Sensorimotor restriction affects sleep-related motor memory consolidation through altered slow oscillation–spindle coupling

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Abstract

Sleep benefits memory consolidation through periodic sleep spindle activity and associated memory reactivations. The temporal organization of spindles in "trains" is considered a critical sleep mechanism for the timed and repeated reactivation of memories. Also, evidence suggests that a timely phase-locking between slow oscillations (SO) and spindles facilitates learning-related synaptic plasticity. Here, we investigated the contribution of the clustering and coupling of spindles with SO in motor memory consolidation by experimentally promoting local synaptic depression in sensorimotor cortical regions, through upper-limb immobilization, following motor sequence learning. Our results reveal that the cluster-based organization of sleep spindles is independent of daytime sensorimotor experience, while leading to distinct overnight behavioral outcomes. Interestingly, immobilization induced a phase shift in the SO-spindle coupling for spindles occurring in trains but not when isolated outside trains. We demonstrate that spindle trains may promote skill-specific strengthening of motor memories, while isolated spindles may instead create memory-instability conditions leading to enhanced skill transfer.

Introduction

Behind every learned motor skill lies a period of practice and rehearsal[1,2]. However, although the repeated practice of a motor skill is crucial for its initial acquisition, developing an effective movement representation is not only a result of practice[3]. The newly formed memory continues to be processed "offline" during waking and sleeping hours following practice. This offline period offers a privileged time window for memory consolidation, which relates to the process whereby newly acquired and relatively labile memories are transformed into enhanced and more stable memory traces[4,5]. Following its initial acquisition, the memory trace is thought to be dynamically maintained during wakefulness and actively reprocessed during a subsequent sleep period, allowing its strengthening and thus influencing its generalizability[3,6–8]. A daytime nap or a night of sleep has long been shown to play a crucial role in the strengthening and transformation of motor memories during consolidation[9–12].

Another important aspect of motor learning involves the ability to generalize the acquired skill to another one or another effector, such as inter-limb transfer. This fundamental principle underlies our capacity to perform unpracticed motor skills or learn new ones more easily[7]. While the role of sleep in the skill-specific strengthening of motor memories during consolidation has been widely studied, evidence also showed that it can promote their generalization[7,13,14]. Indeed, recent theoretical evidence suggests that learning contexts creating conditions of memory instability may be critical for the generalization of motor skills[7,14]. Instability is thought to make a memory vulnerable to interference and even forgetting, with the loss of detailed knowledge reducing memory specificity and enabling the development of generalizable knowledge[7]. Generalization is expected to develop during episodes where the memory is unstable, following its initial formation or reactivation, thus allowing the transfer of skills across related tasks or situations. Hence, it remains to be determined whether sleep consolidation mechanisms may offer optimal conditions for motor memory strengthening and memory-instability conditions supporting the creation of generalized knowledge[4,7,15].

Growing evidence suggests that brief bursts of sigma-like thalamocortical oscillations, known as sleep spindles, during non-rapid eye movement (NREM) sleep play a critical role in the offline covert reactivation of motor memories during consolidation, resulting in over-night/nap memory improvements[9,11,14,16–19]. Sleep spindle activity is an electrophysiological hallmark of NREM-stage2 (NREM2) sleep involving short (0.3-2 sec) synchronous bursts of waxing and waning 11-16 Hz oscillations[8,14,18]. Boutin and colleagues (2020)[8] recently developed a framework for motor memory consolidation that outlines the essential contribution

of the clustering and hierarchical rhythmicity of spindle activity during this sleep-dependent process. More specifically, sleep spindles tend to cluster in 'trains' on a low-frequency time scale of approximately 50 seconds (~0.02 Hz infraslow rhythm) during NREM sleep, interspersed with relative spindle-free periods where sporadic isolated spindles may occur. This NREM sleep infraslow periodicity has been considered a crucial neurophysiological regulatory mechanism providing transient temporal windows of optimal neural conditions for the reactivation of memory traces through spindle activity[20–22]. In addition to the periodic clustering of spindles, recent findings unveiled a recurring pattern where spindles tend to reoccur approximately every 3-4 seconds during trains (~0.2-0.3 Hz mesoscale rhythm). In theory, such inter-spindle intervals during trains represent periods of refractoriness that are assumed to be crucial for the timely and organized segregation of spindle-induced memory reactivations, thus regulating the cyclic interference-free reprocessing of memory traces for efficient memory consolidation[8,18,23,24].

Accompanying this multi-scale rhythmicity of spindle activity, it has been proposed that cross-frequency coupling and hierarchical nesting of NREM sleep rhythms may be critical for memory consolidation. Current trends postulate that slow oscillations (SO; 0.5-1.25 Hz) confer a temporal window for spindles to occur in their excitable up-states and that a timely phase-locking facilitates the induction of persistent synaptic plastic changes[25–31]. At the synaptic level, post-learning neuroplasticity has further been shown to be mediated by inter-hemispheric sleep regulations over localized primary sensorimotor regions[19,32,33], thus suggesting that lateralized and hemisphere-specific local regulations of both SOs and spindles may reflect learning-dependent brain plasticity and skill consolidation during sleep.

Slow wave activity (SWA; 0.5-4 Hz) is now established as a marker of homeostatically regulated sleep pressure and synaptic downscaling during NREM sleep[34]. In an elegant study, Huber and colleagues (2006)[33] transiently promoted local synaptic depression in sensorimotor regions using a short-term upper-limb immobilization paradigm. Interestingly, results revealed that temporary upper-limb immobilization induced local homeostatic changes (i.e., reduced spectral power mainly in the 0.5-1 Hz low-SWA frequency range, which corresponds to the SO frequency band) in the contralateral affected-limb sensorimotor cortex that were associated with deteriorations in motor performance. Moreover, Debarnot and colleagues (2021)[35] revealed that sleep spindle activity is also locally affected by immobilization-induced synaptic depression. Recent studies further demonstrated that the strength of the SO-spindle coupling depends on prior sensorimotor experience and structural brain integrity[28,29,36]. A timely phase-locking between SOs and spindles is expected to induce persistent, learning-related synaptic plastic

changes (see [37] for a review). Hence, considering that SOs and spindles are involved in synaptic consolidation, the potential contribution of spindles' clustering and the strength of the SO-spindle coupling in the strengthening and generalization of motor skills during consolidation remains to be explored.

Using a short-term upper-limb immobilization procedure, combined with the recording of behavioral and electroencephalographic (EEG) night sleep measures, the aim of the present study was twofold: (i) to determine the effects of daytime sensorimotor experience on local sleep spindle expression (i.e., clustering and rhythmicity) and the integrity of the cross-frequency coupling between SOs and spindles, and (ii) to evaluate their functional contribution in the consolidation and generalization of motor skills. Adopting a time-based clustering approach, we hypothesized that the rhythmic occurrence of spindles in trains would confer favorable conditions for efficient reprocessing and consolidation of the memory trace, supported by an effective plasticity-dependent SO-spindle coupling compared to spindles occurring in isolation. Isolated spindles are instead expected to impair skill-specific motor memory strengthening through ineffective, non-recurring memory reactivations[18] or impaired SO-spindle coupling, thus rendering the memory trace unstable and prone to generalization.

Methods

RESSOURCES AVAILABILITY

Lead contact

Further information should be directed to and will be fulfilled by the lead contact, Arnaud Boutin (arnaud.boutin@universite-paris-saclay.fr).

Data and code availability

The data that support the results of this study are available from the corresponding author upon reasonable request and under a formal data-sharing agreement.

Sleep EEG data were processed using the MATLAB R2021b software from The MathWorks (Natick, MA) and the open-source Brainstorm and EEGLAB software. The codes for the detection and clustering of sleep spindles are available at the following GitHub repositories: https://github.com/arnaudboutin/Spindle-detection and https://github.com/arnaudboutin/Spindle-clustering. The codes for slow oscillations and infraslow-mesoscale analyses are available at https://github.com/arnaudboutin/Spindle-SO-

package. The codes used to perform other analyses are available from the corresponding author upon request.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Thirty healthy volunteers (16 females, mean age: 25.4 ± 4 years) were recruited by local advertisements and were randomly and equally divided into two groups: an immobilization group (IMMO; n = 15, 7 females, mean age: 25.9 ± 4 years) and a control group without immobilization (CTRL; n = 15, 9 females, mean age: 25.0 ± 4 years). All participants met the following inclusion criteria: aged between 18 and 35 years, right-handed (Edinburgh Handedness Inventory[38]), medication-free, without history of mental illness, epilepsy, or head injury with loss of consciousness, sleep or neurologic disorders, and no recent upper extremity injuries. The experimental protocol was approved by the "Comité de Protection des Personnes Sud-Ouest et Outre-Mer III" (ID-RCB: 2020-A01465-34) and conformed to relevant guidelines and regulations. All participants gave written informed consent before inclusion. Participants were asked to maintain a regular sleep-wake cycle and to refrain from all caffeine- and alcohol-containing beverages 24 hours prior to the experimentation.

METHOD DETAILS

Experimental design and motor sequence task

Participants sat on a chair at a distance of 50 cm in front of a computer screen. The motor task consisted of performing as quickly and accurately as possible an 8-element finger movement sequence by pressing the appropriate response keys on a standard French AZERTY keyboard using their left, non-dominant hand fingers. The sequence to be performed (B-C-N-V-C-B-V-N, where C corresponds to the little finger and N to the index finger) was explicitly taught to the participant before training.

Test and practice blocks consisted of repeating the 8-element sequence for 30 seconds. Each block began with the presentation of a green cross in the center of the screen accompanied by a brief 50-ms tone. In case of occasional errors, participants were asked “not to correct errors and to continue the task from the beginning of the sequence” (see [14] for a similar procedure). At the end of each block, the color of the green imperative stimulus turned red, and participants were then required to look at the fixation cross during the 30-s rest period. This experimental protocol was designed to control the duration of practice during the test and practice blocks.

Stimuli presentation and response registration were controlled using the MATLAB R2019b software from The MathWorks (Natick, MA) and the Psychophysics Toolbox extensions[39].

Each participant completed two visits at the Sleep and Vigilance Center of the Hotel-Dieu Hospital (Fig. 1). The first visit served as a polysomnographic (PSG) screening and acclimatization night, whereas the second visit consisted of the experimental night following motor sequence learning and the daytime immobilization or non-immobilization control procedure. Assignment to the immobilization or control group was randomized across participants. The first visit started at 9:00 pm; the experimental design was explained and all the forms were provided. After the participants had prepared for the night, the EEG equipment was set up and they were invited to sleep. The sleep-EEG recording started approximately at 10:30 pm. The EEG cap was removed the next morning at 6:30 am, just after awakening. The experimental procedure began at 7:30 am for all participants to minimize the possible impact of circadian and homeostatic factors on individual performance and give them time to shower and have breakfast. The experimental procedure for the first visit comprised three main phases: familiarization, acquisition, and post-test (Fig. 1). First, participants underwent a brief familiarization phase during which they were instructed to repeatedly and slowly perform the 8-element sequence until they accurately reproduced the sequence three consecutive times. This familiarization was intended to ensure that participants understood the instructions and explicitly memorized the sequence of finger movements. The actual acquisition phase consisted of physically performing 16 blocks of the 8-element motor sequence with their left (non-dominant) hand fingers. The first two blocks of this acquisition phase were used as a pre-test to evaluate baseline performance. Approximately five minutes after the end of the acquisition phase, all participants performed a post-test phase consisting of two blocks. This test was briefly preceded by a physical warm-up phase (i.e., slow-paced production of the sequence three consecutive times) to ensure that the correct sequence had been practiced. After the post-test (at 8:00 am), one-half of the participants had their left upper limb immobilized for 13 hours (IMMO group), while the other-half (CTRL group) had no restrictions on the use of their left limb. The immobilization kit consisted of an orthopedic splint immobilizing the wrist and 4 fingers (DONJOY brand, "Comfort Digit" model) and an immobilization sling (DONJOY brand, GCI model). To ensure that immobilization was carried out correctly and maintained throughout the day, an accelerometer (GENEactiv Original) was placed on each participant's wrists, whether immobilized or not.

The second visit took place at 9:00 pm following the immobilization (or control) procedure at the Sleep and Vigilance Center of the Hôtel-Dieu hospital. After removing the

immobilization in the IMMO group, all participants were administered another physical warm-up phase before performing a pre-night test consisting of two blocks of the 8-element sequence before sleep time. The EEG equipment was then set up for the second recording night. The next morning, at around 7:30 am and approximately 1 hour after awakening, participants carried out a physical warm-up phase before performing a post-night test consisting of two blocks of the 8-element sequence. Finally, the generalizability of the learned motor skill was assessed by conducting two transfer tests. An inter-manual transfer test was first administered to all participants to investigate skill generalizability toward the transfer from one limb to another. In this inter-manual transfer test, participants were required to perform two blocks on the original 8-element motor sequence with their unpracticed, dominant right-hand fingers (same keypresses). A transfer test with a new (unpracticed) 8-element sequence of stimuli (V-C-N-V-N-B-C-B) was then presented to differentiate sequence learning from generalized practice effects[40,41]. All participants were requested to perform this new motor sequence using their left (non-dominant) hand fingers. Each of these transfer tests was preceded by a brief familiarization phase during which participants were asked to perform the corresponding 8-element sequence (original or new) with the required hand slowly until they reproduced it correctly three times in a row.

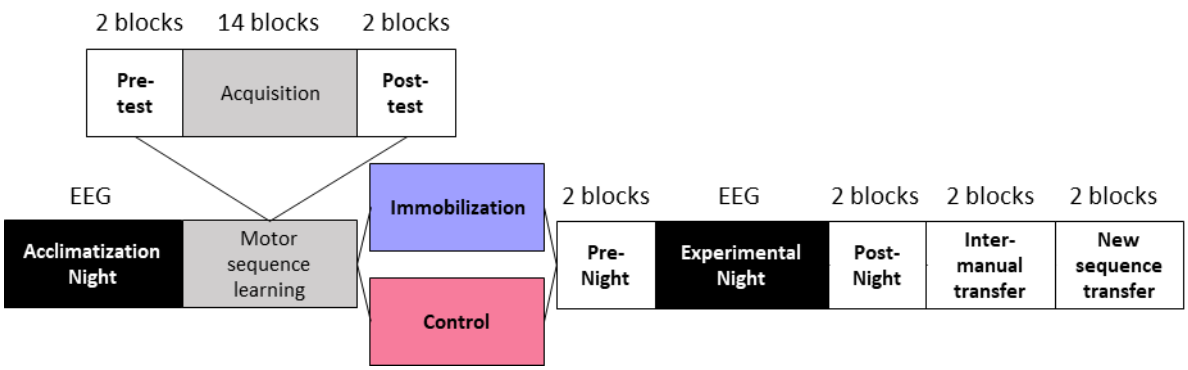

Fig. 1. Experimental design. Sleep EEG recordings of 30 participants were acquired during two consecutive nights. Between the two nights, in the morning, participants were trained on an 8-element finger movement sequence. Immediately after training, one-half of the participants were right-hand immobilized (n = 15, IMMO group) for a period of 13 hours, while the other half were not (n = 15, CTRL group). Following the immobilization or control procedure, all participants were tested on the trained motor sequence during pre-night and post-night tests. They were then evaluated on two transfer tests: an inter-manual transfer test (same motor sequence performed with the right-hand fingers), and a new motor-sequence test (unpracticed motor sequence performed with the left-hand fingers). EEG: Electroencephalography.

EEG-EMG data acquisition and pre-processing

EEG recordings. EEG was acquired using a 64-channel EEG cap (actiCAP snap BrainProducts Inc.) with slim-type electrodes (5kΩ safety resistor) suitable for sleep

recordings. For reliable sleep stage scoring, electrooculography (EOG) and electromyography (EMG) recordings were added using bipolar Ag-AgCl electrodes. The EOG components were recorded by placing a pair of electrodes laterally to both eyes. EMG bipolar electrodes were placed over the chin. All EEG, EMG, and EOG data were recorded using two battery-powered 32-channel amplifiers and a 16-channel bipolar amplifier (respectively, BrainAmp and BrainAmp ExG, Brain Products Inc.). All signals were recorded at a 1-kHz sampling rate with a 100-nV resolution. Electrode-skin impedance was kept below 5 kΩ using Abralyt HiCl electrode paste to ensure stable recordings throughout all experimental phases.

EEG data were down-sampled to 250 Hz, bandpass filtered between 0.5 and 50 Hz to remove low-frequency drift and high-frequency noise, and re-referenced to the linked mastoids (i.e., TP9 and TP10). EOG and EMG data were respectively bandpass filtered between 0.3-35 Hz and 10-100 Hz.

QUANTIFICATION AND STATISTICAL ANALYSIS

All subjects were included in statistical analyses (IMMO: n = 15; CTRL: n = 15), except as noted. Indeed, due to the absence of events of interest during some sleep EEG epochs, statistical power may be reduced for some specific analyses; this will be outlined where appropriate. The significant threshold was set at 0.05 for all analyses.

The sample size in each group was determined based on previous studies[9,42,43]. Error measurements refer to the standard error of the mean (SEM) for non-circular data, and the circular standard deviation otherwise.

Behavioral analysis

Response time (RT) was measured as the interval between two consecutive keypresses during each practice block. This performance index was described as more sensitive to the effects of sleep and the expression of relative performance gains[44,45]. Analyses regarding the number of accurately typed sequences are available in Supplementary Data (Fig. S1). Also, since participants were asked to start over from the beginning of the sequence if they made any error during task production, RTs from error trials (i.e., erroneous key presses) were excluded from the analyses. To better reflect individual performance on the motor sequence task, mean RT performance was computed on accurately typed sequences (see [12,46] for a similar procedure). Individual RTs were then averaged to obtain an overall estimation of the performance for each block. Motor skill consolidation was assessed by analyzing the RT performance changes (in percentages) from the pre-night to the post-night test blocks. The inter-manual transfer capacity

was quantified by the RT performance changes (in percentages) between the post-night and the inter-manual transfer test blocks. Finally, the generalization of learning, or the capacity of transferring sequence knowledge to a new (unpracticed) sequence, was quantified by the RT performance changes (in percentages) between the post-night and the new-sequence transfer test blocks.

Mixed ANOVAs with the between-subject factor CONDITION (IMMO, CTRL) and the within-subject factor BLOCK were performed on the RT data to assess the difference between groups in the magnitude of overnight motor skill consolidation and generalization. Holm post-hoc comparisons were performed in case of significant effects or interaction.

Actimetry analysis

To ensure that the upper-limb was correctly immobilized, two accelerometers were placed on the participants' wrists. The positions of both wrists were collected during the day between the acclimatization and experimental nights at a rate of 100 Hz. The velocity was computed across the day and averaged, giving us a mean velocity score for each upper-limb. This score allows us to compare the degree of use of the immobilized left limb with the contralateral right limb for each participant in the IMMO group, as well as with the non-immobilized upper-limbs of participants in the CTRL group. A mixed ANOVA was performed with the between-subject factor CONDITION (IMMO, CTRL) and the within-subject factor LATERALITY (Left, Right). Holm post-hoc comparisons were performed in case of significant effects or interaction.

EEG analysis

Power spectrum density. The artifact-free EEG signal was sleep-stage scored according to AASM guidelines[47]. Each 30-second epoch was visually scored as either NREM stages 1-3, REM, or wake. As described by Huber et al. (2006)[33], the immobilization procedure should induce local power decreases over sensorimotor regions mainly in the slow-oscillation frequency band (0.5-1.25 Hz) during the experimental night, at least in the first 20 minutes of sleep. Hence, for each participant and all electrodes, we restricted the EEG power spectrum density to the slow oscillation frequency band (0.5-1.25 Hz in steps of 0.25 Hz), computed using the Welch method (4-s Hamming window with 50% overlap between windows). The average power of the slow oscillation frequency band was obtained for all artifact-free NREM2 epochs of the first 20 minutes of sleep. A Student-t permutation test (1000 permutations) was performed to compare the power of the slow oscillation frequency band on each channel

between the IMMO and CTRL groups. The statistical map was then corrected for multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate[48] (Ntest = 63).

Spindle detection. The detection of sleep spindle events was conducted using all artifact-free NREM2 sleep epochs over electrode C4 of the acclimatization and experimental night. This electrode was chosen based on the a priori hypothesis that immobilization of the left upper limb should affect the right sensorimotor cortex[33,35]. Discrete sleep spindle events (i.e., onset and offset) were automatically detected using a wavelet-based algorithm (see [18,19] for further details). Spindles were detected at the C4 derivation by applying a dynamic thresholding algorithm (based on the mean absolute deviation of the power spectrum) to the extracted wavelet scale corresponding to the 11-16 Hz frequency range and a minimum window duration set at 300 ms[18,19,49] (see [8] for a review). Events were considered sleep spindles only if they lasted 0.3-2 seconds, occurred within the 11-16 Hz frequency range, and with onsets during NREM2 sleep periods. Visual inspections in random samples were done to ensure the correct detection of sleep spindles.

Spindle clustering. Sleep spindles may be split into two categories: clusters of two or more consecutive and electrode-specific spindle events interspaced by less than or equal to 6 seconds were categorized as trains, in comparison to those occurring in isolation (i.e., more than 6 seconds between two consecutive spindles detected on the same electrode)[8,18]. Hence, spindles belonging to trains were categorized as *grouped spindles*, and those occurring in isolation were categorized as *isolated spindles*. To evaluate the role of this temporal organization regarding sleep-related skill consolidation and generalizability, the proportion of grouped spindles defined by the total number of grouped spindles divided by the total number of spindles was computed. Hence, the greater the number of isolated spindles, the lower the proportion of grouped spindles. Pearson correlation analyses were performed to evaluate the relationship between the proportion of grouped spindles and the magnitude of skill consolidation, inter-manual skill transfer capacity, and generalization of learning to a new sequence.

Slow oscillation detection. Slow oscillation events were detected for each participant separately from sleep spindle events. The method used is similar to that described by Staresina et al. (2015)[30]. First, the EEG data were bandpass filtered between 0.5-1.25 Hz (two-pass finite

impulse response [FIR] bandpass filter). Only the artifact-free NREM2 and NREM3 epochs were considered. All zero-crossings were determined in the filtered signal, and SO candidates were identified as two successive positive-to-negative zero-crossings (i.e., down-states followed by up-states). SO events were retained if their duration was between 0.8 and 2 sec. Finally, the amplitude of the remaining SO events was determined. They were classified as SO if they met the amplitude criteria (≥ 75 % percentile of all SO amplitudes). Visual inspections in random samples were done to ensure the correct SO detection.

Phase-amplitude coupling. Sleep spindles and slow oscillations were classified as concomitant events if the onset of a sleep spindle occurred within the time interval of a slow oscillation (i.e., co-occurrence of SO and spindles). For each of these concomitant events, a Hilbert transform was applied to the extracted time window surrounding the SO onset (-1 to 2.5 seconds around the SO onset) to extract the instantaneous phase angle of the SO component (0.5-1.25 Hz) corresponding to the spindle power peak (11-16 Hz). For each participant, the average (preferred) phase θ between all concomitant events was given by the synchronization index (SI).

$$SI = \frac{1}{n} \sum_{k=1}^{n} e^{i\theta_k}$$

Where n is the number of concomitant events, θ_k is the SO phase value corresponding to the spindle power peak of the concomitant event k. The SI is a complex number whose angle corresponds to the preferred phase of this synchronization, and its absolute value gives the average length of the resultant vector, from which the circular standard deviation is calculated. For illustration purposes, time-frequency maps in the sleep spindle frequency band were provided with the superimposed SO-filtered signal. For each extracted time window (-1 to 2.5 seconds around the SO onset), a time-frequency decomposition across the 10-20 Hz frequency band was performed using an eight-cycle Morlet wavelet (FWHM: 3 seconds for a central frequency of 1 Hz). Finally, the grand average TF map and SO-filtered signal were computed for each participant.

For each group, the distribution of the preferred phase was tested by the Rayleigh test. It is noteworthy that a non-uniform distribution of the preferred phase is an indicator of coupling[50]. Watson-Williams tests were then used to compare the preferred phase of SO-spindle coupling between groups.

Temporal organization of sleep spindles. Two rhythms govern the temporal dynamics of sleep spindle activity: spindles tend to cluster on a low-frequency time scale of about 50 seconds (~0.02 Hz infraslow rhythm), during which spindles iterate every 3-4 s (~0.2-0.3 Hz mesoscale rhythm). *Infraslow rhythm.* The procedure to highlight the infraslow oscillation is similar to that described by Lecci et al. (2017)[22]. To summarize, a continuous wavelet transform was performed on the artifact-free EEG data during the full night of sleep. The power time course was calculated in the 11-16 Hz range in steps of 0.2 Hz using a four-cycle Morlet wavelet (full width at half maximum; FWHM: 1.5 second for a central frequency of 1 Hz) and extracted for all NREM2 sleep epochs. The average power in the spindle frequency band was calculated at each time point. A symmetric 4-second moving average was applied on the power time course to all consecutive 30-second NREM2 epochs, followed by its standardization, to reduce the temporal resolution and highlight the infraslow oscillation. A second continuous wavelet transform was performed on the power time course of the spindle frequency band, at a frequency resolution of 0.001 Hz in the 0.001-0.12 Hz range, and applied to all NREM2 epochs lasting more than 120 seconds (constituting a NREM2 period). Lastly, the spectral profile for each participant was computed by averaging the spectrum across all NREM2 periods with a 0.5-second time step, weighted by their duration. The final spectral profile per subject was obtained by normalizing it to its own mean. To gather the spectral profile peak for each participant and its corresponding frequency, a Gaussian fit (three terms) was performed, and the maximal value of the fitted curve was extracted. This maximal value, corresponding to the infraslow rhythm peak, was then compared between groups. *Mesoscale rhythm.* The same procedure was applied to study the mesoscale rhythm, except for the symmetric moving average which was not applied. After computation of the average power time course in the spindle frequency band, a second continuous wavelet transform was applied to all consecutive 30-second NREM2 epochs at a frequency resolution of 0.01 Hz in the 0.1-0.6 Hz range. Lastly, the spectral profile for each participant was computed by averaging the spectrum across all NREM2 periods with a 0.5-second time step, weighted by their duration. The final spectral profile was obtained by normalizing it to its own mean. A Gaussian fit (three terms) was performed, and the maximal value of the fitted curve was extracted. This maximal value, corresponding to the mesoscale rhythm peak, was then compared between groups.

Independent Student's t-tests were performed between the IMMO and CTRL groups to compare the effects of the short-term upper-limb immobilization on the infralow and mesoscale rhythm peaks, respectively.

Results

Control of the immobilization procedure

To control for the correct immobilization of the left upper limb in the IMMO group, a mixed ANOVA was performed on the mean velocity score computed from the accelerometer with the between-subject factor CONDITION (IMMO, CTRL) and the within-subject factor LATERALITY (Left, Right). The analysis revealed significant main effects for the factor CONDITION ($F(1,28) = 21.0$, $p < 0.001$, $\eta^2p = 0.43$), and LATERALITY ($F(1,28) = 164$, $p < 0.001$, $\eta^2p = 0.85$), as well as a significant CONDITION x LATERALITY interaction ($F(1,28) = 111$, $p < 0.001$, $\eta^2p = 0.80$). Holm post-hoc tests revealed that the velocity score of the left limb was lower in the IMMO group than in the CTRL group ($p < 0.001$), as well as compared to the right limb of the CTRL group ($p < 0.001$) and IMMO group ($p < 0.001$), which did not differ from each other ($p = 0.75$). These findings thus guarantee compliance with the immobilization procedure in the IMMO group, whose participants used their left upper-limb significantly less than their right upper-limb, and less than both upper-limbs in the CTRL group.

Behavioral results.

Figure 2 shows the mean response times measured for each test block. Analyses regarding the number of accurately typed sequences are available in Supplementary Data (Fig. S1). A mixed ANOVA using a CONDITION (IMMO, CTRL) x BLOCK (pre-test, post-test) factorial design, with repeated measures on the BLOCK factor, was first performed to ensure that the two groups did not differ in terms of initial performance and at the end of the acquisition phase. The analysis revealed a significant effect of the factor BLOCK ($F(1, 28) = 102$, $p < 0.001$, $\eta^2p = 0.79$), indicating performance improvements during training for both groups, from the pre-test to the post-test. However, the analysis failed to detect a significant effect of the factor CONDITION ($F(1, 28) = 0.93$, $p = 0.34$, $\eta^2p = 0.03$), nor a BLOCK x CONDITION interaction ($F(1, 28) = 0.44$, $p = 0.51$, $\eta^2p = 0.02$), suggesting that performance on tests administered before and after the immobilization procedure did not differ between participants in the IMMO ($TR_{pre\text{-}test} = 568 \pm 58$ ms, $TR_{post\text{-}test} = 332 \pm 28$ ms) and CTRL groups ($TR_{pre\text{-}test} = 644 \pm 60$ ms, $TR_{post\text{-}test} = 376 \pm 35$ ms).

To compare changes in performance over the 13-hour retention interval, a mixed ANOVA was performed using a CONDITION (IMMO, CTRL) x BLOCK (post-test, pre-night) factorial design, with repeated measures on the factor BLOCK. The analysis revealed a significant main effect of the factor BLOCK ($F(1, 28) = 7.30$, $p = 0.012$, $\eta^2p = 0.21$), suggesting

a deterioration in performance during the day for both the CTRL ($TR_{post\text{-}test}$ = 376 ± 35 ms, $TR_{pre\text{-}night}$ = 417 ± 39 ms) and IMMO groups ($TR_{post\text{-}test}$ = 332 ± 28 ms, $TR_{pre\text{-}night}$ = 381 ± 48 ms). However, the analysis failed to detect a main effect of the factor CONDITION ($F(1, 28) = 0.60$, $p = 0.44$, $\eta^2p = 0.02$) and a CONDITION x BLOCK interaction ($F(1, 28) = 0.04$, $p = 0.84$, $\eta^2p < 0.01$).

To compare changes in performance after the experimental night, a mixed ANOVA was performed using a CONDITION (IMMO, CTRL) x BLOCK (pre-night, post-night) factorial design, with repeated measures on the factor BLOCK. The analysis revealed a significant main effect of the factor BLOCK ($F(1, 28) = 29.4$, $p < 0.001$, $\eta^2p = 0.51$). However, the analysis failed to detect a main effect of the factor CONDITION ($F(1, 28) = 0.25$, $p = 0.62$, $\eta^2p < 0.01$) and a CONDITION x BLOCK interaction ($F(1, 28) = 0.74$, $p = 0.40$, $\eta^2p = 0.03$). Thus, both groups improved after the night, but overnight performance changes were not significantly different between the CTRL ($TR_{pre\text{-}night}$ = 417 ± 39 ms, $TR_{post\text{-}night}$ = 346 ± 33 ms) and IMMO groups ($TR_{pre\text{-}night}$ = 381 ± 48 ms, $TR_{post\text{-}night}$ = 328 ± 33 ms).

Finally, to compare skill generalizability between groups, a mixed ANOVA was performed using a CONDITION (IMMO, CTRL) x BLOCK (post-night, inter-manual transfer, new-sequence transfer) factorial design, with repeated measures on the factor BLOCK. Two participants in the IMMO group were removed from this analysis as they did not perform the new-sequence transfer test. The analysis revealed a significant effect of the factor BLOCK ($F(2, 52) = 30.9$, $p < 0.001$, $\eta^2p = 0.54$) and a significant CONDITION x BLOCK interaction ($F(2, 52) = 4.64$, $p = 0.014$, $\eta^2p = 0.15$). The analysis failed to detect a significant main effect of the factor CONDITION ($F(1, 26) = 0.19$, $p = 0.67$, $\eta^2p < 0.01$). Concerning the CONDITION x BLOCK interaction, Holm's post-hoc comparisons revealed significant performance decreases from the post-night test to the inter-manual transfer test in the CTRL group only ($TR_{post\text{-}night}$ = 346 ± 33 ms, $TR_{inter\text{-}manual}$ = 446 ± 50 ms, $p < 0.001$), but not in the IMMO group ($TR_{post\text{-}night}$ = 315 ± 33 ms, $TR_{inter\text{-}manual}$ = 377 ± 30 ms, $p = 0.17$). Also, post-hoc comparisons revealed impaired skill transfer toward a new motor sequence for both experimental groups, as expressed by significant performance decreases from the post-night test to the new-sequence transfer test ($TR_{new\text{-}sequence}$ = 479 ± 42 ms, $p < 0.001$ and $TR_{new\text{-}sequence}$ = 446 ± 38 ms, $p < 0.001$ for the IMMO and CTRL groups, respectively). Finally, the IMMO group showed a significant difference in performance between the inter-manual transfer test and the new-sequence transfer test ($p = 0.002$), in contrast to the CTRL group ($p = 1$).

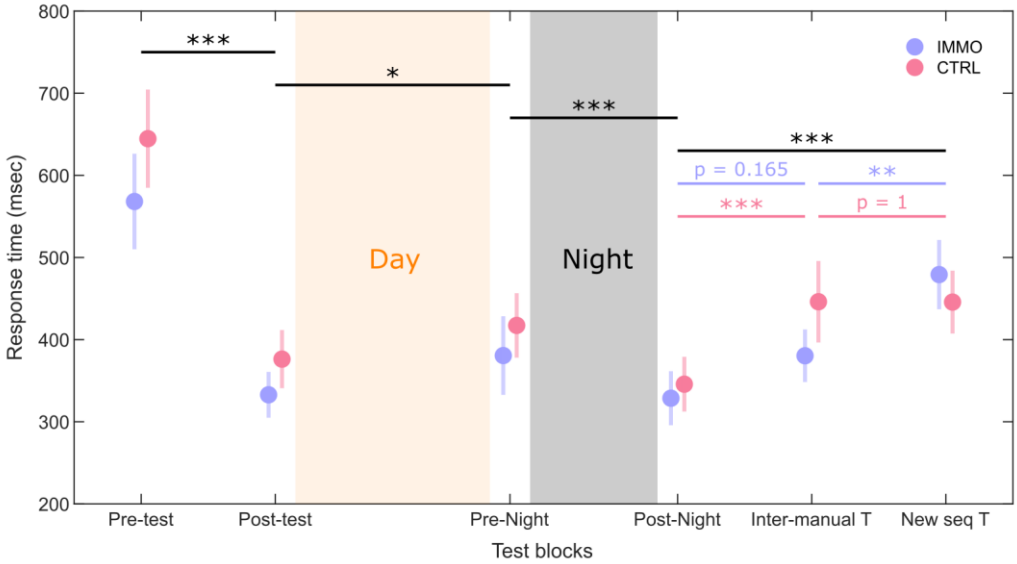

Figure 2. Behavioral data. Mean response time for each test block in the IMMO group (purple) and CTRL group (pink). The colored circles represent the group average, and the bar represents the standard error of the mean (n = 15 for each group and each test block, with the exception of the transfer test blocks with n = 13 for the IMMO group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Inter-manual T: Inter-manual transfer; New seq T: new-sequence transfer.

Slow oscillation spectral power.

To confirm the induction of synaptic depression by the short-term sensorimotor restriction, a Student-t permutation test for independent samples (1000 permutations) was performed to obtain the statistical topographic map of the spectral power difference in the slow oscillation frequency band between the IMMO and CTRL groups (Fig. 3). The analysis revealed significant decreases in SO power in the IMMO group than in the CTRL group on multiple electrodes, which are primarily located over the sensorimotor cortex contralateral to the immobilized limb, after correction for multiple comparisons using the Benjamini-Hochberg procedure[48] to control the false discovery rate (Ntest = 63). Hence, the present result shows a general decrease in the spectral power of slow oscillations during the first 20 minutes of NREM2 sleep following the 13 hours of upper-limb immobilization. We also performed supplementary analyses on the C4 derivation, showing that the difference in the SO power decrease between the two groups fades when considering the whole night (Fig. S2.).

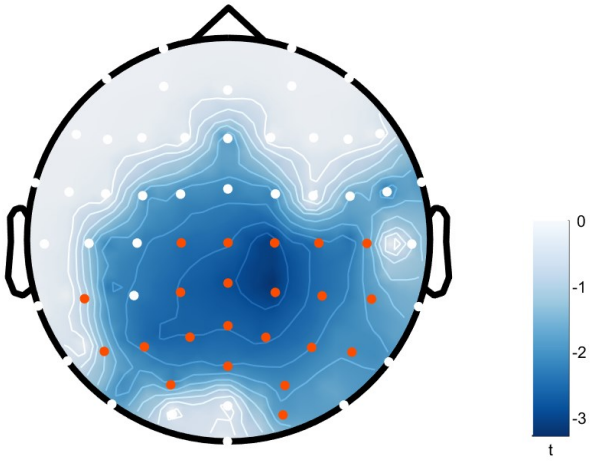

Figure 3. Spectral power of the slow oscillation frequency band. Topographical statistical maps of the difference in SO (0.5–1.25 Hz) spectral power between the IMMO and CTRL groups during the first 20 minutes of NREM2 sleep. The color bar represents the t-test values. Negative t values (blue) represent lower spectral power for the IMMO group (n = 15) compared with the CTRL group (n = 15). White areas represent non-significant difference between groups after permutation test (1000 permutations). The red dots highlight the electrodes with statistical difference after correction for multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (Ntest = 63).

Skill consolidation/generalization and sleep spindles

To assess the specific role of grouped and isolated sleep spindles in the skill consolidation process, correlation analyses were performed between the proportion of grouped over isolated spindles during NREM2 sleep, extracted at scalp derivation C4, and the magnitude of post-night performance changes (percentage of performance changes from the pre-night test to the post-night test). One participant in the CTRL group did not show grouped spindles in the first 20 minutes of NREM2 sleep and was therefore excluded from the correlation analysis. A significant positive relationship was observed between the proportion of grouped spindles during the first 20 minutes of NREM2 sleep and the magnitude of post-sleep performance changes in the CTRL group only (r = 0.58, p = 0.031) but not in the IMMO group (r = -0.10, p = 0.72) (Fig. 4A). In contrast, no significant relationship was found when the proportion of grouped spindles was computed over the whole night, either in the CTRL group (r = -0.23, p = 0.41) or in the IMMO group (r = 0.03, p = 0.92).

We also assessed the role of grouped spindles in the ability to generalize the acquired skill to another one or another effector, such as inter-limb transfer. To that end, we separately correlated the proportion of grouped over isolated spindles during NREM2 sleep, extracted at scalp derivation C4, with the magnitude of i) inter-manual skill transfer (percentage of performance changes from the post-night test to the inter-manual transfer test) and ii) skill

transfer toward a new sequence (percentage of performance changes from the post-night test to the new-sequence transfer test). As mentioned previously, two participants of the IMMO group did not undergo the new-sequence transfer test and were therefore excluded from these analyses. When analyzing the first 20 minutes of NREM2 sleep, no significant relationship was found between the proportion of grouped spindles and the magnitude of inter-manual skill transfer in the IMMO group ($r = -0.20$, $p = 0.47$) and the CTRL group ($r = -0.21$, $p = 0.48$) (Fig. 4B). Similarly, no significant relationship was found when the proportion of grouped spindles was computed over the whole night in the CTRL ($r = 0.23$, $p = 0.41$) and IMMO groups ($r = -0.27$, $p = 0.33$). Interestingly, though, the analysis revealed a significant negative relationship between the proportion of grouped spindles and the magnitude of skill transfer to the new motor sequence in the IMMO group only ($r = -0.70$, $p = 0.008$) but not in the CTRL group ($r = -0.33$, $p = 0.26$) (Fig. 4C). No significant relationship was found when considering the whole night, either in the CTRL group ($r = 0.04$, $p = 0.89$) or in the IMMO group ($r = -0.32$, $p = 0.29$).

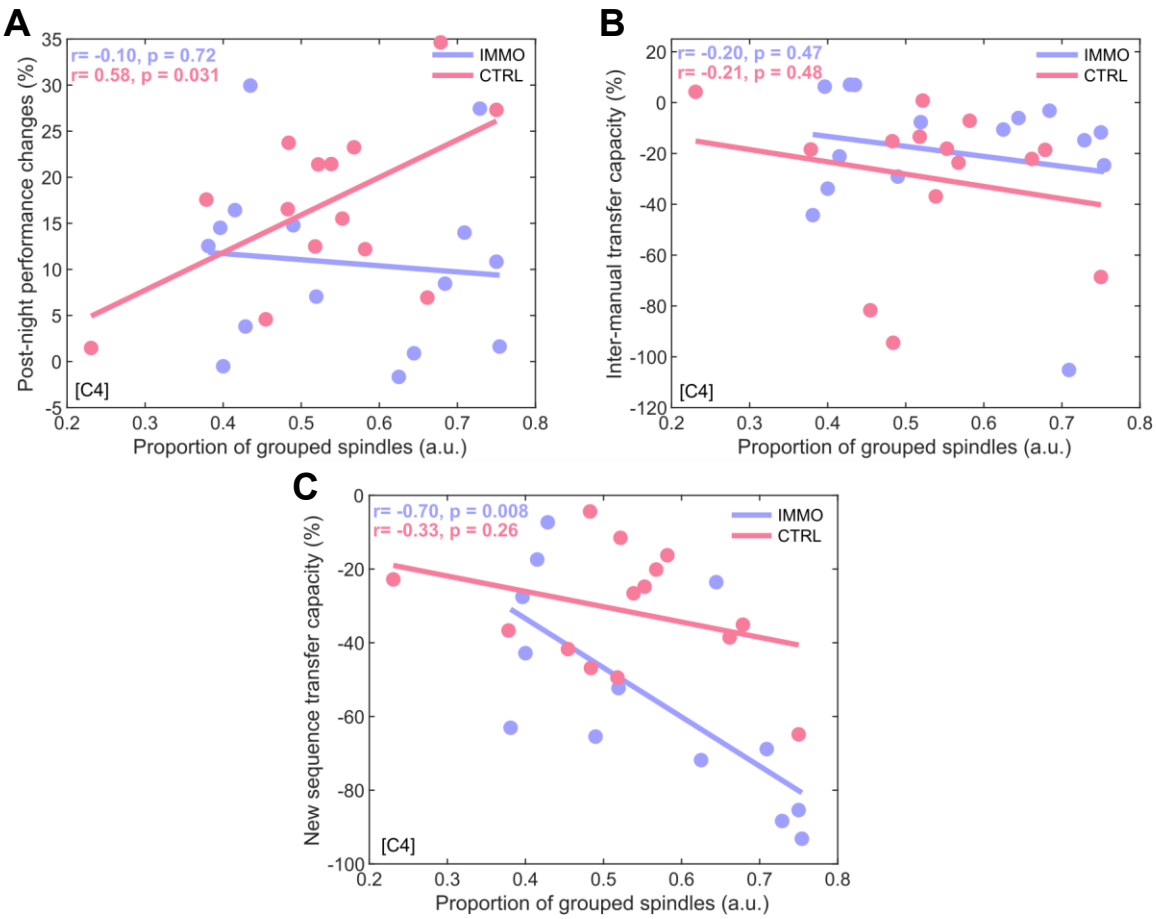

Figure 4. Role of grouped spindles in motor skill consolidation and generalization. (A) Relationship between the proportion of grouped spindles over isolated spindles (a.u.), extracted at scalp derivation C4, and the magnitude of post-night performance changes (in %) on the learned motor sequence in the IMMO (n = 15) and CTRL (n = 14) groups. **(B)** Relationship between the proportion of grouped spindles over isolated spindles (a.u.), extracted at scalp derivation C4, and the magnitude of inter-manual skill transfer (in %) in the IMMO (n = 15) and CTRL (n = 14) groups. **(C)** Relationship between the proportion of grouped spindles over isolated spindles (a.u.), extracted at scalp derivation C4, and the magnitude of skill transfer toward a new sequence (in %) in the IMMO (n = 13) and CTRL (n = 14) groups. Individual data (colored circles) and trend lines are provided, along with Pearson's r and associated p-values.

Phase-amplitude coupling

Phase-amplitude coupling analyses were performed to assess whether the upper limb's transient immobilization influences the coupling between slow oscillations and sleep spindles. These analyses were first performed on the SO-spindle concomitant events detected during the first 20 minutes of NREM2 sleep, extracted at scalp derivation C4 (Fig. 5A and B). Participants without spindles coupled to slow oscillations during this period were removed from the current analysis (2 in the IMMO group, 4 in the CTRL group). The Rayleigh and the Watson-Williams tests were applied to test the non-uniformity of the preferred coupling phases and to compare them between the IMMO and CTRL groups. Analyses indicated a non-uniform coupling in both

the IMMO group ($\theta = 0$ rad, Rayleigh $Z = 9.54$, $p < 0.001$) and the CTRL group ($\theta = -0.83$ rad, Rayleigh $Z = 7.97$, $p < 0.001$). Also, a significant difference in the preferred coupling phase was observed ($F(1,22) = 11.9$, $p = 0.002$) during the first 20 minutes of NREM2 sleep, showing a delay in the coupling of slow oscillations and sleep spindles for participants in the IMMO group compared to their CTRL group counterparts.

The limited number of participants showing SO-spindle coupling with both types of spindles (grouped and isolated) during the first 20 minutes of NREM2 sleep does not allow us to perform such clustering analyses. Therefore, separate analyses of the SO-spindle coupling for grouped and isolated spindles were only performed on all artifact-free NREM2 epochs over the entire sleep recording (whole night) (Fig. 5A and C).

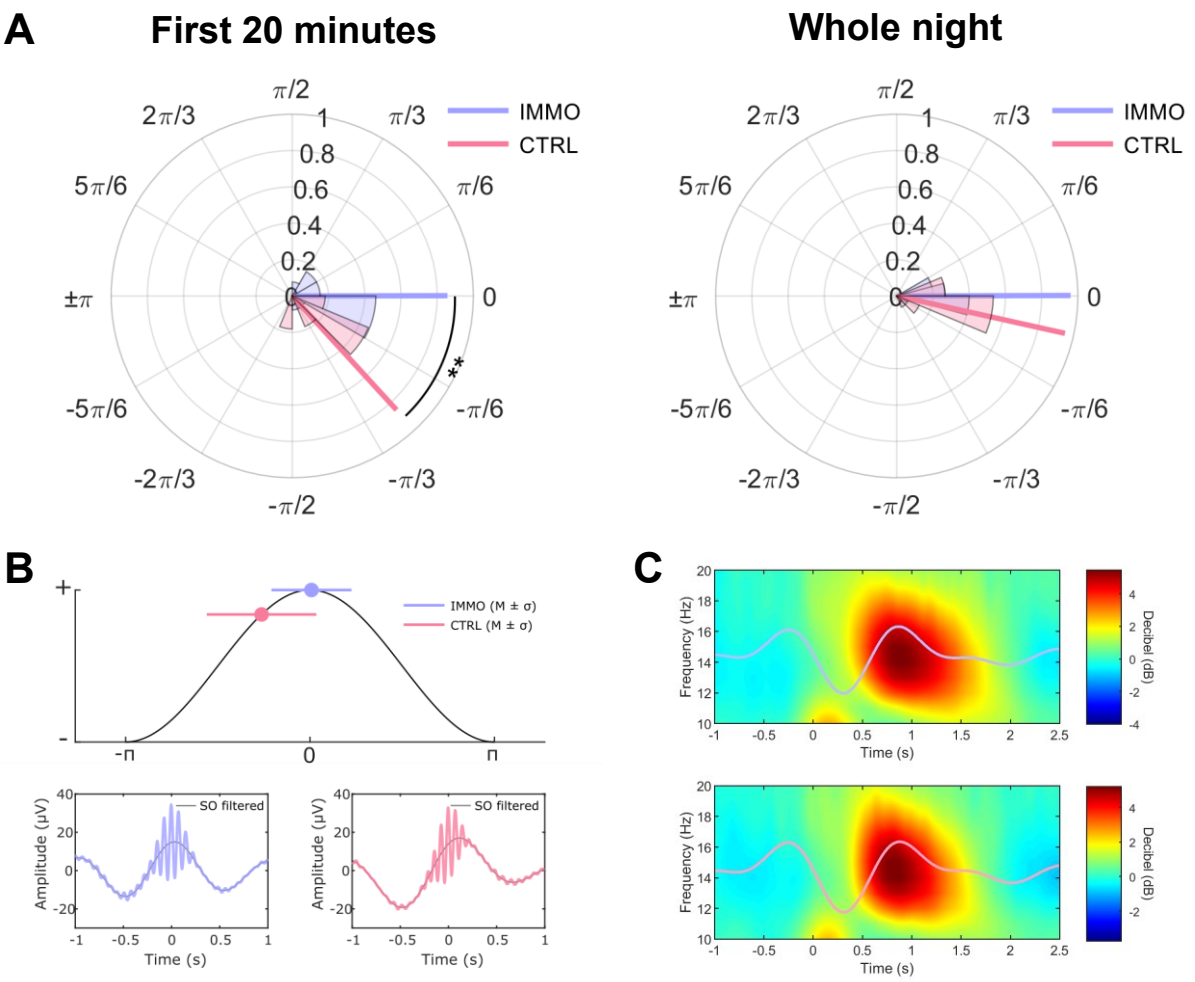

Figure 5. Phase-amplitude coupling of slow oscillation and sleep spindles. (A) Preferred phase in radians (and mean length of the resultant vector) of the slow oscillation at the peak power of sleep spindles, detected at scalp derivation C4, for the IMMO (purple) and CTRL (pink) groups during NREM2 sleep when considering the first 20 minutes (left) and the whole night (right). Probability density plots of phases (histograms) are also provided. **(B)** Top. Representation of the mean preferred phase and the circular standard deviation of the SO-spindle coupling for the IMMO and CTRL groups on a simulated slow oscillation. Bottom. Peak-locked sleep spindle group average across all concomitant events during the first 20 minutes of NREM2 sleep, with the bandpass-filtered SO trace (in gray) superimposed to highlight the SO-spindle coupling in both the IMMO (left) and CTRL (right) groups. **(C)** Time-frequency maps (in dB) in the 10-20 Hz frequency range of all concomitant events detected at scalp derivation C4 during all NREM2 epochs over the whole night, using epoch windows ranging from -1 to +2.5 sec around slow oscillation onset. Time-frequency maps were displayed with the average bandpass-filtered SO trace superimposed to illustrate the high synchronization between sleep spindle power and slow oscillation phase in the IMMO (top) and CTRL (bottom) groups. ** $p < 0.01$

First, for all NREM2 sleep spindles, a non-uniform SO-spindle coupling was found for participants in the IMMO group ($\theta = 0$ rad, Rayleigh Z = 13.8, $p < 0.001$) and in the CTRL group ($\theta = -0.22$ rad, Rayleigh Z = 13.6, $p < 0.001$). No significant group difference in the

preferred phase was observed ($F(1,28) = 3.77$, $p = 0.062$), showing the absence of phase shift in the SO-spindle coupling between the IMMO and CTRL groups when considering all NREM2 sleep spindles of the whole night.

Second, for all NREM2 grouped spindles, a non-uniform SO-spindle coupling was also found for participants in the IMMO group ($\theta = 0.24$ rad, Rayleigh $Z = 8.90$, $p < 0.001$) and in the CTRL group ($\theta = -0.23$ rad, Rayleigh $Z = 12.2$, $p < 0.001$). A significant group difference in the preferred phase was observed ($F(1,28) = 4.25$, $p = 0.049$), suggesting that a phase shift in the SO-spindle coupling between the IMMO and CTRL groups is present when considering only grouped spindles.

Third, for all NREM2 isolated spindles, a non-uniform SO-spindle coupling was also found for participants in the IMMO group ($\theta = -0.08$ rad, Rayleigh $Z = 13.9$, $p < 0.001$) and in the CTRL group ($\theta = -0.23$ rad, Rayleigh $Z = 13.6$, $p < 0.001$). Interestingly, though, no significant group difference in the preferred phase was observed ($F(1,28) = 1.76$, $p = 0.20$), showing the absence of phase shift in the SO-spindle coupling between the IMMO and CTRL groups when considering only isolated spindles.

These results suggest that synaptic depression induced by transient immobilization delayed the onset of the power peak of spindles occurring in trains (grouped spindles) but not when occurring in isolation. The same analyses were performed on the pre-experimental acclimatization night to ensure this difference was due to the immobilization procedure. No difference was observed between the IMMO and CTRL groups, either when considering all spindles, grouped and isolated spindles, as well as the first 20 minutes of NREM2 sleep or the whole night. Detailed analyses of the acclimatization night are provided in supplementary data (Fig. S3). Similar analyses were also carried out on other electrodes (see Supplementary data, Fig. S4). This SO-spindle shift between the IMMO and CTRL groups was only found for a few electrodes, mainly located over the right sensorimotor cortex when considering grouped spindles, suggesting a local effect of the immobilization on the SO-spindle coupling.

Temporal organization of sleep spindles

The temporal organization of sleep spindles is governed by an infraslow (~0.02 Hz) and a mesoscale rhythm (~0.2–0.3 Hz). The infraslow and mesoscale spectral profiles were computed for all artifact-free NREM2 sleep epochs during the whole experimental night (Fig. 6). An independent Student t-test was then performed between the IMMO and CTRL groups to compare the effect of the immobilization on the peak power's frequency of the infralow periodicity. The analyses failed to reveal a significant difference ($t(1, 28) = 1.05$, $p = 0.30$, d =

0.39), suggesting that the periodicity of spindle trains (infraslow rhythm) did not differ between the IMMO (P = 0.017 ± 0.004 Hz) and CTRL groups (P = 0.019 ± 0.005 Hz).

An independent Student t-test was also performed between the IMMO and CTRL groups to compare the effect of the immobilization on the peak power's frequency of the mesoscale periodicity. The analyses failed to reveal a significant difference (t(1,28) < 0.01, p = 1, d < 0.01), suggesting that the periodicity of spindles within trains (mesoscale rhythm) did not differ between the IMMO (P = 0.21 ± 0.03 Hz) and CTRL groups (P = 0.21 ± 0.04 Hz). The present results accord with previous findings, confirming the hierarchical multi-scale periodicity in sleep spindle activity with spindle-band power fluctuations at frequencies close to 0.02 Hz and 0.2–0.3 Hz. Here, the immobilization procedure did not affect the double periodicity of the spindle-band power during NREM2 sleep.

In addition, similar analyses were performed for the acclimatization night (see Supplementary data, Fig. S5). These supplementary analyses failed to detect significant differences, highlighting the stability of the multi-scale sleep spindle periodicity before and after the immobilization procedure.

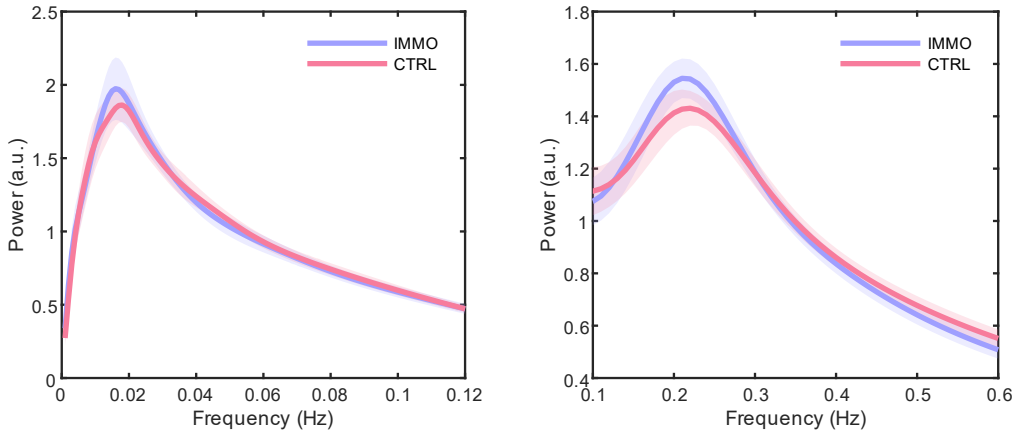

Figure 6. Multi-scale fluctuations of spindle-band power. Grand average of the spectral profile at the scalp derivation C4, ranging from 0.001 to 0.12 Hz (left panel) and 0.1 to 0.6 Hz (right panel) frequency range of the sleep spindle frequency band (11–16 Hz) power fluctuation during NREM2 sleep episodes across the whole night. The peaks around 0.02 Hz and 0.2 Hz for both the IMMO (purple) and CTRL (pink) groups highlight the multi-scale periodicity of spindle-band power at an infraslow and mesoscale rhythm, respectively.

Discussion

In the current study, we examined (i) the effects of daytime sensorimotor experience on local sleep spindle expression (i.e., clustering and rhythmicity) and the integrity of the cross-frequency coupling between SOs and spindles, and (ii) their functional contribution in the

consolidation and generalization of motor skills. On the one hand, our findings corroborate previous work[32,33] by showing the induction of local synaptic depression following immobilization, as reflected by significant local decreases in the SO spectral power during the first 20 minutes of the night in the IMMO group compared to the CTRL group (IMMO-CTRL contrast). Specifically, the decrease in SO power was more pronounced over the sensorimotor cortex of the immobilized limb. However, despite this immobilization-induced synaptic depression, our results did not reveal any significant difference between the IMMO and CTRL groups regarding spindle clustering and rhythmicity: sleep spindles tend to cluster on a low-frequency time scale of about 50 seconds (~0.02 Hz rhythm), during which spindles iterate every 3-4 seconds (~0.2-0.3 Hz rhythm), irrespective of the initial learning context. On the other hand, alterations of the coupling between SOs and spindles during NREM2 sleep have been shown to occur locally over the affected sensorimotor cortex (i.e., contralateral to the immobilized limb) mainly during the first 20 minutes of sleep but not when considering the whole night. Interestingly, immobilization induced a phase shift in the SO-spindle coupling for grouped spindles but not for isolated spindles, supporting the hypothesis of their functional differences in memory consolidation. Finally, performance on the transfer tests reveals that the representation of the consolidated skill differs between the IMMO and CTRL groups. More specifically, our results accord with recent theories assuming that sleep spindles occurring in trains (grouped spindles) play a more critical role than isolated ones in motor skill consolidation. In contrast, isolated spindles may impair skill consolidation by providing conditions of memory instability that support the creation of generalized knowledge.

At the behavioral level, our findings revealed that both groups deteriorated performance from the end of training to the delayed test session (after 13 hours of wakefulness). It has previously been shown that early learning results in a degradation of performance throughout the day, potentially due to a reduction in the signal-to-noise ratio of the newly acquired memory trace and retroactive interference from the learning of new information[51]. This result may also be attributed to the "early boost" phenomenon, which could have yielded enhanced performances during the 5-minute delayed post-training test, with performances returning to baseline a few hours later[52]. However, transient immobilization of the upper limb did not significantly worsen performance deterioration during the day. This result may be surprising at first glance, but the deleterious effect of immobilization has mainly been shown on mental rotation[53,54] and pointing tasks[33,55], in contrast to motor sequence tasks[56]. Indeed, it has been shown that applying low-frequency repetitive transcranial magnetic stimulation (rTMS), a procedure that interferes with local cortical function, 30 minutes after motor sequence learning

over the primary motor cortex (M1) reduced the "early boost" phenomenon. In contrast, rTMS applied 4 hours after motor sequence learning over M1 did not affect task performance[57], thus highlighting the functional role of M1 in the early stage of motor skill consolidation but not in the more delayed stages. Therefore, the delayed synaptic depression induced by limb immobilization locally over the contralateral sensorimotor cortex may not have been early enough to disrupt the early (boost) phase of motor skill consolidation.

Likewise, disruption of the sensorimotor cortex by transient limb immobilization did not affect the later stages of motor skill consolidation, expressed behaviorally by the magnitude of (overnight) skill retention performance. In contrast, both groups improved performance from the pre-night test to the post-night test session. We conjecture that sensorimotor restriction may have induced the reactivation of additional unaffected brain regions during sleep, acting as a compensatory consolidation mechanism to mitigate the deleterious effects of local synaptic depression. Indeed, current results indicated that the IMMO group did not perform significantly worse on the inter-manual transfer test than on the post-night test. At the same time, significant performance decreases were found for the CTRL group. It suggests that the representation of the consolidated memory differs between groups, although similar behavioral outcomes were expressed at the post-night test. This latter finding is consistent with previous work suggesting that immobilization of the nondominant limb does not impair the performance of the dominant limb[56,58], and in cases of prolonged immobilization, it may even lead to improved performance in the opposite limb[59]. This hypothesis is also supported from a neurophysiological perspective. The transient immobilization of a limb, and potentially the overuse of the opposite limb, reduces inter-hemispheric inhibition produced by the hemisphere of the immobilized limb[60] and, in turn, increases the excitability of the hemisphere of the non-immobilized limb. Thus, the 13 hours of immobilization in our study, combined with a night of sleep and the associated consolidation processes, contributed to enhanced inter-manual transfer performance in the IMMO group.

At the brain level, our findings corroborate previous work[32,33] by showing local significant decreases in the SO spectral power during the first 20 minutes of NREM2 sleep in the IMMO group compared to the CTRL group (IMMO-CTRL contrast). Specifically, the decrease in SO power was more pronounced over the sensorimotor cortex of the immobilized limb. Interestingly, though, this early-night difference in SO power decreases between the two groups dissipates progressively throughout the night (Fig. S2). Hence, slow-wave activity may be viewed as a sensitive marker of synaptic efficacy, which is modulated by daytime sensorimotor experience[32,33,61]. In our study, the decrease in slow oscillation activity, which corresponds to the low-SWA frequency range, indicates that transient daytime sensorimotor

restriction through limb immobilization has promoted local synaptic depression during the following night, reducing synaptic efficacy in the sensorimotor cortex of the immobilized limb.

Despite this immobilization-induced synaptic depression, our results did not reveal any significant difference between the IMMO and CTRL groups regarding spindle clustering and rhythmicity. As previously shown, current results confirmed the clustering and temporal organization of spindle activity during NREM2 sleep, irrespective of the initial learning context: spindles tend to cluster on a low-frequency time scale of about 50 seconds (~0.02 Hz rhythm), during which spindles iterate every 3-4 seconds (~0.2-0.3 Hz rhythm)[8,14,18,20,62]. Thus, the temporal organization of spindles in trains appears to be an inherent clocking sleep mechanism independent of prior sensorimotor experience, regulated in a learning-independent manner and coordinated with autonomic nervous system-dependent parameters[8,14,18,20,22,63]. Yet, an in-depth analysis of spindle clustering revealed a significant positive relationship between the proportion of grouped spindles (ratio grouped/isolated spindles) during the first 20 minutes of sleep and the overnight performance changes for the CTRL group only, and not for the IMMO group. The results in the CTRL group accord with the recent findings of Boutin and colleagues[8,14,18,19]. Notably, this relationship was observed locally over the motor cortex contralateral to the immobilized limb, and no significant correlation was found when considering all NREM2 sleep periods across the entire night. Hence, this suggests that local synaptic depression through transient immobilization of the limb may have affected one of the mechanisms involved in spindle clustering, reducing or modifying the beneficial effects of repeated reactivation and reprocessing of memory traces during sleep consolidation. From a theoretical perspective, we may assume that sleep spindles selectively reactivate memory traces in a (pre-)defined temporal order that depends on the degree of synaptic plasticity (synaptic depression/potentiation) induced by daytime sensorimotor experience. In line with the synaptic homeostasis hypothesis[34] that emphasizes the role of sleep in regulating synaptic weight in the brain, which is supposed to be maximal at the beginning of sleep and then progressively returning to baseline, brain regions with higher synaptic efficacy following learning should be processed first during subsequent sleep.

In contrast, however, we demonstrate a significant negative relationship between the proportion of grouped spindles and the magnitude of skill transfer to the new motor sequence in the IMMO group only, not in the CTRL group. This latter result suggests that spindles occurring in isolation during NREM2 sleep may facilitate skill transfer or its generalizability toward a new (related) motor skill. This finding aligns with recent studies supporting the hypothesis that sleep spindles might also be related to forgetting[18,64–66]. More specifically,

Boutin and colleagues (2024)[18] recently suggested that spindles occurring in trains can strengthen memory representations through their timed reactivations, while in contrast, spindles occurring in isolation may instead activate sleep mechanisms promoting memory-instability conditions leading to the clearance or decreased accessibility of the memory content. At the same time, recent theoretical evidence suggests that memory instability may be critical for motor skill generalization[7]. Thus, immobilization-induced synaptic depression may have led to impaired reprocessing of the memory trace through local alterations of spindle clustering during consolidation, giving way to its weakening by isolated spindles and, consequently, its generalization. Altogether, our results accord with recent theories assuming that sleep spindles occurring in trains (grouped spindles) play a more critical role than isolated ones in motor skill consolidation by offering optimal conditions for motor memory strengthening, while isolated spindles may instead promote memory-instability conditions supporting the creation of generalized knowledge[4,7].

Finally, alterations of the coupling between SOs and spindles during NREM2 sleep have been shown to occur locally over the affected sensorimotor cortex (i.e., contralateral to the immobilized limb) mainly during the first 20 minutes of sleep. This result suggests that synaptic depression induced by transient immobilization delayed the onset of the power peak of sleep spindles. Interestingly, this delay is only observed for grouped spindles and not for isolated spindles, and it persists throughout the night. An argument in favor of the functional dissociation between grouped and isolated spindles comes from the recent study of Solano et al. (2022)[67], who demonstrated that the proportion of grouped spindles coupled to slow oscillations increased locally in the sensorimotor cortex of the trained effector, thus suggesting that the SO-spindle coupling relies on previous sensorimotor experience. Hence, it is likely that altered local synaptic efficacy at the cortical level disrupted the SO-spindle coupling during spindle trains only. However, the origin of this altered phase shift is uncertain, as immobilization-induced cortical neuroplasticity is assumed to occur early in the night[33]. In a previous study, Helfrich and colleagues (2018)[29] reported a phase shift in sleep spindle coupling in older adults, compared to young adults, which was associated with a memory consolidation deficit. This phase shift was linked to a structural alteration of the gray matter of the medial prefrontal cortex. In addition, it was described that the structural integrity of the thalamocortical fascicle influences sleep spindle density[68]. For example, daytime learning and sensorimotor experience can rapidly induce white matter changes[69], increasing sleep spindle production during the subsequent night[68]. Based on the observation that sensorimotor restriction also induces white matter structural changes[70], the transient alteration of white matter bundles may

be a potential cause of the SO-spindle phase shift. The latter hypothesis remains speculative, and further evaluations of the effects of sensorimotor restriction on sleep spindle expression are needed. Although the origin of this altered phase shift is uncertain, the specificity of this effect on grouped spindles highlights the potential existence of neurobiological mechanisms underlying and modulating the generation of grouped and isolated spindles, supporting the hypothesis of their functional differences in memory consolidation[71].

Conclusion

Our findings confirm the multiscale periodicity of sleep spindle activity regarding spindle clustering and rhythmicity. We further revealed that this temporal cluster-based organization of sleep spindles is not dependent on daytime sensorimotor experience. Furthermore, by experimentally inducing a phase shift in the coupling between slow oscillations and sleep spindles occurring in trains, but not in isolation, our results support the theoretical assumption that the two types of sleep spindles may have distinct functional roles in memory consolidation. While sleep spindles occurring in trains (grouped spindles) may be involved in motor skill consolidation by offering optimal conditions for memory strengthening, isolated spindles may instead be involved in the weakening or even forgetting of memories by creating conditions of memory instability, which may in turn favor the development of generalized knowledge.

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