Dissociations between spontaneous EEG features and the Perturbational Complexity Index in the minimally conscious state

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Abstract

The analysis of spontaneous EEG is a cornerstone in the assessment of patients with disorders of consciousness (DoC). Alterations in specific frequency bands have been reported, including a predominance of delta power in vegetative state/unresponsive wakefulness syndrome (VS/UWS) patients in contrast with a predominance of alpha activity in minimally conscious state patients (MCS). Although preserved EEG patterns are highly suggestive of consciousness even in unresponsive patients, moderately or severely abnormal patterns are difficult to interpret. Indeed, growing evidence shows that consciousness can be present despite either large delta or reduced alpha activity in spontaneous EEG. Quantifying the complexity of EEG responses to direct cortical perturbations (Perturbational Complexity Index; PCI) may complement the observational approach and provide a reliable assessment of consciousness even when spontaneous EEG features are inconclusive. To systematically test this hypothesis, we compared PCI to EEG spectral measures in the same population of MCS patients (n=40) hospitalized in rehabilitation facilities. We found a remarkable EEG background variability across MCS patients as compared to healthy controls and a non-negligible number of patients with predominant delta and highly reduced alpha power in spontaneous EEG. Conversely, PCI values invariably suggested a capacity for consciousness in all MCS patients, consistent with the presence of clearly discernible, albeit fleeting, behavioral signs of awareness. These results confirm that spontaneous EEG rhythms may dissociate from the actual capacity for consciousness and suggest that a perturbational approach can effectively compensate for this pitfall with practical implications for the individual patient's stratification and tailored rehabilitation.

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Abstract

The analysis of spontaneous EEG is a cornerstone in the assessment of patients with disorders of consciousness (DoC). Alterations in specific frequency bands have been reported, including a predominance of delta power in vegetative state/unresponsive wakefulness syndrome (VS/UWS) patients in contrast with a predominance of alpha activity in minimally conscious state patients (MCS). Although preserved EEG patterns are highly suggestive of consciousness even in unresponsive patients, moderately or severely abnormal patterns are difficult to interpret. Indeed, growing evidence shows that consciousness can be present despite either large delta or reduced alpha activity in spontaneous EEG. Quantifying the complexity of EEG responses to direct cortical perturbations (Perturbational Complexity Index; PCI) may complement the observational approach and provide a reliable assessment of consciousness even when spontaneous EEG features are inconclusive.

To systematically test this hypothesis, we compared PCI to EEG spectral measures in the same population of MCS patients (n=40) hospitalized in rehabilitation facilities. We found a remarkable EEG background variability across MCS patients as compared to healthy controls and a non-negligible number of patients with predominant delta and highly reduced alpha power in spontaneous EEG. Conversely, PCI values invariably suggested a capacity for consciousness in all MCS patients, consistent with the presence of clearly discernible, albeit fleeting, behavioral signs of awareness.

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Keywords: TMS, PCI, delta, alpha, MCS

Abbreviations

BA = Brodmann's area; CRS-R = Coma Recovery Scale Revised; DoC = disorders of consciousness; EEG = electroencephalogram; EOG = electrooculogram; HC = healthy control; ICA = independent component analysis; MCS = minimally conscious state; Mi = mildly abnormal pattern; Mo = moderately abnormal pattern; NREM = non-rapid eye movement; PCI = perturbational complexity index; PCImax = maximum perturbational complexity index; PSD = power spectral density; PSDn = normalized power spectral density; Se = severely abnormal pattern; TEP = transcranial magnetic stimulation evoked potentials; TMS = transcranial magnetic stimulation; VS/UWS = vegetative state / unresponsive wakefulness syndrome

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Author contribution

MM, SS Conceptualization

AC, CD, GH, MR, MF, PDT, SC, SR, SS: Investigation

AC, SC Writing - Original draft

AC, MM, SS Writing - Review and Editing

AC, GH, SC Formal analysis

AV, GD, JN, MR, SS Supervision

Data availability

Both raw and analyzed data will be shared upon motivated request.

Introduction

In severely brain-injured patients, sensory input and motor output pathways can be impaired because of central or peripheral lesions. Thus, the clinical assessment of patients with disorders of consciousness (DoC), which routinely relies on behavioral responses to standardized sensory stimulation (e.g., Coma Recovery Scale - Revised, CRS-R; (Giacino et al., 2004)), may underestimate their capacity for consciousness. It is therefore recommended to complement the behavioral assessment of DoC with brain-based measures (Claassen et al., 2019; Edlow et al., 2021; Kondziella et al., 2016). For example, the detection of event-related neural responses may indicate residual cognitive abilities (e.g., (Faugeras et al., 2012)) or even capacity of command following (e.g., (Curley et al., 2018; Monti et al., 2010)). However, these approaches have rather low sensitivity in detecting consciousness, possibly because they still rely on sensory processing and because they require cognitive abilities that are often impaired after severe brain injury (Comanducci et al., 2020).

A classic approach to assess the integrity of thalamocortical functions independently of sensory, motor and cognitive functions is the evaluation of spontaneous electroencephalographic (EEG) rhythms. In view of its widespread availability and affordability, the EEG is still considered a fundamental tool for the instrumental assessment of both diagnosis and prognosis of DoC (Bai et al., 2020; Comanducci et al., 2020; Rossi Sebastiano et al., 2021), as also recently highlighted by the American Academy of Neurology and the European Academy of Neurology (Giacino et al., 2018a, 2018b; Kondziella et al., 2020). This proposition is consistent with the long-standing evidence of a correlation between spontaneous EEG rhythms and the level of consciousness across multiple conditions. For example, slow EEG waves in the delta range are usually found when consciousness fades during deep NREM (non-rapid eye movement) sleep and anesthesia in healthy subjects (Murphy et al., 2011), whereas faster oscillations in the alpha range with a posterior spatial predominance and reactivity to eye opening are characteristic of quiet physiological wakefulness (Berger, 1935). Similar differences can be found when narrow-band spectral power measures are applied to brain injured patients with disorders of consciousness (for a review see (Bai et al., 2017; Duszyk-Bogorodzka et al., 2022; Wutzl et al., 2021)). Overall, vegetative state/unresponsive wakefulness syndrome (VS/UWS) patients are characterized by a predominance of delta power, whereas minimally conscious state (MCS) patients, conscious brain-injured patients and healthy controls show progressively larger predominance of alpha activity. These features, as assessed by visual (Bagnato et al., 2015; Estraneo et al., 2016; Forgacs et al., 2014; Schiff, 2016) as well as quantitative (Engemann et al., 2018; Sitt et al., 2014) analysis of EEG, have been proposed to complement behavior-based diagnosis and have been demonstrated to improve the detection of covert cognition in patients with very limited or no motor responses. This general correlation notwithstanding, the link between narrow-band spectral EEG features and the global state consciousness has been recently questioned. For example, as recently highlighted by Frohlich and coworkers (Frohlich et al., 2021), several conditions exist in which consciousness can be preserved despite a predominance of slow delta activity on spontaneous EEG (Darmani et al., 2021; Frohlich et al., 2020; Gaskell et al., 2017; Gökyiğit and Calişkan, 1995; Ostfeld et al., 1960; Purdon et al., 2015). On the other hand, reduced alpha power has been often reported in different conscious states, such as during ketamine-induced dissociative experiences (Purdon et al., 2015; Vlisides et al., 2018), dreaming (Esposito et al., 2004) and certain psychedelic states (Timmermann et al., 2019) as well as in patients with locked-in syndrome (Babiloni et al., 2010). These discrepancies may explain why some clinically MCS patients may show a severely abnormal EEG at visual inspection (Estraneo et al., 2016; Forgacs et al., 2014; Schiff, 2016) and be classified as VS/UWS by quantitative EEG methods (Engemann et al., 2018; Sitt et al., 2014).

A complementary way of probing neuronal dynamics within thalamocortical networks without engaging sensory, motor and cognitive functions involves recording the EEG responses triggered by a direct cortical perturbation with transcranial magnetic stimulation (TMS). These responses, called TMS-evoked potentials (TEPs), reflect the reactivity of the neuronal population at the stimulation site as well as remote and re-entrant activations from connected populations with different electrophysiological properties (Massimini et al., 2005; Rosanova et al., 2009). In this way, TEPs may be used to assess, by a causal perspective, to what extent distributed and differentiated groups of neurons interact as a whole to produce complex dynamics. Based on theoretical reasoning (Tononi and Edelman, 1998) and empirical evidence (reviewed in (Sarasso et al., 2021)), this kind of complexity, arising from the coexistence of functional integration and functional differentiation, is considered a reliable marker of consciousness. Thus, specific TMS-EEG-based measures, such as the Perturbational Complexity Index (PCI), have been developed to capture at once the integration and differentiation of brain responses (Massimini et al., 2009) and to assess recovery of consciousness in patients emerging from coma (Casali et al., 2013). This perturbational approach has demonstrated unprecedented sensitivity in detecting residual capacity for consciousness in severely brain-injured patients (Casarotto et al., 2016; Sinitsyn et al., 2020), thereby representing a viable candidate to integrate the classic EEG observational approach. However, apart from a few incidental reports (Casarotto et al., 2016; Darmani et al., 2021; Rosanova et al., 2012; Sarasso et al., 2015; Sinitsyn et al., 2020), a direct, systematic comparison between measures of spontaneous EEG activity and complexity of TEPs in the same population has never been performed.

Here, we quantify both spontaneous EEG features and PCI values in a large population of MCS patients, who show clearly discernible, albeit fleeting, behavioral evidence of consciousness. The results of this analysis reinforce the notion that the alteration of specific rhythms of spontaneous EEG may, in some instances, dissociate from the capacity for consciousness in severely brain-injured patients. More importantly, a direct comparison between TEPs and spontaneous EEG features shows that measuring brain complexity by a perturbational approach can compensate for the limitations of observational spectral measures.

Materials and Methods

Participants

This study involved 40 severely brain-injured patients (28 previously reported in (Casarotto et al., 2016)) with either prolonged (> 28 days) or chronic (> 3 months in non-traumatic and > 12 months in traumatic cases) DoC, diagnosed as MCS after repeated behavioral evaluations (4 times, every other day, for 1 week) with the CRS-R (Giacino et al., 2004). Patient data were collected within a multimodal diagnostic assessment detailed in (Willacker et al., 2022). We did not recruit patients with DoC during the acute phase to minimize the potential effects of anesthetic drugs and septic-dysmetabolic complications on EEG activity. **Table S1** in the **Supplementary material** reports detailed demographic and clinical information. In addition, this study also involved a group of 40 healthy control (HC) subjects. In each participant, we collected (i) at least 5 minutes of continuous spontaneous EEG and (ii) EEG responses to TMS of at least 2 cortical sites, within the same experimental session. During both spontaneous and TMS-evoked EEG recordings, the participants were required to stay awake with their eyes open; whenever patients showed behavioral signs of drowsiness, recordings were momentarily interrupted to apply the CRS-R arousal facilitation protocol (Giacino et al., 2004) as in previous reports (Casarotto et al., 2016; Rosanova et al., 2012).

Data collection

Healthy controls were recruited at the Department of Biomedical and Clinical Sciences, University of Milan (Prot. n. 609/07/27/05/AP). Patients with DoC were recruited at the Intensive Rehabilitation Unit (IRU) and long-term facility of Fondazione Don Carlo Gnocchi ONLUS (ethics committee section "IRCCS Fondazione Don Carlo Gnocchi'' of ethics committee IRCCS Regione Lombardia, Prot. n. 32/2021/CE_FdG/FC/SA). EEG was recorded with either of the following TMS-compatible amplifiers according to local availability: Brainamp DC (Brain Products GmbH, Germany) or eXimia (Nexstim Ltd, Finland), respectively equipped with a 62-channel and a 60-channel EEG cap following the standard 10-20 montage. In all the recordings, reference and ground electrodes were located on the forehead and two additional channels were used to record the electrooculogram (EOG) in a diagonal montage. Impedance at all electrodes was kept below 5 kOhm. When using the eXimia amplifier, raw EEG data were collected at 1450 Hz sampling rate and with a hardware filtering bandwidth between 0.01 and 350 Hz. When using the Brainamp DC amplifier, spontaneous EEG data were collected at 1000 Hz sampling rate and with a hardware filtering between 0.016 and 250 Hz, whereas EEG responses to TMS were collected at 5000 Hz with a hardware filtering bandwidth between DC and 1000 Hz.

TMS was delivered with a Focal Bipulse 8-Coil (mean/outer winding diameter ca. 50/70 mm, biphasic pulse shape, pulse length ca. 280 µs, focal area of the stimulation hot spot 0.68 cm^2; Nexstim Ltd., Finland). Stimulation targets were selected using a neuronavigation software (NBS system, Nexstim Ltd, Finland) bilaterally within the middle-caudal portion of the superior frontal gyrus (Brodmann's area BA6 and BA8) and within the superior parietal lobule (BA7), about 1 cm lateral to the midline to avoid stimulating over lateral scalp muscles (Mutanen et al., 2013). In MCS patients, stimulation targets spatially close to or overlying cortical lesions were deliberately skipped because they are not expected to produce any measurable EEG response (Gosseries et al., 2015; Lioumis and Rosanova, 2022). During TMS stimulation, all the participants wore in-ear earphones that continuously played a masking noise to prevent auditory EEG responses elicited by the TMS click sound (Russo et al., 2022). EEG responses to TMS were visually monitored in real-time using a dedicated software tool (rt-TEP, (Casarotto et al., 2022)) to reduce the impact of major muscle artifacts and to detect the presence of early and local measurable components specific for the stimulation site. The precise location, orientation and intensity of TMS were adjusted based on the real-time feedback of rt-TEP at the beginning of each experimental session in order to obtain an evoked response - in average reference - in the first 50 ms after the pulse with a peak-to-peak amplitude larger than 10 µV in the channel closest to the stimulation site after averaging 20 trials.

Data analysis

Visual analysis of clinical standard EEG. Before visual assessment, raw recordings were band-pass filtered between 0.5-40 Hz, downsampled to 500 Hz and re-referenced to the standard double banana clinical montage. Spontaneous EEG data recorded in MCS patients were visually analyzed according to the neurophysiological descriptors previously reported in (Estraneo et al., 2016; Forgacs et al., 2014): predominant background frequency, the preservation of an anteroposterior gradient and the presence of any diffuse/focal slowing. Accordingly, the EEG pattern was qualitatively classified into four main categories (i.e., normal, mildly abnormal, moderately abnormal, severely abnormal).

Quantitative EEG analysis. Spectral power of spontaneous EEG data was computed both in healthy controls and MCS patients after applying the following pre-processing steps: (i) band-pass filtering of raw EEG data between 0.5 and 40 Hz and notch filtering at 50 Hz; (ii) epoching into 2-s-long windows; (iii) rejection of artifact-contaminated channels and trials by visual inspection; (iv) downsampling at 500 Hz; (v) re-referencing to the average reference; (vi) Independent Component Analysis (ICA) to reduce ocular, muscle and cardiac artifacts; (vii) cubic spline interpolation of bad channels. Power spectral density (PSD) in each channel was estimated using the Welch's method applied to single EEG epochs weighted with a Hanning window of 2 s (50% overlap). The PSD in each frequency bin was then normalized by the average power across the entire spectrum (0.5-40 Hz) in order to better compare among frequency bands irrespective of the broad-band total power, which might be affected by interindividual anatomical variability, particularly relevant in the case of brain-injured patients. Average of normalized PSD (PSDn) over frequency bins pertaining to the delta (0.5-4 Hz) and alpha (8-13 Hz) ranges was computed and averaged across a region-of-interest cluster of channels (i.e., P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2), according to a regional approach previously used for the computation of spectral measures in DoC (Babiloni et al., 2010, 2009; Leon-Carrion et al., 2008; Naro et al., 2016; Rossi Sebastiano et al., 2015). In our study, the parieto-occipital cluster was specifically selected also to maximize the quantification of alpha activity, as it is the most prominent rhythm over posterior regions in the healthy awake state. For the sake of completeness, the results obtained from the all-channels average are also reported in the **Supplementary Material (Figure S3,S4).**

TEPs. EEG responses to TMS were preprocessed according to the following pipeline: (i) (for Brainamp data only): (a) removal of the pulse artifact by replacing the time window between -2 and +5 ms with a mirrored version of the baseline signal between -2 and -9 ms followed by a moving average filter of 4 ms time span; (b) high-pass filtering at 0.01 Hz with a 1st order IIR filter; (ii) high-pass filtering at 0.1 Hz with a 3rd order Butterworth IIR filter; (iii) epoching between -800 and +800 ms around the TMS pulse; (iv) rejection of artifact-contaminated channels and trials by visual inspection; (v) re-referencing to the average reference; (vi) ICA to reduce ocular and muscle artifacts; (vii) low-pass filtering at 45 Hz (plus a notch filtering at 50 Hz in cases of severe contamination by line noise artifact); (viii) cubic spline interpolation of bad channels. TMS-evoked potentials were then analyzed to compute PCI using a fully automatic procedure detailed in (Casali et al., 2013), which included the following steps: downsampling of scalp recordings at 362.5 Hz, estimation of cortical current density, bootstrap-based statistical analysis to detect the spatiotemporally significant cortical activations, computation of normalized algorithmic complexity. For each subject and patient, the maximum PCI value (PCI_{max}) obtained across stimulation sites was retained for further analysis.

Results

Population characteristics

Data from one HC subject were discarded because of excessive contamination by muscular and movement artifacts. One MCS patient was excluded because of significant skull abnormality associated with a breach rhythm, producing outlier measurements of EEG voltage and spectral power. Thus, the following results refer to 39 participants per group.

HC subjects and MCS patients did not significantly differ in terms of age (Wilcoxon rank sum test: P = .087; **Figure S1A**) and sex (z-test for proportions: P = 0.45; **Figure S1B**). In this sample of brain-injured patients, vascular etiology (n = 21) was predominant, although anoxic (n = 7) and traumatic (n = 11) injuries were also represented. The best total CRS-R score spanned between 7 and 15 (**Figure S2A**) and included both MCS- (n = 24) and MCS+ (n = 15) diagnostic categories, respectively characterized by lower and higher behavioral evidence of command following (Bruno et al., 2011). Most of the post-anoxic patients were diagnosed as MCS- (**Figure S2B**).

MCS patients display variable EEG patterns and spectral profiles

Visual analysis of clinical standard EEG showed that most MCS patients (56.4%) were characterized by a Moderately Abnormal background (Mo), consisting of a dominant theta (4–7Hz) rhythm possibly associated with a moderate regional slowing mostly in the theta range and occasionally in the delta range as well (**Figure 1A**). Nonetheless, a minority of MCS patients showed either a Mildly Abnormal (Mi) background (25.6%), with a posterior dominant rhythm faster than 7 Hz, or a Severely Abnormal (Se) background (18%), with diffuse and dominant delta oscillations slower than 4 Hz (**Figure 1A**).

Narrow-band spectral power measures were significantly different across EEG categories, both considering posterior delta PSDn (**Figure 1B';** Kruskal-Wallis H(2)=19.47, P < .0001; post-hoc comparisons: Wilcoxon ranksum test, all P < 0.05 Bonferroni-corrected) and posterior alpha PSDn (**Figure 1B'';** Kruskal-Wallis H(2)=26.07, P < .00001; post-hoc comparisons: Wilcoxon ranksum test, all P < 0.05 Bonferroni-corrected) (for all-channel PSDn results see **Figure S3A',A''** in the **Supplementary material**). In particular, EEG patterns from mildly to moderately to severely abnormal were progressively characterized by an increasing contribution of delta power (**Figure 1B'**) and by a decreasing contribution of alpha power to posterior PSDn (**Figure 1B''**).

When merging MCS patients across EEG categories, posterior PSDn was significantly higher in the delta range (Wilcoxon rank sum test, P < .0005) and lower in the alpha range (Wilcoxon rank sum test, P < .00001) as compared to HC subjects (**Figure 1B',B''**). Notably, all the MCS patients with a Severely Abnormal background as well as about half of the MCS patients with a Moderately Abnormal background, showed outlier values of posterior PSDn as compared to HC subjects, i.e., larger than the maximum value and smaller than the minimum value in the delta and alpha ranges respectively. Hence, abnormal values of posterior PSDn were measured in a considerable fraction of MCS patients. Similar results were obtained for all-channel PSDn values (**Figure S3A',A''** in the **Supplementary material**).

The amount of posterior PSDn in the delta and alpha ranges were inversely correlated (**Figure S4A'**) both in HC subjects (red circles; Spearman correlation rho=-0.56, P < 0.0005) and MCS patients (gray circles; Spearman correlation rho=-0.80, P < 0.00001) (for all-channel PSDn results see **Figure S4A''**). Conversely, PCI_{max} did not significantly correlate with the posterior PSDn neither in the delta (Spearman correlation rho=0.11, P = 0.50) nor in the alpha (Spearman correlation rho=-0.04, P = 0.81) range (**Figure S4B**).

PCI detects MCS patients irrespectively of spontaneous EEG features

The ratio of posterior PSDn between the delta and the alpha range was computed to synthesize into a single measure the narrow-band spectral characteristics of spontaneous EEG. The delta/alpha ratio significantly increased with the progressive severity of spontaneous EEG category (Kruskal-Wallis H(2)=25.66, P < .00001; post-hoc comparisons: Wilcoxon ranksum test, all P < 0.05 Bonferroni-corrected): the observation of significant differences between all pairs of EEG categories further highlights the variability of spectral EEG features among MCS patients (**Figure 2A**, gray circles).

Conversely, the PCI_{max} values of MCS patients were not significantly different among EEG categories (Kruskal-Wallis H(2)=0.42, *P* = 0.81; **Figure 2B'**, gray circles) and fell above the previously validated empirical cutoff (PCI* = 0.31) for consciousness detection (**Figure 2B''**) (Casali et al., 2013; Casarotto et al., 2016; Sinitsyn et al., 2020).

The delta/alpha ratio computed in HC subjects showed a clear-cut predominance of alpha over delta power in the spontaneous EEG (**Figure 2A**, red circles). In addition, the PCI_{max} values computed in HC subjects fully overlapped with the previously reported distribution for consciousness ((Casarotto et al., 2016) **Figure 2B''**).

At the group level, PCI_{max} values were significantly lower (Wilcoxon rank sum test, *P* < .0001) in MCS patients as compared to HC subjects, in agreement with a previous report (Casarotto et al., 2016).

Overall, group results suggested that PCI_{max} in MCS patients reliably indexes their state of consciousness, irrespective of a high inter-individual variability in the characteristics of spontaneous EEG. **Figure 3** highlights this finding by showing comprehensive results from two representative patients, who were selected based on their opposite spectral characteristics of spontaneous EEG (**Figure 1B',B''**): patient #26 (brown-filled circle) with largest delta power, associated with lowest alpha power and severely abnormal background, and patient #17 (green-filled circle) with largest alpha power, associated with low delta power and mildly abnormal background. In both these patients, high-complexity TEPs (**Figure 3A**) coexist with either a faster (green traces) or a slower (brown traces) background, as evident from spontaneous EEG recordings in a bipolar clinical montage (**Figure 3B'**) and from posterior broad-band PSDn (**Figure 3B''**).

Discussion

We performed a systematic comparison between spontaneous EEG activity and the complexity of EEG responses to TMS in a large group of minimally conscious patients admitted in IRU. The present findings indicate that clinically stable MCS patients show a remarkable degree of EEG background alteration: although the moderately abnormal pattern, which represents an intermediate degree of abnormality, is prevalent at the group level, both mildly and severely abnormal patterns, which lay at opposite sides, are also well represented in our sample. Such variability highlighted by the clinical EEG assessment is confirmed by quantitative spectral measures, whereby the prevalence of delta and alpha power showed large across-subject variations. Conversely, TMS invariably elicited high-complexity EEG responses in the same population of MCS patients, indicating the preservation of causal interactions among functionally active portions of the thalamocortical system irrespective of the ongoing EEG background. Exploring the relationships between spontaneous EEG and PCI and their potential dissociations is relevant both by a conceptual and by a practical standpoint.

On average, the amount of power in the delta band tends to be inversely correlated with PCI and the level of consciousness. This is not surprising if one considers that a common underlying mechanism of both slow waves and decreased complexity is cortical bistability, that is the tendency of cortical neurons to plunge into a silent OFF-period after an initial activation (Steriade et al., 1993). Indeed, on one hand, bistability is known to underlie slow (delta) EEG waves (Sanchez-Vives et al., 2017) and on the other hand it plays a critical role in breaking down the chain of causal interactions, thus leading to low PCI values (Arena et al., 2021; D'Andola et al., 2018; Rosanova et al., 2018). Consistently, bistability and slow waves are a common marker of loss of consciousness in physiological and pathological conditions (Ni Mhuircheartaigh et al., 2013; Schiff, 2016). During sleep and anesthesia, cortical bistability is engendered by physiological dampening of activating systems or by increased inhibition. In DoC patients, it is favored by a broad withdrawal of excitatory synaptic activity across the cerebral cortex, following widespread cortical and white matter lesions as well as disruption of key subcortical nuclei and ascending activating systems (Edlow et al., 2021; Schiff, 2016).

However, interesting dissociations between EEG delta power, complexity and the level of consciousness are also possible. For example, high delta power in the scalp EEG can be recorded in rare cases of generalized non-convulsive status epilepticus (Gökyiğit and Calişkan, 1995) or in awake patients with Angelman syndrome (Frohlich et al., 2020), who also show high complexity in spontaneous EEG (Frohlich et al., 2022). In addition, a dramatic shift of spectral power towards low frequencies in spite of preserved consciousness can be found in adults after the administration of tiagabine (Darmani et al., 2021). Notably, also in this case, complexity, as measured by PCI, remains above threshold. The most parsimonious explanation for these dissociations is the presence, within an otherwise functional network, of local foci of bistability and slow waves that prevail at the scalp level due to volume conduction (Frohlich et al., 2021). These slow waves are likely to dominate in terms of amplitude and power in the ongoing EEG but are averaged out by perturbational measures (e.g. repeated single-pulse TMS stimuli), which extract the underlying complexity of deterministic interactions, hence explaining the dissociation. A similar situation may occur in conscious brain-injured patients. Indeed, focal and multifocal brain injury are often associated with the local intrusion of slow waves in the perilesional cortex (Butz et al., 2004; Cassidy et al., 2020; Gloor et al., 1977; Nuwer et al., 1987; Russo et al., 2021; Walter, 1937). Again, depending on the localization and extent of the lesional pattern, these slow waves may dominate the spontaneous scalp EEG, leading to a pattern of diffuse slowing. However, as shown by recent work in conscious patients with focal lesions (Sarasso et al., 2020), applying TMS-EEG reveals that these alterations are only local and that they do not prevent the emergence of high complexity patterns in the rest of the thalamocortical network. This line of reasoning provides a parsimonious explanation for the apparently paradoxical finding of patients who were conscious and showed high PCI in spite of a severely abnormal EEG and dominant delta power (**Figure 1B'**, **Figure 4** brown traces).

Similar to delta power, alpha power is another feature of the spontaneous EEG that is considered as a key element for the discrimination of conscious patients by both standard (Estraneo et al., 2016; Forgacs et al., 2014; Schiff, 2016) and quantitative assessments (Engemann et al., 2018; Sitt et al., 2014). Indeed, the presence of alpha oscillations with a preserved anterior-posterior gradient can be taken as an indication of consciousness, even in unresponsive brain-injured patients (Forgacs et al., 2014; Kondziella et al., 2020). On the other hand, a growing body of evidence challenges the view that preserved alpha power is necessary for consciousness. For example, a marked reduction of EEG spectral power in the alpha range is often observed in conditions when consciousness is present, albeit potentially disconnected from the external environment, such as REM sleep (Baird et al., 2018; Benca et al., 1999; Esposito et al., 2004), ketamine administration at subanesthetic dosage (Vlisides et al., 2017), and during serotonergic-induced psychedelic states with psilocybin and LSD (Schartner et al., 2017; Timmermann et al., 2019). In parallel, studies have shown that the complexity of EEG responses to TMS is relatively unaffected by spectral changes in alpha power, as long as subjects are conscious. For example, PCI is unchanged when alpha power is reduced by eyes opening (Casali et al., 2013), and it remains above threshold when alpha is attenuated during REM sleep (Massimini et al., 2010), psilocybin (Smallridge et al., in press), ketamine at both sub- (Farnes et al., 2020) and anesthetic doses (Sarasso et al., 2015). Such marked dissociation between ongoing alpha oscillations and TMS-evoked EEG responses in conscious subjects parallels the present findings of MCS patients showing alpha reduction and high PCI values.

Although, as shown here, TMS-EEG measures of complexity can index the presence of consciousness even in cases where the spontaneous EEG shows major deviation from the norm, it is important to highlight some limitations and caveats. First, TMS-EEG is a complex technique that currently requires a rather bulky equipment, time and dedicated expertise as well as absence of contraindications to TMS, thus limiting its diffusion in the typical clinical setting. Second, the superior sensitivity of TMS-EEG relies on the adherence to precise criteria during recording such as (i) the accurate selection of cortical targets distant from structural lesions, possibly exploiting a neuronavigation system; (ii) a constant monitoring of the subject's level of arousal (eyes opening) and, in case of low arousal, the application of sensory stimulation according to the CRS-R arousal protocol during the recording (Giacino et al., 2004); (iii) the collection of high signal-to-noise TEPs (10 µV in the first 50 ms, as indicated in (Casarotto et al., 2022, 2016)), (iv) the acquisition of a sufficient number of trials (120-150) to extract the deterministic response out of spontaneous variability, especially large in the presence of high-amplitude slow waves (Parks et al., 2016).

In summary, jointly with previous evidence, the present work bears conceptual relevance as a useful reminder of the possible dissociations between observable ongoing brain dynamics and the complexity of underlying causal structures (Sarasso et al., 2021). At the same time, it has practical relevance for quantitative methods aimed at inferring consciousness in DoC patients based on brain activity (Frohlich et al., 2022). For example, the large heterogeneity of EEG activity in conscious brain-injured patients in both training and test dataset may affect the diagnostic performance of machine learning-based approaches (Amiri et al., 2022; Engemann et al., 2018) and explain the low sensitivity (below 70%) of multivariate EEG classifier. In this vein, the present results advocate the use of a perturbational approach as a valid integration of the observational EEG assessment to improve diagnosis of DoC, especially relevant in the post-acute rehabilitation path.

Conflict of interest

Marcello Massimini is co-founder and share-holder, whereas Silvia Casarotto, Simone Sarasso and Mario Rosanova are advisors and share-holders of Intrinsic Powers, a spin-off of the University of Milan. The other authors declare no conflict of interest.

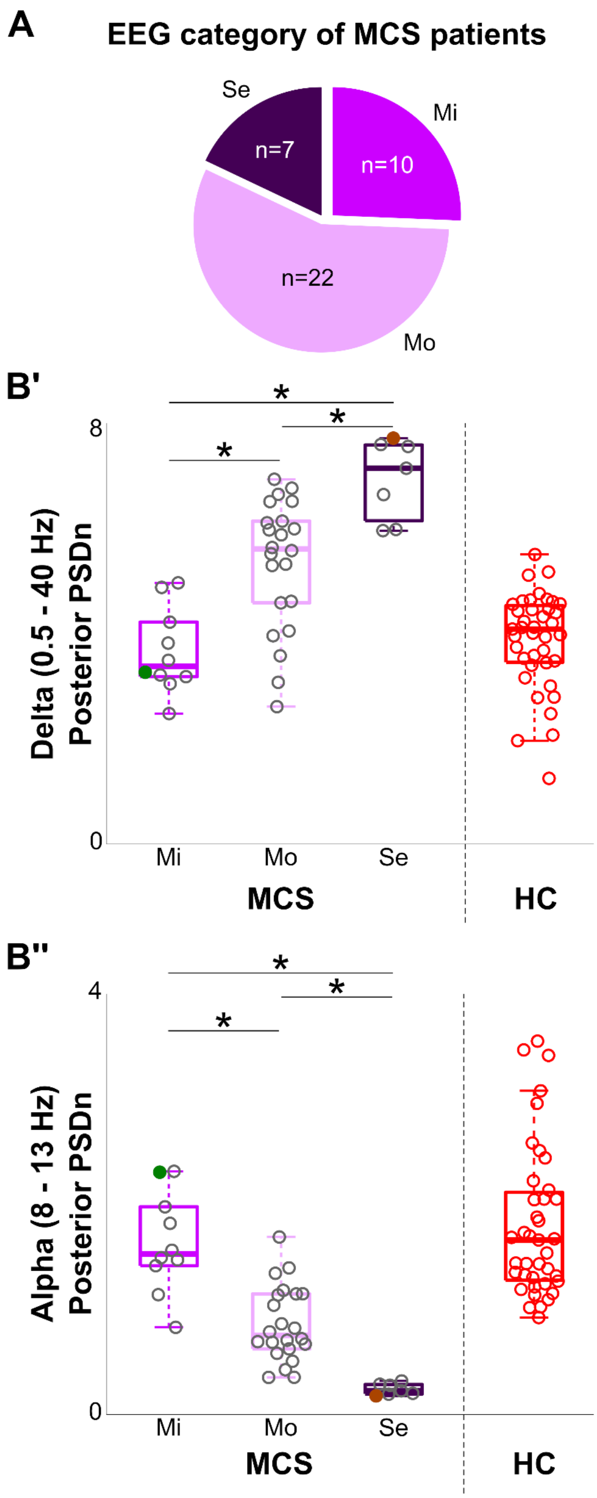

Figure 1. A. Distribution of minimally conscious state (MCS) patients across EEG background categories (i.e., Mi = mildly abnormal (purple); Mo = moderately abnormal (light purple); Se = severely abnormal (dark purple)) according to visual analysis of clinical standard EEG. **B.** Normalized power spectral density (PSDn) averaged over frequency bins in the delta (**B'**) and alpha (**B''**) ranges and averaged across a posterior cluster of channels (i.e., P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2). Gray circles represent single MCS patients, subdivided according to the EEG background category. Two representative patients have been highlighted (filled colored circles) based on opposite spectral characteristics of spontaneous EEG: patient #26 (brown) with largest delta power, associated with lowest alpha power and severely abnormal background, and patient #17 (green) with largest alpha power, associated with low delta power and mildly abnormal background. Red circles represent single HC subjects. The boxes bound the interquartile range divided by the median. Statistical analysis showed significant differences between each pair of EEG background category both in the delta (Kruskal-Wallis H(2)=19.47, $P < .0001$; post-hoc comparisons: Wilcoxon ranksum test, all $P < 0.05$ Bonferroni-corrected) and alpha (Kruskal-Wallis H(2)=26.07, $P < .00001$; post-hoc comparisons: Wilcoxon ranksum test, all $P < 0.05$ Bonferroni-corrected) posterior PSDn. In addition, posterior PSDn was significantly higher in the delta range (Wilcoxon rank sum test, $P < .0005$) and lower in the alpha range (Wilcoxon rank sum test, $P < .00001$) in MCS patients merged across EEG categories as compared to HC subjects.

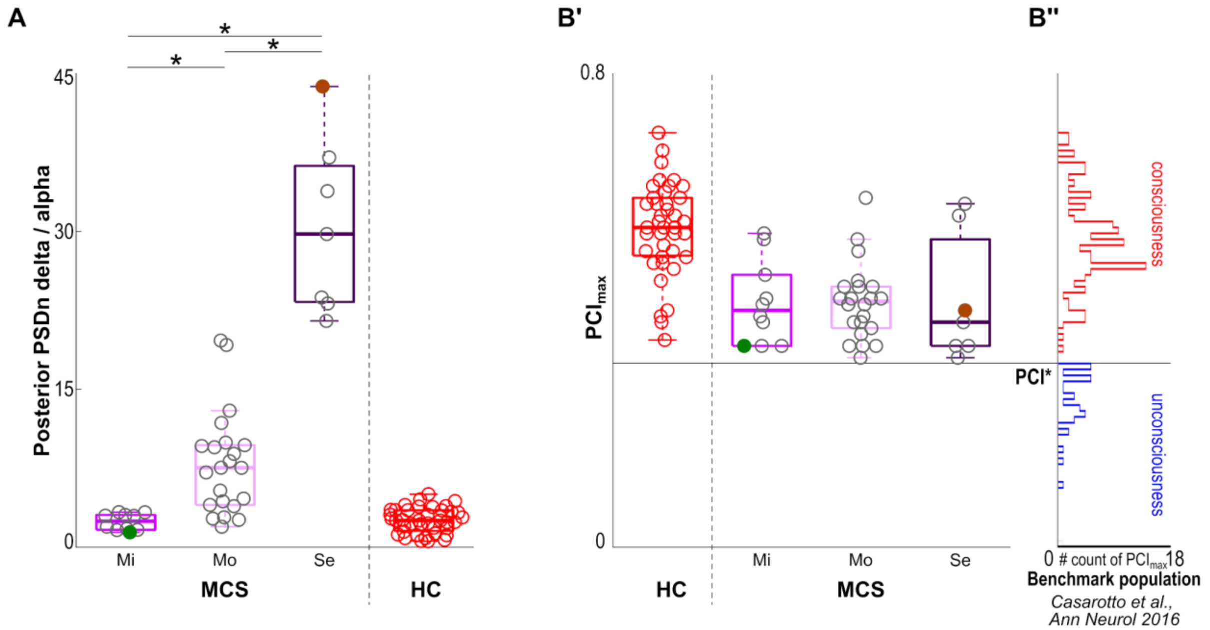

Figure 2. A. Ratio between posterior normalized power spectral density (PSDn) in the delta (0.5 - 4Hz) range and in the alpha (8 - 13 Hz) range. Gray circles represent single minimally conscious state (MCS) patients, subdivided according to the EEG background category (i.e., Mi = mildly abnormal (purple); Mo = moderately abnormal (light purple); Se = severely abnormal (dark purple)). Red circles represent single healthy control (HC) subjects. The boxes bound the interquartile range divided by the median. Statistical analysis showed significant differences between each pair of EEG background category (Kruskal-Wallis $H(2)=25.66$, $P < .0001$; post-hoc comparisons: Wilcoxon ranksum test, all $P < 0.05$ Bonferroni-corrected). In addition, HC subjects displayed significantly lower values than MCS patients merged across EEG categories (Wilcoxon rank sum test, $P < .0001$). **B.** Individual PCI_{max} values (i.e., individual maximum value of the perturbational complexity index across stimulation sites) measured in HC subjects (red circles) and MCS patients (gray circles), subdivided according to the EEG background category. The boxes bound the interquartile range divided by the median. At the group level, PCI_{max} values in HC subjects were significantly higher than in MCS patients merged across EEG categories (Wilcoxon rank sum test, $P < .0001$). In MCS patients, PCI_{max} values were not significantly different among EEG categories (Kruskal-Wallis $H(2)=0.42$, $P = 0.81$) and always fell above the empirical cutoff $PCI^*=0.31$ previously validated to discriminate between models of consciousness (**B''**, red histogram) and unconsciousness (**B''**, blue histogram) reported in a previous large-scale study (Casarotto et al., 2016). In all panels, filled colored circles correspond to the same patients highlighted in **Figure 1B**.

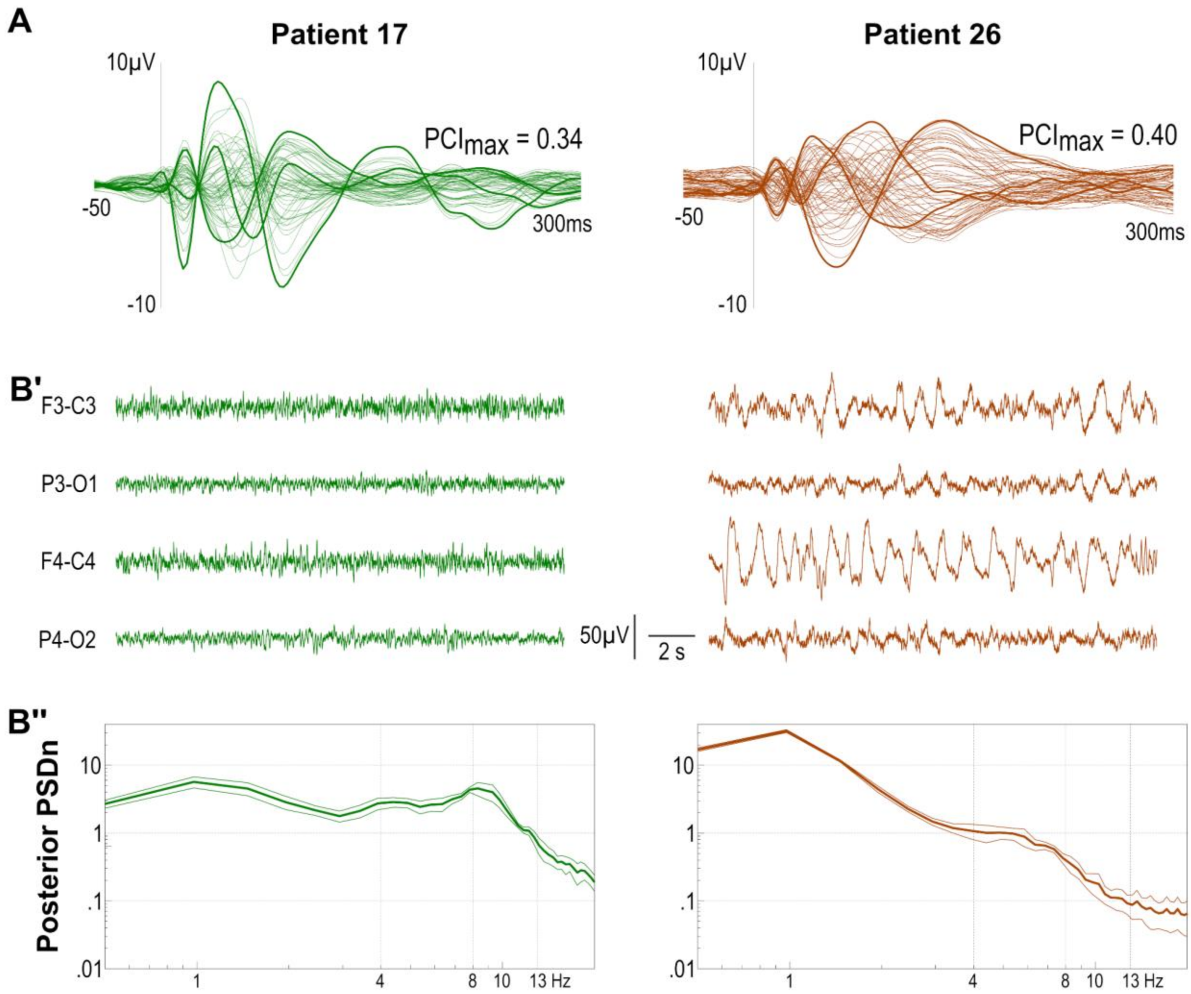

Figure 3. Data from two representative minimally conscious state (MCS) patients, selected for their opposite spectral characteristics, as highlighted in **Figure 1B**: patient #17 (green traces) with largest alpha power, associated with low delta power and mildly abnormal background patient and patient #26 (brown traces) with largest delta power, associated with lowest alpha power and severely abnormal background. **A.** Butterfly plot of average TMS-evoked potentials and the corresponding PCI_{max} value (i.e., individual maximum value of the perturbational complexity index across stimulation sites) in the two patients. **B'.** 20-second continuous spontaneous EEG recordings from 4 bipolar derivations (i.e., F3-C3, P3-O1, F4-C4, P4-O2) **B''.** Normalized power spectral density (PSDn) averaged over a posterior cluster of channels (i.e., P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2).

Supplementary material

Patient ID	Clinical diagnosis	age (years) /sex	Etiology	Time since injury (months)	CRS-R best total score	Aud	Vis	Mot	Oro	Com	Vig	EEG category	PCI_{max}	Posterior PSDn delta (0.5-4 Hz)	alpha (8-13 Hz)
1	MCS+	71,4/m	V	15,1	7	3	0	2	1	0	1	Mo	0,34	4,62	1,15
2	MCS-	56,5/m	V	11,3	8	2	3	1	1	0	1	Mo	0,44	6,94	0,35
3	MCS-	84,2/f	V	129,1	9	2	3	1	1	0	2	Mi	0,34	3,50	1,14
4	MCS-	53,1/m	A	128,9	9	1	3	2	1	0	2	Mo	0,5	3,08	1,18
5	MCS-	61,2/m	T	50,4	9	2	3	1	1	0	2	Mo	0,34	5,88	0,72
6	MCS-	73,2/f	V	17,3	9	2	3	2	1	0	1	Se	0,32	5,99	0,20
7	MCS-	59,2/m	A	12,4	9	2	3	1	1	0	2	Mi	0,52	3,05	1,56
8	MCS-	59,0/f	V	1,1	9	1	3	2	1	0	2	Mo	0,42	6,14	0,69
9	MCS-	27,5/f	V	5,0	9	2	3	2	1	0	1	Se	0,58	7,15	0,19
10	MCS-	43,7/f	T	5,4	9	1	3	2	1	0	2	Mo	0,42	4,59	1,69
11	MCS-	22,7/m	T	4,8	10	1	3	4	1	0	1	Mo	0,59	6,52	0,86
12	MCS-	53,8/f	V	87,6	10	1	3	3	1	0	2	Mi	0,38	4,97	1,49
13	MCS-	49,7/m	A	41,1	10	2	3	2	2	0	1	Mo	0,38	5,64	0,58
14	MCS-	73,4/f	A	69,5	10	1	3	4	1	0	1	Mo	0,41	5,52	0,43
15	MCS-	24,3/m	T	33,3	10	2	3	2	1	0	2	Mi	0,53	3,18	1,82
16	MCS+	20,8/m	T	43,9	10	3	3	1	1	0	2	Mo	0,52	6,52	0,69
17	MCS-	26,0/f	T	33,4	10	2	2	4	1	0	1	Mi	0,34	3,27	2,30
18	MCS+	38,7/m	V	8,5	10	3	3	2	1	0	1	Se	0,38	5,96	0,28
19	MCS-	63,7/m	V	3,7	10	1	3	4	1	0	1	Mo	0,45	5,33	0,71
20	MCS-	55,1/f	V	36,9	11	2	3	2	2	1	1	Mo	0,38	5,98	0,51
21	MCS+	67,4/m	V	4,4	11	3	3	2	1	1	1	Mi	0,34	3,22	1,98
22	MCS+	73,0/m	V	2,3	11	2	3	2	1	1	2	Mo	0,44	6,11	1,14
23	MCS-	72,7/m	V	14,4	11	2	3	2	2	0	2	Mo	0,42	6,65	0,67
24	MCS+	32,0/f	T	46,0	11	3	3	2	1	0	2	Mi	0,41	3,82	2,31
25	MCS-	54,3/m	A	5,3	11	2	2	4	1	0	2	Mo	0,36	5,30	1,15
26	MCS-	62,7/f	V	3,8	11	2	3	3	1	0	2	Se	0,4	7,72	0,18
27	MCS-	50,8/f	V	14,6	12	2	3	4	1	0	2	Mo	0,37	6,77	0,35
28	MCS-	77,5/f	V	15,0	12	2	3	4	2	0	1	Mo	0,32	3,96	1,39
29	MCS+	55,9/m	T	11,7	13	0	4	5	2	0	2	Mi	0,42	4,88	1,47
30	MCS+	66,4/m	V	15,9	13	3	3	3	1	0	2	Se	0,34	6,65	0,29
31	MCS+	54,1/m	V	16,6	13	3	3	3	1	0	2	Mo	0,44	3,58	0,82
32	MCS-	49,9/m	T	93,2	13	2	4	4	1	0	2	Se	0,34	7,57	0,32
33	MCS+	46,5/m	A	56,7	14	3	1	4	3	1	2	Mo	0,39	4,05	1,04
34	MCS+	66,1/f	V	13,2	14	4	0	5	3	1	1	Mo	0,41	5,58	0,79
35	MCS+	44,2/f	V	13,7	14	4	5	2	1	0	2	Mi	0,39	4,22	1,42
36	MCS+	72,2/m	V	1,2	14	3	5	2	2	1	1	Mi	0,46	2,48	0,83
37	MCS-	32,0/m	A	1,2	14	2	3	5	2	0	2	Se	0,56	7,60	0,22
38	MCS+	43,7/m	T	10,4	15	3	4	5	1	0	2	Mo	0,42	6,00	0,62
39	MCS+	32,6/m	T	33,7	15	3	3	5	2	0	2	Mo	0,34	2,61	1,34

Table S1. Demographic and clinical information concerning minimally conscious state (MCS) patients, classified as MCS- or MCS+ according to lower and higher behavioral evidence of command following respectively (Bruno et al., 2011). m = male; f = female. Etiology was classified as anoxic (A), traumatic (T) and vascular (V). Behavioral assessment according to the Coma Recovery Scale Revised (CRS-R; (Giacino et al., 2004) involved the following subscales: Aud = auditory function, Vis = visual function, Mot = motor function, Oro = oromotor/verbal function, Com = communication, Aro = arousal. According to visual analysis of clinical standard EEG (Estraneo et al., 2016; Forgacs et al., 2014), each patient was assigned an EEG background category among the following: mildly abnormal (Mi), moderately abnormal (Mo), severely abnormal (Se).

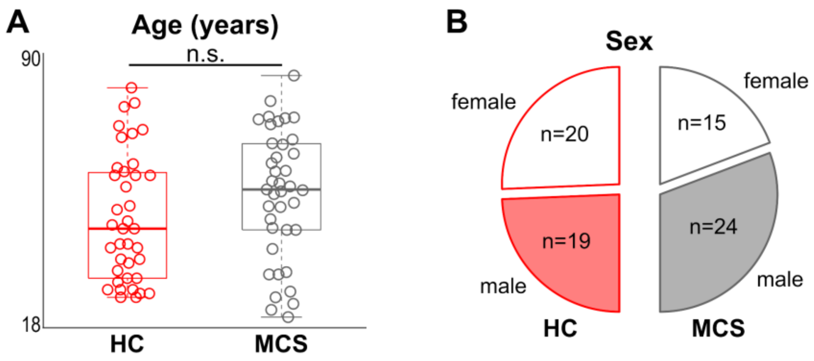

Figure S1. A. Age of single healthy control (HC) subjects (red circles) and minimally conscious state (MCS) patients (gray circles). The boxes bound the interquartile range divided by the median. Age was not significantly different (n.s.) in the two populations (Wilcoxon rank sum test: *P* = .087). **B.** Amount of male and female individuals in HC and MCS populations. The two groups did not significantly differ in terms of sex (z-test for proportions: *P* = 0.45).

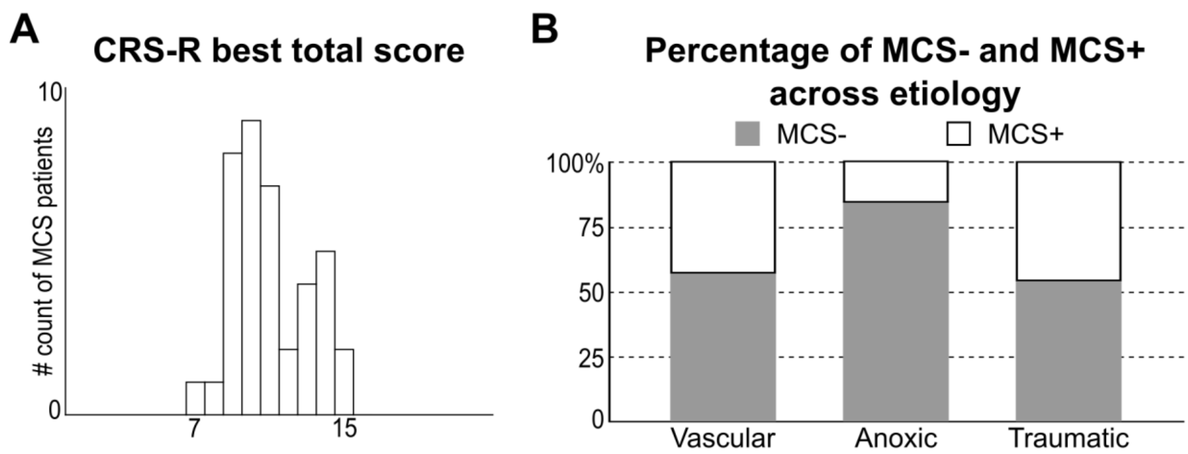

Figure S2. A. Histogram of the best total score of the Coma Recovery Scale Revised (CRS-R) in minimally conscious state (MCS) patients. **B.** For each etiology, percentage of MCS- and MCS+ diagnostic categories, as assessed from lower and higher behavioral evidence of command following (Bruno et al., 2011), respectively.

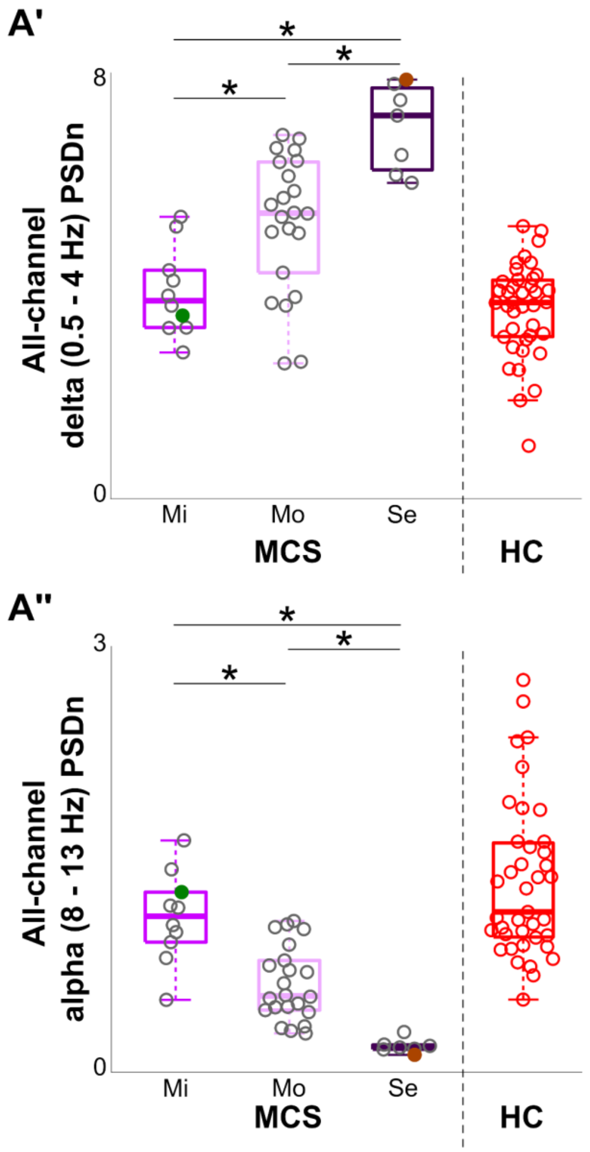

Figure S3. Normalized power spectral density (PSDn) averaged over frequency bins in the delta (**A'**) and alpha (**A''**) ranges and averaged across all EEG channels. Gray circles represent single minimally conscious state (MCS) patients, subdivided according to the EEG category. Filled colored circles indicate the same representative patients highlighted in **Figure 1B** in the main text. Red circles represent single healthy control (HC) subjects. The boxes bound the interquartile range divided by the median. Statistical analysis showed significant differences between each pair of EEG category both in the delta (Kruskal-Wallis H(2)=17.62, $P < .0001$; post-hoc comparisons: Wilcoxon ranksum test, all $P < 0.05$ Bonferroni-corrected) and alpha (Kruskal-Wallis H(2)=24.08, $P < .00001$; post-hoc comparisons: Wilcoxon ranksum test, all $P < 0.05$ Bonferroni-corrected) all-channel PSDn. All-channel PSDn was significantly higher (Wilcoxon rank sum test, $P < .00001$) in the delta range and significantly lower (Wilcoxon rank sum test, $P < .00001$) in the alpha range in MCS patients merged across EEG categories as compared to HC subjects.

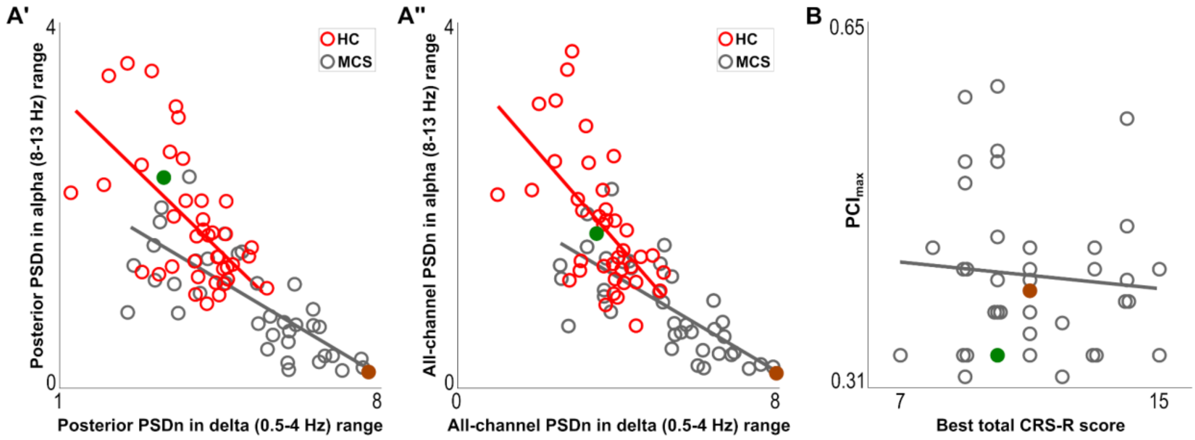

Figure S4. A. Scatterplot of normalized power spectral density PSDn averaged over a posterior cluster of channels (i.e., P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) (**A'**) and averaged over all-channels (**A''**) in the alpha and delta ranges in healthy control (HC) subjects (red circles) and minimally conscious state (MCS) patients (gray circles). These variables were inversely correlated both in HC subjects (Spearman correlation rho=-0.56, $P < 0.0005$ for posterior PSDn and rho=-0.63, $P < 0.00005$ for all-channel PSDn) and in MCS patients (Spearman correlation rho=-0.80, $P < 0.00001$ for posterior PSDn and rho=-0.80, $P < 0.00001$ for all-channel PSDn). **B.** Scatterplot of PCI_{max} values (i.e., maximum individual perturbational complexity index value across stimulation sites) and best total score of the Coma Recovery Scale Revised (CRS-R) in MCS patients (gray circles). These variables were not significantly correlated (Spearman correlation rho = -0.07, $P = 0.65$). In all panels, filled colored circles correspond to the same patients highlighted in **Figure 1B** in the main text.

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