REM Sleep Preserves Affective Response to Social Stress—Experimental Study

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Abstract

Sleep's contribution to affective regulation is insufficiently understood. Previous human research has focused on memorizing or rating affective pictures and less on physiological affective responsivity. This may result in overlapping definitions of affective and declarative memories and inconsistent deductions for how rapid eye movement sleep (REMS) and slow-wave sleep (SWS) are involved. Literature associates REMS theta (4–8 Hz) activity with emotional memory processing, but its contribution to social stress habituation is unknown. Applying selective sleep stage suppression and oscillatory analyses, we investigated how sleep modulated affective adaptation toward social stress and retention of neutral declarative memories. Native Finnish participants ($N = 29$; age, $M = 25.8$ years) were allocated to REMS or SWS suppression conditions. We measured physiological (skin conductance response, SCR) and subjective stress response and declarative memory retrieval thrice: before laboratory night, the next morning, and after 3 d. Linear mixed models were applied to test the effects of condition and sleep parameters on emotional responsivity and memory retrieval. Greater overnight increase in SCR toward the stressor emerged after suppressed SWS (intact REMS) relative to suppressed REMS (20.1% vs 6.1%; $p = 0.016$). The overnight SCR increase was positively associated with accumulated REMS theta energy irrespective of the condition ($r = 0.601$; $p = 0.002$). Subjectively rated affective response and declarative memory recall were comparable between the conditions. The contributions of REMS and SWS to habituation of social stress are distinct. REMS theta activity proposedly facilitates the consolidation of autonomic affective responses. Declarative memory consolidation may not have greater dependence on intact SWS relative to intact REMS.

Key words: affective regulation; REM sleep; sleep suppression; slow-wave sleep; SO–spindle coupling; theta power

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Significance Statement

Disrupted sleep is a common problem with negative effects on affective regulation. While research indicates that rapid eye movement sleep (REMS) has a central role in off-line affective processing, the mechanisms are not well defined. We used selective sleep stage suppression to investigate how disrupted sleep- and stage-specific neural activity modulated the affective responsivity toward a self-conscious stressor inducing shame. We show that theta band oscillatory activity during REMS is especially important for preserving the physiological stress response overnight. Understanding sleep-driven affective regulation facilitates development of applications aiming at improving mental well-being.

Introduction

Sleep stages contribute distinctively to processing recent experiences and memories. Nonrapid eye movement sleep (NREMS) and especially its deepest stage, slow-wave sleep (SWS), facilitate declarative memory consolidation via synchronized occurrence

of slow oscillations (SOs) and sleep spindles (Klinzing et al., 2019). On the other hand, emotional processing is often attributed to rapid eye movement sleep (REMS). According to the sleep-to-remember, sleep-to-forget (SRSF) hypothesis, REMS contributes specifically to the depotentiation of the emotional charge linked to memories (i.e., "forgetting"; Walker and van der Helm, 2009). This dilution of potentially distressing experiences has been suggested to be important for mental health (Wassing et al., 2016), and some empirical evidence supports the hypothesis (Gujar et al., 2011; Rosales-Lagarde et al., 2012). However, a number of studies claim the opposite, i.e., that REMS preserves the affective component of memories (Lara-Carrasco et al., 2009; Pace-Schott et al., 2011; Baran et al., 2012; Werner et al., 2015, 2021). To better understand emotional regulation during REMS, further experimental studies with variable affective induction and active manipulation of potential mechanisms are warranted.

A powerful experimental method for disentangling sleep stage-specific effects is selective sleep stage deprivation/suppression. In human studies, suppressing nocturnal REMS induced increased amygdala response toward social exclusion (Glosemeyer et al., 2020), impaired fear extinction (Spoormaker et al., 2012), and habituation to threatening visual stimuli (Rosales-Lagarde et al., 2012). However, contrasting findings also exist. For instance, REMS deprivation decreased subjective arousal ratings toward aversive pictures the next morning (Lara-Carrasco et al., 2009; Werner et al., 2021), although in one study, the short-term effect was inverted after 2 d (Werner et al., 2021).

Most of these earlier suppression studies have examined memory retention, or subjective rating, of affective pictures, leaving it open how the physiological stress response is modulated during sleep. There is little evidence on how sleep explains the adaptation to, or preservation of, physiological affective response in humans. Only one study (Spoormaker et al., 2012) investigated how sleep stage suppression influenced physiological stress response. In that study, REMS-deprived participants showed impaired fear memory extinction reflected by increased electrodermal response. In addition, previous sleep suppression studies have not examined how neural activity markers in sleep, such as spectral power in electroencephalography (EEG), influence emotional reactivity overnight.

Animal models have provided evidence that EEG dynamics predict affective processing in intact sleep. Intracortical recordings in rats suggest that especially theta ($\sim$4–8 Hz) oscillations during REMS stand out in this regard. For instance, increased REMS theta coherence between the amygdala and hippocampus or medial prefrontal cortex predicted stronger fear memory consolidation (Popa et al., 2010). Another study found that REMS theta activity increased after successful avoidance task training (Fogel et al., 2009). Theta activity is proposedly linked to pontine–geniculo–occipital waves (Hutchison and Rathore, 2015) reflecting hippocampal–amygdala synchrony (Karashima et al., 2010) and promoting synaptic plasticity processes and emotional learning (Mavanji et al., 2004; Datta et al., 2008). Pontine–geniculo–occipital waves are unmeasurable in humans with scalp EEG. In human studies, EEG-measured REMS theta power (Prehn-Kristensen et al., 2013; Kim et al., 2020) and its right-hemisphere lateralization (Nishida et al., 2009; Sopp et al., 2017) have predicted better retention of especially emotional memory content.

To delineate sleep's impact on emotional processing, an experiment should also assess declarative memory. Declarative memory consolidation depends on the oscillatory components of NREMS, particularly SWS (Léger et al., 2018; Klinzing et al., 2019). Numerous studies have shown that sleep spindles (Clemens et al., 2005) and their coupling to SOs (Helfrich et al., 2018; Mikutta et al., 2019; Muehlroth et al., 2019; Halonen, Kuula, Antila, et al., 2021; Weiner et al., 2024) predict memory retention. Given this, it is rather unexpected that selective suppressing of SWS has not conclusively resulted in impaired memory retention in previous studies. For example, the retention of verbal (Genzel et al., 2009; Casey et al., 2016) or visual (Wiesner et al., 2015) memories was not differentially affected by SWS or REMS suppression. In one study, however, suppressed SWS impaired visuospatial memory (Casey et al., 2016). In these studies, either sleep spindles did not predict memory outcome (Casey et al., 2016), or their relevance in learning was N2-specific (Genzel et al., 2009). Examination of SO–spindle coupling in sleep suppression studies is lacking overall.

In an earlier study, a higher overnight REMS percentage predicted preserved physiological response to social stress-related shame (Halonen, Kuula, Makkonen, et al., 2021). The current study went further to investigate how selective sleep stage suppression, REMS or SWS, affected habituation to a stressor inducing self-conscious affect. To better delineate the affective processing component, we also included a declarative memory task. For that purpose, we applied a memory paradigm using novel metaphors, which has been shown to depend on SO–spindle coupling in SWS and N2 (Halonen, Kuula, Antila, et al., 2021).

We assumed that manipulating REMS would impact off-line affective processing and that the overnight change would be associated with REMS theta activity. However, the direction of the effect cannot be predicted based on existing human studies. Regarding declarative memory, we did not expect suppression effects. Instead, we assumed that SO-coupled spindle events would positively predict postsleep performance and that N2 would compensate for reduced SWS coupling events.

Materials and Methods

Participants. The sample consisted of 29 young adults (14 males, 15 females, self-disclosed; age, $M = 25.8$ years; $SD = 4.5$ years; range, 19.0–36.4 years) living in the capital area of Finland. Seven participants were psychology undergraduates, thirteen were recruited from academic hobby societies, and nine were recruited from a local sports club. Compensation of €100 was provided to all participants. Measurements were performed in May–November 2021. The participants answered questionnaires for background information and physical and mental well-being. None of the

participants met the exclusion criteria (severe anxiety, depression or sleep disorders, or major neurological conditions). Participants were randomly allocated to either REMS suppression (REMS$_{SUPPR}$; $n = 15$) or SWS suppression (SWS$_{SUPPR}$; $n = 14$) condition, however, such that sex distribution did not differ between the conditions. Participants were blind to the condition.

All participants provided written informed consents prior to participation. The study protocol was approved by Helsinki University Hospital Ethics Committee (HUS/1390/2019), and all components of the study were conducted in accordance with the Declaration of Helsinki and its later amendments.

Study flow and sleep suppression. Participants were asked to maintain a regular sleep rhythm for 3 d prior to the study. On Day 0 (Fig. 1), participants visited the laboratory to obtain a ZMAX device (Hypnodyne) for in-home sleep recording (Baseline night). Briefing was given via Zoom. The following day (Day 1), at the laboratory, participants sang a karaoke version of Abba's Dancing Queen, which was recorded. After spending the day in their normal daily routines, the participants arrived at the laboratory at 9 P.M. and underwent memory encoding, the first karaoke playback and finally the first metaphor retrieval. The evening test session took ~45 min. Polysomnography (PSG) was setup. Sleep opportunity was given between 11 P.M. and 7 A.M. Depending on the suppression condition (REMS$_{SUPPR}$ / SWS$_{SUPPR}$), the research assistant woke up the participant when distinct REMS-related rapid eye movements or obvious SWS (i.e., the majority of an epoch consisting of approximately <~1 Hz oscillations) were detected on the real-time PSG. The awakenings were done via in-room loudspeaker with progressive intensity: (1) a melody (marimba), (2) calling the participant's name, (3) asking to sit, or (4) presenting simple math problems. If the participant did not wake up with, e.g., marimba, or drifted to the to-be-suppressed sleep stage in a few minutes, a higher intensity step (e.g., calling their name) was applied. On Day 2 morning (>30 min after awakening), a second memory retrieval and karaoke playback took place. On Day 5 (~10 A.M.), the final metaphor retrieval and karaoke playback were administered (Fig. 1). The test sessions on Day 2 and Day 5 took ~25 min each.

Memory task. The memory task was run with the Presentation software 22.0 (Neurobehavioral Systems). The task material consisted of 60 novel verbal metaphors, obtained from a study examining psycholinguistic dimensions (Herkman and Servie, 2013). The metaphors were divided into two subsets of 30 metaphors each: pre- and postsleep recall. We matched the subsets in terms of the normative ease (Herkman and Servie, 2013) dimension (means, 3.88 and 3.84; *t* test; $p = 0.854$), with ranges of 2.39–5.29 and 2.42–5.34 in the subsets. This dimension has been shown to be associated with recall probability (Halonen, Kuula, Antila, et al., 2021).

During encoding, 60 written metaphors were shown on a laptop screen, e.g., "Touch is an insect," for 3,000 ms with a 1,000 ms interval. Participants were instructed to form a mental image of the metaphors. Participants underwent three successive encoding rounds.

In the cued recall, participants were shown the beginning of a metaphor, e.g., "Touch is an", in a random order and were prompted to type the missing word (e.g., "insect"). Responses were manually scored so that 1 point was given for a correct word and 0.5 points if the response was a synonym (e.g., "bug") or a higher/lower abstraction (e.g., "mosquito").
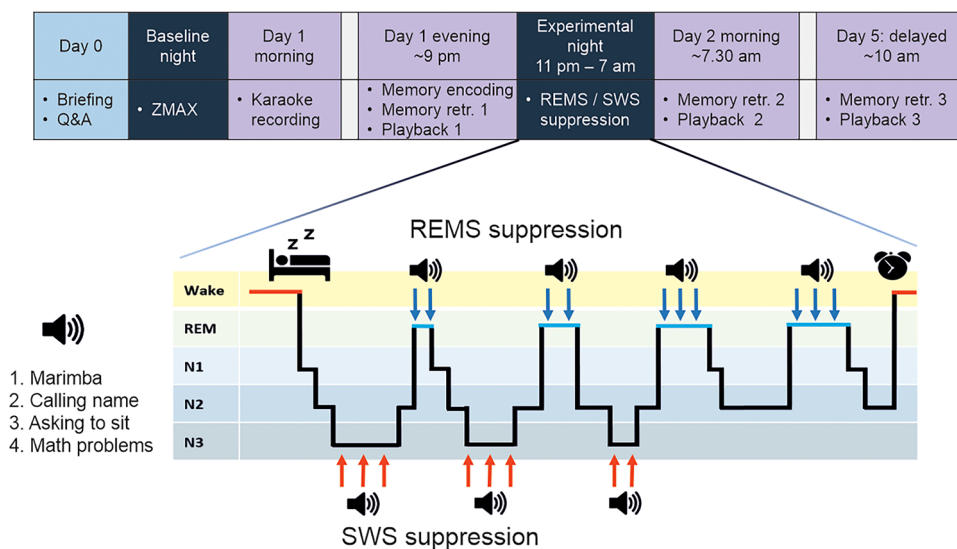
Figure 1. An overview of the study process and the suppression protocol. During the experimental night, sleep was disrupted when participants entered the sleep stage of their respective suppression condition (REMS$_{SUPPR}$ / SWS$_{SUPPR}$). In all playbacks, participants' own singing was played to them via loudspeakers, and physiological and subjective stress responses were measured. In memory retrievals, a subset of encoded metaphors was tested with cued recall.

Recall was tested ~30 min (Day 1), ~12 h (Day 2), and 4 d (Day 5) after encoding (Fig. 1). The presentation order of pre- and postsleep subsets was counterbalanced. On Day 1 and 2 retrievals, one subset (30 metaphors) was tested, and Day 5 retrieval was administered with all 60 metaphors. Recall outcome was the percentage of correct answers out of all trials. Due to technical problems, one participant's recall data was lost in all retrievals and another participant's data in Day 2 retrieval.

Self-conscious affect. To induce shame, we used a karaoke-based paradigm. On Day 1 morning (Fig. 1), the participants sang a karaoke version of Abba's Dancing Queen without hearing their own voice through headphones, promoting out-of-tune singing. The experimenter created a ~1.5 min compilation of the recording without background music using the Audacity 3.0.2 software. The compilation consisted of three selected clips with 5 s silent intervals between the clips.

Both physiological and subjective affective responses to the karaoke playback were measured on Day 1 evening, Day 2 morning, and Day 5 (Fig. 1). Physiological response was assessed with the skin conductance level (SCL). SCL is a measure of tonic electrical conductivity of the skin, and its phasic changes (skin conductance responses, SCRs) reflect autonomic arousal (Braithwaite et al., 2013) and are used experimentally as an accurate marker of stress (Rahma et al., 2022). SCL was measured from the middle and ring fingers of the nondominant hand using a galvanic skin sensor, connected to a Brain Products QuickAmp amplifier (Brain Products). SCL was recorded at a 500 Hz sampling rate and analyzed using MATLAB R2022b (MathWorks). Baseline SCL was obtained from the participant sitting quietly for 5 min; the average baseline measure was calculated after 2 min of quiet sitting, ending 20 s before the playback start (averaged baseline SCL = baseline start +120 s : baseline end −20 s). Next, the participants listened to their singing compilation via a loudspeaker, the experimenter being present. SCL during the playback was averaged. The playback was followed by a 5 min period of quiet sitting. SCR was calculated by dividing playback SCL by baseline SCL and controlling for baseline SCL. Finally, SCR values were square root transformed due to skewedness (Braithwaite et al., 2013).

Subjective embarrassment rating was enquired after each playback: (1) "How ashamed did you feel during the playback?" and (2) "How stressful was it to listen to the playback?". Rating scale was 0–4 (none–highly). Subjective embarrassment was the mean value of the two ratings and was calculated separately for each playback. No explicit memory tests were administered regarding the karaoke task (singing or playback).

PSG and sleep fragmentation. All recordings were performed using SOMNOscreen HD (SOMNOmedics) with online monitoring. Gold cup electrodes were attached at six EEG locations (frontal, F3, F4; central, C3, C4; occipital, O1, O2) and at the mastoids (A1, A2). The electrooculogram and the electromyogram were measured using disposable adhesive electrodes (Ambu Neuroline 715, Ambu), two locations for the electrooculogram and three for the electromyogram. An online reference Cz and a ground electrode on the forehead were used. The sampling rate was 256 Hz. PSG data were scored manually using the DOMINO program (v2.9; SOMNOmedics) in 30 s epochs into N1, N2, N3 (SWS), REMS, and wake (American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events). Additionally, in the sleep epochs, we visually identified artifact segments, i.e., pronounced EEG bursts caused by an extracerebral source, e.g., muscle activity and body movements and short arousals (Kane et al., 2017).

To enable comparison between the suppression conditions, we calculated sleep fragmentation. REMS fragmentation was defined as the time spent in either wake, NREMS, or arousals during REMS episodes divided by REMS duration. The first REMS epoch denoted the start of a REMS episode, and the episode ended with the first of at least four continuous minutes of wake or NREMS. SWS episodes and fragmentation were defined otherwise similarly, but SWS episodes concluded with a REMS epoch or 4 min of continuous wake, N1 or N2.

Sleep spindle detection. The PSG signals were converted to EDF format in the DOMINO software (SOMNOmedics) and then further preprocessed using EEGLAB 14.1.2b (Delorme and Makeig, 2004) running on MATLAB R2018a. Prescored epochs with major (>8 s) artifacts or contact impedance >30 kOhm in the target electrode were excluded from further analyses. All artifact-free signals were digitally bandpass filtered off-line from 0.2 to 35 Hz [Hamming windowed sinc zero-phase finite impulse response (FIR) filter; cutoff, −6 dB], at 0.1 and 35.1 Hz, respectively, and rereferenced to the average signal of the mastoid electrodes.

In this study, we confined our examination to fast sleep spindles due to their documented tendency for SO coupling and their role in memory consolidation (Mölle et al., 2011; Rasch and Born, 2013; Muehlroth et al., 2019; Halonen et al., 2022). Fast sleep spindles were detected in central electrodes according to their topological distribution (De Gennaro and Ferrara, 2003). We used individual frequency bands for spindle detection. First, for each participant, we detrended their power spectral density (PSD) curve and extracted the PSD peak frequency within the sigma range (9–16 Hz). In case two sigma peaks were visually observed, we selected the one with a higher frequency. The sample's mean peak frequency for fast spindle detection was 13.47 (SD, 0.56) Hz. Next, we detected sleep spindles with an automated algorithm adapted from Ferrarelli et al. (2007) using the Wonambi EEG analysis toolbox (Piantoni and O'Byrne, 2023; https://wonambi-python.github.io/). EEG data were bandpass filtered with a zero-phase equiripple Chebyshev FIR filter in individual mean peak frequency ±2 Hz. The threshold values for finding the channel-wise spindle peak amplitude were defined by multiplying the mean channel amplitude (µV) by 5. The putative spindle's amplitude was required to stay over the mean channel amplitude multiplied by 2 for 250 ms in both directions from the peak. The maximum cutoff for spindle length

was 3.0 s. We ran the detection separately for N2 and SWS epochs and excluded spindle-like bursts that occurred during artifacts. Fast spindle density was calculated by dividing the total spindle number by minutes spent in N2/SWS.

SO detection. SOs were detected in central electrodes with an algorithm adapted from Ngo et al. (2015) using the Wonambi toolbox (Piantoni and O'Byrne, 2023). The EEG signal was first low-pass filtered at 3.5 Hz. All negative and positive amplitude peaks were identified between consecutive positive-to-negative zero-crossings. Zero-crossing intervals within 0.8–5 s (0.2–1.25 Hz) were included. Mean values for positive and negative peak potentials were calculated, and events were denoted as SOs where the negative peak was lower than the mean negative peak multiplied by 0.9 and where the positive-to-negative peak amplitude difference exceeded the mean amplitude difference multiplied by 0.9. We ran the detection separately on N2 and SWS epochs.

SO–spindle coupling. We identified fast spindles where the amplitude peaked within a SO cycle (SO-coupled spindles). Next, we bandpass filtered the EEG signal to 0.2–1.25 Hz, and after Hilbert transformation, extracted the instantaneous phase at the SO-coupled spindle peaks. Channel-wise resultant vector length and circular mean phase were calculated using Python implementation of the CircStat toolbox (Berens, 2009; https://github.com/circstat/pycircstat). Finally, we calculated the number of SO-coupled spindles that peaked within ±0.5 radians from the individual mean phase (optimally coupled spindles, OC-spindles). Resultant vector length and OC-spindle values between central electrodes were averaged. The procedure was conducted separately on N2 and SWS epochs (Figure 2).

Power spectral density. PSD was calculated for N2, REMS, and SWS epochs in frontal channels using "spectopo" function of EEGLAB with a window length of 1,024 samples (4 ms) and an overlap of 50% resulting in bins with 0.25 Hz resolution. We averaged the PSD values into 1 Hz bins between 0 and 30 Hz and further between F4 and F3 electrodes. Next, we z-scored the stage-specific 1 Hz PSD bin values across all bins and participants and linearly transferred them into positive range (minimum value of 1) and summed the accumulated power across all REM/SWS epochs (PSD energy). The REMS/SWS bin-wise sum scores were controlled for mean PSD (across 0–30 Hz) of which the effects of the suppression condition and sleep stage duration were partialled out. This was done in order to control for amplitude variation due to variable conductance (e.g., skull and skin properties). Finally, we averaged the 1 Hz bins to SO delta (≤ 4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), beta1 (16–22 Hz), and beta2 (22–30 Hz) ranges.

Questionnaires. Prior to participation, the participants filled in questionnaires for shame-proneness (Test of Self-Conscious Affect-3, Finnish version; Tangney et al., 2000), depression symptoms (Beck Depression Inventory II; Beck et al., 1996), and anxiety symptoms (Generalized Anxiety Disorder 7; Williams, 2014). Additionally, the highest achieved education level was queried (1, primary/lower secondary education; 2, upper secondary education; 3, bachlor's degree; 4, master's degree).

Participants were screened for their experience in performing and singing with the questions "Previous performing experience, e.g., speeches, presentations, acting, or singing?" and "Do you sing at leisure time or work?" on a scale of 1–5 (none–very much). The mean score was denoted as "singing experience."

Statistical power. No a priori power analyses were conducted. However, the sample size (*N* = 29) paralleled experimental studies applying mixed (Rosales-Lagarde et al., 2012; Spoormaker et al., 2012; Halonen, Kuula, Makkonen, et al., 2021)
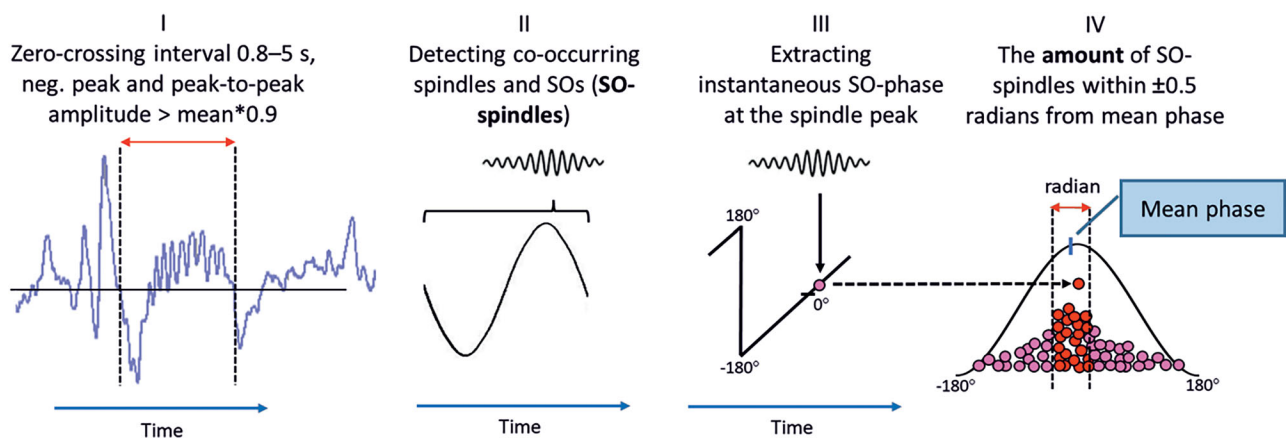

Figure 2. Quantifying SO–spindle coupling. *I*, Detecting SOs. *II*, Detecting spindles that peak within the SO cycle. *III*, After Hilbert transformation, extracting the SO phase at the instant of the spindle peak. *IV*, Averaging the phase of all SO–spindles and calculating the number of spindles that peak within ±0.5 radian from the mean phase.

or within-subject designs (Werner et al., 2015). Sensitivity analysis with G*Power 3.1.9.2 (Faul et al., 2007) showed a minimum detectable effect size of $f = 0.43$ (large; calculated "as in SPSS") for interaction and within-factors in a 2 (between) × 3 (within) mixed ANOVA ($N = 29$; two-tailed $\alpha = 0.05$; power = 0.8). For between-factors, the minimum detectable effect size of $f = 0.56$ (bordering very large), suggesting an increased possibility for false-negatives (Type 2 error). For bivariate correlations in one sample ($N = 29$), the medium–large effect size of $r \geq 0.44$ is required (two-tailed $\alpha = 0.05$; power = 0.8).

Statistics. Data were analyzed using IBM SPSS Statistics for Windows, version 29.0 (IBM), or R, version 4.3.2.

The distributions of the dependent and independent variables were investigated for violations of normality (visually and with Shapiro–Wilk test) and Hartigan's dip test for unimodality (Hartigan and Hartigan, 1985) due to our dichotomous suppression paradigm. Residual scatterplots were visually investigated for heteroscedasticity. The equality of variances between the conditions in all dependent variables was tested with Levene's test. Observations were considered outliers if they deviated at least 2.5 SD from the sample mean in its static variable (e.g., SCR on Day 1) and in its change score (e.g., SCR change between Days 1 and 2) or fitted versus residual value.

In preliminary analyses, we examined whether emotional response (SCR and subjective) and memory scores are associated with age and questionnaire scores (Pearson's correlation) or differed between sexes (one-way ANOVA). The associations between baseline night sleep duration and Day 1 evening measurements (baseline SCL, SCR, subjective embarrassment, and metaphor recall) were tested with Pearson's correlation. χ^2 was used to test sex distribution between the conditions (REMS$_{SUPPR}$/SWS$_{SUPPR}$). Condition differences in age, questionnaire scores, sleep, and oscillatory variables were tested with one-way ANOVA.

We used linear mixed model (LMM; restricted maximum likelihood and random ID intercept) for the main analyses regarding the effects of suppression condition and sleep parameters on emotional response and memory retrieval. LMMs are robust against slight violations of distributional assumptions (Schielzeth et al., 2020) and avoid listwise deletion when a value is missing/excluded.

Baseline versus playback SCL difference in the three measurements (Day 1, Day 2, and Day 5) was tested with a "2 × 3" structure LMM. LMM was used to test the main effect of time (repeated variable, three levels) and suppression condition (between-subjects, two levels) and their interaction ("time × condition") on SCR, subjective embarrassment, and metaphor recall. LMM was also used to examine the main effects and time interactions of PSD energy values during REMS and SWS on both SCR and subjective embarrassment, as well as the main effects and time interactions of spindle/SO–spindle variables on metaphor recall. We ensured LMM covariance structure fit with corrected Akaike's information criterion (Hurvich and Tsai, 1989). Regarding LMMs on emotional response, "Factor analytic: First order" showed the best fit. In metaphor recall "Compound Symmetry: Heterogeneous" was applied.

Significant LMM effects were followed up with repeated-measure *t* tests (change between two measurements) or linear regressions (condition differences and slope between change score and independent variable). The associations between overnight SCR change and PSD energy 1 Hz bins were explored with Pearson's correlation.

All analyses on subjective embarrassment and metaphor recall were controlled for sex. LMMs testing the "time × condition" interaction on SCR were first run unadjusted and rerun using the number of awakenings as a covariate. Regarding subjective embarrassment, we reran the "time × condition" interaction with baseline night sleep duration as a covariate.

The nominal level of statistical significance was set at $\alpha = 0.05$. The preliminary analyses and condition comparisons in sample characteristics were run without multiple-test correction. Bonferroni's correction was applied on LMMs with PSD energy (across six frequency bands) and on LMMs with spindle density and OC-spindle number (two tests). Follow-up tests were Bonferroni-corrected (repeated measures in the whole sample/within condition across three/six tests; regressions on static values/change scores across three/two tests).

As supplementary information, nonparametric equivalents for the follow-up analyses were run including the outliers: Friedmann's test for repeated-measure testing in the whole sample and within-condition, Mann–Whitney *U* test for between-condition comparisons in emotional responses and their change, and Spearman's correlation for the associations between PSD values and emotional response change. Bonferroni's corrections were applied as in the parametric follow-up tests, but PSD band associations were corrected over six tests.

Results

Sample characteristics and condition

Total sleep times were similar between the suppression conditions, but REMS$_{SUPPR}$ had 48% less REMS than SWS$_{SUPPR}$ ($p < 0.001$), and SWS$_{SUPPR}$ had 50% less SWS than REMS$_{SUPPR}$ ($p < 0.001$). Significant sleep/oscillatory differences between the conditions were found in N2 duration, REMS/SWS fragmentation, SO-coupled spindle number in SWS, and PSD energy values in REMS and SWS. Questionnaire scores or highest achieved education did not differ between the conditions, but singing experience and the number of forced awakenings were higher in the SWS$_{SUPPR}$ condition (Table 1).

Examining the distributions revealed extreme outliers: in one participant, both Day 1 SCR and overnight SCR change exceeded the sample mean by 3.4 SD and 3.0 SD, respectively. Another participant showed +2.8 SD and +3.0 SD in

Table 1. Sample characteristics and sleep parameters

	Mean (SD)		
	REMS$_{SUPPR}$ ($n = 15$)	SWS$_{SUPPR}$ ($n = 14$)	p
Age (y)	26.3 (4.4)	25.3 (4.8)	0.571
BDI-II	6.0 (5.7)	6.4 (6.5)	0.877
GAD-7	3.6 (3.4)	3.5 (3.3)	0.936
TOSCA 3 Shame	42.3 (11.4)	41.6 (13.2)	0.892
Education	2.3 (0.8)	2.6 (0.9)	0.348
Singing experience	2.3 (0.9)	3.1 (0.9)	0.015*
Sleep characteristics			
TST baseline night	7:05 (0:38)	7:13 (0:31)	0.539
TST experimental	6:04 (0:27)	6:10 (0:19)	0.548
N1	0:42 (0:16)	0:32 (0:12)	0.052
N2	3:03 (0:30)	3:27 (0:30)	0.039*
SWS	1:36 (0:29)	0:48 (0:21)	<0.001***
REMS	0:43 (0:19)	1:23 (0:18)	<0.001***
WASO	0:53 (0:21)	0:49 (0:20)	0.548
REMS Fragmentation %	42.4 (21.3)	7.1 (8.1)	<0.001***
SWS fragmentation %	13.5 (9.0)	39.2 (17.3)	<0.001***
Forced awakenings, N	10.1 (3.3)	12.7 (2.9)	0.031*
Awakening strength	1.9 (0.2)	1.6 (0.3)	0.002**
REMS power spectral density energy			
SO delta	122.2 (42.5)	206.2 (35.2)	<0.001***
Theta	104.5 (56.4)	219.4 (49.1)	<0.001***
Alpha	109.3 (54.7)	227.8 (51.9)	<0.001***
Sigma	143.1 (62.9)	247.4 (49.0)	<0.001***
Beta 1	125.3 (48.9)	292.7 (54.0)	<0.001***
Beta 2	125.3 (37.5)	252.4 (71.5)	<0.001***
SWS power spectral density energy			
SO delta	311.8 (161.2)	157.8 (92.4)	0.004**
Theta	275.6 (133.6)	160.8 (88.1)	0.012*
Alpha	277.7 (193.1)	165.2 (91.9)	0.058
Sigma	353.4 (183.4)	207.8 (102.1)	0.014*
Beta 1	287.1 (131.9)	182.2 (84.8)	0.018**
Beta 2	307.1 (124.7)	192.3 (87.5)	0.008**
SWS SO–spindle			
Fast spindle N	373.5 (142.7)	190.5 (115.7)	<0.001***
Fast spindle density	4.0 (1.6)	4.2 (1.7)	0.762
SO–spindle N	82.4 (32.7)	39.1 (25.0)	<0.001***
Resultant vector length	0.53 (0.10)	0.55 (0.19)	0.709
N2 SO–spindle			
Fast spindle N	927.1 (341.1)	1,037.9 (312.2)	0.371
Fast spindle density	5.0 (1.2)	5.0 (1.1)	0.947
SO–spindle N	137.2 (41.9)	149.9 (48.9)	0.458
Resultant vector length	0.37 (0.17)	0.41 (0.20)	0.564

BDI, Beck Depression Inventory; GAD-7, Generalized Anxiety Disorder 7 questionnaire; TOSCA, Test of Self-Conscious Affect; TST, total sleep time; SWS, slow-wave sleep; N1-2, sleep stage 1–2; REMS, rapid eye movement sleep; WASO, wake after sleep onset; SO, slow oscillation.
***<0.001.
**<0.01.
*<0.05.

Day 2 SCR and overnight SCR change, respectively. In this participant, SWS PSD energy values exceeded the sample mean by 3.2 SD on average (2.6–3.7 SD). We excluded these outlying SCR and PSD values from LMM and follow-up analyses. Extended Data Figure 3-1*A–D* shows the excluded variables. Subjective embarrassment at Day 5 was not normally distributed (Shapiro–Wilk $p = 0.016$), and the Day 2 to Day 5 change was not normally ($p < 0.001$) or unimodally (Hartigan's test $p < 0.008$) distributed.

Preliminary analyses showed no significant associations between background variables (age, questionnaire scores) and SCR/memory scores (all $p \geq 0.05$). Females had a significantly higher metaphor recall percentage on Day 1 (68.8% vs 53.0%), Day 2 (68.1% vs 47.1%), and Day 5 (65.5% vs 41.5%) retrievals (all $p \leq 0.046$) than males, as well as higher subjective embarrassment ratings on Day 2 (1.37 vs 0.64; $p = 0.021$) and Day 5 (1.13 vs 0.46; $p = 0.029$). Sleep duration on the baseline night correlated significantly (negatively) with subjective embarrassment ($r = -0.373$; $p = 0.046$), but not other evening measures ($p \geq 0.190$).

Stress induction and SCR

SCL was significantly higher during the playback than the baseline ($F_{(2,27.676)} = 61.301$; $p < 0.001$) in all measurements (post hoc $p < 0.001$; Cohen's $d \geq 1.333$; Fig. 3A). Regarding SCR, we found a significant time effect ($F_{(2,24.425)} = 21.906$; $p < 0.001$), indicating that SCR varied between Days 1, 2, and 5 measurements. Bonferroni-corrected follow–up tests showed that SCR was higher on Day 2 ($M = 15.54$; $SD = 2.62$) than on Day 1 ($M = 13.75$; $SD = 1.98$) and Day 5 ($M = 12.73$; $SD = 1.69$; $t_{(26)} = 4.973$, $p < 0.001$, Cohen's $d = 0.96$ and $t_{(27)} = 6.573$, $p < 0.001$, Cohen's $d = 1.35$, respectively) and on Day 1 than Day 5 ($t_{(27)} = 3.170$; $p = 0.011$; Cohen's $d = 0.60$; Fig. 3B). No significant difference was found in overall (across all measurements) SCR between REMS$_{SUPPR}$ and SWS$_{SUPPR}$ conditions (main effect $F_{(1,25.952)} = 0.091$; $p = 0.766$).

The "condition × time" interaction was significant ($F_{(2,24.332)} = 4.209$; $p = 0.027$). Controlling for the amount of awakenings did not change the significance status of "condition × time" ($F_{(2,23.576)} = 4.284$; $p = 0.026$). Following up the interaction by comparing the SCR change between the conditions showed that the overnight SCR change (Day 1 to Day 2) was higher in SWS$_{SUPPR}$ ($M = 2.69$; $SD = 1.30$; percentual increase, $M = 20.1\%$; $SD = 9.3\%$) than in REMS$_{SUPPR}$ ($M = 0.84$; $SD = 1.97$; $M = 6.1\%$; $SD = 13.6\%$; $t_{(1,25)} = 2.906$; $p = 0.016$; $R^2 = 0.253$), but the conditions not differ in the Day 2 to Day 5 SCR
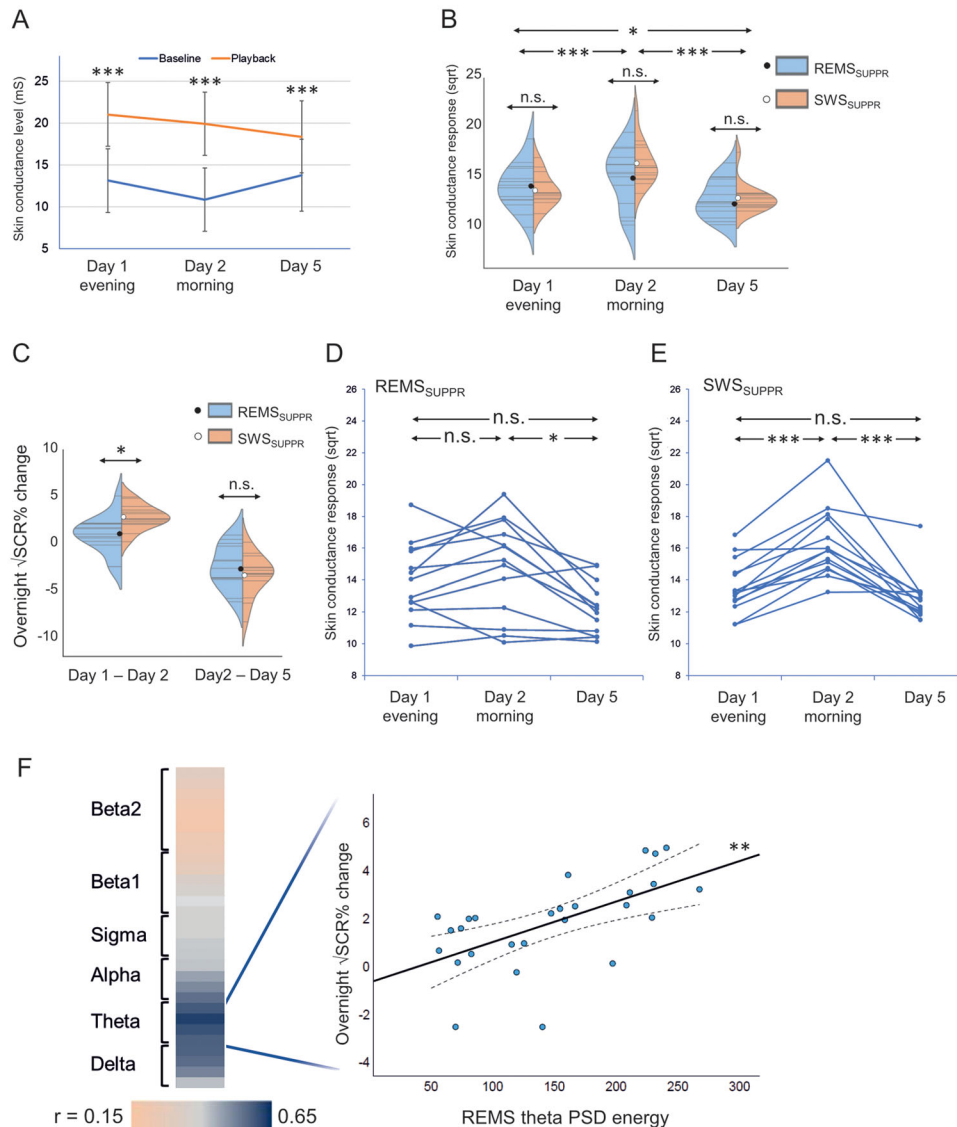

Figure 3. Skin conductance, suppression condition, and REMS oscillations. The playback of the karaoke recording induced a significant rise in SCL in all measurements ($p < 0.001$, Bonferroni-corrected; **A**). Violin plots illustrate individual (gray lines) and condition-wise (blue/orange) distributed SCRs with mean values (black/white dot) across the three measurements (**B**). Overnight change in SCR was higher in SWS$_{SUPPR}$ than in REMS$_{SUPPR}$ ($p = 0.016$, Bonferroni-corrected; **C**). Individual trajectories of SCRs in REMS$_{SUPPR}$ (**D**) and in SWS$_{SUPPR}$ (**E**). The scatterplot illustrates the positive association between REMS theta power spectral density (PSD) energy and overnight SCR change ($p = 0.002$, Bonferroni-corrected), and the heatmap displays Pearson's correlation strength across 0–30 Hz for REMS (**F**). Error bars and dashed lines: 95% confidence interval. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; Bonferroni-corrected. Extended Data Figure 3-1 displays the excluded outliers.

change (REMS$_{SUPPR}$, $M = -2.28$; SD $= 2.38$; $M = -15.9\%$; SD $= 10.8\%$; SWS$_{SUPPR}$, $M = -3.47$; SD $= 2.18$; $M = -20.5\%$; SD $= 10.7\%$; $t_{(26)} = -1.373$; $p = 0.181$; $R^2 = 0.068$; Fig. 3C).

Within-condition repeated–measure tests showed that in REMS$_{SUPPR}$, the SCR was significantly higher on Day 2 ($M = 14.82$; SD $= 2.92$) than on Day 5 ($M = 12.68$; SD $= 1.92$; $t_{(13)} = 3.600$; $p = 0.019$; Cohen's $d = 0.962$). Day 1 SCR ($M = 13.94$; SD $= 2.31$) did not significantly differ from Day 2 ($t_{(12)} = -1.530$; $p = 0.912$; Cohen's $d = 0.424$) or Day 5 measurements ($t_{(13)} = 2.673$; $p = 0.106$; Cohen's $d = 0.714$; Fig. 3D). In SWS$_{SUPPR}$, Day 2 SCR ($M = 16.26$; SD $= 2.14$) was significantly higher than Day 1 ($M = 13.57$, SD $= 1.65$; $t_{(13)} = 7.752$; $p < 0.001$; Cohen's $d = 2.072$) and Day 5 SCRs ($M = 12.79$; SD $= 1.46$; $t_{(13)} = 5.958$; $p < 0.001$; Cohen's $d = 1.592$). No significant difference was found between Day 1 and Day 5 measurements ($t_{(13)} = 1.735$; $p = 0.638$; Cohen's $d = 0.464$; Fig. 3D). Between the conditions, SCR did not differ significantly on Day 1 ($t_{(1,26)} = -0.485$; $p = 1.000$; $R^2 = 0.009$), Day 2 ($t_{(1,26)} = 1.484$; $p = 0.450$; $R^2 = 0.078$), or Day 5 ($t_{(1,27)} = 0.170$; $p = 0.866$; $R^2 = 0.001$; Fig. 3E).

In sum, selective sleep stage suppression elicited pronounced overnight SCR increase in the condition with suppressed SWS (intact REMS) compared with that in REMS-suppressed condition.

SCR and power spectral density

PSD energy in any frequency band was not significantly associated with overall SCR in REMS or SWS (Table 2). In REMS, significant "time × PSD" interaction on SCR was found regarding theta band ($F_{2,26.166} = 7.002$; $p = 0.023$), indicating that theta PSD energy is associated with SCR change between measurements. No other significant time interactions were found regarding PSD energy in REMS or SWS (all $p \geq 0.165$, Bonferroni-corrected; Table 2).

Follow-up examination on the significant "time × REMS theta PSD" interaction showed a robust positive association between REMS theta PSD energy and overnight SCR change ($t_{(1,25)} = 3.762$; $p = 0.002$; $R^2 = 0.336$) but not Day 2 to Day 5 SCR change ($t_{(1,26)} = -1.475$; $p = 0.304$; $R^2 = 0.077$). Figure 3F displays the scatterplot between REMS theta PSD energy and overnight SCR change and heatmapped correlation strengths between bin-wise (0–30 Hz) PSD energy and overnight SCR change. See Extended Data Figure 3-1E for the REMS theta–overnight SCR change including the excluded outliers.

Subjectively rated embarrassment

Subjectively rated embarrassment differed according to time ($F_{(2,28.029)} = 27.058$; $p < 0.001$). Follow-up repeated–measure tests showed that subjectively rated embarrassment was higher on Day 1 ($M = 1.83$; SD $= 0.93$) than on Day 2 ($M = 1.02$; SD $= 0.86$; $t_{(28)} = 7.097$; $p < 0.001$; Cohen's $d = 1.32$) and Day 5 ($M = 0.81$, SD $= 0.82$) [$t_{(28)} = 7.089$, $p < 0.001$, Cohen's $d = 1.32$]. Additionally, embarrassment on Day 2 was rated higher than on Day 5 ($t_{(28)} = 2.820$; $p = 0.027$; Cohen's $d = 0.52$; Fig. 4). Suppression conditions did not differ in overall (across all measurements) subjective embarrassment between the suppression conditions ($F_{(1,26.000)} = 0.216$; $p = 0.646$). "Condition × time" interaction was not significant unadjusted ($F_{(2,27.024)} = 2.056$; $p = 0.148$) or after controlling for baseline night sleep duration ($F_{(2,27.011)} = 1.252$; $p = 0.302$). In sum, subjectively rated embarrassment decreased in all measurements, while suppression condition did not induce significant differences.

Table 2. LMM results: emotional response by condition and power spectral density energy values

	SCR				Subjective embarrassment			
	Main effect		X time		Main effect		X time	
	F	p	F	p	F	p	F	p
Condition	0.091	0.766	4.209	0.027*	0.216	0.646	2.056	0.148
REMS PSD								
SO delta	0.010	1.000	4.191	0.165	0.080	1.000	2.548	0.581
Theta	0.019	1.000	7.002	0.023*	0.069	1.000	3.658	0.236
Alpha	0.833	1.000	3.325	0.314	0.249	1.000	2.002	0.927
Sigma	0.316	1.000	1.575	1.000	0.252	1.000	1.635	1.000
Beta1	0.152	1.000	1.062	1.000	0.219	1.000	2.518	0.596
Beta2	0.115	1.000	0.597	1.000	0.299	1.000	2.412	0.652
SWS PSD								
SO delta	0.017	1.000	0.135	1.000	2.025	1.000	1.589	1.000
Theta	0.004	1.000	0.065	1.000	1.673	1.000	1.878	1.000
Alpha	0.000	1.000	0.217	1.000	1.020	1.000	1.491	1.000
Sigma	0.008	1.000	0.115	1.000	2.266	0.869	2.818	0.468
Beta1	0.005	1.000	0.325	1.000	2.698	0.678	3.872	0.202
Beta2	0.065	1.000	0.397	1.000	2.992	0.576	4.840	0.098

X time, interaction between the examined variable and time (three measurements); REMS, rapid eye movement sleep; SWS, slow-wave sleep; PSD, power spectral density. REMS/SWS PSD p values Bonferroni-corrected across six tests.
*$p < 0.05$. See Extended Data Table 2-1 for nonparametric tests including outliers.
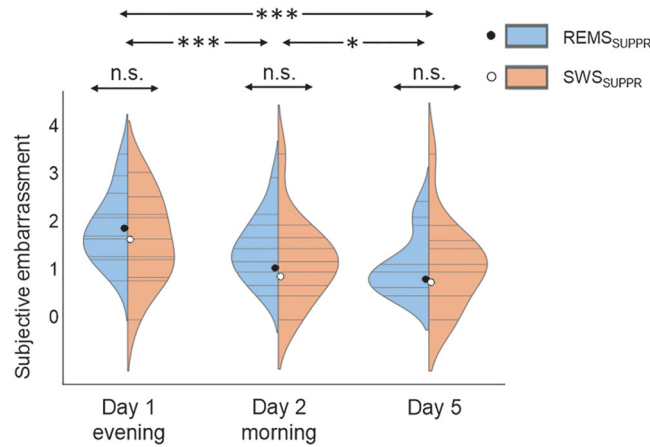
Figure 4. Subjective embarrassment. A violin plot illustrating individual (gray lines) and condition-wise (blue/orange) distributed embarrassment ratings with mean values (black/white dot) across the three measurements.
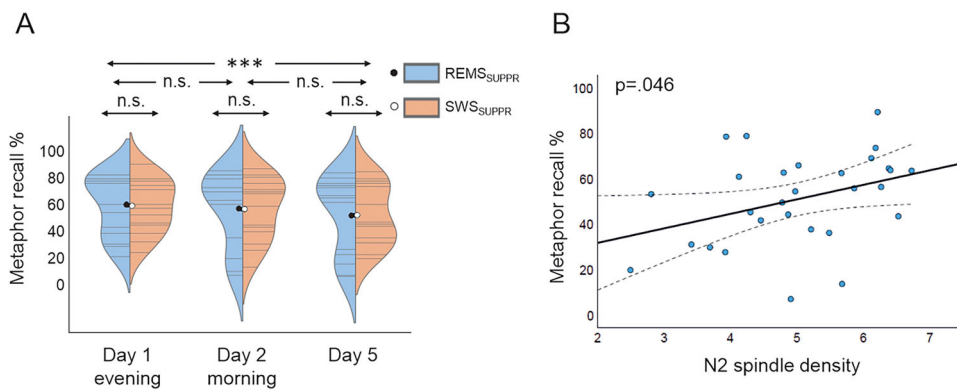

Figure 5. Metaphor recall. A violin plot illustrates individual (gray lines) and condition-wise (blue/orange) distributed metaphor recall percentages with mean values (black/white dot) across the three measurements (**A**). N2 fast spindle density predicted positively overall metaphor recall (averaged across all retrievals; $p = 0.046$, Bonferroni-corrected) (**B**). Dashed lines: 95% confidence interval.

Regarding REMS/SWS PSD energy, none of the frequency bands showed a significant main effect on subjective embarrassment ($p \geq 0.576$). The "time × PSD energy" interaction was not significant in either REMS ($p \geq 0.236$) or SWS ($p \geq 0.098$) in any frequency band. See Table 2 for full results. We explored how excluding the marginally nonoutlier in SWS PSD (Extended Data Figure 3-1D, second highest dot) affected the LMMs regarding PSD and emotional responsivity. No changes in significance status emerged (all $p \geq 0.429$, Bonferroni-corrected; data not shown).

Metaphor recall and NREMS oscillations

Time had a significant main effect on metaphor recall ($F_{(2,26.671)} = 12.508$; $p < 0.001$). Follow-up paired-sample t tests showed that the Day 1 recall percentage ($M = 61.4\%$; SD $= 21.1\%$) was significantly higher than that of Day 5 ($M = 54.4\%$; SD $= 25.7\%$; $t_{(27)} = 4.817$; $p < 0.001$; Cohen's $d = 0.910$) but not on Day 2 ($M = 58.8\%$; SD $= 25.4\%$; $t_{(26)} = 1.433$; $p = 0.492$; Cohen's $d = 0.276$). The difference between Day 2 and Day 5 was not statistically significant ($t_{(26)} = 1.371$; $p = 0.546$; Cohen's $d = 0.264$). The condition did not have a significant main effect ($F_{(1,24.978)} = 0.056$; $p = 0.816$) nor time interaction ($F_{(2,25.666)} = 0.456$; $p = 0.639$) on the metaphor recall percentage (Figure 5A).

Fast spindle density or OC-spindle number in SWS did not associate significantly with the overall recall percentage ($F_{(1,25.003)} = 4.630$, $p = 0.081$ and $F_{(1,24.975)} = 2.757$, $p = 0.219$, respectively). Their interaction with time was also not significant ($F_{(2,25.828)} = 0.659$, $p = 1.000$ and $F_{(2,25.752)} = 1.386$, $p = 0.536$, respectively). The main effect of fast spindle density in N2 sleep was significant ($F_{(1,25.012)} = 5.876$; $p = 0.046$), i.e., N2 spindle density associated positively with the overall recall percentage (Fig. 5B), but its time interaction was not ($F_{(2,25.803)} = 0.719$; $p = 0.994$). No significant findings were observed regarding N2 OC-spindles (main effect, $F_{(1,24.989)} = 5.583$; $p = 0.052$; time interaction, $F_{(2,25.747)} = 0.867$; $p = 0.864$).

Thus, suppression condition, or its impact on accumulated SO–spindle events, did not associate with significant differences in metaphor recall.

Discussion

To better understand the role of REMS and SWS in off-line affective and declarative memory processing, we applied overnight selective sleep stage suppression. As assumed, the two sleep suppression conditions resulted in different outcomes regarding the modulation of emotional response from the pre- to postsleep period. A greater pre- to postsleep increase in physiological reactivity to an emotional stressor was observed after suppressed SWS (intact REMS), relative to suppressed REMS (intact SWS), providing evidence that affective processing is sleep stage-dependent. The overnight increase in emotional responsivity toward the stressor was positively associated with REMS theta activity. The retention of neutral declarative material did not differ between the suppression conditions and was not associated with (SO–)spindle parameters.

The physiological response toward the emotional stressor increased significantly between evening- to-morning measurements in the condition with suppressed SWS, i.e., intact REMS. We interpret this from the perspective of REMS quality, as the overnight SCR change was strongly associated with the accumulated REMS theta (4–8 Hz) energy, beyond any other oscillatory band. Indeed, REMS and theta energy seemed to preserve (or even strengthen) the affective component of the stress memory. This aligns with studies linking the amount of REMS to elevated/preserved subjective or physiological postsleep emotional response (Lara-Carrasco et al., 2009; Pace-Schott et al., 2011, 2015; Baran et al., 2012; Gilson et al., 2015; Werner et al., 2015, 2021; Halonen, Kuula, Makkonen, et al., 2021). The significance of REMS theta oscillations in off-line affective memory consolidation is supported by animal models and studies on emotional learning in humans (Nishida et al., 2009; Popa et al., 2010; Prehn-Kristensen et al., 2013; Sopp et al., 2017; Kim et al., 2020). During REMS, theta generation is tied to pontine–geniculo–occipital waves (Hutchison and Rathore, 2015), which instantiate limbic activity and facilitate emotional memory processing (Mavanji et al., 2004; Datta et al., 2008; Karashima et al., 2010).

However, the effect of REMS on affective memories is not univocal in the previous literature. Our results contradict the forgetting part of the SRSF hypothesis, which argues that especially REMS serves to detune the affective charge from recent memories (Walker and van der Helm, 2009). In support of the SRSF hypothesis, several studies associate the amount of REMS with lower postsleep affective response (Gujar et al., 2011; Rosales-Lagarde et al., 2012; Spoormaker et al., 2012; Wassing et al., 2019). The discrepancy appears to not be related to the type of stress assessment (i.e., subjective or physiological). For example, SCR toward conditioned fear of electric shocks in the study by Spoormaker et al. (2012) was detuned via REMS, whereas another study (Halonen, Kuula, Makkonen, et al., 2021) reported a positive association between REMS and karaoke playback-induced SCR. Importantly, these apparently opposite findings urge one to consider what is being consolidated by REMS. In the earlier karaoke study (Halonen, Kuula, Makkonen, et al., 2021), and in the present study, REMS proposedly strengthened the memory of social stress. On the other hand, Spoormaker et al. (2012) administered a presleep extinction task for a previously formed fear memory. The next day, cueing the fear memory elicited elevated SCR in those whose REM sleep was deprived, relative to intact sleepers. Thus, REMS may either detune (Spoormaker et al., 2012) or strengthen the physiological emotional response, as evidenced by conditioned fear of electric shocks (Menz et al., 2013) and social stress (Halonen, Kuula, Makkonen, et al., 2021). This bidirectionality may adaptively depend on the presleep conditions.

From an evolutionary perspective, fully and rapidly downscaling the charge of all emotional experiences may not be favorable—affective charge ensures the readiness to respond rapidly to threat in natural environments. Sleep may then have an adaptive function in the regulation of affect, such as fear. However, human emotional landscape is more complicated. To put the study in a related context, we applied the concept of social stress linked to shame and social self-representation, equally assumed to have a social evolutionary origins (Tracy and Robins, 2004) and to be a factor in mood disorders like depression (Kim et al., 2011). Indeed, threats to the social self have been shown to induce physiological stress responses, as evidenced in studies applying the Trier Social Stress Test (Ruiz et al., 2010) and the out-of-tune singing paradigm as in the current study (Wassing et al., 2019; Halonen, Kuula, Makkonen, et al., 2021). Shame, in the short term, may motivate "repairing" actions in social contexts (Xing et al., 2021), thus serving adaptivity. In our study, the effect of REMS in preserving the physiological response was short-lived, as no evident sleep effects remained after a few days. Likely, the intervening time and normalized sleep attenuated any excess response to it.

Subjective embarrassment decreased substantially between the evening and morning playbacks. While this would point to sleep-related habituation of subjective stress, the interpretation may be more complex. First, neither the suppression condition (REMS/SWS) nor sleep PSD measures influenced the change in subjective embarrassment from pre- to postsleep measurements. The finding contradicts the SRSF hypothesis claiming that specifically REMS and theta would attenuate the affective response toward the stressor (Walker and van der Helm, 2009). One possibility is that not only REMS, but also SWS, contributed to the regulation of the subjective affect. Evidence of this mechanism is provided by Talamini et al. (2013), showing how overnight emotional habituation to subjectively rated emotional distress was associated with a compensatory response and trait characteristics of SWS. An alternative explanation is that merely repeating the stressor was the main cause of the attenuation. In a previous study using a similar stressor, subjective embarrassment ratings decreased pronouncedly between the first and second playbacks, during a period spent awake (Halonen, Kuula, Makkonen, et al., 2021). Habituation by repetition may have overshadowed any sleep (stage)-specific effects in the present study. Finally, subjective postsleep ratings verged on a floor effect, and especially the measurements (and change) regarding Day 5 embarrassment were compromised by nonnormal and nonunimodal distribution. These statistical

properties, along with a small sample's ($N = 29$) inclination to underdetect sublarge effects, may also explain the nonsignificant findings.

Regarding the retention of declarative memory content, the differences between the suppression conditions were negligible. Previous studies provide solid evidence that SWS, sleep spindles, and their synchronized activity have significance in declarative memory consolidation (Plihal and Born, 1997, 1999; Clemens et al., 2005; Helfrich et al., 2018; Léger et al., 2018; Klinzing et al., 2019; Mikutta et al., 2019; Muehlroth et al., 2019; Halonen, Kuula, Antila, et al., 2021; Halonen et al., 2022). In the current study, while SO–spindle coupling events were markedly reduced via SWS suppression, this did not result in statistically significant retention differences relative to intact SWS (suppressed REMS). This suggests that the impact of (SO-coupled) sleep spindles during SWS in memory consolidation is not dose-dependent. In line with this, another SWS/REMS suppression study reported no declarative memory difference between the conditions (Wiesner et al., 2015). The authors proposed that the residual sleep was sufficient to consolidate the memorized items. Indeed, the amount of SWS may not be consequential for memory retention, as a review concludes (Cordi and Rasch, 2021).

A previous study with SWS/REMS deprivation found that N2 spindles predicted verbal learning (Genzel et al., 2009). Thus, we expected that N2 spindle/SO–spindle activity would "compensate" for the suppressed SWS events, but no evidence on this was observed. A possible explanation for the lack of associations and intercondition differences may be that memory consolidation is not dependent on a single sleep stage. According to the sequential hypothesis (Giuditta, 2014), novel memories are processed and consolidated across successively occurring SWS and REMS episodes. Such intact duets were absent in our study. Experimental evidence suggests that establishing new semantic schemas is dependent on REMS that follows SWS (Batterink et al., 2017). Thus, in our study, consolidating novel semantic metaphors may have been disrupted by suppressing either SWS or REMS, not only SWS.

We found that NREMS oscillations predicted overall (including presleep) recall performance for declarative memory content. Specifically, fast spindle density during N2 associated with better memory outcome across the whole sample. Sleep spindle activity possibly reflects learning ability beyond memory consolidation, as evidenced in earlier studies (Gais et al., 2002; Berner et al., 2006). Both encoding and retrieval require coordinated activity of the thalamus and neocortex (Staudigl et al., 2012; Wagner et al., 2019), and the properties of these structures and their white matter connectivity impact the generation and propagation of spindles (Piantoni et al., 2013; Fernandez and Lüthi, 2020). NREMS oscillations may thus mirror the capacity for initial learning and successful memory retrieval (Staudigl et al., 2012; Wagner et al., 2019).

Strengths of our study include a comparative paradigm with suppressing both REMS and SWS, enabling the examination of both the affective and mnemonic impact of disrupted sleep. Additionally, measuring oscillatory activity enabled investigating the (dose-dependent) relevance of the modulated activity on off-line processing.

Some limitations must also be addressed. First, not having a control condition with undisturbed sleep prevented us from observing nonspecific impacts of disrupted sleep on stress reactivity, and thus, generalizing the results to undisturbed sleep should be done with caution. However, the results converge with a previous study, where the amount of undisrupted REMS predicted higher postsleep SCR toward a similar stressor (Halonen, Kuula, Makkonen, et al., 2021). Additionally, none of the participants had intact sleep cycles. Suppressing either SWS or REMS disrupts sequential off-line processing (Giuditta, 2014), preventing the complementary influence of these sleep stages on the establishment of new memory schemas (Batterink et al., 2017). Second, the sample size was small, limiting statistical power and increasing the possibility of false findings (Button et al., 2013). Especially between-condition comparisons were low-powered, which may have led to an inadequate account of the suppression effects. Third, we did not have a control stimulus for affective response. It remains unclear whether the observed response was specific to own-singing playback, i.e., self-conscious affect. However, in a previous study (Wassing et al., 2019), REMS-driven affective modulation was specific to participants' own singing, and not to a professional singer's performance. Finally, the suppression conditions differed in singing experience and number of awakenings. While optimally such parameters should be matched between conditions, statistically controlling for their effects on SCR did not change the outcome.

Conclusions

This study contributed to understanding of emotional and declarative off-line processing in several ways. Selective suppression of REMS or SWS suggests that the physiological response toward self-conscious emotional stressor is preserved when REMS remains intact and SWS is disrupted and that REMS theta activity likely facilitates the preservation of the affective charge of the memory. The retention of declarative memories was not impaired by suppressed SWS to any greater extent than by suppressed REMS, suggesting that off-line memory consolidation is not dose-dependently related to NREMS oscillations only.

Data Accessibility

Research materials are available from the corresponding author on reasonable request. The scrips used to create study variables were implemented and executed with Python 3.9 or MATLAB R2022b. The scripts are found as Extended Data Figure 1-1 and are available on https://version.helsinki.fi/sleep-mind/karaoke-2024.

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