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Moving Toward Conscious Pain Processing Detection in Chronic Disorders of Consciousness: Anterior Cingulate Cortex Neuromodulation

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Abstract: **It has been assumed that patients with chronic disorders of consciousness (DOC) do not feel pain, but it is possible that some of them just cannot report it. Modulation of γ-band oscillatory activity (γBO) in centroparietal areas (considered as a marker of either subjective pain perception processes or pain-related motor behavior preparation) by part of the anterior cingulate cortex (ACC) has been proposed to be suggestive of conscious pain perception and could therefore be used to assess the maintenance of some level of conscious pain perception in patients with DOC. Hence, we used a repetitive transcranial magnetic stimulation (rTMS) approach in an attempt to trigger frontoparietal output. We enrolled 10 healthy participants (HC), 10 patients in a minimally conscious state (MCS), and 10 with unresponsive wakefulness syndrome (UWS), who underwent a 1-Hz rTMS protocol over ACC. Before and after the neurostimulation paradigm, we measured the pain-rating assessment (pVAS), γBO, latency, and the amplitude of cortical nociceptive potentials evoked by transcutaneous electric sinusoidal stimuli (EEP). In all the HC and MCS and in 2 of the UWS subjects, rTMS increased γBO and reduced the EEP amplitude, whereas pVAS scoring improved in the HC. Our findings provide some evidence about conscious pain processing even in patients with severe DOC and show that rTMS over ACC may be a useful approach to better investigate the level of conscious impairment.**
Perspective: ***Patients with DOC may not be able to respond to pain stimuli, although they may feel it. The possibility of detecting residual pain perceptions by means of a noninvasive neuromodulation paradigm, studying the correlation between the ACC and centroparietal γBO, may help clinicians to better assess pain in such individuals.***

Key words: *Repetitive transcranial magnetic stimulation, anterior cingulate cortex, chronic disorders of consciousness, γ-band oscillations, pain processing.*

The issue of nociception and pain perception in patients with chronic disorders of consciousness (DOC) is of extreme ethical and clinical importance because such individuals have a limited repertoire of pain-related clinical expressions. Patients with unresponsive wakefulness syndrome (UWS) show a wide disconnectivity of the frontoparietal networks (including the pain matrix) and the underlying complex thalamocortical network, which results in a loss of awareness.[21,41,58,59,63,75,89] Hence, nociceptive stimulus processing cannot access a higher conscious level,[6,7,50] ie, UWS individuals cannot experience pain, and only the nociceptive reflex responses can be detected.[34,49,79] On the other hand, patients in a minimally conscious state (MCS) can show purposeful motor behaviors (beyond the reflexive), which are a marker of better preserved cortical/subcortical integrative functions.[76] Nevertheless, it has been suggested that some UWS patients might somehow experience pain, although they are unable to clearly communicate it as a result more of an extreme cognitive/motor output failure than of severe connectivity impairment.[8,31]

Hence, some specific approaches to detect pain perception in patients with DOC are mandatory. To this end, many electrophysiological paradigms have been implemented, besides functional neuroimaging approaches, eg the analysis of cortical evoked responses/activations from laser/electric peripheral nerve/cutaneous stimulation. The main problem with such approaches is reliable correlation among the evoked response, the pain perception, and the subjective relevance of the stimuli. Because MCS and UWS may show

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similar cortical evoked responses,[19,20] it is hypothesized either that UWS patients can somehow experience pain or that such evoked responses may represent merely automatic neural processing rather than evidence of awareness preservation.[6,19,20,41,42,51,91] Hence, it is important to prove a clear association between brain response and conscious pain processing to better assess pain perception. In healthy individuals, cortical pain processing mechanisms (depending on wide frontoparietal networks)[12] can be studied through analysis of nociceptive-induced γ-band oscillatory activity (γBO) within centroparietal areas, without any substantial need for the individual's participation or cognitive/motor output.[36,66] Nociceptive-induced γBO may represent a marker of conscious and subjective pain perception, the access of the neural process regarding nociceptive and salient stimuli to a higher conscious level, or the summary effects of bottom-up and top-down processes concerning pain modulation.[77,81]

Within the wide frontoparietal network, the anterior cingulate cortex (ACC) could have a striking role in pain processing and gating at the cortical level.[14,88] Triggering the ACC could allow the γBO to be modulated and, therefore, could shed some light on pain processing and pain-gating mechanisms at the cortical level.[29,32,53] Repetitive transcranial magnetic stimulation (rTMS), which is a useful and noninvasive tool to modulate brain endogenous activities, may be of some help. It has been shown that the use of enlarged-diameter coils or multicoil devices can deliver high energy amounts with sufficient focality[25,38,84] and so overcome the problems related to target depth, stimulation focality, seizure induction, and interference with activation of cutaneous afferents.[1,73,84]

Hence, we hypothesized that γBO could help in differentiating MCS from UWS, in addition to clinical assessment (because the γBO power modulation induced by nociceptive stimuli may depose conscious pain perception).[36,72] Moreover, γBO modulation after an rTMS protocol over the ACC (which presumes partial integrity of the cingulate-sensorimotor networks[22,28]) may further support DOC differential diagnosis, thus allowing us to identify patients with functional locked-in syndrome within UWS. We quantified the aftereffects of the ACC-rTMS protocol by means of a clinical-electrophysiological approach with regard to the modulation of centroparietal γBO power and the somatosensory/nociceptive evoked potential amplitude/latency (elicited by sine-wave electric transcutaneous stimulation).

Methods

Participants

We enrolled 10 healthy control individuals (HC) (mean age 55.8 $\pm$ 5.7 years) and 20 patients with severe DOC (10 MCS and 10 UWS), after hypoxic-ischemic or traumatic brain damage, who met the criteria for a vegetative state (VS)/MCS diagnosis, according to the Multi-Society Task Force on PVS[79] and Giacino and coworkers,[34] and the following exclusion criteria: preexisting severe neurological or systemic diseases; critical conditions, such as inability to breathe independently or hemodynamic instability; administration of other modifying cortical-excitability drugs than L-DOPA and baclofen; presence of epileptic history, pacemakers, aneurysm clips, neurostimulators, brain/subdural electrodes, or other electric/electromechanical devices; and presence of electroencephalographic (EEG) suppression-burst pattern. The patient's DOC level was further assessed using the JFK Coma Recovery Scale-Revised (CRS-R).[34,45] Detailed demographic and clinical characteristics are reported in Table 1. The local ethics committee approved the present study, and written informed consent was obtained from either the HC or the legal guardian of each patient.

Study Design

HC were seated on a comfortable reclining chair; for patients with DOC, the experimental procedure was carried out at the patient's bedside. First, the participants' electrical evoked potentials (EEPs) and the related γBO power were evaluated. Then, they performed the real (A) and the sham (B) ACC-rTMS, according to a random A/B scheme, with an inter-TMS interval of 2 hours. Both the participants and the experimenters who analyzed the data (A.N., A.L.) were blinded to the stimulation procedure. We measured the effects of rTMS on EEP and γBO power immediately after the end of the rTMS (T_0), and after 30 (T_{30}) and 60 minutes (T_{60}).

Clinical Assessment

The neurological examination predominantly showed a pattern of spastic tetraparesis. Patients were clinically evaluated also through the JFK CRS-R, which was administered daily for 30 days before study enrollment by at least 2 independent neurologists skilled in DOC to assess a true and stable UWS condition. CRS-R is a reliable and standardized scale that integrates behavioral and clinical assessments; includes the current diagnostic criteria for coma, VS, and MCS; and allows the patient to be assigned to the most appropriate diagnostic category. It consists of 29 hierarchically organized items divided into 6 subscales concerning auditory, visual, motor, oromotor, communication, and arousal processes. The total score ranges from 0 to 23. Scores of $\leq$2 on the auditory, motor, and oromotor/verbal subscales, $\leq$1 on the visual subscale, and 0 on the communication subscale are consistent with the diagnosis of VS. Thus, the CRS-R seems to be an appropriate measure for characterizing levels of consciousness and for monitoring neurobehavioral functional recovery.[33,35]

In HC, the pain intensity rating was measured by means of a visual analog scale for pain (pVAS).[57] The pVAS is a continuous scale consisting of a horizontal line 100 mm long, and 2 vertical lines with verbal descriptors, one for each extreme symptom, ie, "no pain" (0 mm) and "pain as bad as it could be" or "the worst imaginable pain" (100 mm). The higher the score, the greater the pain intensity rated, with the following cut points: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm).[78]

Table 1. DOC Patients' Clinical-Demographic Characteristics

DOC	Cause	Gender	Age (y)	BI Onset (mo)	CRS-R	MRI Pattern	Treatment
MCS	A	M	32	6	16	WMH	L-DOPA
	A	F	72	6	17	WMH	L-DOPA, baclofen
	A	M	51	18	15	WMH	L-DOPA
	T	M	44	4	19	R-F h	
	A	F	72	6	18	WMH	
	T	F	70	33	15	R-Fb h	
	T	M	33	18	13	Multiple h	L-DOPA
	A	M	38	4	17	WMH	
	T	F	43	33	16	R-F h	L-DOPA, baclofen
	T	M	63	15	19	multiple h	L-DOPA
Mean ± SE			52 ± 5	14 ± 4	16 ± 1		
UWS	A	F	43	10	6	WMH	L-DOPA
	A	M	40	24	7	WMH	
	A	**M**	**45**	**9**	**6**	**WMH**	
	T	F	54	20	4	DAI, b-F h	L-DOPA
	T	**F**	**48**	**11**	**4**	**DAI, SAH, L-Fp h**	**Baclofen**
	A	M	69	13	7	WMH	
	T	F	62	16	4	DAI	Baclofen
	T	F	64	17	5	DAI	
	T	M	55	15	5	R-Fb h	L-DOPA
	A	M	58	13	5	WMH	Baclofen
Mean ± SE			54 ± 3	15 ± 2	5 ± .5		

Abbreviations: BI, brain injury; A, postanoxic; M, male; WMH, white matter hyperintensity; F, female; T, posttraumatic; R-F, right-frontal; h, hemorrhagic lesion; R-Fb, right-frontobasal; DAI, diffuse axonal injury; b-F, bilateral-frontal; SAH, subarachnoid hemorrhage; L-Fp, left-frontopolar.
NOTE. Real rTMS-responsive UWS patients are marked in **boldface**. Data are reported as individual values and group means ± SE.

TMS Device, Procedure, and Configuration

rTMS was delivered through a Magstim rapid-rate stimulator with a double-cone coil (Magstim Co Ltd, Whitland, Dyfed, UK). The coil consists of 2 angled winding conformations, which increases the stimulating power of the deep brain areas. We chose this coil because previous studies demonstrated a modulation of cognitive function. We first measured the active motor thresholds, ie, the minimum TMS intensity able to elicit a stable motor evoked potential of 100 μV peak-to-peak amplitude from a relaxed tibialis anterior muscle in at least 5 of 10 consecutive sweeps.[71] We adopted this approach because the motor leg representation is placed in the depths of the central sulcus, approximately at the anterior part of the medial cingulate cortex. We delivered 600 stimuli at a frequency of 1 Hz and at an intensity of 110% of the active motor thresholds. The current in the coil flowed in an anterior to posterior direction, with the coil loops put lateral to the midline. The stimulation site was individually set for each participant according to a previous morphological magnetic resonance imaging (MRI) study, and it was approximately ~2 cm anterior to one-third of the nasion-inion distance.[37] For the sham protocol, we used a spacer-equipped coil that was similar to the real one.

Peripheral Electric Stimulation

Transcutaneous sine-wave electrical stimulation has been used to activate specific nociceptive fibers and to evoke specific cortical components compatible with the ultralate laser evoked potential, using juxtathreshold intensities and different frequencies of stimulation (eg, in the current perception threshold protocol). Nevertheless, there are conflicting reports concerning this approach.[15,27,30,47,62,69,85] However, the use of EEG analysis procedures, such as those applied in laser evoked potential studies, may be useful to overcome such problems.

In analogy to previous studies,[27,47] the electric stimuli were applied to the dorsum of the right hand, with a horizontal disposition of electrodes from the thenar to hypothenar eminences, 3 cm apart, and were delivered through a Digitimer Stimulator (Digitimer, Welwyn Garden City, Hertfordshire, UK). Transcutaneous sine-wave electric stimuli (of 1 millisecond duration) were delivered in 3 different blocks, repeated twice (5 minutes each), and in random order. These blocks consisted of 30 stimuli of 5 Hz sinusoidal current delivered at .1 Hz (for C-fibers), 30 of 250 Hz at .1 Hz (δ), or 250 of 2,000 Hz at 3 Hz (α). Skin impedance was kept below 5 kΩ. Voltage was automatically adjusted by the stimulator to keep the intensity constant. The stimulus intensity was set at 1.5 times the HC mean perceptual threshold (3.5 ± .3, 4.2 ± .6, and 6.1 ± 1.1 mA, respectively). To avoid generation of harmonic frequencies at the onset of the electrical current and habituation phenomena, the intensity of each stimulus was increased and decreased at the beginning and end of the stimulation and shifted during the 20-μA stimulation. After each trial, the HC individuals were asked to rate perceived pain through the pVAS. The scale was quantified in a range of 0 to 100% for statistical analysis.

EEP and γBO Power

EEG was continuously recorded using Ag/AgCl electrodes placed at Cz and C3′, serving as active electrodes,

and FPz as reference (according to the 10/20 system). The ground electrode was set at Fz. Signals were filtered at 5 Hz to 2 kHz for 2,000-Hz stimulation, .3 to 30 Hz for 250-Hz stimulation, and .1 to 5 Hz for 5-Hz stimulation, with a notch filter, and sampled at 512 Hz (Micromed, Mogliano Veneto, Italy). In our study, we applied more selective band-pass filters in an attempt to identify early (Aβ), late (Aδ), and ultralate (C) components, in combination with specific electric sinusoidal current stimulation frequencies (2,000, 250, or 5 Hz). The electro-oculogram was registered to monitor ocular artifacts through bipolar derivations using further Ag/AgCl electrodes. The registered trials were epoched (from −100 to 200 milliseconds for Aβ-EEP and from −100 to 1500 milliseconds for Aδ- and C-EEP), and thus averaged offline to obtain Aβ-, Aδ-, and a C-EEP.

To better characterize the electric stimulation aftereffects, EEG data were analyzed through a free release of the EEGLAB toolbox[24] to estimate the γBO power. EEG epochs were refiltered at 70 to 120 Hz (being γ-band, this interval is potentially related to pain processing).[36,46] A notch filter (50 Hz) was also applied. After artifact rejection (visual inspection and independent component analysis), a fast Fourier transform was computed and then averaged across epochs under the same conditions. Power spectra were estimated for all frequency bins (2 Hz maximum bin width) for either Aβ-EEPs or Aδ/C-EEPs. Recordings were Hamming-windowed to control for spectral leakage. Broadband power changes were obtained by averaging the power values of the frequency range chosen for analysis. The γBO power was quantified using an event-related desynchronization/synchronization procedure according to the Pfurtscheller formula.[67]

Statistical Analysis

Each clinical (VAS, CRS-R) and electrophysiological (EEP amplitude and latency, γBO power) parameter was compared at baseline among each group through unpaired-sample t-tests. To assess rTMS effects, we applied separated 3-way repeated-measure analyses of variance, implying time (4 levels: T_{PRE}, T_0, T_{30}, T_{60}) and protocol (2 levels: real and sham rTMS) as within-subject factors, and group (3 levels: UWS, MCS, and HC) as a between-subject factor. The Greenhouse-Geisser method was used if necessary to correct for nonsphericity. In all conditions, the normal distribution of the data was evaluated with the Kolmogorov-Smirnov test (for all $P > .2$). Conditional on a significant F-value, post hoc paired t-tests (adjusted for multiple comparisons using the Bonferroni method) were used to investigate significant main effects and interactions. For all statistical tests, $P < .05$ was considered significant. All data are given as mean or percent change ± standard error (SE). Pearson's correlation analysis was carried out to assess a possible relationship among the clinical and electrophysiological parameters.

Results

No side effects were observed in HC and DOC patients, either during or after the experiment. We did not detect significant changes in vital signs, nystagmus induction, sweating, and CRS-R arousal scale worsening.

EEP and γBO Power Baseline Findings

The transcutaneous electric stimulation yielded a pVAS score of 4.4 ± .3 and clear EEPs in HC at baseline. Detailed data are reported in Table 2. There was a correspondence in terms of latency and morphology between the Aβ-EEP and the N20 component of somatosensory evoked potentials, the Aδ-EEP and the N2P2 component of Aδ-fiber laser evoked potentials, and the C-EEP and the ultralate laser evoked potentials.[17,83]

Patients with DOC showed a reduction in EEP amplitudes and γBO power and an increase in EEP latencies, compared with HC (more detailed data in Table 2). UWS patients showed more delayed EEP latencies, reduced

Table 2. Baseline EEP and γBO Power Raw Values, With HC/MCS/UWS Comparisons (Unpaired t-Tests)

				P		
	HC	*MCS*	*UWS*	*HC/MCS*	*HC/UWS*	*MCS/UWS*
Aβ-EEP						
Latency (ms)	35 ± 1	59 ± 4	68 ± 6	<.001	<.001	<.001
Amplitude (μV)	9 ± 1	3 ± 1	1 ± 1	NS	<.001	.005
Aδ-EEP						
Latency (ms)	285 ± 4	624 ± 88	923 ± 48	<.001	.003	.002
Amplitude (μV)	24 ± 3	18 ± 2	4 ± 2	NS	.04	.004
C-EEP						
Latency (ms)	910 ± 25	1172 ± 54	1564 ± 59	<.001	<.001	.004
Amplitude (μV)	13 ± 2	9 ± 1	2 ± 1	NS	.009	.02
γBO power						
Aβ-EEP	21 ± 1	5 ± 1	2 ± 2	.04	<.001	<.001
Aδ/C-EEP	−12 ± .2	−18 ± .4	−25 ± .9	.001	<.001	<.001

Abbreviations: γBO power, event-related γ-band power; NS, not significant.
NOTE. Data are reported as mean ± SE.

EEP amplitudes, and γBO power than MCS patients. We did not find any significant difference concerning the causes of DOC. Nevertheless, the small sample size limits such significance.

rTMS Neurophysiological Aftereffects

We observed short-lasting rTMS aftereffects (limited to T_0) only after the application of the real rTMS in all the HC and MCS individuals and in 2 UWS patients (numbers 3 and 5) (Fig 1). These 2 patients showed real rTMS aftereffects that were comparable with MCS participants. On the other hand, the sham rTMS was ineffective (Fig 2). EEP latencies were not modified by real rTMS in either HC or DOC patients. Real rTMS was particularly effective in increasing the Aδ/C-γBO power (Time × Group × Protocol interaction: $F_{(6,162)} = 9.7$, $P < .001$; HC: $t_{(1,9)} = 7.8$, $P < .001$; MCS: $t_{(1,9)} = 6.4$, $P < .001$) and reducing the Aβ-γBO power (Time × Group × Protocol interaction: $F_{(6,162)} = 7.8$, $P < .001$; HC: $t_{(1,9)} = -4.9$, $P = .001$; MCS: $t_{(1,9)} = -3.5$, $P = .006$), the Aδ-EEP amplitude (HC: $t_{(1,9)} = -4.9$, $P = .001$), and the C-EEP amplitude (HC: $t_{(1,9)} = -6.9$, $P < .001$). The 2 rTMS-responsive UWS individuals were not significantly different from the other UWS patients at baseline in either the clinical or the electrophysiological parameters.

HC reported a reduction of pVAS score only after real rTMS (T_0: 2.5 ± .3; Time × Protocol interaction: $F_{(3,27)} = 9.4$, $P < .001$; $t_{(1,9)} = 3.6$, $P = .006$).

With regard to the correlation analysis after real rTMS at T_0, we observed that the lower the pVAS score, the higher the Aδ/C-γBO power in HC. Moreover, the higher the Aδ/C-γBO power, the lower the Aδ- and C-EEP amplitudes. Detailed data are reported in Table 3.

Discussion

The present study measured the nociceptive-induced cortical γBO and investigated the modulatory effects of a double-cone coil rTMS protocol over ACC on EEP and γBO power characteristics.

Our findings in the HC group suggest that γBO power and its modulation (which have been related to conscious pain perception)[12,72] could be a reliable marker of pain perception, with regard to pVAS, γBO power, and EEP amplitude effects and correlations.

Consequently, our γBO power findings suggest a conscious pain perception in all the MCS patients but not in the UWS patients. MCS participants showed an Aδ/C-γBO power increase that was paralleled by an Aβ-γBO power and Aδ/C-EEP amplitude decrease, whereas UWS participants did not show such aftereffects. rTMS could have reliably induced an ACC output to sensorimotor areas, leading to a reduced pain rating (as suggested by the reduction pVAS score at T_0 in HC), preferential cognitive pain processing, and, probably, motor output modulation (ie, the selective Aδ/C-γBO power increase and Aβ-γBO power decrease suggest functional reorganization of the sensorimotor area)[40] in MCS but not in UWS individuals. In addition, because rTMS aftereffects were different in terms of Aβ/Aδ/C-γBO power, EEP amplitude, and pVAS scoring, we may hypothesize on a role of the ACC in distinguishing between noxious and innocuous stimuli.[10]

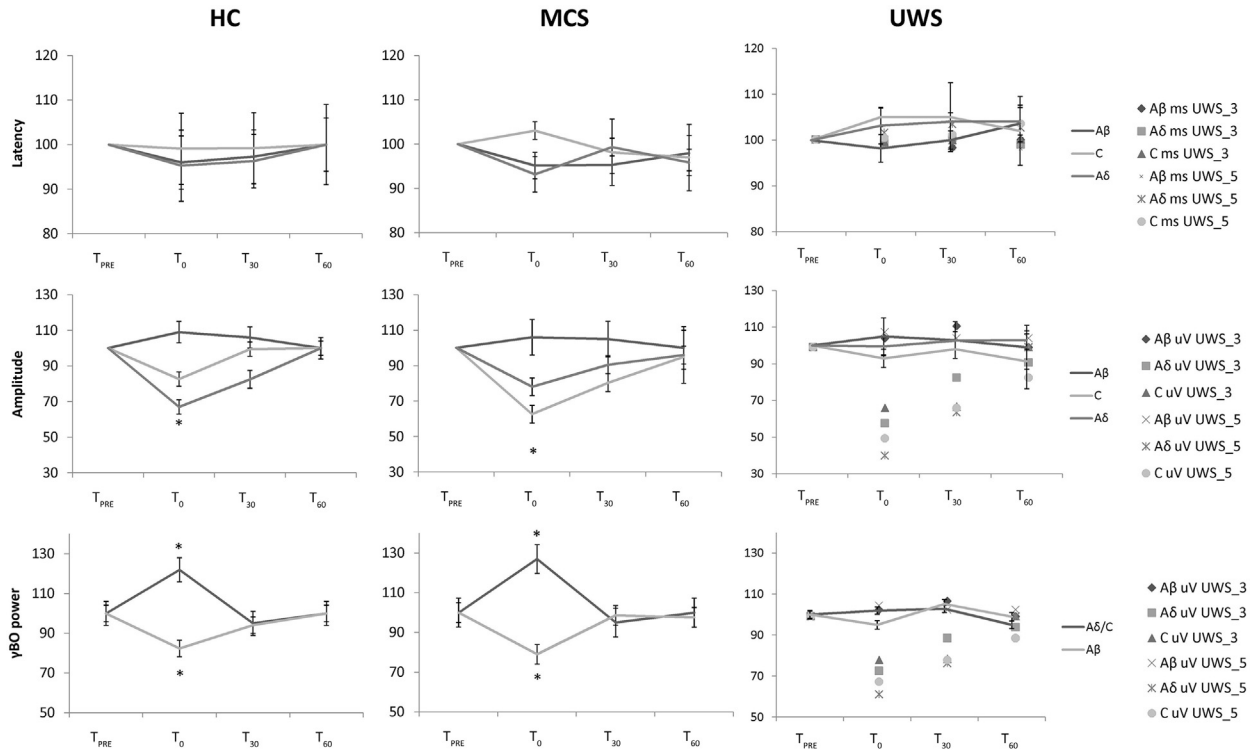

Figure 1. Time course of the EEP latencies, amplitudes, and γBO power in HC and DOC patients after real rTMS. The EEP amplitude and γBO-power aftereffects were significant only in HC and MCS at T_0 (*). In addition, 2 UWS patients (dots in the third column) showed some clear aftereffects. Values are expressed as a percentage of the unconditioned value. Error bars refer to SE.

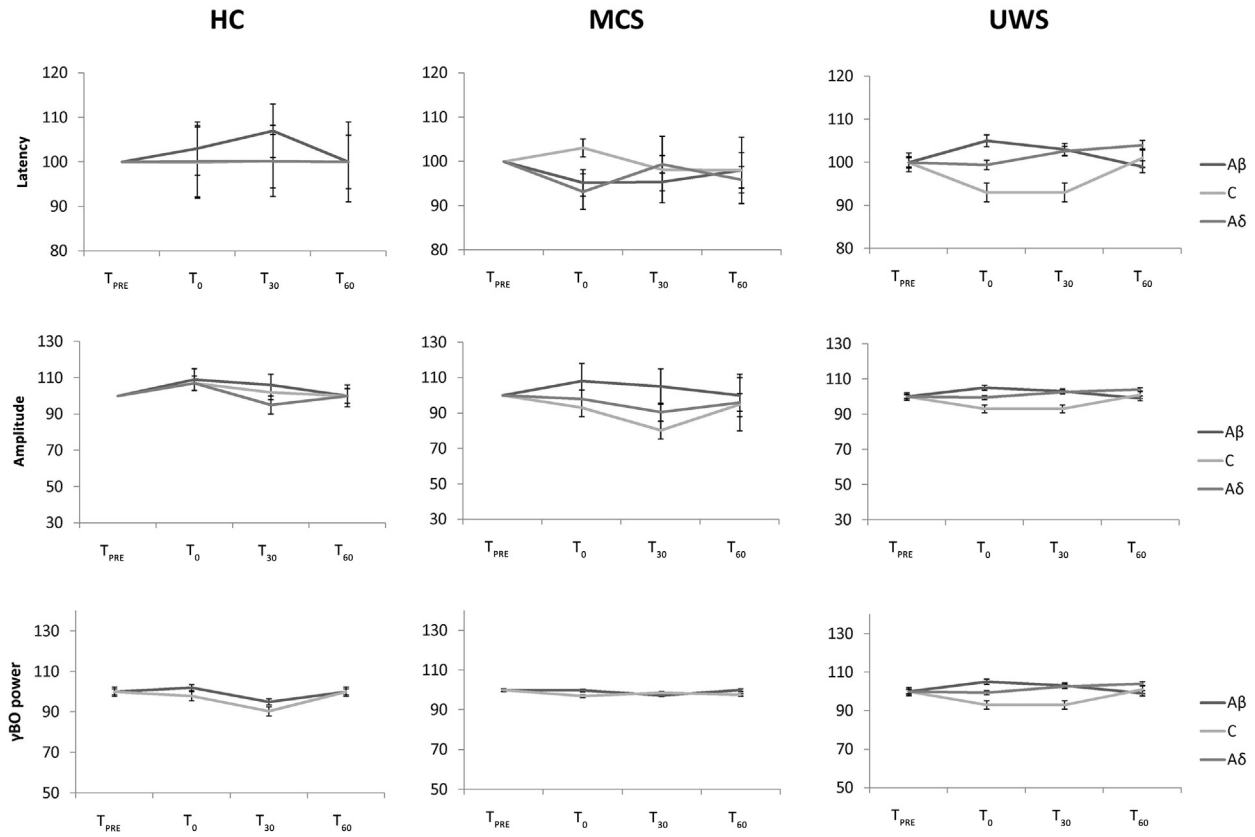

Figure 2. Time course of the EEP latencies, amplitudes, and γBO power in HC and DOC patients after sham rTMS. We did not observe any significant aftereffect. Values are expressed as a percentage of the unconditioned value. Error bars refer to SE.

The dependence of γBO power modulation on ACC stimulation is corroborated by the lack of sham rTMS aftereffects and the fact that nonnociceptive somatosensory evoked potentials and EEP latencies were not strongly affected by the real rTMS protocol, thus ruling out a nonspecific excitability increase or a connectivity modification within the sensorimotor area.

An important finding in our work was the identification of 2 rTMS-responder UWS patients who showed electrophysiological effects comparable with MCS patients. We could thus hypothesize that such patients had a functional locked-in syndrome rather than a UWS, ie, their frontoposterior connectivity was still susceptible to plastic modulation compared with the other UWS patients (who instead showed functional connectivity impairment).

Another interesting finding in our work concerns the potential pain-relieving effect of ACC stimulation, as suggested by the reduction in pain rating after real rTMS in HC. Opioid-mediated analgesia results in significant changes in the activity of the cingulate cortex, beyond other areas of the medial pain system,[13,43] and an opioid modulation of thalamocortical loops related to the ACC was also reported.[87] Therefore, the cingulate cortex may be involved in endogenous opioid production, thus affecting the pain experience.[70]

The ACC represents a structurally and functionally heterogeneous region, essentially organized in a supracallosal or cognitive part and a subcallosal or visceral part.[56,87] In particular, the supracallosal area shows bidirectional connections with the medial dorsolateral prefrontal cortex via basal ganglia,[3,4,86] and it is involved in several cognitive activities, such as emotional awareness,[54] reward anticipation, decision making, empathy, impulse control, emotion,[44,60] error detection,[26] and pain processing.[9] The ACC deals with physical pain detection[18] and processing,[68] and there are several reports suggesting a role of ACC in coding stimulus intensity, differentiating between noxious and innocuous stimuli, and selecting different motor outcomes.[11,61] In particular, the midanterior cingulate is more likely to be involved in executive processing (response selection and monitoring) and control of attention.[2,5,48]

Although our data show a link between the ACC-induced output and the centroparietal γBO (given the clinical-electrophysiological correlations), the fact that

Table 3. Correlation Analyses at T_0, Concerning pVAS (Only in HC) and Aδ/C-γBO

	R	P
pVAS		
Aδ/C-γBO power	−.752	.01
Aδ/C-γBO power		
C-EEP amplitude	−.511	.006
Aδ-EEP amplitude	−.839	<.001

Abbreviation: γBO power, event-related γ-band power.

this correspondence could be a marker of ongoing consciousness generation and maintenance, requiring multiple and integrated brain activations,[22,23] is debatable. Although it has been shown that γBO in the centroparietal region could be related to pain processing, regardless of the stimulus repetition or the attention level,[36,42,66,91] and that it may have a key role in the sensorimotor transformation of pain (given the correlation with reaction time speediness in animal and human experimental models),[40,90] the pain-induced γBO may also be related to automatic attention-related pain processing.[82]

Nonetheless, a correlation between activation of the ACC and the pain-control parietal networks has been shown previously.[16,28,52] The ACC-rTMS aftereffects allow us to hypothesize a residual preservation of the frontoparietal networks involving cingulate-sensorimotor networks,[28] activation of which may indicate the access of sensory information processing to higher conscious levels.[22]

Hence, we may argue that the frontoposterior output we induced could be related to conscious pain processing. In addition, it is conceivable that the clinically defined UWS patients, who showed MCS-like rTMS aftereffects, should instead be considered having functional locked-in syndrome.

Although the ACC networks have not been completely defined, combined TMS/positron emission tomography studies concerning frontocortical-ACC connectivity and ACC regional cerebral blood flow could support the hypothesis that the rTMS could have modulated the ACC and a wider frontocingulate network (including frontocaudate and other extracingulate excitatory corticostriatal projections).[64,65] Moreover, the role of dopaminergic and cholinergic systems should be taken into account.[65] Nonetheless, the ACC-rTMS aftereffects do not seem to be related to L-DOPA administration, because all our MCS patients showed these aftereffects independently of having received or not received the drug. We may hypothesize that different patterns of corticobasal ganglia damage could have produced different L-DOPA effects in ACC-rTMS findings.[39]

However, the role of other metabolites of L-DOPA, the possibility that dopamine secondarily alters the EEG by affecting central noradrenergic or serotonergic neurons, and the correlations between the contemporaneous administration of baclofen and L-DOPA and ACC modulation remain to be explored.[80]

The small sample size, the few electrodes used to register cortical EEP, and the lack of supplemental TMS measures represent the main limiting factors of our work. More detailed neuroanatomical and neurophysiological data, by means of functional neuroimaging[55] or TMS-EEG approaches,[74] are necessary to confirm our rTMS aftereffects.

Our findings offer some new clues about pain processing in patients with severe DOC and show that ACC-rTMS could help in differentiating MCS from UWS and could be a promising approach to assessing conscious pain perception in patients with DOC, and even UWS patients, allowing more adequate analgesic treatment to be either initiated or adapted.

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