Behavioral/Cognitive

Increased Sleep Spindles in Regions Engaged during Motor Learning Predict Memory Consolidation

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Sleep spindles play a critical role in sleep-dependent memory consolidation. Although spindles can occur widely across the cortex, they are often focal. Focal spindles may promote plasticity in distinct circuits to consolidate specific memory types. In this study, we simultaneously acquired EEG and magnetoencephalography (MEG) data from 25 healthy adults (six females) during daytime naps to investigate whether learning the finger tapping motor sequence task (MST) preferentially increases spindle density (#/min) within cortical regions engaged during task performance and whether these task-related spindle increases predict performance improvement measured after the nap. We employed a novel algorithm to project EEG/MEG signals into source space using anatomical constraints from MRI and detected spindles in cortical regions. Compared with a baseline nap, MST training preferentially increased spindle density in regions engaged while learning, which in turn predicted postnap performance improvement. Learning during training and postnap improvement were not correlated, suggesting that they reflect discrete processes. They also had different neural correlates. Whereas learning during training correlated with spindle density increases in motor execution regions, postnap improvement correlated with increases in motor planning regions. We speculate that spindles in motor execution regions represent the memory, while those in planning regions enhance future performance. Our findings demonstrate that spindle expression is influenced by prior learning and support the theory that spindles in task-related regions promote the neural plasticity necessary for motor memory consolidation. We propose that spindles in task-related regions may be more sensitive biomarkers of learning and sleep-dependent memory consolidation than those occurring elsewhere.

Key words: electroencephalography; learning; magnetoencephalography; memory consolidation; sleep; sleep spindles

Significance Statement

Sleep spindles, brain rhythms necessary for consolidating new memories, are deficient in several disorders and are tractable treatment targets. Although spindles can occur widely across the cortex, they are frequently regionally specific. In this study, using EEG and magnetoencephalography, we found that motor learning preferentially increases spindles in cortical regions engaged during learning and that these regionally specific increases predict performance improvement measured after sleep. Our findings demonstrate that spindle expression is influenced by prior learning and support the theory that spindles in regions engaged during learning promote the brain plasticity necessary for motor memory consolidation. We propose that spindles in task-related regions may be more sensitive indicators of new learning and sleep-dependent memory consolidation than those occurring elsewhere.

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Introduction

Sleep spindles, defining EEG oscillations of nonrapid eye movement Stage 2 sleep (N2), play a critical role in sleep-dependent memory consolidation (Stickgold and Walker, 2007). Spindle deficits are seen in several neurodevelopmental, neuropsychiatric, and neurodegenerative disorders characterized by cognitive impairment (Manoach and Stickgold, 2019). Findings that increasing spindles, either pharmacologically or with noninvasive brain stimulation in humans or optogenetically in rodents, can improve memory suggest spindles as a promising physiological target for the development of cognitive-enhancing therapies (Kaestner et al., 2013; Manoach et al., 2020). Here we investigated changes in spindles in response to learning to better understand sleep-dependent memory consolidation, how it might go awry in disorders, and what to target for treatment.

Spindle density (#/min) is highly heritable, exhibits considerable variability among individuals, and remains stable across multiple nights and naps (De Gennaro et al., 2008; Purcell et al., 2017; Mylonas et al., 2020), leading to its description as an "electrophysiological fingerprint" (De Gennaro et al., 2005). Although spindles can occur widely across the cortex, they are more often restricted in spatial extent (Andrillon et al., 2011; Nir et al., 2011; Piantoni et al., 2017). This focality is hypothesized to facilitate the circuit-specific plasticity that underlies memory consolidation (Cox et al., 2014; Cox et al., 2018). Here, our goal was to provide a spatially precise characterization of spindles in relation to motor learning and memory.

Using simultaneously acquired EEG and magnetoencephalography (MEG) recordings, we examined changes in spindle density from a baseline nap to a nap that followed training on the finger tapping motor sequence task (MST), an extensively validated probe of sleep-dependent memory consolidation (Fischer et al., 2002; Walker et al., 2002; Walker et al., 2003a,b; Kuriyama et al., 2004; Fischer et al., 2005; Nishida and Walker, 2007). We expected MST training to preferentially increase spindle density in cortical regions that were engaged during task performance and that these increases would predict improved MST performance after the nap. This would demonstrate that spindle expression is influenced by prior learning and suggest that spindles promote the neural plasticity necessary for memory consolidation in a regionally specific manner.

These hypotheses are based on prior scalp EEG, intracranial EEG, and MEG findings that memory consolidation is associated with focal spindle activity, the location of which depends on the task. For example, the overnight retention of verbal learning correlates with spindles at left frontocentral EEG electrodes, whereas retention of visuospatial learning correlates with spindles at parietal electrodes (Clemens et al., 2005, 2006). Learning also increases spindle activity in task-relevant regions (Johnson et al., 2012), and these focal increases predict performance improvements after sleep (Bang et al., 2014; Solano et al., 2022). On the MST, performance improvement after a nap correlates with an asymmetry of spindle density in the scalp electrode overlying the motor cortex contralateral (vs ipsilateral; C4 > C3) to the hand that performed the task (Nishida and Walker, 2007). Similarly, using MEG source localization, post-sleep MST improvement correlates with increased spindle frequency (sigma) activity in the contralateral supplementary motor area (SMA; Tamaki et al., 2013).

This study builds on prior work by detecting spindles rather than sigma activity, estimating their cortical sources rather than inferring them from scalp sensors, and evaluating them across the cortical surface rather than only at predefined regions of interest. We used a novel algorithm to detect spindles in cortical source space using high-density EEG/MEG recordings during daytime naps (Mylonas et al., 2022a). Based on their biophysical characteristics, EEG and MEG are differentially sensitive to spindle sources (Dehghani et al., 2010a,b; Dehghani et al., 2011a,b). While EEG tends to capture widespread spindles, MEG is more likely to detect focal spindles, particularly in somatosensory and motor regions (Piantoni et al., 2016; Krishnan et al., 2018; Mylonas et al., 2022a). Together they allow a more accurate source estimation than either technique alone (Sharon et al., 2007).

Materials and Methods

Participants

Thirty-one healthy adults, recruited from the community through advertisements, were screened to exclude those with diagnosed sleep disorders, treatment with sleep medications, pregnancy, substance abuse or dependence within the past 6 months, and a history of mental illness (The Mini-International Neuropsychiatric Interview; Sheehan et al., 1998), diagnosed neurological disorder, or head injury with sustained loss of consciousness or neurological sequelae. The data from two participants who dropped out before study completion and four participants who had <10 min of artifact rejected N2 sleep were excluded from all analyses (Mylonas et al., 2022a; Kurz et al., 2024). The 25 participants (age, 29 ± 6 years; range 21–42 years; 19 males and 6 females) who successfully completed the EEG/MEG and MRI scanning visits and produced valid nap data were included in analyses. All participants gave written informed consent and were paid for participation. The study was approved by the Partners Human Research Committee. All but three participants were right-handed (two left-handed; one ambidextrous) based on the Edinburgh Handedness Inventory (White and Ashton, 1976).

Materials and design

Experimental design. Participants completed three MEG/EEG nap visits separated by at least 1 week (Fig. 1). They were asked not to consume caffeine or alcohol on study days. Each visit began at ~11 A.M. with questionnaires and then lunch, followed by wiring for polysomnography. All scans started at ~2 P.M., to take advantage of the circadian dip (Monk, 2005). The adaptation nap scan started with 5 min of quiet rest followed by a 90 min nap opportunity to acclimate the participants to sleeping in the MEG scanner. During quiet rest, participants were instructed to maintain fixation on a cross in the center of the screen, which they viewed through a mirror attached to the MEG dewar. The adaptation visit was followed by an identical baseline visit or the MST visit in a counterbalanced order. During the MST scan, participants had 5 min of quiet rest, were trained on the MST, and then had a second 5 min of quiet rest followed by the 90 min nap opportunity. Following the nap, participants were taken from the scanner and allowed to watch movies or read. Four hours after MST training, they returned to the scanner for MST testing. Ten minutes later, they trained on a new MST sequence. Approximately 1 week after the final nap, participants returned for an MRI scan.

The finger tapping MST. The MST involves pressing four numerically labeled keys on a keypad with the fingers of the left hand, repeating a five-digit sequence (e.g., 4-1-3-2-4) as quickly and accurately as possible for twelve 30 s typing trials with interleaved 30 s rest breaks (Walker

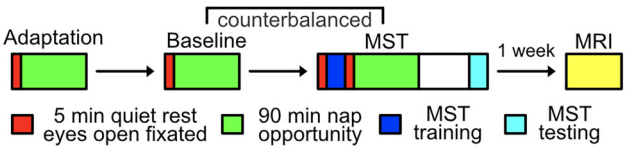

Figure 1. Study overview. Adaptation, baseline, and MST EEG/MEG nap visits were separated by at least a week and followed by an MRI scan. The order of baseline and MST sessions was counterbalanced across participants. *MST, motor sequence task.*

et al., 2002). During typing, the computer screen is green with the numeric sequence displayed at the top, and a dot appears below it with each keystroke going from the left to the right. Upon reaching the end of the screen, a dot disappears with each keystroke from the right to the left. During the breaks, the numeric sequence remains on, but the screen is red, and written out numbers count down the seconds until the next trial. Three seconds before the display turns green again, the numbers are replaced by three beeps to alert the participant to get ready to begin typing. Before starting, participants were informed that they would receive a $0.10 bonus for each correct sequence they typed.

MST performance indices. MST performance is measured as the number of correct sequences per typing trial, which reflects both speed and accuracy. The outcome measures are (1) learning during training, calculated as the percentage increase in correctly typed sequences from the first training trial to the average of the last three training trials, and (2) overnap improvement, calculated as the percentage increase in correctly typed sequences from the average of the last three training trials to the average of the first three test trials (Walker et al., 2002).

Data acquisition and processing

Simultaneous EEG and MEG acquisition. Data were recorded using a 306-channel whole–head Elekta-Neuromag MEG system (Elekta Oy, now MEGIN, Croton Healthcare) in a magnetically shielded room (IMEDCO) simultaneously with 70 channels of EEG, submental electromyography, and two electrooculography electrodes. All signals were digitized at 600 Hz. The MEG sensors are arranged as triplets at 102 locations; each location contains one magnetometer and two orthogonal planar gradiometers. Locations of the EEG electrodes and ∼200 head shape points were recorded using a 3D digitizer (Polhemus FASTRAK). Four head position index (HPI) coils were used to continuously track the position of the head relative to the MEG sensor array.

MRI acquisition. Anatomical MRI was acquired with a 3 T Siemens Trio whole-body high–speed imaging device equipped for echo planar imaging (Siemens Medical Systems) and a 32-channel head coil. Anatomical images were acquired using a 3D rf-spoiled magnetization-prepared rapid gradient echo sequence (TR, 2,530 ms; TE, 1.7/3.6/5.5/7.3 ms; flip angle, 7°; FOV, 256 mm; 176 in-plane sagittal 1 mm isotropic slices; scan duration, 6 min and 12 s). To construct the boundary element model surface for each participant's EEG/MEG source estimation, we acquired a multiecho multiflip angle (5°) FLASH pulse sequence [610 Hz per pixel; TR, 20 ms; TE, $1.89 + 2n$ ms ($n = 0$–7); 128 in-plane sagittal slices sized 1×1.33 mm; 1.33 mm thickness].

MEG and EEG data preprocessing. EEG/MEG data were notch-filtered at 60 Hz and bandpass filtered at 0.3–35 Hz for sleep and 0.3–50 Hz for wake. Electrodes displaying significant artifacts were spherically interpolated. EEG data were then rereferenced to the grand average. MEG data were processed using the signal-space separation (SSS) method (Uusitalo and Ilmoniemi, 1997) to suppress environmental noise and correct for head movements in the scanner using the HPI coils.

Sleep scoring. EEG sleep recordings were divided into 30 s epochs and visually scored according to standard criteria (Iber et al., 2007) as WAKE, REM, N1, N2, and N3 by expert raters (Table 1). Although spindles also occur during N3 sleep, we restricted our analyses to N2 since only 4 of the 25 participants had > 10 min of N3 during both the baseline and MST naps. In four participants, we substituted the adaptation nap for the baseline nap due to a lack of sufficient (>10 min) artifact-free N2 sleep during the baseline nap.

MRI-anatomically constrained combined MEG/EEG source estimation. Coregistration of EEG and MEG sensors to each participant's structural MRI was implemented in MNE-C using the digitized electrodes, fiducials, HPI coils, and head shape points. MRI reconstruction and tissue segmentation were performed using FreeSurfer (Dale et al., 1999; Fischl et al., 1999). The FreeSurfer-derived cortical surface tessellation was decimated to a regular source dipole grid with 3 mm spacing between adjacent source locations, corresponding to ∼18,500 dipoles. EEG/MEG forward solutions were computed using the three-layer boundary element method (Hämäläinen and Sarvas, 1989) using inner skull, outer skull, and scalp surfaces from segmentations of the FLASH images. The cortically constrained minimum-norm estimate of the cortical currents (MNE; Dale and Sereno, 1993; Hämäläinen et al., 1993) was computed with source orientations fixed perpendicular to the local cortical surface and a regularization factor of 0.1. Noise covariance estimates were calculated using the EEG/MEG data from a 5 min pre-MST resting-state scan filtered at 100–140 Hz. We used dynamical statistical mapping (Dale et al., 2000) to reduce the MNE inverse solution bias toward superficial cortical sources. FreeSurfer was used to automatically divide the cortex into 72 regions (Fischl et al., 2004). After discarding "medial wall" and "corpus callosum," these regions were further divided into a total of $N = 448$ similarly sized cortical parcels (Fig. S1) using FreeSurfer (Khan et al., 2018). The resulting source space time courses of the quiet rest, MST training, and artifact-free N2 sleep were then computed in these 448 regions. To align the signs of the time series across vertices, we used the singular value decomposition of the data. The sign of the dot product between the first left singular vector and all other time series in a label was computed. If this sign was negative, we inverted the time series before averaging.

Table 1. Mean ± standard deviation and differences in sleep quality and architecture at the baseline versus MST visits

Sleep quality	Baseline nap	MST nap	*P* value*
Time in bed	94 ± 6 min	91 ± 7 min	0.85
Total sleep time	66 ± 22 min	65 ± 20 min	0.65
Sleep onset latency	6 ± 7 min	7 ± 7 min	0.85
Wake after sleep onset	22 ± 21 min	19 ± 22 min	0.61
Sleep efficiency	70 ± 19%	68 ± 23%	0.66
Sleep architecture (min)			
N1	14 ± 6 min	13 ± 6	0.75
N2	37 ± 20 min	38 ± 24	0.88
N3	11 ± 12 min	10 ± 16	0.77
REM	4 ± 7 min	4 ± 6	0.89

*p value for paired t test (df = 24).

Detecting spindles in source space. Spindles were automatically detected in the 12–15 Hz bandpass-filtered EEG/MEG source space data at each cortical region [see Mylonas et al. (2022a) for details] using a wavelet-based algorithm (Wamsley et al., 2012; Mylonas et al., 2020). This detector has been validated against visual inspection in healthy people, individuals with schizophrenia, and adolescents with autism spectrum disorder (Wamsley et al., 2012; Mylonas et al., 2022b). The threshold for spindle detection was set to nine times the median signal amplitude of the artifact-free epochs as this maximizes the between-class variance for spindles versus nonspindle classification (Otsu, 1979), based on data from healthy participants from a previous study (Wamsley et al., 2012). Spindle density was defined as the average number of spindle events detected per minute across all N2 epochs.

Identification of the task region. To identify regions engaged during MST training, we first compared 1–40 Hz global power (averaged across all parcels) between the 12 concatenated 30 s MST typing trials and the rest before MST training at each 1 Hz bin using paired t tests. This range was selected to include frequencies that show movement-related power suppression (Pfurtscheller and Lopes da Silva, 1999). This analysis showed a significant global power reduction from 9 to 26 Hz ($t_{(24)} > 3.6$; $p < 0.001$) during typing versus rest (Fig. 2*A*), corresponding to suppression of the mu/beta rhythm (10–30 Hz; Pfurtscheller et al., 2003). Second, we identified the parcels showing significant power changes from rest to typing at any frequency bin within the 9–26 Hz range using paired t tests. This analysis found significant power suppression in 102 parcels, which we designated as the task region. These include bilateral primary and secondary motor and somatosensory cortices, medial premotor cortices including the SMA, and cingulate, precuneus, temporal, and insular cortices (Fig. 2*B*). These regions overlap with those previously found to be involved in motor sequence execution in functional

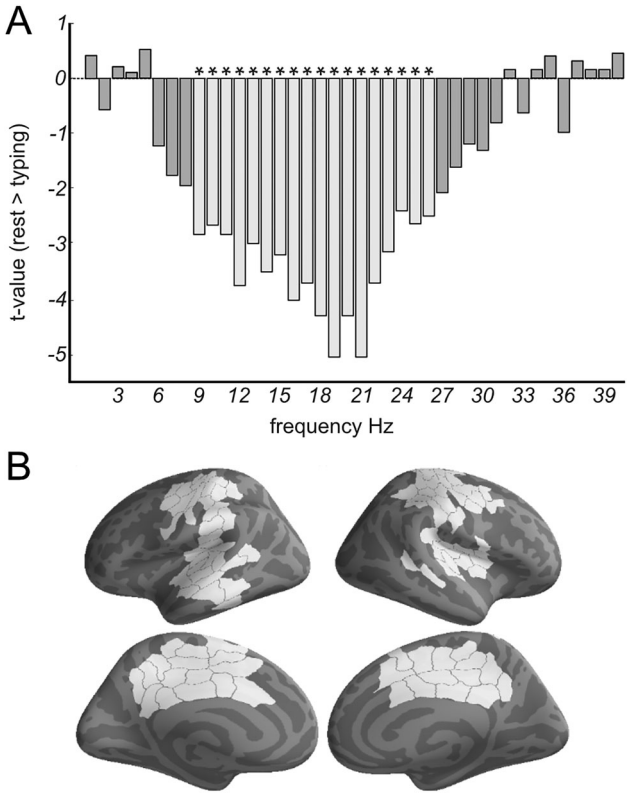

Figure 2. The task region. ***A***, Bars indicate paired *t* tests of global power during MST typing versus pre-MST rest from 1 to 40 Hz. Asterisks denote significance after correction for multiple comparisons. Negative *t* values indicate less power during MST typing than during pre-MST resting state (i.e., suppression). ***B***, The 102 brain parcels forming the task region are shown in white.

MRI (Karni et al., 1995; Debas et al., 2010; Barakat et al., 2013; Gregory et al., 2014) and EEG and MEG studies (Tamaki et al., 2013; Pollok et al., 2014; van der Cruijsen et al., 2021).

Statistical analyses

We identified parcels showing changes in spindle density during the MST nap compared with the baseline nap using paired *t* tests. We also evaluated the relations of MST outcome measures (learning during training and overnap improvement) with spindle density (MST and baseline naps) and spindle density changes (MST vs baseline) using correlation analyses. All statistical tests were corrected for multiple comparisons using the family-wise error rate (FWER; Nichols and Holmes, 2002). We created null distributions by permuting the data and repeating the tests 10,000 times. For paired *t* tests, the data were permuted by randomly relabeling the condition (task-related parcel or not; resting state or MST; spindle density difference in the baseline or MST nap). For correlations of behavioral outcomes (learning during training; overnap improvement) with spindle density or spindle density change, the behavioral outcome values were randomly shuffled. The permuted statistic was calculated for each of the 448 (all cortical) or 102 (within the task region) parcels. For each permutation, the maximum absolute statistic across all included parcels was used to create the final null distribution for a given statistic. The 95th percentile of the resulting null distribution corresponds to FWER corrected two-sided $p < 0.05$. To determine whether spindle density changes and correlations with behavior preferentially occurred in parcels within the task region, we applied the same FWER threshold for the 102 parcels within the task region to parcels outside of the task region and performed χ^2 tests to compare the proportion of parcels that surpassed this threshold.

Neurosynth-derived word clouds. To understand the functional relevance of the topological maps that resulted from our analyses, we used Neurosynth (https://neurosynth.org/). Neurosynth links every cortical vertex of a topological map to a database of thousands of fMRI studies to generate a ranked and weighted list of functional terms associated with this map. We generated word clouds for each map using the Python package word_cloud (https://github.com/amueller/word_cloud). The size of each term in the word cloud corresponds to how often it was found in the Neurosynth terms lists associated with the parcels in that map.

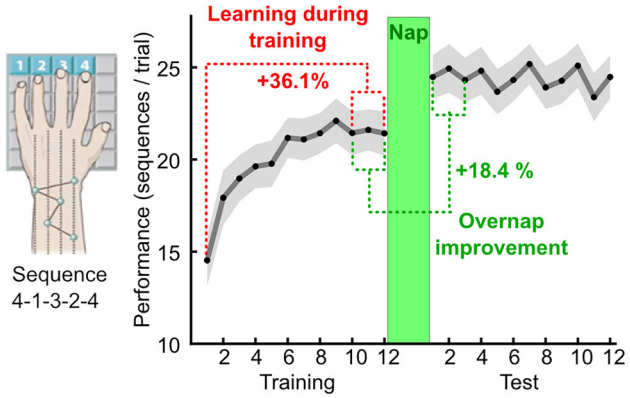

Figure 3. MST performance. Left, Participants repeatedly type a five-digit sequence (e.g., 4-1-3-2-4) by pressing four numerically labeled keys on a keypad with their left hand. Right, Black dots show the average number of correct sequences per trial. Shading represents the standard error. Learning during training (red-dotted line) and overnap improvement (green-dotted line) are shown.

Results

MST performance

Participants showed significant learning during MST training (mean ± SEM, 36 ± 6%; $t_{(24)} = 6.89$; $p = 2.1^{-7}$) and overnap improvement (18 ± 7%; $t_{(24)} = 3.03$; $p = 0.005$; Fig. 3). Consistent with previous findings (Walker et al., 2003b; Manoach et al., 2004), learning during training and postsleep improvement were not correlated ($r = -0.012$; $p = 0.93$), suggesting that they reflect discrete processes of motor learning.

MST training induces spindle density increases preferentially in task-related regions

A significantly greater proportion of parcels within the task region (18/102, 17.6%; Fig. 4*A*; Fig. S2; Table S1) than outside the task region (10/346, 2.9%) showed increased spindle density during the post-MST versus baseline nap ($\chi^2_{(1,\ N=448)} = 26.8$; $p = 2.2^{-7}$). These parcels include bilateral regions of primary motor cortex (M1) corresponding to hand/arm areas; regions of M1 corresponding to mouth/face areas in the left hemisphere, ipsilateral to the hand that performed the MST; and contralateral (right) premotor cortex and SMA. The Neurosynth word cloud emphasizes terms associated with finger movement. No parcels showed significant reductions in spindle density in the MST nap compared with the baseline nap.

Learning during training predicts increases in spindle density preferentially in task-related regions

Greater learning during training correlated with increased spindle density during the MST versus baseline nap. These correlations were disproportionately seen in task region parcels (11/102, 10.8%; Fig. 4*B*; Fig. S3; Table S2) relative to nontask region parcels (7/346, 2.0%; $\chi^2_{(1,\ N=448)} = 15.83$; $p = 0.0001$). Significant parcels included contralateral (right) hand and arm areas of M1 and bilateral somatosensory cortex, but no motor planning regions. The

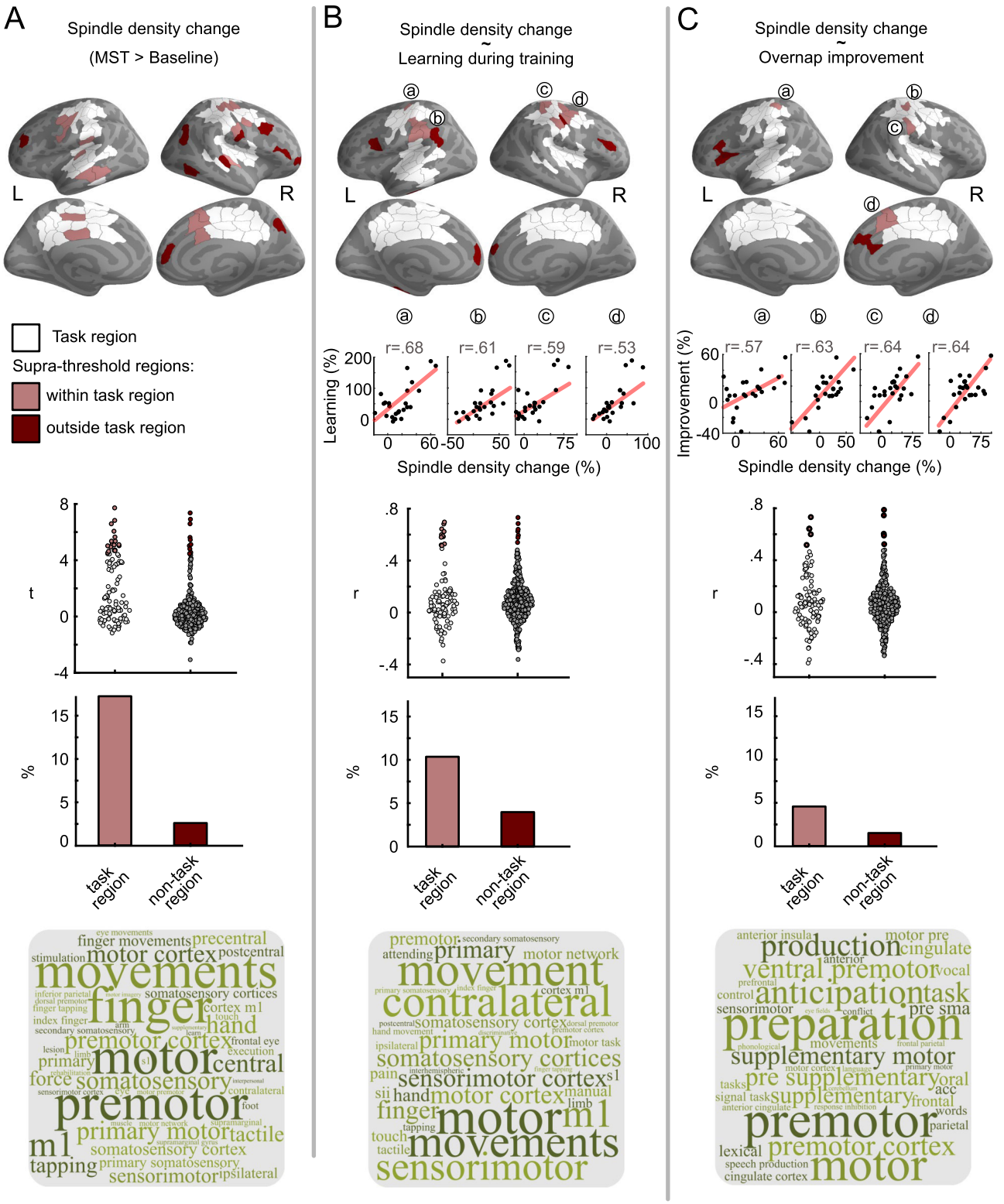

Figure 4. Spindle density changes from the baseline nap to the MST nap and their relations with MST performance. Top row, ***A***, Spindle density changes from the baseline nap to the MST nap. ***B***, Correlations of spindle density changes with learning during training. ***C***, Correlations of spindle density changes with overnap improvement. Inflated brains with pink and crimson parcels show differences or correlations within and outside of the task region, respectively. Second row, ***B/C***, Scatterplots of the relations of spindle density changes from (***B***) the baseline to the MST nap with learning during training or (***C***) overnap improvement for selected parcels. The red line is the best fit of a robust regression. Third row, Distribution of the relevant statistic for significant parcels within and outside the task region. Gray dots represent subthreshold parcels. Fourth row, Proportion of suprathreshold parcels within and outside the task region. Bottom row, Neurosynth word cloud terms associated with the MNI coordinates of the significant cortical parcels in the task region (pink areas), sized by their frequency of mentions.

Neurosynth word cloud emphasizes terms associated with motor execution. No parcels showed significant inverse relations of spindle density changes with learning during training.

Increases in spindle density after MST training predict greater overnap improvement preferentially in task-related regions

Correlations of increased spindle density during the MST versus baseline nap with overnap improvement were disproportionately seen for parcels within the task region (Fig. 4*C*; Fig. S4; Table S3; 5/102, 4.9%; outside the task region, 5/346, 1.4%; $\chi^2_{(1,\ N=448)} = 4.48$; $p = 0.034$). Significant task region parcels included primary and secondary somatosensory cortices and medial premotor and supplementary motor cortices, but not M1 (Fig. S4; Table S3). All but one parcel (secondary somatosensory cortex) was in the contralateral hemisphere. Importantly, three of the five parcels (all contralateral) also showed significant spindle density increases as a result of learning the MST. This indicates that learning-induced increases in spindle density predict the amount of performance improvement measured after the nap. The Neurosynth word cloud emphasizes terms associated with motor planning and preparation. No parcels showed significant inverse relations of spindle density changes with overnap improvement.

Interestingly, learning during training and overnap improvement significantly correlated with spindle density increases in a distinct and nonoverlapping set of parcels. Our plots of the correlations for all parcels in which spindle density increases correlated with either learning during training or overnap improvement shows that this spatial dissociation is not simply a thresholding issue (Fig. 5). Parcels showing suprathreshold correlations with learning during training show much lower correlations with overnap improvement and vice versa. In addition, none of the parcels in which spindle density increases correlated with either learning during training (Fig. 4*B*) or overnap improvement (Fig. 4*C*) also showed correlations with total improvement (i.e., the sum of learning during training and overnap improvement; all $|r| \leq 0.2$; $p \geq 0.31$, uncorrected). These findings complement our behavioral findings in supporting the hypothesis that learning during training and overnap improvement reflect discrete processes of motor learning.

Spindle density during the MST nap, but not the baseline nap, correlates with both greater learning during training and overnap improvement

In addition to evaluating spindle density changes from the baseline to the MST nap, we examined whether MST performance correlated with spindle density during the MST and baseline naps individually. Better learning during training predicted higher spindle density during the MST nap in contralateral hand/arm areas of M1 and contralateral primary sensorimotor cortices (Fig. 6*A*; Fig. S5; Table S4). Similarly, overnap improvement correlated with spindle density exclusively within the task region during the MST nap including the face/mouth area of ipsilateral M1 and the contralateral premotor cortex and SMA (Fig. 6*B*; Fig. S6; Table S5). There were no significant relationships between spindle density during the baseline nap and subsequent MST performance in the task region ($|r| < 0.25$; $p > 0.42$).

No parcels outside of the task region showed suprathreshold spindle density changes from the baseline to the MST nap or correlations of spindle density or spindle density changes with learning or overnap improvement after multiple-comparison correction for all 448 cortical parcels.

Discussion

Consistent with our hypotheses, learning a motor task led to increased sleep spindle density in regions engaged while learning, which in turn predicted performance improvement measured after sleep. These findings demonstrate that spindle expression is influenced by prior learning and provide strong support for the theory that spindles facilitate the neural plasticity necessary for procedural motor memory consolidation in a regionally specific manner. The greater spatial resolution afforded by our methods may explain why in this study we were able to detect increases in spindle density from baseline sleep to the sleep that follows learning, while in our

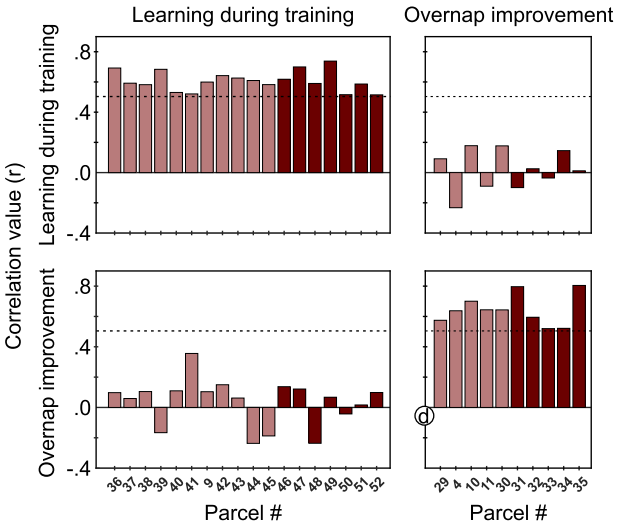

Figure 5. Spatial dissociation of parcels showing correlations of spindle density change with learning during training versus overnap improvement. Barplots show correlation (*r*) values for parcels that showed suprathreshold correlations of spindle density change with either learning during training (left column) or overnap improvement (right column). The dotted line indicates the significance threshold for task-related parcels. Pink bars indicate parcels within the task region; crimson bars indicate parcels outside of the task region. Parcel numbers correspond to Tables S2 and S3.

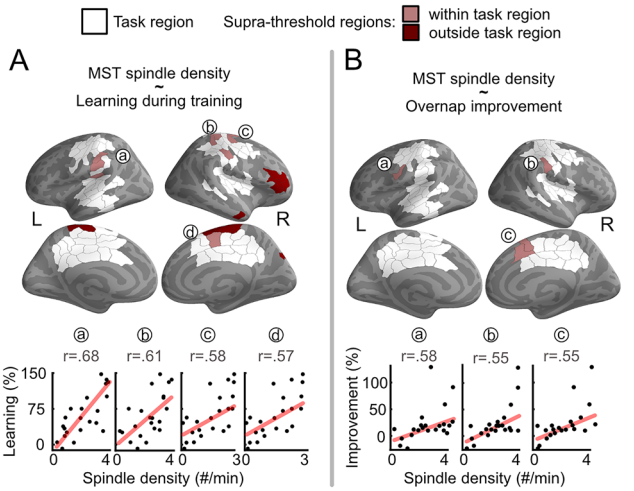

Figure 6. Spindle density in relation to MST performance. Top row, Inflated brains showing suprathreshold correlations of spindle density during the post-MST nap with (***A***) learning during training and (***B***) overnap improvement within (pink) and outside (crimson) the task region. Bottom row, Scatterplots showing the relations of postlearning spindle density with (***A***) overnap improvement or (***B***) learning during training for representative parcels. The red line is the best fit of a robust regression.

prior work with scalp EEG alone we were not (Mylonas et al., 2022b). These findings lead us to propose that regionally specific spindles may be more sensitive biomarkers of sleep-dependent memory consolidation than widespread spindles.

As in prior work, learning during training and overnap improvement were not correlated suggesting that they reflect discrete processes of motor learning (Walker et al., 2003b; Manoach et al., 2004). This hypothesis is supported by our finding that the amount of learning during training significantly correlated with spindle increases in a number of task-related regions that differed from and did not overlap with those in which spindle increases predicted memory consolidation. While learning during training correlated with increased spindles in regions involved in motor execution, including the contralateral M1 hand area, but not in motor planning regions, memory consolidation correlated with spindle increases in contralateral motor planning regions (i.e., premotor cortex and SMA), but not in M1. One possible functional interpretation of this spatial dissociation is that regions in which spindle increases correlate with initial learning may represent and stabilize memories of recent experience, while regions in which spindle increases predict overnap improvement may support sleep-dependent consolidation processes that render performance faster, less effortful, and less dependent on voluntary attention (i.e., more automatic; Kuriyama et al., 2004; Walker et al., 2005; Manoach and Stickgold, 2009). Prior findings that postsleep MST improvement correlates with increased spindle frequency (sigma) activity in the SMA also support the role of this region in memory consolidation processes (Tamaki et al., 2013).

In addition to identifying regions showing spindle density increases from the baseline nap to the MST nap in relation to MST performance (both learning and consolidation), we also examined the correlations of MST performance with spindle density during each nap individually. MST performance correlated with spindle density in task-related regions during the MST nap, but not during the baseline nap. This supports the hypothesis that learning a new task induces increases in spindle density in task-related regions and does not merely reflect that individuals with higher intrinsic spindle density in task-related regions have a greater capacity to learn and remember. Although spindle parameters are stable and heritable signatures of the sleep EEG (Purcell et al., 2017; Mylonas et al., 2020), their expression is influenced by learning. Future work is needed to disentangle the complex interactions of trait-like spindle density and the ability to leverage mechanisms of neural plasticity in the service of memory consolidation.

In addition to increasing spindles in M1 hand regions, MST training also increased spindle density in M1 regions where facial movements, including mouth control, are represented (Fig. 2). Moreover, higher spindle density in the ipsilateral (left) M1 face area during the MST nap correlated with overnap improvement (Fig. 6). This is similar to findings of our previous functional connectivity MRI study showing that enhanced connectivity of bilateral M1 face areas with the contralateral M1 hand area during the wakeful rest that followed MST training predicted subsequent overnight improvement (Gregory et al., 2014). These bilateral face regions are associated with tasks that involve both speech production (Brown et al., 2005; Ghosh et al., 2008) and covert speech (Shergill et al., 2002; Callan et al., 2006). In that study, although not instructed to do so, participants reported that they covertly vocalized the sequence during MST training, presumably to guide their sequential finger movements. We hypothesized that the increase in connectivity during the wakeful rest following learning prepares motor memory for later consolidation by sleep. The present study builds on this result by showing that during the sleep that follows MST training, spindle density in the left M1 face region predicts memory consolidation. Collectively, these studies support the hypothesis that the wakeful resting brain represents motor memories via modulations of motor network connectivity for later consolidation by sleep via spindles.

In the present study, we restricted our examination of spindles to N2 sleep due to the limited amount of N3 sleep observed in our sample. This might reflect that N2 tends to predominate during afternoon naps and that lying in the confined MEG environment may have led to lighter sleep. Spindle parameters differ in N2 and N3 (Cox et al., 2018), and N2 spindle density more reliably correlates with sleep-dependent MST improvement (Nishida and Walker, 2007; Albouy et al., 2013). The role of N3 spindles in motor memory consolidation could be investigated in overnight sleep studies.

In summary, we found that (1) training on a procedural motor memory task leads to sleep spindle density increases preferentially within cortical regions engaged while learning, (2) the amount of learning during training correlates with the magnitude of spindle increases within task-related regions associated with motor execution, and (3) increases in spindle density within task-related regions associated with motor planning predict overnap improvement. The different functional associations of regions that correlated with spindle increases for initial learning versus memory consolidation suggest that they play different roles in memory processing. Regions associated with learning may stabilize the memory, while those associated with consolidation may be involved in processes that improve future performance, making it more automatic. Collectively, these findings suggest that spindle expression dynamically reflects recent motor learning in some regions and contributes to its consolidation and enhancement in other regions.

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