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Effect of High-Frequency Repetitive Transcranial Magnetic Stimulation on Brain Excitability in Severely Brain-Injured Patients in Minimally Conscious or Vegetative State

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ABSTRACT

Background: Repetitive transcranial magnetic stimulation (rTMS) induces prolonged functional changes in the cerebral cortex in normal conditions and in altered states of consciousness. Its therapeutic effects have been variously documented.
Objective: The aim of this study was to investigate the reactivity of electroencephalography (EEG) and the clinical response in six severely brain-injured patients in an altered state of consciousness (minimally conscious state [MCS] or vegetative state [VS]). EEG rhythm and brain excitability were measured before and after a protocol of high-frequency rTMS.
Methods: All six patients underwent clinical and neurophysiological evaluation before rTMS and immediately thereafter. EEG data in resting state were acquired at the beginning of the exam (T_0), after rTMS (T_1), and 38 min after rTMS (T_2). From these data the power values were computed using Fast Fourier Transform.
Results: rTMS over the motor cortex induced long-lasting behavioral and neurophysiological modifications in only one patient in MCS. No significant clinical or EEG modifications were detected in any of the other patients, except for changes in motor threshold and motor evoked potential amplitude over the stimulated motor areas.
Conclusions: The main finding of the study is the correlation between EEG reactivity and clinical response after rTMS. Reappearance of fast activity and an increase in slow activity were noted in the one patient with transitory arousal, whereas no significant reliable changes were observed in the other patients showing no clinical reactivity.

Background

A persistent vegetative state (PVS) refers to a disorder of consciousness in which severely brain-injured patients remain in a state of wakefulness without detectable awareness. In this extended state of unconsciousness, accompanied by nearly normal cycles of sleeping and waking, the brainstem and thalamus are relatively spared, but cortical functional connectivity is limited or absent. The electroencephalogram (EEG) of PVS patients in a resting state is generally characterized by an increase of slow EEG oscillations (delta and theta rhythms) and a decrease of fast alpha oscillations [1]. In response to sensory inputs, Laureys et al. reported that an electrical stimulation of the median nerve could activate the primary somatosensory cortex, but not higher-order multimodal areas that appear disconnected in vegetative patients [2].

Persistently vegetative individuals have no signs of awareness of themselves or their environment. Some may progress to a permanent vegetative state (VS), generally 3 months after an anoxic brain event and 12 months after brain trauma, while others may progress to a minimally conscious state (MCS), in which integrated but undersustained cortical functions are retained [3,4]. If the disorder

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persists for longer than 12 months after severe traumatic brain injury, the state is generally considered to be immutable and no treatment has been shown to accelerate recovery or improve functional outcome [5,6]. Nonetheless, some studies have shown unexpected preservation of large-scale cerebral networks in MCS patients, a condition characterized by definite behavioral evidence of awareness of self or the environment [7–11].

Neurostimulation to restore cognitive and physical functions is an innovative and promising technique for treating patients with severe brain injury. Deep brain stimulation (DBS) has been proposed as an experimental therapeutic strategy that might produce consistent and sustained effects of maintaining excitatory activity within functionally disconnected forebrain neurons [12]. It is used in treating Parkinson's disease, essential tremor, or depression but it has not been tested in clinical trials. Besides its invasiveness associated with surgical risks and complications, another major barrier to its wider use is the syndromic heterogeneity and variance of subjects who might benefit from DBS. Furthermore, the selection of potential recipients of DBS is limited by the current inability to estimate cerebral function based on bedside examination [13].

Among currently available non-invasive painless stimulation techniques, single-pulse transcranial magnetic stimulation (TMS) has been demonstrated to be effective for assessing motor cortex excitability and the integrity of conduction along the central and peripheral motor pathways. Similarly, repetitive transcranial magnetic stimulation (rTMS) has been shown to induce prolonged functional changes in cerebral cortex in normal conditions and therapeutic effects in different diseases [14–17]. Several studies suggest that the thalamocortical system can be engaged in rapid causal interactions [18–22]. One way to study this phenomenon is to perturb directly a subset of cortical neurons with TMS and monitor the brain's reaction using electroencephalography (EEG) [23–27].

To date, few studies have focused on the use of TMS in patients with impaired consciousness [28,29]. Recent advances in EEG-TMS co-registration have shed new light on EEG reactivity in humans [30–32]. For instance, Babiloni et al. demonstrated a relationship between alpha EEG rhythm and conscious awareness [33]. They showed that the parietal and occipital source power of alpha rhythm was high in the normal subjects, low in the PVS patients who recovered some level of consciousness at 3 months follow-up, and practically null in the PVS patients who did not recover. Their findings suggest that the sources of alpha rhythm are related to the outcome of PVS patients at 3 months follow-up. Corroborating this hypothesis, our recent study reported the reactivity of a single MCS patient after brain stimulation, in which an increase in the alpha band was correlated with functional improvement [34]. Also Louise-Bender Pape et al. reported results of a 10 Hz rTMS protocol applied to a MCS patient. They highlighted the therapeutic effect of rTMS concluding that thirty application of rTMS protocol may promote clinically significant neurobehavioral recovery in chronic severe traumatic brain injury [29].

Generally, in behaviorally awake but unresponsive VS patients, TMS triggers a simple, local slow response that indicates a breakdown in effective connectivity, similar to that observed in unconscious sleeping or anaesthetized patients [35–37]. In contrast, in MCS patients, who show fluctuating signs of non-reflexive behavior, TMS seems to trigger complex activations that sequentially involve distant cortical areas ipsilateral and contralateral to the site of stimulation.

Evidence from electrophysiological studies of stimulation over a healthy primary motor cortex (M1) suggests that there is a progressive increase in the excitability of local circuits during rTMS, but not only. Remote changes in cortical and subcortical activity, including associative regions such as the thalamus, caudate nucleus, and putamen, may be involved in stimulation. The nature of the remote effect of TMS is not well understood. The presumed net facilitatory effect on neural activity in remote regions may be produced by trans-synaptic or direct activation of cortico-cortical, or cortico-subcortical neurons [38].

Hypothesis

On this basis, we hypothesized that rTMS could be a useful means to investigate behavioral responsiveness in MCS patients, with possible implications for non-invasive therapy, since the majority of such patients show a consistent presence of residual network properties underlying the expression of fragmentary behavioral patterns [2,19].

The aim of this study was to investigate EEG reactivity and clinical response in 6 severely brain-injured patients in a state of altered consciousness. EEG rhythms and brain excitability were measured before and after a protocol of high-frequency rTMS.

Methods

Patients

Six patients (5 men, 1 woman; mean age, 48 years, ±standard deviation [SD] 19.4 years) in VS or MCS, admitted to the Neurorehabilitation Center of San Camillo Hospital, Venice, between April and July 2008 or resident in an adjacent nursing home during the same period, met the study inclusion criteria: absence of contraindications to TMS; stability of vital parameters; and >12 months since injury event [4,39]. The clinical characteristics of the enrolled patients are shown in Table 1.

Clinical features were assessed with the Disability Rating Scale (DRS) and the JFK Coma Recovery Scale (JFK CRS-R) [40,41]. The JFK CRS-R scale consists of 23 items in six subscales addressing auditory, visual, motor, oromotor, communication and arousal functions. The CRS-R subscales comprise hierarchically arranged items associated with brainstem, subcortical and cortical processes. The lowest item on each subscale represents reflexive activity, while the highest item represents cognitively mediated behaviors.

Table 1
Clinical profiles.

Case	Age	Gender	Clinical diagnosis	Etiology	MRI findings	Months since injury	DRS
1	70	M	MCS	Hemorrhagic	Right thalamic and intraventricular hemorrhage	48	27
2	37	F	VS	Traumatic	Subdural hematoma and diffuse cortical lesions	34	26
3	67	M	VS	Hemorrhagic	Multifocal bifrontal lesions	31	29
4	29	M	MCS	Traumatic	Multifocal bifrontal lesions	94	24
5	38	M	MCS	Traumatic	Pontomesencephalic lesion	36	23
6	27	M	VS	Hemorrhagic	Right centroparietal hematoma	12	28

DRS = Disability Rating Scale.

Table 2
JFK CRS-R response profile.

Case	Patients											
	1 (MCS)		2 (VS)		3 (VS)		4 (MCS)		5 (MCS)		6 (VS)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Auditory	2	4	1	1	2	2	2	2	1	1	0	0
Visual	2	4	1	2	2	2	1	2	1	1	0	0
Motor	3	6	2	2	3	3	3	3	3	3	2	2
Verbal	1	1	1	1	2	2	2	2	1	1	0	0
Communication	0	0	0	0	0	0	0	0	0	0	0	0
Arousal	2	3	2	2	2	2	2	2	2	2	0	0

JFK CRS-R = JFK Coma Recovery Scale.

In accordance with the Declaration of Helsinki, written informed consent to participate in the study was obtained from the patient's caregivers. The study design and protocol were approved by the Local Ethical Committee of San Camillo Hospital.

Clinical and neurophysiological evaluation were performed by the same neurologist before each rTMS session and immediately thereafter.

Stimulation procedures

rTMS was performed using a Magstim-Rapid2 Stimulator (Magstim Company Ltd, London, UK) which generates a maximum magnetic field of 2.2 T. TMS was delivered through a figure-of-eight focal coil oriented so that the induced electric current flowed in a posterior–anterior direction over the left/right primary motor cortex (M1) based on the presence of motor evoked potentials (MEPs). The primary motor cortex has been used extensively for rTMS studies because the effects of stimulation on motor system are easy to quantify by measuring the size of MEPs. In addition motor areas are densely interconnected with prefrontal cortical areas and subcortical structures, creating an important functional network to investigate.

MEPs were recorded from the left/right thenar eminence (TE) muscle with Ag/AgCl surface electrodes fixed to the skin with a belly-tendon montage. The amplified and band-pass filtered (50 Hz–20 kHz) electromyographic (EMG) signal was fed into a Medtronic EMG Machine at a sampling rate of 5 KHz. The coil was placed tangentially with respect to the scalp, with the handle pointing backwards and laterally at a 45° angle away from the midline. The stimulation coil was positioned with the handle pointing backward and over the optimal scalp position to obtain the highest motor evoked potential (MEP), corresponding approximately between C3/C4 and P3/P4 in all patients. Induced currents were directed postero-anteriorly. The motor threshold (MT) intensity was approached from individual suprathreshold levels by reducing the stimulus intensity in 1% steps. MT intensity was defined as the lowest stimulator output intensity capable of inducing MEPs of at least 50 μV peak-to-peak amplitude in relaxed TE muscles in at least half of 10 trials over the optimal scalp position [42]. Stimulus intensities are expressed as a percentage of maximum stimulator output.

Generally, the click associated with the coil discharge propagates through air and bone and can elicit an auditory N1–P2 complex at latencies of 100–200 ms [43,44]. In this study, we inserted earphones to mask the coil-generated click in all patients to avoid any effect of clicks in the modulation of cortical oscillatory activities. A loud white noise (90 dB) was played through the insert earphones to mask the coil-generated click [45].

Each patient underwent a session of 1000 stimuli delivered in 10 trains of 20 Hz rTMS at MT [46]. Each train lasted 5 s with a 20 s inter-train pause. The magnetic stimulation was administered in accordance with safety guidelines [39].

Experimental design

Spontaneous EEG acquisition and magnetic stimulation were performed during the same experimental session, which consisted of 5 conditions (steps):

1. baseline EEG acquisition (3 min) at T_0, rest motor threshold (rMT) and hot-spot detection over the left/right M1 area (left for patients nos. 1, 2, 3, and 6; right for patients nos. 4 and 5), MEP at 120% of MT.
2. rTMS over the left/right M1 area: 5 s of stimulation followed by 20 s of rest repeated 10 times; rMT detection and MEP at 120% of MT.
3. EEG acquisition (3 min) at T_1;
4. rest (35 min);
5. EEG acquisition (3 min) at T_2.

EEG was recorded during all steps; the computer triggered the magnetic pulses by insertion of a marker in a track of the multichannel EEG recording system. Data acquired at T_0, T_1 and T_2 were used to compute the power values.

EEG data recordings

EEG data were acquired using a magnetic resonance (MR)-compatible EEG amplifier (SD MRI 32, Micromed, Treviso, Italy) and a cap providing 21 TMS-compatible Ag/AgCl coated electrodes (diameter 8 mm; thickness 0.5 mm) with 2 mm slits to interrupt eddy currents, positioned according to a 10/20 system. The reference was placed anterior to Fz and the ground posterior to Fz, as in previous studies using the same acquisition setup [47,48]. The EEG data were acquired at a rate of 1024 Hz using the SystemPlus software package (Micromed, Italy). 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.

EEG data analysis

Data were processed using an average reference. EEG recordings were band-pass filtered from 1 to 30 Hz using a Finite Impulse Response (FIR) filter. A baseline correction was also applied to all channels. EEG epochs with ocular, muscular and other types of artifact were visually identified and manually rejected. Three conditions were selected for the analysis: EEG in resting state acquired at the beginning of the exam (T_0), after rTMS (T_1) and 38 min after rTMS (T_2). Segmentation into non-overlapping epochs of 2 s was applied to all channels and for each condition. A mean of 56.3 epochs of 2 s (about 112 s) were used for the analysis at T_0, 45 (90 s) at T_1 and 60 (120 s) at T_2. A Fast Fourier Transform (FFT) was applied to non-overlapping epochs, each containing 2048 data points, with maximum resolution (0.5 Hz), and then averaged across epochs under the same conditions. Density power spectra were estimated for all frequencies between 0 and 512 Hz, then relative power (%) was estimated for delta (1–4 Hz), theta (5–8 Hz), alpha (8–12 Hz) and beta (13–30 Hz) frequency.

ANOVA for repeated measures was applied to relative powers, MT and MEP, with the factor "time" (T_0, T_1, T_2); the sphericity assumption was assessed with Mauchly's test. Greenhouse–Geisser epsilon adjustments for non-sphericity were applied where appropriate. Post-hoc paired *t*-test adjusted for multiple comparisons with Bonferroni method was used. Statistical significance was set at $P < 0.05$.

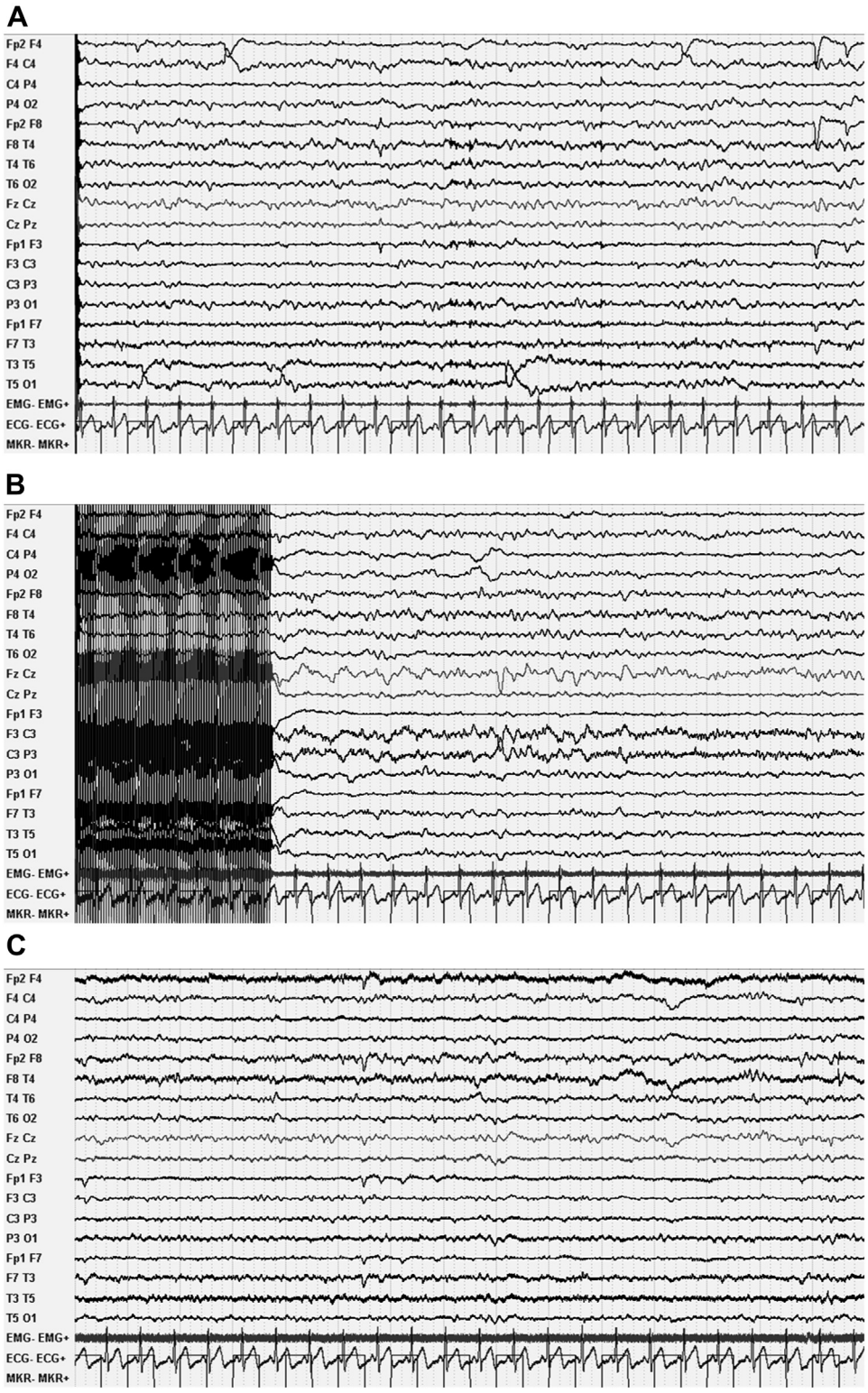

Figure 1. Raw data from patient no. 6: EEG during rest condition T_0 (A), immediately after rTMS T_1 (B), 38 min after rTMS T_2 (C) (EEG amplitude 70 μV/cm; EMG amplitude 100 μV/cm; ECG amplitude 300 μV/cm).

Results

Clinical effects

A good clinical response was observed in only one of the 6 patients. A detailed account of the patient findings was the focus of a separate study and reported independently [34]. After magnetic stimulation, a remarkable improvement in reactivity was noted, with active arousal mechanisms, eyes focused on examiner, and small functional movements of the hand and arm on command. The clinical effects lasted 6 h confirmed hourly by repeated JFK CRS-R assessment [34]. Significant changes in the JFK CRS-R subscores

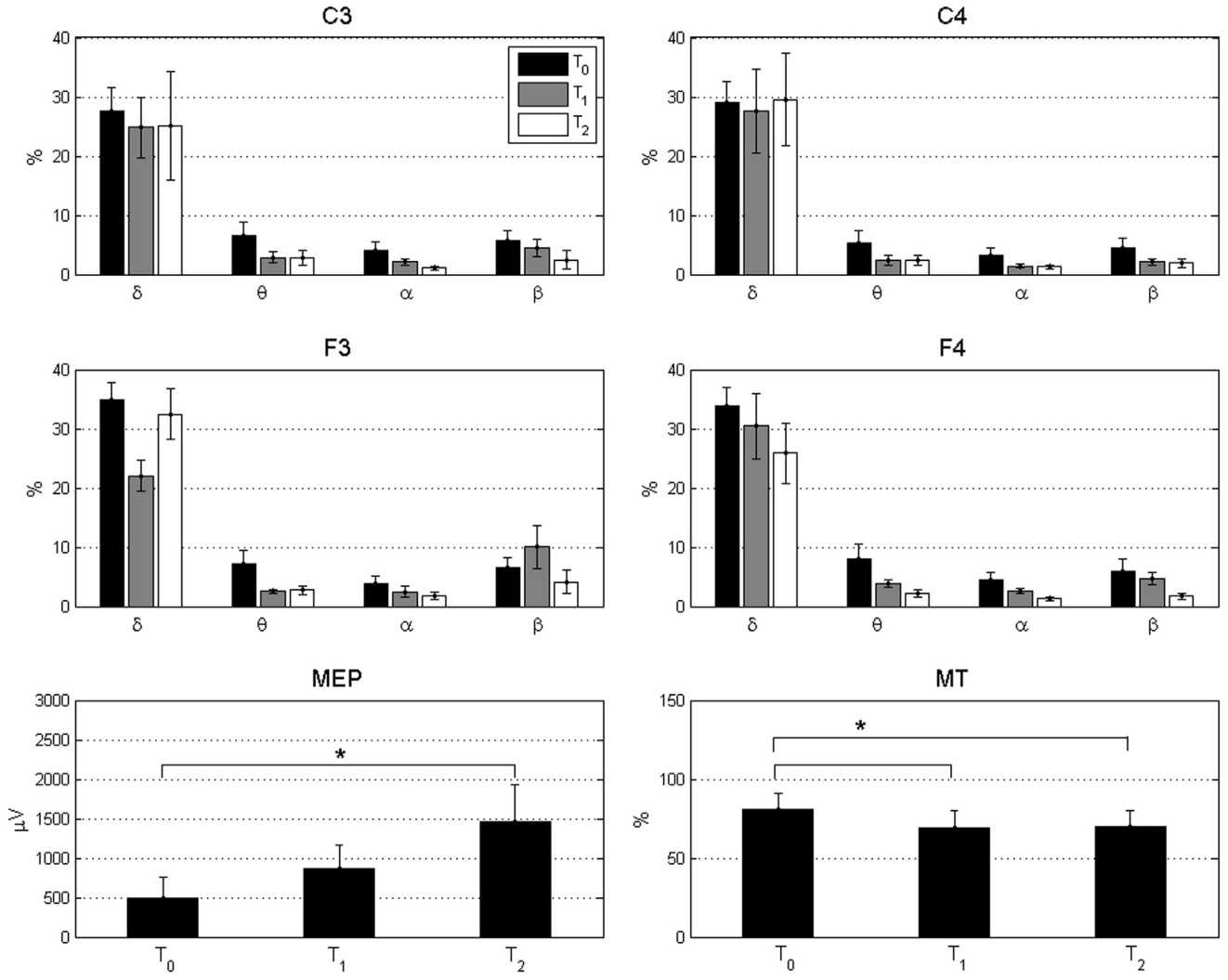

Figure 2. Grand average (5 patients) of delta, theta, alpha and beta relative powers (%) at T_0, T_1, T_2. Bars represent standard error. Mean values of MEP (μV) and MT (%) at T_0, T_1, T_2.

are shown in Table 2 (patient no.1). None of the other patients showed any clinically remarkable response.

Neurophysiological effects: EEG reactivity

No reliable, marked changes were noted in 5 patients after transcranial stimulation in spontaneous EEG activity among T_0, T_1 and T_2 (Fig. 1). A power decrease for all power bands, albeit not significant, was observed immediately after stimulation and after 35 min, except for C4 and F3 where the delta power increased at T_2 and for F3 where the beta power increased at T_1 (Fig. 2).

In the one patient with clinical arousal (patient no. 1) we noted good EEG reactivity: immediately after rTMS there was an increase in signal amplitude that was maintained even after 38 min (Fig. 3). A persistent increase in all rhythms, in delta rhythm in particular, was observed at both T_1 and T_2. A power increase over baseline was also observed in the alpha and the beta range (Fig. 4). The percentage of the increase was lower than in the delta range, but it was more evident at T_1 than at T_2.

Neurophysiological effects: motor threshold and MEP amplitude

The MT, measured before rTMS in order to determine the stimulation intensity, showed a significant decrease after brain stimulation ($F(1.007,4.028) = 34.801$, $P < 0.05$), associated with a significant increase in MEP amplitude ($F(2,10) = 7.964$, $P < 0.05$) in all 6 patients (Fig. 2). The decrease in MT was statistically significant between T_0 and T_1 ($P < 0.05$) and between T_0 and T_2 ($P < 0.05$), while the increase in MEP was significant only between T_0 and T_2 ($P < 0.05$ not corrected). Summarizing, the decrease in MT and the increase in MEP amplitude were present at T_1, after rTMS, and at T_2 (after 38 min). Increased motor excitability was observed also in the 5 patients who showed no EEG reaction to brain stimulation.

Discussion

In this study, a single session of 20-Hz rTMS over the motor cortex induced some significant electrophysiological changes in motor threshold and MEP amplitude in 6 patients in the vegetative or minimally conscious state. In only one MCS patient, however, were long-lasting (up to 6 h) behavioral and EEG modifications associated with changes in motor excitability [34].

The isolated increase in MEP amplitude without changes in EEG activity after rTMS might be related to short-range connectivity, perhaps reflecting a functional correlate of strictly local activity over the motor cortex [14,15,17]. If so, this could further confirm the hypothesis that unambiguous vegetative state patients can activate primary cortices but not higher-order multimodal areas that appear disconnected [21,49].

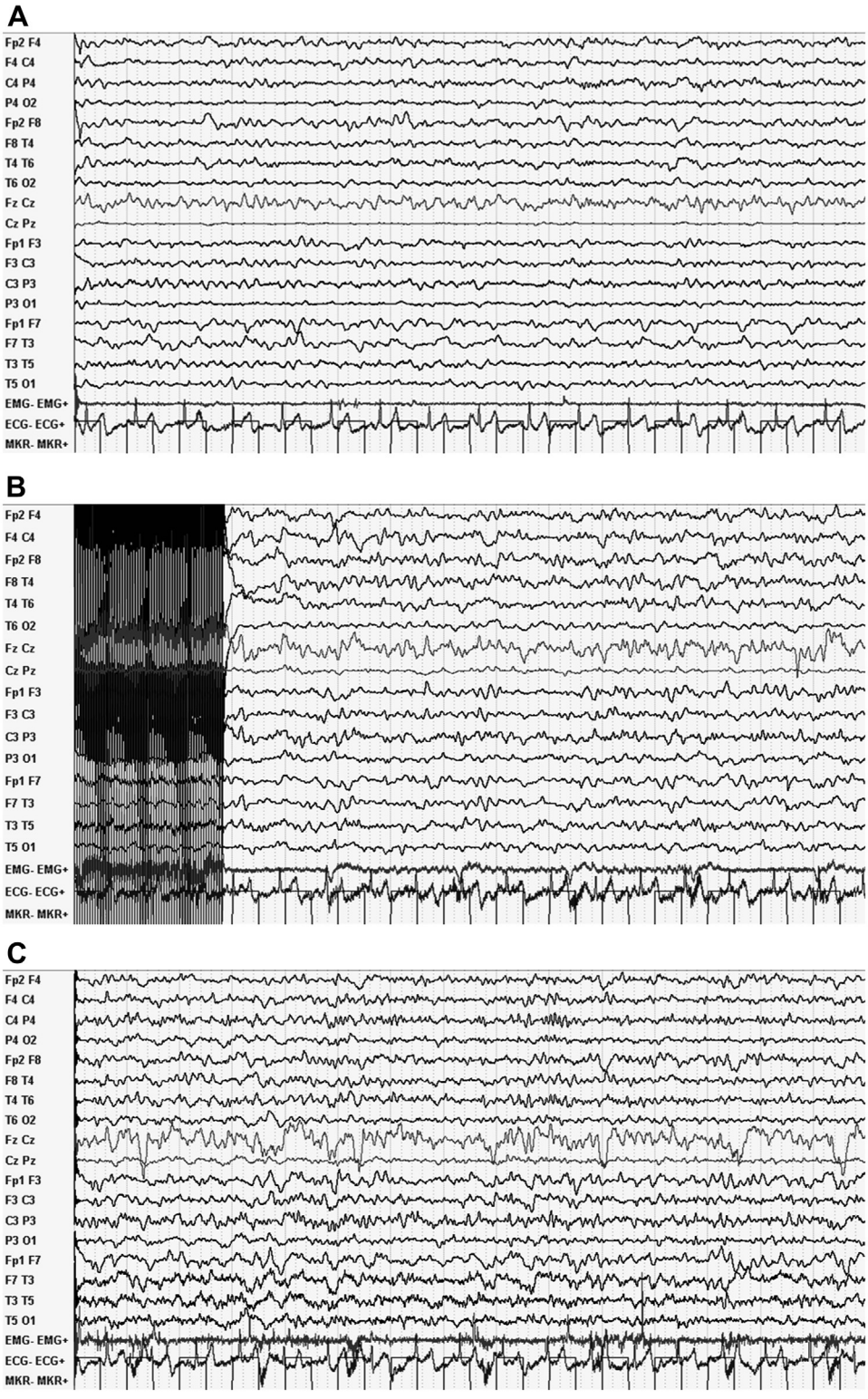

Figure 3. Raw data from patient no. 1: EEG during rest condition T_0 (A), immediately after rTMS T_1 (B), 38 min after rTMS T_2 (C) (EEG amplitude 70 μV/cm; EMG amplitude 100 μV/cm; ECG amplitude 150 μV/cm).

Yet, we observed a correlation between EEG reactivity and clinical response in a MCS patient after brain stimulation. The EEG changes were related to the reappearance of faster activity (alpha and beta) and the increase in slow waves (delta and theta), which could represent a phenomenon of rTMS-induced excitatory neuromodulation. Modulation of cortical excitability with rTMS can indeed influence behavior. Enhancement of motor cortex excitability seems to speed up procedural learning in preserved brains [50]. Repetitive TMS, more so than single TMS, can induce significant perturbations, with deep effects on subcortical regions, including increased dopamine release and long-lasting effects of synchronization of slow and fast brain oscillatory activity [51–53].

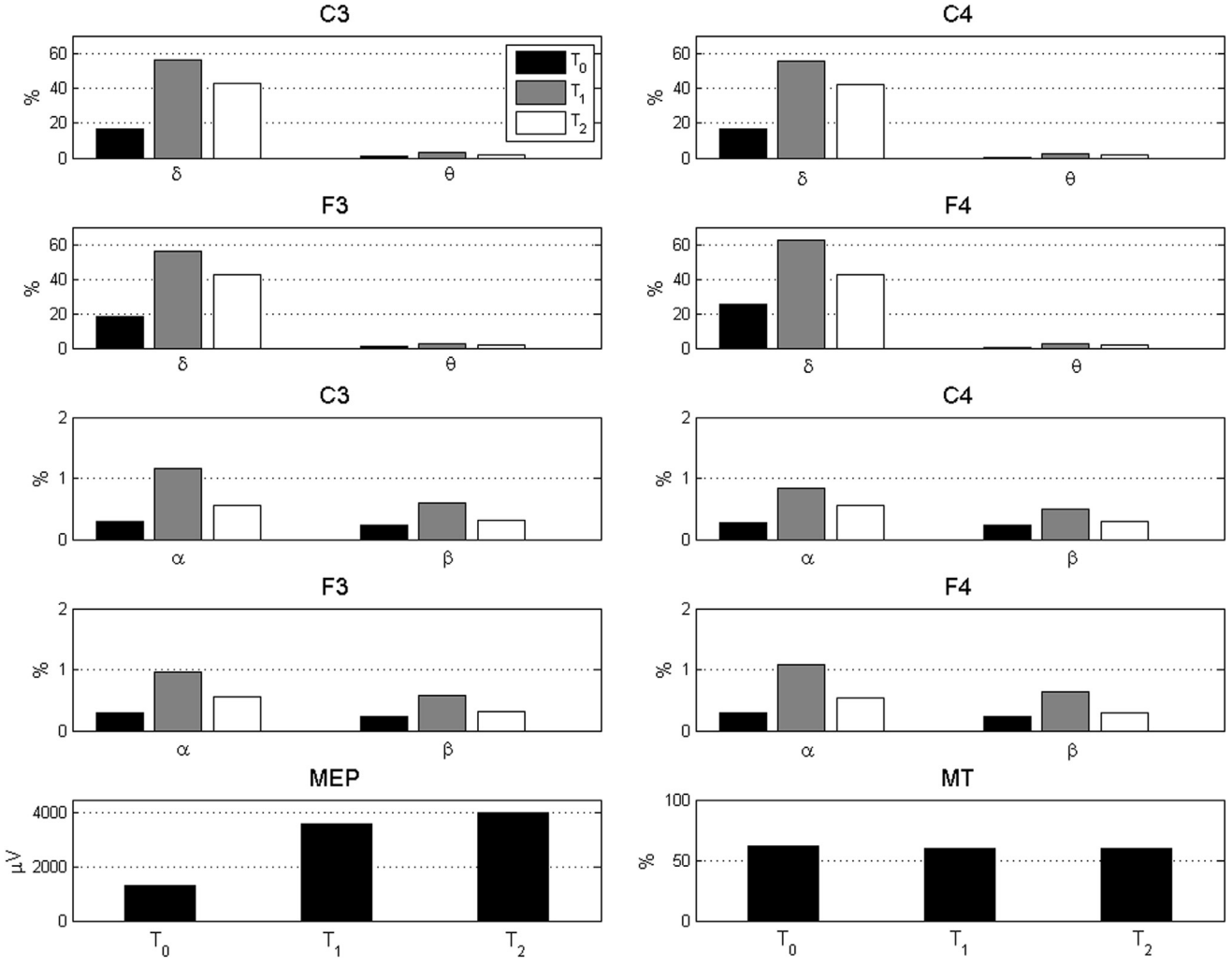

Figure 4. Patient no. 1. Delta, theta, alpha and beta relative powers (%) at T_0, T_1, T_2. Values of MEP (μV) and MT (%) at T_0, T_1, T_2.

Moreover, only the high-frequency stimulation can produce a better desynchronization of EEG rhythms. Indeed, some studies have investigated how TMS can modulate oscillatory activity when delivered over M1. In those studies, TMS-induced oscillations have been recorded after single pulse, as well as low and high-frequency stimulation. The effects of rTMS on cortical excitability appear to depend on the combination of stimulus frequency and duration: short high-frequency rTMS seems to be more effective than longer trains, and low-frequency rTMS requires longer applications [30]. Thus, single-pulse TMS induces a short topographically restricted intensity-dependent synchronization [45]; TMS at 1 Hz frequency can cause dose-dependent increase in power of oscillatory activity and high-frequency trains (20 Hz) result in a progressively increasing modulatory effect, likely due to a temporal summation of the effects induced by each single pulse, able to bring to resonance the activity of a growing number of neurons of the targeted sensory-motor network [54].

The mechanisms underlying rTMS on brain activity could also be related to: a change in excitability not only at the cortical level but also in deep structures and synchronization between spared areas of the cerebral network, as documented by the increase in oscillatory activity in the patient with arousal as compared to the unreactive patients [28,35,37]. A possible explanation for the effect of neurostimulation is the so-called mesocircuit hypothesis according to which large-scale forebrain dysfunction may arise as a result of at least three general mechanisms: 1) widespread death of forebrain neurons (i.e., sufficient to produce brain death or permanent VS); 2) widespread deafferentation and disconnection of neurons; and 3) "circuit"-level functional disturbances due to the loss of these neuronal connections [12].

The use of non-invasive rTMS stimulation to induce changes in brain oscillatory activity in such patients represents a new, additional tool for brain investigation which may allow to select patients eligible for neurostimulation. The central thalamus and the frontal lobe are closely linked through their direct cortico-thalamic connections, including the supplementary motor, anterior cingular, premotor and prefrontal cortex, and indirect links through the frontal cortical-striato pallidal-thalamocortical loop systems. Nearly a decade ago, Strafella et al. reported that rTMS can increase brain excitability and induce the release of dopamine by acting over the circuit. This could be the major hypothesis to explain the long-lasting effect of brain stimulation [51].

There is mounting evidence that consciousness depends not only on some specific circuits, but also on the capacity of remote brain regions to interact through cortico-cortical and cortico-thalamo-cortical connections [21,22,55–57]. The effective connectivity measured by TMS/EEG could therefore distinguish between conditions in which consciousness is present (alert wakefulness,

dreaming) and those in which consciousness is reduced or lost (sleep and anesthesia) [35,36,58].

This preliminary study has some limitations. Well-designed studies with larger sample size and more detailed data are needed to confirm these conclusions. Our patient population was heterogeneous, representing patients with different kind of lesions and diagnosis. The effect of rTMS was found positive in only one patient with thalamic lesion, whereas the other patients suffered from multifocal cortical or subcortical lesions and showed no changes in clinical assessment and oscillatory brain activity. Therefore, we cannot exclude that the lack of EEG changes may depend on the localization of brain damage. However, this finding suggests that different neuronal populations are involved in the electrophysiological phenomena induced by rTMS and that these neurons may be affected differentially. Increasing the number of patients could clarify this point and could lead to identify a predictor of the clinical and electrophysiological response to rTMS based on the electrical cortical activity or neuroimaging data recorded at the baseline. Patient assessment including clinical and neurophysiological tests was repeated 35 min after rTMS. We did not investigate how long the rTMS-induced effects could last, except in the one patient who manifested some clinical improvement and was clinically evaluated for 8 h. Nonetheless, our study encourages broader research programs, with studies using rTMS and longer cognitive follow-up designed to clarify the duration of the cognitive effects of stimulation and the impact of therapy on MCS patients.

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