Title

Electrophysiological signatures of targeted memory reactivation during sleep are not accompanied by motor performance improvements in older adults

Authors

Judith Nicolas[1,2,*], Julie Carrier[3,4], Stephan P. Swinnen[1,2], Julien Doyon[5], Geneviève Albouy[1,2,6,*], Bradley R. King[6]

Affiliation

[1] Department of Movement Sciences, Movement Control and Neuroplasticity Research Group, KU Leuven, 3001 Leuven, Belgium
[2] LBI - KU Leuven Brain Institute, KU Leuven, 3001 Leuven, Belgium
[3] Center for Advanced Research in Sleep Medicine, Centre Intégré Universitaire de Santé et de Services Sociaux du Nord-de-l'Ile de Montréal, Montreal, QC, Canada
[4] Department of Psychology, Université de Montréal, Montreal, QC, Canada
[5] McConnell Brain Imaging Centre, Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2T5.
[6] Department of Health and Kinesiology, College of Health, University of Utah, Salt Lake City, UT 84112, USA

[*] **Corresponding authors**

Judith Nicolas and Genevieve Albouy
Movement Control and Neuroplasticity Research Group
Department of Movement Sciences, KU Leuven
Tervuursevest 101 - Box 1501, 3001 Leuven, BELGIUM
Judith.nicolas@kuleuven.be; Genevieve.albouy@kuleuven.be

Abstract (250 words max)

Targeted memory reactivation (TMR) during post-learning sleep enhances memory consolidation in young adults by modulating electrophysiological markers of plasticity (i.e., slow waves (SW) and slow/sigma oscillation coupling). Interestingly, older adults are known to exhibit deficits in motor memory consolidation, an impairment that has been linked to age-related degradations in the same sleep features sensitive to TMR interventions. We thus hypothesized that TMR would enhance motor memory consolidation in older adults via the modulation of these electrophysiological markers. Seventeen healthy older participants (age range: 50-74) were trained on a bimanual motor task involving two sequences that were cued by different sounds. Participants were retested after a 90-minute nap and the following morning. During non-rapid eye movement sleep of the post-learning nap, two different auditory cues were played: one sound associated to a learned – and thus reactivated – sequence and one control sound not associated to learning. Results indicated that changes in performance did not differ between reactivated and non-reactivated sequences. Yet, analyses of electrophysiological data revealed that TMR increased SW density. Moreover, phase-amplitude coupling between slow (0.5-2 Hz) and beta (around 20 Hz) oscillations before the SW trough was strengthened by the presentation of the unassociated sounds. Furthermore, the increase in slow/sigma (12-16 Hz) oscillation coupling around 1.75 sec post-SW trough for unassociated (as compared to associated) sounds was related to higher TMR-induced performance enhancement. Our results collectively demonstrate that, in older adults, TMR did not impact motor performance but modulated sleep-related markers of plasticity likely involved in protection against irrelevant information.

Key words (10 max)

Aging, Motor learning, Targeted Memory Reactivation, Memory Consolidation, Sleep, Slow waves, Spindles, Sigma oscillations, Slow oscillations

1. Introduction

The development and maintenance of the incredibly vast repertoire of human movement depends on the integrity of the motor memory system, which facilitates the learning, consolidation, and retrieval of motor skills. There is considerable evidence in young adults indicating that sleep following initial learning provides a privileged window for the newly acquired memory to be consolidated into a stable, longer-term trace [1, 2]. Similar sleep-related benefits appear to be predominantly absent in healthy older adults [3, 4, 5, 6 but see 7, 8], a result that is, at least partially, linked to age-related degradations in sleep macro- and micro-architecture [5, 9]. Accordingly, the development and implementation of interventions enhancing sleep-facilitated memory consolidation in older adults offers a promising avenue to alleviate aging-associated deficits in learning and memory processes.

One approach that has the potential to enhance sleep-facilitated consolidation is targeted memory reactivation (TMR) [10, 11, 11]. Briefly, TMR consists of associating a sensory stimulus (e.g., a sound) with to-be-learned material (e.g., word pairs or a sequence of movements) during an initial encoding session. This stimulus is subsequently replayed during a post-learning sleep episode and is thought to reactivate the recently acquired memory trace. In young adults, TMR has proven to be effective in boosting consolidation processes following declarative [e.g. 13, 14, 15, 16] and motor [e.g. 17, 18, 19, 20] learning. These beneficial effects at the behavioral level are paralleled by specific changes at the electrophysiological level. Specifically, our recent research in the motor memory domain in young adults revealed that TMR modulates Slow Wave (SW – high amplitude waves in the 0.5–2 Hz frequency band) characteristics, such as density and peak-to-peak (PTP) amplitude. Additionally, an increase in coupling between slow oscillation phase and amplitude of sigma – 12 to 16 Hz – oscillations at the SW peak was related to higher effect of TMR on motor performance. Conversely, sounds that were not associated to learning strengthened SW/sigma coupling during the descending phase of the SW. These results collectively suggested that an increase of SW activity precisely coordinated with sigma oscillations is crucial for motor memory consolidation processes in young adults.

Interestingly, this previous research demonstrated that TMR modulated specific sleep features that have been previously linked to the aforementioned deficits in sleep-related memory consolidation in older adults. For example, aging is known to be accompanied by decreases in NREM 3 sleep duration as well as alterations in sleep-specific electrophysiological markers of plasticity [21]. Indeed, the number of sleep spindles decreases with age, and they exhibit smaller amplitude, shorter duration and lower frequency [5, 22, 23]. Slow wave density and amplitude also decrease [24, 25]; and, the coupling between SW and spindles exhibits age-related degradations [26]. This previous research collectively raises the intriguing possibility that TMR may be an effective avenue to enhance sleep-facilitated consolidation in healthy older adults via the modulation of these sleep features (e.g., slow and sigma oscillations as well as their phase amplitude coupling). The goal of this study therefore was to investigate auditory TMR-induced modulations of motor memory consolidation processes at the behavioral and electrophysiological levels in healthy older adults. We hypothesized that TMR would enhance motor memory consolidation in this population; and this boost would be linked to alterations in slow and sigma oscillation activity, as well as their coupling, during post-learning sleep.

2. Materials and Methods

The current study employed nearly identical experimental procedures and data analyses as our previous pre-registered study that examined effects of TMR in healthy young adults [27](only sound presentation devices differed).

2.1. Participants

This protocol was approved by the local Ethics Committee (B322201525025) and conducted according to the declaration of Helsinki [28]. Participants gave written informed consent before participating in this study. Monetary compensation was given for participants' time and effort. Healthy older adults (50 years and older) were recruited from Leuven (Belgium) and the surrounding area to serve as participants. Inclusion criteria were: 1) no previous extensive training with a musical instrument or as a professional typist, 2) free of medical, neurological, psychological, or psychiatric conditions, including depression and anxiety as assessed by the Beck's Depression [29] and Anxiety [30] Inventories, 3) no indications of abnormal sleep, as assessed by the Pittsburgh Sleep Quality Index [31]; 4) not considered extreme morning or evening types, as quantified with the Horne & Ostberg chronotype questionnaire [32]; and, 5) free of psychoactive or sleep-affecting medications.

Twenty-four older participants initiated the study protocol. Participation was terminated if sleep duration during the habituation nap was insufficient (less than 10 minutes; N = 3) or if the Montreal Cognitive Assessment [33] revealed signs of cognitive impairment (i.e., score below 26 ; N = 2). An additional participant was excluded due to experimental error and one individual did not complete the entire protocol. In total, 17 participants completed the study and were included in data analyses (see participant characteristics in Table 1).

Table 1. Participant characteristics

N	17 (8 females)
Age (yrs)	62.7 ranging from 50 to 74
Edinburgh Handedness	95.2 [88.7 - 100]
Epworth Sleepiness Scale	5.4 [3.7 – 7]
Beck Depression Scale	1.7 [0.3 – 3]
Beck Anxiety Scale	1.6 [0.3 – 3.1]
PSQI	3.4 [2.4 – 4.4]
Chronoscore (CRQ)	57.9 [53.8 – 62]
MOCA	28.8 [28.2 – 29.4]

Notes. Values are means [lower and upper limit of the 95% Confidence Interval (CI)]. PSQI = Pittsburgh Sleep Quality Index; CRQ = Circadian Rhythm Questionnaire; MOCA = Montreal Cognitive Assessment.

2.2. General design

This study employed a within-participant design (Figure 1). First, to increase the probability of falling asleep during the experimental nap, all participants completed a 90-minute habituation nap monitored with polysomnography (PSG, see below for details) in the early afternoon. Approximately one week later, participants returned to the laboratory to complete the experimental protocol. Participants were instructed to maintain a regular sleep/wake schedule for the 3 days leading up to this experimental session (see Table 2 for results based on averaged data from the objective actigraph and subjective sleep diary data acquired over this interval). On the first experimental day (day 1), two motor sequences were

learned simultaneously in the pre-nap session. A different sound was associated to each of these two sequences during task performance. During the subsequent nap episode, one of these two sounds was presented. This sound is referred to as the *associated* sound that was linked to the *reactivated* sequence. The sequence that was learned during the pre-nap session but not reactivated during the subsequent nap interval served as the control (*non-reactivated*) condition at the behavioral level. At the electrophysiological level, the control condition consisted of a new sound played during the nap. This sound was thus not linked to any learned material (*unassociated* sound). The experimental nap was monitored with PSG. Online monitoring of the sleep data was performed by an experimenter, affording the opportunity to send auditory stimulations during NREM2-3 stages (see below for details). Thirty minutes after the end of the nap, performance on the reactivated and non-reactivated sequences was tested (i.e., post-nap retest). The following morning (day 2), after a night of sleep spent at home (monitored with actigraphy and sleep diary; see Table 2), performance on the two sequences was again assessed (i.e., post-night retest). Objective (Psychomotor Vigilance Task [34]) and subjective (Stanford Sleepiness Scale [35]) measures of vigilance were assessed at the beginning of each behavioral session (results presented in Table 2). General motor execution was also tested with a random variant of the motor task (described below) at the beginning of the pre-nap session and at the end of the post-night session.

The effect of TMR on consolidation was assessed at the behavioral level by comparing the changes in performance between the reactivated and non-reactivated sequences and at the electrophysiological level by comparing the neurophysiological responses to the associated (i.e., reactivated) and unassociated auditory cues.

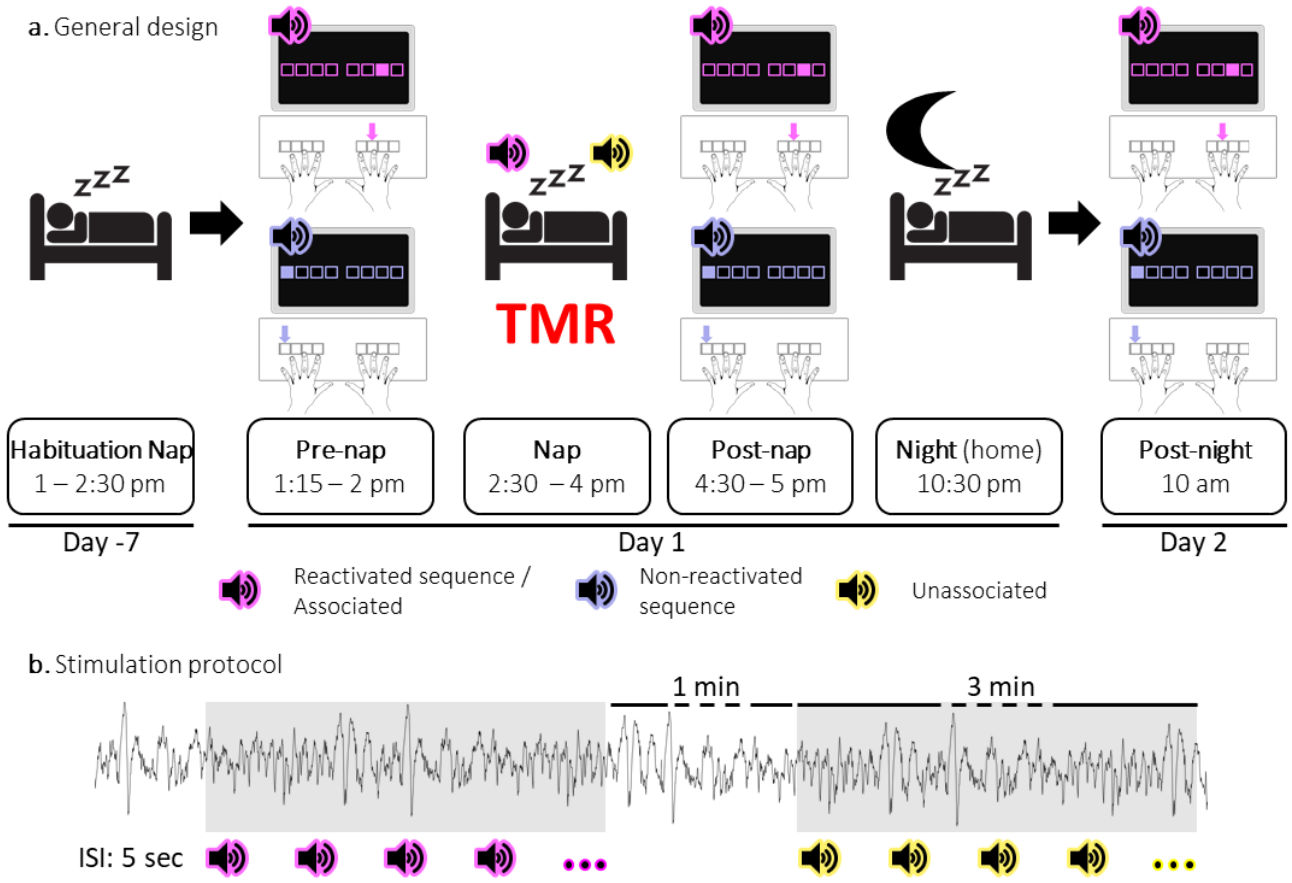

Figure 1: Experimental protocol *(Figure and caption were adapted from [27] under the Creative Commons Attribution (CC BY) license.* ***a. General design****. Following a habituation nap that was completed approximately one week prior to the experiment, participants underwent a pre-nap motor task session, a 90-minute nap episode monitored with polysomnography during which targeted memory reactivation (TMR) was applied and a post-nap retest session. Participants returned to the lab the following morning to complete an overnight retest (post-night). The times provided represent the schedule for an exemplar participant. During the motor task, two movement sequences were learned simultaneously and were cued by two different auditory tones. For each movement sequence, the respective auditory tone was presented prior to each sequence execution (i.e., one tone per sequence). One of these specific sounds was replayed during the subsequent sleep episode (****Reactivated****) and the other one was not (****Non-reactivated****). During the NREM 2-3 stages of the post-learning nap, two different sounds were presented. One was the sound associated (****Associated****) to one of the previously learned sequences, i.e., to the reactivated sequence, and one was novel, i.e., not associated to any learned material (****Unassociated****).* ***b. Stimulation protocol.*** *Stimuli were presented during three-minute stimulation intervals of each cue type alternating with a silent 1-minute period (rest intervals). The inter-stimulus interval (ISI) was of 5 sec. The stimulation was manually started when participants reached NREM sleep and stopped when participants entered REM sleep, NREM1 or wakefulness.*

2.3. Stimuli and tasks

2.3.1. Acoustic stimulation

Sound presentation was conducted using speakers positioned at the bed of the participant during the nap and on the desk, facing the participants on both sides of the computer, during the motor task sessions. Three different 100-ms sounds (see [27] for details on sound characteristics) were pseudo-randomly assigned to the three conditions (reactivated/associated, non-reactivated, and unassociated) for each participant to ensure that the associations between sounds and conditions were evenly distributed across participants. At the start of the experiment, the auditory detection threshold of each sound was determined using a transformed 1-down 1-up staircase procedure [36, 37] with the speakers positioned in the nap configuration and the participant lying down in the bed. The sound pressure level was set to 1000% of the individual auditory threshold during motor task performance and at 140% of the threshold during the nap, thus limiting the risk of awakening [38].

2.3.2. Motor Task

Motor learning and memory consolidation processes were probed using a bimanual serial reaction time task (SRTT) [39]. In the present study, the SRTT consisted of eight squares presented horizontally on the screen meridian. Each square corresponded to one of the 8 fingers (no thumbs) associated to one of the eight keys on a specialized keyboard. During blocks of task practice, participants were instructed to press the key matching the location of a green filled square that appeared on the screen as fast and as accurately as possible. The next square changed to green with no response-to-stimulus interval. Each practice block concluded after 64 key presses; 15-sec rest blocks followed, indicated by the outline of the squares changing from green to red.

The order of the key presses either followed a pseudo-random or a sequential pattern, the former assessing general motor execution and the latter, motor sequence learning. Two different eight-element sequences were learned simultaneously in the sequential SRTT. Specifically, each practice block consisted of four repetitions of a specific sequence (e.g.,

sequence A: 1 6 3 5 4 8 2 7, where 1 through 8 correspond to the left pinky to the right pinky in left-to-right progression) and 4 repetitions of the other sequence (e.g., sequence B: 7 2 6 4 5 1 8 3). Repetitions of the same sequence were separated by 1 sec-intervals while the two different sequences within the same block were separated by 2 seconds. The order of the two sequences was randomized within each block of practice. A different sound was associated to each motor sequence and was played before the first key press of the sequence to be performed. The sequence-condition (conditions reactivated and non-reactivated; sequences A and B) as well as the sound-sequence associations (sounds 1, 2 and 3; sequence A, sequence B, and control sound presented during nap) were randomized and participants were pseudo-randomly assigned to one of these combinations. For the random SRTT, the order of the eight keys was shuffled for each eight-element repetition; thus, the number of each key press was constant across all random and sequential blocks.

During the pre-nap session, participants completed 4 blocks of the random SRTT followed by the sequential SRTT, consisting 16 blocks of training and 4 blocks of post-training test. The post-training test assessed the end of training performance and took place after a 5-min break allowing the dissipation of physical and mental fatigue [40]. Only 4 blocks of the sequential SRTT were completed during the post-nap session, avoiding extensive task practice before the final overnight retest. Last, the post-night session consisted of 16 blocks of the sequential SRTT followed by 4 blocks of the random SRTT.

A generation task was performed between the training and post-training test blocks as well as after the post-night session. The goal of this task was to assess explicit knowledge of the learned sequences as well as the strength of the association between auditory cues and sequences. Participants were instructed to self-generate the motor sequence corresponding to the presented auditory cue. Specifically, a cue was presented and the participant attempted one sequence and this process was repeated 4 times per cue-sequence pairing. The order of the starting sequence was randomized. During the generation task, participants were instructed to focus on accuracy and not be concerned with the speed of their responses. The percentage of correct key presses (i.e., key pressed in its correct ordinal position) was computed per attempt and per sequence. Generation accuracy was computed by averaging across attempts separately for each time point (pre-nap and post-night sessions) and sequence.

2.4. Polysomnography and Targeted Memory Reactivation protocol

The habituation and experimental naps were monitored with a digital sleep recorder (V-Amp, Brain Products, Gilching, Germany; bandwidth: DC to Nyquist frequency) and digitized at a sampling rate of 1000 Hz. Standard electroencephalographic (EEG) recordings were made from Fz, C3, Cz, C4, Pz, and Oz according to the international 10-20 system. A2 was used as the recording reference and A1 as a supplemental individual EEG channel. The recording ground consisted of an electrode placed on the middle of the forehead. Bipolar vertical and horizontal eye movements (electrooculogram: EOG) were recorded from electrodes placed above and below the right eye and on the outer canthus of both eyes, respectively. Bipolar submental electromyogram (EMG) recordings were made from the chin. Electrical noise was filtered using a 50 Hz notch. Impedance was kept below 5kΩ for all electrodes. During the habituation nap, Fz, Pz, Oz, and the vertical EOG were omitted. NREM2-3 sleep stages were visually detected during the experimental nap by a researcher monitoring the PSG recordings (guidelines from the American Academy of Sleep Medicine [41]). Auditory cues were then sent when NREM2-3 sleep stages were reached. Each type of auditory cue

(associated or unassociated) was presented every 5 seconds during a 3-minute-long stimulation interval (Figure 1b). Stimulation intervals of each cue type were separated by 1-minute silent periods (rest intervals). Whenever the experimenter detected REM sleep, NREM1 or wakefulness, the stimulation was stopped manually and resumed when the participant reached NREM2-3 again.

2.5. Analysis

The open-source software R [42, 43] was used to perform statistical tests which were considered significant for p-values < 0.05. Corrections for multiple comparisons was conducted with the False Discovery Rate (FDR) when necessary [44]. In the event of the violation of sphericity, Greenhouse-Geisser corrections were applied. Repeated measures ANOVAs, Student t-tests or Wilcoxon signed-rank tests (when non-normal distribution was detected using the Shapiro-Wilk test) were used to perform contrast tests and F, t and V (or W) statistics were respectively reported. Effect sizes, calculated using G*power [45], are reported for significant comparisons. Pearson or Spearman tests were used for correlation analyses (t and S statistics are respectively reported as well as rho values when significant). Nonparametric Cluster Based Permutations (CBP) tests [46] implemented in fieldtrip toolbox [47] were used for high dimensional time and time-frequency data analyses (e.g. ERP, TF and PAC analyses). For CBP contrast analyses, *Cohen's d* is reported while rho is reported for CBP correlations.

2.5.1. Behavior

2.5.1.1. Preprocessing

Speed (correct response time - RT - in ms) and accuracy (% correct responses) were used to assess m*otor performance* on both the random and sequential SRTT. Mean RT of each block was computed separately for each condition. RTs from individual correct trials were excluded from the analyses if they were greater than 3 standard deviations above or below the participant's mean correct response time for that block (3.9% in the random SRTT data and 1.1% in the sequential SRTT data). Consistent with our previous work [27], our primary dependent variable of interest was speed (but see Figure S1 in supplementary file for sequence accuracy data).

2.5.1.2. Statistical analyses

Data from the initial training session (speed and accuracy) were analyzed to determine whether the two conditions significantly differed before the TMR intervention. Two two-way rmANOVAs were performed on the sequential SRTT performance with block (1st rmANOVA on the 16 blocks of the pre-nap training and 2nd rmANOVA on the 4 blocks of the pre-nap test) and condition (reactivated vs. non-reactivated) as within-subject factors. We also tested potential baseline differences between sequences A and B irrespective of the reactivation condition using similar analyses (results presented in Figure S2 in supplementary file).

Overall performance changes examined whether improvement in movement speed was specific to the learned sequences as opposed to general improvement of motor execution. The overall performance change of the sequential SRTT was computed as the relative change between the first 4 blocks of the pre-nap training and the last 4 blocks of post-night training collapsed across reactivated and non-reactivated sequences whereas the overall performance change of the pseudo-random version of the SRTT was computed as the

relative change between the 4 blocks of the pre-nap session and the 4 blocks of the post-night session.

Our behavioral analysis of primary interest tested whether sequential SRTT offline changes differed between reactivation conditions after a nap and a night of sleep. To do so and similar to our previous research [27], *post-nap offline changes in performance* on the sequential SRTT were computed as the relative change in RT between the post-training test during the pre-nap session (namely the 3 last blocks of practice, see result section for details) and the post-nap session (4 blocks of practice). *Post-night offline changes in performance* were computed as the relative change in RT between the post-training test during the pre-nap session and the first 4 blocks of practice during the post-night session. The offline changes were computed separately for the two sequence conditions (reactivated and the non-reactivated). Positive offline changes reflect performance improvements (i.e., decreased RT) from pre-nap test to post-nap or post-night retests. A rmANOVA was performed on the offline changes in performance with condition (reactivated vs. non-reactivated) and time-point (post-nap vs. post-night) as within-subject factors.

For the purposes of brain-behavior correlation analyses and consistent with our pre-registered referent paper in young adults [27], a *TMR index* was computed as the difference in offline changes in performance - averaged across time points (no interaction between the condition and time-point factors, see results for details) - between the reactivated and non-reactivated sequences. A positive TMR index reflects an advantage for the reactivated as compared to the non-reactivated sequence.

To assess possible confounding factors impacting our analyses of primary interest, we tested whether vigilance differed between our experimental sessions. Specifically, one-way rmANOVAs were performed on both the median RTs of the Psychomotor Vigilance Task and the Stanford Sleepiness Scale score with session as 3-level factor (pre-nap, post-nap, and post-night). Moreover, a one-way rmANOVA was performed on the sleep duration (quantified via actigraphy and sleep diaries) during the 3 nights before and the night between the two experimental sessions. The results presented in Table 2 indicate that vigilance did not differ among sessions and that participants followed a regular sleep schedule prior to and between the experimental sessions.

2.5.2. Electroencephalography

2.5.2.1. Offline sleep scoring

Offline sleep stage scoring was performed by a certified sleep technologist according to criteria defined in [48] using the software SleepWorks (version 9.1.0 Build 3042, Natus Medical Incorporated, Ontario, Canada). Data were visually scored in 30-second epochs and band pass filtered between 0.3 and 30 Hz for EOG, 0.3 and 35 Hz for EEG signals and 10 and 100 Hz for EMG. A 50 Hz notch filter was also applied (details about scored data are in Table 2).

2.5.2.2. Preprocessing

Functions supplied by the fieldtrip toolbox [47] were used to preprocess the EEG data. Data were screened manually by 30-sec epoch for cleaning. Data segments contaminated with

muscular activity or eye movements were excluded. Data were re-referenced with the averaged signal from A1 and A2 and then band-pass filtered between 0.1-30 Hz.

2.5.2.3. Event-related analyses

Auditory-evoked potentials and oscillatory activity were computed using the fieldtrip toolbox on down-sampled data (100 Hz). Segmentation of the data into auditory cue time-locked epochs was obtained separately for the associated and unassociated cues (from one second before to three seconds after the onset of the auditory cue with a correction for onset-trigger lags). Note that 18.1 % [95% CI: 8.7 – 28.8] of the NREM2-3 stages trials were discarded during data cleaning.

Event-related potentials (ERP) were extracted at the channel-level. Individual ERPs were baseline corrected with a mean amplitude subtraction computed on -0.3 to -0.1 sec relative to cue onset time window. ERP data were averaged across all 6 EEG channels as our low-density EEG montage did not allow fine topographical analyses. In a first step, we used CBP approaches on ERP data computed across conditions (associated and unassociated) to identify specific time windows during which significant brain activity was evoked by the auditory stimulation (i.e., where ERPs were significantly different from zero). Results showed that across conditions, ERP was significantly different from zero between 0.37 and 0.53 sec at the peak and between 1.03 and 1.35 sec at the trough of the potential (peak cluster p-value= 0.042 (*Cohen's d* = 0.65); trough cluster p-value= 0.006 (*Cohen's d* = 0.7)). In a second step, ERP amplitude was then averaged within these specific time-windows for the two conditions separately and compared using one-tailed paired Wilcoxon signed-rank tests. The hypothesis was that the associated, as compared to unassociated, stimulation intervals would evoke larger potential amplitudes.

Event-related oscillatory activity was computed by extracting the Time-Frequency Representations (TFRs) of the power spectra separately for the two experimental conditions and for each channel. The power was estimated using Hanning taper/FFT approach between 5 and 30 Hz with an adaptive sliding time window of five cycles length per frequency ($\Delta t = 5/f$; 20-ms step size). To highlight the power modulation following the auditory cue onsets, baseline relative change of power correction was performed on individual TFRs (baseline from -0.3 to -0.1 sec relative to cue onset). An average of all 6 EEG channels was then computed. TFR locked to auditory cues were compared between the associated and the unassociated conditions using a CBP test from 0 to 2.5 sec relative to cue onset and between 5 to 30 Hz.

2.5.2.4. Sleep-event detection

Preprocessed cleaned data were down-sampled to 500 Hz and were transferred to the python environment. *S*low waves and spindles were detected automatically in NREM2-3 sleep epochs on all the channels with algorithms implemented in the YASA open-source Python toolbox [49]. Events were defined as SWs if they met the following criteria adapted from [51]: frequency between 0.5 and 2 Hz, a duration of the negative peak between 0.3 and 1.5 sec, a duration of the positive peak between 0.1 and 1 sec, an amplitude of the negative peak between 40 and 300 µV, an amplitude of the positive peak between 10 and 200 µV and an PTP amplitude between 75 and 500 µV (see Figure S3 in supplementary file for similar results when implementing detection algorithms for age-adapted criteria). Events were defined as spindles if they met the criteria adapted from [51] including a duration between 0.5

and 3 sec and a frequency between 12 and 16 Hz. For further details on the SW and spindle detection algorithms (e.g. amplitude threshold), please see [52] for the original paper and [27] for our recent research that employed the algorithms.

SWs and spindles were detected in the stimulation intervals of both associated and unassociated cues as well as during rest intervals (i.e., NREM 2-3 epochs without auditory stimulation). Three participants did not show any SWs during the associated cue stimulation (N=1), unassociated cue stimulation (N=1) or rest intervals (N=1). The three participants were thus excluded from the analyses with detected SWs (i.e., sleep event detection and the SW-locked phase amplitude coupling described below). For detected SWs, the mean PTP amplitude (µV) as well as the density (number per total time in minutes spent in stimulation or rest intervals) were extracted for each participant and condition. For spindles, amplitude, frequency and density were extracted for each participant and condition. One-tailed paired Student t-tests or Wilcoxon signed-rank tests were used to compare these different dependent variables. The hypothesis was that the associated, as compared to unassociated, stimulation intervals would show higher values. As spindle characteristics were comparable between associated and unassociated stimulation conditions (see present results and [27]), we collapsed across conditions and compared to rest intervals using two-tailed Student t-tests or Wilcoxon signed-rank tests.

2.5.2.1. Phase-amplitude coupling

Down-sampled (500 Hz) data of the 6 EEG channels were averaged together (but see Figure S4 in supplementary file for channel level data), and then transferred to the python environment. The Event-Related Phase-Amplitude Coupling (ERPAC) method [53] implemented in the TensorPac [54] open-source Python toolbox was used to investigate the modulation of the coupling between the phase of the 0.5-2 Hz oscillatory signal and the amplitude of the signal in the 7-30 Hz frequency range in relation to either the *auditory cue onset* or *the negative peak of the SWs* (detected on the Fz electrode). The raw data were filtered between 0.5-2 Hz and 7-30 Hz. Next, the complex analytical form of each signal was obtained using the Hilbert transform. The phase of the signal from -1 to 3 sec around the auditory cue onset and from -1 to 2 sec around the negative peak of the SWs was extracted from the filtered signal within the 0.5-2 Hz slow oscillation (SO) frequency band. The amplitude of the signal was also extracted in the windows described above and between 7 and 30 Hz with 0.5 Hz step size. The ERPAC approach affords the computation of the strength of the SO/sigma coupling at each time point of the analysis window (i.e., every 2ms). ERPAC computed separately for the two sound conditions (cue locked and SW-locked) and for the rest intervals (SW-locked only) were compared using CBP tests.

The preferred phase (PP), reflecting whether the amplitude of the signal in a given frequency band is modulated by the phase of the signal in another band, was also computed using tensorPac [54]. These analyses were restricted to the phase of the SO and the amplitude of the signal in the sigma band after the cue onset (associated vs. unassociated) and around the negative peak of the SW (associated vs. unassociated vs. rest). The amplitude was binned according to 72 phase slices. The phase bin at which the amplitude is maximum is therefore defined as the preferred phase. The circular statistical analyses of the PP were performed using the CircStat toolbox [55] using Rayleigh test for non-uniformity and Watson-Williams multi-sample test for equal means.

2.5.2.2. Correlational analyses

Similar to our pre-registered previous research [27], we performed correlations between the TMR index and the following EEG-derived data: (1) the difference between the densities of SWs detected during the associated and unassociated cue stimulation intervals using one-sided Spearman correlations; (2) the difference between the densities of spindles detected during the associated and unassociated cue stimulation intervals using one-sided Spearman correlation; (3) the relative change between the amplitude of the negative peak of the ERP following the associated and unassociated auditory cues using one-sided Spearman correlation; (4) the difference in auditory-locked sigma band power (0-2.5 sec relative to cue onset and from 12 to 16 Hz) between the associated and unassociated auditory cues using CBP test; and, (5) the difference between SO phase and sigma oscillation amplitude (12-16 Hz) coupling strength during the associated and unassociated stimulation intervals in relation to the cue onset and to the SW event using CBP test. For all one-sided tests, we predicted that the TMR index would be positively correlated with the EEG-derived metrics. Moreover, following the pre-registered analyses pipeline of the referent paper [27], we tested whether the TMR index was correlated to the generation accuracy during the pre-nap generation task (Pearson's correlation).

3. Results

3.1. Behavioral data

Participants accurately performed the SRTT, as indicated by only 4.1% and 3.4% incorrect trials on the random and sequential task variants, respectively (see Figure S1 for detailed analyses). There was a strong and significant difference between the improvement in movement speed over the course of sequential SRTT practice as compared to the pseudo-random SRTT (t = 8.4, df = 16, p-value = 2.8e-7, *Cohen's d* = 2.1, Figure 2a). This indicates that the observed performance improvements during the sequential task variant can be attributed to sequence-specific learning as opposed to general improvement of motor execution.

Analyses of the pre-nap training data indicated that participants learned the two sequence conditions (reactivated and non-reactivated sequences) to a similar extent during initial learning (16 blocks of training; main effect of block: F(15, 240) = 7.4, p-value = 2.42e-6, η^2 = 0.32; main effect of condition: F(1, 16)= 5.5e-5, p-value = 0.99; block by condition interaction: F(15, 240)= 1, p-value = 0.41; Figure 2a). Before computing offline changes in performance, we assessed whether participants reached stable and similar performance levels between conditions during the pre-nap test. Results showed that while performance reached similar levels between conditions (4 blocks; main effect of condition: F(1,16) = 1.1, p-value = 0.31; block by condition interaction: F(3,48) = 2.1, p-value = 0.11), asymptotic performance levels were not reached, as shown by a significant block effect (F(3,48) = 3.1, p-value = 0.036, η^2 = 0.16). Similar to our referent study [27] and to meet the performance plateau pre-requisite to compute offline changes in performance, first block of the pre-nap test driving this effect was removed from further analyses. Performance on the remaining 3 blocks was stable, as indicated by a non-significant block effect (F(2,32) = 0.7, p-value = 0.93) and block by condition interaction (F(2,32)= 2.6, p-value = 0.087). Consistent with above, the main effect of condition was not significant (F(1,16) = 1.6, p-value = 0.22) when only including this subset of blocks. Altogether, these results indicate that a performance plateau was ultimately reached and both sequence conditions were learned similarly (Figure 2a).

For each condition separately, post-nap and post-night offline changes in performance were then computed as the relative change in speed between the three plateau blocks of the pre-nap test and the first four blocks of the post-nap and post-night retests, respectively (Figure 2b). A rmANOVA performed on offline changes in performance with time-point (post-nap vs. post-night) and condition (reactivated vs. non-reactivated) as within-subject factors highlighted a marginally significant time-point main effect ($F(1,16) = 3.3$, p-value = 0.086). Yet, our primary factor of interest, condition, did not reveal a significant main effect nor an interaction with time-point (condition effect: $F(1,16) = 2.9$, p-value = 0.11; condition by time-point interaction: $F(1,16) = 1.3$, p-value = 0.28).

These results indicate that TMR did not impact motor memory consolidation, as measured by the offline change in performance, in older adults. Offline changes were slightly larger at the post-night as compared to the post-nap test regardless the reactivation condition.

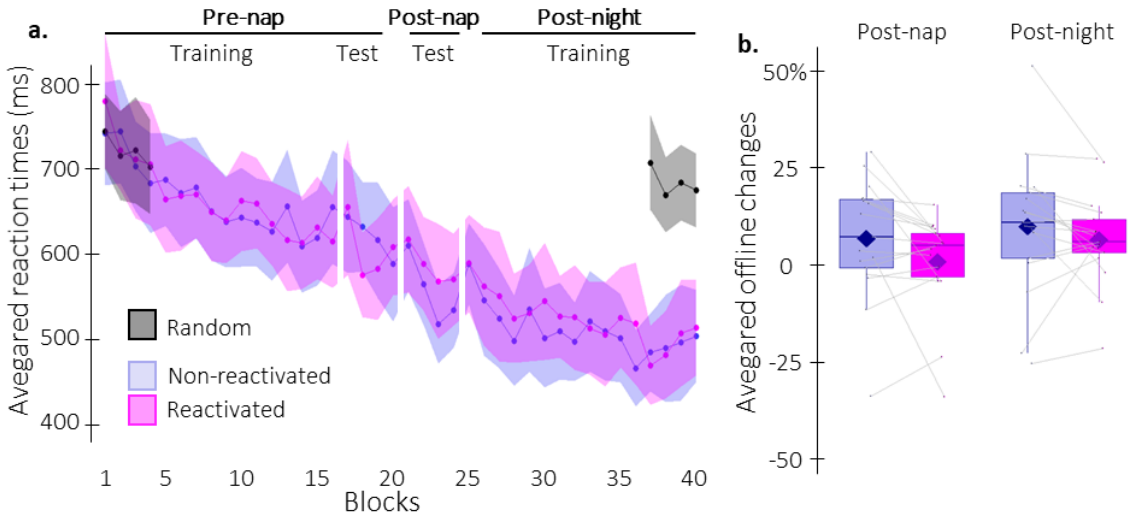

Figure 2: Behavioral results. a. Performance speed *(mean reaction time in ms +/- standard error in shaded regions) across participants plotted as a function of practice blocks during the pre- and post-nap sessions for the random SRTT (black overlay) and for the reactivated (magenta) and the non-reactivated (blue) sequences. Results on performance accuracy can be found in Figure S1 in the supplementary file.* ***b. Offline changes in performance speed*** *(% change) averaged across participants for reactivated (magenta) and non-reactivated (blue) sequences and for post-nap and post-night time-points. There was no significant effect of condition (reactivated vs. non-reactivated) or condition by timepoint (post-nap vs. post-night) interaction. Box: median (horizontal bar), mean (diamond) and first(third) as lower(upper) limits; whiskers: 1.5 x interquartile range.*

3.2. Electrophysiological data

Sleep characteristics resulting from the offline sleep scoring as well as the distribution of auditory cues across sleep stages are shown in Table 2. Results indicate that participants slept on average 49.6 minutes during the nap and that the majority of the cues (80.7%) were accurately presented in NREM sleep. The number of associated cues, however, was significantly higher than the number of unassociated cues ($t = 2.4$, $df = 16$, p-value = 0.03, *Cohen's d* = 0.6). Although unexpected, it is worth emphasizing that the purpose of the unassociated cues was to serve as a control for the electrophysiological analyses. Given that the number of cues sent in the stages of interest were approximately 120 (see Table 2 for

details), it is unlikely that this comparably small difference in the number of trials included in the event-related EEG analyses (mean difference of 11.4 stimuli) influenced the results.

Table 2. Sleep and vigilance scores (N=17)

Sleep duration (hrs)			
Mean for each of the 4 nights (Night 4 being between the 2 experimental days)			
Night 1	8.1 [7.7 – 8.5]		
Night 2	8 [7.7 – 8.5]		
Night 3	8.2 [7.9 – 8.5]		
Night 4	8 [7.6 – 8.4]		
One-way rmANOVA result	F(16,48) = 0.4; p-value = 0.73		
St. Mary's Sleep	*Night 3*	*Night 4*	*Student t test result*
Quality	4.8 [4.3 – 5.3]	5.2 [4.5 – 5.8]	t = -1.3, df = 17, p-value = 0.21
Duration (hrs)	7.7 [7.2 – 8.2]	7.5 [6.9 – 8.2]	t = 0.6, df = 17, p-value = 0.54
Psychomotor Vigilance Task [a] (ms)			
Pre-nap	281.7 [275.4 – 288.1]		
Post-nap	280.8 [262.7 – 289]		
Post-night	279.4 [271.5 – 287.2]		
One-way rmANOVA result	F(16,32) = 0.3; p-value = 0.76		
Stanford sleepiness score			
Pre-nap Session	1.9 [1.6 – 2.2]		
Post-nap Session	2.1 [1.7 – 2.6]		
Post-night Session	2.2 [1.8 – 2.6]		
One-way rmANOVA result	F(16,32) = 1; p-value = 0.38		
Daytime sleep characteristics			
Time allowed to sleep (min)	91.6 [89.3 – 92.9]		
Total Sleep Time (min)	49.6 [39.4 – 55.8]		
Sleep Efficiency [b] (%)	54.4 [45 – 63.8]		
Stage 1 Latency (min)	9.6 [5.9 – 13.4]		
Time awake (min)	25.4 [17.4 – 33.5]		
Stage 1 duration (min)	16.3 [12.8 – 19.8]		
Stage 2 duration (min)	42.8 [35.5 – 50.2]		
Stage 3 duration (min)	4.1 [0.6 – 7.6]		
REM duration (min)	2.6 [0.3 – 5]		
Participants reaching			
Stage 3	N = 9		
REM	N = 6		
Number of Auditory cues	*All*	*Associated*	*Unassociated*
During all stages	294.9 [237.5 – 352.3]	151.6 [120.9 – 182.4]	143.2 [115.7 – 170.7]
During wake	19 [3.8 – 34.2]	11.4 [1.8 – 20.9]	7.6 [1.2 – 14.1]
During Stage1 Sleep	33.1 [17.3 – 48.9]	13.7 [7.7 – 19.7]	19.4 [8.4 – 30.4]
During Stage2 and 3 Sleep	241.6 [188 – 295.2]	126.6 [99.5 – 153.6]	115 [87.5 – 142.5]
During REM Sleep	1.2 [0 – 3.1]	0 [0 – 0]	1.2 [0 – 3.1]
Accuracy[c] (%)	80.7 [70.6 – 90.8]	82.8 [72.7 – 93.1]	78.6 [67.8 – 89.4]

Notes. Values are means [lower and upper limit of the 95% Confidence Interval - CI]. REM: Rapid Eye Movement. [a] Means of individual median RTs. [b] Sleep efficiency was computed as the relative change between the time asleep (namely in stage 2 and 3 and in REM sleep) and the total time in bed (specifically, from lights off to lights on). [c] Percentage of auditory cues sent during Stage 2 and Stage 3 Sleep.

3.2.1. Event-related analyses

Between-condition comparisons using Wilcoxon signed-rank test showed that neither the peak amplitude of the ERP nor the trough was significantly different (V=51, p-value = 0.24; V = 56, p-value = 0.35, respectively) following associated as compared to unassociated cues. Further, CBP tests on the auditory evoked *oscillatory* activity did not highlight any significant clusters between the two auditory cues.

3.2.1. Sleep event detection

Slow waves (SWs) and spindles were detected automatically on all EEG channels in all NREM2-3 sleep epochs. The detection tool identified on average 82.9 [95% CI: 31.6 – 134.3] slow waves and 91.9 [95% CI: 67.9 – 116] spindles averaged across channels during the nap episode (see Table S1 in supplementary file for the number of events detected on each channel and each condition).

Concerning the detected SWs, the peak-to-peak (PTP, Figure 3a) amplitude was not different between the three conditions (associated vs. unassociated: t = -0.04, df = 13, p-value = 0.52; associated vs. rest: t = 0.55, df = 13, p-value = 0.59; unassociated vs. rest: t = 0.6, df = 13, p-value = 0.56). However, the density of the SWs (Figure 3b) during the associated condition was greater as compared to both the unassociated stimulation and the rest intervals (associated vs. unassociated: V = 86, p-value = 0.018 (0.026 FDR Corrected), r = 0.56; associated vs. rest: V =96, p-value = 0.004 (0.012 FDR-corrected), r = 0.79). The density of the SWs during unassociated stimulation and rest intervals was not different (V = 68, p-value = 0.36 (0.36 FDR-corrected)). Note that these results were similar when using age-adapted criteria for SW detection [25] (Figure S3 in supplementary file).

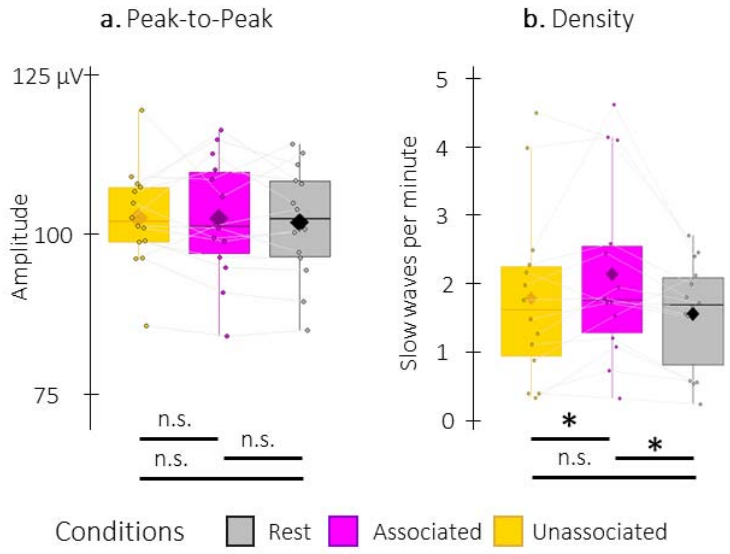

Figure 3: Detected Slow Waves *(SWs)**. a.*** *Peak-to-peak SW amplitude (µV) did not differ between conditions.* ***b.*** *SW density (number of SWs per total time in minute spent in stimulation or rest intervals) was higher during associated stimulation as compared to rest intervals and to unassociated stimulation. Box: median (horizontal bar), mean (diamond) and first(third) as lower(upper) limits; whiskers: 1.5 x interquartile range; *: p-value < 0.05; n.s.: not significant.*

Sleep spindle density, amplitude and frequency did not differ between associated and unassociated stimulation intervals (density: V = 76, p-value = 0.52; amplitude: V = 83, p-value = 0.39; frequency: t = 0.17, df = 16, p-value = 0.43). Consistent with our earlier work [27], the two conditions were then pooled together and compared to the characteristics of spindles detected during the rest intervals (Figure 4). Spindle density was significantly lower (V = 3, p-value = 7.6e-5, r = 0.94) during the auditory stimulation intervals as compared to the rest intervals. The difference in the averaged frequency of the detected spindles was marginally significant, as spindles in the auditory stimulation intervals tended to have a lower frequency than those occurring in the rest intervals (t = -2.1, df = 16, p-value = 0.05, *Cohen's d* = 0.51). Spindle amplitude was not significantly different between the auditory stimulation and rest intervals (V = 97, p-value = 0.35).

Altogether, these results indicate that the associated stimulation resulted in an increase in SW density as compared to the unassociated stimulation and rest. Furthermore, auditory stimulation altered spindle features (density and frequency) as compared to rest regardless the nature of the sound (associated or unassociated).

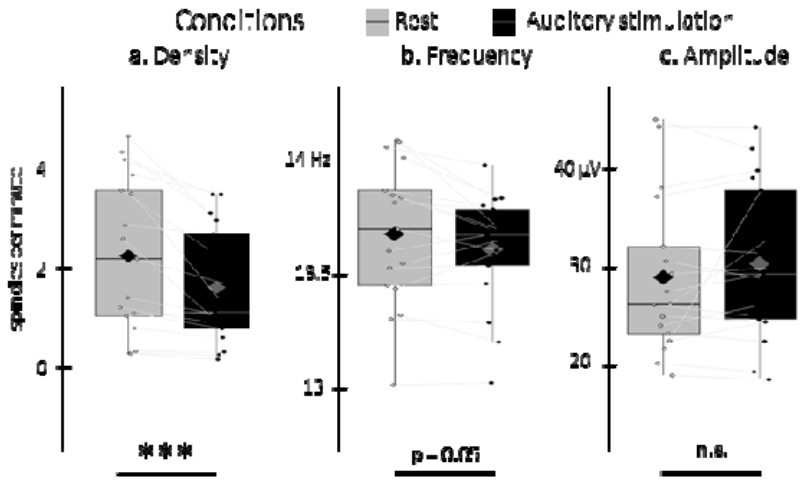

Figure 4: Detected spindles. a. *Spindle density (number of spindles per total time in minute spent in stimulation or rest intervals) was lower during stimulation intervals (irrespective of sound type; black) as compared to rest (grey) intervals.* ***b.*** *Spindle frequency (Hz) was lower during stimulation intervals as compared to rest intervals.* ***c.*** *Spindle amplitude (µV) did not differ between conditions. All spindle features were averaged across channels. Box: median (horizontal bar), mean (diamond) and first(third) as lower(upper) limits; whiskers: 1.5 x interquartile range; ***: p-value < 0.001; n.s.: not significant*

3.2.1. Phase-amplitude coupling

We investigated whether the phase of the slow oscillations in the 0.5-2 Hz frequency band was coupled to the amplitude of sigma (12-16 Hz) oscillations following either the auditory cue or the negative peak of the detected (i.e., spontaneous) SWs. The analyses presented below focus on the comparison between conditions but see Figure S5 in supplementary file for coupling analyses performed within each stimulation condition and at rest.

The cue-locked preferred coupling phase, which represents the phase at which the maximum amplitude is observed, did not significantly differ between conditions ($F(1,32)= 0.03$, p-value = 0.86). This suggests that the stimulation conditions did not influence the coupling between the phase of the slow oscillations and the amplitude of sigma oscillations at the auditory cue (Figure S5 in supplementary file). Event-related phase-amplitude coupling (ERPAC) analyses were performed across channels on the 7-30 Hz frequency range. The ERPAC values locked to the *auditory cues* were compared between the two stimulation conditions. The CBP test did not highlight any significant clusters (cluster p-values > 0.2).

The preferred phases around the negative peak of the SW were not significantly different between conditions (associated vs. unassociated: $F(1,42) = 1e-4$, p-value = 0.99; associated vs. rest: $F(1,42) = 0.01$, p-value = 0.75; unassociated vs. rest: $F(1,42) = 0.1$, p-value = 0.72; see Figure S5 in supplementary file). Comparison of the ERPAC locked to the *negative peak of the SWs* between stimulation conditions revealed no significant cluster (Figure 5a). Neither did the comparison between rest and associated stimulation intervals (all cluster p-values > 0.28). However, a significant cluster was observed between the ERPAC during unassociated stimulation intervals and during the rest intervals (cluster threshold = 0.025, cluster p-value = 0.02; Cohen's d = 0.89; Figure 5b). This cluster was observed between 19 and 20.5 Hz and -0.53 to -0.07 sec locked to the negative peak of the SW. Altogether, these results suggest that the coupling between slow oscillations and beta (but not sigma) oscillations was stronger just before the onset of the SW during the unassociated as compared to the rest intervals but that the preferred coupling phase was not modulated by the type of auditory cue.

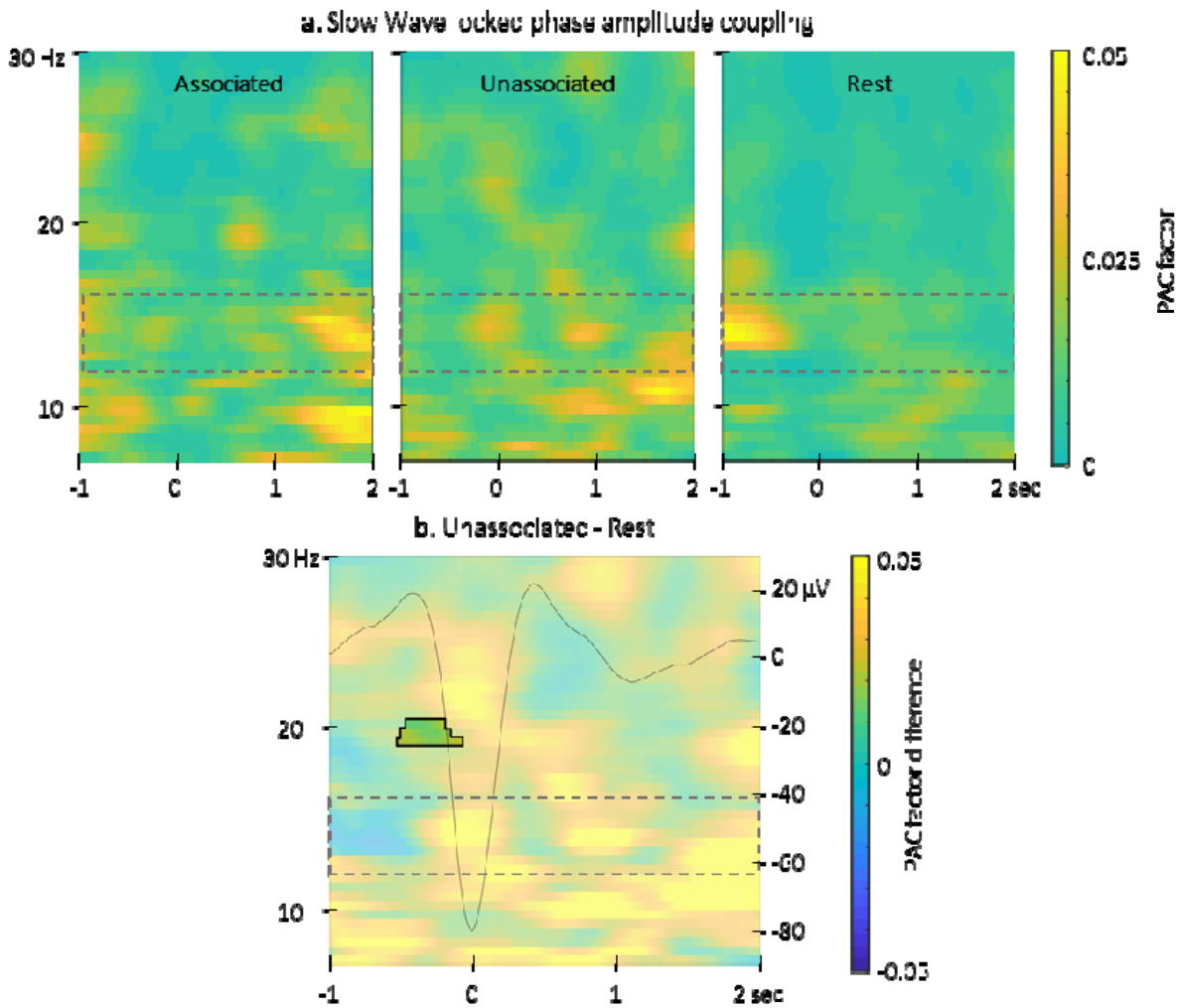

Figure 5: Event related phase-amplitude coupling locked to the detected slow wave negative peaks. a. *Time-Frequency Representation (TFR) of group average coupling strength between the phase of the 0.5-2 Hz frequency band and the amplitude from -1 to 2 sec (x-axis) relative to SW negative peak and from 7 to 30 Hz (y-axis) for the three interval types.* ***b.*** *ERPAC was significantly higher around the SWs detected during the unassociated stimulation intervals as compared to those detected during the rest intervals in the highlighted cluster. Dashed frames indicate the sigma frequency band of interest. Superimposed on the TFR in panel b (black line): SW grand average across individuals and conditions (y-axis on right).*

3.3. Correlational analyses

Correlation analyses between the TMR index (i.e., the difference in offline changes in performance between the reactivated and the non-reactivated sequences) and the density of either the SW or the spindles did not yield any significant results (density of spontaneous SW: S = 276, p-value = 0.08; density of spontaneous spindles: S = 540, p-value = 0.09). The correlational CBP analysis between the TMR index and the difference in oscillatory activity elicited by the different auditory cues did not highlight any significant clusters (all cluster p-values > 0.6). Generation accuracy of the reactivated sequence during the pre-nap generation task was also not significantly correlated to the TMR index (t = 0.6, df = 17, p-value = 0.53).

With respect to ERPAC-TMR index correlation analyses, no significant correlation was observed between the TMR index and the *auditory-locked* ERPAC (all cluster p-values > 0.6). In contrast, CBP correlational tests performed between the TMR index and the *SW-locked* ERPAC difference (associated - unassociated) revealed a significant cluster in the 13.5-18 Hz frequency band and 1.34 and 2 sec post SW-trough. The ERPAC was negatively correlated with the TMR index (cluster threshold = 0.01, cluster p-value = 0.001, r_s = -0.69; Figure 6a). For illustration purposes, we extracted the difference in ERPAC in the significant cluster. The resulting scatter plot (Figure 6b) indicates that the stronger the phase-amplitude coupling during unassociated as compared to associated stimulation intervals, the lower the TMR index. Note that, however, this result doesn't remain significant without the two extreme (but not defined as outlier) participants with TMR values inferior to -10.

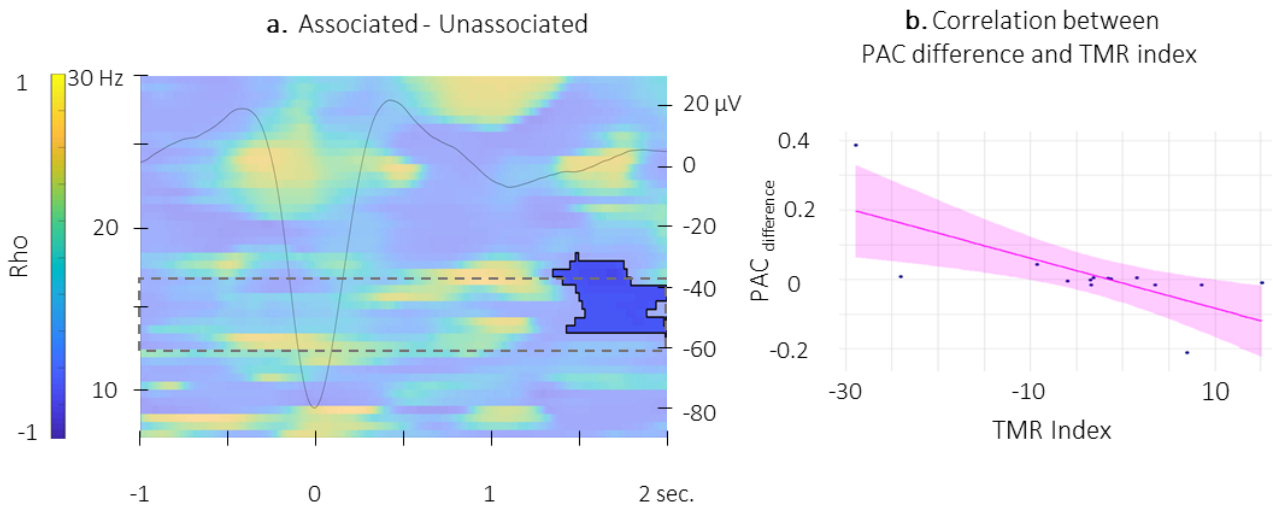

Figure 6. Correlation between SW-locked event related phase-amplitude coupling difference and TMR Index*. (a) Time-Frequency Representation (TFR) of the rho values resulting from the correlation between the TMR index and the difference between the SW-locked ERPAC during the associated vs. unassociated stimulation intervals (N = 14). Highlighted, the positive cluster in which the TMR index is significantly correlated with the difference in SW-locked ERPAC (cluster-based permutation test). Superimposed on the TFR (black line): SW grand average across individuals and conditions. Dashed frame highlights the sigma frequency band of interest. (b) Depiction of the negative correlation between the SW-locked, ERPAC difference (1.34–2 s post negative peak, 13.5–18 Hz) and the TMR index (dots represent individual datapoints).*

4. Discussion

This study examined the impact of auditory TMR on the behavioral and electrophysiological correlates of motor memory consolidation in older adults. Despite the lack of a TMR-induced advantage on motor performance, our electrophysiological analyses revealed that the experimental intervention elicited modulations of brain activity. Specifically, slow wave density was greater during the associated stimulation intervals as compared to the unassociated stimulation and rest intervals. Unassociated sound stimulation intervals, as compared to rest, exhibited a higher coupling between slow oscillation phase and beta band amplitude (around 20 Hz) at the descending phase of the SW. Last, there was a significant negative relationship between the associated/unassociated difference in slow oscillation phase / sigma band amplitude coupling and the reactivation-induced behavioral advantage.

Contradictory to our predictions, results from the current study showed that sleep-related offline changes in motor performance were not modulated by TMR in older individuals. This null effect is in contrast to the TMR-induced behavioral advantage we recently demonstrated with the same protocol in young adults [27]. One could speculate that the lack of effect on the performance of the reactivated sequence in older individuals is the lower number of stimulations delivered during the post-learning nap episode as compared to our previous research in young adults (241.6 vs. 349.5 [27]). Although feasible, we contend that this explanation is unlikely, as a recent meta-analysis reported that the beneficial effect of TMR on memory is not correlated with the number of stimulations provided during the post-learning sleep episode [12]. We reproduce this result in the current research: there was no correlation between the number of TMR stimulations and offline performance changes for the reactivated sequence ($r = 0.1$, $p = 0.67$).

An alternative potential explanation for this pattern of results could be linked to the learning session prior to our manipulation. Specifically, it is possible that the protocol requiring participants to simultaneously learn two movement sequences mitigated initial encoding of the sequences. This might have in turn compromised subsequent sleep-facilitated consolidation and consequently the impact of the TMR intervention. Such an explanation would be in line with previous research demonstrating that the initial learning process in older adults is particularly susceptible to increases in task complexity [9, 56, 57] and that the effect of post-learning sleep critically depends on performance levels achieved at the end of initial training [8, 58, 59, 60]. To further explore this possibility, we compared data from older adults in the current study to those acquired from younger individuals who completed an identical protocol [27]. Results show that the learning magnitude of older adults was significantly lower than in young adults (see Figure S6 in supplementary file, for details), providing some indirect support for this explanation. To more conclusively examine the possibility that degradations in the initial encoding of older adults potentially diminished the impact of the TMR intervention, additional experimental groups consisting of older adults with extended training protocols would be necessary.

It is also possible that the presentation of sounds during the post-learning nap boosted consolidation in older adults independently of the specific memory trace associated to the cue. The idea of a general boost of consolidation processes via sound presentation during the nap is in line with the only study, to the extent of our knowledge, that investigated TMR during sleep in older adults [61]. Their results showed that sounds boosted consolidation of all previously-acquired material (i.e., the reactivated learned material and the non-reactivated learned material) as compared to a group undergoing a nap without sounds. Thus, although

our results demonstrate that the TMR intervention did not trigger enhanced consolidation of the reactivated sequence specifically, we cannot rule out the possibility that the presentation of sounds boosted sleep-related consolidation processes – across reactivated and non-reactivated sequences – in our older population. Supplemental experimental groups without any acoustic stimulation would be necessary to definitively support this explanation. Interestingly, this speculation would also be consistent with extensive research indicating that, in older adults, non-specific acoustic or non-invasive brain stimulation during a post-learning nap resulted in a memory consolidation benefit [62, 63] and altered sleep features [64].

Additional insights into the lack of TMR-effect on memory consolidation can be gleaned from our electrophysiological analyses. The null effect at the behavioral level could be attributed to a maladaptive response from the aging sleeping brain. For example, one could argue that the sleeping brain of older adults failed to differentiate between associated and unassociated sounds, as indicated by no differences between some of the electrophysiological responses to the two types of acoustic cues. For example, even though significant brain activity was evoked by auditory stimulations, the evoked potentials were not significantly modulated by the type of auditory cue. Similarly, the peak-to-peak amplitude of the detected SWs was equivalent in the two stimulation intervals. Additionally, the strength of the coupling between the phase of the slow oscillations and the amplitude of the sigma oscillations was not significantly different between the associated and unassociated stimulation. Interestingly, all of these electrophysiological events were modulated by the presentation of the cue associated to the memory trace in young adults [27]. However, and inconsistent with the proposal of age-related alterations in the brain's ability to differentiate cues, our results did demonstrate that the density of the SWs increased specifically during associated stimulation intervals. We thus assert that TMR can modulate the characteristics of specific sleep oscillations, but these neural responses appear to be less ubiquitous than previously observed in young adults. This, in turn, may preclude the emergence of a TMR-induced behavioral enhancement.

In line with this explanation, our electrophysiological analyses demonstrated that the unassociated cue stimulation elicited a modulation in slow/beta phase amplitude coupling as compared to rest. In our previous research in young adults [27], similar effects were observed during unassociated stimulation intervals whereby the phase of the slow oscillation was specifically coupled with the amplitude of the signal in the 14-18 Hz frequency band (when compared to associated internals) and in the 13.5-20 Hz frequency band (when compared to rest intervals) in a similar time window (i.e. descending phase of the slow wave). We suggested that this modulation of oscillatory activity *before the onset of the SW negative peak* might prevent the processing of unassociated/irrelevant sounds during post-learning sleep and, in turn, be reflected by a decrease in the amplitude of the slow electrophysiological responses during unassociated sound intervals. Supporting this speculation, the increase in PAC strength during unassociated stimulation intervals in young adults was negatively correlated with SW characteristics (density and peak-to-peak), such that higher coupling was related to lower SW amplitude and density [27]. In the present older adult study, we found results that echo these previous findings in young, albeit in a more restricted frequency band. Specifically, PAC was higher for unassociated stimulation intervals – as compared to rest - during the descending phase of the SW in the beta band (19 – 20.5 Hz). Similar to this previous research, we performed additional exploratory analyses testing for potential relationships between this slow/beta coupling observed during

unassociated stimulation intervals and SW characteristics. Results showed that the increase of PAC strength also negatively correlated with SW density and peak-to-peak amplitude (see Figure S7 in supplementary file, for details). These results are collectively in line with a sensory gating role of spindle activity / sigma oscillations [65, 66] and suggest that the aging brain also possesses protective mechanisms against interference from irrelevant stimuli. It is worth noting that the frequency that was modulated in older adults was restricted to the beta band and thus did not involve the sigma band which is most commonly linked to sleep-facilitated consolidation [26, 27, 67]. The role of beta in motor learning and memory consolidation processes is not without precedent, however. Previous research has demonstrated higher beta spectral power, in addition to higher sigma power and spindle activity, during sleep epochs following motor sequence learning as compared to following a control motor task [68].

Our results also demonstrated that the difference in slow/sigma oscillation phase-amplitude coupling between the associated and unassociated stimulation intervals was negatively linked to the TMR index. Specifically, for approximately 0.6 sec around 1.7 sec after the negative peak of the SW (1.2 sec. after the positive peak), the increase in slow/sigma phase-amplitude coupling for unassociated (as compared to associated) sounds was related to higher TMR-induced performance enhancement. Given this link between greater coupling during the *unassociated* intervals and a behavioral benefit, we contend that this result is also in line with the protection processes discussed above. Namely, the aging sleeping brain possesses mechanisms which can mitigate potential interference from irrelevant stimuli. It is worth emphasizing that this finding must be taken with caution, as the result did not hold after removing participants with extreme, yet not outlier, TMR indices (see results).

In conclusion, targeted memory reactivation in older adults did not specifically improve motor performance of the presumably reactivated motor memory trace. The electrophysiological analyses suggest that the responses to auditory stimulation during post-learning sleep are partially preserved in the aging brain. While associated sound presentation resulted in an increase in slow-wave density, unassociated sounds modulated the properties of slow/beta coupling at the trough of the slow oscillation. Additionally, greater coupling between slow/sigma oscillations following the negative SW peak during unassociated intervals was associated to a TMR-induced performance enhancement. These findings collectively suggest that the temporal coordination between SWs and sigma/beta oscillations plays a role in the protection of memories against irrelevant stimuli.

References

1. Born J, Wilhelm I. System consolidation of memory during sleep. *Psychological Research*. 2012;76(2):192-203. doi:10.1007/s00426-011-0335-6

2. King BR, Hoedlmoser K, Hirschauer F, Dolfen N, Albouy G. Sleeping on the motor engram: The multifaceted nature of sleep-related motor memory consolidation. *Neuroscience & Biobehavioral Reviews*. 2017;80:1-22. doi:10.1016/j.neubiorev.2017.04.026

3. Spencer RMC, Gouw AM, Ivry RB. Age-related decline of sleep-dependent consolidation. *Learn Mem*. 2007;14(7):480-484. doi:10.1101/lm.569407

4. Wilson JK, Baran B, Pace-Schott EF, Ivry RB, Spencer RMC. Sleep modulates word-pair learning but not motor sequence learning in healthy older adults. *Neurobiology of Aging*. 2012;33(5):991-1000. doi:10.1016/j.neurobiolaging.2011.06.029

5. Fogel SM, Albouy G, Vien C, et al. fMRI and sleep correlates of the age-related impairment in motor memory consolidation: Age-Related Sleep-Dependent Impaired Memory. *Human Brain Mapping*. 2014;35(8):3625-3645. doi:10.1002/hbm.22426

6. Fang Z, Smith DM, Albouy G, et al. Differential Effects of a Nap on Motor Sequence Learning-Related Functional Connectivity Between Young and Older Adults. *Front Aging Neurosci*. 2021;13:747358. doi:10.3389/fnagi.2021.747358

7. Tucker M, McKinley S, Stickgold R. Sleep Optimizes Motor Skill in Older Adults: SLEEP AND MOTOR MEMORY IN OLDER ADULTS. *Journal of the American Geriatrics Society*. 2011;59(4):603-609. doi:10.1111/j.1532-5415.2011.03324.x

8. King BR, Saucier P, Albouy G, et al. Cerebral Activation During Initial Motor Learning Forecasts Subsequent Sleep-Facilitated Memory Consolidation in Older Adults. *Cerebral Cortex*. Published online January 21, 2016:bhv347. doi:10.1093/cercor/bhv347

9. King BR, Fogel SM, Albouy G, Doyon J. Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. *Frontiers in Human Neuroscience*. 2013;7. doi:10.3389/fnhum.2013.00142

10. Rudoy JD, Voss JL, Westerberg CE, Paller KA. Strengthening Individual Memories by Reactivating Them During Sleep. *Science*. 2009;326(5956):1079-1079. doi:10.1126/science.1179013

11. Rasch B, Buchel C, Gais S, Born J. Odor Cues During Slow-Wave Sleep Prompt Declarative Memory Consolidation. *Science*. 2007;315(5817):1426-1429. doi:10.1126/science.1138581

12. Hu X, Cheng LY, Chiu MH, Paller KA. Promoting memory consolidation during sleep: A meta-analysis of targeted memory reactivation. *Psychological Bulletin*. 2020;146(3):218-244. doi:10.1037/bul0000223

13. Fuentemilla L, Miró J, Ripollés P, et al. Hippocampus-Dependent Strengthening of Targeted Memories via Reactivation during Sleep in Humans. *Current Biology*. 2013;23(18):1769-1775. doi:10.1016/j.cub.2013.07.006

14. Oudiette D, Antony JW, Creery JD, Paller KA. The Role of Memory Reactivation during Wakefulness and Sleep in Determining Which Memories Endure. *Journal of Neuroscience*. 2013;33(15):6672-6678. doi:10.1523/JNEUROSCI.5497-12.2013

15. Schreiner T, Rasch B. Boosting Vocabulary Learning by Verbal Cueing During Sleep. *Cerebral Cortex*. 2015;25(11):4169-4179. doi:10.1093/cercor/bhu139

16. Cairney SA, Guttesen A á V, El Marj N, Staresina BP. Memory Consolidation Is Linked to Spindle-Mediated Information Processing during Sleep. *Current Biology*. 2018;28(6):948-954.e4. doi:10.1016/j.cub.2018.01.087

17. Antony JW, Gobel EW, O'Hare JK, Reber PJ, Paller KA. Cued memory reactivation during sleep influences skill learning. *Nature Neuroscience*. 2012;15(8):1114-1116. doi:10.1038/nn.3152

18. Laventure S, Fogel S, Albouy G, et al. Motor memory consolidation potentiated by exposition to a conditioned stimulus in stage 2 sleep. In: ; 2013:poster.

19. Schönauer M, Geisler T, Gais S. Strengthening Procedural Memories by Reactivation in Sleep. *Journal of Cognitive Neuroscience*. 2014;26(1):143-153. doi:10.1162/jocn_a_00471

20. Cousins JN, El-Deredy W, Parkes LM, Hennies N, Lewis PA. Cued Reactivation of Motor Learning during Sleep Leads to Overnight Changes in Functional Brain Activity and Connectivity. Battaglia FP, ed. *PLOS Biology*. 2016;14(5):e1002451. doi:10.1371/journal.pbio.1002451

21. Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. Meta-Analysis of Quantitative Sleep Parameters From Childhood to Old Age in Healthy Individuals: Developing Normative Sleep Values Across the Human Lifespan. *Sleep*. 2004;27(7):1255-1273. doi:10.1093/sleep/27.7.1255

22. Carrier J, Land S, Buysse DJ, Kupfer DJ, Monk TH. The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). *Psychophysiology*. 2001;38(2):232-242. doi:10.1111/1469-8986.3820232

23. Fitzroy AB, Kainec KA, Spencer RMC. Ageing□related changes in nap neuroscillatory activity are mediated and moderated by grey matter volume. *Eur J Neurosci*. 2021;54(9):7332-7354. doi:10.1111/ejn.15468

24. Carrier J, Viens I, Poirier G, et al. Sleep slow wave changes during the middle years of life: Changes in slow waves with age. *European Journal of Neuroscience*. 2011;33(4):758-766. doi:10.1111/j.1460-9568.2010.07543.x

25. Rosinvil T, Bouvier J, Dubé J, et al. Are age and sex effects on sleep slow waves only a matter of electroencephalogram amplitude? *Sleep*. Published online September 15, 2020:zsaa186. doi:10.1093/sleep/zsaa186

26. Helfrich RF, Mander BA, Jagust WJ, Knight RT, Walker MP. Old Brains Come Uncoupled in Sleep: Slow Wave-Spindle Synchrony, Brain Atrophy, and Forgetting. *Neuron*. 2018;97(1):221-230.e4. doi:10.1016/j.neuron.2017.11.020

27. Nicolas J, King BR, Levesque D, et al. Sigma oscillations protect or reinstate motor memory depending on their temporal coordination with slow waves. *eLife*. 2022;11:e73930. doi:10.7554/eLife.73930

28. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191. doi:10.1001/jama.2013.281053

29. Beck AT, Steer RA, Ball R, Ranieri WF. Comparison of Beck Depression Inventories-IA and-II in Psychiatric Outpatients. *Journal of Personality Assessment*. 1996;67(3):588-597. doi:10.1207/s15327752jpa6703_13

30. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology*. 1988;56(6):893-897. doi:10.1037/0022-006X.56.6.893

31. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research*. 1989;28(2):193-213. doi:10.1016/0165-1781(89)90047-4

32. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol*. 1976;4(2):97-110.

33. Nasreddine ZS, Phillips NA, BÃ©dirian V, et al. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment: MOCA: A BRIEF SCREENING TOOL FOR MCI. *Journal of the American Geriatrics Society*. 2005;53(4):695-699. doi:10.1111/j.1532-5415.2005.53221.x

34. Dinges DF, Powell JW. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. *Behavior Research Methods, Instruments, & Computers*. 1985;17(6):652-655. doi:10.3758/BF03200977

35. Hoddes E, Dement W, Zarcone V. *Development and Use of Stanford Sleepiness Scale (SSS)*. Vol 9.; 1972.

36. Levitt H. Transformed Up□Down Methods in Psychoacoustics. *The Journal of the Acoustical Society of America*. 1971;49(2B):467-477. doi:10.1121/1.1912375

37. Leek MR. Adaptive procedures in psychophysical research. *Perception & psychophysics*. 2001;63(8):1279-1292.

38. Sterpenich V, Schmidt C, Albouy G, et al. Memory Reactivation during Rapid Eye Movement Sleep Promotes Its Generalization and Integration in Cortical Stores. *Sleep*. 2014;37(6):1061-1075. doi:10.5665/sleep.3762

39. Nissen MJ, Bullemer P. Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology*. 1987;19(1):1-32. doi:10.1016/0010-0285(87)90002-8

40. Pan SC, Rickard TC. Sleep and motor learning: Is there room for consolidation? *Psychological Bulletin*. 2015;141(4):812-834. doi:10.1037/bul0000009

41. Berry. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.5.*; 2018.

42. R Core Team. R: A language and environment for statistical computing. Published online 2020. https://www.R-project.org/

43. RStudio Team. RStudio: Integrated Development for R. Published online 2020. http://www.rstudio.com/

44. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)*. 1995;57(1):289-300.

45. Faul F, Erdfelder E, Lang AG, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*. 2007;39(2):175-191.

46. Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. *Journal of Neuroscience Methods*. 2007;164(1):177-190. doi:10.1016/j.jneumeth.2007.03.024

47. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Computational Intelligence and Neuroscience*. 2011;2011:1-9. doi:10.1155/2011/156869

48. Berry RB, Gamaldo CE, Harding SM, et al. AASM Scoring Manual Version 2.2 Updates: New Chapters for Scoring Infant Sleep Staging and Home Sleep Apnea Testing. *Journal of Clinical Sleep Medicine*. 2015;11(11):1253-1254. doi:10.5664/jcsm.5176

49. Vallat R. raphaelvallat/yasa: v0.1.9. Published online February 5, 2020. doi:10.5281/ZENODO.3646596

50. Dube J, Lafortune M, Bedetti C, et al. Cortical Thinning Explains Changes in Sleep Slow Waves during Adulthood. *Journal of Neuroscience*. 2015;35(20):7795-7807. doi:10.1523/JNEUROSCI.3956-14.2015

51. Gaudreault PO, Gosselin N, Lafortune M, et al. The association between white matter and sleep spindles differs in young and older individuals. *Sleep*. 2018;41(9). doi:10.1093/sleep/zsy113

52. Vallat R, Walker MP. An open-source, high-performance tool for automated sleep staging. *eLife*. 2021;10:e70092. doi:10.7554/eLife.70092

53. Voytek B, D'Esposito M, Crone N, Knight RT. A method for event-related phase/amplitude coupling. *NeuroImage*. 2013;64:416-424. doi:10.1016/j.neuroimage.2012.09.023

54. Combrisson E, Nest T, Brovelli A, et al. Tensorpac: An open-source Python toolbox for tensor-based phase-amplitude coupling measurement in electrophysiological brain signals. Schneidman-Duhovny D, ed. *PLoS Comput Biol*. 2020;16(10):e1008302. doi:10.1371/journal.pcbi.1008302

55. Berens P. **CircStat**: A *MATLAB* Toolbox for Circular Statistics. *J Stat Soft*. 2009;31(10). doi:10.18637/jss.v031.i10

56. Howard DV, Howard JH, Japikse K, DiYanni C, Thompson A, Somberg R. Implicit Sequence Learning: Effects of Level of Structure, Adult Age, and Extended Practice. *Psychology and Aging*. 2004;19(1):79-92. doi:10.1037/0882-7974.19.1.79

57. Bennett IJ, Howard JH, Howard DV. Age-Related Differences in Implicit Learning of Subtle Third-Order Sequential Structure. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 2007;62(2):P98-P103. doi:10.1093/geronb/62.2.P98

58. Kuriyama K, Stickgold R, Walker MP. Sleep-dependent learning and motor-skill complexity. *Learn Mem*. 2004;11(6):705-713. doi:10.1101/lm.76304

59. Hauptmann B, Reinhart E, Brandt SA, Karni A. The predictive value of the leveling off of within session performance for procedural memory consolidation. *Cognitive Brain Research*. 2005;24(2):181-189. doi:10.1016/j.cogbrainres.2005.01.012

60. Fitzroy AB, Kainec KA, Seo J, Spencer RMC. Encoding and consolidation of motor sequence learning in young and older adults. *Neurobiology of Learning and Memory*. 2021;185:107508. doi:10.1016/j.nlm.2021.107508

61. Johnson BP, Scharf SM, Verceles AC, Westlake KP. Sensorimotor performance is improved by targeted memory reactivation during a daytime nap in healthy older adults. *Neuroscience Letters*. 2020;731:134973. doi:10.1016/j.neulet.2020.134973

62. Westerberg CE, Florczak SM, Weintraub S, et al. Memory improvement via slow-oscillatory stimulation during sleep in older adults. *Neurobiology of Aging*. 2015;36(9):2577-2586. doi:10.1016/j.neurobiolaging.2015.05.014

63. Ladenbauer J, Ladenbauer J, Külzow N, et al. Promoting Sleep Oscillations and Their Functional Coupling by Transcranial Stimulation Enhances Memory Consolidation in Mild Cognitive Impairment. *The Journal of Neuroscience*. 2017;37(30):7111-7124. doi:10.1523/JNEUROSCI.0260-17.2017

64. Schneider J, Lewis PA, Koester D, Born J, Ngo HVV. Susceptibility to auditory closed-loop stimulation of sleep slow oscillations changes with age. *Sleep*. 2020;43(12):zsaa111. doi:10.1093/sleep/zsaa111

65. Dang-Vu TT, Bonjean M, Schabus M, et al. Interplay between spontaneous and induced brain activity during human non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences*. 2011;108(37):15438-15443. doi:10.1073/pnas.1112503108

66. Schabus M, Dang-Vu TT, Heib DPJ, et al. The Fate of Incoming Stimuli during NREM Sleep is Determined by Spindles and the Phase of the Slow Oscillation. *Front Neur*. 2012;3. doi:10.3389/fneur.2012.00040

67. Helfrich RF, Lendner JD, Mander BA, et al. Bidirectional prefrontal-hippocampal dynamics organize information transfer during sleep in humans. *Nat Commun*. 2019;10(1):3572. doi:10.1038/s41467-019-11444-x

68. Morin A, Doyon J, Dostie V, et al. Motor sequence learning increases sleep spindles and fast frequencies in post-training sleep. *Sleep*. 2008;31(8):1149-1156.