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ABSTRACT

During non-rapid eye movement (NREM) sleep, a global decrease in synaptic strength associated with slow waves would enhance signal-to-noise ratio (SNR) of neural responses during subsequent wakefulness. To test this prediction, thirty-two human volunteers were trained to a coarse orientation discrimination (COD) task, either in the morning or the evening. They were retested after 8 hours of wakefulness or sleep, respectively. Performance was enhanced only after a night of sleep, in the absence of any change in the abundance of NREM slow waves but in proportion to the number of slow waves 'initiated' in lateral occipital areas during post-training NREM sleep. The sources of these waves overlapped with the lateral occipital complex (LOC), in which responses to the learned stimulus, as assessed by functional magnetic resonance imaging (fMRI), were selectively increased the next morning. This response enhancement was proportional to REM sleep duration. These results provide an example of local sleep in which local initiation of slow waves during NREM sleep predicts later skill improvement and foreshadows locally enhanced neural signals the next day. In addition, REM sleep also promotes local learning-dependent activity, possibly by promoting synaptic plasticity.

INTRODUCTION

Slow waves (SWs) represent a paramount feature of NREM sleep. They occur in close temporal relationship with fluctuations in neuronal membrane potential, which alternates at low frequency between depolarized levels during which neurons fire intensely ('UP' state), and a hyperpolarized state during which firing rate is reduced ('DOWN') (Steriade and McCarley, 2005). Transitions between UP and DOWN states occur synchronously in large neuronal populations and result in the alternation of ON and OFF states (Vyazovskiy et al., 2009), or activated and silent states (Volgushev et al., 2006).

Slow wave activity (SWA, the electroencephalogram (EEG) power between 0.75 and 4Hz), as well as the slope of SWs have been proposed as markers of neuronal synchronization which reveal changes in synaptic efficacy across the sleep/wake cycle (Esser et al., 2007). They increase with the duration of the preceding wake and decrease during NREM sleep (Riedner et al., 2007; Vyazovskiy et al., 2009). Likewise, in rodents, synaptic efficacy assessed by evoked responses *in vivo* (Vyazovskiy et al., 2008) or miniature excitatory post-synaptic currents (mEPSCs) from cortex slices (Liu et al., 2010) increases after waking and decreases after sleep. Also, molecular markers are consistent with synaptic potentiation of glutamatergic neurotransmission during wakefulness and its depression during sleep (Vyazovskiy et al., 2008). These results are currently framed in a hypothesis assuming a net synaptic potentiation during wakefulness and a downscaling of synaptic strength during NREM sleep (Tononi and Cirelli, 2006).

This hypothesis makes three predictions about the influence of NREM sleep on memory. First, NREM sleep is locally modulated by the experience acquired during preceding wakefulness. This prediction is supported by the effects of local interventions (Huber et al., 2007; Huber et al., 2008). However, its influence on learning has been so far tested using a visuo-motor task (Huber et al., 2004) that involves large-scale brain circuits (Krakauer et al., 2004), making it difficult to assess local learning. By contrast, visual perceptual learning results from local plasticity in relevant neural populations of the visual system (Schwartz et al., 2002; Zhang et al., 2010) and allows to test the influence of local learning on subsequent sleep. We used a COD task to assess interactions between local learning and NREM sleep changes. Second, the hypothesis assumes that synaptic downscaling during NREM sleep would increase SNR in the relevant brain circuits the following day. Although, at the behavioral level, NREM sleep was associated with enhanced (Huber et al., 2004) and less variable performance (Hill et al., 2008), this prediction has never been directly tested in terms of local brain activity. Using fMRI, we assessed whether neural responses to the

learned task increased above noise level after a night of sleep. Finally, the hypothesis states that "the homeostatic regulation of SWA is tied to the amount of synaptic potentiation that has occurred during previous wakefulness" (Tononi and Cirelli, 2006). We show that the site of initiation of SWs during post-training NREM sleep is another parameter that relates to local learning.

MATERIAL AND METHODS

Ethics Statement

The study was approved by the Ethics Committee of the Academic Hospital and Faculty of Medicine of the University of Liège.

Participants

Thirty-two young healthy volunteers participated to this study (age: 18-30, 16 males). They were recruited through advertisement on the University intranet. The absence of medical, traumatic, psychiatric or sleep disorders was established by a semi-structured interview. None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (Johns, 1991), nor of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse et al., 1989). All participants had normal scores on the 21-item Beck Anxiety Inventory and the 21-item Beck Depression Inventory II (Beck et al., 1988). They were right-handed as indicated by the Edinburgh Inventory (Oldfield, 1971). Extreme chronotypes according to the Horne and Östberg morningness-eveningness questionnaire (Horne and Ostberg, 1976) and Munich Chronotype Questionnaire (Roenneberg et al., 2003) were excluded. They were non-smokers, moderate caffeine (≤ 2 U caffeine /d), and alcohol (≤ 2 U alcohol /wk) consumers. None were on medication. No caffeine was allowed during the experiment.

Design overview

Volunteers were trained to perform a COD task in which they had to discriminate the orientation of orthogonal gratings displayed peripherally and occluded by increasing levels of noise (decreasing SNR, Figure 1A-B). Previous experiments have shown that nocturnal sleep enhances this perceptual skill (Matarazzo et al., 2008). Volunteers were divided into two groups, depending on whether initial training took place in the morning or in the evening. Volunteers were retested for behavioral performance after a period of wakefulness or sleep, respectively (Figure 1C). Functional MRI measurements assessed the responses to learned and control stimuli during 3 testing sessions: at baseline (before training), during immediate testing (right after training) and during a delayed testing session (Figure 1C).

Task Description

The task was coded using Matlab 7.1 (The MathWorks Inc., Natick, USA), with Cogent 2000 (version 1.25; http://www.vislab.ucl.ac.uk/cogent.php). The stimuli were generated by a DELL workstation (Dell Inc., USA) and were projected on a MRI compatible screen via a Sanyo projector (Sanyo Corporation, Japan), with a display area covering 10 degrees of the volunteers' visual field. Stimuli were presented with maximum contrast, mean luminance of 45 cd/m^2 and a maximum illuminance of 50 lux at 90 cm, at a 1024x768 pixels resolution and 50 Hz refresh rate.

Volunteers were instructed to discriminate the orientation of a sinusoidal grating (2 cycles/° grating, 2° in diameter) placed at 5° eccentricity in the lower visual quadrant (Figure 1A). The phases of the gratings were randomized across trials to make sure that participants discriminated orientation instead of local luminance cues. The stimuli were embedded in variable background sinusoidal noise covering the entire screen. A central cross was always presented in order to ensure visual fixation, as assessed by the infrared eye-tracking system. Each trial consisted of a

150 ms presentation of a grating embedded in background noise, followed by an answer prompt embedded in different background noise. Volunteers had 1250 ms to give their answer via a MRI-compatible keypad held in their right hand (Figure 1B).

Two versions of the COD task were used for this study. One version aimed at inducing perceptual learning and was used in the TRAINING session. It was also briefly used during the FAMILIARIZATION session. During these sessions, only two orthogonal orientations were presented (22.5° and 112.5° from the horizon, which were referred to as 'right' and 'left' orientations for the volunteers) in the lower right quadrant (Figure 1A, upper panel). Noise level was adapted to individual performance following a 4/1 transformed Up–Down staircase procedure (Wetherill and Levitt, 1965), and matching the luminance distribution of the sinusoidal grating (sinusoidal noise). The initial noise level was set at 10%, corresponding to a SNR of 0.9. For a given SNR X, (1 - X) * 100% grating pixels were replaced by the noise pattern (e.g., for the 0.9 SNR, 10% of the grating pixels were occluded by the noise pattern). After 4 consecutive correct answers, the current SNR was reduced by 6%, whereas it was increased by 6% in case of a single wrong answer. No feedback was given. The SNR at the beginning of a given block was equal to the SNR achieved at the end of the preceding block.

Another version of the task was used during TESTING sessions, to assess learning-related changes in regional brain responses. In this version, 3 trial types were presented. In the first trial type, stimuli were identical to those described in the training session and are referred to as 'trained orientation and quadrant', and thus named QTOT (Quadrant Trained, Orientation Trained). The second trial type consisted of stimuli displayed in the lower right quadrant but at a different orientation: a 45° shift was applied to learning stimuli resulting in 67.5° or 157.5° orientations (Quadrant Trained, Orientation Untrained: QTOU; Figure 1A, lower right panel). The third trial type consisted of stimuli displayed at the learned orientation (22.5° and 112.5°) but in the lower *left*

quadrant (Quadrant Untrained, Orientation Trained: QUOT; Figure 1A, lower left panel). Stimulus orientation and position was randomly varied across blocks. SNR were adapted to the individual SNR achieved at the end of the familiarization session.

In addition, for testing sessions, two categories of stimuli were used, characterized by their low or high SNR. These SNRs were equal to the individual SNR achieved at the end of the familiarization session, decreased or augmented by 10%. For instance, a final SNR of 0.35 in the familiarization session would result in testing SNR values of 0.25 and 0.45 in the test sessions. This method allowed us to present the volunteers with two sets of SNR values; low SNR values (HIGH noise, HN) are consistently harder to discern than higher SNR values (LOW noise, LN). Testing sessions lasted for about 20 minutes and comprised 90 blocks of 8 trials, equally divided in 30 blocks per type of stimulus.

Volunteers were always informed of which type of block they were about to begin, as well as whether SNR would be modulated (staircase during training or 2 fixed levels during testing). In both cases, pauses between blocks randomly varied from 15 to 35 seconds.

Experimental design

Experimental design is illustrated on Figure 1C.

For 7 days before the beginning of the first scanning session, volunteers were instructed to follow strict sleeping schedules with no daytime naps. Their sleep/wake cycle was assessed by sleep diaries and actigraphic recordings (Actiwatch, Cambridge Technology, UK). Participants who showed deviations for more than 15 minutes from their habitual sleep-wake schedules were excluded from the study.

During the first habituation night in the laboratory, a polygraphic sleep recording was obtained to rule out sleep disorders and familiarize participants to experimental settings. During the second

night, sleep polygraphy was recorded using 64-channel EEG, 2-channels electrooculograms and chin electromyogram. These data served as baseline to assess the influence of training on the post-training sleep activity.

Volunteers were assigned to two experimental groups for this study. Both groups underwent the same experimental sessions although at different times of day in the following order (Figure 1C):

- The FAMILIARIZATION session lasted about 4 minutes and consisted of one block of 100 trials. SNR was adjusted using the staircase procedure. The performance level achieved at the end of this session was used to generate the HN and LN trials of the TESTING sessions.
- A BASELINE TESTING (TEST SESSION 1) session took place before training. No difference was expected between brain responses to QTOT, QTOU and QUOT stimuli, since volunteers had not yet been consistently trained to any stimulus. This session, as all TESTING sessions, lasted for about 20 minutes and consisted of 90 blocks of 8 trials.
- A FIRST TRAINING session was conducted to induce perceptual learning and compute behavioral performance. This session lasted for about 25 minutes and consisted of 7 blocks of 70 trials.
- An IMMEDIATE TESTING session (TEST SESSION 2) took place after training. This session was aimed at assessing the early modifications of brain responses induced by perceptual learning, if any.
- A DELAYED TESTING session (TEST SESSION 3) took place 8 (WAKE) or 12 hours (SLEEP) after training. This session allowed us to assess the changes in brain responses to the learned stimulus that would occur after several hours of wakefulness (group WAKE) or sleep (group SLEEP).

- A SECOND TRAINING session was conducted right after the DELAYED TESTING session, in order to estimate the change in perceptual performance (SNR) achieved by volunteers after a period of wakefulness or sleep.

In the SLEEP group, participants came to the lab at 7:00 PM, were instructed about the task, then underwent the first scanning session (starting around 8:00 PM). Afterwards, they were placed in a dimly lit room (< 5 lux), where they were fitted with a 64-channel electrode net. They were subsequently put to bed in accordance with their usual sleep time, typically around 11:30 PM. After 8 hours of sleep, participants were woken up (typically around 7:30 AM); they could then take a shower and eat a light breakfast. After performing a Psychomotor Vigilance Task (PVT; (Dinges and Powell, 1985)), they were placed in the scanner around 8:30 AM for the last sessions. Participants left the lab around 10:30 AM.

In the WAKE group, participants came to the lab at around 8:00 or 10:00 AM (according to their individual sleep/wake schedule), were instructed about the task and underwent the first scanning session. They were then immediately placed in a dimly lit room (< 5 lux) for 8 hours. They were instructed to stay in a semi-supine position, performed hourly PVTs and were not allowed to read, watch movies or TV. A closed circuit camera system allowed the experimenter to verify that participants were awake and were not stimulated in any way. Light snacks were administered at noon and right before the end of the 8 h rest period. Between 6:30 and 8:30 PM, participants performed the last scanning sessions. They left the laboratory between 8:00 and 10:00 PM.

Behavioral Analysis

We computed the geometric mean of the SNR value of the reversal points for each block of 70 trials in the training sessions. Statistical analyses consisted of a repeated-measures ANOVA with

individual blocks and sessions as within-subject factors and groups (SLEEP vs WAKE) as between-subjects factor, followed by planned comparisons testing the main effect of sessions and the group by session interaction across blocks. Only the last six blocks of each session were included in the analysis to exclude the warm-up effect observed during the first block.

fMRI Data Acquisition and Analysis

Functional MRI time series were acquired during all training and testing sessions with a Siemens Allegra 3T MRI Scanner (Siemens, Erlanger, Germany). Blood Oxygen Level Dependent (BOLD) signal was recorded using multislice T2* weighted fMRI images which were obtained with a gradient echo-planar imaging sequence using axial slice orientation (32 slices; voxel size: 3.4 x 3.4 x 3 mm^3 with 30% of gap; matrix size 64 x 64 x 32; repetition time = 2130 ms; echo time = 40 ms; flip angle = 90°). Structural brain images consisted of a T1-weighted 3D MDEFT image (repetition time = 7.92 ms, echo time = 2.4 ms, time of inversion = 910 ms, flip angle = 15°, field of view 230 x 176 cm^2, matrix size = 256 x 224 x 176, voxel size = 1 x 1 x 1 mm^3;(Deichmann, 2006). An eye-tracking system (LRO5000, ASL, Oxford, UK) with a sampling rate of 60 Hz was used to monitor eye gaze during all sessions.

Functional volumes were analyzed with Statistical Parametric Mapping 5 (SPM5; www.fil.ion.ucl.ac.uk/spm/software/spm5/). The three initial scans were discarded to account for magnetic saturation effects. Remaining scans were corrected for head motion, co-registered, spatially normalized to a canonical echo-planar imaging template conforming to the Montreal Neurological Institute (MNI), and spatially smoothed (Gaussian kernel, 8 mm full-width at half-maximum). A first level analysis accounting for fixed effects within subjects and a second level analysis accounting for random effects (RFX) were conducted. A general linear model was used to estimate brain responses to the different types of stimuli that were presented during the testing

sessions. Visualization of the correctly identified stimuli were modeled as stick functions and convolved with the canonical hemodynamic response function. More specifically, we modeled 6 types of responses, two for each of the three stimuli types presented during testing sessions (QTOT, QTOU, QUOT). Each trial type was further split with respect to the level of noise (HN or LN) embedded in the stimuli. Regressors derived from incorrect and absent responses, as well as realignment parameters, were included in the design matrix and considered covariates of no interest. High-pass filtering was implemented in the matrix design using a cutoff period of 128 s to remove low frequency drifts from the time series. Serial correlations in the fMRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood algorithm.

In each subject, the effects of interest were then tested by linear contrasts, generating statistical parametric maps. The main contrast of interest assessed the **learning effect** by comparing the cerebral responses to QTOT and QTOU (for HN and LN) and estimating the changes in these responses between sessions 1 and 3, as well as 2 and 3 (sessions by learning interactions). Summary statistic images for the contrasts of interest were spatially smoothed (6 mm full-width at half-maximum) and entered in a second level analysis to account for inter-subject differences (RFX model): we computed two-sample t-tests to assess whether these differences were statistically significant between groups (SLEEP vs WAKE). Because procedural learning was shown to be associated with spindles and REM sleep, further RFX tested whether the overnight changes in BOLD responses were linearly related to the changes in spindle density and REM sleep duration between baseline and post-training nights.

The resulting set of voxel values for each contrast constituted maps of the t-statistics with a threshold set at $p_{uncorrected} = 0.001$. Statistical inferences were performed after correction for multiple comparisons at a threshold of $p = 0.05$. Corrections for multiple comparisons (Family

Wise Error method) were based on the Gaussian random field theory and computed on the entire brain volume or on small spherical volumes (10 mm radius) around a priori locations of activation. Activations were expected in occipital areas responsible for orientation coding and object-from-texture discrimination (Orban et al., 1997; Appelbaum et al., 2006).

To conform to the downscaling hypothesis, any modification of learning-related responses mediated by sleep should be observed only within areas recruited during learning in the first place. Therefore, data acquired during the first TRAINING session were analyzed using the same mixed-effects model as described above for TESTING sessions. The fixed-effects design matrix modeled the main effect of stimulus presentation and its linear modulation by the stimulus SNR. This regressor accounted for learning effect during the TRAINING session. Because during TRAINING sessions, only QTOT stimuli were presented, this learning effect is estimated differently than the learning effect reported for TESTING sessions (QTOT-QTOU). The second-level RFX consisted of a one-sample t test assessing the learning effect during training. Statistical inferences were conducted as in the main analysis.

EEG data acquisition and analysis

For baseline and post-training night sleep, our aim was to obtain a high density EEG recording during the first NREM cycle, as changes in regional SWA were reported mainly during this period (Huber et al., 2004). EEG signals were acquired via 64 ring-type electrodes fitted into a net plugged into a Quickamp (Brain Products GmbH, Gilching, Germany) 72-channel amplifier (64 EEG, bipolar vertical and horizontal electrooculograms and chin electromyogram), and recorded via BrainVision Recorder (BrainVision LLC, USA). During recording, EEG electrodes were referenced to the mean signal of all channels. Electrode-skin impedance was kept below 10 kOhm. Recordings were digitalized at 500 Hz with a bandpass filter from DC to Nyquist frequency and a 50 Hz notch filter.

Electrode positions were measured by a positioning system (Zebris Gmbh, Germany) to allow for 3D reconstruction of scalp currents (for instance, SWs).

Sleep periods were scored using standard criteria with the "fMRI Artifact Removal and Sleep Scoring Toolbox" (FASST, http://www.montefiore.ulg.ac.be/~phillips/FASST.html) (Leclercq et al., 2011). The EEG files were segmented to isolate the first NREM sleep cycle for each volunteer. Power spectra were computed using a fast Fourier transform on successive 4 s epochs, overlapping by 2 s and weighted by a Hanning window. For each electrode, relative power in the delta band (0.5 - 4 Hz) was normalized to the mean absolute power over all electrodes (Huber et al., 2004). Repeated-measures ANOVAs were conducted with electrodes and nights (baseline vs post-training) as within-subjects factors. We also correlated the individual overnight improvement in performance with the SWA computed at each electrode over the first NREM sleep period.

The FASST was also used to semi-automatically detect and characterize NREM sleep SWs and spindles during the first NREM cycle. Slow wave detection was based on the detection parameters by Massimini et collaborators (Massimini et al., 2004), and identified the peak negativity of each wave on the scalp, as well as the trajectory followed by each slow oscillation across electrodes. The detection algorithm was applied to potentials locally averaged over four non-overlapping areas of the scalp (region 1: F3, F1, Fz, F2, F4, FC1, FC2; region 2: FC5, FC3, C5, C3, C1, CP3, CP5; region 3: FC6, FC4, C6, C4, C2, CP6, CP4, and region 4: CP1, CPZ, CP2, P3, P1, Pz, P2, P4) areas. The criteria for slow oscillation detection were as follows: a negative zero crossing and a subsequent positive zero crossing separated by 0.3–1.0 sec, a negative peak between the two zero crossings with voltage less than -80 μV, and a negative-to-positive peak-to-peak amplitude > 140 μV. This analysis allowed us to identify the waves and their trajectories, by determining which scalp electrode positions were crossed by each waves. We also identified the site of 'initiation' of each

wave (between quotes), operationally defined as the electrode position associated with the earliest peak negativity. This initiation site is obviously different from the actual initiation site of ON states which can only be identified by intracortical multicellular recordings (à la (Chauvette et al., 2010). For each slow wave, parameters such as amplitude and maximum slope were computed for both baseline and post-training nights. Repeated-measures ANOVAs were conducted with electrodes and night (baseline vs post-training) as within-subject factors. We correlated the individual overnight improvement in performance with the number of SWs propagating through or 'initiated' at each electrode.

In further analyses of SWs, we identified the current sources associated with the peak negativity of waves which we thought related to learning (see Results), using the SPM toolbox (SPM8, www.fil.ion.ucl.ac.uk/spm/software/spm8/). For each volunteer, electrode positions were co-registered to the individual structural MR, by specifying 3 fiducial points (left and right ear, nasion) derived from electrode position measurement. Forward (EEG BEM model) and inverse (based on multiple sparse priors algorithm) models were then applied to each individual EEG signal. For each volunteer, a mean wave 'initiated" over PO7 was generated by averaging the individual waves 'initiated' over PO7. Finally, the projected EEG activity during the 1000 ms period centered on the peak negativity of average wave was obtained as a 3D NIFTI image per each volunteer.

These images were then analyzed with the non-parametric statistical testing (SnPM) toolbox (http://www2.warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/software/snpm/) of SPM in order to visualize any consistent focal points of origin for the mean slow oscillations across all subjects.

Spindles were detected following the procedure proposed by Mölle and collaborators (Molle et al., 2002), on Fz, Cz and Pz (referenced to mean mastoids). Data were bandpass filtered between 11–15 Hz using linear phase FIR (finite impulse response) filters (-3 dB at 11.1 and 15.9 Hz). Filtering

was performed in both forward and reverse directions, which results in zero-phase distortion and doubling of the filter order. The root mean square of the filtered signal was calculated using a time window of 0.25 sec. Sleep spindles were identified by thresholding the spindle root mean square signal at its 95th percentile. Spindles were subsequently visually checked for correct detection.

Statistical analyses of EEG data consisted of repeated-measures ANOVA on SWA, or the number of waves 'crossing' or 'initiated' at each electrode during the first NREM cycle with electrode and night (baseline vs post-training) as within-subject factors. Degrees of freedom of the ANOVA were adjusted using the Greenhouse–Geisser (GG) method. Uncorrected F values are reported together with the GG epsilon and corrected p values.

Simple linear regressions were used to assess the dependency between individual overnight improvement in performance (i.e., the individual difference in mean SNR between sessions) and (1) the number of SWs crossing or (2) 'initiated' at each scalp positions during test night, (3) the change in spindle density or (4) the change in REM sleep duration from baseline to test night. Statistical inferences were conducted after Bonferroni correction for multiple comparisons at $p < 0.05$.

RESULTS

Demographic data

After excluding 4 volunteers for technical issues and 5 for non-compliance to regular sleep rhythms, 11 participants were included in the SLEEP group (7 women) and 12 in the WAKE group (6 women, mean age: 23 years ± 6 months). According to actigraphic recordings and sleep diaries, sleep duration did not differ between groups for the 5 days prior to the testing day (average: 8 hours and 17 minutes ± 22 minutes, ($t(21) = -0.58$; $p = 0.15$). On average, volunteers in the SLEEP group spent 7 hours and 53 minutes (± 19 minutes) and 7 hour 58 minutes (± 24 minutes) in the

laboratory during the baseline and post-training nights, respectively. Volunteers in the WAKE group spent an average of 8 hours and 2 minutes (± 12 minutes) during the baseline night ($t(21) = -0.69$; $p = 0.49$, relative to SLEEP group).

Behavioral Results

We checked that changes in performance at retest were not due to differences in alertness at different time-of-day (evening for the WAKE group, morning for the SLEEP group). Objective alertness as assessed by reaction times during a PVT before final retest did not differ between groups ($t(18) = -1.25$; $p = 0.349$).

We then assessed perceptual learning with a repeated-measures ANOVA testing for the effects of group, training session, and block (the last six out of seven blocks of each training session) on the SNR of noise-filled gratings: the lower the SNR, the better was the performance (Figure 2A). The main effects of session ($F(1,12) = 7.79$; $p = 0.011$) and block ($F(5,105) = 13.859$; $p < 10^{-6}$) were statistically significant, while the main effect of group was not ($F(1,21) = 1.687$; $p = 0.208$). However, the session by group interaction ($F(1,21) = 6.391$; $p = 0.019$), as well as the block by group interaction ($F(5,105) = 6.731$; $p < 10^{-5}$) were statistically significant. In contrast, the session by block interaction ($F(5,105) = 0.862$; $p = 0.509$) and the triple session by block by group interaction ($F(5,105) = 0.558$; $p = 0.732$) were not significant. Planned comparisons showed that performance was significantly better in the SLEEP than in the WAKE group during the second session ($p = 0.007$), but not during the initial training session ($p = 0.837$). In the SLEEP group, performance increased significantly overnight ($p = 0.001$) whereas performance did not change significantly between sessions for the WAKE group ($p = 0.850$). The data showed that an offline gain in performance between sessions could be observed 8 hours after initial training, without any

further practice. However, this gain was observed only when this interval contained a night of sleep.

Finally, the gain in performance was not correlated with changes in spindle density or REMS duration between baseline and post-training nights.

Functional MRI results

The changes in regional brain responses induced by perceptual learning were assessed by contrasting responses to stimuli with trained orientations presented in the trained quadrant with highly noisy stimuli (SNR < 30%) presented in the same quadrant but with untrained orientations (QTOT > QTOU, see Materials and Methods and Table 2).

No significant learning effect (QTOT-QTOU) was detected before training, or during the immediate post-training session, neither in the WAKE group nor the SLEEP group. In neither group were there any significant changes in learning-related responses from baseline to immediate post-training session.

A significant learning effect was observed in the left inferior lateral occipital cortex (coordinates: -38 -92 -12 mm, Z = 3.41, p_{svc} = 0.033, Figure 2B, green), during the delayed testing session (session 3), the day after training in the SLEEP group, but not in the post-training evening in the WAKE group. The area overlapped with brain areas significantly recruited during training as performance increases (i.e., SNR decreases; local peak voxel : coordinates : -30 -92 -6, Z = 3.27, p_{svc} = 0.017; Figure 2B, yellow).The learning effect in the delayed testing session was significantly larger in the SLEEP than in the WAKE group (trial type (QTOT vs QTOU) x group (SLEEP vs WAKE) interaction during session 3; -42 -90 – 8, Z = 3.63, p_{svc} = 0.009). The changes in learning effect from immediate to delayed testing were significant in the SLEEP group in the same occipital area (trial type (QTOT vs QTOU) x session (2 vs 3) in SLEEP ; -32 -90 -8, Z = 3.56, p_{svc} = 0.003), and significantly larger in the latter than in the WAKE group (trial type (QTOT vs QTOU) x session (2 vs 3) x group (SLEEP vs

WAKE) interaction; -38 -88 - 8; Z = 3.23; p_{svc} = 0.029, Figure 2B). At a lenient statistical threshold ($P_{uncorrected}$ < 0.05), a learning effect was detected in the contralateral homologous area in the right occipital cortex (ipsilateral to the learned stimulus, 44 -82 -12; Z = 3.15) when comparing activations between SLEEP and WAKE groups for trained versus untrained orientations between the first and last testing sessions (trial type (QTOT vs QTOU) x session (2 vs 3) x group (SLEEP vs WAKE) interaction).

These results indicate that brain responses to HN stimuli selectively presented at the trained orientation and quadrant were enhanced 8 hours after initial training, but only if the interval was filled with nocturnal sleep. Activity estimates confirm that learning-related responses were significantly enhanced during the delayed testing session (in the morning, after a full night of sleep) relative to the immediate testing session (in the evening) in the SLEEP group, whereas learning-related responses did not significantly change across sessions in the WAKE group (Figure 2B).

These results were observed for HN stimuli. The same contrasts, for the LN condition, did not show any significant differences in brain responses. We assume that only HN stimuli were associated with changes in brain responses because stimuli during training sessions were almost exclusively presented at HN level with low SNRs. This high level of noise thus supported a greater level of perceptual learning.

Because sleep-dependent consolidation has been related to spindles and REM sleep and for the sake of completeness, we looked whether the changes in learning-related responses (QTOT-QTOU) were related to modifications in spindle density or REM sleep duration between baseline and test nights. The change in learning-related responses between immediate and delayed testing was not correlated with the change in spindle density between baseline and post-training nights. By contrast, learning-related responses in the same area (coordinates : -40 -92 1; Z = 3.65; p_{svc} =

0.017) were significantly enhanced during delayed testing, relative to immediate testing, in proportion to the change in REM sleep duration between baseline and post-training nights (Figure 2C).

Sleep EEG Results

The data of one subject were lost and the analyses were run on 10 participants' data. Sleep parameters in the SLEEP group are summarized in Table 1. No significant differences in the sleep architecture were observed.

Repeated-measures ANOVA on SWA during the first NREM cycle with electrode and night (baseline vs post-training) as within-subject factors revealed a significant effect of electrode ($F(63,504) = 51.75$; $p < 10^{-6}$; GG $\varepsilon = 0.052$), whereas neither the effect of night ($F(1,8) = 0.874$, $p = 0.38$; GG $\varepsilon = 1$) nor the electrode by night interaction ($F(63,504) = 0.441$; $p = 0.99$; GG $\varepsilon = 0.026$) were significant (Figure 3A). The individual overnight improvement in performance did not significantly regress with SWA computed over the first NREM sleep period for any of the electrodes.

The repeated-measures ANOVA conducted on the total number of waves crossing each scalp electrode position during the first NREM cycle revealed a significant effect of electrode ($F(63,504) = 123.41$; $p < 10^{-6}$; GG $\varepsilon = 0.047$) but did not show any significant effect of night ($F(1,8) = 0.13$; $p = 0.72$; GG $\varepsilon = 1$) nor any electrode by night interaction ($F(63,504) = 0.28$; $p = 1$; GG $\varepsilon = 0.002$; Figure 3B). By summing waves respectively over frontal and occipital electrodes, the number of detected waves was expectedly larger in frontal (1279 ± 234) than occipital electrodes (389 ± 76), confirming the frontal predominance of SWs. For none of the electrodes did the individual overnight improvement in performance regress with the number of SWs the trajectory of which crossed the electrode scalp positions.

The repeated-measures ANOVA conducted on the number of waves 'initiated' at each electrode (i.e., electrode with the earliest peak negativity, see Materials and Methods) during the first NREM cycle revealed a significant effect of electrode ($F(63,504) = 20.75$; $p < 10^{-6}$; GG $\varepsilon = 0.083$). As expected, most of the waves 'initiated' in frontal areas (frontal electrodes: 89 ± 12) and less frequently from occipital areas (occipital electrodes: 37 ± 9). However, there was not any significant night effect ($F(1,9) = 0.04$; $p = 0.83$; GG $\varepsilon = 1$) nor any electrode by night interaction ($F(63,504) = 0.73$; $p = 0.93$; GG $\varepsilon = 0.073$; Figure 3C).

However, and most importantly, the number of slow oscillations 'initiated' at PO7 during the first NREM cycle was significantly correlated to the individual overnight performance gain ($F(1,8) = 28.11$; $p = 0.00078$, Bonferroni correction over 64 electrodes; $r^2 = 0.75$; Figure 3D-E). The correlation remained significant after discarding the two best performers ($p = 0.04$, $r^2 = 0.55$). This correlation was not significant for any other electrode during post-training night and no such correlation was observed during the baseline night. These results suggest that the initiation of SWs is related to offline memory processing selectively in occipital areas. In addition, occipital slow wave initiation does not reflect a general ability for visual perceptual learning as it was related to performance gains specifically during NREM sleep following training.

This result is all the more intriguing that the number of SWs 'initiated' from PO7 was not significantly different from that recorded in PO8 neither during baseline night [paired t test, $t(8) = 0.60$, $p = 0.56$], nor post-training night [paired t test, $t(8) = 1,34$, $p = 0.21$].

The current sources associated with the averaged slow wave 'initiated' at PO7 during the first NREM period, computed over the entire group, were located in bilateral occipito-temporal areas and, on the left side, showed an excellent consistency (peak voxel of reconstructed slow-waves activity coordinates: -40 -90 -10 mm) with the location of enhanced learning-related BOLD responses observed after sleep (Figure 3F). This result is remarkable given that the signals were

obtained at different time points in the process of memory consolidation, in 2 different states of vigilance, and with 2 different imaging techniques. It shows that SWs 'initiated' in lateral occipital areas during post-training NREM sleep not only predict individual overnight gains in performance but recruit brain areas that selectively enhance their response to the learned stimulus the next morning.

DISCUSSION

Normal volunteers were trained to discriminate two orthogonally-oriented gratings embedded in increasing levels of noise, either in the morning or in the evening. They were retested after 8 hours of wakefulness or sleep, respectively. Performance was enhanced after 8 hours only in the SLEEP group. This gain in performance was specifically predicted by the number of SWs 'initiated' in occipito-temporal areas during NREM sleep. The next morning (i.e., only in the SLEEP group), an overlapping area in the left occipital cortex showed enhanced neural responses selectively for the trained orientations. This response enhancement was proportional to individual REM sleep changes from baseline to post-training night. Collectively, these findings suggest that in the same occipital area, the local initiation of learning-related SWs during post-training NREM sleep foreshadows the enhancement of learning-related responses the next morning, and that REM sleep favorably influences this increase.

Increased performance only occurs after nocturnal sleep and is predicted by locally 'initiated' NREM sleep SWs

Confirming our previous findings (Matarazzo et al., 2008), orientation discrimination was enhanced 8 hours after initial training only if this interval included nocturnal sleep. An equal period of quiet daytime wakefulness did not lead to any change in performance. We conclude that

the mere passing of time cannot account for the behavioral results. Although a circadian effect cannot be ruled out, we provide evidence for a direct influence of NREM sleep SWs on delayed perceptual performance. During the post-training night, the number of SWs specifically 'initiated' at PO7 with sources in bilateral occipital areas predicted the overnight performance gain although the total number of waves crossing occipital electrode scalp positions or 'initiated' in occipital areas did not change from baseline to post-training night.

During later testing, local neural responses are selectively enhanced above noise level, only for trained orientations, only after sleep and in proportion to REM sleep

Consistent with behavioral results, changes in responses specific to learned orientations, relative to novel untrained orientations, were observed after training only in the SLEEP group. This result indicates that only sleep can lead to offline memory processing resulting in increased signal-to-noise neural responses the next morning. The influence of sleep is further suggested by two pieces of experimental evidence. First, the changes in fMRI activations paralleled the behavioral changes, which in turn were significantly related to SWs 'initiated' locally during post-training NREM sleep. Second, the between-session enhancement of responses to the learned stimuli was spatially consistent with the source of local SWs spontaneously generated during the interleaved night of sleep. These findings suggest that a local offline memory processing takes place during post-training NREM sleep and leads the next morning to both improved perceptual skills and enhanced local neural responses reflecting an increased SNR for the learned stimulus, in keeping with the predictions of the downscaling hypothesis.

The enhancement of occipital learning-related responses was proportional to the change in REM sleep during post-training night, relative to baseline night. This result suggests the participation of

REM sleep in perceptual learning although the overnight gain in performance did not correlate with REM sleep, in contrast to previous reports (Karni et al., 1994; Mednick et al., 2003).

The identified brain area is compatible with the LOC, a set of areas involved in shape and object-related processing (Malach et al., 1995). Object recognition is thought to rely in part on extracting shapes from temporally-structured figure-ground configurations (Appelbaum et al., 2006), a process involved in our COD task. The LOC is also activated by fine orientation discrimination (Orban et al., 1997). After sleep, neural populations coding for trained orientations enhanced their responses whereas in the populations coding for untrained orientations, responses did not change or changed to a significantly smaller extent. Perceptual learning would thus be supported by a limited neural population, specifically responsive to the learned orientation.

The initiation of SWs as a marker of local learning during NREM sleep

Perceptual learning is currently thought to be associated with Hebbian plastic changes in sensory cortices under the combined influence of attention, neuromodulatory systems (Raiguel et al., 2006; Roelfsema et al., 2010) and local horizontal connections within each area. Experience would locally induce a net increase in synaptic strength, either through synaptic potentiation or by the growth of dendritic spines and axonal boutons (Stettler et al., 2006; Holtmaat et al., 2008). It can be assumed that learning the COD task increased local synaptic strength and, following the hypothesis of synaptic homeostasis, this should result in changes in local SWs during post-training NREM sleep. Contrarily to this prediction, perceptual learning was not associated with any change in SWA, number of SWs crossing occipital electrode positions, or number of locally 'initiated' waves. (Gilbert et al., 2009). However, these results are consistent with the fact that orientation learning is associated with changes in orientation tuning of individual neurons and not necessarily

with an increase in the population of neurons responding to learned orientations (Schoups et al., 2001).

Nevertheless, during the post-training night, the number of SWs 'initiated' in occipital areas predicted the overnight gain in performance. The initiation of cortical activity during a slow oscillation is still debated and could result from intrinsic properties of layer V neurons (Sanchez-Vives and McCormick, 2000), state transitions occurring in small neuronal ensembles (Cossart et al., 2003) or spontaneous synaptic events switching the whole network into an active state (Timofeev et al., 2000). In the latter case, the switch from DOWN to UP state would be mediated by spontaneous synaptic events that appear probabilistically in any neuron and are reflected as mEPSCs (Chauvette et al., 2010). Neurons with potentiated synapses, which generate more frequent mEPSCs, are best suited for switching local neural populations into UP state. If these processes apply to humans, initiation of slow oscillations would randomly recruit local neurons during the baseline night (according to their respective synaptic potentiation during the previous waking period), whereas it would more consistently involve populations coding for the trained orientation during post-training night, which are more prone to generate mEPSCs (Liu et al., 2010). At the level of neuronal populations coding for the learned orientations, this enhanced participation in local slow oscillation is likely to optimize synaptic homeostasis and increase subsequent responses to learned orientations, relative to other orientations. Testing this hypothesis is beyond current fMRI resolution but it leads to the testable prediction that the probability of UP state initiation should change differentially between neural ensembles depending on the respective synaptic potentiation induced by orientation learning during the previous waking period. The generality of these findings should be ultimately investigated using other perceptual learning paradigms.

CONCLUDING REMARKS

We provide an example of a local learning, which is supported by an orientation-selective neural population within lateral occipital areas. This perceptual learning seems associated with a change in local slow wave initiation during post-training NREM sleep, without any modification in standard NREM sleep parameters, such as SWA. Indeed, the next morning, the selective gain in performance for trained stimuli is predicted by SWs locally 'initiated' in occipital areas. In addition, during delayed testing the next morning, brain responses selectively evoked by learned stimuli are enhanced in the same occipital areas, suggesting that slow-waves dependent processes foreshadow this increase in SNR. These results support the downscaling hypothesis and extend its scope by emphasizing the potential importance of NREM sleep slow wave initiation as a marker of local sleep pressure induced by learning. However, it is also true that the results, based on BOLD signal, do not identify the underpinning mechanisms which could equally involve protein synthesis induced by learning, or local metabolic changes related to energy restoration or elimination of adenosine**.** Finally, occipital responses were increased in proportion to REM sleep duration, supporting the view that REM sleep is also associated with substantial local neural plasticity, potentially in relation with enhanced synaptic potentiation (Ribeiro et al., 1999; Ribeiro et al., 2007; Ravassard et al., 2009).

AUTHOR CONTRIBUTIONS

LuMa, PM and RV designed and coded the task, analyzed and interpreted the data. LuMa, EB and PM co-wrote the manuscript. All other authors helped with data acquisition and provided valuable input during data analysis. The authors thank Igor Timofeev for his input about the cellular mechanisms of slow wave initiation during NREM sleep.

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FIGURE LEGENDS

Figure 1. Protocol

A. Stimuli. Top panel: Trained quadrant and orientation (QTOT), one of the 2 possible trained stimuli (112.5°, 5° eccentricity in the lower right quadrant) is displayed at 90% SNR. Lower left panel: Untrained quadrant but trained orientation (QUOT), a 22.5° orientation stimulus is displayed in the left lower quadrant at 90% SNR. Lower right panel: Trained quadrant but untrained orientation (QTOU), a 157.5° orientation stimulus is displayed in the lower right quadrant at 90% SNR.

B. Time course of a trial (30% SNR). Note the fixation cross and the answer prompt ("Left or Right?")

C. Experimental design. Upper row: SLEEP group, lower row: WAKE group. IT: Initial Training. Tr: Training session. Te: Testing session

Figure 2. Behavioral and fMRI results

A. SNR thresholds achieved during block 2 to 7 during behavioral ('training') sessions, in WAKE (red) and SLEEP (blue) groups.

B. **Left-hand panel,** left lateral occipital area showing a significantly larger increase in learning effect (QTOT-QTOU) from immediate (session 2) to delayed (session 3) testing in the SLEEP than in the WAKE group (red), displayed in sagittal (left) and transverse (right) sections, at $P < 0.001$ uncorrected and superimposed on the learning effect observed during training (yellow) and the structural MR scan of a typical volunteer, normalized to the MNI space. **Right-hand panel,** parameter estimates of learning-related responses in this area during sessions 2 and 3 in SLEEP (blue) and WAKE (red) groups. * Significant within-session learning effect (QTOT-QTOU; $p < 0.05$, corrected for multiple comparisons on small volume

of interest). ** Significant change in learning effect from immediate to delayed testing in SLEEP group ($p < 0.05$, corrected for multiple comparisons on small volume of interest). *** Significant session by group interaction ($p < 0.05$, corrected for multiple comparisons on small volume of interest). n.s., not significantly different from zero.

C. Sagittal (left-hand panel) and coronal (right-hand panel) display of the areas showing enhanced response during delayed testing in proportion to REM sleep duration (orange), superimposed on the group by session interaction (green), the learning effect observed during training (yellow) and the structural MR scan of a typical volunteer, normalized to the MNI space (Displays at $P < 0.001$ uncorrected). L = Left; P = Posterior.

Figure 3. EEG and fMRI results

A. Scalp distribution of SWA during the first NREM cycle of baseline (left panel) and post-training (right panel) nights.
B. Scalp distribution of the number of SWs crossing each electrode position during the first NREM cycle of baseline (left panel) and post-training (right panel) nights.
C. Scalp distribution of the number of SWs 'initiated' at each electrode position during the first NREM cycle of baseline (left panel) and post-training (right panel) nights.
D. Scalp distribution of squared correlation coefficients (r^2) between the number of SWs 'initiated' at each electrode position during the first NREM cycle of post-training night, and the overnight gain in performance.
E. Correlation between the number of SWs 'initiated' at PO7 during the first NREM cycle of post-training night and the overnight gain in performance
F. Coregistration of the current sources of SWs 'initiated' at PO7 during the first NREM cycle of post-training night (red) and the area showing an overnight increase in learning response during fMRI testing (green) averaged across all subjects.

TABLES

Table 1. Sleep parameters derived from the baseline and post-training nights in the SLEEP group.

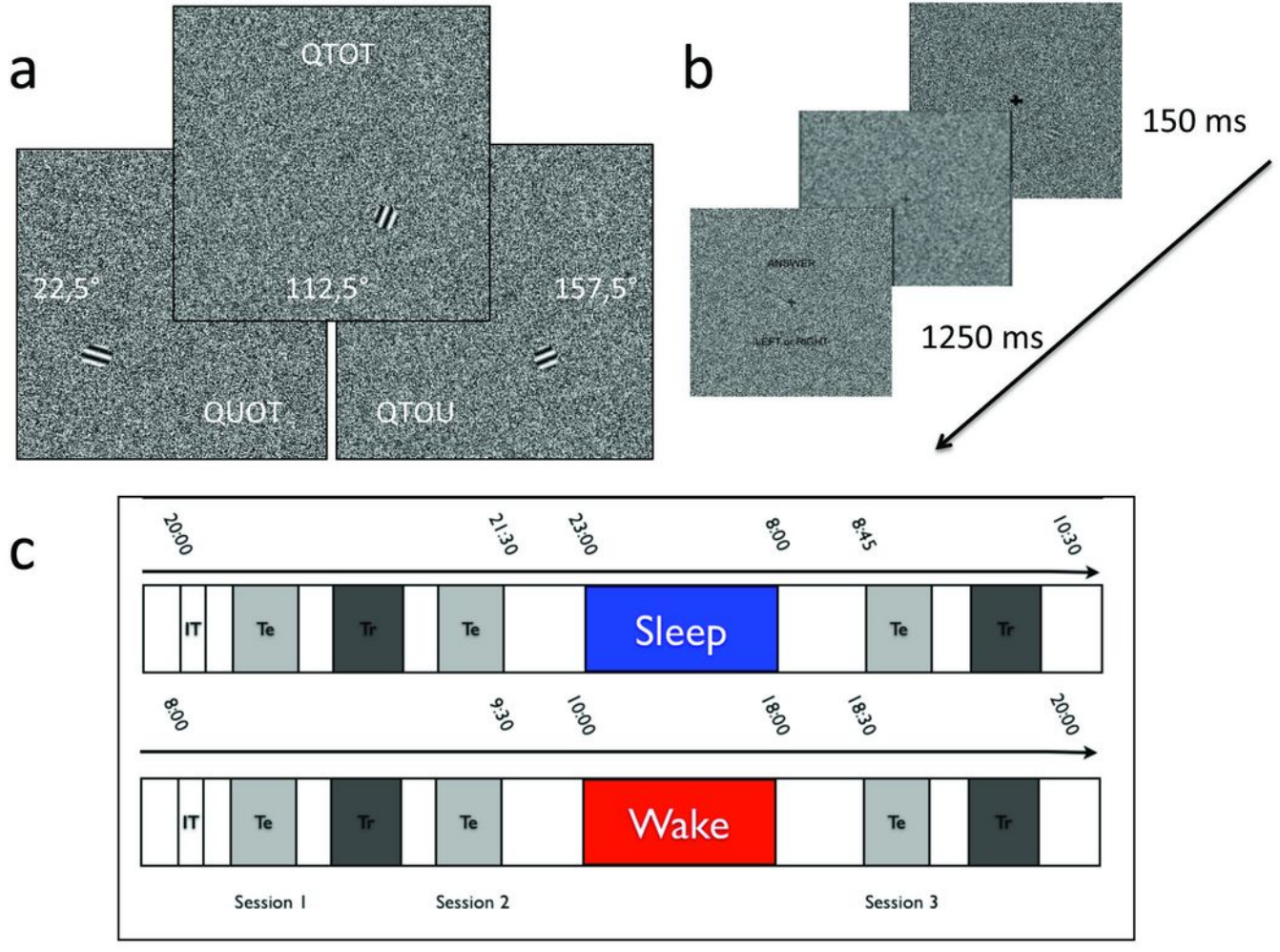
Sleep efficiency is defined as (N2+N3+R/total sleep time (TST))*100.

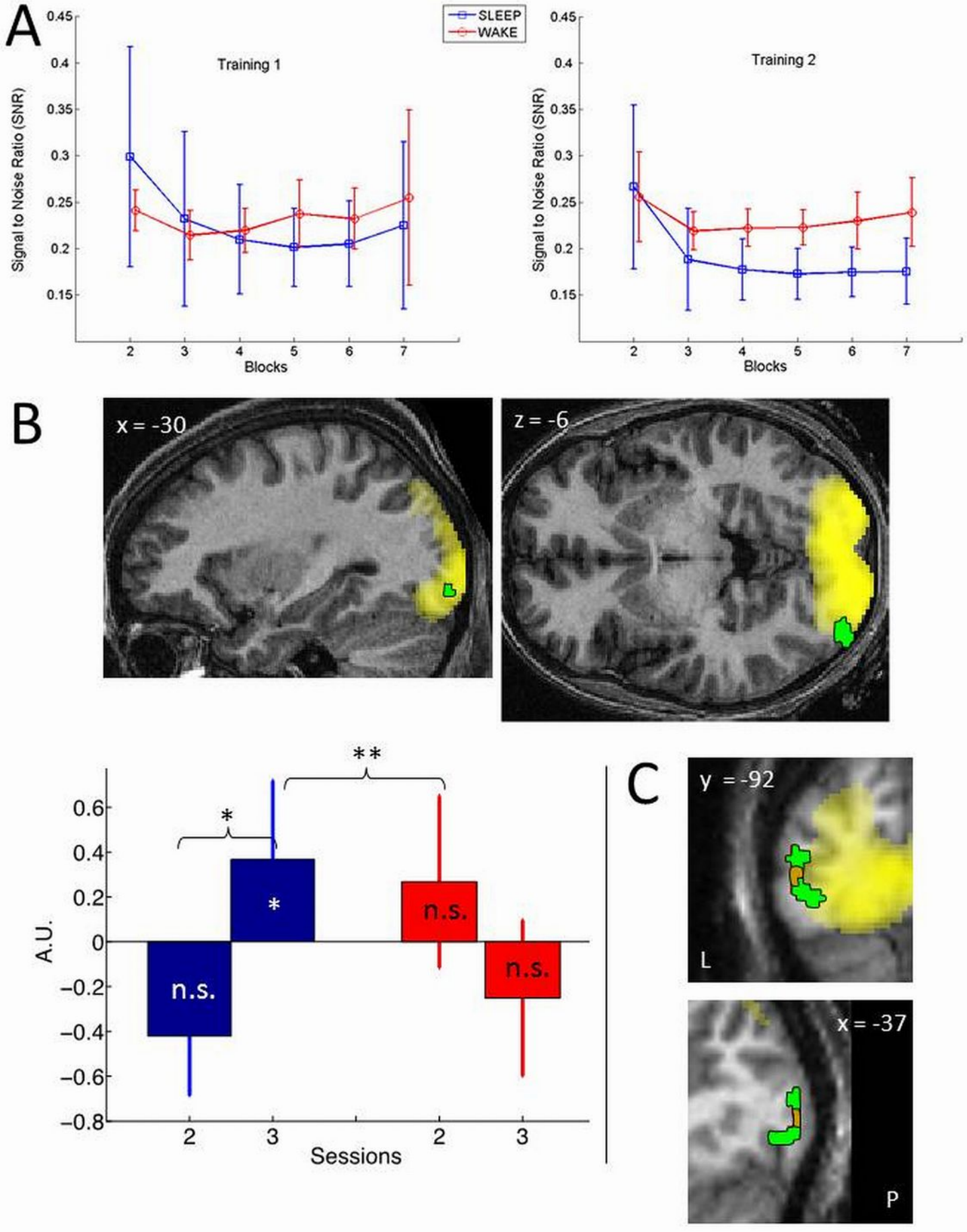
Parameter	dimension	Baseline	Post-training	p
N2 latency	min	27.1 ± 19.27	13.75 ±7.31	0.23
REMS latency	min	112.3 ±34.76	115.3 ± 35.46	0.86
TST	min	406.9 ± 79.2	436.1 ± 71.6	0.82
Sleep efficiency	%	82.9 ± 16.3	86.4 ± 14.0	0.56
Duration N2	min	175.9 ± 41.4	222.4 ± 66.2	0.11
Duration N3	min	141.1 ± 31.9	138.6 ± 48.5	0.89
Duration REM Sleep	min	89.9 ± 30.8	75.3 ± 22.3	0.26
Duration N2 (% TST)	%	35.5 ± 8.6	43.9 ± 12.8	0.13
Duration N3 (% TST)	%	28.4 ± 6.5	27.4 ± 9.9	0.81
Duration REM sleep (% TST)	%	18.1 ± 6.3	14.9 ± 4.5	0.22
Total Number of SWs		1933.9 ±25.2	1879.8 ± 21.4	0.72
Number of SWs propagating through PO7		475.8 ± 275.5	378.9 ± 193.8	0.34
Number of SWs 'initiated' at PO7		47.22 ± 43.9	35.2 ± 20.0	0.41
Number of SWs 'initiated' at PO8		41,56 ± 33.8	43.89 ± 21.96	0.22
Spindle density	min^{-1}	2.84 ± 1.29	3.05 ± 1.27	0.70

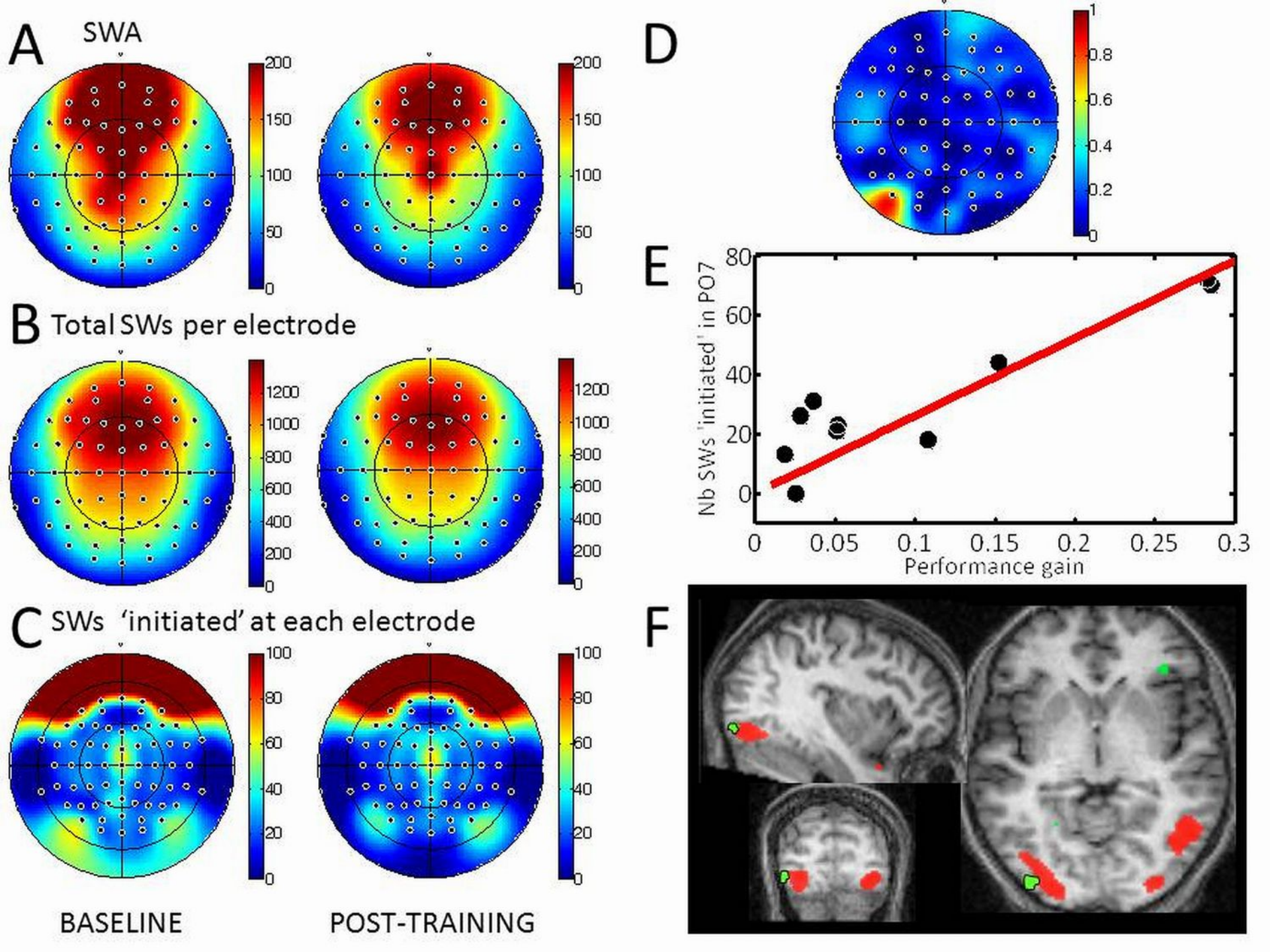
Table 2. Summary of the neural correlates of learning effects. See results and figure 2.

n.s. : not significant

Brain area	**x (mm)**	**y (mm)**	**z (mm)**	**Z score**	**P_{unc}**	**p_{svc}**
Local peak voxel significantly related to learning during initial training (inversely proportional to SNR)						
Left inferior lateral occipital cortex	-30	-92	-6	3.37	<0.001	0.017
Trial type (QTOT>QTOU) by group (SLEEP>WAKE) interaction during the delayed testing session (session 3)						
Left inferior lateral occipital cortex	-42	-90	-8	3.63	<0.001	0.009
Learning effect (QTOT>QTOU) during the delayed testing session (session 3) in the SLEEP group						
Left inferior lateral occipital cortex	-38	-92	-12	3.41	<0.001	0.033
Trial type (QTOT>QTOU) by session (SESSION 3>SESSION2) interaction in the SLEEP group						
Left inferior lateral occipital cortex	-32	-90	-8	3.56	<0.001	0.003
Trial type (QTOT>QTOU) by session (SESSION 3>SESSION2) by group (SLEEP>WAKE) interaction						
Left inferior lateral occipital cortex	-38	-88	-8	3.23	<0.001	0.029
Right inferior lateral occipital cortex	44	-82	-12	3.15	<0.05	n.s.
Peak voxel with significant changes in learning-related (QTOT>QTOU) responses in proportion to REM sleep duration						
Left inferior lateral occipital cortex	-40	-92	1	3.65	<0.001	0.017


a
QTOT
22,5°
112,5°
157,5°
QUOT
QTOU
b
150 ms
ANSWER
LEFT or RIGHT
1250 ms
c
20:00
21:30
23:00
8:00
8:45
10:30
IT
Te
Tr
Te
Sleep
Te
Tr
8:00
9:30
10:00
18:00
18:30
20:00
IT
Te
Tr
Te
Wake
Te
Tr
Session 1
Session 2
Session 3


A
SLEEP
WAKE
Training 1
Training 2
Signal to Noise Ratio (SNR)
Blocks
B
x = -30
z = -6
C
y = -92
L
x = -37
P
A.U.
Sessions
n.s.
*
**


A
SWA
200
150
100
50
0
B
Total SWs per electrode
1200
1000
800
600
400
200
0
C
SWs 'initiated' at each electrode
100
80
60
40
20
0
BASELINE
POST-TRAINING
D
1
0.8
0.6
0.4
0.2
0
E
Nb SWs 'initiated' in PO7
80
60
40
20
0
0
0.05
0.1
0.15
0.2
0.25
0.3
Performance gain
F