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Dissociations between spontaneous electroencephalographic features and the perturbational complexity index in the minimally conscious state

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Abstract

The analysis of spontaneous electroencephalogram (EEG) is a cornerstone in the assessment of patients with disorders of consciousness (DoC). 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 seek empirical evidence of this hypothesis, we compared PCI with EEG spectral measures in the same population of minimally conscious state (MCS) patients ($n = 40$) hospitalized in rehabilitation facilities. We found a remarkable variability in spontaneous EEG features across MCS patients as compared with healthy controls: in particular, a pattern of predominant delta and highly reduced alpha power—more often observed in vegetative state/unresponsive wakefulness syndrome (VS/UWS) patients—was found in a non-negligible number of MCS patients. Conversely, PCI values invariably fell above an externally validated empirical cutoff for consciousness in all MCS patients, consistent with the presence of clearly discernible, albeit fleeting, behavioural signs of awareness. These results confirm that, in some MCS patients, spontaneous EEG rhythms may be inconclusive

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; PCI_{max}, 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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about 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.

KEYWORDS
alpha, delta, electroencephalogram, MCS, PCI

1 | 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 behavioural 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 behavioural 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 longstanding 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 non-rapid eye movement (NREM) sleep and anaesthesia 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 DoC (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 (HCs) 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 behaviour-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, spontaneous EEG features are not always decisive for the diagnosis of DoC patients, and the pathophysiological 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 & 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 & 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 potential 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 the sensitivity of spontaneous EEG metrics and of TEPs' complexity in the same population of MCS patients, who show inconsistent but verifiable behavioural signs of consciousness, has never been performed.

Here, we perform such comparison and find that measuring brain complexity by a perturbational approach with TMS-EEG is more sensitive to recovery of consciousness than observational EEG spectral measures.

2 | MATERIALS AND METHODS

2.1 | 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 behavioural 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 anaesthetic drugs and septic-dysmetabolic complications on EEG activity. Table S1 reports detailed demographic and clinical information. In addition, this study also involved a group of 40 HC subjects, who were selected based on the following inclusion criteria: age range 20–80 years, absence of neurological or psychiatric diagnoses, not currently using central nervous system-active medications, ability to provide informed consent and follow study procedures. Subjects with a history of significant neurological trauma, epilepsy, substance abuse and conditions affecting scalp integrity were excluded. In each participant, we collected (i) at least 5 min 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 behavioural 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).

2.2 | Data collection

HCs 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 .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 .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 .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 & 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 artefacts 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.

2.3 | Data analysis

2.3.1 | Visual analysis of clinical standard EEG

Before visual assessment, raw recordings were band-pass filtered between .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 analysed 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 and severely abnormal).

2.3.2 | Quantitative EEG analysis

Spectral power of spontaneous EEG data was computed both in HCs and MCS patients after applying the following pre-processing steps: (i) band-pass filtering of raw EEG data between .5 and 40 Hz and notch filtering at 50 Hz; (ii) epoching into 2-s-long windows; (iii) rejection of artefact-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 artefacts; (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 (.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 (.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, and 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 Figures S3 and S4.

2.3.3 | TEPs

EEG responses to TMS were preprocessed according to the following pipeline: (i) (for Brainamp data only): (a) removal of the pulse artefact 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 .01 Hz with a 1st order IIR filter; (ii) high-pass filtering at .1 Hz with a 3rd order Butterworth IIR filter; (iii) epoching between −800 and +800 ms around the TMS pulse; (iv) rejection of artefact-contaminated channels and trials by visual inspection; (v) re-referencing to the average reference; (vi) ICA to reduce ocular and muscle artefacts; (vii) low-pass filtering at 45 Hz (plus a notch filtering at 50 Hz in cases of severe contamination by line noise artefact); (viii) cubic spline interpolation of bad channels. TEPs were then analysed 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 (see details in the Supporting Information, 'PCI computation' section). For each subject and patient, the maximum PCI value (PCI_{max}) obtained across stimulation sites was retained for further analysis. Individual PCI_{max} values were compared with an empirical cutoff ($PCI^* = .31$) previously validated in a large benchmark population (Casarotto et al., 2016), which included 540 TMS-EEG recordings performed in the following conditions: (i) conscious wakefulness in healthy subjects as well as in stroke, emergence from MCS and locked-in syndrome patients; (ii) disconnected consciousness in healthy subjects either dreaming or anesthetized with ketamine, who provided a delayed conscious report upon awakening despite behavioural unresponsiveness during the measurement; (iii) unconsciousness in healthy subjects during deep non-rapid-eye-movement sleep and during anaesthesia with midazolam, propofol and xenon, who were unresponsive and did not provide any delayed conscious report upon awakening. In this benchmark population, PCI* discriminated between consciousness, even if disconnected, and unconsciousness with 100% accuracy. Validation studies have shown that MCS patients provided PCI_{max} values higher than PCI* in 94.7% (Casarotto et al., 2016) and 92.3% (Sinitsyn et al., 2020) of the cases, demonstrating a remarkable sensitivity of this empirical cutoff for the potentiality for consciousness in brain-injured patients who show minimal signs of conscious behaviour often fluctuating over time.

3 | RESULTS

3.1 | Population characteristics

Data from one HC subject were discarded because of excessive contamination by muscular and movement artefacts. 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: $P = .45$; Figure S1B). In this sample of brain-injured patients, vascular aetiology ($n = 21$) was predominant, although anoxic ($n = 7$) and traumatic ($n = 11$) injuries were also represented. The best total CRS-R score spanned between

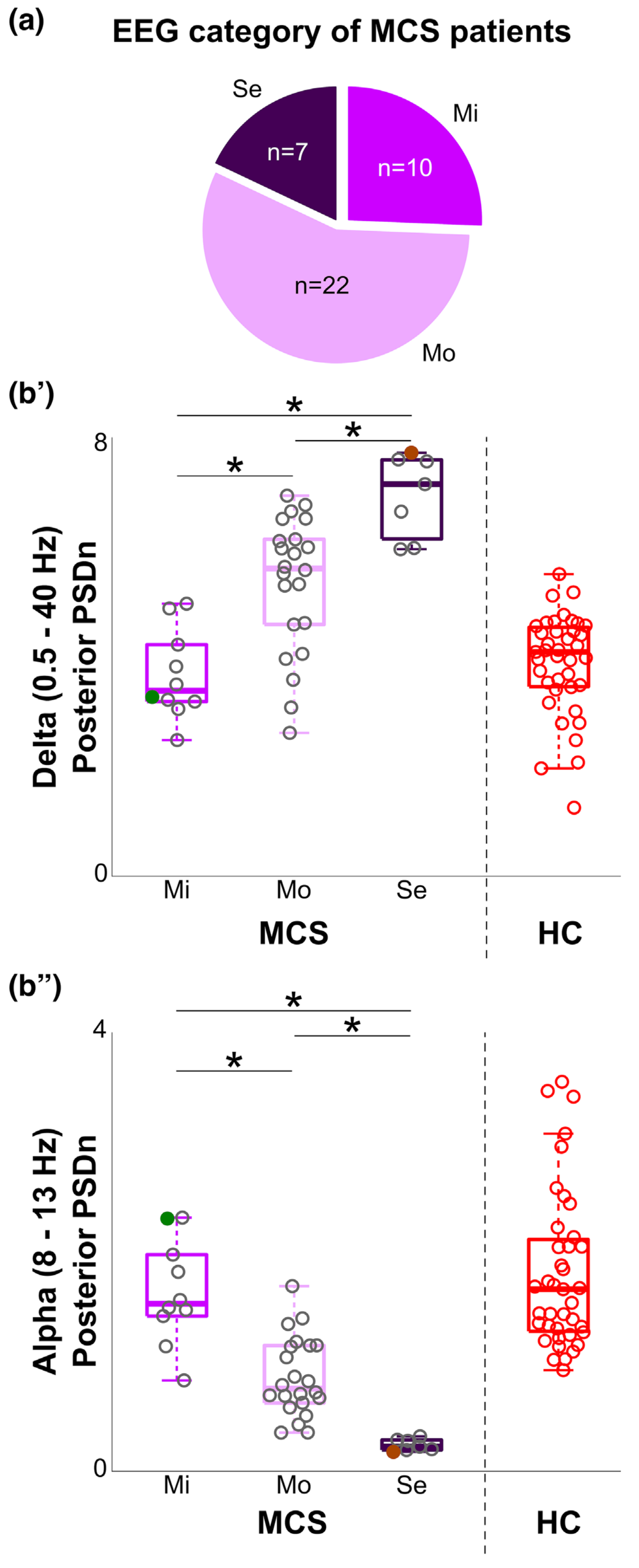

FIGURE 1 Legend on next page.

FIGURE 1 (a) Distribution of minimally conscious state (MCS) patients across electroencephalogram (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 and O2). Grey circles represent single MCS patients, subdivided according to the EEG background category. Two representative patients have been highlighted (filled coloured 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 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 both in the delta (Kruskal–Wallis H(2) = 19.47, $P < .0001$; post hoc comparisons: Wilcoxon rank sum test, all $P < .05$ Bonferroni-corrected) and alpha (Kruskal–Wallis H(2) = 26.07, $P < .00001$; post hoc comparisons: Wilcoxon rank sum test, all $P < .05$ 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 with HC subjects.

7 and 15 (Figure S2A) and included both MCS− ($n = 24$) and MCS+ ($n = 15$) diagnostic categories, respectively, characterized by lower and higher behavioural evidence of command following (Bruno et al., 2011). Most of the post-anoxic patients were diagnosed as MCS− (Figure S2B).

3.2 | 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 (4–7 Hz) 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 rank sum test, all $P < .05$ Bonferroni-corrected) and posterior alpha PSDn (Figure 1b″; Kruskal–Wallis H(2) = 26.07, $P < .00001$; post hoc comparisons: Wilcoxon rank sum test, all $P < .05$ Bonferroni-corrected) (for all-channel PSDn results, see Figure S3A′,A″). 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″). Accordingly, the slope of the aperiodic component of PSD, previously applied to DoC patients in (Colombo et al., 2023), was significantly different across EEG categories (Kruskal–Wallis H(2) = 10.32, $P < .01$), with steeper values (Bonferroni-corrected $P < .05$) associated to severely as compared to mildly abnormal pattern. Individual recordings from two representative patients showing largest delta and largest alpha posterior PSDn, respectively, (filled coloured circles) are shown in greater detail below.

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 with 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 with HC subjects, that is, 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″). However, neither alpha (Spearman correlation rho = −.02, $P = .89$) nor delta (Spearman correlation rho = −.02, $P = 0{=}.88$) posterior PSDn, nor their ratio (Spearman correlation rho = −.03, $P = .88$) significantly correlated with the best total CRS-R score in MCS patients. Accordingly, a receiver operating characteristic (ROC) analysis showed that none of these spectral measures was able to discriminate between MCS+ and MCS− patients (area under the curve = 61.4%, 64.7% and 60.0% for posterior alpha, delta and delta/alpha PSDn, respectively).

The amount of posterior PSDn in the delta and alpha ranges was inversely correlated (Figure S4A′) both in HC subjects (red circles; Spearman correlation rho = −.56, $P < .0005$) and MCS patients (grey circles; Spearman correlation rho = −.80, $P < .00001$) (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 = .11, $P = .50$) nor

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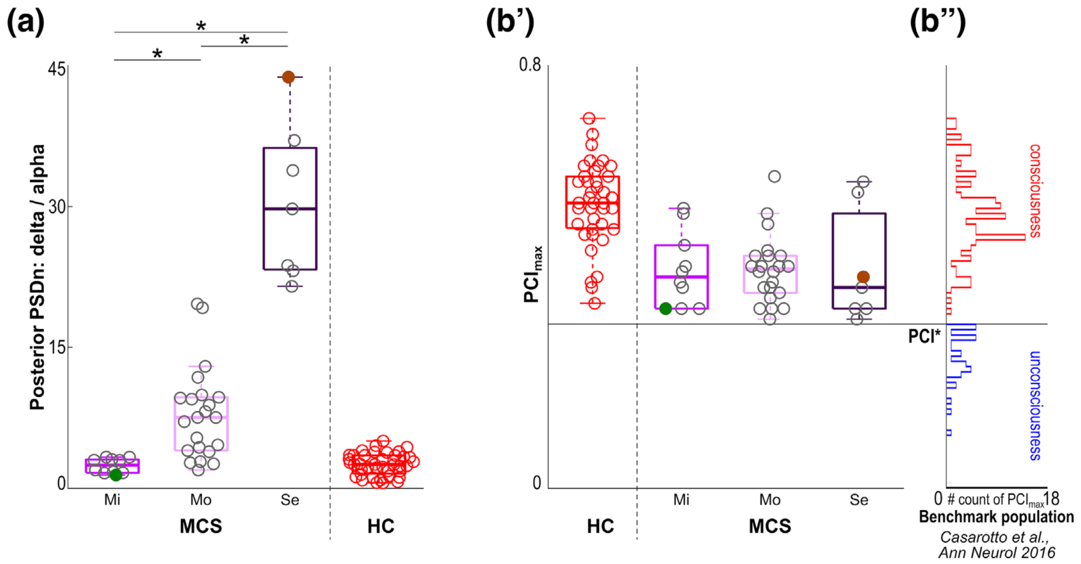

FIGURE 2 (a) Ratio between posterior normalized power spectral density (PSDn) in the delta (.5–4 Hz) range and in the alpha (8–13 Hz) range. Grey circles represent single minimally conscious state (MCS) patients, subdivided according to the electroencephalogram (EEG) background category (i.e., Mi = mildly 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 rank sum test, all $P < .05$ 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 PCImax values (i.e., individual maximum value of the perturbational complexity index across stimulation sites) measured in HC subjects (red circles) and MCS patients (grey 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) = .42, $P = .81$) and always fell above the empirical cutoff PCI* = .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 coloured circles correspond to the same patients highlighted in Figure 1b.

in the alpha (Spearman correlation rho = −.04, $P = .81$) range.

3.3 | 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 rank sum test, all $P < .05$ 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, grey circles). Conversely, the PCI_{max} values of MCS patients were not significantly different among EEG categories (Kruskal–Wallis H(2) = .42, $P = .81$; Figure 2b′, grey circles) and fell above the previously validated empirical cutoff (PCI* = .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 with HC subjects, in agreement with a previous report (Casarotto et al., 2016). Interestingly, an ROC analysis, performed to evaluate whether any of the considered neurophysiological measures allowed to discriminate between HC subjects and MCS patients, showed that PCI_{max} outperformed the other spectral EEG measures (Figure S5). However, PCI_{max} did not significantly correlate with the best total CRS-R score of MCS patients (Figure S4B; Spearman correlation rho = −.07, $P = .65$) and did not significantly differ

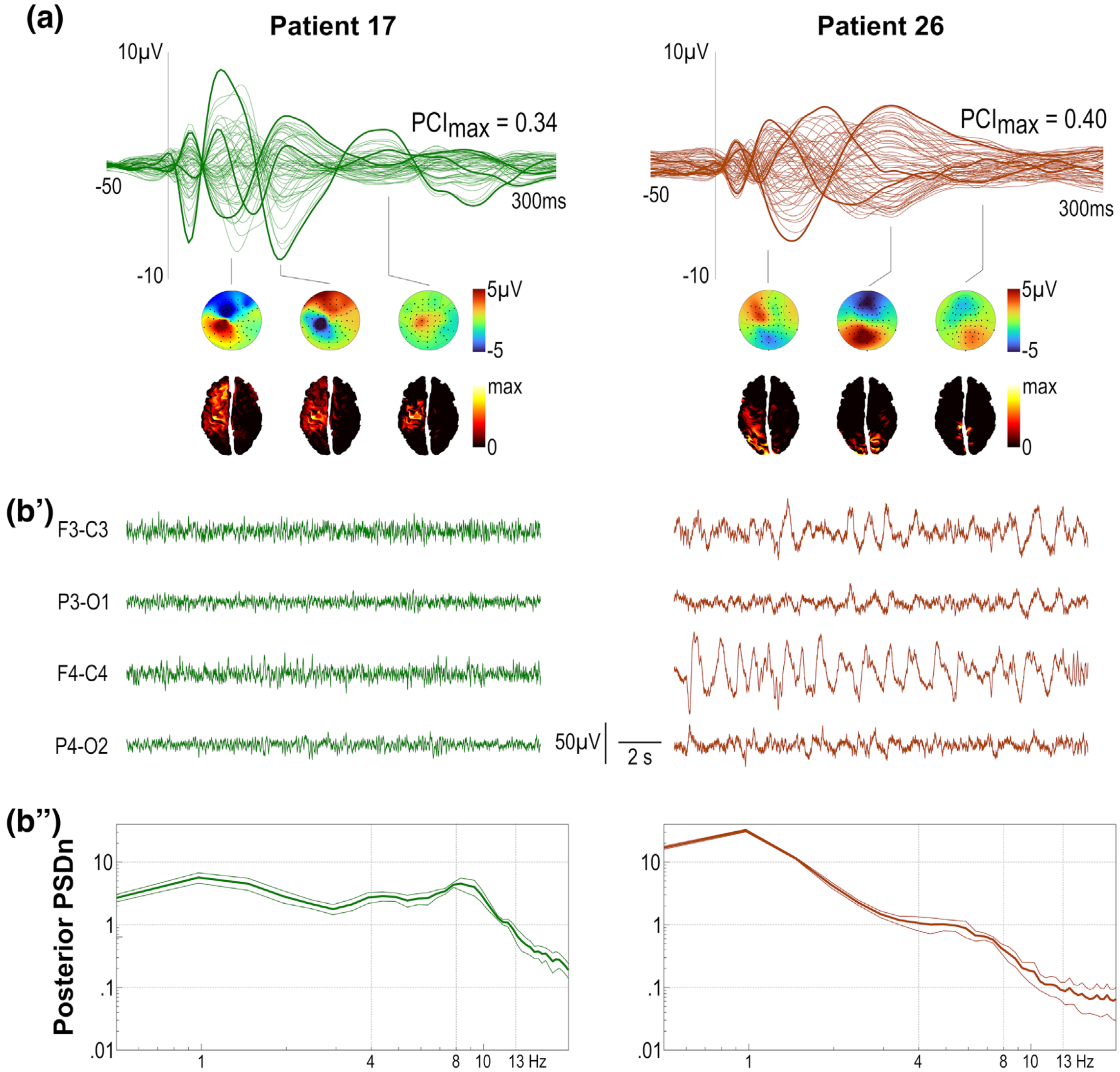

FIGURE 3 Data from two representative minimally conscious state (MCS) patients, selected for their opposite spectral characteristics, as highlighted in Figure 1b: patient #17 (green traces) with largest alpha power, associated with low delta power and mildly abnormal background patient, and patient #26 (brown traces) with largest delta power, associated with lowest alpha power and severely abnormal background. (a) Butterfly plot of average transcranial magnetic stimulation (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. Topographical maps of scalp voltages as well as cortical maps of significant current density are shown for selected time points. (b′) Twenty-second continuous spontaneous electroencephalogram (EEG) recordings from 4 bipolar derivations (i.e., F3-C3, P3-O1, F4-C4 and 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 and O2).

between MCS+ and MCS− categories (Wilcoxon rank sum test, $P = .83$). Moreover, a ROC analysis showed that MCS− and MCS+ patients cannot be effectively classified using PCI_{max} (area under the curve = 52.2%).

Overall, group results suggested that PCI_{max} in MCS patients reliably indicated a state of consciousness, irrespective of their background 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′′).

4 | DISCUSSION

We compared visual and spectral features of spontaneous EEG with 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: the moderately abnormal pattern, which represents an intermediate degree of abnormality, is prevalent at the group level; nonetheless, both mildly and severely abnormal patterns, which lay at opposite sides, are also well represented in our sample. These observations deriving from the clinical EEG assessment are 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 the 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 anaesthesia, cortical bistability is engendered by physiological dampening of activating systems or by increased inhibition. In DoC patients, it is favoured 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, divergent results among 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 & 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 contrasting measures 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. 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 3 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 lysergic acid diethylamide (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 anaesthetic 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.

It must be noted that the spontaneous EEG features considered in this work represent only a small fraction of the many measures that have been used to characterize background EEG activity in DoC patients. Thus, in future comparisons, it would be important to include additional measures, such as the ABCD model (Franzova et al., 2023) and a systematic evaluation of EEG reactivity (here performed only in a limited subset of 11 patients).

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 remind that this technique currently requires dedicated expertise as well as absence of contraindications to TMS, thus limiting its diffusion in the typical clinical setting. Also, 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 this perspective, 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., 2023; 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.

AUTHOR CONTRIBUTIONS

Silvia Casarotto: Formal analysis; investigation; writing—original draft. **Gabriel Hassan:** Formal analysis; investigation. **Mario Rosanova:** Investigation; supervision. **Simone Sarasso:** Conceptualization; investigation; supervision; writing—review and editing. **Chiara-Camilla Derchi:** Investigation. **Pietro Davide Trimarchi:** Investigation. **Alessandro Viganò:** Supervision. **Simone Russo:** Investigation. **Matteo Fecchio:** Investigation. **Guya Devalle:** Supervision. **Jorge Navarro:** Supervision. **Marcello Massimini:** Conceptualization; writing—review and editing. **Angela Comanducci:** Formal analysis; investigation; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

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. Simone Russo is the Chief Medical Officer of Manava Plus. The other authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.16299.

DATA AVAILABILITY STATEMENT

The dataset used during the current study is available upon reasonable request.

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REFERENCES

Amiri, M., Fisher, P. M., Raimondo, F., Sidaros, A., Hribljan, M. C., Othman, M. H., Zibrandtsen, I., Albrechtsen, S. S., Bergdal, O., Hansen, A. E., Hassager, C., Højgaard, J. L. S., Jakobsen, E. W., Jensen, H. R., Møller, J., Nersesjan, V., Nikolic, M., Olsen, M. H., Sigurdsson, S. T., … Kondziella, D. (2023). Multimodal prediction of residual consciousness in the intensive care unit: The CONNECT-ME study. *Brain*, *146*, 50–64. https://doi.org/10.1093/brain/awac335

Arena, A., Comolatti, R., Thon, S., Casali, A. G., & Storm, J. F. (2021). General anesthesia disrupts complex cortical dynamics in response to intracranial electrical stimulation in rats. *eNeuro*, *8*, ENEURO.0343-20.2021. https://doi.org/10.1523/ENEURO.0343-20.2021

Babiloni, C., Pistoia, F., Sarà, M., Vecchio, F., Buffo, P., Conson, M., Onorati, P., Albertini, G., & Rossini, P. M. (2010). Resting state eyes-closed cortical rhythms in patients with locked-in-syndrome: An eeg study. *Clinical Neurophysiology*, *121*, 1816–1824. https://doi.org/10.1016/j.clinph.2010.04.027

Babiloni, C., Sarà, M., Vecchio, F., Pistoia, F., Sebastiano, F., Onorati, P., Albertini, G., Pasqualetti, P., Cibelli, G., Buffo, P., & Rossini, P. M. (2009). Cortical sources of resting-state alpha rhythms are abnormal in persistent vegetative state patients. *Clinical Neurophysiology*, *120*, 719–729. https://doi.org/10.1016/j.clinph.2009.02.157

Bagnato, S., Boccagni, C., Sant'Angelo, A., Prestandrea, C., Mazzilli, R., & Galardi, G. (2015). EEG predictors of outcome in patients with disorders of consciousness admitted for intensive rehabilitation. *Clinical Neurophysiology*, *126*, 959–966. https://doi.org/10.1016/j.clinph.2014.08.005

Bai, Y., Lin, Y., & Ziemann, U. (2020). Managing disorders of consciousness: The role of electroencephalography. *Journal of Neurology*, *268*, 4033–4065. https://doi.org/10.1007/s00415-020-10095-z

Bai, Y., Xia, X., & Li, X. (2017). A review of resting-state electroencephalography analysis in disorders of consciousness. *Frontiers in Neurology*, *8*, 471. https://doi.org/10.3389/fneur.2017.00471

Baird, B., Castelnovo, A., Riedner, B. A., Lutz, A., Ferrarelli, F., Boly, M., Davidson, R. J., & Tononi, G. (2018). Human rapid eye movement sleep shows local increases in low-frequency oscillations and global decreases in high-frequency oscillations compared to resting wakefulness. *eNeuro*, *5*(e0293–18), 2018. https://doi.org/10.1523/ENEURO.0293-18.2018

Benca, R. M., Obermeyer, W. H., Larson, C. L., Yun, B., Dolski, I., Kleist, K. D., Weber, S. M., & Davidson, R. J. (1999). EEG alpha power and alpha power asymmetry in sleep and wakefulness. *Psychophysiology*, *36*, 430–436. https://doi.org/10.1111/1469-8986.3640430

Berger, H. (1935). Das Elektrenkephalogramm des Menschen. *Naturwissenschaften*, *23*, 121–124. https://doi.org/10.1007/BF01496966

Bruno, M.-A., Vanhaudenhuyse, A., Thibaut, A., Moonen, G., & Laureys, S. (2011). From unresponsive wakefulness to minimally conscious PLUS and functional locked-in syndromes: Recent advances in our understanding of disorders of consciousness. *Journal of Neurology*, *258*, 1373–1384. https://doi.org/10.1007/s00415-011-6114-x

Butz, M., Gross, J., Timmermann, L., Moll, M., Freund, H.-J., Witte, O. W., & Schnitzler, A. (2004). Perilesional pathological oscillatory activity in the magnetoencephalogram of patients with cortical brain lesions. *Neuroscience Letters*, *355*, 93–96. https://doi.org/10.1016/j.neulet.2003.10.065

Casali, A. G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K. R., Casarotto, S., Bruno, M.-A., Laureys, S., Tononi, G., & Massimini, M. (2013). A theoretically based index of consciousness independent of sensory processing and behavior. *Science Translational Medicine*, *5*, 198ra105. https://doi.org/10.1126/scitranslmed.3006294

Casarotto, S., Comanducci, A., Rosanova, M., Sarasso, S., Fecchio, M., Napolitani, M., Pigorini, A., Casali, A. G., Trimarchi, P. D., Boly, M., Gosseries, O., Bodart, O., Curto, F., Landi, C., Mariotti, M., Devalle, G., Laureys, S., Tononi, G., & Massimini, M. (2016). Stratification of unresponsive patients by an independently validated index of brain complexity. *Annals of Neurology*, *80*, 718–729. https://doi.org/10.1002/ana.24779

Casarotto, S., Fecchio, M., Rosanova, M., Varone, G., D'Ambrosio, S., Sarasso, S., Pigorini, A., Russo, S., Comanducci, A., Ilmoniemi, R. J., & Massimini, M. (2022). The rt-TEP tool: Real-time visualization of TMS-evoked potentials to maximize cortical activation and minimize artifacts. *Journal of Neuroscience Methods*, *370*, 109486. https://doi.org/10.1016/j.jneumeth.2022.109486

Cassidy, J. M., Wodeyar, A., Wu, J., Kaur, K., Masuda, A. K., Srinivasan, R., & Cramer, S. C. (2020). Low-frequency oscillations are a biomarker of injury and recovery after stroke. *Stroke*, *51*, 1442–1450. https://doi.org/10.1161/STROKEAHA.120.028932

Claassen, J., Doyle, K., Matory, A., Couch, C., Burger, K. M., Velazquez, A., Okonkwo, J. U., King, J.-R., Park, S., Agarwal, S., Roh, D., Megjhani, M., Eliseyev, A., Connolly, E. S., & Rohaut, B. (2019). Detection of brain activation in unresponsive patients with acute brain injury. *The New England Journal of Medicine*, *380*, 2497–2505. https://doi.org/10.1056/NEJMoa1812757

Colombo, M. A., Comanducci, A., Casarotto, S., Derchi, C.-C., Annen, J., Viganò, A., Mazza, A., Trimarchi, P. D., Boly, M., Fecchio, M., Bodart, O., Navarro, J., Laureys, S., Gosseries, O., Massimini, M., Sarasso, S., & Rosanova, M. (2023). Beyond alpha power: EEG spatial and spectral gradients robustly stratify disorders of consciousness. *Cerebral Cortex*, *33*, 7193–7210. https://doi.org/10.1093/cercor/bhad031

Comanducci, A., Boly, M., Claassen, J., De Lucia, M., Gibson, R. M., Juan, E., Laureys, S., Naccache, L., Owen, A. M., Rosanova, M., Rossetti, A. O., Schnakers, C., Sitt, J. D., Schiff, N. D., & Massimini, M. (2020). Clinical and advanced neurophysiology in the prognostic and diagnostic evaluation of disorders of consciousness: Review of an IFCN-endorsed expert group. *Clinical Neurophysiology*, *131*, 2736–2765. https://doi.org/10.1016/j.clinph.2020.07.015

Curley, W. H., Forgacs, P. B., Voss, H. U., Conte, M. M., & Schiff, N. D. (2018). Characterization of EEG signals revealing covert cognition in the injured brain. *Brain*, *141*, 1404–1421. https://doi.org/10.1093/brain/awy070

D'Andola, M., Rebollo, B., Casali, A. G., Weinert, J. F., Pigorini, A., Villa, R., Massimini, M., & Sanchez-Vives, M. V. (2018). Bistability, causality, and complexity in cortical networks: An in vitro perturbational study. *Cerebral Cortex*, *28*, 2233–2242. https://doi.org/10.1093/cercor/bhx122

Darmani, G., Nieminen, J. O., Bergmann, T. O., Ramezanpour, H., & Ziemann, U. (2021). A degraded state of consciousness in healthy awake humans? *Brain Stimulation*, *14*, 710–712. https://doi.org/10.1016/j.brs.2021.04.012

Duszyk-Bogorodzka, A., Zieleniewska, M., & Jankowiak-Siuda, K. (2022). Brain activity characteristics of patients with disorders of consciousness in the EEG resting state paradigm: A review. *Frontiers in Systems Neuroscience*, *16*, 654541. https://doi.org/10.3389/fnsys.2022.654541

Edlow, B. L., Claassen, J., Schiff, N. D., & Greer, D. M. (2021). Recovery from disorders of consciousness: Mechanisms, prognosis and emerging therapies. *Nature Reviews. Neurology*, *17*, 135–156. https://doi.org/10.1038/s41582-020-00428-x

Engemann, D. A., Raimondo, F., King, J.-R., Rohaut, B., Louppe, G., Faugeras, F., Annen, J., Cassol, H., Gosseries, O., Fernandez-Slezak, D., Laureys, S., Naccache, L., Dehaene, S., & Sitt, J. D. (2018). Robust EEG-based cross-site and cross-protocol classification of states of consciousness. *Brain*, *141*, 3179–3192. https://doi.org/10.1093/brain/awy251

Esposito, M. J., Nielsen, T. A., & Paquette, T. (2004). Reduced alpha power associated with the recall of mentation from stage 2 and stage REM sleep. *Psychophysiology*, *41*, 288–297. https://doi.org/10.1111/j.1469-8986.00143.x

Estraneo, A., Loreto, V., Guarino, I., Boemia, V., Paone, G., Moretta, P., & Trojano, L. (2016). Standard EEG in diagnostic process of prolonged disorders of consciousness. *Clinical Neurophysiology*, *127*, 2379–2385. https://doi.org/10.1016/j.clinph.2016.03.021

Farnes, N., Juel, B. E., Nilsen, A. S., Romundstad, L. G., & Storm, J. F. (2020). Increased signal diversity/complexity of spontaneous EEG, but not evoked EEG responses, in ketamine-induced psychedelic state in humans. *PLoS ONE*, *15*, e0242056. https://doi.org/10.1371/journal.pone.0242056

Faugeras, F., Rohaut, B., Weiss, N., Bekinschtein, T., Galanaud, D., Puybasset, L., Bolgert, F., Sergent, C., Cohen, L., Dehaene, S., & Naccache, L. (2012). Event related potentials elicited by violations of auditory regularities in patients with impaired consciousness. *Neuropsychologia*, *50*, 403–418. https://doi.org/10.1016/j.neuropsychologia.2011.12.015

Forgacs, P. B., Conte, M. M., Fridman, E. A., Voss, H. U., Victor, J. D., & Schiff, N. D. (2014). Preservation of electroencephalographic organization in patients with impaired consciousness and imaging-based evidence of command-following. *Annals of Neurology*, *76*, 869–879. https://doi.org/10.1002/ana.24283

Franzova, E., Shen, Q., Doyle, K., Chen, J. M., Egbebike, J., Vrosgou, A., Carmona, J. C., Grobois, L., Heinonen, G. A., Velazquez, A., Gonzales, I. J., Egawa, S., Agarwal, S., Roh, D., Park, S., Connolly, E. S., & Claassen, J. (2023). Injury patterns associated with cognitive motor dissociation. *Brain*, *146*, 4645–4658. https://doi.org/10.1093/brain/awad197

Frohlich, J., Bird, L. M., Dell'Italia, J., Johnson, M. A., Hipp, J. F., & Monti, M. M. (2020). High-voltage, diffuse delta rhythms coincide with wakeful consciousness and complexity in Angelman syndrome. *Neuroscience of Consciousness*, *6*, niaa005. https://doi.org/10.1093/nc/niaa005

Frohlich, J., Chiang, J. N., Mediano, P. A. M., Nespeca, M., Saravanapandian, V., Toker, D., Dell'Italia, J., Hipp, J. F., Jeste, S. S., Chu, C. J., Bird, L. M., & Monti, M. M. (2022). Neural complexity is a common denominator of human consciousness across diverse regimes of cortical dynamics. *Communications Biology*, *5*, 1374. https://doi.org/10.1038/s42003-022-04331-7

Frohlich, J., Toker, D., & Monti, M. M. (2021). Consciousness among delta waves: A paradox? *Brain*, *144*, 2257–2277. https://doi.org/10.1093/brain/awab095

Gaskell, A. L., Hight, D. F., Winders, J., Tran, G., Defresne, A., Bonhomme, V., Raz, A., Sleigh, J. W., & Sanders, R. D. (2017). Frontal alpha-delta EEG does not preclude volitional response during anaesthesia: Prospective cohort study of the isolated forearm technique. *British Journal of Anaesthesia*, *119*, 664–673. https://doi.org/10.1093/bja/aex170

Giacino, J. T., Kalmar, K., & Whyte, J. (2004). The JFK coma recovery scale-revised: Measurement characteristics and diagnostic utility. *Archives of Physical Medicine and Rehabilitation*, *85*, 2020–2029. https://doi.org/10.1016/j.apmr.2004.02.033

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., Barbano, R., Hammond, F. M., Laureys, S., Ling, G. S. F., Nakase-Richardson, R., Seel, R. T., Yablon, S., Getchius, T. S. D., Gronseth, G. S., & Armstrong, M. J. (2018a). Comprehensive systematic review update summary: Disorders of consciousness. *Neurology*, *91*, 461–470. https://doi.org/10.1212/WNL.0000000000005928

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., Barbano, R., Hammond, F. M., Laureys, S., Ling, G. S. F., Nakase-Richardson, R., Seel, R. T., Yablon, S., Getchius, T. S. D., Gronseth, G. S., & Armstrong, M. J. (2018b). Practice guideline update recommendations summary: Disorders of consciousness. *Neurology*, *91*, 450–460. https://doi.org/10.1212/WNL.0000000000005926

Gloor, P., Ball, G., & Schaul, N. (1977). Brain lesions that produce delta waves in the EEG. *Neurology*, *27*, 326–333. https://doi.org/10.1212/wnl.27.4.326

Gökyiğit, A., & Calişkan, A. (1995). Diffuse spike-wave status of 9-year duration without behavioral change or intellectual decline. *Epilepsia*, *36*, 210–213. https://doi.org/10.1111/j.1528-1157.1995.tb00982.x

Gosseries, O., Sarasso, S., Casarotto, S., Boly, M., Schnakers, C., Napolitani, M., Bruno, M.-A., Ledoux, D., Tshibanda, J.-F., Massimini, M., Laureys, S., & Rosanova, M. (2015). On the cerebral origin of EEG responses to TMS: Insights from severe cortical lesions. *Brain Stimulation*, *8*, 142–149. https://doi.org/10.1016/j.brs.2014.10.008

Kondziella, D., Bender, A., Diserens, K., van Erp, W., Estraneo, A., Formisano, R., Laureys, S., Naccache, L., Ozturk, S., Rohaut, B., Sitt, J. D., Stender, J., Tiainen, M., Rossetti, A. O., Gosseries, O., Chatelle, C., on behalf of the EAN Panel on Coma, & Disorders of Consciousness. (2020). European Academy of Neurology guideline on the diagnosis of coma and other disorders of consciousness. *European Journal of Neurology*, *27*, 741–756. https://doi.org/10.1111/ene.14151

Kondziella, D., Friberg, C. K., Frokjaer, V. G., Fabricius, M., & Møller, K. (2016). Preserved consciousness in vegetative and minimal conscious states: Systematic review and meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *87*, 485–492. https://doi.org/10.1136/jnnp-2015-310958

Leon-Carrion, J., Martin-Rodriguez, J. F., Damas-Lopez, J., Barroso y Martin, J. M., & Dominguez-Morales, M. R. (2008). Brain function in the minimally conscious state: A quantitative neurophysiological study. *Clinical Neurophysiology*, *119*, 1506–1514. https://doi.org/10.1016/j.clinph.2008.03.030

Lioumis, P., & Rosanova, M. (2022). The role of neuronavigation in TMS–EEG studies: Current applications and future perspectives. *Journal of Neuroscience Methods*, *380*, 109677. https://doi.org/10.1016/j.jneumeth.2022.109677

Massimini, M., Boly, M., Casali, A., Rosanova, M., & Tononi, G. (2009). A perturbational approach for evaluating the brain's capacity for consciousness. *Progress in Brain Research*, *177*, 201–214. https://doi.org/10.1016/S0079-6123(09)17714-2

Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., & Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science*, *309*, 2228–2232. https://doi.org/10.1126/science.1117256

Massimini, M., Ferrarelli, F., Murphy, M., Huber, R., Riedner, B., Casarotto, S., & Tononi, G. (2010). Cortical reactivity and effective connectivity during REM sleep in humans. *Cognitive Neuroscience*, *1*, 176–183. https://doi.org/10.1080/17588921003731578

Monti, M. M., Vanhaudenhuyse, A., Coleman, M. R., Boly, M., Pickard, J. D., Tshibanda, L., Owen, A. M., & Laureys, S. (2010). Willful modulation of brain activity in disorders of consciousness. *The New England Journal of Medicine*, *362*, 579–589. https://doi.org/10.1056/NEJMoa0905370

Murphy, M., Bruno, M.-A., Riedner, B. A., Boveroux, P., Noirhomme, Q., Landsness, E. C., Brichant, J.-F., Phillips, C., Massimini, M., Laureys, S., Tononi, G., & Boly, M. (2011). Propofol anesthesia and sleep: A high-density EEG study. *Sleep*, *34*, 283–291. https://doi.org/10.1093/sleep/34.3.283

Mutanen, T., Mäki, H., & Ilmoniemi, R. J. (2013). The effect of stimulus parameters on TMS-EEG muscle artifacts. *Brain Stimulation*, *6*, 371–376. https://doi.org/10.1016/j.brs.2012.07.005

Naro, A., Bramanti, P., Leo, A., Cacciola, A., Bramanti, A., Manuli, A., & Calabrò, R. S. (2016). Towards a method to differentiate chronic disorder of consciousness patients' awareness: The low-resolution brain electromagnetic tomography analysis. *Journal of the Neurological Sciences*, *368*, 178–183. https://doi.org/10.1016/j.jns.2016.07.016

Ni Mhuircheartaigh, R., Warnaby, C., Rogers, R., Jbabdi, S., & Tracey, I. (2013). Slow-wave activity saturation and thalamocortical isolation during propofol anesthesia in humans. *Science Translational Medicine*, *5*, 208ra148. https://doi.org/10.1126/scitranslmed.3006007

Nuwer, M. R., Jordan, S. E., & Ahn, S. S. (1987). Evaluation of stroke using EEG frequency analysis and topographic mapping. *Neurology*, *37*, 1153–1159. https://doi.org/10.1212/wnl.37.7.1153

Ostfeld, A. M., Machne, X., & Unna, K. R. (1960). The effects of atropine on the electroencephalogram and behavior in man. *The Journal of Pharmacology and Experimental Therapeutics*, *128*, 265–272.

Parks, N. A., Gannon, M. A., Long, S. M., & Young, M. E. (2016). Bootstrap signal-to-noise confidence intervals: An objective method for subject exclusion and quality control in ERP studies. *Frontiers in Human Neuroscience*, *10*, 50. https://doi.org/10.3389/fnhum.2016.00050

Purdon, P. L., Sampson, A., Pavone, K. J., & Brown, E. N. (2015). Clinical electroencephalography for anesthesiologists. *Anesthesiology*, *123*, 937–960. https://doi.org/10.1097/ALN.0000000000000841

Rosanova, M., Casali, A. G., Bellina, V., Resta, F., Mariotti, M., & Massimini, M. (2009). Natural frequencies of human corticothalamic circuits. *The Journal of Neuroscience*, *29*, 7679–7685. https://doi.org/10.1523/JNEUROSCI.0445-09.2009

Rosanova, M., Fecchio, M., Casarotto, S., Sarasso, S., Casali, A. G., Pigorini, A., Comanducci, A., Seregni, F., Devalle, G., Citerio, G., Bodart, O., Boly, M., Gosseries, O., Laureys, S., & Massimini, M. (2018). Sleep-like cortical OFF-periods disrupt causality and complexity in the brain of unresponsive wakefulness syndrome patients. *Nature Communications*, *9*, 4427. https://doi.org/10.1038/s41467-018-06871-1

Rosanova, M., Gosseries, O., Casarotto, S., Boly, M., Casali, A. G., Bruno, M.-A., Mariotti, M., Boveroux, P., Tononi, G., Laureys, S., & Massimini, M. (2012). Recovery of cortical effective connectivity and recovery of consciousness in vegetative patients. *Brain*, *135*, 1308–1320. https://doi.org/10.1093/brain/awr340

Rossi Sebastiano, D., Panzica, F., Visani, E., Rotondi, F., Scaioli, V., Leonardi, M., Sattin, D., D'Incerti, L., Parati, E., Ferini Strambi, L., & Franceschetti, S. (2015). Significance of multiple neurophysiological measures in patients with chronic disorders of consciousness. *Clinical Neurophysiology*, *126*, 558–564. https://doi.org/10.1016/j.clinph.2014.07.004

Rossi Sebastiano, D., Varotto, G., Sattin, D., & Franceschetti, S. (2021). EEG assessment in patients with disorders of consciousness: Aims, advantages, limits, and pitfalls. *Frontiers in Neurology*, *12*, 649849. https://doi.org/10.3389/fneur.2021.649849

Russo, S., Pigorini, A., Mikulan, E., Sarasso, S., Rubino, A., Zauli, F. M., Parmigiani, S., d'Orio, P., Cattani, A., Francione, S., Tassi, L., Bassetti, C. L. A., Lo Russo, G., Nobili, L., Sartori, I., & Massimini, M. (2021). Focal lesions induce large-scale percolation of sleep-like intracerebral activity in awake humans. *NeuroImage*, *234*, 117964. https://doi.org/10.1016/j.neuroimage.2021.117964

Russo, S., Sarasso, S., Puglisi, G. E., Dal Palù, D., Pigorini, A., Casarotto, S., D'Ambrosio, S., Astolfi, A., Massimini, M., Rosanova, M., & Fecchio, M. (2022). TAAC—TMS adaptable auditory control: A universal tool to mask TMS clicks. *Journal of Neuroscience Methods*, *370*, 109491. https://doi.org/10.1016/j.jneumeth.2022.109491

Sanchez-Vives, M. V., Massimini, M., & Mattia, M. (2017). Shaping the default activity pattern of the cortical network. *Neuron*, *94*, 993–1001. https://doi.org/10.1016/j.neuron.2017.05.015

Sarasso, S., Boly, M., Napolitani, M., Gosseries, O., Charland-Verville, V., Casarotto, S., Rosanova, M., Casali, A. G., Brichant, J.-F., Boveroux, P., Rex, S., Tononi, G., Laureys, S., & Massimini, M. (2015). Consciousness and complexity during unresponsiveness induced by propofol, xenon, and ketamine. *Current Biology*, *25*, 3099–3105. https://doi.org/10.1016/j.cub.2015.10.014

Sarasso, S., Casali, A. G., Casarotto, S., Rosanova, M., Sinigaglia, C., & Massimini, M. (2021). Consciousness and complexity: A consilience of evidence. *Neuroscience of Consciousness*, *7*, 1–24. https://doi.org/10.1093/nc/niab023

Sarasso, S., D'Ambrosio, S., Fecchio, M., Casarotto, S., Viganò, A., Landi, C., Mattavelli, G., Gosseries, O., Quarenghi, M., Laureys, S., Devalle, G., Rosanova, M., & Massimini, M. (2020). Local sleep-like cortical reactivity in the awake brain after focal injury. *Brain*, *143*, 3672–3684. https://doi.org/10.1093/brain/awaa338

Schartner, M. M., Carhart-Harris, R. L., Barrett, A. B., Seth, A. K., & Muthukumaraswamy, S. D. (2017). Increased spontaneous MEG signal diversity for psychoactive doses of ketamine, LSD and psilocybin. *Scientific Reports*, *7*, 46421. https://doi.org/10.1038/srep46421

Schiff, N. D. (2016). Mesocircuit mechanisms underlying recovery of consciousness following severe brain injuries: model and predictions. In M. M. Monti & W. G. Sannita (Eds.), *Brain function and responsiveness in disorders of consciousness* (pp. 195–204). Springer International Publishing. https://doi.org/10.1007/978-3-319-21425-2_15

Sinitsyn, D. O., Poydasheva, A. G., Bakulin, I. S., Legostaeva, L. A., Iazeva, E. G., Sergeev, D. V., Sergeeva, A. N., Kremneva, E. I., Morozova, S. N., Lagoda, D. Y., Casarotto, S., Comanducci, A., Ryabinkina, Y. V., Suponeva, N. A., & Piradov, M. A. (2020). Detecting the potential for consciousness in unresponsive patients using the perturbational complexity index. *Brain Sciences*, *10*, 917. https://doi.org/10.3390/brainsci10120917

Sitt, J. D., King, J.-R., El Karoui, I., Rohaut, B., Faugeras, F., Gramfort, A., Cohen, L., Sigman, M., Dehaene, S., & Naccache, L. (2014). Large scale screening of neural signatures of consciousness in patients in a vegetative or minimally conscious state. *Brain*, *137*, 2258–2270. https://doi.org/10.1093/brain/awu141

Steriade, M., Amzica, F., & Nuñez, A. (1993). Cholinergic and noradrenergic modulation of the slow (approximately 0.3 Hz) oscillation in neocortical cells. *Journal of Neurophysiology*, *70*, 1385–1400. https://doi.org/10.1152/jn.1993.70.4.1385

Timmermann, C., Roseman, L., Schartner, M., Milliere, R., Williams, L. T. J., Erritzoe, D., Muthukumaraswamy, S., Ashton, M., Bendrioua, A., Kaur, O., Turton, S., Nour, M. M., Day, C. M., Leech, R., Nutt, D. J., & Carhart-Harris, R. L. (2019). Neural correlates of the DMT experience assessed with multivariate EEG. *Scientific Reports*, *9*, 16324. https://doi.org/10.1038/s41598-019-51974-4

Tononi, G., & Edelman, G. M. (1998). Consciousness and complexity. *Science*, *282*, 1846–1851. https://doi.org/10.1126/science.282.5395.1846

Vlisides, P. E., Bel-Bahar, T., Lee, U., Li, D., Kim, H., Janke, E., Tarnal, V., Pichurko, A. B., McKinney, A. M., Kunkler, B. S., Picton, P., & Mashour, G. A. (2017). Neurophysiologic correlates of ketamine sedation and anesthesia. *Anesthesiology*, *127*, 58–69. https://doi.org/10.1097/ALN.0000000000001671

Vlisides, P. E., Bel-Bahar, T., Nelson, A., Chilton, K., Smith, E., Janke, E., Tarnal, V., Picton, P., Harris, R. E., & Mashour, G. A. (2018). Subanaesthetic ketamine and altered states of consciousness in humans. *British Journal of Anaesthesia*, *121*, 249–259. https://doi.org/10.1016/j.bja.2018.03.011

Walter, W. G. (1937). The electro-encephalogram in cases of cerebral tumour. *Proceedings of the Royal Society of Medicine*, *30*, 579–598. https://doi.org/10.1177/003591573703000526

Willacker, L., Raiser, T. M., Bassi, M., Bender, A., Comanducci, A., Rosanova, M., Sobel, N., Arzi, A., Belloli, L., Casarotto, S., Colombo, M., Derchi, C. C., Fló Rama, E., Grill, E., Hohl, M., Kuehlmeyer, K., Manasova, D., Rosenfelder, M. J., Valota, C., & Sitt, J. D. (2022). PerBrain: A multimodal approach to personalized tracking of evolving state-of-consciousness in brain-injured patients: Protocol of an international, multicentric, observational study. *BMC Neurology*, *22*, 468. https://doi.org/10.1186/s12883-022-02958-x

Wutzl, B., Golaszewski, S. M., Leibnitz, K., Langthaler, P. B., Kunz, A. B., Leis, S., Schwenker, K., Thomschewski, A., Bergmann, J., & Trinka, E. (2021). Narrative review: Quantitative EEG in disorders of consciousness. *Brain Sciences*, *11*, 697. https://doi.org/10.3390/brainsci11060697

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