first 10 sec of odor stimulation. Similar but weaker changes were visible during the entire 30-sec period of odor presentation, suggesting that external reactivation cues might be most effective in facilitating consolidation processes shortly after stimulation onset, with longer stimulation periods not providing additional benefits.

Whereas it is widely accepted that the neocortical slow oscillation exerts a top–down influence on thalamic and hippocampal activity, which synchronizes thalamo-cortical spindles and hippocampal memory reactivations to the slow oscillation up state (Mölle & Born, 2011; Ji & Wilson, 2007; Wolansky, Clement, Peters, Palczak, & Dickson, 2006), it is currently a matter of debate whether hippocampal memory reactivations and associated sharp-wave ripples can exert a converse "bottom–up" control on spindles and the neocortical slow oscillation. Correlational analyses of the temporal relationships between hippocampal and neocortical activity revealed increases in sharp-wave ripples associated with the developing slow oscillation up state, consistent with the view that sharp-wave ripples contribute to the induction and maintenance of widespread depolarization in cortical networks characterizing the slow oscillation up state (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009; Mölle, Yeshenko, Marshall, Sara, & Born, 2006; Sirota, Csicsvari, Buhl, & Buzsáki, 2003). However, those analyses basically remain inconclusive with regard to the direction of the influence between neocortex and hippocampus. Assuming that congruent odor presentation during SWS specifically acts to enhance hippocampal memory reactivations and the number of associated sharp-wave ripples (although see Bendor & Wilson, 2012), the present data can be taken as a first hint that hippocampal reactivations by producing an enhanced information transfer to higher cortical networks indeed causally contribute, in a bottom–up manner, to the formation of cortical slow oscillation up states. The effect expressing itself mainly in a steeper slow oscillation slope (and in corresponding increases in delta power) rather than in increased slow oscillation amplitude suggests that hippocampal reactivation primarily contributes to synchronizing activity in distributed neocortical networks during the excitable up state of the slow oscillation. Such influence might help optimizing plastic synaptic processes underlying the storage of reactivated memory information in neocortical regions, although this scenario is in need of further experimental elaboration.

The power changes in the delta band during odor administration are specific to the reactivation of memory-related contents, as no such changes were observed when participants were exposed to a different odor during sleep than during learning. In addition, our study confirms that unspecific olfactory stimulation during SWS is not sufficient to reactivate memories previously associated with an olfactory context. Only when the same odor was present during learning and subsequent SWS, memory retrieval was improved on the next day. Such context specificity of cueing is known from previous research on olfactory context effects during wakefulness: When participants learned word lists or pictures in a certain odor context, retrieval performance was improved only in the presence of the

same odor but not with an odor different from that present during learning (Smith, 1992; Schab, 1990; Cann & Ross, 1989). Concurring with the present findings, the positive or negative valence of the odors was unrelated to the context-related memory improvement in these studies. Furthermore, individual valence ratings of the odors did not correlate with subsequent changes in memory or sleep parameters. Thus, as expected, odor reexposure during sleep shares properties known from context effects on memory recall during wakefulness, although active retrieval is omitted during sleep.

Although we cannot fully exclude any possible effects of arousals on changes in memory and oscillatory activity, such effects are unlikely given our findings from several additional analyses, namely, visual scoring of EEG arousals, analyses of arousal-related power bands, and analyses of frequencies higher than 16 Hz. Most importantly, the number of arousals was comparable in all experimental groups. Participants who received the same odor during learning and during sleep showed the same amount of arousals than participants who received a different odor during sleep than during learning and participants who were presented with an odorless vehicle. Arousal counts were also comparable when considering only high-frequency arousals with frequencies larger than 16 Hz. Furthermore, groups did not differ in power of any of the frequency bands that are classically associated with arousal responses, namely, in the theta, alpha, beta, and gamma frequency bands. The oscillations for which the congruent odor induced an increase in power, that is, the delta band, fast spindle band, and slow oscillation slopes, are not typically related to arousal responses. On the contrary, some researchers even define the suppression of delta power and spindle power as a marker of arousals (e.g., Cho, Joo, Koo, & Hong, 2013; Wulbrand, McNamara, & Thach, 1998). Additionally, all of the observed changes in oscillatory activity as well as in memory performance were only evident on cueing with the memory-related congruent odor but not with the unrelated incongruent odor, excluding any unspecific arousal effects on odor stimulation.

Taken together, reexposure during SWS to an odor that was already present during prior learning improves memory, enhances fast spindle power, and shifts SWA toward faster oscillations. The latter is reflected in an increase in the slopes of the slow oscillation, which points to increased synchrony at the neuronal level. Thus, we present novel evidence that experimentally induced reactivations of hippocampus-dependent memory shape slow delta activity associated with the consolidation process. The exact mechanisms of this shaping influence need to be elaborated in rodent models of hippocampal memory consolidation.

Acknowledgments

We thank Ingeborg Schroer and Christina Fuhrmann for assistance in data collection and analysis and Maurice Göldi and Peter O'Connor for help in programming. This work was supported by grants from the Swiss National Foundation (PP00P1_133685) and the German Research Foundation (DFG, SFB 654).

Reprint requests should be sent to Björn Rasch, Division of Cognitive Biopsychology and Methods, Department of Psychology, University of Fribourg, Rue P.-A.-Faucigny 2, CH-1701 Fribourg, Switzerland, or via e-mail: Bjoern.Rasch@unifr.ch.

REFERENCES

Antony, J. W., Gobel, E. W., O'Hare, J. K., Reber, P. J., & Paller, K. A. (2012). Cued memory reactivation during sleep influences skill learning. *Nature Neuroscience, 15,* 1114–1116.

ASDA (American Sleep Disorders Association and Sleep Disorders Society). (1992). Atlas Task Force report EEG arousals: Scoring rules and examples. *Sleep, 15,* 173–184.

Bendor, D., & Wilson, M. A. (2012). Biasing the content of hippocampal replay during sleep. *Nature Neuroscience, 15,* 1439–1444.

Bergmann, T. O., Mölle, M., Diedrichs, J., Born, J., & Siebner, H. R. (2012). Sleep spindle-related reactivation of category-specific cortical regions after learning face–scene associations. *Neuroimage, 59,* 2733–2742.

Bölsterli, B. K., Schmitt, B., Bast, T., Critelli, H., Heinzle, J., Jenni, O. G., et al. (2011). Impaired slow wave sleep downscaling in encephalopathy with status epilepticus during sleep (ESES). *Clinical Neurophysiology, 122,* 1779–1787.

Cann, A., & Ross, D. A. (1989). Olfactory stimuli as context cues in human memory. *The American Journal of Psychology, 102,* 91–102.

Carskadon, M. A., & Herz, R. S. (2004). Minimal olfactory perception during sleep: Why odor alarms will not work for humans. *Sleep, 27,* 402–405.

Cho, J. R., Joo, E. Y., Koo, D. L., & Hong, S. B. (2013). Let there be no light: The effect of bedside light on sleep quality and background electroencephalographic rhythms. *Sleep Medicine, 14,* 1422–1425.

Chu, S., & Downes, J. J. (2000). Long live Proust: The odour-cued autobiographical memory bump. *Cognition, 75,* B41–B50.

Chu, S., & Downes, J. J. (2002). Proust nose best: Odors are better cues of autobiographical memory. *Memory & Cognition, 30,* 511–518.

De Gennaro, L., Ferrara, M., & Bertini, M. (2001). EEG arousals in normal sleep: Variations induced by total and selective slow-wave sleep deprivation. *Sleep, 24,* 673–679.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience, 11,* 114–126.

Diekelmann, S., Büchel, C., Born, J., & Rasch, B. (2011). Labile or stable: Opposing consequences for memory when reactivated during waking and sleep. *Nature Neuroscience, 14,* 381–386.

Drosopoulos, S., Wagner, U., & Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learning & Memory, 12,* 44–51.

Fogel, S. M., Nader, R., Cote, K. A., & Smith, C. T. (2007). Sleep spindles and learning potential. *Behavioral Neuroscience, 121,* 1–10.

Fridlund, A. J., & Cacioppo, J. T. (1986). Guidelines for human electromyographic research. *Psychophysiology, 23,* 567–589.

Gais, S., Mölle, M., Helms, K., & Born, J. (2002). Learning-dependent increases in sleep spindle density. *The Journal of Neuroscience, 22,* 6830–6834.
Gottfried, J. A. (2010). Central mechanisms of odour object perception. *Nature Reviews Neuroscience, 11,* 628–641.
Herz, R. S., & Cupchik, G. C. (1995). The emotional distinctiveness of odor-evoked memories. *Chemical Senses, 20,* 517–528.
Herz, R. S., & Engen, T. (1996). Odor memory: Review and analysis. *Psychonomic Bulletin & Review, 3,* 300–313.
Herz, R. S., & Schooler, J. W. (2002). A naturalistic study of autobiographical memories evoked by olfactory and visual cues: Testing the Proustian hypothesis. *The American Journal of Psychology, 115,* 21–32.
Huber, R., Ghilardi, M. F., Massimini, M., Ferrarelli, F., Riedner, B. A., Peterson, M. J., et al. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nature Neuroscience, 9,* 1169–1176.
Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience, 10,* 100–107.
Kessels, R. P., de Haan, E. H., Kappelle, L. J., & Postma, A. (2001). Varieties of human spatial memory: A meta-analysis on the effects of hippocampal lesions. *Brain Research Brain Research Reviews, 35,* 295–303.
Laird, D. A. (1934). Some normal odor effects and associations of psychoanalytic significance. *Psychoanalytic Review, 21,* 194–200.
Little, J. T., Johnson, D. N., Minichiello, M., Weingartner, H., & Sunderland, T. (1998). Combined nicotinic and muscarinic blockade in elderly normal volunteers: Cognitive, behavioral, and physiologic responses. *Neuropsychopharmacology, 19,* 60–69.
Lorig, T. S. (2000). The application of electroencephalographic techniques to the study of human olfaction: A review and tutorial. *International Journal of Psychophysiology, 36,* 91–104.
Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature, 444,* 610–613.
Mölle, M., Bergmann, T. O., Marshall, L., & Born, J. (2011). Fast and slow spindles during the sleep slow oscillation: Disparate coalescence and engagement in memory processing. *Sleep, 34,* 1411–1421.
Mölle, M., & Born, J. (2011). Slow oscillations orchestrating fast oscillations and memory consolidation. *Progress in Brain Research, 193,* 93–110.
Mölle, M., Yeshenko, O., Marshall, L., Sara, S. J., & Born, J. (2006). Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *Journal of Neurophysiology, 96,* 62–70.
Ngo, H.-V. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*.
Nishida, M., & Walker, M. P. (2007). Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PloS One, 2,* e341.
Oudiette, D., Antony, J. W., Creery, J. D., & Paller, K. A. (2013). The role of memory reactivation during wakefulness and sleep in determining which memories endure. *The Journal of Neuroscience, 33,* 6672–6678.
Oudiette, D., & Paller, K. A. (2013). Upgrading the sleeping brain with targeted memory reactivation. *Trends in Cognitive Sciences, 17,* 142–149.
Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuroscience, 12,* 919–926.
Plihal, W., & Born, J. (1999). Effects of early and late nocturnal sleep on priming and spatial memory. *Psychophysiology, 36,* 571–582.
Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews, 93,* 681–766.
Rasch, B., Büchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science, 315,* 1426–1429.
Rechtschaffen, A., & Kales, A. (1968). *A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects*. Los Angeles: Brain Information Service/Brain Research Institute, University of California.
Riedner, B. A., Vyazovskiy, V. V., Huber, R., Massimini, M., Esser, S., Murphy, M., et al. (2007). Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep, 30,* 1643–1657.
Ritter, S. M., Strick, M., Bos, M. W., van Baaren, R. B., & Dijksterhuis, A. (2012). Good morning creativity: Task reactivation during sleep enhances beneficial effect of sleep on creative performance. *Journal of Sleep Research, 21,* 643–647.
Rudoy, J. D., Voss, J. L., Westerberg, C. E., & Paller, K. A. (2009). Strengthening individual memories by reactivating them during sleep. *Science, 326,* 1079.
Saletin, J. M., Goldstein, A. N., & Walker, M. P. (2011). The role of sleep in directed forgetting and remembering of human memories. *Cerebral Cortex, 21,* 2534–2541.
Schab, F. R. (1990). Odors and the remembrance of things past. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 16,* 648–655.
Schab, F. R. (1991). Odor memory: Taking stock. *Psychological Bulletin, 109,* 242–251.
Schabus, M., Dang-Vu, T. T., Albouy, G., Balteau, E., Boly, M., Carrier, J., et al. (2007). Hemodynamic cerebral correlates of sleep spindles during human non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences, U.S.A., 104,* 13164–13169.
Schimicek, P., Zeitlhofer, J., Anderer, P., & Saletu, B. (1994). Automatic sleep-spindle detection procedure: Aspects of reliability and validity. *Clinical EEG (Electroencephalography), 25,* 26–29.
Sirota, A., Csicsvari, J., Buhl, D., & Buzsáki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences, U.S.A., 100,* 2065–2069.
Smith, D. G. (1992). Verbal memory elicited by ambient odor. *Perceptual and Motor Skills, 74,* 339.
Sommer, T., Rose, M., Gläscher, J., Wolbers, T., & Büchel, C. (2005). Dissociable contributions within the medial temporal lobe to encoding of object-location associations. *Learning & Memory, 12,* 343–351.
Vyazovskiy, V. V., Cirelli, C., & Tononi, G. (2011). Electrophysiological correlates of sleep homeostasis in freely behaving rats. *Progress in Brain Research, 193,* 17–38.
Vyazovskiy, V. V., Olcese, U., Lazimy, Y. M., Faraguna, U., Esser, S. K., Williams, J. C., et al. (2009). Cortical firing and sleep homeostasis. *Neuron, 63,* 865–878.
Wilhelm, I., Diekelmann, S., Molzow, I., Ayoub, A., Mölle, M., & Born, J. (2011). Sleep selectively enhances memory expected to be of future relevance. *The Journal of Neuroscience, 31,* 1563–1569.
Willander, J., & Larsson, M. (2006). Smell your way back to childhood: Autobiographical odor memory. *Psychonomic Bulletin & Review, 13,* 240–244.

Wolansky, T., Clement, E. A., Peters, S. R., Palczak, M. A., & Dickson, C. T. (2006). Hippocampal slow oscillation: A novel EEG state and its coordination with ongoing neocortical activity. *The Journal of Neuroscience, 26,* 6213–6229.

Wulbrand, H., McNamara, F., & Thach, B. T. (1998). Suppression of sigma spindle electroencephalographic activity as a measure of transient arousal after spontaneous and occlusion-evoked sighs and startles. *Pediatric Research, 44,* 767–773.

Yaroush, R., Sullivan, M. J., & Ekstrand, B. R. (1971). Effect of sleep on memory: II. Differential effect of the first and second half of the night. *Journal of Experimental Psychology, 88,* 361–366.

Zeitlhofer, J., Gruber, G., Anderer, P., Asenbaum, S., Schimicek, P., & Saletu, B. (1997). Topographic distribution of sleep spindles in young healthy subjects. *Journal of Sleep Research, 6,* 149–155.

Zelano, C., & Sobel, N. (2005). Humans as an animal model for systems-level organization of olfaction. *Neuron, 48,* 431–454.