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Wakefulness delta waves increase after cortical plasticity induction

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HIGHLIGHTS

- iTBS-induced (intermittent theta burst stimulation) plasticity increases delta EEG.
- Delta waves emerge as effectors of cortical plasticity in wakefulness besides sleep.
- In patients affected by brain lesions, the meaning of slow EEG waves can be reinterpreted.

ABSTRACT

Objective: Delta waves (DW) are present both during sleep and in wakefulness. In the first case, DW are considered effectors of synaptic plasticity, while in wakefulness, when they appear in the case of brain lesions, their functional meaning is not unanimously recognized. To throw light on the latter, we aimed to investigate the impact on DW exerted by the cortical plasticity-inducing protocol of intermittent theta burst stimulation (iTBS).
Methods: Twenty healthy subjects underwent iTBS (11 real iTBS and nine sham iTBS) on the left primary motor cortex with the aim of inducing long-term potentiation (LTP)-like phenomena. Five-minute resting open-eye 32-channel EEG, right opponens pollicis motor-evoked potentials (MEPs), and alertness behavioral scales were collected before and up to 30 min after the iTBS. Power spectral density (PSD), interhemispheric coherence between homologous sensorimotor regions, and intrahemispheric coherence were calculated for the frequency bands ranging from delta to beta.
Results: Real iTBS induced a significant increase of both MEP amplitude and DW PSD lasting up to 30 min after stimulation, while sham iTBS did not. The DW increase was evident over frontal areas ipsilateral and close to the stimulated cortex (electrode F3). Neither real nor sham iTBS induced significant modifications in the PSD of theta, alpha, and beta bands and in the interhemispheric coherence. Behavioral visuo-analogic scales score did not demonstrate changes in alertness after stimulations. No correlations were found between MEP amplitude and PSD changes in the delta band.
Conclusions: Our data showed that LTP induction in the motor cortex during wakefulness, by means of iTBS, is accompanied by a large and enduring increase of DW over the ipsilateral frontal cortex.
Significance: The present results are strongly in favor of a prominent role of DW in the neural plasticity processes taking place during the awake state.

1. Introduction

Delta waves (DW, <4 Hz) are the most prominent electroencephalographic (EEG) feature of human non-rapid eye movement (NREM) sleep, which have their origin in cortical layers. Several studies proposed them as sensors for weighing synaptic efficacy and possible effectors of sleep-dependent synaptic plasticity (for a review, see Tononi and Cirelli, 2012). This evidence relies on several animal experiments demonstrating that DW recorded over the scalp are the EEG counterpart of near-synchronous transitions between up and down states involving large populations of cortical neurons (Steriade et al., 1993, 2001). Large-scale simulations (Esser et al., 2007) and human studies (Riedner et al., 2007; Vyazovskiy et al., 2009) show that the amplitude and slope of DW are proportional to the number of cortical neurons entering such up/down states near-synchronously. This synchrony is directly related to the number and strength of synaptic connections among them. The data indicating that DW can be effectors of sleep cortical

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plasticity come mainly from high-density EEG studies in humans. For instance, sleep DW increase locally over the parietal cortex following learning of a visuomotor task (Huber et al., 2004). On the contrary, the arm immobilization during the day is followed by reduced sleep DW over the contralateral sensorimotor cortex, which goes in parallel with a decrease of motor performance and of sensory responses evoked by the stimulation of the nerve of the arm, consistently with the induction of a synaptic depression (Huber, 2006). Along this line, neuromodulatory techniques (i.e., paired-associative stimulation) able to induce synaptic cortical plasticity change the DW amount during sleep (Huber et al., 2008). DW changes triggered by the induction of cortical plasticity mainly occur in the stimulated regions, but are not necessarily confined to the site of the stimulation (Huber et al., 2007; Bergmann et al., 2008; De Gennaro et al., 2008). On the other hand, spontaneous DW during NREM sleep originate at a well-defined site (more frequently in prefrontal–orbitofrontal regions) and propagate in an orderly fashion to the rest of the scalp as a traveling wave (Massimini et al., 2004). In wakefulness, DW are almost absent in physiological conditions, but they appear when a subcortical brain lesion occurs requiring an intact cortex (Gloor et al., 1977; Steriade et al., 1993, 2001). Therefore, wakefulness DW are interpreted as a lesional sign, despite conclusive data about their functional significance still being missing. From a mere physical point of view, an increase of DW may originate from a higher number of synchronously oscillating neurons or from a stronger activity of such neurons. Both of these theories converge towards the hypothesis of a focused information processing, which might aim to induce local or network plasticity (Carmichael and Chesselet, 2002; Topolnik et al., 2003; Mazevet et al., 2003; Assenza et al., 2013). Although wake (injury-related) and sleep DW do not share generating pathways and topographical distribution, they are similar due to their EEG frequency and their neocortical origin; furthermore, they share the association with cortical plasticity phenomena. This evidence led us to investigate the causality of the linkage between wake DW and neuronal plasticity in humans. Therefore, we noninvasively induced cortical plasticity to explore changes of the EEG slow activity during wakefulness. Intermittent theta burst stimulation (iTBS), a robust neuromodulatory technique able to induce a reliable and prolonged shift in cortical excitability via long-term potentiation (LTP)-like plastic phenomena (Huang and Kandel, 2005; Di Lazzaro et al., 2008), was provided to healthy individuals to test our hypothesis.

2. Materials and methods

The study was approved by the Ethics Committee of Campus Bio-Medico University. Informed written consent was obtained from all subjects. We enrolled 20 right-handed healthy subjects (11 males). All subjects were right handed as self-reported. None of the subjects was taking drugs acting on the central nervous system.

2.1. Experimental design

The main aim of the study was to evaluate the effects of iTBS on brain activity and connectivity by means of EEG. To this end, we collected resting-state EEG and motor-evoked potentials (MEPs) produced by transcranial magnetic stimulation (TMS) before and after a real iTBS ($N = 11$, age 25 ± 5 years, six males) and a sham iTBS ($N = 9$, age 25 ± 4, five males). The change of MEP amplitude is widely accepted as an indirect measure of the effects of neuromodulatory techniques on brain excitability and, thus, of the induced motor cortical plasticity (Di Lazzaro et al., 2008). We identified four time points (Fig. 1): T_0, before iTBS (corresponding to the baseline); T_1, immediately after iTBS; T_2, 15 min after iTBS; and T_3, 30 min after iTBS. These time points were chosen to evaluate the long-lasting iTBS-dependent modulation of EEG activity and MEP. In order to estimate possible fluctuations of vigilance/attention, the following behavioral scales were administered at each time point (T_0, T_1, T_2, and T_3): sleepiness and anxiety visual analog scale (VAS) scales (ranging from 0 to 10) and Stanford Sleepiness Scale (Hoddes et al., 1971). All the experimental procedures were performed in a quiet room with the subject lying supine on a bed, with eyes opened, wearing earplugs that masked the TMS stimulus noise. Subjects were instructed to abstain from caffeine/alcohol and to maintain their regular sleep/wake schedule for at least 3 days before the experimental session. iTBS was applied over the left dominant hemisphere, whereas the activity/connectivity EEG modulations were evaluated bilaterally. Experimental sessions started at 10:00 a.m. with the placement of the EEG cap. The achievement of impedances of all electrodes below 5 kΩ required on average 20–30 min. After this technical adjustment, the first EEG recording started.

2.2. Transcranial magnetic stimulation

TMS was carried out in accordance with the safety guidelines suggested by Rossi and Hallett (2009). Considering the influence of ovarian hormones on human cortical excitability (Smith et al., 2002), the experiments with female subjects were always performed during the early follicular phase (Days 5–10, Day 1 being the first menstrual day). We employed a Rapid Magstim stimulator (Magstim Company, Dyfed, UK) connected to an eight-shaped coil with an inner diameter of 70 mm for each wing. The TMS pulse was always delivered with the coil tangentially placed to the scalp with the handle pointing anteromedially from the midline at 45°. Employing a biphasic waveform, we induced an anteroposterior followed by posteroanterior (AP–PA) current in the brain (Kammer et al., 2001). Muscle twitches triggered by TMS were recorded from the opponens pollicis (OP) of the right hand. The EMG signal was collected using Ag–Cl surface electrodes arranged in a standard tendon–belly montage, amplified, and recorded by a BrainAmp System (BrainProducts GmbH, Munich, Germany) via 1–2000-Hz filter setting with a 5-kHz sampling rate. The time window in which the poststimulus analysis was performed was set to 50 ms.

2.3. Excitability modulation assessment

After positioning the EEG cap, the hot spot for the right OP primary motor cortex (M1) and the resting motor threshold (rMT) were identified according to international guidelines (Rossini et al., 1994). We also collected the active motor threshold (aMT) corresponding to the lowest stimulator intensity able to produce an MEP amplitude of 200 μV during a 10% maximum voluntary contraction of the OP muscle (Di Lazzaro et al., 2005). At T_0, T_1, T_2, and T_3, the left M1 excitability was assessed by applying 15 single TMS pulses (inter stimulus interval of 5 s on average, 10% jittered), using a stimulator intensity output set to 120% of rMT. The maximal peak-to-peak MEP amplitude was analyzed off-line using Matlab 2011 (The Mathworks, Inc., Natick, MA, USA).

2.4. Intermittent theta burst stimulation

iTBS was delivered using the same stimulation equipment (stimulation intensity set to 80% of aMT). Real iTBS consisted of bursts of three pulses delivered at 50 Hz (20 ms between each pulse) repeated at 5 Hz (200 ms between each burst). The bursts have been combined in trains where each train consists of 10 bursts and lasts 2 s. Twenty trains have been repeated every 10 s

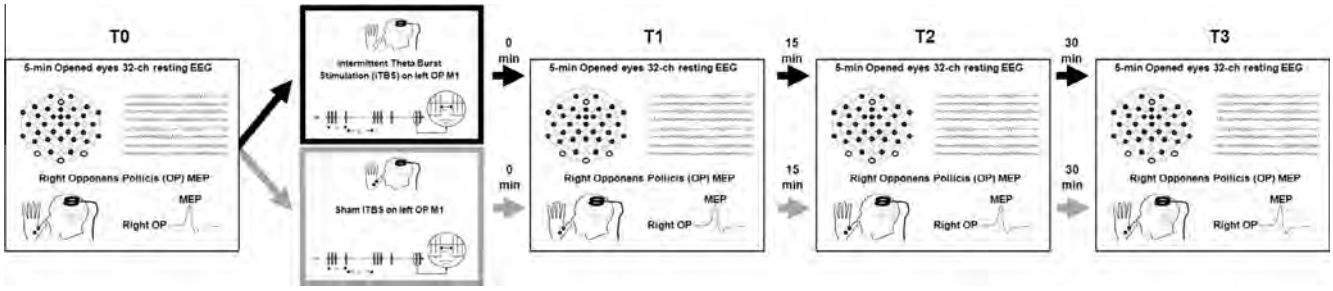

Fig. 1. Scheme of the experiment. Five minutes of 32-channel resting open-eye EEG and motor-evoked potential (MEP) of right opponens pollicis (OP) muscle were recorded at T_0, immediately after (T_1), 15 min (T_2) after, and 30 min (T_3) after intermittent theta burst stimulation (iTBS; 13 healthy subjects) or sham iTBS (9 healthy subjects) applied on OP scalp hot spot. Changes of EEG power spectral density and interhemispheric coherence between homologous regions were analyzed all over the scalp.

(inter-train interval of 8 s) for a total of 190 s when 600 pulses have been delivered (Suppa et al., 2008). The Rapid Magstim stimulator does not support the iTBS stimulation protocol for stimulus intensities >35% of the maximal stimulator output; thus, we had to rule out from the study each subject with aMT >42% of the maximal stimulator output (two female subjects having an aMT of 50% and 52%, respectively, were ruled out after this screening). In our subjects' group, the stimulation intensity was 30 ± 2% (mean ± standard deviation) of the maximal stimulator output.

Sham stimulation was provided with the same experimental setup, but the coil touching the scalp of the subject was not plugged to the stimulator, and another coil, tilted by 90° on the sagittal plane, was made to lean over the first coil and was connected to the stimulator to provide the real acoustic/mechanical stimuli of iTBS stimulation at a fixed intensity of 30% of the maximal stimulator output (Talelli et al., 2012).

2.5. EEG recording

A 32-channel TMS-compatible EEG (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) recording was acquired at rest with eyes opened (5 min) for each time point. We used scalp electrodes mounted on an elastic cap, according to the 10–20 international system (Fp1, Fp2, F3, F4, C3, 226 C4, P3, P4, O1, O2, F7, F8, P7, P8, T7, T8, FZ, CZ, PZ, FC1, FC2, CP1, CP2, FC5, FC6, FT9, FT10, FCZ, CP5, CP6, TP9, and TP10) and a binaural reference. Vertical and horizontal electrooculographs were recorded bipolarly in order to control eye movement-related artifacts. The impedance of all electrodes was kept below 5 kΩ. EEG data were sampled at 5000 Hz (pre-sampling analogical band-pass filter set at 0.48–256 Hz, BrainAmp System).

2.6. EEG data analysis

EEG data analysis was performed off-line by means of Matlab 2011 (The Mathworks, Inc., Natick, MA, USA). Power spectral density (PSD) analysis for delta (1–4 Hz), theta (4–8), alpha (8–12), and beta (12–33) bands was performed employing the Matlab toolbox EEGLab (Delorme and Makeig, 2004). In order to increase the local specificity, the scalp current density transformation was applied before power calculation.

Cortico-cortical coherence (COH) was focused on the signal coupling between homologous sensory–motor regions and evaluated for all the possible combinations of interhemispheric (F3C3–F4C4, C3P3–C4P4, CP1CP5–CP2CP6, and FC1FC5–FC2FC6) and intrahemispheric (F3C3–C3P3, F4C4–C4P4, FC1FC5–CP1CP5, and FC2FC6–CP2CP6) bipolar derivation. The resting-state EEG recordings were visually inspected to exclude epochs affected by artifacts. In particular, because eye movements and DW share the same frequency band, it is usually very difficult to disentangle the two, even employing the most advance computational technique of artifact rejection. Being well aware of such an issue, we took care of manually discarding segments of EEG corresponding to eye movements. This is the reason why only about 4 min of resting EEG out of 5 min, for each time point, were analyzed for each subject. PSD was calculated through a standard Fourier fast transform approach using the Welch technique and a Hanning windowing function (window 1024 ms, overlap 0.5). COH was computed using a fixed number of 120 averaged epochs. PSD and COH were estimated for all the following frequency bands: delta (1–4 Hz), theta (4.5–7.5 Hz), alpha (8–13 Hz), and beta (13.5–33 Hz).

2.7. Statistical analysis

Excitability: After checking the frequency distribution of MEP amplitudes by means of the Kolmogorov–Smirnov test for each time point (T_0, T_1, T_2, and T_3) and group (*Real* and *Sham* iTBS), we evaluated whether the baselines (T_0) differed between the two groups by means of an independent sample *t*-test. Then, we computed a repeated-measure ANOVA with factor *Time* (T_0 vs. T_1) as a within-subject factor and *Stimulation* as a between-subject factor. Paired sample *t*-tests were then performed to assess the differences between consecutive time points (T_2 vs. T_1 and T_3 vs. T_2) in the *Sham* and *Real* groups, distinctively.

EEG: We applied a parametric analysis within EEGLab (Delorme and Makeig, 2004) with *Time* (T_0 vs. T_1) as the within-subject factor and *Stimulation* as the between-subject factor for each frequency band (delta, theta, alpha, and beta).iTBS-related modulations on COH for each frequency band and behavioral scales were evaluated using a similar approach.

In order to investigate the reciprocal link among excitability, EEG activity, and connectivity, we computed Pearson's correlation coefficients after checking for putative violations of the underlying assumptions. For all the statistical analyses, a *p*-value <0.05 was considered significant and the alpha inflation due to multiple comparisons was realized according to the Bonferroni–Holmes procedure.

3. Results

3.1. iTBS effects on brain excitability (Fig. 2)

At T_0, in the real iTBS group, the mean MEP amplitude across subjects was 614.81 ± 123.35 μV (mean ± standard error), and in the sham iTBS group the average MEP amplitude across subjects was 542.61 ± 151.17, without a significant difference ($p = 0.715$).

Neither the factor *Time* nor the factor *Stimulation* was significant ($p = 0.738$ and $p = 0.075$, respectively), but we found a significant *Time*-by-*Stimulation* interaction ($F(3.51) = 3.366$, $p = 0.026$). At T_1, the MEP amplitude increased after real iTBS

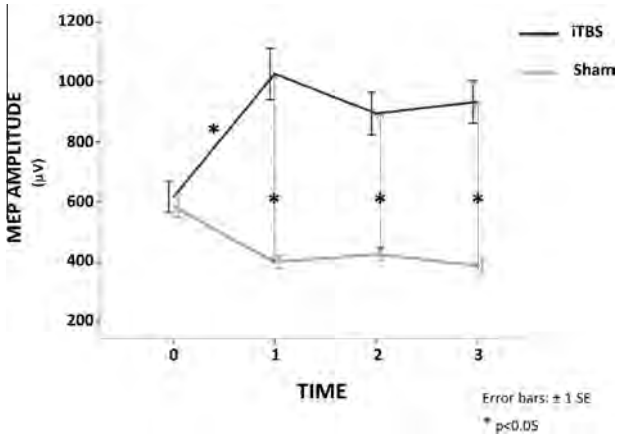

Fig. 2. Motor-evoked potential amplitude after iTBS. Absolute amplitude (μV) of opponens pollicis motor-evoked potential (MEP) in the real iTBS group (black line) and in the sham iTBS group (gray line) at the four time points: 0 = before iTBS; 1 = immediately after iTBS; 2 = 15 min after T_1; 3 = 30 min after T_1. Data are shown as mean ± 1 standard error. SE = standard error.

(T_1 = 1027.29 ± 243.20 μV and T_0 = 614.82 ± 125.34 μV, paired sample *t*-test $t = 2.255$, $p = 0.048$), demonstrating the efficacy of real iTBS in inducing a shift in cortical excitability. As expected, in the sham group, iTBS did not change the MEP amplitude (T_0 = 597.86 ± 159.55 μV; T_1 = 413.82 ± 53.60 μV; paired sample *t*-test $t = -1.283$, $p = 0.240$). At T_1, the MEP amplitude was higher after real iTBS than after sham iTBS (paired sample *t*-test $t = 2.463$, $p = 0.032$). At T_2, the MEP amplitude was higher after real iTBS than after sham ($t = 11.549$, $p = 0.050$). At T_3, the MEP amplitude was higher after real iTBS than after sham ($t = 10.929$, $p = 0.028$). Overall, we observed a clear increase of brain excitability after real iTBS compared to baseline and sham iTBS.

3.2. iTBS effects on brain activity

3.2.1. Delta band

We found a *Time*-by-*Stimulation* interaction suggesting that iTBS produced different effects on delta-band PSD in the two groups. This effect was more evident over the left frontal region – F3 electrode ($p = 0.013$) – ipsilateral and close to the stimulated cortex. In Fig. 3, we report the Real and Sham groups' delta-band PSD at T_1, T_0, and $T_1 - T_0$, and the statistical values. No delta-band EEG differences were found comparing the two groups along subsequent time points.

3.2.2. Power spectral band modulation from theta to beta bands (Fig. 4)

No effects of sham and real iTBS stimulation on EEG PSD for theta, alpha, and beta frequency bands were found throughout the scalp ($p > 0.05$).

3.2.3. iTBS effects on brain connectivity – all frequency bands

We did not found any significant modulation of intrahemispheric and interhemispheric coherence across different frequency bands.

3.2.4. Relation between changes of brain excitability and brain activity

We applied Pearson's correlation coefficients between delta PSD and MEP changes induced by iTBS. We did not found any significant correlation ($p > 0.08$).

3.2.5. Behavioral scales

No changes of sleepiness or anxiety were found among the different time points.

4. Discussion

Our experiment demonstrates an increase of DW in the cortex close to that undergoing LTP via iTBS during wakefulness.

4.1. Plasticity induction

iTBS is a reliable neuromodulatory technique able to induce transient LTP-like cortical plasticity (Huang and Kandel, 2005). Plastic effects are obtained enhancing the excitability of excitatory inputs to pyramidal neurons, showed by an increase of MEP amplitude (Di Lazzaro et al., 2008). In the present study, iTBS significantly increases the MEP amplitude, although less evidently than we previously showed (Di Lazzaro et al., 2011). Nevertheless, the present results are well conceivable in the light of the recent work from Hamada and colleagues (Hamada et al., 2013), who reported that only a minority of participants out of a large population have the "expected" increase of cortical excitability after iTBS and that individual responses can be considerably variable. The induced plasticity in our healthy subjects lasted at least the whole duration of the experiment, that is, 30 min after iTBS administration, in accordance with previous reports (for a review, see Thut and Pascual-Leone, 2010).

4.2. Resting EEG changes induced by iTBS

A single TMS pulse is able to instantaneously synchronize EEG oscillatory activity in the delta band with a corresponding PSD increase (Manganotti et al., 2013). The numerous attempts to establish the effects of neuromodulatory TMS on resting EEG, by means of repetitive protocols, obtained so far inhomogeneous results, possibly because of the high heterogeneity of the stimulation protocols employed (Okamura et al., 2001; Strens et al., 2002; Schutter et al., 2003; Klimesch et al., 2003; Griskova et al., 2007; Brignani et al., 2008; Fuggetta et al., 2008; Grossheinrich et al., 2009; Noh et al., 2012; Vernet et al., 2013). Okamura and colleagues found an increased frequency and amplitude of the total EEG spectrum, including the delta band, after 3 s of frontal 10-Hz repetitive TMS (rTMS), but did not evaluate, in parallel, the possible induced cortical plasticity. Griskova et al. (2007) administered a 10-Hz rTMS over the left dorsolateral prefrontal cortex (DLPFC) and induced a bilateral huge and selective increase (about 200%) of the delta band power, exactly as in the present study. They did not evaluate MEP modulation and speculated that the delta power increase was related to a hypothetic hemodynamic modification of regional cerebral blood flow. Grossheinrich et al. (2009) applied continuous TBS (cTBS, a TBS paradigm able to decrease cortical excitability), iTBS, and sham stimulation over the DLPFC of healthy individuals and observed a specific alpha power enhancement only after iTBS. However, in this study, delta-band analysis was performed considering an unusual frequency range (1.5–6 Hz), which also included some of the "classic" theta band (Niedermeyer and Lopes Da Silva, 2005), which, in our experiment, has not been significantly modified by iTBS. Furthermore, with TBS sessions being delivered only 1 h apart and intermixed with neuropsychological assessments, it was not possible to rule out cognitive contamination of the EEG activity. Finally, the iTBS aftereffects are estimated to last up to 1 h (for a review, see Thut and Pascual-Leone, 2010) and a possible intersession reciprocal adulteration should be considered. Other authors have not included the analysis of the EEG delta band in their investigations and have only focused on higher bands, probably because of their strong involvement in cognitive and motor functions (Klimesch et al., 2003; Fuggetta et al., 2008; Brignani et al., 2008; Noh et al., 2012; Vernet et al., 2013). To the best of our knowledge, the present experiment is

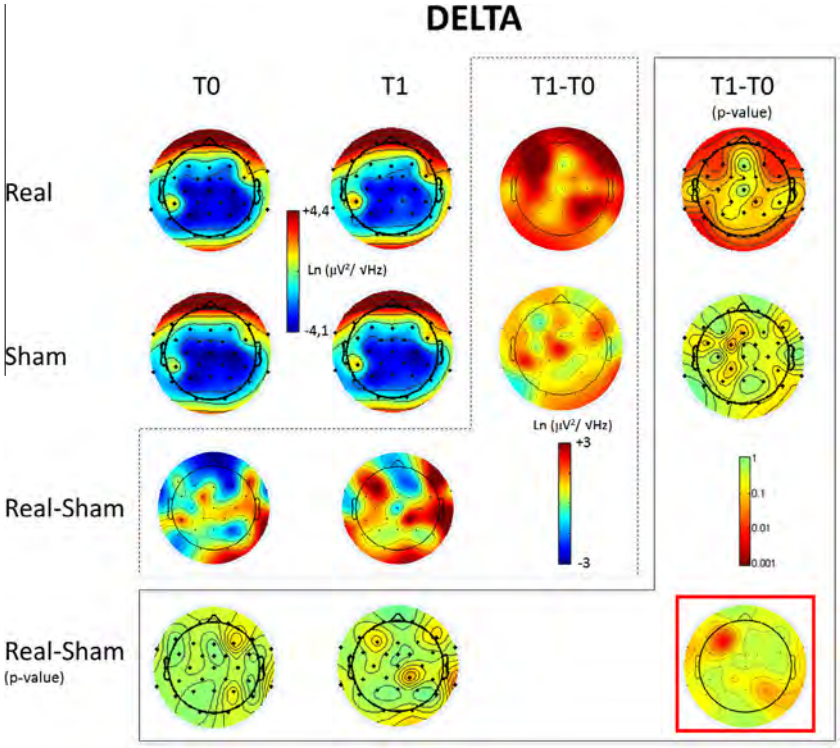

Fig. 3. PSD variation after iTBS in delta EEG frequency band. Mean EEG PSD in the delta frequency before (T_0) and immediately after real and sham iTBS (T_1). In the dashed line, PSD differences between T_1 and T_0 for real and sham iTBS (vertical line) and between real and sham iTBS at T_0 and at T_1 (horizontal line). In the continuous line, *p*-value for differences between T_1 and T_0 for real and sham iTBS (vertical line, paired samples) and between real and sham iTBS in T_0 and T_1 (horizontal line, unpaired samples). In the red box in the lower right corner, *p*-value of the Time-by-Stimulation effect. Note the significant effect is confined to the F3 electrode, ipsilateral and close to the stimulated cortex. PSD: power spectral density. iTBS: intermittent theta burst stimulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the first one evaluating EEG power spectral modifications in a frequency range from delta to beta bands, caused by the induction of cortical plasticity via neuromodulatory techniques. We found a selective EEG power increase in the delta band, as in the work by Griskova et al., and, different from other reports, we did not observe any clear change in theta, alpha, or beta bands. Noh et al. (2012) observed that cTBS increased theta and beta band power over the stimulated areas and Klimesch reported (2003) an enhancement of task-related alpha desynchronization after rTMS. These experiments required a cognitive task execution between EEG recordings, so that an effect on attentive motor cortical networks cannot be completely ruled out. In Vernet et al. (2013), an alpha and beta band increase was found after cTBS without any intermingled motor task, but they acquired EEG data with closed eyes, therefore increasing the total amount and sensitivity of the alpha power band. Furthermore, they recorded EEG activity after 10–30 single-pulse TMS as their main goal was to study TMS-induced EEG potentials and synchronization, while we performed the EEG recording before TMS evaluation. These methodological discrepancies might account for the different results observed across studies, in particular for alpha and beta band changes. The studies by Fuggetta et al. (2008) and Brignani (2008) also reported modulation of alpha and beta activity but their experimental setups were completely different from the one we adopted, as they recorded EEG activity between single-train rTMS stimuli and single-pulse TMS, respectively, to study EEG perturbation during TMS.

The selectivity of the iTBS effect on the delta power may subtend an activation of a neuronal oscillator specific for the delta band. Indeed, repetitive TMS protocols were demonstrated to synchronize EEG oscillations (Veniero et al., 2012) with a high topographical selectivity. This is the case of DW in frontal areas, which are the main source of the so-called slow traveling wave of sleep (Ferri et al., 2005; Murphy et al., 2009). Over these regions, there is a higher chance of triggering, by means of TMS pulses, slow waves resembling those of physiological NREM sleep (Massimini et al., 2004).

4.3. Topography of DW increase

We observed a focal increase of DW (Fig. 3) over the frontal cortex, ipsilateral and close to the stimulated site. A possible explanation of this effect may come from the shift of motor cortex excitability obtained by LTP-like phenomena induced by iTBS as expressed by the increased amplitude of the corresponding MEP. An alternative interpretation may involve changes of neuronal synchronization/networking as independent from excitability modifications. In our cohort, iTBS did not modify either interhemispheric or intrahemispheric COH, an index of neuronal functional coupling (Mima, 2004; Di Pino et al., 2012; Pellegrino et al., 2012), suggesting that EEG changes would act without synchronizing huge neural populations (Thut et al., 2011; Veniero et al., 2012). Indeed, with the methods used in the present study, it is not possible to rule out effects on synchronization among small clusters of neurons located in the stimulated hemisphere. Furthermore, as reported in the previous paragraph, the DW increase in frontal areas may occur from an iTBS-induced activation of cortical oscillators specific for the delta band, which reside in frontal areas as demonstrated in sleep (Massimini et al., 2004). The simple activation of oscillators, instead of a network modulation, may also explain the lack of an increase in cortical coherence and its dissociation with respect to the power spectrum density.

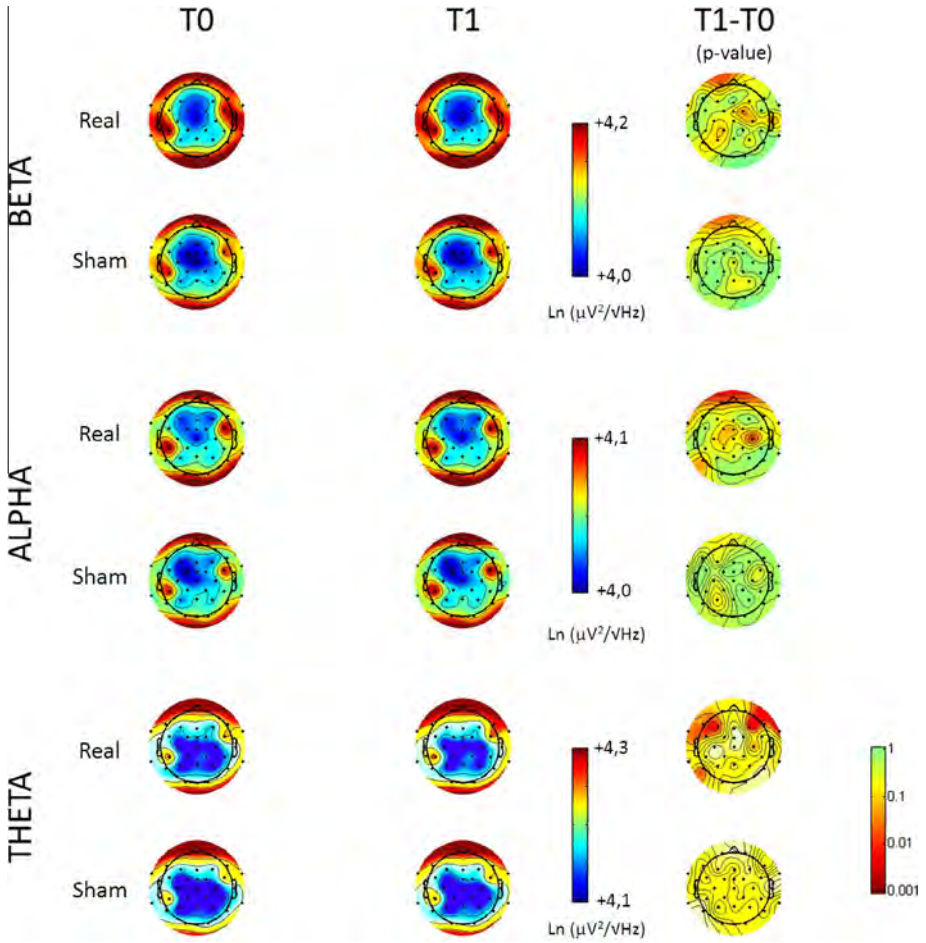

Fig. 4. Scalp distribution of PSD in theta, alpha, and beta bands after iTBS. Mean EEG PSD in beta, alpha, and theta frequency bands before (T_0, left column) and immediately after real and sham iTBS (T_1, central column). In the right column, *p*-value for differences between T_1 and T_0 for real and sham iTBS were provided. PSD: power spectral density. iTBS: intermittent theta burst stimulation.

4.4. Can DW during wakefulness be a sign of plasticity?

Our results support the working hypothesis that, also during wakefulness, an increase of DW occurs in parallel with the induction of an LTP-like plasticity. The possibility that iTBS may affect delta activity arises from Tononi's synaptic homeostasis hypothesis (SHY) – DW during sleep are sensors of synaptic weight and possible effectors of sleep-dependent synaptic plasticity (Tononi and Cirelli, 2012) – seen in the frame of our experience about the prognostic value of EEG activity in stroke patients (Graziadio et al., 2012; Pellegrino et al., 2012; Assenza et al., 2013).

In other words, DW above lesional and contralateral areas may be not merely a marker of network dysfunction but more a sign of neuronal rearrangement phenomena accompanying the acute and chronic phases of recovery (Tecchio et al., 2007; Assenza et al., 2009, 2013; Di Lazzaro et al., 2010). Accordingly, DW could be an epiphenomenon of cortical ongoing plasticity during wakefulness as during sleep and of the attempt of the cortex to reestablish a near-physiological functioning. Animal studies provide further data supporting the "active" role of DW in the awake state. In stroke rats, Carmichael and Chesselet (2002) demonstrated that DW in the contralesional hemisphere might function as an attraction guide for interhemispheric fiber sprouting. Finally, spontaneous physiological DW during sleep and lesion-induced DW during wakefulness share common features: both of them have cortical origin (Riedner et al., 2007 for a review and Ball et al., 1977, respectively) and reflect an oscillating state of synchronous hyper- and hypo-activation of a large group of neurons (Steriade, 2006; Topolnik et al., 2003).

In conclusion, our data documented that the cortical plasticity, produced by iTBS, in awake subjects is accompanied by an increase in EEG delta activity in the frontal areas ipsilateral and close to the stimulated cortex. The time frame of the increase is in line with the time needed by the LTP-like plasticity to arise. These results confirm the prominent role of DW in the processes behind neural plasticity, and extend it, beyond sleep, to the wakefulness. Present data may open new scenarios in the interpretation of scalp EEG slow wave components in patients affected by brain lesions, considered so far only a negative sign of the damage.

Conflict of Interest

None.

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