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Original Article

ORIGINAL ARTICLE

Neural fatigue due to intensive learning is reversed by a nap but not by quiet waking

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Abstract

Do brain circuits become fatigued due to intensive neural activity or plasticity? Is sleep necessary for recovery? Well-rested subjects trained extensively in a visuo-motor rotation learning task (*ROT*) or a visuo-motor task without rotation learning (*MOT*), followed by sleep or quiet wake. High-density electroencephalography showed that *ROT* training led to broad increases in EEG power over a frontal cluster of electrodes, with peaks in the theta (mean ± *SE*: 24% ± 6%, $p = 0.0013$) and beta ranges (10% ± 3%, $p = 0.01$). These traces persisted in the spontaneous EEG (sEEG) between sessions (theta: 42% ± 8%, $p = 0.0001$; beta: 35% ± 7%, $p = 0.002$) and were accompanied by increased errors in a motor test with kinematic characteristics and neural substrates similar to *ROT* (81.8% ± 0.8% vs. 68.2% ± 2.3%; two-tailed paired *t*-test: $p = 0.00001$; Cohen's $d = 1.58$), as well as by score increases of subjective task-specific fatigue (4.00 ± 0.39 vs. 5.36 ± 0.39; $p = 0.0007$; Cohen's $d = 0.60$). Intensive practice with *MOT* did not affect theta sEEG or the motor test. A nap, but not quiet wake, induced a local sEEG decrease of theta power by 33% (SE: 8%, $p = 0.02$), renormalized test performance (70.9% ± 2.9% vs 79.1% ± 2.7%, $p = 0.018$, Cohen's $d = 0.85$), and improved learning ability in *ROT* (adaptation rate: 71.2 ± 1.2 vs. 73.4 ± 0.9, $p = 0.024$; Cohen's $d = 0.60$). Thus, sleep is necessary to restore plasticity-induced fatigue and performance.

Statement of Significance

Intensive motor learning in well-rested subjects induces brain fatigue evidenced by an increase in EEG activity in the theta range recorded during rest and localized over brain areas previously involved in learning. Moreover, after learning performance, error rate increases in a test that uses those same brain areas. Intensive practice without learning *does* not produce these effects. A nap, but not quiet wake, renormalized brain activity and test performance while consolidating learning. Thus, brain circuits become fatigued due to plasticity and learning. Sleep is necessary for recovery.

Key words: plasticity; training; movement; fatigue; EEG; quiet wake

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Introduction

Can the intensive activation of neural circuits lead to neural fatigue under physiological conditions, as indexed by persistent alterations in neural activity and negative consequences on performance? If so, is fatigue mostly due to activity or plasticity? And can the brain be restored by quiet wakefulness (rest) or only by sleep? Surprisingly, we still do not know the answer to these basic questions.

We know, however, that staying awake too long leads to persistent changes in neural activity and performance impairments that can only be restored by sleep. Recent studies in animals have shown that impaired performance after extended wake is associated with the occurrence of "local sleep"—bursts of low-frequency activity and neuronal OFF periods similar to those of sleep [1]. Presumably, the occurrence of OFF periods leads to performance impairment because it interferes with the proper activation of neural circuits. Human subjects kept awake for more than 20 h and engaged in intensive task performance also show a local, task-specific increase in slow frequencies (5–9 Hz) in the spontaneous EEG (sEEG), which is accompanied by a deterioration of test performance [2, 3]. Intracranial recordings in sleep-deprived humans also found that performance lapses are associated with local activity in the low frequencies (2–10 Hz) during wake and with delayed, attenuated spiking responses of individual cortical neurons [4]. These and other studies of prolonged sleep deprivation [5–7] show that extended wake leads to performance errors associated with increased EEG power in low frequencies.

In this study, we asked whether neural fatigue is triggered in well-rested brains without sleep deprivation. We did so by requesting subjects to engage in three sessions of an intensive visuomotor rotation learning task (*ROT*) during the morning hours. Fatigue was assessed both subjectively and through recordings of local changes in EEG activity and indexed by the performance of a motor test employing neural circuits similar to *ROT* [8–11]. We further asked whether intensive neural activity alone is sufficient to trigger neural fatigue, or whether learning and plasticity are necessary. We did so by comparing the effects of *ROT* on EEG activity and performance to those of a kinematically equivalent visuomotor task that did not involve rotation learning (*MOT*). Finally, we asked whether periods of quiet wakefulness are sufficient to restore neuronal activity and performance, or whether sleep is necessary. We did so by requiring subjects to either sleep or rest quietly for an hour upon the completion of *ROT*, after which their EEG activity and performance were assessed again.

Methods

Subjects

A total of 49 healthy subjects completed the study (age range: 20–35 years, mean ± *SD* = 24.0 ± 4.0 years, 26 women). All participants were right-handed, without a history of sleep or medical disorders and color vision impairment; they reported sleeping an average of 7–8 h/night for at least a week before the experiment, with consistent bed and rise times, as verified by sleep diaries. The study was approved by the local Institutional Review Board (IRB). Each participant signed an IRB-approved informed consent form.

Experimental design

Subjects arrived in the lab around 8:00 am and were fitted with a hd-EEG cap, to start the data collection by 9:00 am. Alcohol and caffeine-containing beverages were not allowed starting the night before and throughout each experiment. As described in Figure 1, during the morning, 36 subjects (age range: 20–33 years, mean ± *SD* = 23.6 ± 3.5 years, 19 women) performed a baseline consisting of 2-min eyes open sEEG, followed by *mov*, a reaching test (see below and Figure 1, C) and *mem*, a working memory and attentional test (see below and Figure 1, C). After the baseline, participants underwent three 45-min blocks of *ROT*, a motor adaptation task (see below and Figure 1, A and B); each *ROT* block was followed by a 2-min recording of sEEG with eyes open, *mem* and *mov* tests. At the end of the three blocks, subjects had lunch and afterward, 20 subjects were asked to take a 90-min nap (nap group), while 16 subjects (quiet wake group) were asked to stay awake and to rest quietly for 90 min lying down with their eyes closed, listening to audiobooks, and performing guided meditation. To minimize the influence of sleep inertia on cognitive functions, two 2-min eyes open sEEG recordings were performed about 30 min from the end of the 90-min quiet wake or nap interval. Experimenters took turns to enforce as much as possible adherence to the protocol throughout the entire experiment. In particular, during all tasks and EEG recordings, the experimenters alerted participants when signs of drowsiness were detected. For 11 participants (3 subjects in the awake group and 8 subjects in the nap group) EEG data was not available due to technical problems during collection and storage. The behavioral data of these participants were still used in the analysis of task performance. Therefore, EEG data were analyzed in 25 subjects (13 in the awake group and 12 in the nap group), while performance data were analyzed in 36 subjects (16 in the awake group and 20 in the nap group). The sample size was determined based on the behavioral results of pilot data in nine subjects (specifically, performance in *ROT* and *mov*). We concluded that at least 12 participants per group were required to have power of at least 0.9 to identify effects similar to those observed in the pilot study (Cohen's $d = 1.066$ for *ROT* and $d = 1.13$ for *mov*) at the $\alpha = 0.05$ significance level.

For the control experiment, we recruited 13 subjects (7 women) whose age matched that of the *ROT* group (range: 20–35 years, mean ± *SD* = 25.3 ± 5.3 years, two-tailed unequal variance *t*-test: $t = -0.22$; $p = 0.830$). As for the *ROT* session, after a baseline, subjects performed three 1-h blocks of *MOT*, a simple reaching movement task (see below), each followed by two 2-min recordings of sEEG with eyes open and by *mem* and *mov* tests (Figure 1, A). Since this control experiment was performed to ascertain whether local theta power increases over the frontal area were specific to learning in *ROT*, the *MOT* session included only the three morning blocks.

ROT

In this motor adaptation task (Figure 1, B), a circular array of eight targets (4 cm from a central starting point) was presented on a screen together with a cursor indicating the hand position. Targets lighted up in a random, unpredictable order, one every 1.5 s; they were presented in 21 sets of 56 with 30-s inter-set intervals. Subjects were asked to make out-and-back movements with their right hand by moving a cursor on a digitizing tablet and reaching the highlighted target "as soon as possible" and

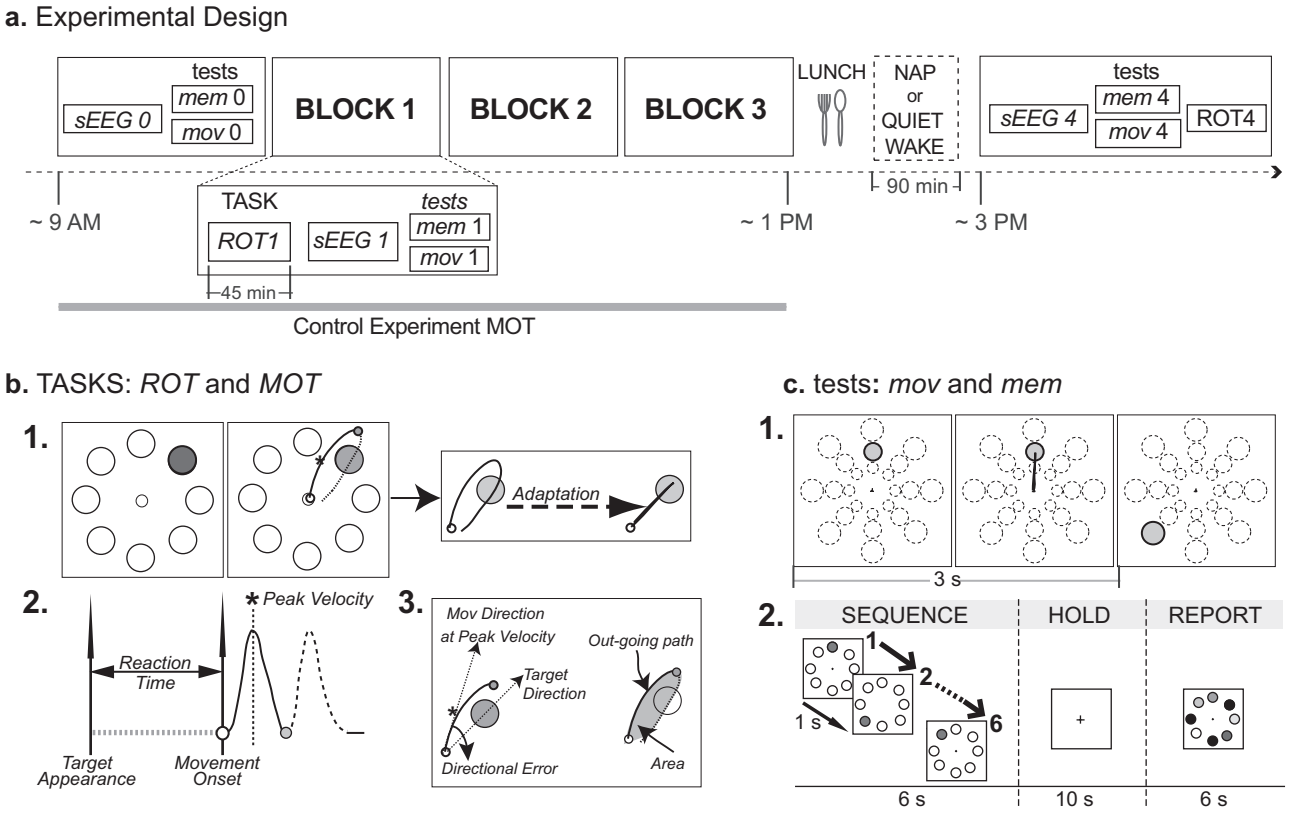

Figure 1. Experimental design, tasks and tests. (A) Experimental design of the *ROT* session. The *MOT* session (grey bar) encompassed only a baseline and three blocks. (B) *ROT* and *MOT* tasks. (1) In both tasks, the target was presented on the screen (left) and the movement trajectory was displayed (center). In the inset (right), an example of adaptation to an imposed visuo-motor adaptation in *ROT*. (2 and 3) Measures of each movement. (2) Temporal profile of velocity with the asterisk representing peak velocity of the outgoing movement. Reaction time is the time difference between target appearance and movement onset. (3) Directional error at peak velocity and normalized hand path area are represented for a movement. (C) *mov* (1) and *mem* (2) tests. (1) Target array used for mov. Instructions and movements are the same of *MOT* and *ROT*. (2) In mem, a sequence of targets was presented and after an interval, subjects reported the order of target appearance.

"as fast and as accurately as possible," thus minimizing reaction and movement time, but avoiding anticipation. Unbeknownst to subjects, the direction of the cursor on the screen was rotated relative to the direction of the hand on the tablet in incremental steps of 10°, 20°, or 30° each, either clockwise or counterclockwise, starting from 0° (no rotation of the cursor) up to a maximum of 60°. For each rotation step, subjects performed two sets of movements (112 movements). The first and the last sets were performed without any rotation imposed. The rotation steps for the sets were: Block 1: 10°, 20°, 30°, 40°, 50°, 60°, 50°, 40°, 30°, 20°, 10°; Block 2: 20°, 30°, 10°, 30°, 40°, 20°, 10°, –10°, –30°, –20°, 10°; Block 3: –10°, 10°, –20°, 0°, –20°, –30°, –10°, 10°, 20°, 0°, –10°; Block 4: –10°, –20°, –30°, –40°, –50°, –60°, –50°, –40°, –30°, –20°, –10°. Importantly, all the *ROT* blocks ended with three sets of movements without rotations to avoid interference with performance in the *mov* test (see below). Mean directional errors at the end of each block in the last set were similar to those of the first set (two-tailed paired *t*-test: $N = 36$, $t < 0.22$; $p > 0.9$ for each block), suggesting that subjects' performance at the end of the *ROT* blocks was back to baseline levels and thus, there was no interference of rotation adaptation in the subsequent *mov* tests (see below).

MOT

In this control motor reaching task, target array and presentations were the same as in *ROT*. Subjects were asked to make out-and-back movements with the same instructions as for *ROT*. However, no cursor rotations were imposed. Subjects performed a total of 20 sets of 56 movements in every block. A total of three blocks were performed (Figure 1, A and B).

mov

This motor test shares characteristics of *ROT* without the rotation adaptation component (Figure 1, C). Targets at three different distances (4, 7, and 10 cm) and eight directions (45° separation) appeared on a screen in non-repeating, unpredictable order at 3-s interval. Instructions were as for *ROT* and *MOT*, and subjects reached the target with out-and-back movements on a digitizing tablet. The total testing time for *mov* was approximately 5–6 min (96 targets).

mem

This learning test involves encoding visual sequences without any motor components (Figure 1, C). After three warning flashes, five or six out of eight targets successively lightened up on a screen for 250 ms at a 1-s time interval; subjects were asked to memorize the target sequence, to hold it in memory for 10 s, to verbalize it and be ready for the next sequence. For sequence verbalization, a color-coded target array was presented and subjects reported the order by mentioning the corresponding color. Subjects were tested before the session for proper color recognition. The experimenter wrote on a printed form the color order, while responses were also audio recorded. The correctness

of the written records was checked by listening to the audio recording and then scored. Sixteen different sequences were presented in each block for an overall duration of about 8 min. Declarative scores were from 0 (no order recognized) to 100% (correct sequence order). Trials with a score of less than 100% were considered "failed".

Kinematic analyses

Kinematic data were collected with custom-designed software by E.T.T. s.r.l. (MotorTaskManager Genoa, Italy). The analysis was performed on single movements, as previously described [8, 11–14]. For each movement, we computed, among other parameters: directional error at peak velocity, hand-path area (area included in the trajectory normalized by path length, a measure of interjoint coordination and thus of trajectory accuracy), reaction time, movement time, peak, and mean velocity. For each step (which included two sets), we computed the adaptation reached in the last eight movements in percentage as: %Adaptation = (1 – [average DirErr/imposed rotation]) * 100. The mean adaptation rate of a block was measured as the average of all steps in that block. This index of adaptation rate is based on the changes of directional errors normalized by each step of rotation and their starting point [12]. For *mov,* we also computed the number of correct movements, defined as movements with values of reaction time, normalized movement area or directional errors within 1.5 standard deviation of the mean of the baseline *mov0*.

EEG recording and analyses

High-density EEG (256 electrodes; Electrical Geodesic Inc., Hydrocel net, Eugene, OR) was recorded for the entire duration of the sessions with a sampling rate of 250 Hz, using the Net Amp 300 amplifier and Net Station 5.0 software (Electrical Geodesic Inc.). Impedances were maintained below 50 kΩ throughout the whole session. During the recording, the signal was referenced to the vertex (Cz). As mentioned above, sEEG recordings were analyzed in 11 subjects out of 20 in the nap group, and in 13 subjects out of 16 in the quiet wake group.

Preprocessing

Data were preprocessed using the public Matlab toolbox EEGLAB version 14.1.1 [15]; the continuous signal was filtered between 1 and 80 Hz (two-way least-squares FIR) with the addition of a notch filter centered at 60 Hz. The EEG signal was segmented into 4-s epochs. Data were then visually inspected to remove epochs and channels containing artifacts, defined as evident abnormalities of the signal. On average we removed 66.24 ± 27.79 (mean ± *SD*) out of 150 4-s epochs in the sEEG *ROT* session and 51.89 ± 27.19 epochs in the *MOT* experiment. These values are in agreement with previous studies [16]. Also, in the two conditions, we removed 54.2 ± 20.4 and 50.0 ± 25.2 out of 256 channels, respectively. In the *ROT* task, we removed 158.9 ± 82.4 out of 1,176 epochs and 43.7 ± 27.1 channels. *MOT* task recording had 135.8 ± 83.0 epochs rejected and 31.3 ± 18.7 channels. Channels were then replaced with spherical spline interpolation. Stereotypical artifacts, such as blinks, eye movements and motion-related signals were removed using the independent component analysis (ICA) with principal component analysis (PCA)-based dimension reduction. During sEEG data preprocessing, 74.5% of the components for *ROT* and 73.5% for *MOT* were removed. During tasks data preprocessing, 75.7% and 76.6% of the components for *ROT* and *MOT* were removed, respectively. These values are in the range of those reported in previous publications [10, 11, 16]. Electrodes located on the cheeks and neck were removed from the subsequent analysis, resulting in 180 electrodes. After the preprocessing and cleaning steps, the signal was average-referenced.

sEEG analysis

The power spectrum for each block was computed via the fast-Fourier transform function of Fieldtrip (FFT Hanning window) [17] in 0.5 Hz bins. For each subject, the power at each channel was normalized by the baseline, that is, the first sEEG recorded at the beginning of the day, within the following frequency ranges: broad-band (1–55 Hz), theta (4.5–8 Hz), and beta (13–25 Hz), according to the following equation: (sEEGn – sEEG0)/sEEG0.

Differences in the EEG activity between the first and the last morning sessions were assessed using cluster-based non-parametric permutation testing. The same approach was used to ascertain the effect on the sEEG of the 90-min interval of both nap and quiet wake. This non-parametric statistical approach directly addresses the "multiple comparison" problem by incorporating biophysically motivated constraints. In addition, such an approach permits to formulate the null hypothesis (identical probability distribution at different time points) and to control for false alarm rate under the null hypothesis [18]. Specifically, for cluster-based nonparametric permutation testing, nearest neighbor channels were determined via triangulation with three as the minimum number of significant channels for inclusion in a cluster. The reference distribution was created using the Monte Carlo method with 10,000 random iterations and critical alpha of 0.025 at the cluster level [18].

ROT and MOT EEG analysis

After preprocessing, epochs associated with invalid movements, that is, movements whose parameters exceeded two standard deviations of the mean, were rejected. First, we aligned each valid trial (i.e. trials that were not discarded from either EEG or kinematic preprocessing) to the time of movement onset; then, the recordings of the last set of movements of either *ROT1* or *MOT1* were normalized and compared to the first set to define the effects of practice. Frequency representations were computed for the 1–55 Hz range as well as for theta (4.5–8 Hz) and beta (13–25 Hz) ranges using Complex Morlet Wavelets at linearly spaced frequencies (0.5 Hz bins) and a constant time window (1.5 s). The number of wavelets cycles and length were increased as a function of frequency (cycles 3–10; length 2.5–0.17 s). This approach was used to allow for time-frequency analyses. Finally, cluster-based permutation testing was performed to define significant ROIs for the three ranges.

Analysis of the nap/quiet wake period

EEG recorded during the nap and the quiet wake period was scored for sleep stages using an open-source, Matlab based toolbox [19]. Both nap and quiet wake periods were scored by trained experimenters and confirmed by an experienced sleep

scorer using standard guidelines [20]. Recordings were scored in 30-s epochs as follows: wakefulness (W), NREM sleep stage 1 (N1), NREM sleep stage 2 (N2), and NREM sleep stage 3 (N3). REM sleep (R) was not present in either group. For scoring purposes, the contralateral mastoid reference was used and stages were primarily determined from classical derivations from the 10 to 20 montage (F4, F3, C4, C3, P3, P4, O1, O2). The disappearance of the rhythms associated with wakefulness such as posterior alpha oscillations (8–10 Hz) and the occurrence of slow rolling eye movements were indicative of the transition to N1. K complexes and sleep spindles marked the transition to N2. The transition to and the maintenance of N3 were determined by the occurrence of >75 μV slow waves for more than 20% of the epoch.

Statistics

Two-tailed *t*-tests for paired comparisons were used to compare differences in task performance between the first and the last training block of either *ROT* (*ROT1* vs. *ROT3*) or *MOT* (*MOT1* vs. *MOT3*) on the following indices: degree of adaptation (for *ROT* only), movement time, peak velocity, reaction time, and hand-path area. This approach was also used to verify the within-group effects of a nap and quiet wake (*ROT3* vs. *ROT4*) on task measures of adaptation, reaction time, and hand-path area; to compare test performance at baseline to that at the end of the morning in *mov* (number of correct movements, *mov3* vs. *mov0*) and *mem* (number of correct sequences, *mem3* vs. *mem4*); and to compare test performance after a nap and after *mov* (number of correct movements). The distribution of all these indices was normal, as tested with both Shapiro–Wilks tests and Kolmogorov–Smirnov tests (all $p > 0.05$). The only exception was the number of correct sequences in *mem* (both tests $p < 0.05$) and therefore the Mann–Whitney U-test was used for the within-group comparisons for *mem*. Paired *t*-tests with Bonferroni correction for multiple comparisons was also used to find spectral differences between sEEG0 and sEEG3 after both *ROT* and *MOT* and for EEG changes during the two tasks. Significant increases were found in the 5–30 Hz range. Two-tailed unequal variance *t*-tests were used to compare sEEG3 theta power changes between the *ROT* and *MOT* sessions. For all significant results with *t*-tests, we also computed effect sizes with Cohen's *d*. Pearson coefficients with Bonferroni corrections (when appropriate) were used to explore significant correlations between performance measures and sEEG changes; sEEG and *ROT1* EEG practice-related changes; sEEG power changes after a nap and sleep parameters; local power changes occurring during both *ROT1* and sEEG; performance changes and sleep parameters.

Results

A group of well-rested subjects performed three morning sessions of intensive training in a reaching task that requires adaptation to a visually rotated display (*ROT*, Figure 1). Another group of well-rested subjects performed a reaching task (*MOT*) that is kinematically equivalent to *ROT* but does not require motor adaptation [8, 11]. *MOT* mainly involves the activity of sensorimotor areas similarly to *ROT*, but not of frontal areas [8, 11]. The effects of the tasks on brain networks were assessed using two performance tests: (1) *mov* (reaching for random targets), a test with kinematic features and involvement of sensorimotor areas similar to *ROT* and *MOT* and (2) *mem*, a test that involves attention/spatial working memory but not motor activity. In what follows, tasks are always upper case whereas tests are always lower case.

Of note, the ROT training was designed to induce a persistent state of learning across all sessions, without allowing the subjects to learn fully a specific rotation, a goal achieved with frequent and small rotation changes (see Methods section). Thus, subjects' performance remained in early phases of adaptation, a situation associated with activation of the sensorimotor areas and pre-supplementary motor area [9]. This approach differs from the one used in previous works where subjects reached virtually complete and firm adaptation of a specific rotation, which was associated with activation of right parietal areas [8–10, 21].

Intensive training in ROT is accompanied by learning and EEG changes

During the *ROT* blocks, subjects adapted their movements to the rotated display by decreasing their directional error, without awareness of the imposed rotations. Handpath area, an index of trajectory accuracy reflecting inter-joint coordination [22, 23], decreased in the last block compared to the first, suggesting that subjects improved their skills across *ROT* blocks (Table 1). There was also a significant change in reaction time, but no change in the degree of adaptation to the rotated display, peak and mean velocity as well as movement time from the first to last blocks (Table 1).

We then asked whether any EEG changes accompanied rotation adaptation in the *ROT1* block. As shown in Figure 2, A, the broad-band EEG (1–55 Hz) recorded during rotation adaptation compared to the EEG recorded during baseline task without adaptation showed a significant power increase in a cluster of electrodes over a frontal area (mean ± *SE*: 13% ± 2%; cluster $t = 47.96$, $p = 0.0086$). A bin-by-bin analysis showed that the power increase in this cluster was significant in the range from 5 to 30 Hz, with two peaks centered around the theta and beta bands (Figure 2, B). Both peaks involved a frontal cluster of electrodes, more prominently on the left side (theta cluster: mean ± *SE*: 24% ± 6%; cluster $t = 164.96$, $p = 0.0013$; beta cluster: 10% ± 3%; cluster $t = 71.6$, $p = 0.010$; Figure 2, C).

Next, we asked whether rotation adaptation would result in persistent changes that could be detected by recording the spontaneous EEG during the rest periods after *ROT* blocks (sEEG). As shown in Figure 2, D, comparing the resting state broad-band EEG recorded after the last *ROT* block (sEEG3) with that recorded at baseline before *ROT1* (sEEG0) revealed a power increase over a left frontocentral cluster of electrodes (mean ± *SE*: 31% ± 8%; cluster $t = 224.12$, $p = 0.0001$). A bin-by-bin analysis showed that the power increase in this cluster was again significant in a range centered around the theta band (4.5–8 Hz) and the beta band (13–28 Hz; Figure 2, E). Both peaks involved a frontal cluster of electrodes, predominantly on the left side, which overlapped with the larger frontal cluster in both theta (42% ± 8%; cluster $t = 147.14$; $p = 0.0001$) and beta (35% ± 7%; cluster $t = 217.07$; $p = 0.002$) ranges revealed by recordings during *ROT1* (Figure 2, F). These results indicate that rotation learning

Table 1. Mean ± SE of performance and subjective indices of *ROT1* and *ROT3*

	ROT1	*ROT3*					
	Mean ± SE	Mean ± SE	95% CI	*df*	*t*	*P*	Cohen's *d*
N = 36							
Adaptation rate	71.1 ± 0.7	71.2 ± 0.7	1.46, 1.68	35	0.07	0.94	
Peak velocity	38.0 ± 1.1	37.1 ± 0.9	−1.72, 0.06	35	1.94	0.06	
Mean velocity	22.4 ± 0.7	21.9 ± 0.6	−0.96, 0.14	35	1.53	0.14	
Movement time	229.3 ± 5.7	232.5 ± 5.4	−1.46, 8.18	35	1.33	0.19	
Reaction time	252.4 ± 3.2	247.4 ± 2.8	−8.75, −0.78	35	2.63	**0.013**	**0.29**
Handpath area	0.059 ± 0.002	0.050 ± 0.002	−0.011, −0.003	35	3.66	**0.001**	**0.75**
Tiredness	4.22 ± 0.31	5.58 ± 0.33	0.87, 1.85	35	5.68	**0.00002**	**0.72**
Boredom	5.19 ± 0.34	5.69 ± 0.32	−0.12, 1.12	35	1.64	0.11	
Sleepiness	5.28 ± 0.38	5.44 ± 0.31	−0.44, 0.77	35	0.55	0.58	
N = 25							
Adaptation rate	71.4 ± 0.8	71.8 ± 0.8	−1.55, 2.31	24	0.16	0.87	
Peak velocity	38.6 ± 1.2	37.7 ± 1.0	−1.89, 0.05	24	1.96	0.06	
Mean velocity	23.0 ± 0.8	22.3 ± 0.7	−1.38, 0.04	24	1.98	0.059	
Movement time	224.8 ± 5.7	230.1 ± 5.8	−0.33, 10.81	24	1.94	0.064	
Reaction time	252.9 ± 3.9	248.1 ± 3.2	−9.17, −0.29	24	2.20	**0.038**	**0.24**
Handpath area	0.062 ± 0.002	0.052 ± 0.002	−0.015, −0.005	24	4.04	**0.0005**	**0.83**
Tiredness	4.00 ± 0.39	5.36 ± 0.39	0.77, 1.95	24	4.82	**0.0007**	**0.60**
Boredom	5.16 ± 0.41	5.44 ± 0.36	−0.44, 1.00	24	0.80	0.43	
Sleepiness	5.16 ± 0.45	5.12 ± 0.37	−0.65, 0.73	24	0.12	0.91	

Results of *t*-tests comparing the two tasks with corresponding 95% confidence intervals for the difference of means. Significant *p*-values are in bold; effect sizes (Cohen's *d*) were computed for $p < 0.05$. The first part of the table concerns the entire sample of subjects tested (*N* = 36); the second part is about the subgroup of subjects with EEG recordings (*N* = 25). Values of adaptation rate are expressed as a percentage; peak and mean velocities in cm/s; movement and reaction times in ms.

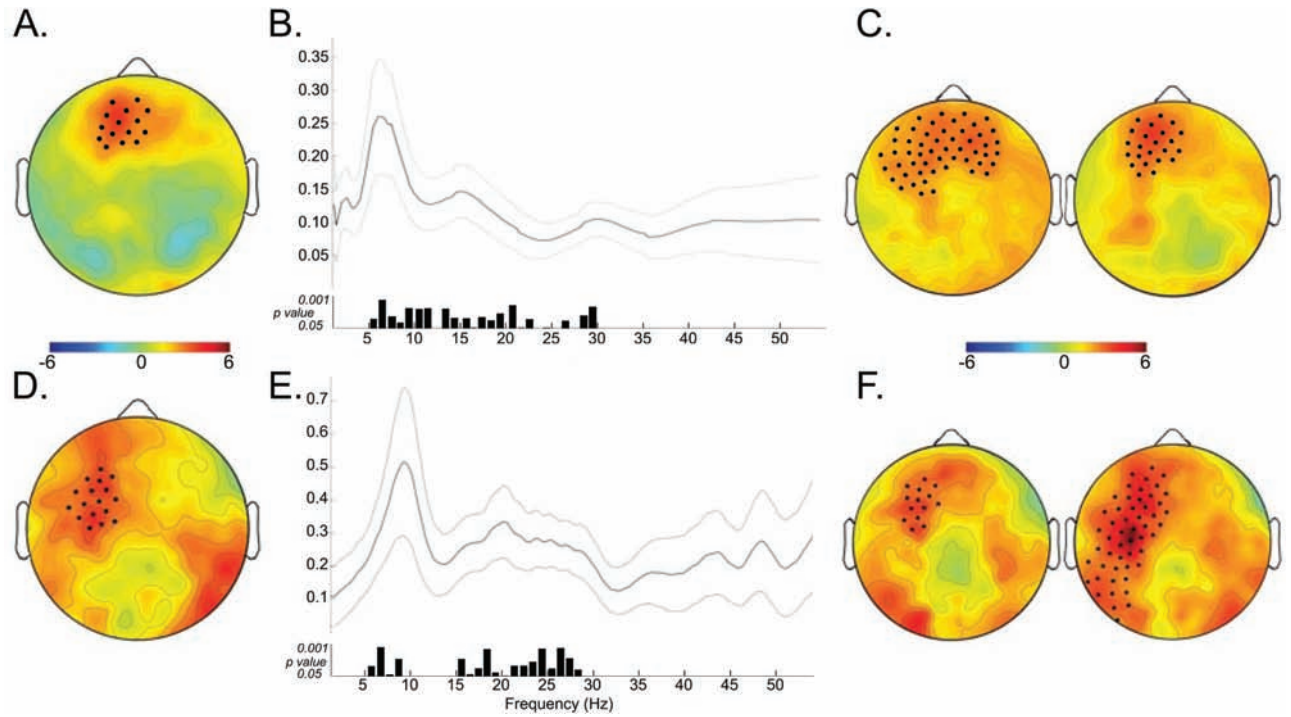

Figure 2. Changes during the ROT task (A–C) and during resting state after three blocks of ROT (sEEG3, D–F). In the T-maps (A, C, D, F), significant clusters of electrodes are highlighted with black dots. (A) T-map of the difference between the average of the sets during *ROT1* adaptation and the initial sets where no rotation was imposed in a broad band from 1 to 55 Hz. (B) Power spectrum (mean, thick solid line, ± standard errors, thin light lines) of the cluster shown in 1 Hz bins. Frequency bins with significant increases between the first and the last set of movements in ROT1 (paired *t*-tests, *p* Bonferroni corrected for multiple comparisons) are shown as black bars. Significant increases were found in the 5–30 Hz range. (C) T-maps for the theta (left) and beta (right) ranges. (D) T-map during sEEG3 compared to the baseline sEEG0 in the 1–55 Hz range. (E) Power spectrum and frequency bins with significant increases compared to the sEEG0 as per (B). Significant increases were found in two bands, 5–8 Hz (theta) and 15–28 Hz (beta). (F) T-maps for the theta (left) and beta (right) ranges.

leaves a persistent trace in the sEEG power over a frontal region involved in rotation learning. Indeed, the local increases in sEEG3 power were positively correlated with the local power increases in both the theta range ($r = 0.61$, $p = 0.001$, 95% confidence interval for r [0.28, 0.81]; Supplementary Figure 1, A) and the beta range ($r = 0.50$, $p = 0.01$, 95% confidence interval for r

[0.13, 0.75]; Supplementary Figure 1, B) during *ROT1*, a session where learning was least contaminated by interference and consolidation, processes that likely were more prominent in later sessions.

The local increase in sEEG3 power in the theta range was positively correlated with the improvement in trajectory accuracy (r = 0.58, p = 0.002, 95% confidence interval for r [0.25, 0.80]; Supplementary Figure 1, C), indicating that it might result from persistent changes associated with plasticity and learning. On the other hand, a persistent increase in sEEG power at low frequencies may also indicate a spontaneous occurrence of neuronal OFF periods, which would lead to signs of fatigue involving the neural circuits involved in *ROT* [1]. If so, we would expect a deterioration of performance after intensive *ROT* training in a test, such as *mov*, that shares similar neural circuits. Indeed, as shown in Figure 3, A, the number of correct movements in *mov* decreased after the last block (*mov3*) compared to baseline (*mov0;* two-tailed paired *t*-test: $t(35) = -5.46$, $p < 0.00001$; 36 subjects; 95% confidence interval [−18.3%, −8.7%]; Cohen's d = 0.95). Similar results were obtained in the subset of 25 subjects in which the EEG was analyzed (mean ± *SE*, *mov0*: 81.8% ± 0.8% vs. *mov3*: 68.2% ± 2.3%; two-tailed paired *t*-test: $t(24) = -5.52$, p = 0.00001, 95% confidence interval [−18.5%, −8.8%]; Cohen's d = 1.16). This deterioration of performance was specific, as it did not occur with *mem*, a test that involves attention/spatial working memory rather than motor performance (*mem3* vs. *mem0*; Mann–Whitney U-test; U = 538.5, z = 1.228, p = 0.22; Figure 3, B). Similar results were obtained in the subset of 25 subjects in which the EEG analysis was performed (*mem0*: 12.28 ± 0.45 vs. *mem3*: 11.56 ± 0.55; U = 250.5, z = 1.15, p = 0.25).

Altogether, these results suggest that intensive training leaves a trace that is confined to areas involved in the learning process, is correlated with the amount of learning, and is associated with performance deterioration in a homologous test but not in a test involving other brain areas. Of note, intensive training also led to an increase in subjective scores of tiredness across blocks, but not of boredom and sleepiness (Table 1).

Intensive motor performance in *MOT* does not affect either performance indices or frontal EEG activity

Next, we determined whether the local EEG changes caused by *ROT* training were specific to learning occurring during visuomotor adaptation or they could also be induced by practicing a simple motor reaching task without the adaptation component [8, 11]. Following the same design of *ROT* (Figure 1, A), we tested 13 subjects with three blocks of *MOT* during the morning. We found no significant changes in handpath area, suggesting that no major learning occurred across blocks (Table 2). Additional analysis of reaction time, movement time, peak and mean velocity, and the subjective scores of tiredness, boredom, sleepiness revealed no practice-related changes (Table 2).

During *MOT1*, we did not find any significant increase in the broad-band EEG power (1–55 Hz; Figure 4, A), even when the analysis was performed with less conservative criteria (i.e. critical alpha of 0.1 and 0.05 at the cluster level instead of 0.025). As a

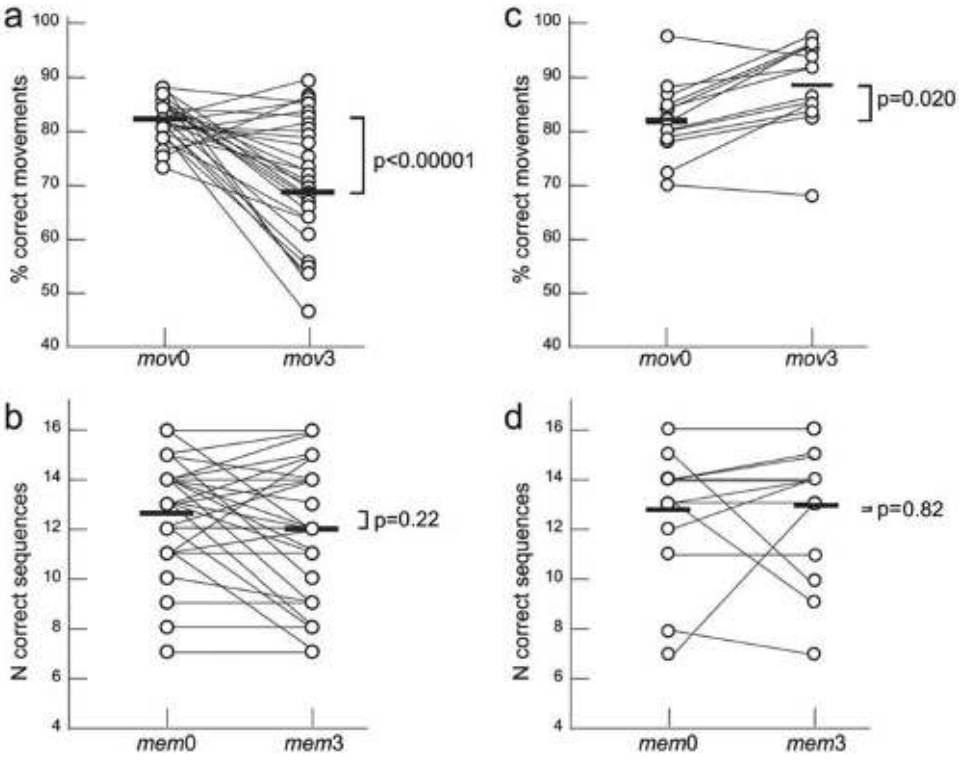

Figure 3. Performance changes in the two tests after *ROT* (left) or *MOT* (right) training. (A) In *mov*, percentages of correct movements decreased during *mov3*, after three *ROT* blocks, compared to baseline in *mov0* (two-tailed paired *t*-test: $t(35) = -5.46$, $p < 0.00001$; 95% confidence interval: [−18.3%, −8.3%]; Cohen's d = 0.95) in 36 subjects. (B) The numbers of correct sequences during *mem* were similar at baseline (*mem0*) and after three *ROT* blocks in 36 subjects (Mann–Whitney U-test, *mem3*; U = 538.5, z = 1.228, p = 0.22). (C) The percentages of correct movements increased during *mov3* after three *MOT* blocks compared to baseline in *mov0* (two-tailed paired *t*-test: $t(12) = 2.65$, p = 0.020; 95% confidence interval: [1.3%, 9.0%]; Cohen's d = 0.82). (D) The numbers of correct sequences during mem were similar at baseline (*mem0*) and after three *MOT* blocks (*mem3*) did not change (N = 13, Mann–Whitney U = 79.5, z = −0.23, p = 0.82).

complement to these results, a direct comparison confirmed that the EEG power increase was higher during *ROT1* than during *MOT1* over the frontal clusters of electrodes previously identified in *ROT1* both in the 1–55 Hz range (13% ± 2% vs. 5% ± 3%; independent samples two-tailed test unequal variance: $t(27.6) = 2.11$, $p = 0.044$; 95% confidence interval: [1%, 15%]; Cohen's $d = 0.77$) as well as in the theta range (24% ± 6% vs. 5% ± 6%; t (29.9) = 2.06, $p = 0.048$; 95% confidence interval: [1%, 38%]; Cohen's $d = 0.73$), but not in the beta range (10% ± 3% vs 16% ± 3%; t (28.3) = 1.27 $p = 0.21$, 95% confidence interval: [–4%, 15%]; Figure 2). These results are further supported by separate cluster t analyses for the theta and beta bands. The results showed a significant cluster of increased power with electrodes overlapping with those of the significant cluster found for *ROT* only for beta (Figure 4, C) but not for theta range (Figure 4, B).

Also, after the three *MOT* blocks, we found no increase in broad-band power (1–55 Hz) when comparing sEEG3 to sEEG0 (Figure 4, D), even using analyses with less conservative criteria (i.e. critical alpha values of 0.1 and 0.05 at the cluster level instead of 0.025). Again, a direct comparison showed that resting state EEG power over the frontal cluster was higher in sEEG3 after *ROT* in the theta range (*ROT*: 42% ± 8% vs. *MOT*: 20% ± 9%, two-tailed unequal variance t-test: $t(31.1) = 2.63$, $p = 0.038$; 95% confidence interval: [16%, 28%]; Cohen's $d = 0.60$) but not in the beta range (*ROT*: 22% ± 7% vs. *MOT*: 15% ± 8%, $t(25.3) = 1.78$, $p = 0.088$; 95% confidence interval: [–4%, 48%]). No significant clusters were found with separate cluster t analyses for both theta and beta bands (Figure 4, E and F).

Finally, after *MOT*, performance in the *mov* test did not deteriorate but improved slightly (Figure 3, C). The performance of *mem* was not affected (Figure 3, D).

Altogether, these findings suggest that the increase in EEG power over a frontal cluster of electrodes, especially in the theta range, both during the task and persisting during the resting state EEG, is due to the intensive visuomotor learning occurring in *ROT* but not in *MOT*. Therefore, it is likely that these EEG traces reflect neural plasticity rather than neural activity without substantial new learning.

An afternoon nap but not an equivalent period of quiet wake renormalizes local EEG changes induced by intensive training

We hypothesized that, if the increase over the frontal cluster in sEEG theta power is due to the cellular consequences of intensive plasticity, only a nap could renormalize sEEG power. If instead the local theta power increase were due to a transient depletion of energy resources, an equivalent period of quiet wake without sleep would be sufficient to restore the sEEG. To distinguish between these two possibilities, after *ROT3* a group of subjects took a nap while another group rested quietly but without sleeping. In the nap group, the mean sleep time was over 60 min with most of sleep spent in NREM stages N2 and N3, indicating that sleep was consolidated and deep. Despite the instructions to stay awake, a minority of subjects of the quiet wake group briefly reached N2 (Tables 3 and 4).

As predicted, in the nap group spontaneous EEG power decreased in the theta range (sEEG4 after the nap compared to sEEG3 before the nap, mean ± SE: –33% ± 8% cluster $t = 58.22$, $p = 0.02$; Figure 5, A) over a left frontal cluster that involved electrodes where theta power increased in sEEG3 (Figure 2). In fact, theta power in sEEG4 returned to baseline levels at sEEG0 (no difference between sEEG4 and sEEG0, two-tailed paired t-test: $t(10) = –0.1$, $p = 0.92$). The theta decrease correlated with slow wave activity (SWA, 0.5–4 Hz) during the nap: the higher the level of SWA during the nap, the greater the theta power decrease in sEEG4 ($N = 12$, $r = –0.80$; $p = 0.002$, 95% confidence interval [–0.42, –0.94]; Supplementary Figure 2, A). In the quiet wake group, theta power did not decrease from sEEG3 to sEEG4 (Figure 5, B) and remained higher than the baseline at sEEG0 ($t(11) = 2.964$, $p = 0.013$; 95% confidence interval: [12%, 55%]; Cohen's $d = 0.74$). A comparison between the nap and quiet wake groups further evidenced a group difference for sEEG4 theta power of the frontal cluster (two-tailed unequal variance t-test: $t(17.5) = 2.46$, $p = 0.025$; 95% confidence interval: [5%, 76%]; Cohen's $d = 0.91$).

Conversely, beta power decreased in a more posterior cluster, over the left centro-parietal area in both the nap group (–30% ± 9%, cluster $t = 67.04$, $p = 0.02$; Figure 5, C) and the quiet wake group (–28% ± 5%, cluster $t = 35.05$, $p = 0.01$; Figure 5, D) in electrodes where beta power increased in sEEG3 (see Figure 2), without group differences ($t(14.2) = 0.154$; $p = 0.88$). Beta power in the sEEG4 cluster reached sEEG0 baseline values in both groups (sEEG4 vs. sEEG0: two-tailed t-tests: nap: $t(10) = 1.8$, $p = 0.11$; quiet wake: $t(11) = –0.195$, $p = 0.85$).

In summary, these results show that the frontal increase in EEG theta power induced by intensive training may be reversed by a nap but not by an equivalent period of quiet wake. Conversely, beta power over the left centro-parietal area decreased both after a nap and after quiet wake.

Table 2. Mean ± SE of performance and subjective indices *of MOT1* and *MOT3*

	MOT1	*MOT3*				
N = 13	Mean ± *SE*	Mean ± *SE*	95% CI	*df*	*t*	*P*
Peak velocity	36.0 ± 1.7	37.3 ± 2.0	–2.12, 4.68	12	0.82	0.429
Mean velocity	21.2 ± 0.9	20.8 ± 0.9	–1.86, 1.18	12	0.49	0.632
Movement time	236.0 ± 7.9	231.8 ± 9.4	–19.30, 10.73	12	0.62	0.546
Reaction time	252.9 ± 6.7	249.5 ± 6.4	–9.35, 2.62	12	1.23	0.244
Handpath area	0.039 ± 0.002	0.038 ± 0.002	–0.0021, 0.0017	12	0.24	0.811
Tiredness	4.61 ± 0.55	4.85 ± 0.54	–1.10, 1.56	12	0.38	0.712
Boredom	5.46 ± 0.62	5.15 ± 0.63	–1.89, 1.28	12	0.42	0.680
Sleepiness	5.84 ± 0.56	6.00 ± 0.59	–0.94, 1.25	12	0.30	0.766

Results of t-tests comparing the two tasks and corresponding 95% confidence intervals for the difference of means. Values of peak and mean velocities are expressed in cm/s; movement and reaction times in ms.

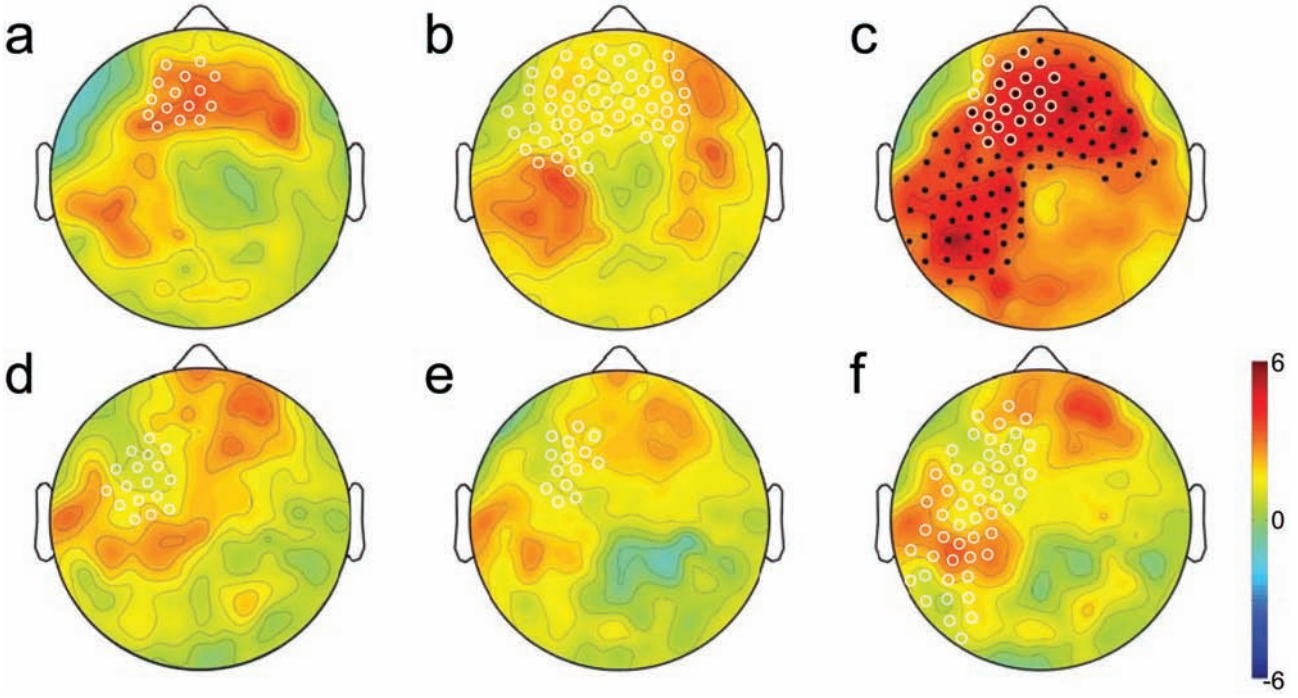

Figure 4. T-maps during the MOT task (A, B, C) and during resting state after three blocks of MOT (sEEG3) (D, E, F). Significant clusters of electrodes are highlighted with black dots. The empty white circles represent the electrodes where significant increases in *ROT* were found (cf. Figure 2). (A–C) T-maps during the performance of *MOT1* in a broad band from 1 to 55 Hz (A), in the theta (B) and beta range (C). A significant cluster was found only for the beta range (cluster $t = 406.32$, $p = 0.0001$) with electrodes overlapping with those of the significant cluster found for *ROT1*. (D–F) T-map during sEEG3 compared to the baseline sEEG0 in the 1–55 Hz band (D), as well as in the theta (E) and beta range (F). No significant clusters were found.

Table 3. Sleep stages duration (percentage of total duration)

Sleep state	Nap (mean ± *SE*)	Quiet wake (mean ± *SE*)
TST	63.46 ± 5.91 (12/12)	18.23 ± 4.00 (13/13)
N1	15.96 ± 4.82 (12/12)	8.62 ± 1.39 (13/13)
N2	27.67 ± 3.98 (12/12)	9.62 ± 3.21 (4/13)
N3	19.83 ± 5.05 (10/12)	0 ± 0.00 (0/13)
SOL	9.29 ± 1.95	–
N2 + N3	47.50 ± 3.25	9.62 ± 3.21

SE, standard error of the mean; TST, total sleep time; N1–N3, NREM stages 1,2,3; SOL, sleep onset latency (first occurrence of N1); N2 + N3 NREM, stages 2 and 3 combined.

Table 4. Sleep characteristics

	Global power (mean ± *SE*)	Frontal ROI (mean ± *SE*)
Normalized N2		
Delta	2.78 ± 0.28	3.03 ± 0.54
Theta	1.76 ± 0.23	1.69 ± 0.17
Alpha	1.59 ± 0.14	1.82 ± 0.23
Spindle (12-16 Hz)	2.02 ± 0.14	2.20 ± 0.20
Beta	1.13 ± 0.06	1.16 ± 0.09
Normalized N3		
Delta	6.90 ± 0.87	8.36 ± 1.86
Theta	2.28 ± 0.23	2.55 ± 0.39
Alpha	1.53 ± 0.17	1.97 ± 0.39
Spindle	1.67 ± 0.18	1.80 ± 0.27
Beta	0.70 ± 0.04	0.70 ± 0.05
Normalized NREM		
Delta	4.37 ± 0.66	5.12 ± 1.18
Theta	1.96 ± 0.23	1.97 ± 0.18
Alpha	1.55 ± 0.14	1.83 ± 0.23
Spindle	1.87 ± 0.15	2.06 ± 0.22
Beta	0.99 ± 0.07	1.03 ± 0.11
Normalized SWE	2.23 ± 0.44	2.66 ± 0.75

Global mean spectra for each frequency band during artifact-free EEG normalized by N1 power for the given band. NREM is the mean power during N2 and N3 sleep. Slow wave energy (SWE) is the mean normalized SWA of N2 and N3 multiplied by their respective durations.

A nap but not quiet wake renormalizes performance and the ability to learn

In the entire nap group ($N = 20$), correct movements increased in *mov4* compared to *mov3* (Figure 6, A), restoring performance to the levels observed in *mov0* ($t(19) = -1.01$, $p = 0.286$). By contrast, in the entire quiet wake group ($N = 16$), there was no restoration of performance ($t(15) = -0.64$, $p = 0.534$; Figure 6, B). Similar results were obtained for the subset of subjects (12 nap and 13 quiet wake) where the EEG was analyzed (mean ± *SE*; nap: 70.9% ± 2.9% vs. 79.1% ± 2.7%; two-tailed paired *t*-test: $t(11) = 2.78$, $p = 0.018$, 95% confidence interval [1.5%, 15.3%]; Cohen's $d = 1.25$; quiet wake: 65.7% ± 3.6% vs. 64.3% ± 4.3%; $t(12) = -0.57$, $p = 0.58$, 95% confidence interval: [–6.6%, 3.8%]). Performance in *mem* did not change in either group (Mann–Whitney U-test, $U = 170.5$, $z = 0.78$, $p = 0.43$; quiet wake: $U = 97$, $z = 1.15$, $p = 0.25$; Figure 6, C and D) and again the results were similar when considering the subgroups for which EEG analysis was performed (nap: 11.9 ± 0.7 vs. 12.9 ± 0.7; $U = 57$, $z = 0.83$, $p = 0.40$; quiet wake: 11.3 ± 0.9 vs. 12.5 ± 0.7; $U = 63$, $z = 1.08$, $p = 0.28$).

The restorative effect of the nap was not confined to test performance but extended to learning ability. In a further learning

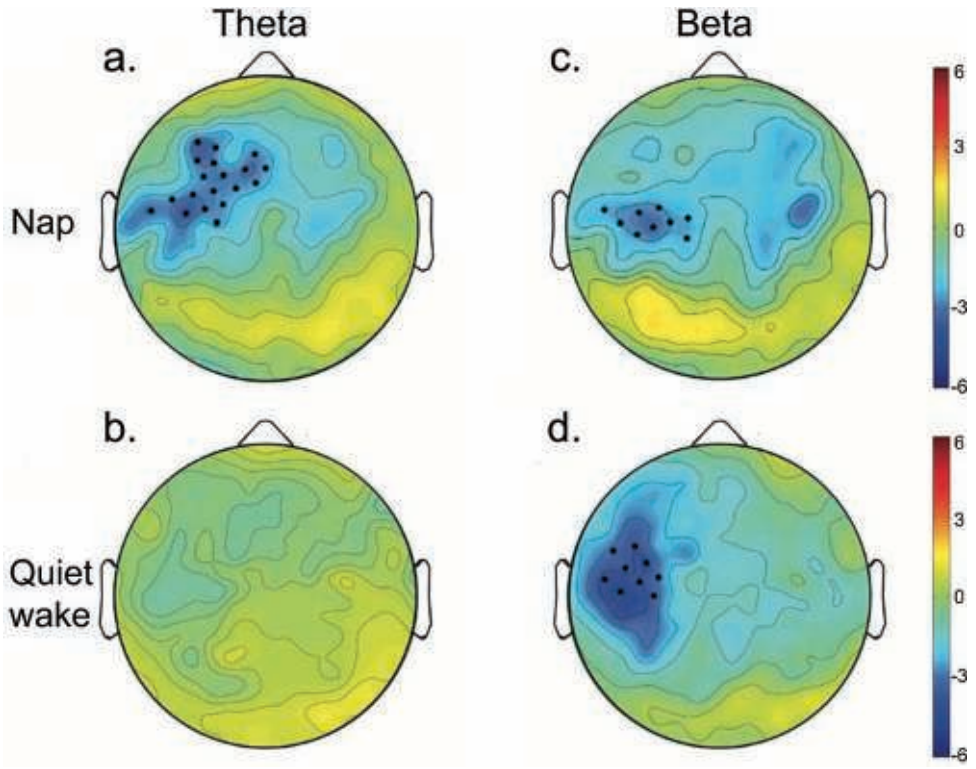

Figure 5. T-maps of the differences between *sEEG4* and *sEEG3* after nap and quiet wake. T-maps are shown for the nap (A, C) and quiet wake groups (B, D) for theta (left column) and beta power ranges (right column).

block performed immediately after the tests (*ROT4*), adaptation rate, trajectory accuracy (i.e. handpath area), and reaction time improved compared to *ROT3* in the nap group, but not in the quiet wake group (Tables 5 and 6).

Finally, we found that SWA during the nap N3 stage was correlated with the improvement in performance: the higher the level of SWA during the nap, the greater the increase of correct movements from *mov3* to *mov4* ($N = 10$, $r = 0.82$, $p = 0.003$, 95% confidence interval: [0.40, 0.96]; Supplementary Figure 2, B). SWA during the nap was also correlated with the improvement in *ROT4* learning compared to *ROT3*: the higher SWA during the nap, the greater the decrease in hand-path area and thus the improvement of trajectory accuracy ($N = 12$, $r = -0.74$, $p = 0.0059$, 95% confidence interval: [−0.30, −0.92]; Supplementary Figure 2, C).

Thus, a nap after intensive learning renormalizes not only the EEG but also performance and the ability to learn, whereas an equivalent period of quiet wake fails to do so. Moreover, the renormalization of the spontaneous EEG, performance, and the ability to learn are correlated with the amount of SWA during the nap.

Discussion

We investigated whether intensive training can induce signs of task-specific neural fatigue in well-rested subjects. Subjects performed three sessions of a visuomotor rotation learning task (*ROT*) during the morning hours, after a full night of sleep. We found that the circuits involved in the learning task displayed changes in the theta and beta ranges that appeared during the task and persisted in the rest EEG at the end of the training. These persistent EEG traces can be characterized as a form of neural fatigue. The results also suggest that the changes in the theta range reflect neural fatigue specifically linked to the occurrence of plastic changes and learning, because they correlated with performance changes and subsided only if subjects were allowed to sleep after training. By contrast, the changes in the beta range may result from an increased neuronal activity even in the absence of synaptic plasticity.

Periods of sustained waking of at least 20 h are associated with a significant increase in theta power that correlates with performance decrements (e.g. [2, 3, 6]). Even within the first 16 h of waking, several studies have found a small increase in theta power that is modulated by the circadian time [6, 24, 25]. In our experimental conditions, however, we have several reasons to think that changes in the theta range reflect neural fatigue due to learning rather than simply time spent awake. First, hd-EEG recorded during task performance revealed an increase in theta power at the end of the third training session, which was localized to frontal areas engaged in learning the task. The location of task-induced theta frequencies was specific to *ROT*, as it did not occur in a group of subjects trained intensively in *MOT*, a simple motor task without rotation learning. This is in agreement with imaging studies showing that, differently from simple motor performance, the state of continuous adaptation in *ROT* is specifically associated with activation of frontal regions, in particular of the pre-supplementary motor area [9, 10]. Second, after the last training session an increase in frontal theta frequencies was observed not just during task performance, but in the spontaneous hd-EEG recorded during periods of inactivity. This finding indicates a persistent change

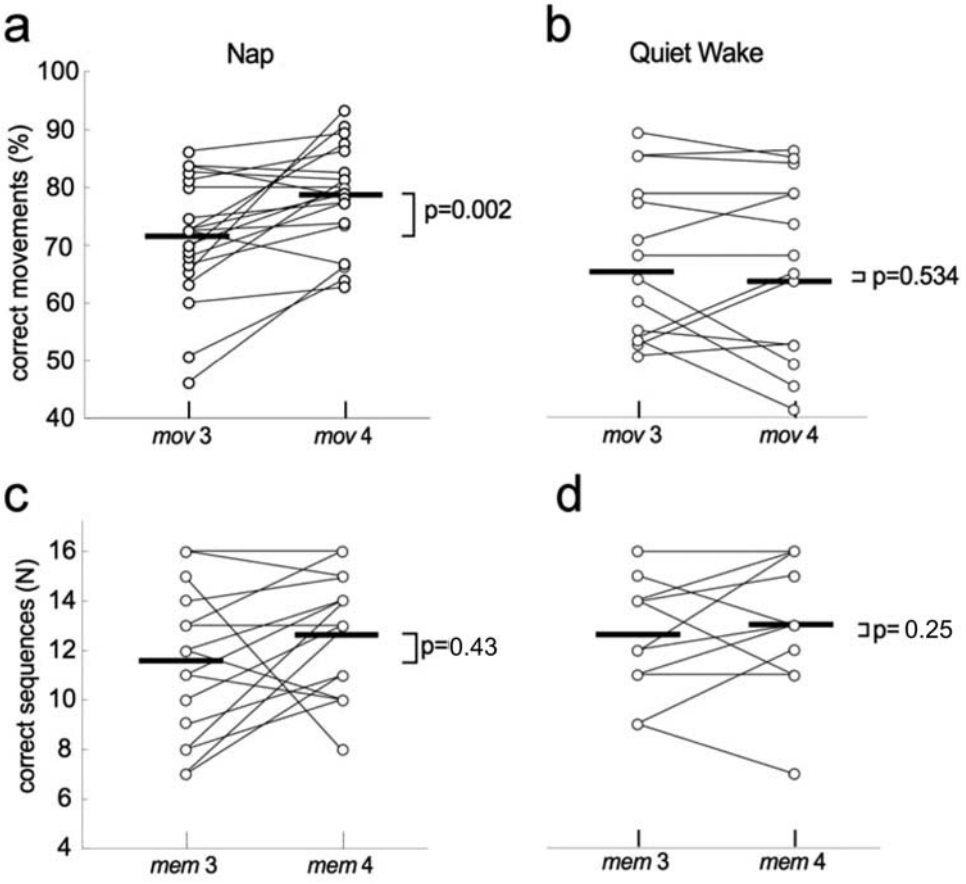

Figure 6. Effect of nap (left) and quiet wake (right) on test performance. (A) and (B) Percentage of correct movements in *mov*3 and *mov*4. (C) and (D) Number of correct sequences in *mem*3 and *mem*4. Comparisons were performed with paired *t*-tests. Only after the nap, correct movements in mov increased significantly (two-tailed paired *t*-test: nap: $t(19) = -3.57$, $p = 0.002$; 95% confidence interval: [3.3%, 11.8%]; Cohen's $d = 0.76$; quiet wake: $t(15) = -0.64$; $p = 0.534$; 95% confidence interval: [−5.5%, 2.8%]). In *mem*, no changes were found in either group (Mann–Whitney U-test, nap: $N = 20$; $U = 170.5$, $z = 0.78$, $p = 0.43$; quiet wake: $N = 16$; $U = 97$, $z = 1.15$, $p = 0.25$).

Table 5. Comparison of *ROT3* and *ROT4* performance in the nap group

	ROT3	*ROT4*					
	Mean ± *SE*	Mean ± *SE*	95% CI	*df*	*t*	*P*	Cohen's *d*
N = 20							
Adaptation rate	71.2 ± 0.8	72.7 ± 0.8	0.24, 3.09	19	2.46	**0.024**	**0.55**
Reaction time	248.0 ± 2.9	242.6 ± 3.0	−9.85, −1.21	19	2.97	**0.008**	**0.41**
Handpath area	0.049 ± 0.002	0.046 ± 0.002	−0.005, −0.001	19	2.21	**0.040**	**0.34**
N = 12							
Adaptation rate	71.5 ± 1.2	73.7 ± 0.9	0.28, 4.08	11	2.47	**0.028**	**0.73**
Reaction time	248.6 ± 5.2	242.3 ± 5.3	−10.27, −2.33	11	3.49	**0.005**	**0.35**
Handpath area	0.0526 ± 0.002	0.0494 ± 0.002	−0.004, −0.002	11	2.32	**0.041**	**0.47**

Mean ± *SE* and results of two-tailed paired *t*-tests with corresponding 95% confidence intervals for the difference in means. Effect sizes (Cohen's *d*) were computed for $p < 0.05$. The first part of the table concerns the entire nap group tested ($N = 20$); the second part is about the subgroup with EEG recordings ($N = 12$). Adaptation rate values are expressed in percentage, values of reaction times in ms.

in neural activity that outlasted the task for as long as the EEG was recorded. Third, the persistent increase in theta frequencies in the EEG over learning-related areas was associated with deteriorated performance specific to the task. Thus, subjects were impaired in a visuomotor test (*mov*) that engages similar circuits as *ROT*. On the other hand, they were not impaired in a test that probed memory for visual sequences. Fourth, subjects reported a feeling of task-specific fatigue but no increase in sleepiness. Fifth, as we will discuss below, EEG slow frequencies and performance impairments could only be reversed by a period of sleep and not by the mere passage of time awake. Together, these observations suggest that the increase in theta power reflects the fact that the neural circuits involved in intensive task learning became functionally fatigued because of increased plasticity. In other words, the theta trace reflects "the cost of plasticity".

Usually, although not always [26], plastic changes are coupled with increased neural activity. In rodents, for instance, motor skill learning leads to increased firing of task-related neurons in the primary motor cortex, long-term potentiation-like

Table 6. Comparison of *ROT3* and *ROT4* performance in the quiet wake group

	ROT3	*ROT4*				
	Mean	Mean	95% CI	*df*	*t*	*P*
N = 16						
Adaptation rate	71.4 ± 1.2	71.3 ± 1.1	−0.95, 0.82	15	0.02	0.983
Reaction time	246.8 ± 3.9	245.3 ± 3.8	−5.72, 2.46	15	0.85	0.409
Handpath area	0.051 ± 0.003	0.053 ± 0.002	−0.002, 0.006	15	0.8	0.439
N = 13						
Adaptation rate	72.1 ± 1.1	71.6 ± 1.3	−1.94, 0.97	12	0.72	0.483
Reaction time	247.7 ± 4.1	244.1 ± 3.0	−7.40, 0.15	12	1.65	0.125
Handpath area	0.0523 ± 0.004	0.0503 ± 0.003	−0.008, 0.003	12	0.84	0.419

Mean ± *SE*, and results of two-tailed paired *t*-tests with 95% confidence intervals for the difference in means. The first part of the table concerns the entire quiet wake group tested (*N* = 16); the second part is about the subgroup with EEG recordings (*N* = 13). Adaptation rate values are expressed in percentage, values of reaction times in ms.

strengthening of cortical connections, and formation and enlargement of spines [27–32]. In our subjects, we assume that intensive training also resulted in both increased neural activity and plasticity. Conceivably, at the cellular level, fatigue might be triggered by increased neural activity even without the occurrence of plastic changes, due to depletion of metabolic resources or persistent changes in ionic concentrations. If so, why do we suggest that the theta trace reflects plasticity rather than simply activity? It is because subjects trained intensively on a visuomotor task that had the same kinematic requirements as *ROT*, but did not require rotation learning (*MOT*), did not show signs of fatigue as indexed by an increase in theta power. Specifically, they did not show an increase in theta frequencies either during task performance or in the spontaneous EEG during inactivity after the third session; they were unimpaired when performing the motor test (*mov*); and they did not report task-specific fatigue. Thus, the theta trace seems to reflect neural fatigue mostly triggered by the requirements of intensive learning and associated neural plasticity, rather than by the requirements of task execution and associated neural activity.

This conclusion is strengthened by the finding that the signs of fatigue as indexed by an increase in theta power could only be reduced by a nap, but not by an equivalent period of quiet wake, consistent with previous evidence showing that naps can provide at least some of the benefits of sleep in terms of memory consolidation and restoration of the ability to learn [21, 33–38]. Specifically, we found that a nap after intensive learning significantly reduced the EEG theta frequencies and restored test performance. The restoration of spontaneous EEG and performance were correlated with the amount of slow-wave activity (SWA, 0.5–4 Hz) during the nap. If fatigue had been caused by excessive neural activity, an hour of inactive wake should have sufficed to restore the task-related neurons, because the firing of most of these cells increases during motor skill learning and returns to low levels as soon as the task is completed [28, 39]. Thus, recovery from the fatigue caused by task-related high firing should be afforded by any condition with low firing (no task), including quiet waking. By contrast, if learning-induced fatigue had been caused by the growth of stronger connections among task-related neurons, those connections would still be strong after the task, requiring more energy, synaptic receptors, membrane lipids, and other supplies, to be maintained. In this case, there is substantial evidence—at the molecular, electrophysiological, and ultrastructural level—that sleep is required to renormalize increases in synaptic strength observed during wake as a consequence of learning [40, 41]. Work in animal models has also shown that sleep SWA is enhanced locally by learning and the induction of synaptic plasticity, rather than by neuronal firing per se [42–45].

In addition to restoring EEG and performance, the nap after intensive learning enhanced the subjects' ability to further improve in *ROT* during a subsequent training session. Again, the enhancement of further learning was correlated with the amount of SWA during the nap. This result is in line with previous work showing that sleep can lead to improved performance and ability to learn, phenomena that are mediated, at least in part, through SWA, synaptic renormalization, and increased signal-to-noise ratio [39, 46, 47].

Rotation learning also resulted in a local increase in beta power during the task, as well as in the spontaneous EEG after training. Contrary to the increase in theta power, however, the increase in beta power was not correlated with changes in performance and, following training, it subsided to the same extent after both a nap and a period of quiet waking. For these two reasons, we suggest that the beta trace may more closely reflect activity-dependent fatigue than plasticity-related fatigue. In line with this hypothesis, in a previous study, a local, frontal increase in beta power was also observed at rest after motor training without rotation learning (*MOT*) [48]. In the current study, we found no changes in beta power after MOT training (sEEG3 vs. sEEG0). However, when EEG changes at rest were compared directly after ROT and after MOT, ROT changes were significantly higher than MOT changes in the theta range but not in the beta range, implying a small (not significant) increase in beta power at rest also after MOT practice. In another study [49], an increase in beta power at rest was found after transcranial magnetic stimulation only in the subjects that showed the expected decrease in cortical excitability after the stimulation, again consistent with the idea that beta power at rest may reflect fatigue due to intense neural activity.

A limitation of the study is that the order in which the tests were presented was fixed, with *mov* always preceding *mem*. We cannot rule out that this design may have contributed to the lack of effect on *mem* performance, and further studies are warranted to confirm this finding. However, we think this is unlikely because the performance in *mov* shared many aspects with the performance in the last sets of *ROT* blocks. Hence this specific design, if anything, should have increased the probability of finding a worsening in *mem* performance, which we did not find. Another limitation is the lack of nap and quiet wake conditions

after *MOT*. However, the *MOT* experiment was mainly used to determine whether the theta changes observed after *ROT* were related to plasticity-linked fatigue and as expected, *MOT* did not result in any visuomotor learning, nor did it cause broad-band changes in the EEG.

In conclusion, while practice may "make perfect," intensive learning may also progressively fatigue local neuronal circuits through increased activity and the accumulation of plastic changes. Even in well-rested subjects, trained in the morning hours and not exposed to sleep deprivation, restoring neural fatigue requires sleep and not just rest. While the mechanisms underlying learning-induced fatigue are currently unknown, animal studies may clarify whether they involve primarily the synaptic compartment, the surrounding glial cells, or the neuropil as a whole.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Data Availability

None.

References

1. Vyazovskiy VV, *et al*. Local sleep in awake rats. *Nature*. 2011;**472**(7344):443–447.
2. Hung CS, *et al*. Local experience-dependent changes in the wake EEG after prolonged wakefulness. *Sleep*. 2013;**36**(1):59–72.
3. Bernardi G, *et al*. Neural and behavioral correlates of extended training during sleep deprivation in humans: evidence for local, task-specific effects. *J Neurosci*. 2015;**35**(11):4487–4500.
4. Nir Y, *et al*. Selective neuronal lapses precede human cognitive lapses following sleep deprivation. *Nat Med*. 2017;**23**(12):1474–1480.
5. Aeschbach D, *et al*. Dynamics of the human EEG during prolonged wakefulness: evidence for frequency-specific circadian and homeostatic influences. *Neurosci Lett*. 1997;**239**(2–3):121–124.
6. Finelli LA, *et al*. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience*. 2000;**101**(3):523–529.
7. De Gennaro L, *et al*. Neurophysiological correlates of sleepiness: a combined TMS and EEG study. *Neuroimage*. 2007;**36**(4):1277–1287.
8. Ghilardi M, *et al*. Patterns of regional brain activation associated with different forms of motor learning. *Brain Res*. 2000;**871**(1):127–145.
9. Krakauer JW, *et al*. Differential cortical and subcortical activations in learning rotations and gains for reaching: a PET study. *J Neurophysiol*. 2004;**91**(2):924–933.
10. Perfetti B, *et al*. Modulation of gamma and theta spectral amplitude and phase synchronization is associated with the development of visuo-motor learning. *J Neurosci*. 2011;**31**(41):14810–14819.
11. Perfetti B, *et al*. Temporal evolution of oscillatory activity predicts performance in a choice-reaction time reaching task. *J Neurophysiol*. 2011;**105**(1):18–27.
12. Moisello C, *et al*. TMS enhances retention of a motor skill in Parkinson's disease. *Brain Stimul*. 2015;**8**(2):224–230.
13. Nelson AB, *et al*. Beta oscillatory changes and retention of motor skills during practice in healthy subjects and in patients with Parkinson's disease. *Front Hum Neurosci*. 2017;**11**:104.
14. Tatti E, *et al*. Beta modulation depth is not linked to movement features. *Front Behav Neurosci*. 2019;**13**:49.
15. Delorme A, *et al*. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods*. 2004;**134**(1):9–21.
16. D'Atri A, *et al*. Electrical stimulation of the frontal cortex enhances slow-frequency EEG activity and sleepiness. *Neuroscience*. 2016;**324**:119–130.
17. Oostenveld R, *et al*. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*. 2011;**2011**:156869.
18. Maris E, *et al*. Nonparametric statistical testing of EEG- and MEG-data. *J Neurosci Methods*. 2007;**164**(1):177–190.
19. Mensen A, *et al*. Optimizing detection and analysis of slow waves in sleep EEG. *J Neurosci Methods*. 2016;**274**:1–12.
20. Silber MH, *et al*. The visual scoring of sleep in adults. *J Clin Sleep Med*. 2007;**3**(2):121–131.
21. Huber R, *et al*. Local sleep and learning. *Nature*. 2004;**430**(6995):78–81.
22. Huber R, *et al*. Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat Neurosci*. 2006;**9**(9):1169–1176.
23. Moisello C, *et al*. Short-term limb immobilization affects motor performance. *J Mot Behav*. 2008;**40**(2):165–176.
24. Cajochen C, *et al*. EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. *Am J Physiol*. 1999;**277**(3 Pt 2):R640–R649.
25. Cajochen C, *et al*. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol*. 2003;**15**(4):432–437.
26. Rossato JI, *et al*. Silent Learning. *Curr Biol*. 2018;**28**(21):3508–3515.e5.
27. Rioult-Pedotti MS, *et al*. Learning-induced LTP in neocortex. *Science*. 2000;**290**(5491):533–536.
28. Costa RM, *et al*. Differential corticostriatal plasticity during fast and slow motor skill learning in mice. *Curr Biol*. 2004;**14**(13):1124–1134.
29. Monfils MH, *et al*. Skilled-learning-induced potentiation in rat sensorimotor cortex: a transient form of behavioural long-term potentiation. *Neuroscience*. 2004;**125**(2):329–336.
30. Xu T, *et al*. Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature*. 2009;**462**(7275):915–919.

31. Hayashi-Takagi A, *et al*. Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature*. 2015;**525**(7569):333–338.
32. Roth RH, *et al*. Cortical synaptic AMPA receptor plasticity during motor learning. *Neuron*. 2020;**105**(5):895–908.e5.
33. Mednick S, *et al*. Sleep-dependent learning: a nap is as good as a night. *Nat Neurosci*. 2003;**6**(7):697–698.
34. Ficca G, *et al*. Naps, cognition and performance. *Sleep Med Rev*. 2010;**14**(4):249–258.
35. Kvint S, *et al*. Acquisition and retention of motor sequences: the effects of time of the day and sleep. *Arch Ital Biol*. 2011;**149**(3):303–312.
36. Lo JC, *et al*. Comparing the effects of nocturnal sleep and daytime napping on declarative memory consolidation. *PLoS One*. 2014;**9**(9):e108100.
37. van Schalkwijk FJ, *et al*. The effect of daytime napping and full-night sleep on the consolidation of declarative and procedural information. *J Sleep Res*. 2019;**28**(1):e12649.
38. Zhang Y, *et al*. Can slow-wave sleep enhancement improve memory? a review of current approaches and cognitive outcomes. *Yale J Biol Med*. 2019;**92**(1):63–80.
39. Gulati T, *et al*. Neural reactivations during sleep determine network credit assignment. *Nat Neurosci*. 2017;**20**(9):1277–1284.
40. Tononi G, *et al*. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*. 2014;**81**(1):12–34.
41. Tononi G, *et al*. Sleep and synaptic down-selection. *Eur J Neurosci*. 2020;**51**(1):413–421.
42. Cirelli C, *et al*. Locus ceruleus control of slow-wave homeostasis. *J Neurosci*. 2005;**25**(18):4503 LP–4511. http://www.jneurosci.org/content/25/18/4503.abstract.
43. Huber R, *et al*. Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*. 2007;**30**(2):129–139.
44. Hanlon EC, *et al*. Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. *Sleep*. 2009;**32**(6):719–729.
45. Rodriguez AV, *et al*. Why does sleep slow-wave activity increase after extended wake? assessing the effects of increased cortical firing during wake and sleep. *J Neurosci*. 2016;**36**(49):12436–12447.
46. Fattinger S, *et al*. Deep sleep maintains learning efficiency of the human brain. *Nat Commun*. 2017;**8**:15405.
47. González-Rueda A, *et al*. Activity-dependent downscaling of subthreshold synaptic inputs during slow-wave-sleep-like activity in vivo. *Neuron*. 2018;**97**(6):1244–1252.e5.
48. Moisello C, *et al*. Practice changes beta power at rest and its modulation during movement in healthy subjects but not in patients with Parkinson's disease. *Brain Behav*. 2015;**5**(10):e00374.
49. McAllister CJ, *et al*. Oscillatory beta activity mediates neuroplastic effects of motor cortex stimulation in humans. *J Neurosci*. 2013;**33**(18):7919–7927.