Assessment of Event-Related EEG Power After Single-Pulse TMS in Unresponsive Wakefulness Syndrome and Minimally Conscious State Patients

Emanuela Formaggio[1] · Marianna Cavinato[1] · Silvia Francesca Storti[2] · Paolo Tonin[1] · Francesco Piccione[1] · Paolo Manganotti[3]

Accepted: 7 November 2015

Abstract In patients without a behavioral response, non-invasive techniques and new methods of data analysis can complement existing diagnostic tools by providing a method for detecting covert signs of residual cognitive function and awareness. The aim of this study was to investigate the brain oscillatory activities synchronized by single-pulse transcranial magnetic stimulation (TMS) delivered over the primary motor area in the time–frequency domain in patients with the unresponsive wakefulness syndrome or in a minimally conscious state as compared to healthy controls. A time–frequency analysis based on the wavelet transform was used to characterize rapid modifications of oscillatory EEG rhythms induced by TMS in patients as compared to healthy controls. The pattern of EEG changes in the patients differed from that of healthy controls. In the controls there was an early synchronization of slow waves immediately followed by a desynchronization of alpha and beta frequency bands over the frontal and centro-parietal electrodes, whereas an opposite early synchronization, particularly over motor areas for alpha and beta and over the frontal and parietal electrodes for beta power, was seen in the patients. In addition, no relevant modification in slow rhythms (delta and theta) after TMS was noted in patients. The clinical impact of these findings could be relevant in neurorehabilitation settings for increasing the awareness of these patients and defining new treatment procedures.

Keywords Transcranial magnetic stimulation · Event-related EEG · Unresponsive wakefulness syndrome · Minimally conscious state

✉ Emanuela Formaggio
emanuela.formaggio@ospedalesancamillo.net

[1] Department of Neurophysiology, Foundation IRCCS San Camillo Hospital, Via Alberoni 70, 30126 Venice, Italy

[2] Department of Computer Science, University of Verona, Verona, Italy

[3] Department of Medicine, Surgery and Health Sciences, Clinical Neurology Unit, Cattinara University Hospital, Trieste, Italy

Introduction

Assessment of non-communicative patients with disorders of consciousness remains a clinical challenge. Modern medicine has increased the chance of survival for patients with severe impairment or injury but also the number of those surviving for prolonged periods in an unresponsive wakefulness syndrome [UWS], previously known as vegetative state, (Laureys et al. 2010) and in a minimally conscious state (MCS). The current gold standard in detecting consciousness relies on clinical assessment derived from standard and functional imaging modalities and behavioral assessment. However, possible confounding factors and the mechanisms underlying impaired brain function may not be fully considered. The absence of behavioral evidence of command-following is not necessarily indicative of the true absence of awareness (Owen et al. 2006).

Recent neuroimaging and electrophysiological studies have demonstrated that some brain-injured patients retain a range of cognitive capacities despite minimal or no behavioural evidence of awareness. Using functional magnetic resonance imaging (fMRI), Monti et al. (2010) evaluated patients with severe brain injury during a motor imagery and a spatial imagery tasks. A small portion of patients with UWS or in MCS had brain activation reflecting some awareness and cognition. Differently, an electroencephalography (EEG) study (Goldfine et al. 2011)

investigating brain response to motor and spatial navigation tasks in three patients reported a task-dependent modulation of EEG, though the patterns of change differed from those of the controls.

An innovative approach for studying brain functioning is by triggering oscillatory brain activity with a perturbation method, e.g. direct stimulation. EEG-transcranial magnetic stimulation (TMS) co-registration has shed new light on EEG reactivity in humans. TMS offers an advantageous way to assess brain function in patients with disorders of consciousness because it does not depend on the integrity of sensory and motor pathways and it does not require active subject participation (Gosseries et al. 2014). When combined with simultaneous EEG recording, TMS allows to investigate the distribution of synaptic activation and to make inferences on local excitability and area-to-area functional connectivity in the central nervous system (Komssi et al. 2002). Moreover, EEG–TMS can be employed at the bedside.

Most studies to date have focused on EEG response to TMS, interpreted as evoked potential (the TMS evoked potential: TEP), (Bonato et al. 2006; Del Felice et al. 2011; Ilmoniemi et al. 1997; Izumi et al. 1997; Paus et al. 1998; Thut et al. 2003; Thut and Pascual-Leone 2010) by investigating the characteristics of cortical reactivity and connectivity. This response (TEP) is time-locked to the event and it is detected by averaging techniques. The basic assumption is that the evoked activity has a relatively fixed time-delay to the stimulus, while EEG activity behaves as additive noise. In fact, the TEP can be used to study the reactivity of a target area by using its amplitude, thus allowing quantification of the state of the brain (Veniero et al. 2010) and tracking the connectivity of both intra-hemispheric and interhemispheric cortical networks (Rosanova et al. 2012). Stimulus-induced rhythmic EEG activity, which is not phase locked to an event, and not detected by the time domain analysis (averaging techniques), but can be detected by frequency analysis (Pfurtscheller and Lopes da Silva 1999). In that case, activity that is not phase-locked to the onset of the TMS pulse is cancelled out due to averaging. It is, however, possible that the TMS pulse induces responses that are not necessarily phase-locked to the onset of the pulse, for example changes in spontaneous oscillatory activity. The application of EEG–TMS co-registration has opened new and intriguing lines of research in the study of the brain response yielding a wealth of data on rhythmic brain activities (Fuggetta et al. 2005, 2008; Manganotti et al. 2012, 2013a; Noh et al. 2012; Thut and Miniussi 2009; Napolitani et al. 2014) and on the connectivity of brain areas during the wake state or sleep (Massimini et al. 2005). Cortical response to TMS has been variously described, but only few studies have examined consciousness disorders (Rosanova et al. 2012; Ragazzoni et al. 2013; Tallus et al. 2013).

The time–frequency analysis is fundamental when the goal is to investigate the several reactive EEG frequencies, each exhibiting unique temporal and topographic patterns in association with functional brain activation. This approach allows defining the time course of the slow and fast oscillations and evaluating the rhythms modifications as well as the TMS-locked response. Accordingly, wavelet-based techniques are being increasingly used for processing non-stationary EEG recordings with respect to oscillatory behavior (Samar et al. 1999) as well as for evaluating the effects of a magnetic (Manganotti et al. 2012, 2013a; Rosanova et al. 2009) or electric stimulation (Formaggio et al. 2013), during robot-assisted motor performance (Formaggio et al. 2015), and during EEG-functional magnetic resonance imaging co-registration (Formaggio et al. 2011, 2014; Storti et al. 2010).

Recently, Manganotti and colleagues used a time–frequency analysis based on the wavelet method to characterize rapid modifications of oscillatory EEG rhythms induced by single-pulse TMS in healthy subjects. Stimulation induced a rapid desynchronization over the frontal and central-parietal electrodes mainly in the alpha and beta bands, followed by a rebound of synchronization, and a rapid synchronization of delta and theta activity (Manganotti et al. 2012). Afterwards, during awake, awake after sleep deprivation and in sleep, they observed a sleep-related slight desynchronization of alpha, mainly over the frontal areas and a widespread increase in theta synchronization applying the same method of analysis to investigate EEG response to TMS (Manganotti et al. 2013a). This method is appealing for detecting dynamic changes in the regional neural oscillatory activity of cortical areas and for correlating the modulation of these induced oscillations to different brain states.

The aim of this study was to investigate the brain oscillatory activities synchronized by single-pulse TMS delivered over the primary motor area (M1) in the time–frequency domain in UWS and MCS patients as compared to healthy controls. These findings could drive the systematic development of neurophysiological and stimulation paradigms to assess brain function and cognitive capacity after severe brain injuries (Manganotti et al. 2013b). Moreover, the clinical impact could be relevant in the neurorehabilitation setting for increasing the awareness of these patients and defining new treatment procedures.

Materials and Methods

Patients and Controls

Five patients (3 women, 2 men; mean age, 59.6 years ± standard deviation [SD] 15.5 years) with UWS or in MCS,

Table 1 Demographic and clinical profiles of patients

No.	Age	Sex	Clinical diagnosis	Aetiology	MRI findings	Months since injury	DRS
1	47	Female	UWS	Anoxic	Parietal-occipital lesions	29	26
2	61	Male	UWS	Anoxic	Diffuse anoxic-ischemic lesion	8	26
3	72	Male	UWS	Haemorrhagic	Right thalamic lesions	72	29
4	41	Female	UWS	Haemorrhagic	Right mesencephalic lesions	12	24
5	77	Female	MCS	Ischemic	Multifocal brainstem and thalamic lesions	27	21

admitted to the Neurorehabilitation Center of IRCCS San Camillo Hospital, Venice, met the study inclusion criteria: absence of contraindications to TMS; stability of vital parameters; and at least 12 months after the injury event (Giacino et al. 2002; Wassermann 1998). The clinical characteristics of the enrolled patients are shown in Table 1. Clinical features were assessed with the disability rating scale (DRS) (Rappaport et al. 1982).

Control data from five healthy subjects (1 woman, 4 men; mean age 25.7 years; ± SD 4.2 years) were obtained in a previous study (Manganotti et al. 2012). In accordance with the Declaration of Helsinki, written informed consent to participate in the study was obtained from the healthy volunteers and the patients' caregivers. The study design and protocol were approved by the Local Ethical Committee of IRCCS San Camillo Hospital.

Transcranial Magnetic Stimulation

TMS was delivered using a Magstim-Rapid2 Stimulator (Magstim Company Ltd, London, UK) and a figure-of-eight focal coil over the left/right M1 based on the presence of motor evoked potentials (MEPs). MEPs were recorded from the left/right thenar eminence muscle with Ag/AgCl surface electrodes fixed to the skin with a belly-tendon montage.

Stimulus intensity was set at 110 % of motor threshold (MT) intensity. The MT intensity was approached from individual suprathreshold levels by reducing the stimulus intensity in 1 % steps (Rossini et al. 1994).

A white noise (90 dB) was played through the earphones to mask the coil-generated click, to avoid any effect of clicks in the modulation of cortical oscillatory activities (Nikouline et al. 1999; Tiitinen et al. 1999) during both resting state and stimulation conditions.

Spontaneous EEG, acquired in resting state condition (the basal EEG), and single-pulse magnetic stimulation were performed during the same experimental session; 50 stimuli were delivered at 110 % of MT (a minimum inter-trial interval (ITI) of 6 s and a maximum of 8 s) over the left/right M1 area. During TMS the computer triggered the magnetic pulses by insertion of a marker in a track of the EEG recording system.

EEG Data Recordings and Analysis

EEG data were acquired at a rate of 1024 Hz as in Manganotti et al. (2012) using a magnetic resonance (MR)-compatible EEG amplifier (SD MRI 32, Micromed, Treviso, Italy) and a cap providing 30 TMS-compatible coated-electrodes positioned according to a 10/20 system. To avoid saturation, the EEG amplifier had a resolution of 22 bits (range, ± 25.6 mV). An anti-aliasing hardware band-pass filter was applied with a bandwidth between 0.15 and 269.5 Hz.

Time–frequency analysis was performed with continuous Morlet wavelet transform in delta (1–4 Hz), theta (4–7 Hz), alpha (8–12 Hz), and beta (13–30 Hz) ranges. A family of complex Morlet wavelets was constructed at 1 Hz frequency intervals. Each wavelet function has a Gaussian distribution in the time (SD: σ_t) and frequency domains (SD: σ_f) around the centre frequency f_0 and it depends on a parameter, the number of oscillations (f_0/σ_f), which has to be chosen by the user. The number of oscillations in each data window can be critical and there is no rule for determining this parameter. We chose these parameters to better investigate power changes as the optimal compromise in time–frequency using the frequency ranges specified above and a temporal window of 5 s. Our wavelet family was computed using a ratio of 4 oscillations for delta, 7 for theta, 12 for alpha, and 30 for beta bands.

Wavelet transform was applied to each epoch of basal EEG (24 epochs) and to each single post-stimulus trial (43 trials). The relative power (%), calculated by dividing the power of the frequency band (delta, theta, alpha, beta) with the power of 1–30 Hz, was estimated for the two conditions (basal and post-stimulus). The reference basal relative power for each frequency band was calculated by averaging wavelet spectra across time and frequency, obtaining one value for each band. The mean and the standard deviation of relative power among epochs were computed. The post-stimulus relative power was computed by averaging wavelet spectra in the frequency ranges of interest, producing a power time course for each trial and each band. Profiles for each subject were averaged from the post-stimulus trials and normalized to the basal value

(expressed as 1). In this way we can appreciate the difference in power within and between groups. The researcher paid special attention to have an EEG in "awake", with eyes open, and without periods of sleep throughout the recording session.

Statistical analysis was performed using SPSS software package (SPSS Inc., Chicago, IL). Analysis of covariance (ANCOVA) with Bootstrap was applied using the four factors "group" (patients, controls), "condition" (basal EEG, post-stimulus), "frequency range" (delta, theta, alpha and beta), "electrodes" (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4), and the "age" as covariate. The mean of not-normalized power in the 5-s interval (one value for each electrode) was considered as the dependent variable.

In order to check whether post-stimulus activity differed significantly from the basal EEG for each group and in order to compare the power time course of the patients to that of healthy subjects, ANCOVA for repeated measures was applied to the relative power with the factors "group" (patients, controls), "condition" (basal EEG, post-stimulus), "time" (10 values for each electrode, the mean of relative power in the 500-ms intervals) and the "age" as covariate.

Grand mean topographic maps showing power at different frequency ranges were computed for the patient and the control groups. The topographical maps allowed us to verify the power distribution on the whole scalp at a specific time-point corresponding to the maximum or minimum of the C3 power time course for each band in the controls. ANCOVA with Bootstrap was applied to normalized relative power computed at the specific time-point, for each band, using the factors "group" (patients, controls) and "electrodes" (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4), and the "age" as covariate.

A post hoc paired sample two-tailed t test adjusted for multiple comparisons with the Bonferroni method was used in all the statistical analysis. Statistical significance was set at $p < 0.05$, corrected.

Results

ANCOVA applied to the average power values in time showed a significant main effect for the factor "frequency range" $F(3575) = 603.331; p < 0.001$ (Bootstrap statistics). There was a significant interaction between "group" and "frequency range" $F(3575) = 70.106;\ p < 0.001;$ "electrodes" and "frequency range" $F(24,575) = 3.396;$ $p < 0.001$; "group" and "condition" and "frequency range" $F(3575) = 3.885; p < 0.05$. There was no significant effect for the factor "group" or for the covariate "age".

Mentioned below are the times/latencies in which EEG modifications were observed and are related to the time in the processed epochs and not to latency with respect to TMS. Trials of 5 s were selected from 35 ms to 5035 ms after the stimulus (Manganotti et al. 2012).

Delta Band

The delta power in the controls was characterized by an increase, with a peak at about 0.5 s, followed by a gradual reduction in power until reaching the basal condition (Fig. 1). This initial synchronization was significant ($p < 0.05$, Bonferroni corrected) in C3, and evident in Fz, F4, C4 and P4. In the patients, the power did not significantly change over time and remained above the basal value for the entire time. The differences between patients and controls were significantly evident ($p < 0.05$, Bonferroni corrected) only in C3 during the last 500 ms.

Theta Band

The theta relative power increased significantly from the basal value in the first 0.2 s only in F4 (Fig. 2) in the controls. The increase was followed by a rapid decrease. A power significant increase in F4 from 7 to 9 s and in the last 500 ms after stimulation was also noted. The only significant differences, as compared to the basal EEG, observed in the patients were the power decrease in F4 from 0.5 to 4.5 s and from 2.5 to 4 s. As observed in the controls, the pattern of increase was also detected in the patients (in P3 and Pz), though no significant changes were found. No significant differences were observed between patients and controls.

Alpha Band

In the controls, TMS induced a marked decrease in power over both sensorimotor areas (C3 and C4) and in Fz and P4, followed by a progressive synchronization in time, especially over the parietal (P3, Pz, and P4) electrodes (Fig. 3). A decrease, as compared to the basal EEG, was also observed in the patients immediately after the stimulation in F3, Fz, and Cz. In Cz the power remained below the basal value during the entire post-stimulus interval. As compared to the controls, no significant differences were found.

Beta Band

In the controls, there was an initial, rapid power decrease in the beta band mainly in Fz, C3 and P4 followed by a marked increase in beta power over the sensorimotor areas, and more specifically, over C4 (Fig. 4). A synchronization was also observed from 4 to 5 s after stimulation in P3, Pz and P4. As noted in the alpha band, an opposite pattern was

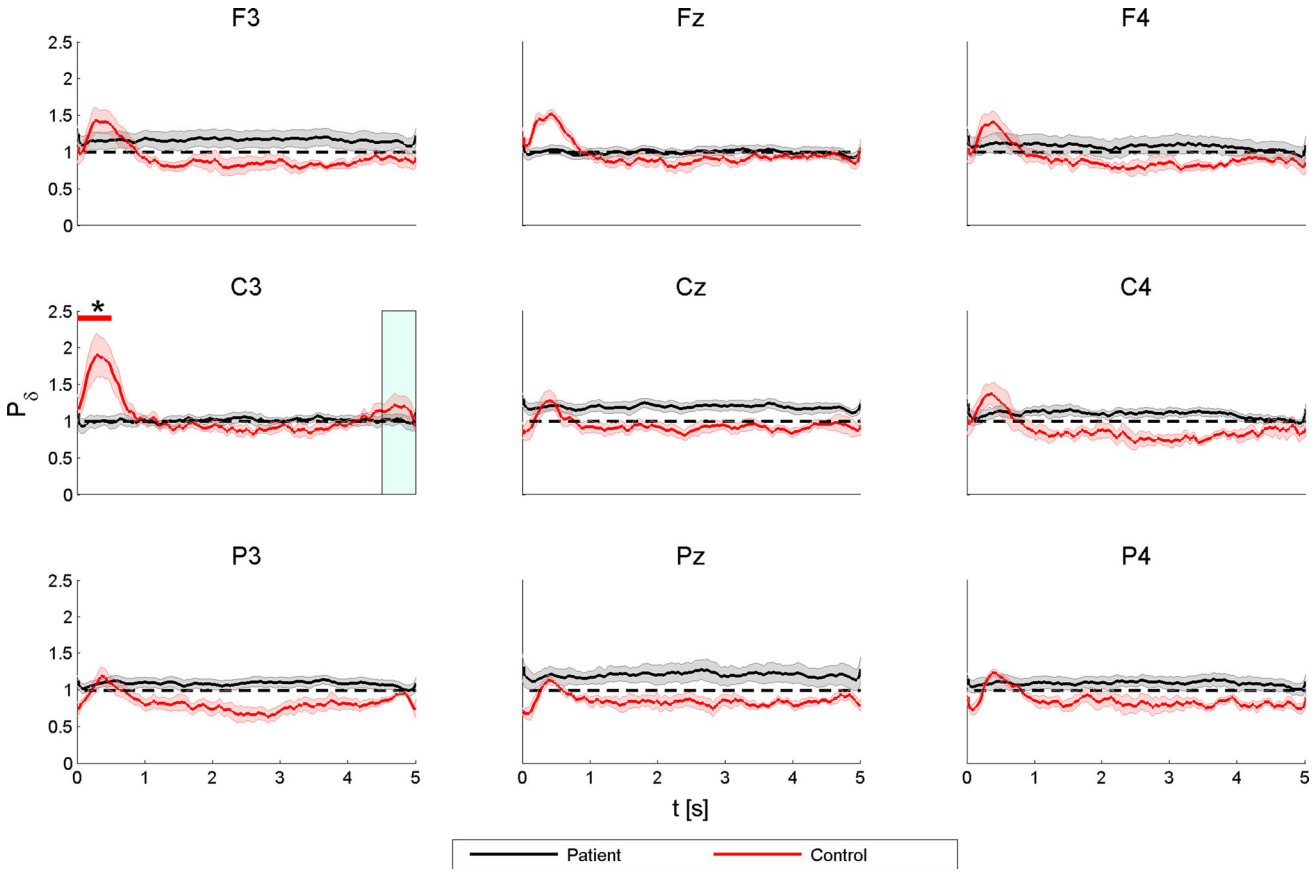

Fig. 1 Average relative wavelet power and standard error in delta range (1–4 Hz), after single-pulse TMS, in controls (*red*) and patients (*black*). (*) above the *bars* indicates values significantly different from basal level. Data are analyzed from 35 ms to 5035 ms after the stimulus onset. The *green* area represents the time interval in which the relative power time course computed in patients differs statistically from that computed in healthy subjects (Color figure online)

observed in the patients immediately after the stimulation, with an increase of power in F4, C3, C4, P3, Pz and P4. No significant differences were observed between patients and controls.

Patients Versus Controls at the Specific Time-Point

ANCOVA applied to normalized relative power computed at the specific time-point showed a significant main effect for the factor "age" in delta ($F(1239) = 42.039$; $p < 0.001$), alpha ($F(1239) = 17.096$; $p < 0.001$), beta ($F(1239) = 35.611$; $p < 0.001$) and "group" in delta ($F(1239) = 96.386$; $p < 0.001$), alpha ($F(1239) = 40.422$; $p < 0.001$), beta ($F(1239) = 61.544$; $p < 0.01$). There was a significant interaction between "group" and "electrodes" in delta range $F(29,239) = 1.461$; $p < 0.05$. A post hoc *t* test showed significant differences between patients and controls in delta for all the electrodes (except for Pz) ($p < 0.05$, Bonferroni corrected).

The grand average maps, computed at the time point corresponding to the maximum (delta and theta bands) or minimum (alpha and beta bands) of the C3 power time course detected in the controls, showed a delta power decrease in the patients as compared to the controls over C3 and over Fz, and a theta decrease over the frontal (Fz, F4), central (Cz) and parietal (P4, PO3) regions (Fig. 5). An opposite pattern was observed in the alpha and beta bands where an alpha power increase was noted over both the sensorimotor cortex (C3, C4) and over the fronto-central electrodes. Power decrease was observed in beta range over frontal (FC5, F3, Fz) and centro-parietal (Cz, Cp1, PO3) electrodes.

Discussion

This study investigated the time course of different patterns of brain oscillatory activity induced by single-pulse TMS in patients with altered state of consciousness. An opposite pattern of EEG power in alpha and beta bands was detected in the patients as compared to the controls. TMS represents an input to the brain that can be helpful to quantify the

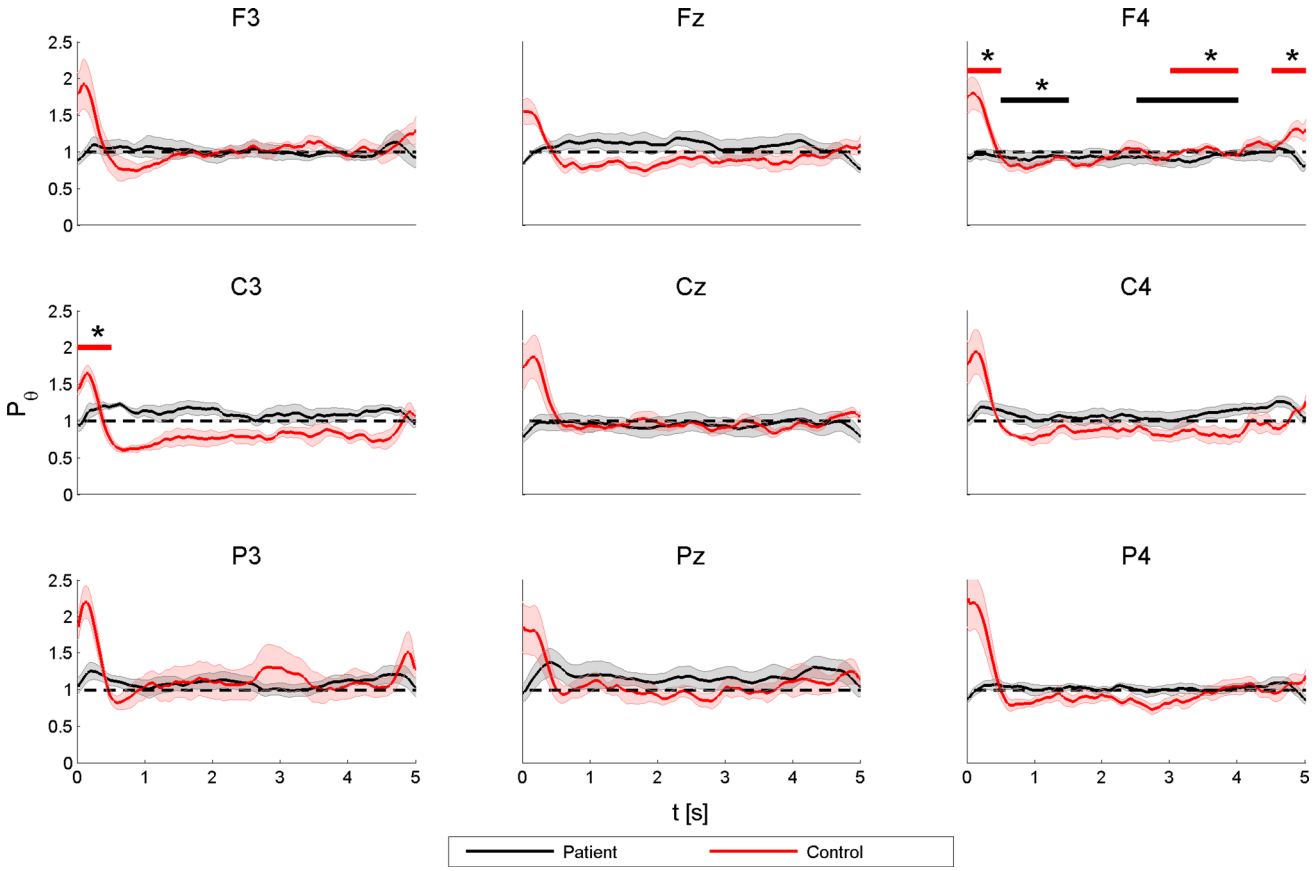

Fig. 2 Average relative wavelet power and standard error in theta range (4–7 Hz), after single-pulse TMS, in controls (*red*) and patients (*black*). (*) above the *bars* indicates values significantly different from basal level. Data are analyzed from 35 to 5035 ms after the stimulus onset. No significant differences were observed between patients and controls (Color figure online)

local response and the dynamic spatial spreading pattern of the induced activity. In particular, analysis of brain oscillatory activity by quantitative EEG can be a useful tool to assess thalamo-cortical function and can help to understand the mechanisms underlying the modifications of the level of consciousness in persons with UWS and MCS.

Different techniques and protocols have been developed and applied to assess neuroplasticity in patients with disorders of consciousness. Combined EEG and TMS has been used to study the effect of a single-pulse TMS on slow evoked potential (TEP). A recent study (Rosanova et al. 2012) applied TMS with high-density EEG to evaluate the effective connectivity in severely brain-injured, non-communicating patients. In the patients with UWS, TMS stimulation triggered a simple, local response indicating a breakdown in effective connectivity, similar to that observed in unconscious sleeping or anaesthetized subjects. In contrast, in MCS patients, TMS triggered complex activations that sequentially involved distant cortical areas ipsilateral and contralateral to the site of the stimulation. In another study, Tallus et al. (2013) compared the brain responses between conscious patients with mild brain injury and controls and found altered brain reactivity and connectivity in the former as compared to the latter. This pattern of response was explained as a compensatory mechanism of recovery. TEP results suggest that cortical reactivity and connectivity are severely impaired in all patients with UWS, whereas in most MCS patients TEPs are preserved but show abnormal features (Ragazzoni et al. 2013). A new measure, the Perturbational Complexity Index (PCI), has been recently developed to assess the brain capacity of consciousness by evaluating the amount of information contained in the integrated response of the thalamo-cortical system to a direct TMS perturbation (Casali et al. 2013). PCI has been shown to provide a data-driven metric that can discriminate the level of consciousness in single subjects under different conditions. In a recent review, the authors summarized that EEG response to TMS collapses when consciousness is lost during deep sleep, anesthesia, and vegetative state, while it recovers when consciousness resurges in wakefulness, during dreaming, in the minimally conscious state or locked-in syndrome (Sarasso et al. 2014).

Differently from those studies, here we assessed the power time-course modifications by comparing changes in

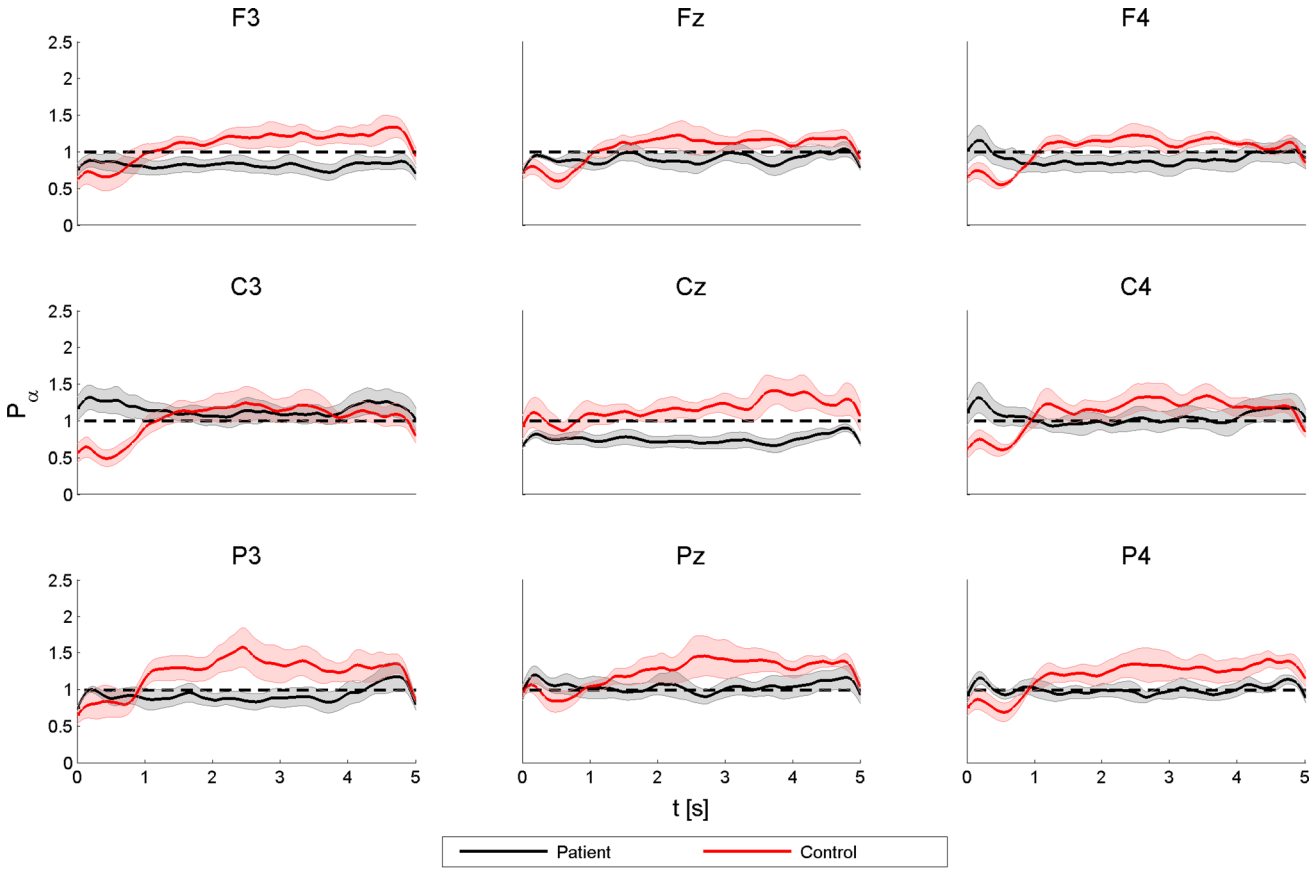

Fig. 3 Average relative wavelet power and standard error in alpha range (8–12 Hz), after single-pulse TMS, in controls (*red*) and patients (*black*). (*) above the *bars* indicates values significantly different from basal level. Data are analyzed from 35 to 5035 ms after the stimulus onset. No significant differences were observed between patients and controls (Color figure online)

EEG oscillatory activity induced by single-pulse stimulation to evaluate the pattern of slow and fast oscillatory activities in terms of frequency modification, compared to the basal EEG. The basal EEG acquisition was performed in a resting state condition, differently from other approaches applied to the study of TEP, where the evoked high amplitude EEG deflections are usually baseline corrected using a very short epoch of some ms preceding the stimulus. Our aim was to compare the changes in oscillatory activity induced on EEG by the stimulation to a reference value (resting state condition), devoid of any external perturbation.

Our results showed that the pattern of EEG changes in the patients differed from that of the controls. Because of the conservative nature of the Bonferroni correction for multiple comparisons, few results reached statistical significance; nevertheless the main modifications of the rhythms were anyway marked. In the controls there was an early synchronization of slow waves (delta and theta) immediately followed by a desynchronization of alpha and beta frequency bands over the frontal and centro-parietal electrodes, whereas an opposite early synchronization, particularly over C3 and C4 for alpha and beta, and over the frontal and parietal electrodes for beta power was seen in the patients. In addition, the patients showed no relevant modifications of slow rhythms (delta and theta) after TMS. The ANCOVA applied to the average power values in time did not show any significant difference between patients and controls, except for delta range at C3 in the last 500-ms interval after stimulation; however, a significant difference between groups was observed at the time point corresponding to the maximum in the healthy subjects in the delta band over all the electrodes.

The ability of TMS to increase slow oscillatory activity immediately after stimulation in controls may indicate enhanced fronto-parietal susceptibility to magnetic field fluctuations in this frequency. EEG theta oscillations during resting state seem to reflect long-range cortical network activity (Destexhe et al. 2007) that increase between prefrontal and parietal neocortical regions during working memory and encoding tasks (Sarnthein et al. 1998; Mizuhara and Yamaguchi 2007), and is seen as a functional binding of widely distributed cortical assemblies (Sauseng et al. 2007). Our TMS appears sufficient to induce a

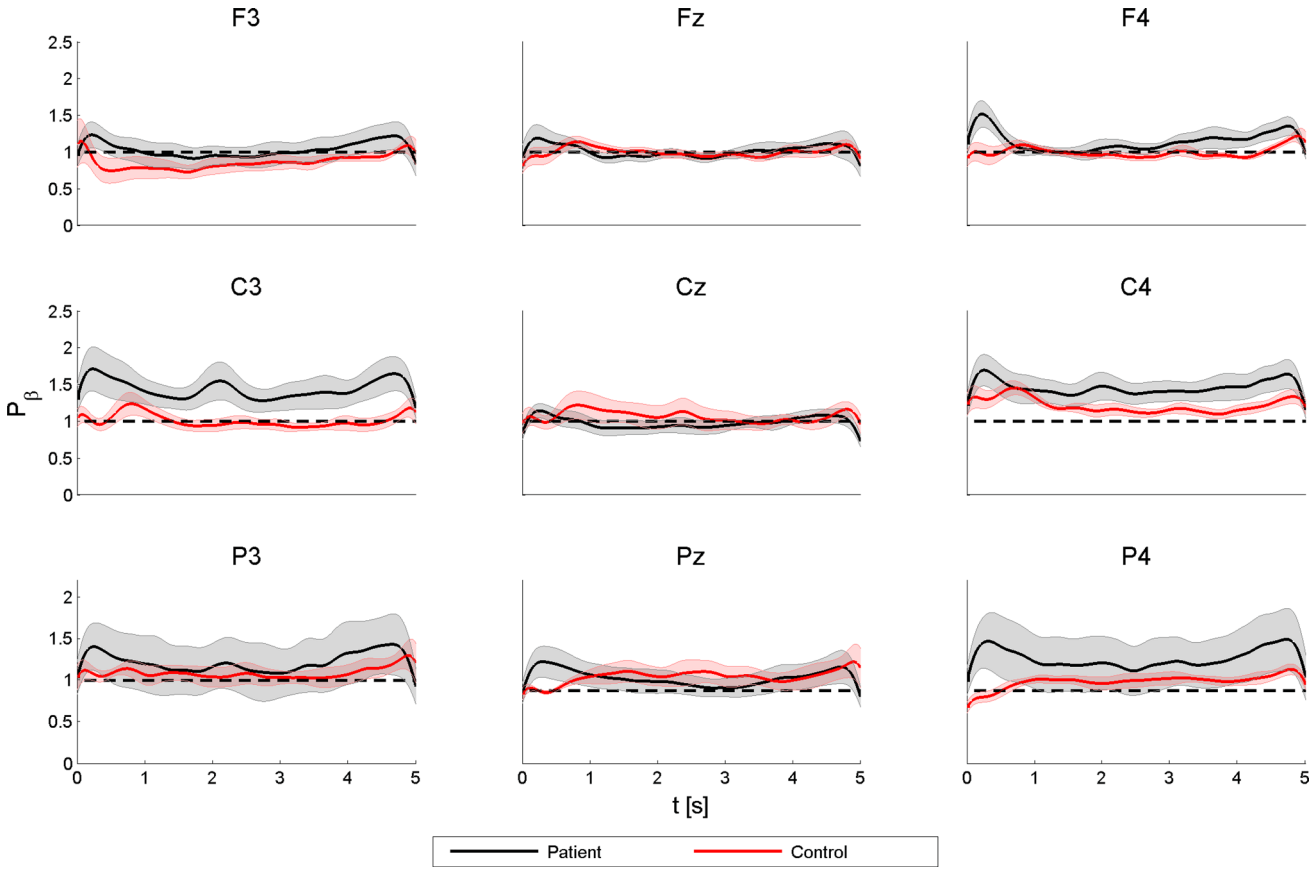

Fig. 4 Average relative wavelet power and standard error in beta range (13–30 Hz), after single-pulse TMS, in controls (*red*) and patients (*black*). (*) above the *bars* indicates values significantly different from basal level. Data are analyzed from 35 to 5035 ms after the stimulus onset. No significant differences were observed between patients and controls (Color figure online)

perturbation of intact cortical networks oscillating in theta frequency range measurable during a short time period in the immediate aftermath of stimulation, but limited to healthy controls. This could be explained by the functional impairment of the fronto-parietal network and its connections of the vegetative state.

Another result that characterized our findings was a secondary desynchronization of faster activity. Berger described the phenomenon of "alpha blocking", alpha power suppression during eyes opening, as a top-down activation process (Berger 1930). This basic finding dating from the earliest work in electroencephalography demonstrates that alpha suppression, in terms of desynchronization, may primarily reflect top-down sensory processing. Alpha waves can be recorded over the visual cortex, above the sensory-motor cortical area (usually referred to mu, or somatosensory alpha rhythm), over the supplementary motor area, as well as above the middle temporal cortex. Alpha synchronization/desynchronization is a common feature of activity of these sensorimotor cortices and play a key role in modulating cognitive functions such as perceptual learning (Sigala et al. 2014). The idling role of alpha oscillations has been replaced by the inhibition hypothesis which states that the amplitude of alpha oscillations is suppressed in specialized sensorimotor areas when processing sensory stimuli while it emerges in areas that are not explicitly involved in the task (Kelly et al. 2006; Thut et al. 2006). Other investigators have found an alpha desynchronization effect correlated with subsequent behavioral performance, suggesting that the alpha decrease is associated with an enhanced excitability of cortical areas in charge of processing stimuli (Snyder and Foxe 2010; Mo et al. 2011). Therefore, alpha event-related desynchronization seems to be a correlate of activated cortical networks related to information processing (Klimesch 1997), selective attention (Suffczynski et al. 2001), and motor preparation (Pfurtscheller et al. 2000).

Fuggetta et al. (2005) observed that single-pulse TMS produces an increase in power in both the alpha and beta bands, in healthy subjects, unlike the self motor finger movement which produces a well-known decrease in alpha and beta powers. In this study, spectral estimation was performed using fast Fourier transform which does not detect dynamic changes. To overcome this limitation,

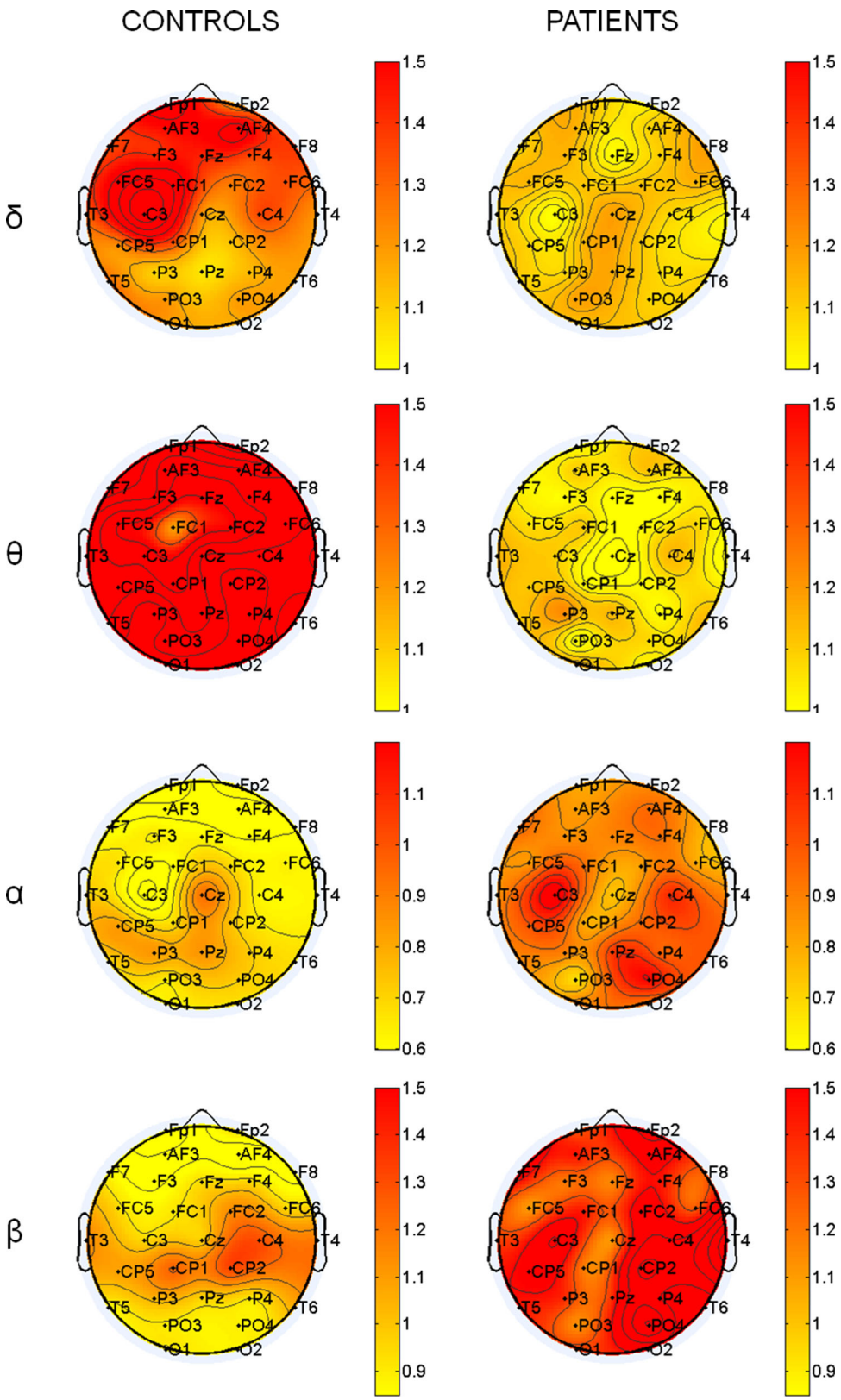

Fig. 5 Grand average relative power maps at one specific time-point (corresponding to the maximum or minimum of the C3 power time course for each band, computed in healthy subjects) for controls and patients

methods that can monitor the temporal variation of EEG power are needed. Rosanova et al. (2009) applied the event-related spectral perturbation, based on Morlet wavelets, to detect alpha and beta dominant oscillations, evoked by TMS, in the occipital and parietal cortex, respectively, in the first 200 ms after stimulus. In this study

we used wavelet-based methods to detect the temporal modulation of brain oscillations in the main frequency bands. The initial decrease of power in the alpha and beta bands, followed by a more prominent increase of power in beta activity over the ipsilateral and contralateral M1, is simultaneously associated with an early increase in delta power over the central and frontal areas, followed by a slow return to background activity.

On the basis of these findings, we may presume that immediately after single-pulses TMS the cortical networks are put in a state of excitation similar to that observed for cognitive processing, inducing a large desynchronization of alpha power (Klimesch et al. 2003). The causal nature of a close relationship between EEG alpha oscillations and human behavior has been pointed out by demonstrating that enhanced alpha power by repetitive trancranial magnetic stimulation (rTMS) can improve cognitive task performance (Klimesch et al. 2003). As explained by Klimesch, the alpha reactivity, measured by event-related desynchronization (ERD), depends on the amplitude of alpha oscillations acquired in a reference period (resting state condition) that precedes the task performance. Cognitive performance is related to the amplitude of ERD which depends on the power in the reference period. Subjects with large alpha power have significant ERD and this is associated with good performance in different tasks. rTMS at alpha frequency can enhance alpha power in the reference period, improving task performance, and can be a useful therapeutic tool for patients with cortical dysfunctions.

On the other hand, the synchronization of fast rhythms (alpha and beta) over sensorimotor areas in patients with disorders of consciousness could support the notion that large amplitudes of synchronized alpha activity reflect a brain state of reduced information processing (Pfurtscheller 2001). This synchronization, accompanied by a non-reactive theta activity, could represent a rearrangement of dynamic cortical patterns in disorders of consciousness.

In a state of unconsciousness, a circuit-based mechanism is known to be capable of producing strong power in the theta range after severe or moderate deafferentation at the level of the neocortex and thalamus. Deafferentation of the neocortex leads to intrinsic membrane oscillations at theta frequencies and deafferentation of the thalamus from its cortical inputs leads to bursting in the wakeful state and increased theta power in the EEG (Llinás et al. 1999; Williams et al. 2013). The absence of modifications in EEG power could be explained by the inability of distributed regions of the brain to interact through divergent cortico-cortical and cortico-thalamo-cortical connections.

Klimesch et al. (1994) provided good evidence that theta power is related to the encoding of new information and to episodic memory and that a task related increase in theta power reflects the successful encoding of new information. Because theta power increases in a large variety of different tasks it seems plausible to assume that theta power reflects at least in part unspecific factors such as e.g., attentional demands, task difficulty and cognitive load (Klimesch et al. 1994). In particular, they demonstrated that theta synchronization and upper alpha desynchronization are related to episodic and semantic memory performance, respectively. Furthermore, slow oscillations have been proposed to play a key role in NREM in supporting the information processing related to the experiences of the previous awake period and slow sleep-associated consolidation of memory (Ji and Wilson 2007).

In light of this, our study further supports the hypothesis for a reduced computational capability, as represented by a lack of theta synchronization/alpha desynchronization after single-pulses TMSs in patients with UWS. From a more general perspective, our findings indicate that a particular pattern of synchronization/desynchronization in different frequency bands after single TMS pulses could be an indicator for encoding processes that are impaired in patients with consciousness disorders.

The novelty of the present method consists in the application of a time–frequency analysis to the rapid and slow brain oscillations induced by TMS in patients with disorders of consciousness. This preliminary study has several limitations. Well-designed studies with a larger sample size and more detailed data are needed to confirm our observations. Our patient population was heterogeneous and included patients with different types of lesions and diagnosis. As well known, EEG frequency changes can occur as a function of age and it would be very interesting to acquire EEG data in a consistent sample of healthy subjects in the same range of ages of the patients. Although in our study the mean age of patients differed from controls, it had no significant effect as a covariate in the statistics applied to the average power values in time and to the mean of relative power in the 500-ms intervals. However, a significant influence of age was observed in all the frequency ranges, except for theta, in the ANCOVA applied to the relative power at a specific time point. Further investigations on larger populations are needed to generalize the results of our study to the clinic, translate insights from research to clinical implications in unconsciousness patients, and find a conclusion. Nonetheless, our findings suggest that different neuronal populations are involved in the electrophysiological phenomena induced by TMS and that these neurons may be affected differentially. Moreover, our data add important insights into the patterns of oscillatory activity during TMS and indicate the potential this new approach to analysis holds in the evaluation of cortical oscillatory activity time changes in patients with disorders of consciousness. The aim of this

study was not to discriminate between conscious and unconscious patients but to describe the different neurophysiological time–frequency responses to TMS as compared to healthy subjects in order to better understand the pathophysiology of loss of consciousness.

Patients with consciousness disorders constitute a major challenge for diagnosis, prognosis and treatment. Certain electrophysiological and neuroimaging methodologies may not be sufficient to select patients for a specific treatment (Yamamoto et al. 2013). The combination of different electrophysiological and neuroimaging techniques (e.g., EEG, magnetoencephalography [MEG], fMRI, perfusion MRI, TMS) could be useful to deepen our understanding of brain function and improve diagnosis. Moreover, the multimodality approach may offer new insights into the physiopathology of consciousness disorders and help to define novel non-invasive treatment procedures. In patients without behavioral responses, non-invasive techniques and new methods of data analysis can complement existing diagnostic tools by providing a method for detecting covert signs of residual cognitive function and awareness.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Berger H (1930) On the electroencephalogram of man. Journal fur Psychologie und Neurologie 40:160–179

Bonato C, Miniussi C, Rossini PM (2006) Transcranial magnetic stimulation and cortical evoked potentials: a TMS/EEG co-registration study. Clin Neurophysiol 117(8):1699–1707

Casali AG, Gosseries O, Rosanova M, Boly M, Sarasso S, Casali KR, Casarotto S, Bruno MA, Laureys S, Tononi G, Massimini M (2013) A theoretically based index of consciousness independent of sensory processing and behavior. Sci Transl Med 5(198):198ra105

Del Felice A, Fiaschi A, Bongiovanni GL, Savazzi S, Manganotti P (2011) The sleep-deprived brain in normals and patients with juvenile myoclonic epilepsy: a perturbational approach to measuring cortical reactivity. Epilepsy Res 96(1–2):123–131

Destexhe A, Hughes SW, Rudolph M, Crunelli V (2007) Are corticothalamic "up" states fragments of wakefulness? Trends Neurosci 30(7):334–342

Formaggio E, Storti SF, Bertoldo A, Manganotti P, Fiaschi A, Toffolo GM (2011) Integrating EEG and fMRI in epilepsy. Neuroimage 54(4):2719–2731

Formaggio E, Storti SF, Tramontano V, Casarin A, Bertoldo A, Fiaschi A et al (2013) Frequency and time-frequency analysis of intraoperative ECoG during awake brain stimulation. Front Neuroeng 6:1. doi:10.3389/fneng.2013.00001

Formaggio E, Storti SF, Boscolo Galazzo I, Bongiovanni LG, Cerini R, Fiaschi A et al (2014) Reproducibility of EEG–fMRI results in a patient with fixation-off sensitivity. Clin EEG Neurosci 45(3):212–217

Formaggio E, Storti SF, Boscolo Galazzo I, Gandolfi M, Geroin C, Smania N et al (2015) Time-frequency modulation of ERD and EEG coherence in robot-assisted hand performance. Brain Topogr 28(2):352–363

Fuggetta G, Fiaschi A, Manganotti P (2005) Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. Neuroimage 27(4):896–908

Fuggetta G, Pavone EF, Fiaschi A, Manganotti P (2008) Acute modulation of cortical oscillatory activities during short trains of high-frequency repetitive transcranial magnetic stimulation of the human motor cortex: a combined EEG and TMS study. Hum Brain Mapp 29(1):1–13

Giacino JT, Ashwal S, Childs N, Cranford R, Jennett B, Katz DI et al (2002) The minimally conscious state: definition and diagnostic criteria. Neurology 58:349–353

Goldfine AM, Victor JD, Conte MM, Bardin JC, Schiff ND (2011) Determination of awareness in patients with severe brain injury using EEG power spectral analysis. Clin Neurophysiol 122(11):2157–2168

Gosseries O, Thibaut A, Boly M, Rosanova M, Massimini M, Laureys S (2014) Assessing consciousness in coma and related states using transcranial magnetic stimulation combined with electroencephalography. Ann Fr Anesth Reanim 33(2):65–71

Ilmoniemi RJ, Virtanen J, Ruohonen J, Karhu J, Aronen HJ, Naatanen R et al (1997) Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. NeuroReport 8:3537–3540

Izumi S, Takase M, Arita M, Masakado Y, Kimura A, Chino N (1997) Transcranial magnetic stimulation-induced changes in EEG and responses recorded from the scalp of healthy humans. Electroencephalogr Clin Neurophysiol 103:319–322

Ji D, Wilson MA (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10:100–107

Kelly SP, Lalor EC, Reilly RB, Foxe JJ (2006) Increases in alpha oscillatory power reflect an active retinotopic mechanism for distracter suppression during sustained visuospatial attention. J Neurophysiol 95.3844–3851

Klimesch W (1997) EEG-alpha rhythms and memory processes. Int J Psychophysiol 26:319–340

Klimesch W, Schimke H, Schwaiger J (1994) Episodic and semantic memory: an analysis in the EEG-theta and alpha band. Electroencephalogr Clin Neurophysiol 91:428–441

Klimesch W, Sauseng P, Gerloff C (2003) Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. Eur J Neurosci 17:1129–1133

Komssi S, Aronen HJ, Huttunen J, Kesaniemi M, Soinne L, Nikouline VV et al (2002) Ipsi- and contralateral EEG reactions to transcranial magnetic stimulation. Clin Neurophysiol 113:175–184

Laureys S, Celesia GG, Cohadon F, Lavrijsen J, León-Carrión J, Sannita WG et al (2010) European task force on disorders of consciousness. Unresponsive wakefulness syndrome: a new name for the vegetative state or apallic syndrome. BMC Med 8:68

Llinás R, Ribary U, Jeanmonod D, Kronberg E, Mitra PP (1999) Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. Proc Natl Acad Sci 96:15222–15227

Manganotti P, Formaggio E, Storti SF, De Massari D, Zamboni A, Bertoldo A et al (2012) Time-frequency analysis of short-lasting modulation of EEG induced by intracortical and transcallosal paired TMS over motor areas. J Neurophysiol 107(9):2475–2484

Manganotti P, Formaggio E, Del Felice A, Storti SF, Zamboni A, Bertoldo A et al (2013a) Time-frequency analysis of short-lasting modulation of EEG induced by TMS during wake, sleep deprivation and sleep. Front Hum Neurosci 7:767. doi:10.3389/fnhum.2013.00767

Manganotti P, Formaggio E, Storti SF, Fiaschi A, Battistin L, Tonin P et al (2013b) Effect of high-frequency repetitive transcranial magnetic stimulation on brain excitability in severely brain-injured patients in minimally conscious or vegetative state. Brain Stimul 6(6):913–921

Massimini M, Ferrarelli F, Huber R, Esser SK, Singh H, Tononi G (2005) Breakdown of cortical effective connectivity during sleep. Science 309(5744):2228–2232

Mizuhara H, Yamaguchi Y (2007) Human cortical circuits for central executive function emerge by theta phase synchronization. NeuroImage 36:232–244

Mo J, Schroeder CE, Ding M (2011) Attentional modulation of alpha oscillations in macaque inferotemporal cortex. J Neurosci 31:878–882

Monti MM, Vanhaudenhuyse A, Coleman MR, Boly M, Pickard JD, Tshibanda L et al (2010) Willful modulation of brain activity in disorders of consciousness. N Engl J Med 362(7):579–589

Napolitani M, Bodart O, Canali P, Seregni F, Casali A, Laureys S, Rosanova M, Massimini M, Gosseries O (2014) Transcranial magnetic stimulation combined with high-density EEG in altered states of consciousness. Brain Inj 28(9):1180–1189

Nikouline V, Ruohonen J, Ilmoniemi RJ (1999) The role of the coil click in TMS assessed with simultaneous EEG. Clin Neurophysiol 110:1325–1328

Noh NA, Fuggetta G, Manganotti P, Fiaschi A (2012) Long lasting modulation of cortical oscillations after continuous theta burst transcranial magnetic stimulation. PLoS ONE 7(4):e35080

Owen AM, Coleman MR, Boly M, Davis MH, Laureys S, Pickard JD (2006) Detecting awareness in the vegetative state. Science 313(5792):1402

Paus T, Jech R, Thompson CJ, Comeau R, Peters T, Evans AC (1998) Dose-dependent reduction of cerebral blood flow during rapid-rate transcranial magnetic stimulation of the human sensorimotor cortex. J Neurophysiol 79:1102–1107

Pfurtscheller G (2001) Functional brain imaging based on ERD/ERS. Vision Res 41:1257–1260

Pfurtscheller G, Lopes da Silva FH (1999) Event-relatd EEG/MEG synchronization and desynchronization: basic principles. Clin Neurophysiol 110:1842–1857

Pfurtscheller G, Neuper C, Krausz G (2000) Functional dissociation of lower and upper frequency mu rhythms in relation to voluntary limb movement. Clin Neurophysiol 111:1873–1879

Ragazzoni A, Pirulli C, Veniero D, Feurra M, Cincotta M, Giovannelli F et al (2013) Vegetative versus minimally conscious states: a study using TMS-EEG, sensory and event-related potentials. PLoS ONE 8(2):e57069. doi:10.1371/journal.pone.0057069

Rappaport M, Hall KM, Hopkins K, Belleza T, Cope DN (1982) Disability rating scale for severe head trauma: coma to community. Arch Phys Med Rehabil 63(3):118–123

Rosanova M, Casali A, Bellina V, Resta F, Mariotti M, Massimini M (2009) Natural frequencies of human corticothalamic circuits. J Neurosci 29:7679–7685

Rosanova M, Gosseries G, Casarotto S, Boly M, Casali AG, Bruno MA et al (2012) Recovery of cortical effective connectivity and recovery of consciousness in vegetative patients. Brain 135:1308–1320

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ et al (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 91:79–92

Samar VJ, Bopardikar A, Rao R, Swartz K (1999) Wavelet analysis of neuroelectric waveforms: a conceptual tutorial. Brain Lang 66:7–60

Sarasso S, Rosanova M, Casali AG, Casarotto S, Fecchio M, Boly M, et al. (2014) Quantifying cortical EEG responses to TMS in (un)consciousness. Clin EEG Neurosci 45(1):40–49

Sarnthein J, Petsche H, Rappelsberger P, Shaw GL, von SA (1998) Synchronization between prefrontal and posterior association cortex during human working memory. Proc Natl Acad Sci USA 95:7092–7096

Sauseng P, Hoppe J, Klimesch W, Gerloff C, Hummel FC (2007) Dissociation of sustained attention from central executive functions: local activity and interregional connectivity in the theta range. Eur J Neurosci 25:587–593

Sigala R, Haufe S, Roy D, Dinse HR, Ritter P (2014) The role of alpha-rhythm states in perceptual learning: insights from experiments and computational models. Front Comput Neurosci 4:8–36

Snyder AC, Foxe JJ (2010) Anticipatory attentional suppression of visual features indexed by oscillatory alpha-band power increases: a high-density electrical mapping study. J Neurosci 30:4024–4032

Storti SF, Formaggio E, Beltramello A, Fiaschi A, Manganotti P (2010) Wavelet analysis as a tool for investigating movement-related cortical oscillations in EEG-fMRI coregistration. Brain Topogr 23(1):46–57

Suffczynski P, Kalitzin S, Pfurtscheller G, Lopes da Silva FH (2001) Computational model of thalamo-cortical networks: dynamical control of alpha rhythms in relation to focal attention. Int J Psychophysiol 43:25–40

Tallus J, Lioumis P, Hämäläinen H, Kähkönen S, Tenovuo O (2013) Transcranial magnetic stimulation-electroencephalography responses in recovered and symptomatic mild traumatic brain injury. J Neurotrauma 30:1270–1277

Thut G, Miniussi C (2009) New insights into rhythmic brain activity from TMS-EEG studies. Trends Cogn Sci 13(4):182–189. Review

Thut G, Pascual-Leone A (2010) A review of combined TMS-EEG studies to characterize lasting effects of repetitive TMS and assess their usefulness in cognitive and clinical neuroscience. Brain Topogr 22(4):219–232

Thut G, Northoff G, Ives JR, Kamitani Y, Pfennig A, Kampmann F et al (2003) Effects of single-pulse transcranial magnetic stimulation (TMS) on functional brain activity: a combined event-related TMS and evoked potential study. Clin Neurophysiol 114:2071–2080

Thut G, Nietzel A, Brandt SA, Pascual-Leone A (2006) Alpha-band electroencephalographic activity over occipital cortex indexes visuospatial attention bias and predicts visual target detection. J Neurosci 26:9494–9502

Tiitinen H, Virtanen J, Ilmoniemi RJ et al (1999) Separation of contamination caused by coil clicks from responses elicited by transcranial magnetic stimulation. Clin Neurophysiol 110:982–985

Veniero D, Maioli C, Miniussi C (2010) Potentiation of short-latency cortical responses by high-frequency ripetitive transcranial magnetic stimulation. J Neurophysiol 104:1578–1588

Wassermann EM (1998) Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation. Electroencephalogr Clin Neurophysiol 108:1–16

Williams ST, Conte MM, Goldfine AM, Noirhomme Q, Gosseries O, Thonnard M et al (2013) Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury. Elife 2:e01157

Yamamoto T, Katayama Y, Obuchi T, Kobayashi K, Oshima H, Fukaya C (2013) Deep brain stimulation and spinal cord stimulation for vegetative state and minimally conscious state. World Neurosurg 80(3–4):S30.e1–9