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Review

Clinical neurophysiology of prolonged disorders of consciousness: From diagnostic stimulation to therapeutic neuromodulation

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HIGHLIGHTS

- The efficacy of neurophysiological stimulation techniques in prolonged DoC is reviewed.
- TMS-EEG appears the most promising neurophysiological diagnostic tool for DoC.
- Non-invasive and invasive stimulations in DoC are of little therapeutic relevance, so far.

ABSTRACT

The identification of signs of awareness in patients with prolonged disorders of consciousness (DoC) after severe brain injury is a challenging task for clinicians. Differentiating on behavioural examination the vegetative state (VS) from the minimally conscious state (MCS) can lead to a high misdiagnosis rate. Advanced neuroimaging and neurophysiological techniques can supplement clinical evaluation by providing physiological evidence of brain activity. However, an open issue remains whether these empirical results are directly or indirectly associated with covert consciousness and limitations emerge for their diagnostic application at the single-patient level. On the therapeutic side, the efficacy of both non-invasive and invasive brain stimulation/modulation trials is matter of debate. The present review provides an updated analysis of the diagnostic and prognostic impact that the different neurophysiological techniques of stimulation [including short-latency evoked potentials, long-latency event related potentials (ERPs), transcranial magnetic stimulation (TMS), TMS-EEG co-registration] offer in prolonged DoC. The results of the therapeutic stimulation techniques are also evaluated. It is concluded that TMS-EEG emerges as the most promising tool for differentiating VS from MCS whereas ERPs allow neurophysiologists to probe covert cognitive capacities of each patient. Significant behavioural improvements in prolonged DoC with brain stimulation techniques are still anecdotical and further treatment options are awaited.

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Contents

1. Introduction

The philosopher David Chalmers (1995) defined the quest for the ultimate theory of consciousness as the "hard problem" of science. For the clinician, the "hard problem" is probing consciousness in non-communicating patients lying in the vegetative state (VS) or in the minimally conscious state (MCS), the most severe conditions along the spectrum of prolonged disorders of consciousness (DoC) from acquired brain injury (Young, 1998). By definition, VS patients exhibit signs of wakefulness but no signs of awareness (non-responsive; Jennett and Plum, 1972), whereas MCS patients show signs of wakefulness and fluctuating signs of awareness (responsive; Giacino et al., 2002), with behavioural interactions of lower (MCS-) or higher (MCS+) level of complexity (Bruno et al., 2011). The diagnosis, based on the clinical examination and bedside behavioural scales, is challenging and can lead to a misclassification (mainly MCS classified as VS) in a significant percentage of cases, estimated between 15 and 40% (Schnakers et al., 2009; Seel et al., 2010). Moreover, minor clinical changes are difficult to be detected in DoC patients (Schnakers et al., 2009). Impairment of motor output, impinging upon the behavioural responses, has been proposed as the main cause leading to the underestimation of the level of consciousness (Giacino et al., 2014). However, advanced neurodiagnostic investigations (including functional magnetic resonance imaging [fMRI], electroencephalography [EEG], event-related potentials [ERPs]) reveal that some complex brain operations are still functioning, implying some degree of awareness, in a significant minority of behaviourally unresponsive patients diagnosed as VS (Laureys et al., 2004a,b; Monti et al., 2010; Cruse et al., 2011; Lehembre et al., 2012). These findings led to the proposal for the less restrictive term "unresponsive wakefulness syndrome" (UWS) that would include behaviourally unresponsive patients with "covert awareness", who demonstrate the integrity of at least some higher order networks in the brain as revealed by functional neuroimaging and/or electrophysiology (Laureys et al., 2010). These markers of neural activity provided by fMRI, EEG or ERPs have also been proposed as surrogates of consciousness and potential alternatives to the clinical examination (Greenberg, 2007). Accordingly, to indicate those patients that are in a VS/UWS state on the behavioural examination but that unambiguously show preserved higher cognitive functions, measured by neurodiagnostic tests, different terms have been proposed: "functional locked-in syndrome" (Giacino et al., 2009), "non-behavioural MCS" (Gosseries et al., 2014), "cognitive motor dissociation" (Schiff and Fins, 2016).

A few caveats however should be considered when examining the results of neurodiagnostic procedures in patients with prolonged DoC. Two types of experimental protocols are used: active tasks, in which patients are asked to perform behavioural/cognitive operations, and passive tasks, in which the patients are submitted to external stimulations with no performance required. Both tasks have their pros and cons: active paradigms document residual consciousness in patients with greater reliability (higher specificity). However, being more demanding they suffer from a lower sensitivity. The reverse is true for passive paradigms (lower specificity but higher sensitivity) (Kondziella et al., 2016). Therefore, to improve the diagnostic accuracy, passive and active tasks of varied and increasing complexity are sometimes applied as part of the same study protocol (Kotchoubey et al., 2005; Coleman et al., 2007; Cavinato et al., 2011; Beukema et al., 2016). However, a major confounding factor is the lack of an objective test ("gold standard", a test that most reliably produces true positives and true negatives; Peterson et al., 2015) for consciousness: the diagnostic conclusions are still based on the bedside clinical examination, therefore reflecting the subjective bias of the observer. It is therefore impossible to objectively evaluate the sensitivity and specificity of neuroimaging and electrophysiological investigations, for both active and passive paradigms, in prolonged DoC (Cruse et al., 2014a). Additionally, arousal/wakefulness (not only awareness) is often severely impaired in VS/UWS and, to a lesser extent, in MCS, as revealed by long-term polygraphic monitoring. The sleep-wake cycle and the sleep architecture (absence of NREM and/or REM stages) can be profoundly disrupted; behavioural signs of arousal/wakefulness (eyes open/closed) are frequently dissociated from their EEG correlates (Isono et al., 2002; Landsness et al., 2011; Cologan et al., 2013; Cruse et al., 2013; de Biase et al., 2014; Arnaldi et al., 2016) and EEG can persist unmodified for many hours. In conclusion, disruption of arousal can significantly reduce the brain's activation to external stimuli or commands and interfere with the assessment of patients.

Because the lesion pattern in prolonged DoC can have a huge impact on the brain responses to diagnostic stimulations and on the outcome of therapeutic stimulations, it is important to summarize the neuroanatomical-pathophysiological basis of the VS/UWS and MCS.

VS/UWS and MCS show similar neuroanatomical substrates, with MCS patients having considerably more preservation of cortical and thalamic integrity (Jennett et al., 2001).

There are three main anatomical patterns:

1. Diffuse cortical and/or thalamic neuronal loss is present in the setting of global ischemia due to cardiac arrest (Adams et al., 2000; Young and Schiff, 2014).
2. Widespread damage to axonal connections, mostly long-range fibers (as opposed to U fibers), best exemplified by diffuse axonal injury (DAI) from trauma (Kinney et al., 1994; Young and Schiff, 2014).
3. The least common is extensive damage to the upper brainstem and thalamus, usually from basilar artery stroke (Ingvar and Sourander 1970).

The common link for these three injury types in VS/UWS is the loss of corticothalamic function, either from cell death, disconnection, or loss of brainstem activation. *In vivo* imaging studies demonstrate that VS/UWS reflects very diffuse corticothalamic dysfunction (Laureys and Schiff 2012). Metabolic studies reveal that VS/UWS is associated with reduction of global metabolic rates 50% or less than healthy controls values (Laureys and Schiff 2012). Interestingly, recent connectome data provided by fMRI identified a specific brainstem-cortical functional network, including a small region in the left pontine tegmentum, the left anterior insula (AI) and the pregenual anterior cingulate cortex (pACC), subserving and linking arousal and awareness (Fischer et al., 2016). The connectivity between AI and pACC was peculiarly disrupted in patients with prolonged DoC, suggesting for these two cortical regions a prominent role in supporting consciousness (Fischer et al., 2016).

Establishing that higher order networks are functioning and interacting in the brain has important implications both for communication with the VS/UWS patient, either one-way or reciprocal, e.g., through brain-computer interfaces in the long-term (Naci et al., 2012), and in the subacute phase for prognosis: individuals showing intact network function or "cognitive responses" have a greater chance of recovering full awareness and interaction with others and their environment (Norton et al., 2012). For some of these patients, there may even be potential treatments, including drugs or brain stimulation (Rosa et al., 2012; Du et al., 2014).

The purpose of this review is to critically summarize the current state of scientific knowledge of prolonged DoC related to the application of advanced neurophysiological investigations. Specifically, we analyze the results provided by the electrophysiological techniques of stimulation, either in their diagnostic applications (i.e., short- and long-latency evoked potentials EPs, transcranial magnetic stimulation TMS, and TMS-EEG co-registration) or in therapeutic trials (non-invasive brain stimulation [NIBS], that comprises repetitive TMS [rTMS], and transcranial direct current stimulation [tDCS]; deep brain stimulation [DBS]; epidural spinal cord stimulation [SCS]). The focus on stimulation is prompted by the fact that the majority of the diagnostic studies conducted on patients with prolonged DoC have used stimuli of different modalities to probe residual consciousness (Kondziella et al., 2016). Of note, these studies rely on a theoretical approach framed by a long series of experiments performed in normal subjects to explore how stimuli gain access to conscious processing and which are the neural signatures of conscious access (Dehaene and Changeux, 2011; Koch et al., 2016). Among the range of treatments to facilitate recovery, stimulation techniques (electrical, magnetic; invasive, non-invasive) are those that more directly aim at modulating neural circuits that mediate arousal and attention (Schiff, 2010). They have been explored in a significant number of VS/UWS and MCS patients and the results warrant a critical analysis. The relevant contribution of functional neuroimaging methods (SPECT, PET, fMRI) for the understanding of prolonged DoC has been acknowledged in a series of review papers (Laureys et al., 2004a,b; Giacino et al., 2006; Owen and Coleman, 2008; Tshibanda et al., 2010; Laureys and Schiff, 2012; Celesia, 2013; Giacino et al., 2014; Kondziella et al., 2016): however, their use in clinical practice is restricted by a series of practical limitations (Harrison and Connolly, 2013), including reduced availability, safety and transports risks for the critical patients, and high cost. The neurophysiological techniques, on the other hand, which are widely available, repeatable, portable at the bedside and less expensive, are more suited for clinical applications. Therefore, only the neurophysiological investigations were considered in our review aiming to provide the clinicians with a timely survey of the real clinical impact of tools used in the standard assessment of patients with prolonged DoC. Finally, although the present analysis is focussed on stimulus-related techniques, we cannot fail to mention the role of two other neurophysiological tests, i.e., ongoing EEG (Menon et al., 1998; Leòn-Carriòn et al., 2008; Bagnato et al., 2010; Cruse et al., 2011; Logi et al., 2011; Forgacs et al., 2014; Sitt et al., 2014; Bagnato et al., 2015; Estraneo et al., 2016) and sleep/wake polysomnography (Isono et al., 2002; Landsness et al., 2011; Cologan et al., 2013; deBiase et al., 2014; Arnaldi et al., 2016; Wislowska et al., 2017), which can provide clinically relevant informations in VS/UWS and MCS patients.

2. Evoked potentials (EPs)

Although the use of sensory EPs in the study of prolonged DoC (namely the VS) dates back to the mid-seventies (Dolce and Sannita, 1973; Kawamura et al., 1975), it was only 20 years later that the neuroscience community realized that EPs could detect, in some of these persistently unresponsive patients, residual cognitive processing unnoticeable on clinical grounds only, possibly implying some covert conscious awareness (Marosi et al., 1993; Glass et al., 1998; Menon et al., 1998; Jones et al., 2000; Kotchoubey et al., 2001; Schiff et al., 2002). These observations and the complementary findings from functional neuroimaging (Laureys and Schiff, 2012) represented a major breakthrough in clinical neurosciences and fueled a long series of studies aiming at detecting latent cognitive capacities in DoC patients (Giacino et al., 2014). All these researches have greatly increased our understanding of the neural correlates of consciousness and also have produced a paradigm shift in the way we look at the pathophysiology of DoC. However, these techniques have limitations and pitfalls and their clinical impact must be critically evaluated. The roles of short-latency and long-latency EPs are separately examined with regard to their respective diagnostic and prognostic powers.

2.1. Short-latency EPs

The term short-latency EPs refers to responses shaped by the physical characteristics of the eliciting stimulus (stimulus-related, exogenous or obligatory potentials) and evoked within a brief interval from the stimulus, ranging from 10 ms for the auditory modality (brainstem auditory EPs, BAEPs) to 60 ms for the somatic sensory modality (somatosensory EPs, SEPs) to 140 ms for visual stimulation (visual EPs, VEPs). They measure, beyond peripheral afferents, the integrity of the central sensory pathways up to the primary sensory cortices. The rationale for using short-latency EPs in patients with prolonged DoC is to obtain a measure of the central nervous system (CNS) lesional load, on the assumption that the extent and pattern of the neurophysiological abnormalities could differentiate VS/UWS from MCS. A point of strength of this neurophysiological technique is the unique capacity for testing the cortical responsiveness of patients, as the judgment of the integrity of the cerebral cortex based solely on clinical examination is a difficult task to face in patients with severe brain injury. However, in contrast to their high specificity for monitoring fast-conducting sensory pathways, short-latency EPs are by their nature blind to the assessment of cognitive functions and cannot provide any direct contribution to the detection of conscious awareness. As far as patients with acute DoC (coma) are concerned, SEPs, supported by compelling scientific evidence, are unanimously acknowledged as reliable predictors of positive/negative outcome (Zandbergen et al., 1998; Logi et al., 2003; Carter and Butt, 2005; Young et al., 2005; Cruse et al., 2014b) and have been included in major practice guidelines (Wijdicks et al., 2006; Guérit et al., 2009). On the other hand, in patients with prolonged DoC, short-latency EPs have been the object of a limited number of studies and their diagnostic and prognostic value remain controversial.

BAEPs turned out being of little help in shaping the clinical judgment: they are usually normal or delayed, with no differences between VS/UWS and MCS (Hansotia, 1985; Isono et al., 2002; Jones et al., 2000; Kotchoubey et al., 2001; Fischer et al., 2010) and have no prognostic implications (Cavinato et al., 2009; Luauté et al., 2010). Middle-latency auditory evoked potentials (MLAEPs), identifiable at latencies between 20 and 50 ms after monoaural click stimulation, are reported being abnormal much more frequently in VS/UWS than in MCS patients (Fischer et al., 2010). Their absence predicts neurological deterioration over a 5-year follow-up (Luauté et al., 2010). Bilateral abolition of cortical SEP components (N20 and the following) appears a frequent finding in VS/UWS patients following anoxia (Fischer et al., 2010; Estraneo et al., 2013), at variance with post-traumatic patients (Cavinato et al., 2009) and MCS patients. Moreover, the presence (at least on one side) of median nerve cortical SEPs reliably predicts long-term recovery of responsiveness in anoxic vegetative patients (Estraneo et al., 2013). Ragazzoni et al. (2013) in a study comparing data from different neurophysiological techniques found no correlation between SEP abnormalities and clinical diagnosis (VS/UWS vs MCS). As for VEPs from flash-stimuli, their latencies can predict long-term outcome in the post-acute VS/UWS (Wijnen et al., 2014a), a positive prognostic power already acknowledged in the study of Hildebrandt et al. (2007). In a recent series including VS/UWS and MCS patients, multimodal stimulus-related EPs (BAEPs, SEPs, VEPs) have shown no significant correlation with the clinical evaluation and the level of consciousness (de Biase et al., 2014).

Undoubtedly short-latency EPs, in particular when used in a multimodal approach, may contribute to the clinical assessment of patients with prolonged DoC, by providing crucial information on the extent and severity of brain damage. Being widely available and easily administered at patient's bedside, they represent an extension of the neurological examination. However, their power in making the differential diagnosis between VS/UWS and MCS is weak. On the contrary, the predictive value of short-latency EPs is supported by a few studies (Luauté et al., 2010; Estraneo et al., 2013; Wijnen et al., 2014a), showing that the presence of SEP and/or VEP cortical components is associated with subsequent better outcome from VS/UWS.

2.2. Long-latency EPs, event-related potentials (ERPs)

ERPs also known as late, slow or "cognitive" evoked potentials represent a class of electrophysiological responses with latencies, from the eliciting stimuli, longer than 100 ms and whose peculiarity is that of reflecting the mass activity of neuron assemblies underpinning a series of cognitive processes (Picton et al., 2000; Duncan et al., 2009). Being generated by synaptic current flows they offer a critical link between cognitive and neural processes[1]. The sequence and latencies of ERP components are related to successive stages of the information-processing stream, spanning from simpler perception to higher-order cerebral processes, such as attention, memory updating, semantic comprehension and other cognitive activities (Polich, 2007; Naatanen et al., 2011). Importantly, the earlier components elicited in the 100–250 ms time interval (such as P1, N1, P2, N2, Mismatch Negativity-MMN, P3a), have been associated with the automatic sensory and perceptual processing of stimuli, operating independently from attention on an unconscious level. Access to conscious awareness is signalled by the appearance over the central-parietal scalp of a later positive component, named P3b (or P300 for its modal latency) peaking between 300 and 500 ms[2], depending on the sensory modality of the eliciting stimulus (Vogel et al., 1998; Dehaene et al., 2003; Lamy et al., 2009; Salti et al., 2015). Although not all experimental evidences support this interpretation (Verleger, 2010; Pitts et al., 2014), P3 b appears at present as one of the most reliable electrophysiological marker of conscious access (Dehaene and Changeux, 2011). By applying ERPs to patients with chronic DoC, researchers were able to investigate different levels of neural organization and to detect residual cognitive processes, not accessible to bedside clinical examination. In addition, capitalizing on the link of P3 to conscious awareness, it was possible to observe electrophysiological signs of covert consciousness in some of VS/UWS patients which were diagnosed as unaware on a behavioural examination. Stimuli in the auditory modality are preferred as they can be easily delivered even in eyes-closed conditions. Many studies applied the so-called "oddball" ERP paradigm in which the subject/patient has to detect the rare target stimuli randomly embedded in a stream of repetitive frequent standard stimuli (Kotchoubey et al., 2005; Perrin et al., 2006; Schnakers et al., 2008; Fischer et al., 2010; Cavinato et al., 2011; Chennu et al., 2013; Ragazzoni et al., 2013; Risetti et al., 2013; Gibson et al., 2016; Real et al., 2016). The appearance of a late P3b signals the conscious identification of the target stimuli. This task is associated with cognitive operations such as selective attention, working memory, stimulus categorization. Unconscious or preconscious processing has been explored using a "passive" version of the "oddball" paradigm to detect the mismatch negativity (MMN), index of echoic memory (Wijnen et al., 2007; Qin et al., 2008; Boly et al., 2011), or the N400 ERP effect as an index of semantic processing (Schoenle and Witzke, 2013; Kotchoubey et al., 2005; Balconi et al., 2013; Steppacher et al., 2013; Rohaut et al., 2015; Beukema et al., 2016). A new ERP paradigm, specifically developed for the study of consciousness in healthy individuals, has gained popularity in the investigations of patients with prolonged DoC: the local-global paradigm (Bekinschtein et al., 2009) in which violations of local (within trials) auditory regularity elicit components MMN and P3a while only violations of global (across trials) regularity evoke component P3b (Faugeras et al., 2011, 2012; King et al., 2013).

It must be emphasized that the recording of earlier ERP components (i.e., N1, P2, N2, MMN, P3a, N400) in prolonged DoC patients does not represent evidence of conscious awareness: they reflect automatic cognitive processes operating at an unconscious level. Indeed, only the presence of a reliable and reproducible P3b implies the possibility of some form of awareness from the patient. Again, the observation in a VS/UWS patient of an electrophysiological correlate of consciousness (i.e., P3b) does not necessarily prove that the patient is conscious: it merely signals the possibility for the presence of some form of consciousness, unless/until any behavioural evidence unequivocally confirms the electrophysiological result (Nachev and Hacker, 2010). A recent study using the local-global paradigm found cognitive ERP components, resembling P3b, in patients deeply unconscious in acute coma following cardiac arrest, treated with hypothermia and sedation (Tzovara et al., 2015): such results question the role of P3b as a marker for consciousness. However, a number of relevant objections raised on the selection of patients and the analysis of electrophysiological

[1] A compelling evidence of ERPs endogenous nature comes from the fact that they (i.e., MMN, P300) can be elicited also by the absence of stimulus (emitted potentials), provided the omitted stimulus is part of a regular temporal pattern of repeating stimuli and is identified as a target (Sutton et al., 1967).

[2] The ERP family of P3 components includes two positive waves, spanning over a 250–600 ms time window: the earlier, frontally centred P3a and the later P3b, largest at central-parietal electrodes. P3a is automatically generated whenever an unexpected/surprising stimulus is presented (novelty P3) and reflects some non-conscious aspects of the orienting response (Friedman et al., 2001). Only the appearance of P3b seems to specifically index when a task-relevant target stimulus gains access to conscious awareness (Dehaene and Changeux, 2011).

Table 1
ERP diagnostic studies in VS/UWS and MCS.

Authors	ERP examined	VS/UWS N°	Response Level 1[a]	Response Level 2[b]	MCS N°	Response Level 1[a]	Response Level 2[b]	Task
Kotchoubey et al. (2005)	MMN, P3b, N400, P600	50	26 (52%)	12 (24%)	38	13 (34%)	14 (36%)	Auditory & semantic oddball
Perrin et al. (2006)	N1, P3b	5	na	3 (60%)	6	na	6 (100%)	SON oddball
Schnakers et al. (2008)	N1, P3b	8	na	0 (0%)	14	na	9 (64%)	SON oddball
Bekinschtein et al. (2009)	MMN, P3b	4	3 (75%)	0 (0%)	4	4 (100%)	3 (75%)	Auditory local-global paradigm
Fischer et al. (2010)	MMN, P3a, P3b	16	3 (19%)	1 (6%)	11	4 (36%)	1 (9%)	Auditory & ON oddball
Cavinato et al. (2011)	N1, P3b	11	na	5 (45%)	6	na	6 (100%)	Auditory & SON oddball
Boly et al. (2011)	MMN	8	8 (100%)	na	13	13 (100%)	na	Auditory oddball
Höller et al., 2011	MMN	16	2 (12%)	na	6	0 (0%)	na	Auditory oddball
Faugeras et al. (2012)	MMN, P3b	24	6 (25%)	2 (8%)	28	9 (32%)	4 (14%)	Auditory local-global paradigm
Chennu et al. (2013)	P3a, P3b	9	1 (11%)	1 (11%)	12	3 (25%)	0 (0%)	Auditory verbal oddball
Ragazzoni et al. (2013)	N1, P3b	8	na	0 (0%)	5	na	0 (0%)	Auditory oddball
Steppacher et al. (2013)	N400, P3b	50	19 (38%)	32 (64%)	39	18 (46%)	29 (74%)	Auditory oddball, Sentences
Rohaut et al. (2015)	N400, LPC	15	1 (7%)	1 (7%)	14	5 (36%)	5 (36%)	Auditory semantic priming paradigm
Beukema et al. (2016)	N1, N400	8	3 (37%)	0 (0%)	8	4 (50%)	0 (0%)	Auditory perceptual & semantic priming paradigm
Gibson et al. (2016)	P3a, P3b	7	2 (28%)	0 (0%)	6	5 (83%)	0 (0%)	Somatosensory oddball paradigm
Real et al. (2016)	N1, P2, P3b	29	na	3 (10%)	16	na	2 (13%)	Auditory oddball Passive/Active
Total number		268	74 (36%)	60 (25%)	226	78 (44%)	79 (38%)	

Abbreviations: N° = number of patients; VS/UWS = Vegetative State/Unresponsive Wakefulness Syndrome; MCS = Minimally Conscious State; MMN = Mismatch Negativity; LPC = Late Positive Component; SON = subject's own name; % = percentage of patients in which ERP components were detected over the total number of patients examined for the exact same ERPs; na= not analyzed in the study.
[a] Response Level 1 indicates detection of ERP components MMN, P3a, N400.
[b] Response level 2 indicates detection of ERP components P3b, LPC (P600).

data strongly challenge the interpretation of these results (Naccache et al., 2015).

The interpretation of ERPs in VS/UWS and MCS patients requires considerable experience from the operator. The morphology of ERPs is often markedly deteriorated and in order to obtain a more reliable evaluation of the responses it has been recommended to integrate the visual identification of waves with statistical analysis methods of the individual ERP components (Fischer et al., 2010; Ragazzoni et al., 2013; Rohaut et al., 2015; Beukema et al., 2016; Gibson et al., 2016). Sometimes, repetitive artefacts or periodic epileptiform discharges on the EEG can synchronize with the stimuli and generate waveforms mimicking the morphology of averaged ERPs. The deceptive impression can arise that a P3 component is present where none exists (Ragazzoni et al., 2011): therefore, the spontaneous EEG should always be scrutinized before averaging the post-stimulus responses.

An abundance of studies with ERPs in prolonged DoC have been produced in the last 20 years and over 500 patients have been examined. Some reports concerned single or few patients or were cohort studies, therefore of limited clinical relevance, although important for a better understanding of prolonged DoC physiopathology (Connolly et al., 1999; Laureys et al., 2004b; Schoenle and Witzke, 2004; Faran et al., 2006; Balconi et al., 2013): they will not be considered here. Table 1 summarizes the results of sixteen studies with relatively larger samples of patients analyzed at the individual level and aiming at differentiating patients with VS/UWS from those with MCS (diagnostic studies). Studies are categorized into two levels, reflecting the different complexity of ERP components analyzed. Level 1 indicates analysis of earlier components (i.e., MMN, P3a, N400) associated with preconscious cognitive functions, such as sensory memory, orienting response or semantic processing.

Level 2 refers to analysis of later components (P3b, LPC, P600) that in normals reflect conscious processing. It is remarkable that about 1 out of 3 VS/UWS patients presented ERP responses at level 1 and/or 2, indicating that a substantial proportion of the patients could harbour some level of cognitive processing and even of conscious awareness (level 2: 25% of the patients examined). Unfortunately, the clinical evidence of a recovery of consciousness was available only for a part of them (Kotchoubey et al., 2005; Faugeras et al., 2012; Steppacher et al., 2013). Another notable fact is that no more than 38% of MCS patients presented with ERP signs of consciousness (level 2), despite behavioural evidence of awareness. Possible explanations for these false negative results are fluctuations in arousal, lack of motivation, fatigue, difficulty in understanding the task, sensory defects, technical factors such as artifacts and latency variability of responses. These remarks are valid even more for VS/UWS patients and suggest as entirely possible the hypothesis that ERPs (and other electrophysiological and functional neuroimaging investigations as well) largely underestimate the cognitive capacities of prolonged DoC patients. Clearly, the diagnostic power of ERPs in differentiating VS/UWS from MCS is weak due to a lack of sensitivity, however some studies confirmed that the detection of P3b represents a highly specific signature of conscious processing (Schnakers et al., 2008; Bekinschtein et al., 2009; Faugeras et al., 2012; King et al., 2013), as only conscious patients present with this electrophysiological response. Of note, ERPs can detect in patients latent cognitive competences that are inaccessible to the clinical examination or are at a higher level than that shown by behavioural performance (Beukema et al., 2016). A new ERP protocol has been proposed recently to probe multiple cognitive functions in a single recording session, an approach alternative to focussing on a specific but elusive neurophysiological marker of consciousness (Sergent et al., 2016). The test, based on the combination of several ERP markers, uses an adaptation of the Posner cueing protocol and explores eight cognitive domains with different levels of complexity. An interesting result of this multidimensional ERP testing was that high-level functions as opposed to low-level functions differentiated MCS from VS/UWS patients. This protocol aims at detecting the residual cognitive capacities (not only signs of consciousness) of the single patient, therefore providing relevant information for rehabilitative programs. The diagnostic value of ERPs can also be improved by repetitive measurements at different times, as it has been reported that the test-retest reliability in DoC patients is low due to fluctuations in responses over time: Schorr et al. (2015) showed that the Krippendorff's alpha coefficient comparing P3 occurrence between days was 0.72 for controls and 0.24 for patients, despite unmodified clinical appearance.

In a recent study, Gibson et al. (2016) found no reliable evidence of a P3b for any 'conscious' patients, including EMCS (Emerging from MCS) patients and 1 in 3 healthy participants who demonstrably follow commands with their behaviour. This suggests that tasks that require sustained attention to index consciousness may ultimately confound consciousness with the ability to complete a lengthy and complex cognitive task (Koch et al., 2016). Interestingly, Gibson et al. (2016) observed that all patients who could follow commands produced a tactile P3a. Crucially, this was true whether the patients could follow behavioural commands (i.e., MCS+) or if they were in a misdiagnosed VS/UWS and only able to demonstrate command-following via mental imagery tasks with fMRI. This result indicates that a distinction based on a multi-modal description of awareness (i.e., behaviour and neuroimaging) rather than a purely behavioural definition may provide a fruitful avenue to more accurately identify the diagnostic utility of these approaches (Cruse et al., 2016). Overall, the sensitivity of published ERP markers of consciousness is poor, with 62% of MCS patients failing to exhibit reliable ERP components linked to consciousness (i.e., Level 2 responses in Table 1). Indeed, these issues are not specific to ERPs, as considerable false negatives are also evident in fMRI methods (see Monti et al., 2010). This Type II error rate (i.e., false negatives) may be reduced in future by employing tasks with lower cognitive demands, monitoring each patient's level of arousal at the time of assessment, and applying advanced statistical analyses. Type I error rate (i.e., false positives) is more difficult to estimate from patient data due to the potential for covert awareness in a significant proportion of VS/UWS patients, and must therefore be estimated from controlled studies of diminished awareness, such as sleep, sedation, and inattention. Nevertheless, as with behavioural methods, protocols of repeated assessment (i.e., replication) will separate the errors from the true results.

The prognostic value of ERPs has been tested mostly in terms of recovery of consciousness and neurological functions with follow-up of one study extending as far as 14 years (Steppacher et al., 2013). The stronger ERP predictor for recovery of consciousness turned out being the presence of MMN, which also provided prognostic information on the functional outcome (Kotchoubey et al., 2005; Wijnen et al., 2007; Qin et al., 2008), with one study not supporting this statement however (Luauté et al., 2010). P3b emerged as a less reliable predictor of outcome (Steppacher et al., 2013; Wijnen et al., 2014b), but in one study its presence predicted recovery of consciousness at 1 year (Cavinato et al., 2009). The presence of N400 was associated with a favourable neurological recovery over a long-term follow-up period (Steppacher et al., 2013). Overall, it can be stated that ERP recordings are helpful in the prognostication of recovery in patients with DoC.

The important issue of pain perception in prolonged DoC patients has been addressed by recording long-latency laser-evoked potentials (LEPs) on the assumption that the presence of cortical responses could reflect the experience of pain. The results were inconclusive, as in one study LEPs were recorded in all patients examined (5 VS/UWS, 4 MCS; de Tommaso et al., 2015) whereas in the other study LEPs were observed in 13 out of 23 VS/UWS patients and in all 15 MCS patients (Naro et al., 2015a). A major problem is that LEPs are not considered as being specifically related to the subjective perception of pain and therefore no inferences can be advanced from LEP results on the experience of pain in the patients (de Tommaso, 2016).

3. Transcranial magnetic stimulation (TMS)

TMS utilizes a spatially restricted magnetic field to noninvasively induce an electric field in a target cortical area (Rossi et al., 2009; Rossini et al., 2015), which –depending from its intensity- activates directly or trans-synaptically cortical pyramidal neurons (Di Lazzaro et al., 2004; Caliandro et al., 2014). TMS represents the gold standard tool to assess the integrity of the human corticospinal tract and to evaluate distinct excitatory and inhibitory circuits of the motor cortex (Rossini et al., 2015). In the study of prolonged DoC, TMS investigations have been based on single-pulse stimulation, that allows to elicit motor evoked potentials (MEPs) and to evaluate the motor threshold, the central motor conduction time, and MEP size changes at different TMS intensities (MEP recruitment curve). The motor threshold is defined as the minimal intensity required to elicit a MEP of <50 μV in the target muscle with 50% probability (Rossini et al. 2015) at rest (resting motor threshold, RMT) or during target muscle contraction (active motor threshold). Moreover, the amplitude of the MEP elicited by a test pulse is modulated by a number of different conditioning stimuli: the conditioning stimulus can be another generally subthreshold TMS pulse delivered to the same scalp position in order to assess intracortical inhibition and facilitation mechanisms (ICI and ICF; Kujirai et al., 1993), or a non-magnetic stimulus (e.g., an electric pulse delivered to a peripheral nerve) to assess afferent inhibition mechanisms (Rossini et al., 2015) of the human motor cortex.

Early studies (for a review see also Lapitskaya et al., 2009), mainly conducted before the introduction of the diagnostic criteria for MCS, used TMS to detect the presence of MEPs in post-comatose patients (Moosavi et al., 1999) or to evaluate prognostic value of MEPs during recovery (Mazzini et al., 1999). Although MEPs were elicited in most of patients defined as consistently unresponsive or minimally responsive (Moosavi et al., 1999), the prognostic value of MEPs for recovery was considered poor (Mazzini et al., 1999).

More recent studies using single- or paired-pulse TMS protocols documented abnormal cortical excitability in prolonged DoC patients. In five VS/UWS patients following traumatic brain injury, Bagnato et al. (2012) evaluated ICI at short interval (2 ms) and ICF at 10 ms intestimulus interval, which are thought to mainly involve $GABA_A$ergic and glutamatergic circuits in the primary motor cortex (M1), respectively (Rossini et al., 2015). Findings showed that both parameters were significantly reduced compared to healthy controls, while no significant differences emerged in the RMT. Two patients who evolved into a MCS were re-tested but no significant changes in such measures were observed.

Lapitskaya et al. (2013) compared a number of TMS-related electrophysiological measures recorded in 24 VS/UWS and 23 MCS patients (with different aetiologies: trauma, anoxic-ischemic encephalopathy, stroke, haemorrhage, and encephalitis) and in a group of healthy controls. The RMT was significantly higher in VS/UWS with respect to MCS group and healthy controls. All patients, mostly in the VS/UWS group, showed lower MEP amplitudes and a narrower MEP recruitment curve, while the central motor conduction time did not differ across groups (Lapitskaya et al., 2013). Moreover, the short latency afferent inhibition (SAI), a mainly cholinergic-mediated phenomenon (Rossini et al. 2015) induced by a peripheral electrical stimulus cohincident with the

TMS pulse at cortical level, was reduced in both VS/UWS and MCS patients compared to healthy controls. Interestingly, a correlation was observed between SAI alterations and the level of consciousness as tested by the Coma Recovery Scale-Revised (CRS-R) total score (Lapitskaya et al., 2013).

In a study designed to investigate the pain-motor plasticity in ten post-anoxic VS/UWS patients by a specific paired laser associative stimulation protocol, Naro et al. (2015b) reported at baseline a RMT similar to that of healthy controls, whereas the central motor conduction time was increased and the MEP morphology was overall abnormal. Similarly to SAI (Lapitskaya et al., 2013), the inhibitory effect on MEP amplitude induced by a conditioning laser stimulus to assess the pain-motor integration, was reduced in patients compared to healthy controls (Naro et al., 2015c).

Finally, using a different approach Pistoia et al. (2013) evaluated the effect of different facilitating conditions on motor cortex excitability in six patients with a diagnosis of VS/UWS. Namely, MEPs were recorded in three experimental conditions: at rest; when patients were asked to open, and close the right hand; or when they were encouraged to imitate a movement performed by the examiner in front of the patient. Such protocol was repeated for 3 consecutive days. Findings showed that the MEP amplitude was significantly increased in the observation/'imitation' compared to the rest condition whereas no significant differences emerged during the verbal instructions. Authors reported that this effect was associated to behavioural improvement in 4 patients (Pistoia et al., 2013). In conclusion, MEPs following TMS reflect selectively the function of M1 and motor pathways but not of other cortical areas. In case of a lesion of M1 or along the corticospinal tract, or in case of severe axonal damage, no MEPs can be gathered. Moreover, in prolonged DoC patients taking CNS-acting drugs that increase the excitability threshold (see Rossi et al., 2009), it might be difficult to obtain reliable MEPs even at the maximal intensity of the stimulator output. These limitations strongly affect the diagnostic and prognostic power of MEPs in prolonged DoC patients.

4. TMS-EEG co-registration

In analogy to the other EP modalities previously described, the degree of corticospinal activation following TMS of the motor cortex can be easily indexed by the amplitude of the MEPs in the target muscles, provided that the cortical area itself or the efferent pathway are not lesioned (Rossini et al., 2015); however, this is obviously not possible when TMS is applied outside the motor cortex.

Recent advances in amplifier technology (Veniero et al., 2009; Virtanen et al., 1999) have allowed the successful co-registration of brain activity during and immediately following TMS without saturation (for revisions see Komssi and Kähkönen, 2006; Miniussi and Thut, 2010; Rogasch and Fitzgerald, 2013; but see also Rogasch et al., 2014; Atluri et al., 2016; Mutanen et al., 2016). These technological developments made possible to record TMS evoked brain responses, or TEPs, that are expression of the direct activation of cortical neurons below the stimulation point; therefore, it reflects the local cortical reactivity of the cerebral cortex to the focal TMS (Komssi and Kähkönen, 2006; Komssi et al., 2007; Miniussi and Thut, 2010). Crucially, the local activation caused by the magnetic pulse diffuses trans-synaptically to connected areas over the ensuing tens of milliseconds (Komssi et al., 2002; Bortoletto et al., 2016): this "wave" can be traced by simultaneous EEG recording, and reflects, rather than the mere temporal or coherence correlation, the rapid causal interactions among multiple groups of neurons, thus closely resembling an effective connectivity phenomenon (Bortoletto et al., 2016). Hence, local and remote EEG responses to TMS (i.e., the TEPs) are considered quantifiable and reproducible (Casarotto et al., 2010) markers of the overall state of the brain (Veniero et al., 2010), provided that TMS is delivered outside a lesioned cortex (Gosseries et al., 2015).

For a reliable clinical application, it is recommended to integrate TMS-EEG measurements with a navigation system for coil positioning onto the desired target brain region, as well as to reduce the variability of the induced currents in the brain (Cincotta et al., 2010). Finally, advanced procedures should be implemented to localize and eventually minimize any sensory stimulation due to the TMS-associated "click" noise that can give origin to evoked responses (ter Braack et al., 2015), even in "apallic" patients (Gosseries et al., 2015).

In the study of DoC, TMS-EEG co-registration has the advantages that delivering TMS directly to the area of interest bypasses the need to access the functionality of the cortex through afferent pathways and primary areas; therefore, providing the opportunity to stimulate, virtually all different cortices directly. Moreover, the procedure can be performed at the bedside without the need of patient's cooperation (Massimini et al., 2009).

The degree of cortical reactivity and effective connectivity (Rosanova et al., 2012; Ragazzoni et al., 2013) depends on the physiological state of the neurons of the stimulated cortex, according to the general concept of state-dependency of brain response to external stimulations (Bortoletto et al., 2015, 2016); therefore, they vary as a function of the neuronal state at the moment of stimulation. Stringent examples of this are represented by specific changes of TEPs amplitude along different phases of the wakefulness/sleep cycle and even during different types of anaesthesia (Massimini et al., 2005; Ferrarelli et al., 2010; Sarasso et al., 2015). On these premises, TEPs appear to conceptually represent an excellent tool for exploring cortical reactivity and tracking the (residual) connectivity of both the intra-hemispheric and inter-hemispheric cortical networks in patients with prolonged DoC (Rosanova et al., 2012; Ragazzoni et al., 2013). A handful of investigations recently appeared on the topic, often with converging pathophysiological implications.

TMS-EEG co-registration provided important clues in improving the differential diagnosis between patients in VS/UWS and MCS. Two recent studies (Rosanova et al., 2012; Ragazzoni et al., 2013) examined a total of 18 VS/UWS patients, 10 MCS patients, 2 locked-in syndrome (LIS) patients. The pattern of TEPs in patients with VS/UWS was clearly different from that of patients with MCS and LIS. In the VS/UWS patients, TMS induced only ipsilateral responses (i.e., in the hemisphere under the stimulating coil: expression of residual cortical reactivity) or no response at all (Fig. 1).

In the MCS and LIS patients, TMS triggered complex activations that, after the local response, sequentially involved distant cortical areas in the stimulated hemisphere and in the contralateral one (11 cases out 12), suggesting the presence of residual intra and interhemispheric effective cortical connectivity. However, when present in patients, TEPs had reduced amplitudes and altered morphologies as compared to responses obtained in healthy subjects.

Overall, given that TEPs strongly correlate with the clinical diagnosis, these results prove that TMS-EEG co-registration, is more useful than other standard neurophysiological techniques, such as SEPs and auditory ERPs, in differentiating VS/UWS from MCS (Ragazzoni et al., 2013) (Table 2).

In the attempt to even better quantify the complexity of local and distant brain responses to the TMS pulse, a new index called Perturbational Complexity Index (PCI) has been introduced (Casali et al., 2013) and recently validated in a large sample of patients (Casarotto et al., 2016). The analysis of the algorithm behind PCI is out of the scope of the present review, and can be found in Casali et al. (2013). However, according to the authors (Casarotto et al. 2016) the PCI can be considered "a measure that

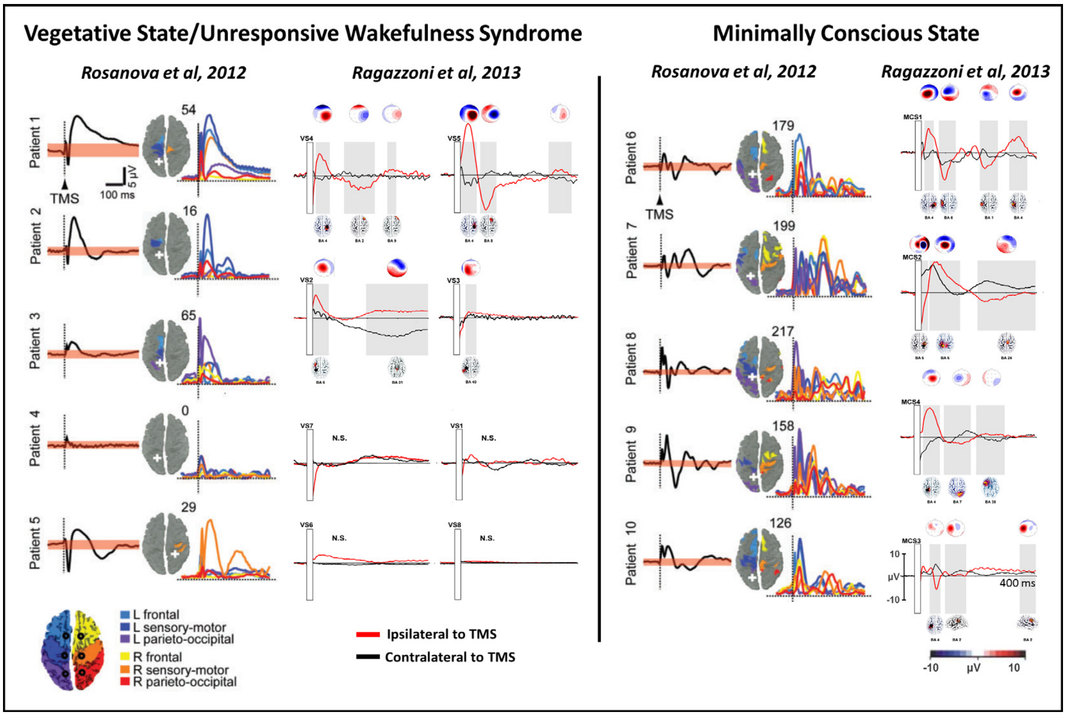

Fig. 1. TEPs recorded in thirteen VS/UWS patients and in ten patients with MCS (modified from Rosanova et al. (2012) and Ragazzoni et al. (2013)). In patients drawn from Rosanova et al., black traces represent TEPs recorded at one electrode under the stimulator, with the significance threshold represented by the pink bands. The current sources are plotted on the cortical surface and colour-coded according to their location in six anatomical macro-areas (the white cross marks the site of stimulation). The number of detected sources is indicated at the top right of each map. The coloured traces represent TMS-evoked cortical currents recorded from each macro-area. In patients from Ragazzoni et al., TEPs are recorded from C3 and C4 electrodes following stimulation of the scalp overlying (left or right) M1. Red traces are from the hemisphere ipsilateral to the site of TMS stimulation whereas black traces are from the contralateral hemisphere. The grey areas indicate the time windows in which the amplitude of the response is above the significance threshold (i.e., signal exceeding >3 SDs the pre-stimulus baseline for at least 20 ms; N.S.: not significant). For each patient, voltage scalp maps are reported on top of the traces (colour bar indicates the amplitude scale) and sLORETA sources (calculated only for the significant TEPs) are presented on bottom. Note how in all VS/UWS patients TMS triggered either a simple, local response (confined to the stimulated hemisphere) or no response at all, whereas in MCS patients responses sequentially involved distant cortical areas over both hemispheres. These results show that cortical reactivity and effective connectivity are severely impaired in all VS/UWS patients but they are much less impaired in MCS patients. The two studies concur to indicate that TMS-EEG very efficiently distinguish VS/UWS from MCS patients.

Table 2
Survey of the different neurophysiological techniques of stimulation to investigate brain function in VS/UWS and MCS.

Technique	Task	Availabiity	Recording /analysis complexity	Information on effective cortico-cortical connectivity	Diagnostic utility	Prognostic utility
Short-latency EPs (BAEPs, SEPs, VEPs)	Passive	++++	+	+	+	++
Long-latency EPs (ERPs)	Passive/ active	+++	+++[a]	++	+++[c]	++
TMS (single/paired pulse)	Passive/ active	+++	+	+	+	+
TMS-EEG	Passive	+	++++[b]	++++	++++[d]	++

Abbreviations: EPs = Evoked Potentials; BAEPs = Brainstem Auditory Evoked Potentials; SEPs = Somatosensory Evoked Potentials; VEPs = Visual Evoked Potentials; ERPs = Event-Related Potentials; TMS = Transcranial Magnetic Stimulation; TMS-EEG = concurrent TMS-EEG recording.
[a] It is recommended to integrate the visual identification of ERPs with methods of statistical analysis.
[b] TMS-compatible EEG amplifiers are required for recording TMS-EEG responses.
[c] Low sensitivity but high specificity estimated for ERPs.
[d] High sensitivity and high specificity estimated for TMS-EEG.

gauges the ability of thalamocortical circuits to integrate information irrespectively of the integrity of sensory processing, motor behaviour and subject's participation", hence well fitting the bedside requirements for disentangling the individual level of consciousness. In this study, PCI has been evaluated in 38 MCS and 43 VS/UWS patients after validation (corrected for brain lesions and behavioural unresponsiveness) in a benchmark population of 150 subjects/patients interrogated on their immediate or delayed conscious experience (including healthy subjects of different age; brain-injured, yet conscious, patients; subjects with referred no conscious or conscious experience upon awakening from NREM sleep or anaesthesia). The PCI, which detected consciousness in 100% of the benchmark population, showed a sensitivity of 94.7% in detecting patients with minimal signs of consciousness, thus greater than the sensitivity of spontaneous EEG conventional analysis (81.6%). Among the VS/UWS population, the PCI index – derived after TMS of multiple scalp sites - identified three subgroups, which were indistinguishable on a behavioural level only: a "no-response" subgroup of 13 patients (30%), a "low complexity" subgroup of 21 patients (49%) and a smaller "high-complexity" subgroup of 9 patients (21%). TMS-EEG responses in the low-complexity subgroup resembled those of healthy unconscious subjects during NREM sleep and anaesthesia (Massimini et al., 2005; Ferrarelli et al., 2010; Sarasso et al., 2015), while brain responses in the high-complexity group resembled those of REM sleep (Massimini et al. 2010) or ketamine anaesthesia, when consciousness is accessible, although in the frame of a disconnection from the external environment (Collier, 1972; Siclari et al., 2013; Sarasso et al., 2015).

A convergent finding across studies from indipendent laboratories is the absence of TEPs in VS/UWS (as well as MCS) patients due to diffuse axonal damage (Rosanova et al. 2012; Ragazzoni et al. 2013): this was evident either when "standard" TEPs analysis was applied (3/15 and 6/13, respectively, in the two cited studies) and in 12/13 patients whose brain PCI showed "no response" (Casarotto et al. 2016). Thus, if replicated in larger sample of patients, the absence of TMS-EEG response might represent a suitable neurophysiological marker of a diffuse axonal damage subtending prolonged DoC.

Only a series of case reports are available about prognostic value of TMS-EEG findings, as far as the recovery of consciousness is concerned (Rosanova et al. 2012). Although PCI should not be regarded as a marker for prognostic purposes, it is noteworthy that 9/43 of the VS/UWS patients subgroup with highest values of PCI (i.e., those showing more complex brain responses) had a more favourable clinical outcome at six months, with a transition from VS/UWS to MCS in 6 out 9 (5/21 among patients in the low complexity group), suggesting that these patients may retain a capacity for consciousness that might be missed looking only at their behavioural responses (Casarotto et al., 2016). Looking at these findings into a neuromodulatory perspective (see the following paragraphs of the current review), the complexity of TMS-EEG responses might find a place as a screening procedure to individuate suitable candidates (i.e., those patients maintaining local reactivity and residual cortico-cortical connectivity) that can enter a neurorehabilitation protocol.

It remains to be determined the clinical utility in DoC patients of automated TMS-EEG "functional cytoarchitecture" cortical mapping (Harquel et al., 2016) as well as the eventual adjunctive utility of merging measures of brain metabolism with TMS-EEG findings (Bodart et al., 2017).

5. Non-invasive brain stimulation (NIBS) as a neuromodulatory tool

No satisfactory pharmacologic treatments are currently available for severe DoC (Gosseries et al., 2011). Invasive neurostimulation techniques such as DBS have been regarded as a potential approach to prolonged DoC treatment in proofs-of-principle studies, but clinical trials are still lacking (for details, see section 6). In addition, ethical and procedural limitations have to be considered in these patients (Giacino et al., 2012; Patuzzo and Manganotti, 2014). Considering these points, NIBS techniques such as rTMS and tDCS have been proposed as an experimental therapeutic strategy in prolonged DoC.

Overall, the rationale behind the use of NIBS as neurostimula tion/neuromodulation approach to treat a given neurological disorder relies on the possibility (a) to produce plastic changes outlasting the stimulation period and (b) to induce effects in brain regions at a distance from the stimulating site by a widespread activation of neural networks. This aims at counteracting the abnormalities in brain circuitry thought to cause specific clinical deficits. Classically, some NIBS protocols such as high-frequency rTMS, intermittent theta burst stimulation, quadripulse magnetic stimulation at interstimulus intervals (ISIs) of 1.5–10 ms, and anodal tDCS are considered to have excitatory effects, whereas other paradigms (e.g., low-frequency rTMS, continuous theta burst stimulation, quadripulse stimulation for ISIs of 30–100 ms, cathodal tDCS) are considered inhibitory (Rossi et al., 2009; Lefaucheur et al., 2014). However, in this context, the terms "excitation" and "inhibition" refer to the balance between excitatory and inhibitory effects on different neural circuitries. Moreover, this dichotomy is challenged by the experimental evidence that several factors such as baseline cortical excitability and patterns of cortical oscillations strongly influence the net amount and persistence of the effects of different NIBS techniques at the individual level (Siebner and Rothwell, 2003; Fertonani and Miniussi 2017; Thut et al., 2017). These results suggest complex interactions of physiological, disease-related, and drug-related homeostatic plastic and metaplastic mechanisms (for a detailed discussion, see Lefaucheur et al., 2014). As to the widespread effects of NIBS, an indirect evidence in support of this concept comes from DBS in Parkinson's disease (PD) (Benninger and Hallett, 2015). Namely, improvement of motor symptoms induced by DBS of the subthalamic nucleus and internal pallidum has been reported to be associated to changes in cerebral activity (Ceballos-Baumann et al., 1999; Eusebio et al., 2011; Limousin et al., 1997) and in motor cortex excitability (Chen et al., 2001; Cunic et al., 2002). This suggests remote effects of DBS on distributed motor circuit connecting motor cortex, basal ganglia and thalamus and opens up the possibility that similar effects may be obtained stimulating other targets within the circuit such as cortical regions easily accessible to NIBS.

The effect of rTMS on distant brain network have been firstly demonstrated by neurophysiological studies that reported a modulation of the M1 excitability induced by conditioning protocols applied over the dorsal premotor cortex (Gerschlager et al., 2001; Munchau et al., 2002; Rizzo et al., 2004). Further evidence came from neuroimaging data showing changes of blood-oxygen-level-dependent signal (Bestmann et al., 2005) and cerebral blood flow (Okabe et al., 2003) after rTMS of the premotor or motor cortex. Moreover, increased dopamine release within basal ganglia has been reported after stimulation of the dorsolateral prefrontal cortex (DLPFC) and M1 (Strafella et al., 2001, 2003). Similarly, tDCS has been demonstrated to exert distant action by modulating different pattern of functional connectivity between cortical and subcortical networks when applied over the M1 (Baudewig et al., 2001; Polanía et al., 2012) or the DLPFC (Peña-Gómez et al., 2012). As in other neurological conditions, the use of NIBS techniques as potential neuromodulatory tools in DoC patients aims to activate the residual connections and the neuroplastic potential. This notwithstanding the intrinsic limitations of NIBS application to DoC, mostly VS/UWS, represented by the severe disconnections between different brain areas and by the cell death, as detailed in the Introduction.

Clinical data on the efficacy of NIBS as neurostimulation/neuro modulation approaches in prolonged DoC mainly derive from small open-label studies and case reports, with only a few cross-over, controlled studies as can be seen from the Table 3. At first, two case reports, using different experimental protocols and site of stimulation, suggested that rTMS might produce some effects in patients with prolonged DoC. Louise-Bender Pape et al. (2009) applied a patterned rTMS over the right DLPFC (300 paired-pulse trains with 100 ms inter-pulse and 5 s inter-train intervals), for 6 weeks in a patient with post-traumatic VS/UWS. A non-significant trend toward behavioural improvement associated with an improvement of auditory pathways conduction has been reported (Louise-Bender Pape et al., 2009). The same research group applied such protocol in two additional VS/UWS patients in order to evaluate safety indicators of the treatment without reporting clinical data (Pape et al., 2014). Afterwards, Piccione et al. (2011) described an arousal with transient increase of meaningful behaviours and EEG changes in a MCS patient who underwent a single session of 20-Hz rTMS applied on the scalp overlaying M1. The effects were

Table 3
Studies evaluating Non-Invasive Brain Stimulation (NIBS) in VS/UWS and MCS.

Study	NIBS technique	Design	Patients		Stimulation protocol				Outcome measures	Results	
			Number, diagnosis and etiology	Time from injury (mean, range)	Target area	Control condition	Treatment duration	Stimulation parameters		Immediate	Follow-up
Louise-Bender Pape et al. (2009)	rTMS	Case report	1 VS (traumatic)	287 days	Right DLPFC	No control	6 weeks (30 sessions)	300 paired-pulse trains; 100 ms inter-pulse and 5 s inter-train intervals	DOCS scale and evoked potentials	Afetr 30 sessions no significant effect	6 weeks afer the treatment: a trend toward significant neurobehavioral gains
Piccione et al. (2011)	rTMS	Case report	MCS (hemorrhagic)	4 years	Left M1	Median nerve stimulation	Single session	20-Hz/90% RMT/ 10 min/1000 pulses	CRS-R, EEG	Behavioural imporving in the 6 h after the rTMS (CRS-R score from 13 to 19) with signs of increased arousal with absolute and relative power increase of the delta, alpha, and beta bands	6 months after, the clinical and neurophysiological conditions of the patient were the same as before the experiment
Manganotti et al. (2013)	rTMS	Open-label	3 VS, 3 MCS (3 traumatic, 3 hemorrhagic)	42.5 months (12–94)	Left or right M1	No control	Single session	20-Hz/120% RMT/ 10 min/1000 pulses	CRS-R, EEG	Long-lasting (up to 6 h) behavioral and EEG modification only in one MCS patient	/
Cincotta et al. (2015)	rTMS	Randomized, controlled, double-blind, cross-over	11 VS (9 post-anoxic, 2 traumatic)	35.4 months (9–85)	Left M1	Sham coil stimulation	5 days (5 sessions)	20-Hz/60% MSO/ 10 min/1000 pulses	CRS-R, EEG	Slight non-significant changes in the aorusal CRS-R subsacale; no significant EEG changes except for sporadic brain reactivity under the stimulation point	No differences between real and sham stimulation after 1 months
Louise-Bender Pape et al. (2014)	rTMS	Case reports	2 VS (traumatic)	188 days and 9 years	Left or righ DLPFC	No control	6 weeks (30 sessions)	300 paired-pulse trains; 100 ms inter-pulse and 5 s inter-train intervals/110% RMT	Only safety indicators; no clinical data	One ictal event in one patient; no other relevant changes in monitored indicators	/
Naro et al. (2015)	rTMS	Controlled study	10 UWS (post-anoxic)	12.2 months (4–15)	Right DLPFC	Sham rTMS only in 3 “responder” patients after 1 week	Single session	10-Hz/90% RMT/1000 pulses (trains of 50 stimuli in 5 s repeated every 20 s)	CRS-R; inhibitory and facilitatory intracortical and interregional TMS measures	3 patients showed a short-lasting increase of the CRS-R_M score (from 2 to 3) and reshaping of brain connectivity immediately after rTMS	No effects 60 min after rTMS
Xie et al. (2015)	rTMS	Non-randomized study	11 VS, 7 MCS, and 2 in coma (following stroke).; 10 patients assigned to the treatment group	Not reported	Right DLPFC	Details on control group not reported	4 weeks (28 sessions)	5 Hz; other details not reported	CRS-R; EEG, EPs	Changes in the alpha power during rTMS treatment with higher CRS-R scoresparticularly in 6 patients	4 weeks after rTMS treatment, 6 patients mainteined improvements
Liu et al. (2016)	rTMS	Controlled study	5 VS, 5 MCS (3 traumatic, 3 hemorrhagic)	5.6 months (1–28)	Left M1	Sham stimulation	Single session	20-Hz/100% RMT/ 10 min/1000 pulses	CRS-R; cerebral hemodynamics of the left middle cerebral arteries	Increase of the peak systolic velocity and the mean flow velocity only in MCS; no CRS-R scores changes	/

Angelakis et al. (2014)	tDCS	Non-randomized controlled study	7 UWS, 3 MCS (5 traumatic, 4 post-anoxic, 1 post-operative infarct)	6 months-10 years	Left DLPFC or left primary sensorimotor cortex	Non-randomized sham stimulation	3 weeks (5 days per week)	Week 1: sham; week 2: anodal tDCS (1 mA, 20 min); week 3: anodal tDCS (2 mA); reference electrode over the right supraorbicular cortex.	CRS-R; EEG	Clinical improvement only in MCS patients (particularly in 1 patients)	After 3 months, one MCS patient received a second cycle of 10 tDCS sessions with furhter improvement
Thibaut et al. (2014)	tDCS	Randomized, controlled, double-blind, cross-over	25 UWS, 30 MCS (25 traumatic, 30 non-traumatic)	33.5 months (7 days-11 years)	Left DLPFC	Sham stimulation	Single session	anodal tDCS/2 mA/20 min; reference electrode over the right supraorbicular cortex	CRS-R	Transient improving in CRS-R total scores only in MCS patients; 13 MCS and 2 UWS patients further showed tDCS-related signs of consciousness	No correlation between tDCS response and patient outcome was observed at 12 months follow-up
Naro et al. (2016a)	tACS	Controlled study	14 UWS, 12 MCS (11 post-anoxic, 15 traumatic)	9.5 months (3–19)	Right DLPFC or frontopolar cortex	tRNS (0.1–640 Hz) over the right DLPFC	Single session	gamma-range (35–140 Hz) tACS/1 mA/10 min; reference electrode over Cz	EEG; CRS-R	Increase of the frontotemporal theta and gamma relative power and of the partial directed coherence measures in MCS patients; no CRS-R changes during or after each experimental session	/
Naro et al. (2016b)	otDCS	Controlled study	10 UWS, 10 MCS (7 post-anoxic, 13 traumatic)	19.5 months (3–72)	Medial cerebellum (half a centimeter below the inion)	Sham stimulation	Single session	5 Hz anodal otDCS/2 mA/10 min; reference electrode over the left buccinator muscle	EEG; CRS-R	In MCS patients, increase of the theta and gamma power and gamma coherence up to 30 min after the stimulation, associated to transient CRS-R amelioration	/

rTMS: repetitive transcranial magnetic stimulation; tDCS: transcranial direct current stimulation; tSCA: transcranial alternating current stimulation; otDCS oscillatory transcranial direct current stimulation; tRNS: transcranial random noise stimulation; VS: vegetative state; UWS: unresponsive wakefulness syndrome; MCS: minimally conscious state; DLPFC: dorsolateral prefrontal cortex; M1: primary motor cortex; DOCS: disorders of consciousness scale; EP: evoked potentials; RMT: resting motor treshold; MSO: maximum stimulation output.

een in the 6 h following the rTMS protocol while no changes emerged after peripheral stimulation applied as a control condition. The same stimulation protocol (i.e., single session 20-Hz rTMS delivered over the scalp corresponding to M1) has been employed in an open-label study investigating EEG reactivity and clinical response in 6 severely brain injured patients with prolonged DoC (VS/UWS and MCS) (Manganotti et al., 2013). Authors reported long-lasting EEG and behavioural changes only in one MCS patient, whereas no significant clinical or EEG modifications were observed in any other of the patients. These early reports in the literature had great resonance in the mass media and created strong expectations among patients' families, raising the need of controlled studies on larger samples.

On this line, Cincotta et al. (2015) conducted the first randomised, double blind, sham-controlled crossover trial in 11 VS/UWS patients (9 post-anoxic, 2 post-traumatic). Real or sham 20-Hz rTMS (10 min stimulation for a total of 1000 pulses) were applied to the left M1 for 5 consecutive days. Clinical data and EEG recordings were collected up to one month after the treatment period. Using a standardized clinical evaluation by the JFK CRS-R (Giacino et al., 2004), no significant behavioural difference was observed between real and sham conditions. In addition, no overall EEG modifications were detected.

Interestingly, when blind evaluation performed by the neurologist and the relatives using the Clinical Global Impression scale (Guy, 1976) were compared, a lack of concordance was seen in single VS/UWS patients. This finding underlies the difficulty to detect minor clinical modifications in prolonged DoC patients (Schnakers et al., 2009). Using a similar 20-Hz rTMS protocol over the left M1, Liu et al. (2016) reported changes of cerebral hemodynamic of the left middle cerebral arteries, as tested by transcranial doppler ultrasound, after a single stimulation session. Namely, an increase of the peak systolic velocity and the mean flow velocity has been observed in MCS but not in VS/UWS patients compared to sham stimulation. No clinical changes in the CRS-R scores were seen (Liu et al., 2016).

After the early case report by Louise-Bender Pape et al. (2009), the effect of rTMS applied over the DLPFC has been evaluated in two recent studies. Naro et al. (2015c) tested the clinical and neurophysiological effect of a single session of 10-Hz rTMS in ten UWS patients. Authors reported no significant clinical changes at group level. However, in 3 patients rTMS induced a transient significant clinical improvement, limited to the motor domain of the CRS-R, associated with a short-lasting reshaping of brain connectivity, as tested by a dual-coil TMS paradigm with the conditioning stimulus over the right pre-motor cortex or the pre-supplementary motor area and the test stimulus over the left M1 (Naro et al., 2015c). In addition, Xie et al. (2015) conducted an open-label study on 20 patients with DoC following stroke (11 VS/UWS, 7 MCS, and 2 coma). The treatment group of ten patients received 28 sessions of 5-Hz rTMS. Authors reported significant increase of the absolute alpha power after the first rTMS session and of the relative alpha power after 2 weeks of treatment. At the behavioural level, patients showed higher scores in the GCS and CRS-R scales between two and four weeks of treatment. However, interpretation of these results appears difficult as no clear clinical data and design details are given.

In the last years, few studies evaluated the clinical and neurophysiological effects of different transcranial electrical stimulation (tES) protocols in DOC patients. Angelakis et al. (2014) evaluated the effect of a 5-day treatment by anodal tDCS in ten patients (3 MCS and 7 UWS). All patients underwent 3 consecutive weeks of treatment including three conditions with a non-randomized design: sham stimulation in the week 1; real anodal tDCS at 1 mA in the week 2 (20 min per day, 5 days per week); and real anodal tDCS at 2 mA in the week 3 (20 min per day, 5 days per week). Half of patients received tDCS over the left DLPFC, the others over the left primary sensorimotor cortex, with the reference electrode placed over the contralateral supra-orbital region. All 3 MCS patients showed clinical improvement within one week after the end of the whole treatment cycle, however the results should be considered with caution due to limitations in the number of studied patients. Authors reported also that one patient who was in a UWS for 6 years before treatment changed status to MCS at 1-year follow-up, but such data can hardly be attributable to tDCS treatment (Angelakis et al., 2014).

A double-blind, sham-controlled study with a crossover design has been conducted by Thibaut et al. (2014). They explored the effect of a 20-min single session of anodal tDCS at 2 mA over the left DLPFC on 55 patients (30 MCS, 25 UWS). Findings showed that tDCS treatment may transiently improve CRS-R total scores in MCS patients compared to sham stimulation. In contrast, no significant effects were seen in UWS patients. Interestingly, authors reported that 13 MCS patients and only 2 out of 25 patients included in the UWS group showed signs of consciousness after tDCS treatment observed neither during the pre-tDCS evaluation nor during the pre- or post-sham evaluation (Thibaut et al., 2014). The same authors conducted a retrospective study to evaluate the relationship between tDCS behavioural responsiveness and structural MRI and fluorodeoxyglucose positron emission tomography data in a subgroup of MCS patients (Thibaut et al., 2015). Patients classified as tDCS-responders showed pattern of grey matter and metabolic preservation in brain areas such as the left DLPFC, the medial-prefrontal cortex, the precuneus, and the thalamus (Thibaut et al., 2015).

Recently, Naro et al. (2016a) used transcranial alternating current stimulation (tACS) to modulate brain oscillation patterns of the gamma band in order to evaluate residual network connectivity in patients with prolonged disorders of consciousness. Twenty-six patients (14 UWS and 12 MCS) and 15 healthy individuals underwent three 10-min single session stimulation protocols in different days: (a) gamma-range (35–140 Hz) tACS over the right DLPFC; (b) gamma-range tACS over the frontopolar cortex; and transcranial random noise stimulation (tRNS; 0.1–640 Hz) over the right DLPFC as an active stimulation control conditions. No sham condition was included. Immediately, 30, and 60 min after the end of each stimulation protocol, 10-min EEG was recorded and CRS-R was performed. At behavioural and clinical level, neither tACS nor tRNS induced significant CRS-R changes either during or after each experimental session. tACS over the right DLPFC induced a significant increase of the frontotemporal theta and gamma relative power and of the partial directed coherence measures in all the healthy participants and MCS patients and in some VS/UWS individuals.

A different stimulation target has been tested by Naro et al. (2016b) to evaluate fronto-parietal network functional connectivity changes induced by cerebellar 5-Hz oscillatory tDCS (otDCS) compared to sham stimulation. Authors reported an increase of the theta and gamma EEG power, on central and frontal electrodes respectively in MCS patients up to 30 min after the stimulation. Moreover, gamma coherence increased within central and, partially, fronto-central electrodes up to 30 min after the stimulation. At clinical level, such changes were associated to transient CRS-R amelioration in the MCS group 30 min after the stimulation, whereas neither clinical nor EEG changes emerged in VS/UWS patients (Naro et al., 2016b).

In summary, the currently available data failed to provide evidence for a therapeutic neuromodulatory effect of NIBS in VS/UWS, at least when conventional magnetic coils and recommended rTMS (Rossi et al., 2009) and tES parameters are employed. Several hypotheses can be advanced to explain lack of NIBS efficacy in these patients. First, differently from what occurs in physiological conditions (Bestmann et al. 2004; Denslow et al., 2005), in VS/UWS the massive derangement of brain connectivity may result in the lack of neural networks acting as an efficient substrate for remote effects of stimulation. Important to this view, a severe alteration of functional inter-regional connectivity has been demonstrated in VS/UWS using simultaneous TMS and EEG recordings (Rosanova et al., 2012; Ragazzoni et al., 2013) and dual site TMS measures (Naro et al., 2015b), as well by the lack of relevant offline EEG modifications at distance from the site of rTMS application (Manganotti et al., 2013; Cincotta et al., 2015). Second, as in most VS/UWS patients the cortical excitability is greatly reduced (Cincotta et al., 2015), another possibility is that the rTMS intensities currently used in accordance with the international safety guidelines (Rossi et al., 2009), could be insufficient. The same could also be hypothesized for the current tES intensities, although recent evidence showed a partially non-linear relationship between intensity and tDCS-induced effects, suggesting that intensity enhancement does not necessarily increase efficacy (Batsikadze et al., 2013; Opitz et al., 2016). At least for rTMS, however, this hypothesis is partially challenged by the local EEG changes underneath the stimulation site observed in some VS/UWS patients (Cincotta et al., 2015). Another possibility is that the targets used so far (i.e., M1 and DLPFC) could not be the most appropriate for NIBS in VS/UWS. Moreover, deeply stimulating magnetic coils such as the H-coil have not been tested yet. Finally, a limit of the present data is the small sample size. Nevertheless, taken together, these studies may help to define the appropriateness of NIBS targets for VS/UWS treatment, in order to optimize the allocation of human and financial resources for rehabilitation.

Unlike VS/UWS, the current preliminary findings support the possibility that neuromodulatory NIBS may have some clinical effects in some MCS patients (Angelakis et al., 2014; Thibaut et al., 2014; Naro et al., 2016b). As most data refer to the *acute* effect of single tES application over the left DLPFC (Thibaut et al., 2014) or the cerebellum (Naro et al., 2016b), the persistence of these effects is still unknown. Nevertheless, these results are in keeping with the hypothesis that some residual plastic capacities may still be present in MCS patients (Monti, 2012). If so, the preservation of sufficient neural networks appears to be the putative substrate of neuroplastic changes in these patients. In accordance with this view, TMS-EEG recordings (Rosanova et al., 2012; Ragazzoni et al., 2013) and EEG changes following tACS (Naro et al., 2016a) have shown a somewhat preserved functional connectivity among different brain areas in MCS patients. Further studies are needed to evaluate whether these NIBS effects in MCS actually represent a therapeutic perspective. In designing these studies, a crucial factor will be matching the sites and modality of stimulation with the patterns of structural and metabolic derangement of recruited MCS patients, in order to optimize their capacity to react to NIBS application.

6. Deep brain stimulation (DBS) and other invasive methods of CNS stimulation

DBS consists in the stereotaxic, reversible, mostly bilateral, implant of stimulating leads in subcortical targets (usually grey nuclei), connected to a controllable pulse generator via subcutaneous cables. They chronically deliver extracellular direct currents of variable pulse frequency, intensity and width (20–200 Hz, 1–6 V, 60–120 μs). Mechanisms of action of DBS are multiple and complex, and not fully known yet, even for diseases –as Parkinson's Disease- where DBS is the gold standard for the treatment of advanced cases (Follett et al., 2010). Most credited mechanisms, especially for frequencies above 100 Hz, are both local and system effects: the former are mainly consequence of excitation/inhibition of both afferent and efferent axonal fibers, rather than body cells (Gradinaru et al., 2009). The latter implies that DBS modifies dynamics of the whole network connected with the discrete region being stimulated (Hammond et al., 2008; Alhourani et al., 2015; see Perlmutter and Mink, 2006; Rosa et al., 2012 for reviews), possibly through synaptic inhibition (Dostrovsky et al., 2000) and "jamming", that is a sort of masking of pathological oscillatory signals sustaining symptoms (de Hemptinne et al., 2015).

The central nuclei of the thalamus are the main target proposed for DBS in prolonged DoC according to the view that DBS at this level may support thalamocortical and thalamostriatal outflow, thereby depolarizing neocortical and striatal neurons of the anterior forebrain mesocircuit, which has a major role in arousal regulation (Schiff, 2016). Such "activating" perspective of DBS hardly reconciles with the abovementioned mechanisms of action of DBS in movement disorders, where there is agreement that DBS –whatever its mechanisms of action- finally resembles the same inhibitory effect that a destructive neural lesion has on symptoms/behaviour (Benazzouz et al., 1993; Dostrovsky et al., 2000; Hammond et al., 2008). Therefore, it is conceptually puzzling that DBS could have been proposed among therapeutic strategies for patients with prolonged DoC, moreover considering that one of the pathological hallmark of the VS/UWS is a disconnection between the thalamus and the neocortex.

In support of this, the majority of previous attempts of DBS of the central thalamus in prolonged DoC patients failed to report clinically meaningful effects in terms of recovered or improved awareness, with effects limited to some evidence of increased arousal, such as occasional eye-opening and changes in autonomic function (i.e., increases in heart rate or blood pressure) (Shah and Schiff, 2010), the latter being a common effect of DBS of several subcortical grey nuclei/structures (subthalamus, periventricular-periaqueductal grey matter, hypothalamus) (Rossi et al., 2016) rather than specific for stimulation of central thalamic nodes.

Most of these attempts to use DBS of the central thalamic nuclei to improve arousal in prolonged DoC patients, including pioneeristic investigations (Hassler et al., 1969; Strum et al., 1979), concerned single case reports (Tsubokawa et al., 1990; Katayama et al., 1991) or small case series (Cohadon, 1985; Cohadon and Richer, 1993; Deliac et al., 1993; Magrassi et al., 2016). Among case reports, it is worth mentioning the unique findings reported by Schiff et al. (2007), concerning a MCS patient following traumatic brain injury (TBI) with leads implanted in the centromedial thalamic nuclei: after DBS performed in a chronic and stable MCS phase, behavioural measurement blindly performed with DBS ON (parameters of stimulation: 100 Hz, pulse width 90 μs, amplitude 4 V right, 4.5 V left electrode contacts) and OFF showed stimulation-related improvement of some scales specifically capturing "cognitively mediated behaviours requiring working memory and sustained attention, such as expressed verbal fluency and semantic retrieval, controlled sensorimotor integration, and communication", together with a broad frontocentral EEG modulation (Schiff et al., 2007).

This encouraging result, that was consistent with the recruitment of fronto-striatal resources within the anterior forebrain mesocircuit, was partly replicated in a larger study (Yamamoto et al., 2010) involving 21 VS/UWS patients (9 TBI, 9 cerebrovascular accident, 3 brain anoxia) who underwent unilateral DBS of the centromedian-parafascicular thalamic nucleus (19 cases) or reticular formation (2 cases) of the less affected side, and using different stimulation parameters (frequency 25 Hz; intensity adjusted individually and pulse width not reported) from the successful case by Schiff et al. (2007). Reported results were emergence from VS and recovered ability to obey some verbal commands occurring in 8/21 VS/UWS patients (at a time ranging 8–19 months following DBS), associated with recovered desynchronization to continuous EEG analysis. These changes were not detected in none of the 86 VS/UWS patients that did not undergo DBS, but were followed up for an overlapping time-span. Most importantly, the eight patients who improved after DBS retrospectively fulfilled strict electrophysiological criteria of preserved brain reactivity to sensory stimulations (thus indicating residual efficiency of thalamo-cortical connections), expressed by the presence of cortical waves during brainstem auditory and somatosensory evoked potentials, in line with previous and following observations of the same group of researchers on the same patients' sample (Yamamoto and Katayama, 2005; Yamamoto et al., 2013). However, it is difficult to ascribe these signs of behavioural improvements to the exclusive effect of DBS, since the implant was carried out 4–8 months following the initial cause of coma, hence well within the period of possible spontaneous recovery at least for TBI and cerebrovascular patients (that, however, did not occur in the "control" VS/UWS patients without DBS).

Another invasive approach that has been introduced in the attempt to improve clinical conditions of prolonged DoC patients is the epidural spinal cord stimulation (SCS) of the cervical dorsal columns. In this case, afferent impulses reach the reticular formation and the thalamus and, through thalamocortical connections, the neocortex (Paradiso et al., 1995) (provide that the afferent pathways are not disconnected). In all available studies (see Della Peppa et al., 2013), stimulation ranged between 25 and 200 Hz (pulse width 0.3–1 ms), was applied in a cyclic mode (15 min on/15 min off, only during daytime for a maximum of 11 h) and was below motor threshold (amplitude 2–15 V).

Besides direct activation of the reticular formation and thalamus by afferent inputs (Visocchi et al., 2001), additional proposed mechanisms of actions of SCS are an increase (Hosobuchi, 1985) or a sort of "redistribution" of the cerebral blood flow (CBF) at the cortical level (Mazzone et al., 1995), possibly through a modulatory action of the autonomic nervous system, as well as the release of hormonal factors, both acting on cerebral haemodynamics regulation (Visocchi et al., 2011). Increase of dopamine and norepinephrine levels have been also documented following chronic SCS (Liu et al., 2008).

Few case studies suggested that increased CBF was paralleled by improvement in some communication skills in patients with MCS (Hosobuchi, 1985; Yamamoto et al., 2013). One successive large prospective, uncontrolled and non-randomized observational study for 20 consecutive years (Kanno et al., 2009) showed that 54% (109 out of 201) of patients classified as being in a permanent VS/UWS, implanted with a cervical (C2-C4) epidural stimulator, recovered stimulation-related signs of awareness of self and surrounding environment. Positive results were particularly evident in younger (<35 years) patients, in those with TBI VS/UWS and when regional CBF was over 20 ml/100 g/min (Kanno et al., 2009). However, the follow up was too short (3.5 months) to verify the real clinical utility of SCS and, most importantly, the evaluation scales were designed "ad hoc" for the study and gave relevance to unspecific behaviours: for example, the detection of a behavioural expression or swallowing food or water when placed in the mouth was considered as an "excellent response", while a "positive response" included eye movements or blink following a visual stimulus.

In conclusion, the extant literature of invasive stimulation in prolonged DoC include some interesting results, both for DBS and SCS, although in the context of studies that have been often poorly controlled for clinical measures and outcomes (see Della Peppa et al., 2013). Therefore, they should overall be considered still preliminary. A roadmap for forthcoming DBS clinical trials has been proposed (Giacino et al., 2012), but DBS (and SCS) controlled clinical trials are still lacking, so the current evidence is not sufficient to recommend large-scale application of invasive brain stimulation in prolonged DoC patients. Our conservative view is that if DBS of central thalamic nuclei (as well as SCS) is offered as an ultimate treatment option in a chronically stable DoC patient, at least a partial functional integrity of thalamo-cortical connections should be overtly and electrophysiologically demonstrated before the surgical implant.

7. Concluding remarks

The clinical "hard problem" of detecting for certainty consciousness in VS/UWS and MCS states has not been resolved yet, but neurosciences have brought lately relevant contributions to identify the neural bases of arousal and wakefulness and so to better focus the questions to be answered. Electrophysiology, with the advent of new techniques, has provided both diagnostic and prognostic clues, accessible at the bedside and therefore complementing the behavioural assessment. Among the many neurophysiological investigations, TMS combined with EEG appears at present as the most promising approach in detecting and tracking recovery of consciousness in prolonged DoC patients, consistently differentiating VS/UWS from MCS. Other neurophysiological techniques are also useful as they can disclose and characterize covert cognitive abilities not accessible through the clinical examination. Less rewarding are the results obtained in therapeutic trials with different approaches of invasive and non-invasive brain stimulation. Clearly, what is still missing is an accepted neurobiological theory of consciousness based on neurophysiological, anatomical and neuropathological evidences, providing a specific marker of awareness to be detected with neurodiagnostic investigations.

Without such a gold-standard "consciousness detector", lone data points of evidence for or against awareness are challenging to interpret. Indeed, guidelines for clinical behavioural assessments of consciousness highlight the necessity of multiple observations before a diagnosis can be reached (Kalmar and Giacino, 2005). In the same way, evidence from multiple research assessments and modalities (e.g., behaviour, neuroimaging, neurostimulation) must be accumulated and weighed before any clinical conclusions can be made (Peterson, 2016). It is therefore an important goal of the research field to identify multiple approaches to detecting awareness that can be combined to improve clinical practice.

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