Neurophysiological correlates of sleepiness: A combined TMS and EEG study

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Changes of cortical and corticospinal excitability as a function of sleep deprivation have been studied, using EEG power maps and several TMS measures in 33 normal subjects before and after a 40-h sleep deprivation (SD).

The effects of SD were independently assessed by subjective and EEG measures of sleepiness, the latter being represented in terms of cortical maps for different frequency bands. Short intracortical facilitation (SICF) and inhibition (SICI) were measured by the paired-pulse TMS technique with different inter-stimulus intervals. Besides standardized motor threshold (MT), lower threshold (LT) and upper threshold (UT) were also determined.

Subjective sleepiness severely increased as a consequence of SD, paralleled by a drastic decrease of alertness. EEG topography showed large increases in delta and theta activity, mainly evident at fronto-central areas. Standard MTs, as well as LTs and UTs, all increased as a consequence of SD. SICF also showed a significant increase as compared to pre-deprivation values, but only in females. The increase of theta activity was strongly associated in the left frontal and prefrontal cortex to a smaller decrease of corticospinal excitability, expressed by MTs, and a larger increase of intracortical facilitation, expressed by SICF.

TMS and EEG measures converge in indicating that SD has severe effects on both cortical and corticospinal excitability, as shown respectively by the increases of slow-frequency EEG power and MTs. The SICF enhancement in females and the results of the combined topographical analysis of EEG and TMS changes are coherent with the hypothesis that cortical TMS-evoked responses are higher as a consequence of a longer wakefulness.

Introduction

Sleep deprivation has severe effects on human alertness and performance. These neurobehavioral deficits are well established, the first evidence dating back to 110 years ago (De Manacine, 1894). Reduced alertness has been shown by means of subjective and objective measures of sleepiness (Curcio et al., 2001; Cluydts et al., 2002). Simple task performance is negatively affected by sleep deprivation, as indexed by tests of reaction time, vigilance and attention (Dinges and Kribbs, 1991). Additionally, complex task performance involving frontal lobe or executive function is particularly impaired (Jones and Harrison, 2001).

The degrading effects of sleep deprivation on alertness and cognitive performance clearly suggest alterations in underlying brain physiology and function. This issue has not been clarified yet by brain imaging studies that investigated in vivo brain activity changes during prolonged wakefulness. One night of sleep deprivation has been associated with a significant decrease in global cerebral metabolic rate for glucose (CMRglu) (Thomas et al., 2000; 2003), with no overall decrease in whole brain metabolism as measured by F-18 deoxyglucose (FDG) (Wu et al., 1991), or with an

However, the lack of an increase in cortical excitability after prolonged wakefulness in males suggests some caution in the generalization of these effects, that deserve further investigation.

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increased activation as measured by fMRI in several cortical (Drummond et al., 2000; 2005; Mu et al., 2005) and thalamic regions (Ayalon et al., 2006; Portas et al., 1998). However, all these studies assessed metabolic changes during performance on different tasks, and the contrasting findings may be related to the task-specific changes in brain response. Moreover, changes in brain responses could be affected by a compensatory recruitment of resources to counteract consequences of sleep deprivation on cognitive performance. On the other hand, the infrequent evidence of comparisons between “resting” conditions after variable schedules of sleep deprivation suggests a more univocal picture, since absolute blood flow (measured with $^{15}$O corrected for changes in arterial pCO2) values were 17.5% higher at the end of the waking day than after a night of sleep (Braun et al., 1997). As an indirect support, it should be mentioned that a recent meta-analysis of 22 PET studies conducted in the same Cyclotron Research Centre shows a positive correlation between regional CBF and hours spent in wakefulness (Christina et al., 2006).

Quantitative analyses of the frequency components of the waking electroencephalogram (EEG) also provide useful parameters for objective assessment of the neurophysiological consequences of sleep loss. It has been shown that EEG frequencies up to 9 Hz progressively increase during 40 h of sustained wakefulness (Aeschbach et al., 1997; Cajochen et al., 1995). Power density in the 0.75- to 9-Hz range also exhibited a wake-dependent increase (Finelli et al., 2000). The topographic analysis of the rise rate of theta power during prolonged wakefulness revealed that the largest increases are centered over frontal cortical areas (Finelli et al., 2000). More recently, the frontal predominance in waking of EEG power in the 2 to 8-Hz range has been confirmed (Tingueyl et al., 2006).

Only a few studies have non-invasively evaluated the effects of sleep deprivation on human motor cortex and corticospinal excitability by means of the transcranial magnetic stimulation (TMS) technique. It is well-known that sleep deprivation leads to sensitization to seizures and this is why it is widely used as an activation test in clinical electroencephalography (Malow, 2004); moreover, sleep loss is considered a risk factor for seizure precipitation even in epileptic patients with good pharmacological control (Dinnor, 2002). Nevertheless, the effects of sleep deprivation on corticospinal excitability have not been elucidated yet. Using the paired-pulse TMS technique (Kujirai et al., 1993), no difference in short latency intracortical inhibition (SICI) and short latency intracortical facilitation (SICF) has been found in two different studies comparing baseline values in the morning with recordings performed at the same time after 24 h of sleep deprivation (Manganotti et al., 2001; 2006). On the other hand, Civardi et al. (2001) showed a reduction of both SICI and SICF after a sleep deprivation. Small samples and circadian factors affecting cortical excitability may account for these contrasting results.

Undoubtedly, studies combining different measures of brain physiology are the best candidate to elucidate the neurophysiological correlates of sleepiness. Therefore, the main aim of the current study was to assess, in a large sample of normal subjects, the effects of one night of sleep deprivation on EEG and TMS measures of cortical activation and corticospinal excitability. The latter issue seems particularly relevant in view of testing one of the predictions of the Synaptic Homeostasis Hypothesis, which claims that sleep plays a role in down-regulating brain synaptic weight (Tononi and Cirelli, 2003, 2006). According to this hypothesis, wakefulness is associated with synaptic potentiation resulting in a net increase in synaptic strength and “safety” of synaptic transmission in several brain circuits, while sleep is associated with the homeostatic regulation (downscaling) of the synaptic weight impinging on neurons. Consequently, the amplitude of TMS-evoked potentials and EEG responses recorded on the scalp—probably mediated via cortico-cortical connections—should be higher at the end of a day of wakefulness and lower after a night of sleep (Tononi and Cirelli, 2003).

The current experiment is also addressed to directly test this hypothesis since an even higher synaptic potentiation, resulting in a coherent pattern of changes in SICI, SICF and motor thresholds (MTs), is expected after sleep deprivation. As a further prediction, the level of sleepiness as measured by changes in slow-frequency EEG activity should be associated to the amount of cortical activation as expressed by changes in TMS measures after the prolonged sleep deprivation.

Materials and methods

Experimental subjects

Thirty-three healthy, young-adult volunteers (18 M and 15 F; age = 24.6±2.4 years) were selected from a university student population to participate in the study. In a clinical interview, the subjects reported the absence of epilepsy or any other neurological condition in themselves and their family history and the absence of any other medical or psychiatric disorder.

Further requirements for inclusion were normal sleep duration (habitual sleep time: 2400–0800±1 h) and schedule, no daytime nap habits, no excessive daytime sleepiness, no other sleep, medical or psychiatric disorder, as assessed by a one-week sleep log and by a clinical interview. Participants were required to avoid napping throughout the experiment; compliance was controlled by actigraphic recordings (AMI Mini motion logger).

The subjects gave their written informed consent; the protocol of the study was approved by the local Institutional Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

Materials

Transcranial magnetic stimulation

The stimulator used was a Magstim 200 Mono Pulse connected to a Bistim module and to a figure-of-eight coil with external wing diameters of 9 cm (Magstim Company Limited, UK). The peak magnetic field produced by such a coil was 2.0 T.

The motor-evoked potentials (MEPs) of the hand muscle were recorded from the ADM muscle. Two Ag-AgCl surface cup electrodes of 9 mm diameter were used: the active electrode was firmly taped over the muscle belly, and the reference electrode over the metacarpophalangeal joint of the little finger after gentle skin cleaning and conductive jelly application in order to lower skin-electrode conductance to less than 10 kΩ. The recorded MEPs were stored and analyzed using an EMG-dedicated software (Myto, EBNeuro, Italy).

EEG recordings

An Esaote Biomedica VEGA 24 polygraph was used for polygraphic recordings. EEG signals were high-pass filtered with a time constant of 0.3 s and low-pass filtered at 30 Hz. The nineteen unipolar EEG derivations of the international 10–20 system (Fp1,
Fp2, F7, F8, F3, F4, Fz, C4, C3, Cz, P3, P4, Pz, T4, T6, T3, T5, O1 and O2) were recorded from scalp electrodes with averaged mastoid reference. Submental EMG was recorded with a time constant of 0.03 s. Bipolar horizontal eye movements were recorded with a time constant of 1 s. Bipolar horizontal EOG was recorded from electrodes placed about 1 cm from the medial and lateral canthi of the dominant eye. Impedance of these electrodes was kept below 5 kΩ.

**Design and procedure**

**General experimental schedule**

Each subject participated in an SD study across 4 consecutive days and nights. The sleep recordings, carried out in a sound-proof, temperature-controlled room, were scheduled in the first night (adaptation), in the second night (baseline sleep) and in the fourth night (recovery sleep).

The subjects’ sleep was undisturbed in all three nights, started at 2400 and ended after 0730 h of accumulated sleep (as online visually checked by expert sleep researchers). Following awakening from baseline sleep, each subject participated in a protocol of 40-h SD with recordings of the waking EEG in 36 sessions at 1 h intervals starting at 1000 am.

When not involved in testing sessions, subjects were allowed to carry out their own preferred activities, such as reading, writing, listening to music, watching TV or playing games, always under the direct supervision of at least one experimenter. Lying down, sleeping and vigorous physical activity were not permitted. Meals were provided to subjects at 0830, 1430 and 1930 h. Non-scheduled light snacks were permitted, while caffeinated beverages, chocolate, alcohol and medications that can influence sleepiness were not allowed during the deprivation protocol. Time information was available to subjects, and light exposure was not strictly controlled for (although the laboratory was constantly illuminated by 4 neon lamps, blinds only in part attenuated the light coming from the outside). The 40-h schedule of SD ended at 2400 h.

**Subjective sleepiness**

Self-rated sleepiness was measured in both conditions (baseline and post-deprivation) by the Karolinska Sleepiness Scale (Akerstedt and Gillberg, 1990) and a Visual Analog Scale for Global Vigor (Monk, 1987) just before the start of each EEG recording session (see below). The Karolinska Sleepiness Scale (KSS) is a 9-U rating scale, ranging between “very alert” and “very sleepy, fighting sleep”, while the Visual Analog Scale for Global Vigor is a paper-and-pencil measure of subjective alertness which combines the scores on four continuous 10-cm scales (alert, sleepy, weary and effort) to obtain a Global Vigor score between 0 and 40 cm.

**Transcranial magnetic stimulation**

Subjects were seated on a comfortable chair in a soundproof room and tested at the same time of day (2230 h) in two identical sessions (baseline and post-deprivation) separated by 48 h. In both conditions, subjects were tested fully relaxed, with very dim lighting and minimal verbal interactions.

The most effective point on the subject’s scalp for eliciting an ADM stimulation was localized by positioning the coil such that the junction region of the figure-of-eight coil was approximately over the central sulcus and moving the coil in 1 cm steps. The coil was positioned tangentially to the scalp and pointing in the anteromedial direction, 45° from the midsagittal axis of the subject’s head, and the handle of the coil posteriorly oriented. In this way, the induced current in the brain flowed perpendicularly to the orientation of the central sulcus, and the lowest MTs were defined (Brasil-Neto et al., 1992; Maeda et al., 2002).

**Motor thresholds**

With the muscle relaxed and monitored by visual feedback, MT was measured in both conditions with the standardized technique as the lowest intensity level of stimulation able to produce at least 5 MEPs with 100 μV of amplitude (peak-to-peak) in 10 consecutive stimulations (Rossini et al., 1994; 1999). After measuring standard threshold (ST), two further points on the intensity scale were defined, by adapting the criteria proposed by Mills and Nithi (1997). First, the maximum intensity at which 10 stimuli all produced no response was found by decreasing intensity in 1% steps; this was defined as the lower threshold (LT). Second, the minimum intensity at which 10 stimuli all produced a positive response was found by increasing the intensity in 1% steps from the lowest level which so far had not resulted in a “no response”; this was defined as the upper threshold (UT).

**Intracortical paired pulses**

According to the intracortical paired-pulse (PP) technique (Kujirai et al., 1993), two magnetic stimuli were delivered in close sequence to the left motor cortex through a single stimulating coil. Thus, the effect of a conditioning stimulus, delivered on the left motor cortex on the MEP amplitude evoked in the ADM muscles by a magnetic test stimulus applied to the same cortex, was assessed. Inter-stimulus intervals (ISIs) between the conditioning and test pulses were: 1, 3, 7, 10, 12 and 15 ms. The intensity of the conditioning shock was set at 70% of the individual MT at muscle rest, while the test one was set at 130%. Eight responses per condition, both test and conditioning pulses, were collected and their peak-to-peak amplitude was measured offline and subsequently averaged. The position of the coil was kept constant throughout each block of stimulations, and the EMG level was monitored to ensure that the muscles were maintained relaxed.

The baseline level of MEP responses (unconditioned responses) was measured with an independent series of 12 test stimuli, administered alone at 130% of the individual motor threshold (test alone). Hence, each intracortical paired-pulse session included 7 blocks: test alone, 1, 3, 7, 10, 12, 15 ms, and their order was randomized between subjects.

**EEG recordings**

Pre- and post-deprivation EEG was recorded at 2330 h, about 1 h after the end of the TMS protocol. In both baseline and post-deprivation conditions (corresponding to a 39-h SD), the EEG was recorded just before the subjects went to sleep while laying in bed, fully relaxed, with very dim light and minimal verbal interactions. Each session consisted of a 5-min eyes-closed period, followed by a 5-min eyes-open period.

The polygraphic signals (19 EEG channels, 1 EOG and 1 EMG) were analogue to digital converted online with a sampling rate of 128 Hz and stored on the disk of a personal computer. Ocular and muscle artefacts were excluded offline on a 2-s basis by visual inspection. Power spectra of 19 derivations were computed by a Fast Fourier Transform (FFT) routine in 2-s epochs across the
following bands: delta (1–4 Hz), theta (5–7 Hz), alpha (8–12 Hz), beta1 (13–15 Hz) and beta2 (16–30 Hz).

Data analysis

Transcranial magnetic stimulation measures

Motor thresholds were compared by means of two-way mixed-design multivariate analyses of variance (ANOVA)s, Gender × Treatment (Pre vs. Post-deprivation), the second factor as a repeated measure. Dependent variables of these ANOVAs were log-transformed ST, LT and UT values. Data were log-transformed in order to obtain a better approximation to gaussianity and a higher homoscedasticity.

MEP amplitude was measured peak-to-peak. Changes in MEP amplitude as a consequence of conditioning pulse administration were expressed in terms of the ratio between conditioned (preceded by conditioning pulse) and unconditioned responses; the ratios were then log-transformed. SICI and SICF were calculated by averaging values respectively at 1–3 ms and at 7, 10, 12 and 15 ms.

Changes in MEP amplitude were compared by the same two-way mixed-design analysis of variance (ANOVA), Gender × Treatment (Pre vs. Post-deprivation), the second factor as a repeated measure. Dependent variables were log-transformed SICI and SICF values. The level of significance was set at \( p \leq 0.05 \).

Subjective and EEG measures of sleepiness

Subjective measures of sleepiness (KSS and Global Vigor scores) were also submitted to a two-way mixed-design analysis of variance (ANOVA), Gender × Treatment (Pre vs. Post-deprivation), with the second factor as a repeated measure. The means of significant interactions were compared by post hoc (Fisher’s PLSD) tests (significance at \( p \leq 0.05 \)).

Log-transformed EEG power maps were computed separately for the eyes-closed and eyes-open conditions. Statistical comparisons for every EEG band and scalp location were carried out by paired two-tailed \( t \)-tests. To correct for multiple comparisons, the Bonferroni correction was applied. Considering the mean correlation between the variables \( r = 0.60 \), the alpha level was then adjusted to \( = 0.006 \) (\( t = 2.93 \)).

Combined TMS and EEG changes after sleep deprivation

The relationship between the extent of EEG changes associated to the increased sleepiness and changes in the TMS measures after SD was examined through a correlational approach. With respect to TMS measures, standard motor threshold (MT), and changes in MEP amplitude at the ISI of the intracortical PP protocol showing the largest difference as a consequence of SD, were considered. Power in theta band was considered as the main measure of sleepiness expressed by EEG activity since it has repeatedly been demonstrated that theta activity is the EEG marker of sleep propensity during extended deprivation (Finelli et al., 2000).

Multiple correlations were carried out for each scalp location, considering changes in corticospinal excitability (AMT) and in cortical facilitation (ΔSICF) as predictors, and changes in EEG activity as criterion (ΔEEG power).

To correct for multiple comparisons, the Bonferroni correction was applied. Considering the mean correlation between the variables \( r = 0.72 \), the alpha level was then adjusted to \( \leq 0.01 \), corresponding to a multiple \( r = 0.51 \) and to a partial \( r = 0.44 \).

Results

Subjective measures of sleepiness

After SD, subjects were significantly more sleepy, as revealed by the KSS scores. The main effect for treatment was significant \( (F_{(1,31)} = 72.05; p < 0.0001) \) with a clear increase of KSS scores after SD [Pre = 2.81 (SE = 0.20); Post = 5.45 (SE = 0.31)]. The main effect for gender and interaction were not significant.

Subjects were also significantly less alert, as measured the Global Vigor scores. Again, only SD affected these scores \( (F_{(1,31)} = 48.28; p < 0.0001; \text{Pre} = 27.74 \text{ (SE = 1.22), Post} = 17.46 \text{ (SE = 1.52)}) \), with no significant effect for gender or interaction.

EEG measures of sleepiness

Topographic distribution of waking EEG power for the selected frequency bands with eyes-closed and -open are illustrated respectively in Figs. 1 and 2. The figures depict EEG power maps for the selected frequency bands, scaled between the maximum and minimum values, in baseline wakefulness just before the onset of sleep recordings and at the same time after about 40 h of SD (first two panels from left), as well as the maps of differences between these two experimental conditions (third panel from left). Sleep deprivation enhanced low-frequency power in both eyes-closed and eyes-open conditions, with similar EEG power topography: the largest increases occurred in the delta and theta bands, and deprivation showed a distinct regional specificity that is reflected in the \( t \)-values maps (right panel).

The inspection of the two baseline maps reveals stable patterns within different frequency bands. The delta and theta bands exhibit a centro-frontal midline predominance of power and minimum values over the temporal regions. In the alpha band the highest values are seen at the fronto-parieto-occipital midline areas reaching the zenith over the right occipital area where also the two beta bands were maximally represented. This frequency-specific regional pattern is roughly maintained after SD, with the largest increases of power in the slow frequencies over the midline regions. Statistical maps reveal significant changes limited to the delta and theta bands (two-tailed \( p = 0.01 \) corresponds to a \( t = 2.74 \)). The significant enhancement of the delta band encompasses most of the centro-frontal sites, spreading also to the parietal areas. The effect of SD on the theta band appears more focal, being particularly pronounced at the frontal areas and at the vertex. The occipital region also exhibits a peak of significant increase of theta power in the eyes-open condition.

Motor thresholds

As shown in Fig. 3, the three measures of motor thresholds point to a coherent pattern of effects as a consequence of SD. ST significantly increased after a 39-h SD [baseline = 1.63 (SE = 0.01), post-deprivation = 1.64 (SE = 0.01); \( F_{(1,31)} = 10.89; p = 0.002 \)]. Similarly, LT increased from baseline = 1.59 (SE = 0.01) to post-deprivation = 1.61 (SE = 0.01) \( (F_{(1,31)} = 4.37; p = 0.04) \), and UT increased from baseline = 1.66 (SE = 0.01) to post-deprivation = 1.67 (SE = 0.01) \( (F_{(1,31)} = 10.10; p = 0.003) \).

There was also an effect for gender, with females showing higher thresholds than males with respect to ST \( (\text{females} = 1.68 \text{ (SE = 0.02), males} = 1.60 \text{ (SE = 0.01); } F_{(1,31)} = 14.28; p = 0.0007) \),
LT [females = 1.65 (SE = 0.02), males = 1.56 (SE = 0.01); $F_{(1,31)} = 14.73; p = 0.0006$] and UT [females = 1.71 (SE = 0.02), males = 1.63 (SE = 0.01); $F_{(1,31)} = 13.55; p = 0.0009$]. The Gender × Treatment interaction was not significant for any measure of motor thresholds.

Intracortical excitability

As detailed in Fig. 4, which shows MEP amplitude changes of male and female subjects across the full range of ISIs considered, intracortical inhibition was found at 1 and 3 ms, while intracortical
facilitation is evident at 7, 10, 12 and 15 ms. Regardless of the experimental manipulation and of gender, a cortical inhibition was found at 1 ms (indicated by the 78.5% MEP decrease) and at 3 ms (59.8% MEP amplitude decrease). The cortical facilitation was expressed by an 11.4% MEP increase at 7 ms, a 39.5% increase at 10 ms, a 42.2% increase at 12 ms and a 40.1% increase at 15 ms.

The ANOVA on the SICI values (ISIs ranging from 1 to 3 ms) did not show any significant main effect or interaction ($F_{1,31} < 1$).

The same ANOVA design carried out on the SICF values (ISIs ranging from 7 to 15 ms) did not show any significant main effect. On the other hand, the SICF increased in females as a consequence of SD. The significant Gender × Treatment interaction ($F_{1,31} = 5.53; p = 0.02$) is explained by the significant enhancement of MEP amplitude in females [from baseline = 0.07 (SE = 0.03) to post-deprivation = 0.18 (SE = 0.04); $p = 0.01$ at the post hoc Fisher’s PLSD test]; this increase was not present in males [baseline = 0.09 (SE = 0.02); post-deprivation = 0.07 (SE = 0.03)].

**Combined EEG and TMS changes**

The topographical distribution of the correlation coefficients between TMS measures and EEG with eyes-closed and -open is

![Fig. 3](image-url) Mean ADM muscle motor thresholds (expressed as a percentage of the stimulator’s output) in male ($n=18$) and female ($n=15$) subjects during the baseline recording session (white bars) and after a 39-h sleep deprivation (black bars). Motor thresholds were measured according to standard procedures (standard threshold, ST), lower threshold (LT, defined as the highest stimulus intensity evoking responses in the relaxed ADM muscle with a probability of 0) and upper threshold (UT, defined as the lowest intensity evoking responses with a probability of 1).

![Fig. 4](image-url) Intracortical excitability curve measured by the paired-pulse technique, as a function of the experimental conditions. Mean changes in MEP amplitude expressed as the ratio between conditioned responses at different inter-stimulus interval (ISI) divided by test (unconditioned) responses, as a function of the different conditions [baseline and after a 39-h sleep deprivation (post-deprivation)]. Standard errors are also reported.
illustrated in Fig. 5. The figure depicts maps of the correlation coefficients between increases of theta activity after SD (ΔEEG power) and, respectively, increases of MTs (ΔMT) and of SICF at the 12-ms ISI (ΔSICF).

The first two panels from the left show the coefficients of the partial correlations, while the third panel reports the coefficients of the multiple correlations.

The increase of theta activity during the extended wakefulness was strongly associated to a less significant decrement of corticospinal excitability (ΔMTs), and a marked increase of intracortical facilitation, expressed by SICF at the 12-ms ISI (ΔSICF).

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The increase of theta activity during the extended wakefulness was strongly associated to a less significant decrement of corticospinal excitability (ΔMTs), and a marked increase of intracortical facilitation, expressed by SICF at the 12-ms ISI (ΔSICF).

The increased percentage of variance of EEG changes due to the increased sleepiness (i.e., maxima in the right side maps of Fig. 5) was explained by the contribution of functionally coherent TMS changes, that is, smaller decreases of corticospinal excitability are paralleled by larger increases of intracortical facilitation.

The maps of partial correlations point to larger contributions of MT changes in the eyes-closed condition (significant at the F7, F1 and T3 sites), and of SICF changes in the eyes-open condition (significant at the F7 site). In both cases, partial correlations show a widespread topographical congruence.

In order to exclude that the topographical pattern of correlations between TMS and EEG measures could be explained as a direct consequence of the TMS stimulation and not a specific effect of SD, the EEG recordings at 21.00 h, corresponding to the last session of another protocol (Marzano et al., 2007), were analyzed and the increase of theta power after a 36-h of extended wakefulness (ΔEEG power) and without TMS was again correlated to the TMS measures. Actually, this session had some procedural differences: (a) subjects were sitting on a comfortable chair; (b) the eyes-closed condition lasted 2 min; and (c) the eyes-open condition lasted 4 min.
As shown by Fig. 6, topographical maps confirm that an increased sleepiness is associated with minor decreases of corticospinal excitability and major increases of intracortical facilitation. Although significantly only in the eyes-closed condition, this topographical pattern mostly resembles what was previously described in Fig. 5 and was found by comparing equivalent experimental conditions. Also in the present case, maxima were found in the frontal and prefrontal regions (significant at the F7 site for the eye-closed condition).

Discussion

This is the first combined analysis of TMS-evoked responses and EEG topography of waking under conditions of normal and increased sleepiness. The present study showed in a large sample of subjects that one night of sleep deprivation profoundly affects cortical activation, as indexed by EEG power and motor threshold measures. This picture of general deactivation is paralleled by the increase of subjective sleepiness, as well as the decrease of subjective alertness. On the other hand, the increase of cortical facilitation in females, expressed by the higher MEP amplitude elicited by paired pulses at longer ISIs after SD, partially confirms the prediction of the synaptic homeostasis hypothesis (Tononi and Cirelli, 2003, 2006). Another indirect support to this hypothesis comes from the results of the combined analysis of EEG and TMS changes, as a consequence of a prolonged wakefulness. In fact, the EEG signature of sleep propensity, that is, the large increase of theta power, corresponds to smaller decreases of corticospinal excitability and larger increases of intracortical facilitation, as induced by transcranial magnetic stimulations.

Waking EEG measures of sleepiness

With specific regard to the effects of sleep deprivation on waking EEG measures, this is the first study reporting on the topographic distribution of EEG power recorded in two different conditions: eyes-closed and eyes-open, and with the largest sample of subjects ever recorded in this kind of experimental protocol. Our results confirm and extend the findings of the only topo-

Effects of sleep deprivation on TMS measures of cortical and corticospinal excitability

Sleep deprivation affected TMS measures in different ways with a decreased corticospinal excitability and an increased intracortical facilitation, although limited to females. As regards motor thresholds, all the three measures showed a coherent behavior, with significant increases as a consequence of SD. The lack of significant changes in motor thresholds as a consequence of SD found in preliminary studies (Civardi et al., 2001; Manganotti et al., 2001, 2006) is probably explained by the small samples and by some confounding effects of circadian factors. On the other hand, the current study considered quite a large sample and measured MTs at the same time of day, after an interval of 48 h, and with the same experimental procedure.

Although MTs reflect the excitability of the corticospinal system, and the exact locus of the changes in excitability along the corticospinal pathway cannot be determined, the about 3% increase found after SD should be mainly explained by the contribution of peripheral factors. Critical for this interpretation, the cortical EEG topography of sleepiness, as indexed by increases of theta activity, negatively correlates with changes in motor threshold, meaning that a higher sleep propensity associated to the fronto-central increase of theta EEG power is predicted by smaller differences in MTs recorded before and after SD. Hence, a relative increase of corticospinal excitability expressed by the parallel increase of three different measures of motor thresholds is related to the heightened slow-frequency EEG activity characterizing states of profound sleepiness, and this “double-face” of corticospinal excitability seems compatible in view of an interpretation of a general effect of sleepiness on peripheral motor responses, while the cortical components of the motor system involved in the MT measures appear to show an at least unchanged activation. Such a dissociation between the central and peripheral components of the motor system has been found after awakenings from REM sleep with an increased cortical excitability parallel to the spinal inhibition of this stage (De Gennaro et al., 2004a,b). The increased activation measured by fMRI in the left frontal gyrus (Brodmann areas 45 and 47) in sleep-deprived subjects, with worsened performances at a verbal learning task (Drummond et al., 2005), also points to a dissociation between cortical and behavioral levels.

More unequivocally, the increased cortical facilitation in females on the SICF values (ISIs ranging from 7 to 15 ms) points to a significant enhancement of MEP amplitude after delivering conditioning pulses. SICF is thought to reflect the activation of excitatory interneurons mediated by glutamatergic NMDA receptors (Liepert et al., 1997; Ziemann et al., 1996; 1998; Schwenkreis et al., 2000). Glutamate levels increase in the rat and human brain after SD (Bettendorff et al., 1996; Murck et al., 2002; Sallanon-Moulin et al., 1994). An increased glutamatergic transmission could tentatively be responsible for both the heightened cortical facilitation during prolonged wakefulness and the potentiation of synaptic strength due to long-term potentiation (LTP) mechanisms, associated to plastic changes occurring during wakefulness.
The CBF higher levels at the end of the waking day than after a night of sleep (Braun et al., 1997) and the positive correlation between regional CBF and time of day (Christina et al., 2006) also supports this interpretation. Hence, the increased cortical facilitation in sleep-deprived subjects lends some support to the synaptic homeostasis hypothesis, which predicts that learning and plasticity processes during wakefulness lead to a net increment of synaptic weight in several cortical circuits (Tononi and Cirelli, 2003; 2006). Overall, the strength of intracortical connections reaches a maximum toward the end of the day. The hypothesis predicted that the amplitude of TMS-evoked potentials on the scalp should be lower after a night of sleep, higher at the end of a day of wakefulness and should show a further increase after a prolonged SD. At least for females, this prediction was directly confirmed. Furthermore, the combined TMS-EEG approach seems coherent with the hypothesis also in the whole sample. In left and midline frontal and prefrontal regions the rise of theta activity is significantly explained by both lower MT changes and higher cortical facilitation. Maxima of these relationship at the left frontal EEG recording site correspond to a 47% and 44% of variance in the eyes-closed and -open conditions and cannot be explained by the consequences of the TMS stimulation itself, since the same association was found in different waking EEG recordings carried out before the TMS protocol.

Even though it is beyond the aims of the current study, TMS measures also predict some aspects of sleep homeostasis. In fact, changes in the TMS measures are significantly related to the homeostatic increase measured during sleep subsequent to SD. Like theta activity after extended wakefulness, the 24% increase of theta EEG power at the left frontal EEG recording site during NREM sleep of the recovery night as compared to the baseline night preceding SD ($F_{(1,32)} = 16.26; p = 0.0003$) is also significantly associated to waking MT and SICF changes. Multiple correlation between $\Delta$MT and $\Delta$SICF vs. $\Delta$EEG power (during NREM sleep) was significant ($r = 0.53; p = 0.006$) with a significant contribution of $\Delta$SICF (partial $r = 0.43; p = 0.01$) and a close-to-significance contribution of $\Delta$MT (partial $r = 0.27; p = 0.09$).

**Gender differences**

The issue of gender differences in brain anatomy and function may represent one of the most important challenges for neuroscientists in next years, after having been neglected so long (Cahill, 2006). Unfortunately, a very scarce information is available on the differences between males and females concerning TMS measures. Females tended to have larger MEP variability (Pitche et al., 2003) and to show a higher transcallosal inhibition than males using the interhemispheric paired-pulse technique (De Gennaro et al., 2004a,b), while no significant gender difference has been found in MTs (Fratello et al., 2006). Most studies simply did not consider this issue, even when they have mixed samples. In the current sample, females showed higher thresholds than males, and the SICF increased only in females as a consequence of SD. These findings are in line with some predictions of the Synaptic Homeostasis Hypothesis (Tononi and Cirelli, 2003; 2006).

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