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To cite this article: Martino Napolitani, Olivier Bodart, Paola Canali, Francesca Seregni, Adenauer Casali, Steven Laureys, Mario Rosanova, Marcello Massimini & Olivia Gosseries (2014) Transcranial magnetic stimulation combined with high-density EEG in altered states of consciousness, Brain Injury, 28:9, 1180-1189, DOI: 10.3109/02699052.2014.920524

To link to this article: http://dx.doi.org/10.3109/02699052.2014.920524

Published online: 06 Aug 2014.

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Transcranial magnetic stimulation combined with high-density EEG in altered states of consciousness

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Abstract

Background: This review discusses the advantages of transcranial magnetic stimulation combined with high-density electroencephalography (TMS-hdEEG) over other current techniques of brain imaging.

Methods and results: Its application was reviewed, focusing particularly on disorders of consciousness, in the perspective of recent theories of consciousness. Assessment of non-communicative patients with disorders of consciousness remains a clinical challenge and objective measures of the level of consciousness are still needed. Current theories suggest that a key requirement for consciousness is the brain’s capacity to rapidly integrate information across different specialized cortical areas. TMS-EEG allows the stimulation of any given cortical area and the recording of the immediate electrical cortical response. This technique has recently been successfully employed to measure changes in brain complexity under physiological, pharmacological and pathological conditions.

Conclusions: This suggests that TMS-EEG is a reliable tool to discriminate between conscious and unconscious patients at the single subject level. Future works are needed to validate and implement this technique as a clinical tool.

Keywords
Consciousness, effective connectivity, electroencephalography, minimally conscious state, transcranial magnetic stimulation, unresponsive wakefulness syndrome, vegetative state

Introduction

Despite considerable progress, patients with disorders of consciousness (DOC) such as those in a vegetative state (recently renamed unresponsive wakefulness syndrome, UWS [1]) or in a minimally conscious state (MCS [2]) still pose a challenge to both medical teams and families. After falling into a coma, patients with UWS recover arousal but not consciousness, whereas patients in MCS show reproducible but fluctuating signs of consciousness (e.g. responses to command, visual pursuit). The diagnosis of consciousness is mainly based on behavioural evaluation, which can give rise to misdiagnoses [3]. This problem is only partially resolved by the introduction of standardized dedicated scales such as the Coma Recovery Scale–Revised (CRS-R [4]). Numerous neuroimaging and electrophysiological paradigms have been developed in recent years, leading to a better understanding of the neural correlates of consciousness, thereby allowing for the detection of differences between patient groups. However, at the single subject level, all these advancements lack sensitivity and/or specificity that are required in a clinical setting. In parallel, new theories of consciousness suggest that a perturbational approach would bring relevant information about the brain’s capacity for consciousness [5]. Transcranial magnetic stimulation (TMS) coupled with high-density electroencephalography (EEG) [6] is a technique that allows the stimulation of any given cortical area and the recording of the immediate electrical cortical response. TMS-EEG has been successfully employed to discriminate between conscious and unconscious patients under physiological, pharmacological and pathological conditions.

This review will discuss the recent findings obtained with TMS-EEG in various states of consciousness, with a particular focus on DOC. These results will be explored along with the most recent theories of consciousness and this study concludes with the perspectives of what TMS-EEG can offer.

Evaluation of consciousness

Current evaluations of the residual level of consciousness rely on behavioural assessments. These evaluations have an intrinsic limitation: they need the subject to produce a
motor output, either to a visual, tactile or auditory stimulus. They also usually require patients to understand the task and be willing to collaborate [7, 8]. This behavioural approach leaves the physician unaware of a possible impairment of motor and/or afferent projections, likely to be present in patients with severe brain injury [8]. In order to bypass these limitations, neuroscientists have developed different techniques to assess consciousness without relying on motor output. Functional magnetic resonance imaging (fMRI) using mental imagery paradigms can detect consciousness via wilful activation of specific brain areas in some patients with DOC [9–12]. However, the lack of response should not be interpreted as an absence of consciousness. In fact, many patients in MCS, despite showing some signs of consciousness at the bedside, do not show task-related brain activation, which might be due to a lack of comprehension, attention, vigilance or motivation [7, 11]. Besides active task procedures, results from passive paradigms using various external stimulations have shown differences at the group level between patients in MCS and UWS, but not at the individual level [13–15]. Ultimately, the brain can be studied at rest, without performing any task and without receiving any stimulation. Recent fMRI studies show that functional connectivity in the default mode network (which is involved in self-related processes such as inner speech and mind wandering) correlates with the level of consciousness in patients with DOC [16, 17]. However, some have questioned the validity of such indirect tools to assume one’s consciousness [18]. While functional neuroimaging has an excellent spatial resolution, it lacks temporal resolution because it cannot record events shorter than several seconds. This can be problematic as the time scale for capturing consciousness is thought to be in the order of hundreds of milliseconds [19]. Moreover, functional connectivity does not provide any causal interpretation.

Conversely, electrophysiological measurements can detect neuronal activities in a timescale of milliseconds. Active paradigms have also been employed with EEG, allowing the detection of wilful activities following specific instruction in several patients otherwise considered as unconscious [20–22]. Many sophisticated EEG analyses have been developed, such as the bispectral index [23, 24], spectral and entropy analyses [25, 26], neuronal complexity [27], causal density [28] and Granger causality [29], but these procedures solely deal with the background neuronal activity and, thus, lack the deterministic value of a perturbational approach.

Although neuroimaging and EEG techniques are able to bypass some of the behavioural evaluations’ limitations and some show promising results at the group level, none can presently be used at the single subject level. Some authors suggest that TMS-EEG is a good candidate to achieve accurate evaluation of consciousness at the single subject level [5, 30].

TMS-EEG techniques

One way to perturb the brain non-invasively is to force neurons in a small area of the cortex to depolarize, with the expectation that this excitation would propagate to other brain regions. TMS allows researchers to do so by means of electromagnetic induction [5]. These magnetic pulses induce secondary ionic currents in the brain that penetrate the membranes of the neurons, resulting in an action potential or excitatory/inhibitory post-synaptic potential. As the magnetic field falls off rapidly with distance from the coil [5, 31], it only directly activates neural elements superficially (that is, on the surface of the cortex) or in the white matter underneath the stimulation site, limiting the number of brain’s structures one can reach. The amount of neurons and the layers that are directly triggered by the TMS remain undetermined. However, experimental [32] and modelling [33] studies strongly suggest that axons, rather than cell bodies, are most likely the targets of the stimulation. Evidently, axons have the lowest threshold for activation to the brief electrical currents induced by TMS. The capacity of TMS to depolarize neurons depends on the ‘activating function’, which causes a sufficient transmembrane current to flow and depolarize the membrane. Stimulation will take place at the point where the spatial derivative of the induced electric field is maximal. From the site of cortical stimulation, the neuronal signal then propagates along intra- and inter-hemispheric association fibres to other cortical areas and to deeper neural structures. However, the neural signal also, via projecting fibres, propagates to sub-cortical structures and sometimes to the spinal cord. The ‘effective connectivity’ measures the causal interaction between the differently specialized brain areas, which is different from ‘functional connectivity’ (that measures temporal correlation) and from ‘structural connectivity’ (that assesses the anatomical links between brain regions) [34].

While TMS systems have been available for a long time, EEG amplifiers have not been compatible and recording artefact-free response was impossible. EEG amplifiers have then been equipped with sample-and-hold circuits to prevent the recording of the powerful TMS-related artefact. This allowed recording the TMS evoked potentials response with milliseconds time scale, which reliably reflects the state of excitability of underlying cortical circuits [6, 35–38]. The EEG cap is often composed of 60 flat open ring carbon electrodes specially designed to further decrease TMS related artefacts, along with one reference, one ground and two electrooculogram electrodes (Figure 1). Muscles artefacts are also reduced to their minimum by stimulating central scalp regions, avoiding the temporal muscles masses [39]. In order to avoid the contamination of the responses by auditory evoked EEG signal, noise masking is applied through earphone playing white noise in the frequency band of the TMS coil ‘click’ [40]. To further standardize stimulation parameters, ensuring reproducibility and accuracy over cortical areas and across subjects, the experimental set-up should be equipped with a stereotactic neuronavigation system (Figure 1) [41]. The neuronavigation system is used with a structural image of the brain of the subject and locates the relative position of the subject’s head and the TMS coil. Moreover, the system may take into account the relative scalp-to-cortex distance to calculate the electric field induced by TMS on the cortical surface. This ensures that the effective stimulation intensity is the same across subjects and stimulation sites. Therefore, the combination of neuronavigation system, TMS and compatible EEG allows the acquisition of...
cortical evoked potentials that are triggered and recorded from cortical structures. The amplitude, spreading and morphology of the evoked potentials are the characteristics considered in order to investigate the brain’s excitability and connectivity.

Regarding the analysis, the EEG data sets are pre-processed to remove any trial contaminated with eye movements, blinks, muscle or movement artefact, before a source-modelling algorithm is applied. Significant signals from EEG sensors are transposed to cortical sources, according to the time sequence and the topography of the registered evoked response. This transposition can be performed on the subject’s own brain imagery or on a standard model [42]. Casali et al. [42] developed a method to further analyse TMS-EEG data with three synthetic indices: significant current density (SCD), significant current scattering (SCS) and phase locking (PL). SCD sums up the amplitude of all significant currents induced by TMS, SCS measures the average distance of significantly activated sources from the stimulated region, while PL reflects the ability to re-set the ongoing phase of cortical oscillations; these indices, therefore, help to assess different aspects of brain responsiveness. Table I summarizes the advantages and disadvantages of the TMS-EEG technique. Considering this evidence, this technique seems to be a promising tool to assess brain functions in both healthy subjects and pathological conditions. Specifically, it can be a useful method in the assessment of non-communicative patients, as discussed in the next sections.

Experimental recordings in healthy awake subjects

Some recent studies employed TMS-EEG to measure cortical excitability (i.e. the amplitude of the initial response to TMS, which can also be assessed by SCD) and effective connectivity (i.e. the causal interaction between the stimulated area and the subsequent activated cortical regions, which can be assessed by SCS) in healthy awake subjects. The responses were recorded during rest and various cognitive tasks such as memory [43, 44], visual attention [45, 46] and motor planning [47].

Massimini et al. [35, 48] were first to demonstrate that, during wakefulness, TMS is followed by multiple low amplitude fast EEG waves, associated with spatially and temporally differentiated patterns of activation. Brain areas also respond differently to a given stimulation, as shown by Rosanova et al. [49], who delivered TMS on different brain regions of healthy awake subjects. Regardless of the

Table I. Advantages and disadvantages of TMS-EEG.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Bypass afferent sensory pathways.</td>
<td>• Dependent on the subject cortical excitability, lowered in case of brain</td>
</tr>
<tr>
<td>• Does not require functioning efferent pathways.</td>
<td>atrophy (it increases scalp-to-cortex distance) and with several drugs</td>
</tr>
<tr>
<td>• Does not require subject active participation.</td>
<td>(including anti-epileptic).</td>
</tr>
<tr>
<td>• Does not require language processing.</td>
<td>• Requires stable state of wakefulness.</td>
</tr>
<tr>
<td>• Highly reproducible within subject.</td>
<td>• Limited spatial resolution.</td>
</tr>
<tr>
<td>• Can be use at the patient’s bedside.</td>
<td>• Acute patients assessment limited by the presence of metallic implant,</td>
</tr>
<tr>
<td>• Sensitive to changes in stimulation parameters.</td>
<td>external CSF drain or uncontrolled epilepsy.</td>
</tr>
<tr>
<td>• Good temporal resolution.</td>
<td>• Requires considerable logistic and subject preparation.</td>
</tr>
<tr>
<td>• Probes effective connectivity.</td>
<td>• Source modelling possibly inaccurate in cases of extensive brain lesions</td>
</tr>
<tr>
<td>• Discrimination between conscious and unconscious conditions.</td>
<td>or scalp deformations.</td>
</tr>
</tbody>
</table>

CSF, Cerebrospinal fluid.
stimulation intensity, TMS evoked alpha band oscillations (8–12 Hz) in the occipital cortex, beta band oscillations (13–20 Hz) in the parietal cortex and fast beta/gamma oscillations (21–50 Hz) in the frontal cortex. TMS-EEG can, therefore, be used to directly investigate the properties of thalamocortical circuits to produce natural oscillations.

Huber et al. [50] showed that the cortical excitability steadily increased in healthy subjects during the course of prolonged wakefulness. Similarly, attention to motor tasks, even without actual movement, causes an increase of local cortical excitability, as assessed by TMS-EEG [47]. During visual attention tasks, an increase of effective connectivity has also been observed between the frontal eye field (the site of stimulation) and posterior brain regions [46]. This effect is thought to reflect a top-down control over these latter regions. Another recent study showed that, during a spatial working memory task, the delivery of TMS on a specific brain area (i.e. the superior parietal lobule, an area known to play a role in working memory) during the delay period also induced a marked increase of both SCD and SCS, as compared to a condition of rest (where subjects fixated a static point) [43]. Moreover, Kundu et al. [44] have recently demonstrated that a long-term training of a working memory visual task resulted, once again, in an increase in SCS in the task-related networks. Further evidence of task-related network specific change of cortical excitability has been provided in another recent study on face recognition, where excitability was modulated in the medial prefrontal cortex, but not in the premotor cortex [51].

In summary, these findings in healthy awake subjects converge to the assumption that cognitive functions induce increased cortical excitability and/or effective connectivity. It is noted that alcohol intake reduces cortical excitability, especially on the frontal and prefrontal areas [52]. Reduced brain excitability and decreased effective connectivity have also been observed in various states of altered consciousness.

### Experimental recordings in altered states of consciousness

#### Physiological and pharmacological states

If awakened from an early non-rapid eye movement (NREM) or slow-wave sleep, subjects report little or no conscious content, while, if awakened from REM sleep, they are able to report dreams, which can be as vivid as conscious experience during wakefulness [53]. Different thalamocortical networks responses have been recorded with TMS-EEG during transition from wakefulness to NREM and REM sleep. In NREM sleep, TMS triggers a larger positive–negative low frequency wave. Depending on stimulation parameters such as intensity, this response can stay local and be transient or can be global and look like typical slow waves [54]. Sleep-stage one induced an intermediate response between wakefulness and NREM sleep and low intensity stimulation evokes a typical slow wave with disappearance of the latter components [35]. In contrast, in REM sleep, the brain’s response to TMS is similar to the one observed during wakefulness, with fast oscillatory components, especially during the first 100–150 milliseconds post-stimulus [48] (Figure 2). These findings suggest that TMS-EEG is able to monitor graded changes in the level of consciousness during normal physiological changes. Note also that, after a night of sleep, cortical excitability decreases, as opposed to the increase observed after prolonged wakefulness [50].

Pharmacological agents inducing a loss of consciousness, such as midazolam at anaesthetic concentrations, have also been investigated with TMS-EEG. Midazolam targets GABA-A receptors exclusively, increasing inhibitory post-synaptic currents and possibly causing its anaesthetic effect [55]. In this condition, TMS evokes a stereotypical large positive–negative wave, similar to the one evoked in NREM sleep, which remains localized to the stimulation site (Figure 2) [36]. Even if the mechanisms of action differ,

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Typical response to TMS in different physiological, pharmacological and pathological conditions. For each condition, cortical responses under the coil (bold black trace) and in seven other brain areas (lighter black traces) following right premotor stimulations (black lightning) are shown. (A) In NREM sleep, depending on stimulation intensity, TMS can trigger either a global or a local response, but both remain stereotypical and slow. In REM sleep, the response is complex, spread in time and space, similar to the one of wakefulness. (B) General anaesthesia responses look like NREM sleep responses. (C) In DOC, the response in patients in UWS is similar to the one in general anaesthesia and NREM sleep. In patients in MCS, the response is similar to the one observed in REM sleep and in wakefulness. TMS, Transcranial magnetic stimulation; REM, Rapid eye movement; NREM, non-REM; UWS, Unresponsive wakefulness syndrome; MCS, Minimally conscious state. Adapted with permission from Sarasso et al. 2014 [76].
similar responses are expected with other pharmacological inhibitory agents.

In summary, during wakefulness and REM sleep the brain is able to sustain long-range and different activity patterns, marked by a complex, diffuse and long-lasting evoked response, whilst during NREM sleep and anaesthesia, when consciousness fades, this ability is lost and, even if the thalamocortical system remains active, it responds to a direct TMS perturbation in a stereotypical and short lasting manner (Figure 2).

Pathological state

Given these promising results in monitoring different levels of consciousness in healthy subjects, TMS-EEG was subsequently performed in patients with DOC. In the two studies published so far, a total of 30 patients were assessed (15 UWS, 13 MCS and two patients with a locked-in syndrome) during acute (less than 30 days post-brain injury), sub-acute (between 1–3 months post-injury) and chronic (more than 3 months post-anoxia and more than 1 year post-trauma) periods [40, 56]. Nine of the 15 patients with an unambiguous diagnosis of UWS showed a simple, local and slow response to TMS, very similar to the one observed in NREM sleep and general anaesthesia. The other six patients with UWS did not show any response to TMS (one sub-acute and five chronic patients). Interestingly, in the Rosanova et al. [40] study, the only three patients who did not show any response to TMS were the ones who suffered from anoxia. Anoxic aetiology might be linked to some extent to the absence of response in UWS patients. The exact implication of the aetiology needs, however, to be further studied on a larger population sample.

In MCS patients, 12 out of 13 showed complex responses with changing patterns of cortical activation over time. These responses were similar to the ones observed in the two patients with locked-in syndrome (conscious patients who are unable to produce any volitional motor output, except for eye movements) and very similar to what is observed in normal wakefulness. One patient in MCS, with an anoxic aetiology, did not show any response to brain stimulation. Only one brain area was stimulated in this patient, so one cannot exclude that, if other brains areas had been perturbed, it would have induced a complex and widespread response. Moreover, the stimulus intensity was fixed as a percentage of the maximum intensity deliverable and not based on actual intensity at the cortical level; thus, the intensity necessary to evoke a response may not have been reached in this patient.

Importantly, Ragazzoni et al. [56] compared the TMS-EEG to other electrophysiological techniques such as somatosensory evoked potentials, oddball auditory ERPs and EEG power spectrum and showed that only TMS-EEG was able to discriminate between patients in UWS and MCS at the group level.

In acute settings, TMS-EEG was also able to detect, in three patients with DOC, changes in cortical responses matching those observed with behavioural evaluation [40]. The responses recorded when the patients were in an unambiguous UWS were very similar to the one observed previously in chronic UWS, as well as in NREM sleep and general anaesthesia. On the other hand, once the patients recovered signs of consciousness and, therefore, were diagnosed as in a MCS, their responses to TMS looked like those observed in patients with locked-in syndrome and in conscious healthy subjects. Once the patients emerged from the MCS (i.e. recovery of functional communication or functional use of objects—EMCS), their brain responses to TMS were also complex, as observed in other conscious states (Figure 2). Interestingly, one patient who had improved to MCS was behaviourally back in an UWS the day of the TMS assessment, but complex and widespread brain responses could still be detected, although, at the bedside, no sign of consciousness could be observed. Two other acute UWS patients who did not recover were also evaluated twice, 1 month apart. Each time, TMS triggered no response in one patient and a simple, slow and local response in the other patient. This suggests that, in the acute setting, TMS-EEG is able to monitor recovery of consciousness or absence thereof and is less sensitive to daily fluctuation in behavioural diagnosis. However, it is still sensitive to the level of arousal, as one patient in MCS showed simple and local slow wave in response to TMS when sleeping, whereas complex brain responses were recorded when awake [40]. In contrast, transition from behavioural sleep to wakefulness was not associated with a modification of the TMS responses in a patient with an UWS (Figure 3).

Differences have also been observed between patients who suffered mild traumatic brain injury and healthy subjects. Some of the patients remained symptomatic, while some fully recovered. None of them had, however, obvious structural lesions on the MRI. In the symptomatic group, stimulation of the dorsolateral prefrontal cortex elicited delayed peaks and increased amplitude of some of the TMS-EEG components [57]. In the asymptomatic group, stimulation of the same site elicited responses of shortened latency. In both mild traumatic brain injury groups, TMS recordings also revealed an increased motor threshold (as defined by the minimum TMS intensity required to elicit a 50 μV response on electromyography in 5 out of 10 stimulations), as well as differences in the effect of increasing stimulation intensity as compared to healthy control. This increased motor threshold has also been observed in patients in UWS and MCS [58].

Patients with psychiatric disorders also show altered brain responses to TMS. For instance, while stimulating frontal and prefrontal brain regions, patients with schizophrenia demonstrated a clear deficit in the production of fast EEG oscillations, as compared to healthy subjects [59]. Frantsseva et al. [60] also found an abnormal widespread activation of the EEG response to TMS in schizophrenia, which was associated with higher global voltage between 400–750 milliseconds post-stimulation, as compared to healthy controls [60]. Both findings revealed a correlation between EEG gamma band abnormalities and positive symptoms in schizophrenia.

TMS-EEG could also be an effective tool to assess altered excitability and connectivity in neurodegenerative diseases. Casarotto et al. [61] demonstrated a lower frontal cortical excitability in patients with Alzheimer’s disease compared to healthy elderly individuals. All these
applications of TMS-EEG clearly demonstrate its ability to investigate the status of various cortical networks. However, it is important to follow clear guidelines in order to accurately perform TMS-EEG assessment, especially in patients with DOC.

**How to conduct a good experiment in patients with disorders of consciousness?**

As patients with DOC often have significant brain lesions, one needs to carefully check the patient brain MRI or CT scan prior to deciding the stimulation target. Stimulation should be performed on intact brain areas, as direct stimulation of a brain lesion is likely to trigger no or very little response, hence increasing the risk of misdiagnosis. Stimulation of several sites can avoid such a risk in the absence of recent neuroimaging data. The experimenter has also to make sure the patient stays awake for the duration of the TMS-EEG, for example with the help of the CRS-R arousal protocol and by positioning seated. If no responses or very little response is observable online, intensity of the stimulation should be increased, as responses are sometimes very subtle in this population.

It is important to acknowledge the risk of inducing a seizure in patients with a history of epilepsy. Although this is rare and was mostly seen with high frequency stimulation (more than 1 Hz) [62], patients with severe brain injuries often had at least one episode of seizure and, thus, are at higher risk to present another one. This issue should be discussed with the local ethic committee, tempered by the facts that (i) the stimulation parameters typically used are less susceptible to trigger a seizure than high frequency repeated stimulations, (ii) patients who had only one contextual seizure and since then have been treated efficiently (i.e. have had none since the treatment was started) are unlikely to present another one. Moreover, TMS-EEG on patients with DOC is performed most of the time in a setting where adequate management is immediately available. The recorded response should also be interpreted in light of the most recent theories of consciousness.

Figure 3. (A) Effect of arousal on TMS-EEG findings in UWS and in MCS. Behavioural sleep or wakefulness does not influence the response observed in the patient in UWS, whereas in the patient in MCS there is a clear difference between eyes opened (EO) and eye closed (EC) conditions. The response under the coil (black trace) and the stimulation site (white cross), with the significance threshold ($p < 0.01$, pink band) are depicted. The 10 most activated sources during the significant post-stimulus activation are plotted and colour-coded according to the location. (B) Effect of increasing level of consciousness. The same graphs are plotted for an acute UWS patient who improved behaviourally until emergence from MCS. Note that the complexity of the responses improves parallelly. (C) Typical findings in a patient with locked-in syndrome (LIS) showing very similar results to those observed in wakefulness, REM sleep and EMCS. EC, eyes closed; EO, eyes opened; UWS, Unresponsive wakefulness syndrome; MCS, Minimally conscious state; EMCS, emergence from minimally conscious state; LIS, Locked-in syndrome; TMS, transcranial magnetic stimulation. Adapted from Rosanova et al. [40] with permission.
Theories of consciousness and the perturbational approach

In order to establish an objective and reliable marker of human consciousness, there is a need to design a theoretical framework that allows making hypothesis about the brain mechanisms underlying conscious experiences. Although different hypotheses exist, the most recent ones, such as the global workspace theory of consciousness [63, 64], the information integration theory of consciousness – IITC [65]—and the cognitive binding theory [66], converge to the necessity of a neural basis of consciousness matching the temporal dynamic of conscious perception and cognitive abilities (as described in Ortinski and Meador [67]), while keeping in mind that these neural processes can be modulated by other brain structures (e.g. the amygdala for emotional stimuli [68]).

According to the IITC [65], consciousness can only arise if its supporting neural network has the ability to differentiate and integrate information. Indeed, the conscious events experienced are at the same time different from any past event and integrated into their context and are impossible to isolate as such. At the neural level, this conscious event results in a specific neuronal activation pattern among an infinite number of possible states. For consciousness to emerge, the brain, therefore, needs an integrated and differentiated network, consisting of several specialized modules that are effectively connected. To infer the brain capacity for consciousness, one would then need to assess effective connectivity between these brain regions and the TMS-EEG technique seems to be the perfect tool to do so, as seen above. The complex and widespread distributed response observed in normal wakefulness, in patients in MCS or during dreams, reflects that the underlying network is integrated (the response spreads across distant cortical regions) and specialized, providing information (the response is complex and not stereotypical). On one hand, if the system’s modules operate as independent sub-sets, being effectively disconnected from the rest of the network, no information can be integrated; on the other hand, if there is no specialization and the system behaves stereotypically, integration occurs without differentiation. In both cases, the loss of information would lead to a loss of consciousness. In NREM sleep, general anaesthesia and UWS, the loss of integration gives rise to the simple, local and short lasting response observed [35, 36, 40]. During general seizures, where the entire cortex is firing anarchically, there is no specialization and, hence, no information, leading to an alteration of consciousness [69].

The aforementioned results are mainly based on qualitative comparisons between response shapes. To implement the TMS-EEG technique in a clinical routine for the assessment of consciousness, there is a need to objectively quantify those responses. This has previously been done, to some extent, with the SCD and SCS indices [42]. However, these indices do not allow the direct comparison between individual subjects and do not reflect complexity per se. A new complexity measure, the Perturbational Complexity Index (PCI), has been recently developed to assess the brain capacity for consciousness. This ingenious measure is in line with the IITC theory and allows comparison of different subjects and different levels of consciousness [70]. Briefly, based on TMS-EEG artefact-free recordings, statistically significant sources are modelled and a binary matrix is generated. This matrix is then compressed using algorithmic complexity compression and normalized by the source entropy, resulting in the PCI. PCI is low in cases of loss of integration (as its time course would be quite short) and in cases of loss of differentiation (because the information contained would be extremely redundant and, thus, highly compressible).

PCI is able to discriminate between conscious and unconscious conditions regardless of the strength and extent in duration of the cortical response, the stimulation site and intensity (given that the latter is sufficient to evoke an EEG response). PCI is also sensitive to graded changes of consciousness (in different sleep stages, degree of general anaesthesia and DOC). In conclusion, as shown in Figure 4, this measure allows clear-cut differences in intracortical effective connectivity between patients with severe brain injury in UWS and those who recover consciousness...
(MCS, EMCS, locked-in syndrome), representing for the first time a reliable assessment of consciousness at the single subject level [70].

**Future perspectives**

TMS-EEG and its derived measures have shown interesting results in the field of DOC. PCI has demonstrated its consistent ability in determining brain’s capacity for consciousness in a significant number of subjects under many conditions. However, it would be interesting to verify the consistency of these results in a larger group of patients and also using different set-ups. To be more easily implemented in a clinical setting, the whole equipment should be more portable: easy to bring and set up at the patients’ bedside. The pre-processing and analyses of TMS-EEG derived measures should ideally be more straightforward, possibly automated and a standard procedure should be available. This would allow different centres to obtain directly comparable measures of consciousness and to elaborate guidelines for the diagnosis of DOC using more than behavioural scales. Larger longitudinal studies of patients with DOC are also needed to confirm the sensitivity to graded changes in consciousness, as only three patients have been studied during the course of their recovery, so far. Early prognostic markers should be implemented to identify earlier patients who are more likely to recover consciousness. The effect of brain injury aetiology on TMS-EEG response and its implication on the prognosis could also be studied. Similarly, TMS-EEG should be used to assess the effect of pharmacological treatments including zolpidem, amantadine and apomorphine, which have been shown to promote functional recovery in a sub-set of patients with DOC [71–73]. This would allow researchers to better understand the mechanisms by which such treatments work and perhaps determine early on the patients who are likely to respond. Repetitive TMS, which has not been discussed in this review, can modulate brain excitability and potentially connectivity. These effects could also be quickly assessed by TMS-EEG.

Finally, TMS-EEG results could be combined with those obtained with other techniques in order to study neural correlates of consciousness. For example, correlating effective connectivity with structural connectivity (using diffusion tensor imaging) or functional connectivity (using EEG or fMRI [74]). In fact, structural data would provide prior information as input for the source-modelling algorithm, possibly increasing its power and making it closer to reality, as effective connectivity is supported by structural connectivity [34]. Intracranial EEG recordings in patients evaluated for pharmacoresistant epilepsy, for instance, would also extend the spatial capacity of this approach and allow studying of the deeper structures of the thalamocortical networks. Previous studies have demonstrated the safety of using TMS with implanted electrodes, although some recommendations have been released; such as avoiding stimulation near electrodes loops and avoiding repetitive TMS in a chronic setting due to lack of data on potential tissue damage [75].

**Conclusion**

The concomitant development of combined TMS-EEG technique and new theories of consciousness has led to a promising evolution in the way of investigating brain processes. In acute and chronic DOC, TMS-EEG is uncovering new aspects of the brain physiology. Most importantly, for the first time, one has the potential to assess the residual level of consciousness of patients with severe brain injuries at the single subject level and at the bedside. Further developments such as the PCI are likely to improve the feasibility of a daily practical application of TMS in evaluation of consciousness. However, as with every new technique, several questions remain to be answered, such as the exact sensitivity and specificity of this tool or its potential prognostic value and the exact physiology of TMS responses, which has not yet been determined.

**Acknowledgements**

The authors would like to thank Simone Sarasso and Chanyoung Kang for their constructive comments about this manuscript.

**Declaration of interest**

This research was funded by the University and University Hospital of Liège, the Léon Fredericq Funds, the Belgian National Funds for Scientific Research (FRS-FNRS), the European Commission (COST, DISCOS, MINDBRIDGE, DECODER), the James McDonnell Foundation, the Mind Science Foundation, Wallonie-Bruxelles International (WBI) the French Speaking Community Concerted Research Action (ARC 06/11-340), the Belgian American Educational Foundation (BAEF) the Foundation Médicale Reine Elisabeth, the Public Utility Foundation ‘Université Européenne du Travail’ and ‘Fondazione Europea di Ricerca Biomedica’. OG received support from NIH grant MH064498 and MH095984 to Bradley R. Postle and Giulio Tononi. OB is a research fellow, OG a postdoctoral researcher and SL a research director at the FNRS.

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