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RESEARCH REPORT

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A neurophysiological profiling of the heartbeat-evoked potential in severe acquired brain injuries: A focus on unconsciousness

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Abstract

Unconsciousness in severe acquired brain injury (sABI) patients occurs with different cognitive and neural profiles. Perturbational approaches, which enable the estimation of proxies for brain reorganization, have added a new avenue for investigating the non-behavioural diagnosis of consciousness. In this prospective observational study, we conducted a comparative analysis of the topological patterns of heartbeat-evoked potentials (HEP) between patients experiencing a prolonged disorder of consciousness (pDoC) and patients emerging from a minimally consciousness state (eMCS). A total of 219 sABI patients were enrolled, each undergoing a synchronous EEG-ECG resting-state recording, together with a standardized consciousness diagnosis. A number of graph metrics were computed before/after the HEP (*Before/After*) using the R-peak on the ECG signal. The peak value of the global field power of the HEP was found to be significantly higher in eMCS patients with no difference in latency. Power spectrum was not able to discriminate consciousness neither *Before* nor *After*. Node assortativity and global efficiency were found to vary with different trends at unconsciousness. Lastly, the Perturbational Complexity Index of the HEP was found to be significantly higher in eMCS patients compared with pDoC. Given that cortical elaboration of peripheral inputs may serve as a non-behavioural determinant of consciousness, we have devised a low-cost and translatable technique capable of estimating causal proxies of brain functionality with an endogenous, non-invasive stimulus. Thus, we present an effective means to enhance consciousness assessment by incorporating the interaction between the autonomic nervous system (ANS) and central nervous system (CNS) into the loop.

Abbreviations: ANS, autonomic nervous system; CRS-R, Coma Recovery Scale-Revised; eMCS, emergence from MCS; GFP, global field power; HEP, heartbeat-evoked potential; MCS, minimally conscious state; PCI, Perturbational Complexity Index; pDoC, prolonged disorders of consciousness; PSD, power spectrum density; sABI, severe acquired brain injuries; TMS, transcranial magnetic stimulation; UWS, unresponsive wakefulness syndrome.

KEYWORDS
ANS-CNS interaction, ECG, EEG, prolonged disorders of consciousness, severe acquired brain injuries

1 | INTRODUCTION

Prolonged Disorders of consciousness (pDoC) are characterized by alteration in arousal and/or awareness due to vascular, traumatic, anoxic or other brain damages lasting more than 28 days. The heterogeneity of behavioural/neural profiles of patients with a pDoC necessitates a standardized assessment, currently relying solely on behavioural responses only (Bayne et al., 2017). Within the current taxonomy of consciousness, coma is characterized by the absence of both arousal and awareness, the unresponsive wakefulness syndrome (UWS; Laureys et al., 2010) by arousal without awareness and the minimally conscious state (MCS; Bruno et al., 2011) by reproducible but non-consistent awareness (although minimal). Finally, patients who present consistent awareness with intentional or functional communication are considered as emergent from the MCS (eMCS). Those patients often enter in a confusional cognitive state before recovering (or not) full or partial cognitive functions.

The Coma Recovery Scale-Revised (CRS-R; Giacino et al., 2005) hierarchically targeting reflexive behaviour and cognitively mediated activity (McDonnell et al., 2015) has been recommended with minor reservations for the clinical diagnosis by the American Congress of Rehabilitation Medicine (Seel et al., 2010). However, given the multitude of factors influencing the misdiagnosis rate (up to 40% in pDoC), vigilance fluctuations (Wannez et al., 2017), impairments in the motor networks (Hakiki, Cecchi, et al., 2022), associated diseases (Thibaut et al., 2014) and diffuse pain (Formisano, Contrada, Aloisi, et al., 2019) need to be acknowledged. To cope with these risks, authors demonstrated how the repeated CRS-R administration (Wang et al., 2020; Wannez et al., 2017), the use of a mirror for the visual pursuit assessment (Cruse et al., 2017) and the inclusion of autoreferential stimuli (Bredart et al., 2006; Hermann et al., 2019; Laureys et al., 2007; Vanhaudenhuyse et al., 2008) improve responsiveness and diagnostic accuracy. In this context, quantitative, non-behaviourally based neuronal correlates of consciousness have also been proposed from neuroimaging (Demertzi et al., 2015; Menon et al., 1998; Owen et al., 2005; Soddu et al., 2012), central (Casarotto et al., 2016; Engemann et al., 2018; Liuzzi, Grippo, Campagnini, et al., 2022; Liuzzi, Hakiki, Draghi, et al., 2023; Sitt et al., 2014) and peripheral (Candia-Rivera et al., 2021; Liuzzi, Campagnini, Hakiki, et al., 2023; Liuzzi, Grippo, Hakiki, et al., 2022; Raimondo et al., 2017; Riganello et al., 2018; Riganello et al., 2021) neurophysiology to probe consciousness in absence of overt behavioural responses. It has been highlighted how the neural responses to visceral inputs contribute to conscious experience in healthy individuals (Liuzzi, Hakiki, Scarpino, et al., 2023; Park et al., 2018; Park & Tallon-Baudry, 2014; Park & Thayer, 2014). Moreover, it has been suggested that cortical modulation of peripheral body functions is influenced by concurrent cognition (Pernice et al., 2019), closing the bidirectional peripheral-central loop. In particular, whenever the input is the cardiac heartbeat, the evoked brain response is termed heartbeat-evoked potential (HEP). Candia-Rivera et al. showed how multivariate models trained on HEP-locked Electroencephalography (EEG) segments improve consciousness diagnosis with respect to random EEG segments, confirming the fundamental role of brain-heart interplay (Candia-Rivera et al., 2021).

Despite the high number of correlates of consciousness found in literature, the only marker successfully distinguishing consciousness levels is the Perturbational Complexity Index (PCI), inspired on the Integrated Information Theory by Tononi et al. (2016). The PCI targets the ability of the thalamocortical system to differentiate and integrate the information efficiently, assessing the spatio-temporal compression complexity of the electrocortical response. However, such approach is based on a combination of transcranial magnetic stimulation (TMS) and EEG, which is more discomfortable, costly and with more exclusion criteria for sABI patients than electrocardiogram (ECG) recordings.

Therefore, following this line of inquiry, in this study, the fundamental hypothesis was based on a parallel between the HEP (cardiac beat) and the TMS-Evoked Potential (TEP). The first is an internally conducted (*endogenous*) stimulus, and the second is an *exogenous* magnetic pulse. In particular, we tested whether the re-organization following the HEP is related to the consciousness level and therefore to the integration capabilities of the brain repertoires. Operationally, we therefore aimed to compare brain metrics (spectral, graph-based, PCI) related to the HEP between patients with/without a pDoC in a large cohort of patients with an sABI.

2 | MATERIALS AND METHODS

2.1 | Study design and data collection

This study is a prospective observational analysis within the framework of the PRABI study (Hakiki, Donnini, et al., 2022). It included sABI patients admitted to IRCCS Fondazione Don Carlo Gnocchi Firenze from 1 January 2020 to 31 December 2022. Inclusion criteria were diagnosis of an sABI and adult age (>18). Approval from the local Ethical Committee was obtained (N. 16606OSS) and enrolment was done following the Helsinki Declaration. Patients were included after obtaining a written consent signed by a legal guardian.

Demographics information (age, sex) and clinical characteristics, including the clinical diagnosis of consciousness at admission, were recorded. The latter was based on the maximal value across at least five consecutive CRS-R evaluations on five different days by skilled evaluators (neurologists, speech therapists) with five or more years of experience, using recommendations to reduce the risk of misdiagnosis (e.g., use of a mirror and caregiver involvement; Formisano, Contrada, Iosa, et al., 2019; Vanhaudenhuyse et al., 2008).

EEG recordings based on the 10–20 International Standard System were performed using a digital machine (Gal NT, EBNeuro). An EEG prewired head cap, with 19 electrodes' (Fp1-Fp2-F7-F8-F3-F4-C3-C4-T3-T4-P3-P4-T5-T6-O1-O2-Fz-Cz-Pz) set was adopted with previously proposed EEG recording parameters (Scarpino et al., 2020) at a sample rate of 128 Hz. A synchronous ECG recording was performed via two electrodes applied to the right and left of the heart at a sample rate of 128 Hz. Closed-eyes polygraphy recordings included an initial 10-min resting-state part and 20 min of randomly administered stimuli (used in clinical practice to assess reactivity, variability and, in general, cortical responsiveness of the patient).

2.2 | Data pre-processing

The resting-state portion of the recording was retained for further analysis. Each 10-min recording underwent visual inspection by expert neurophysiologists to identify excessive movement noise. The patient was included for further analysis only if at least five consecutive minutes of clean resting-state EEG were identified during this review. The starting and ending points of the 5-min section were manually selected and used to crop the polygraphy recording.

EEG unipolar recordings were re-referenced to the grand average and high-pass filtered using the MNE library (Gramfort et al., 2013) (zero-phase FIR filter at 1 Hz with a Hamming's window as suggested by the PREP pipeline; Bigdely-Shamlo et al., 2015). Infinite-impulse-response notch (50 Hz) filtering was subsequently applied to further remove power line disturbance. The initial 5 s of the recording was discarded to reject transitory artefacts. Channels exhibiting persistent excessive or uncorrelated noise were identified based on PREP criteria and interpolated by means of spherical spline interpolation (Freeden, 1984) using the MNE library. Lastly, the extended InfoMax independent component analysis (ICA) method was applied, and the independent components (ICs) were automatically labelled using the MNE-ICALabel library (Li et al., 2022) (Figure 1). Specifically, a neural-network classifier trained on crowd-sourced data, based on the Matlab ICLabel implementation, was used to select the excluded ICs. Only ICs labelled as brain with a confidence higher than 80% were retained. Channel-level data were then reconstructed from the IC spaces after including only brain data. From the clean electrode-wise signal, power spectral density (PSD) frequency peaks were extracted. Evoked data were averaged across trials to obtain the average evoked response per patient, and the results were also reported via topographic interpolation (topo-maps) at steps of 0.1 s. From each averaged evoked data, the global field power was computed as $GFP = std(x_n(t)) \forall t \in T$, representing the spatial standard deviation of the EEG recording, with $x_n(t)$ the EEG recording of the n-th channel. Peak values (in μV) and the related latencies (s) of the GFP were computed for each patient. Additionally, the peak/latency values of the averaged GFP within patient's group were computed.

The ECG signal was initially filtered using a high pass 5th-order Butterworth filter at 0.5 Hz followed by the same notch filter applied to EEG data. Tachogram was extracted using the Python library NeuroKit2, and it was visually inspected for missing beats (Makowski et al., 2021). R-peaks were identified and rejected whenever fell within ±5 s from a manual annotation of EEG artefact added during the recording by the neurophysiopathologist.

2.3 | Functional connectivity estimation

Using the time points of the retained R-peaks (t^*), two different windows (i.e., *Before*: t^*; $t^* + 0.25$ s and *After*: $t^* + 0.25$ s; $t^* + 0.5$ s) were employed to extract segments of synchronous EEG-ECG recordings, conditioned to previous findings on the HER-timing (Luft & Bhattacharya, 2015; Park et al., 2018; Park & Thayer, 2014). Within the interval

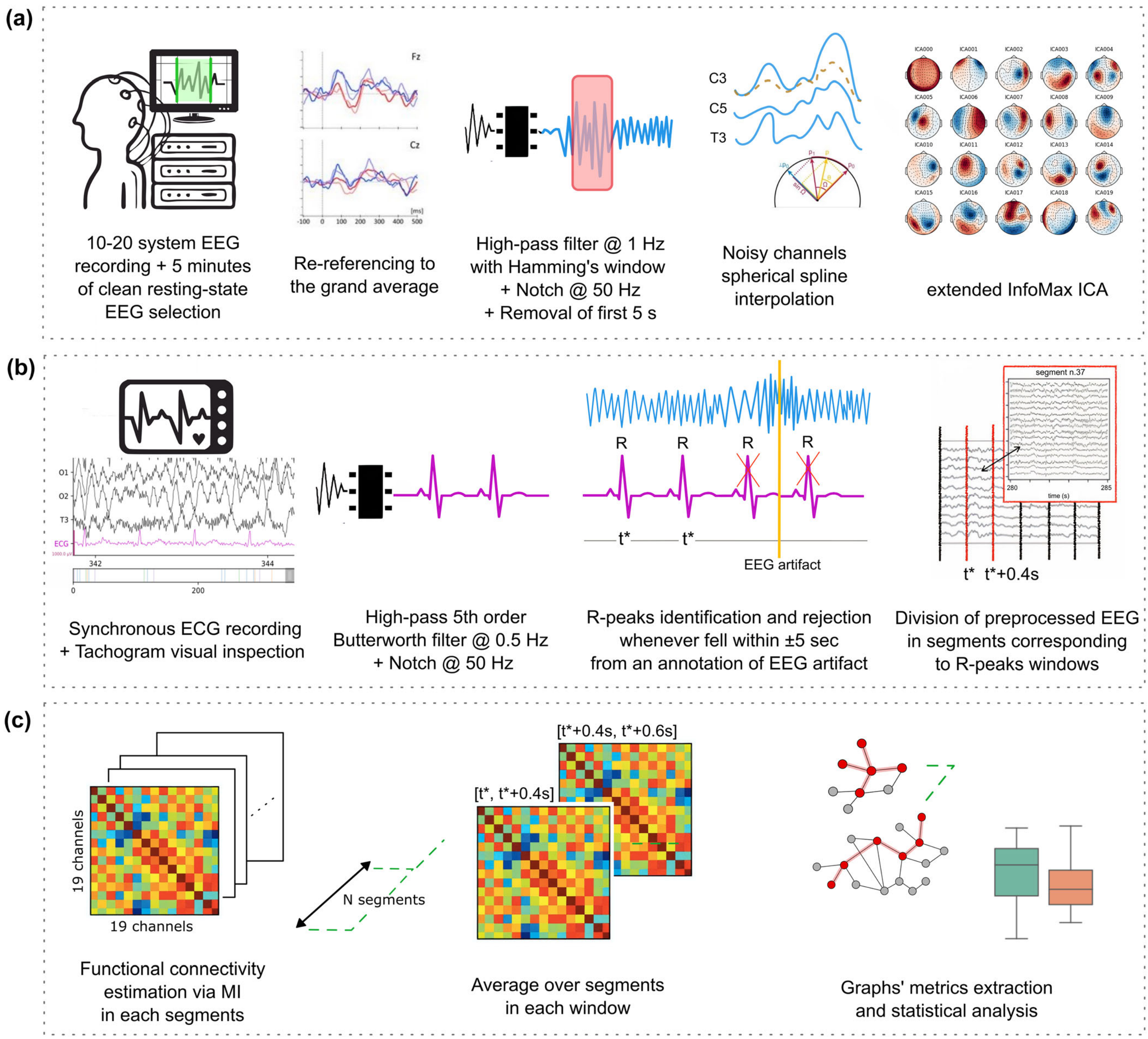

FIGURE 1 Work pipeline. EEG and ECG recording, pre-processing and artefact rejection are reported in panels (a) and (b), respectively. In panel (c), the estimation of functional connectivity matrixes and the related graph metrics (conditioned to the R-peaks) is reported. ciPLV, corrected imaginary phase locking value; ECG, electrocardiography; EEG, electroencephalography; ICA, independent component analysis; PCA, principal component analysis.

After, the peak of the HEP itself and a window of ~0.2 s right after the peak are included. *Before* is termed in this manner, since it corresponds to the portion of data before the HEP peak, but given the inherent cyclicity of the heart rhythm, each *Before* segment can also be considered as a '*Much after*' segment of the previous *After* segment. Each EEG electrode was used as a node for the definition of the brain graph model. Weighted edges were set between each nodes' pairs computing mutual information (MI) following Tzannes and Noonan, resulting in a 19 × 19 connectivity matrix for each segment and each window. In particular, the estimation of probability distributions was done in each window using equi-populated bins as suggested by Magri et al. (2009), with the number of bins following Sturge's rule (Scott, 2009) and previous work on this topic (Liuzzi, Hakiki, Scarpino, et al., 2023). For a detailed explanation of the computation process, we send the reader to Liuzzi et al. (Liuzzi, Hakiki, Scarpino, et al., 2023). Because of the intrinsic (non-negative) undirected nature of the measure, the resulting connectivity matrixes were lower triangular. The values on the main diagonals were set to zero to avoid self-loops. Graph metrics were computed on the weighted connectivity matrix to obtain density-independent values. In particular, via custom Python code and the library NetworkX (Hagberg et al., 2008), the following metrics were computed for each patient and window: edge betweenness centrality, node betweenness centrality, closeness centrality, clustering coefficient, normalized degree assortativity and global efficiency. Global efficiency was computed by taking the inverse of the shortest path length using Dijkstra's method for weighted graphs.

2.4 | PCI computation

PCI was computed using the Python implementation of Comolatti and Casali (Comolatti et al., 2019) using as baseline window *Before* and as response window *After*. As in the manuscript, the percentage of variance accounted for by the selected principal components was set to 0.99, the minimum signal-to-noise ratio to retain a principal component was set to 1.1. Noise control parameter k and the number of steps were set to 1.2 and 100.

2.5 | Statistical analysis

Descriptive statistics were reported in terms of medians and interquartile ranges (IQR) for continuous variables and in terms of counts and percentages for categorical variables. The outcome was set to the presence of a pDoC (versus eMCS patients). Evoked data were then grouped between outcome groups. GFP peaks and related latencies were compared between outcome groups via Mann–Whitney tests. Evoked data, taken at the GFP peaks' timing, were compared between the two groups via Mann–Whitney tests and corrected for multiple comparisons ($N_{ch} = 19$), to evaluate significant differences at the channel level.

Graphs' metrics computed on the full window [t^*; $t^* + 0.5$ s] were compared between pDoC and eMCS patients, through the use of Mann–Whitney (or t-) tests (conditioned to normality results). Then, metrics computed on the two separated segments ([t^*; $t^* + 0.25$ s] and [$t^* + 0.25$ s; $t^* + 0.5$ s]) were compared (between the two segments) via paired-samples Wilcoxon Sum Rank tests (or t-) tests (conditioned to normality results). Lastly, a two-way mixed ANOVA was performed with between-factor set to presence/absence of a pDoC and within-factor set to the computation window. Greenhouse–Geisser corrected p-value is provided conditioned to the results of Mauchly's test of sphericity. Partial η^2 was used as effect size measure. All tests were performed on SPSS v. 27, and significance level α was set to 0.05 for all tests.

3 | RESULTS

3.1 | Cohort and pre-processing

Two-hundred seventy-five patients were enrolled in the study, of which 12 were discarded due to missing clinical data, 34 due to the absence of the ECG recording and 10 due to the limited number of detected R-peaks ($NR_{peaks} < 50$), resulting in 219 sABI patients (Figure 2). The included cohort (median [IQR], age: 66 years [Owen et al., 2005], TPO: 39 [Engemann et al., 2018]) was composed of 119 eMCS patients and 97 patients with a pDoC (Table 1). Recordings had a median length of 5.18 min [IQR = 0.45] with a median number of R-peaks of 355 [IQR = 105.5] and median HR of 74.6 BPM [IQR = 21.41]. No significant differences were found when comparing the number of peaks and HR between pDoC and eMCS patients (Figure 3, $p > 0.05$, Mann–Whitney).

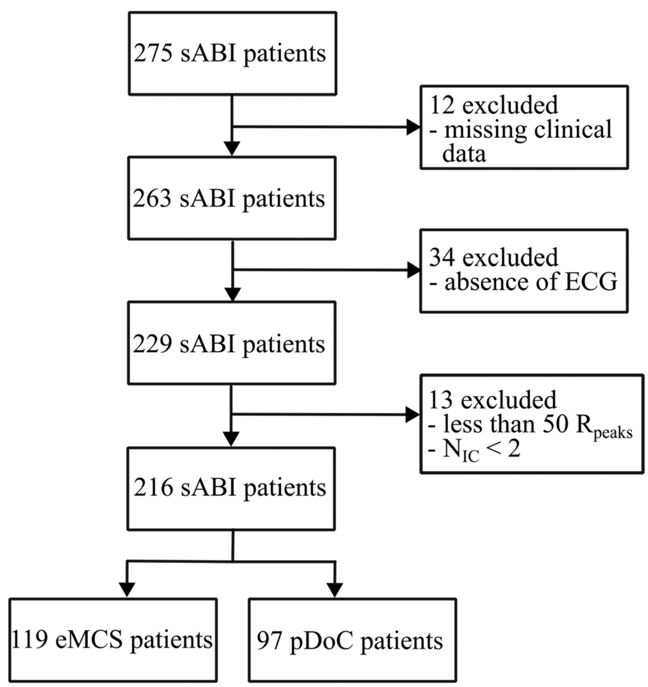

FIGURE 2 Enrolment flowchart with exclusion reasons. eMCS, emergence from minimally conscious state; pDoC, prolonged disorder of consciousness; sABI, severe acquired brain injuries.

Interpolation was performed on 162 channels (3.95% of all channels) with an average of 0.75 channels interpolated per patient ($std = 0.97$). O1, O2 and T5 were interpolated 21 (12.9%), 21 (12.9%) and 18 (11.1%) times, respectively (Figure 4c) with only nine times O1 and O2 being interpolated in the same patient. For what concerns IC removal, the median number of excluded components resulted equal to 13 [IQR = 4], compared with 9 [IQR = 3] when including all ICs independently from the labelling confidence (Figure 4a). However, such more aggressive IC removal strategy did not influence differently eMCS (median 13 [IQR = 3]) and pDoC (median 13 [IQR = 3]) patients (Mann–Whitney $p = 0.123$, $U = 6469.500$, Figure 4b).

TABLE 1 Descriptive statistics of the cohort.

	pDoC (*N* = 97)	eMCS (*N* = 119)
Age, years	68 [19]	65 [19]
Sex, F	37 (38.1)	50 (41.0)
TPO, days	40 [21]	38 [24]
Etiology		
TBI	20 (21.1)	32 (26.7)
Anoxic	11 (11.6)	4 (3.3)
Ischemic	20 (21.1)	23 (19.2)
Vascular	42 (44.2)	53 (44.2)
CRS-R, points	8 [7]	23 [1]
Auditory	2 [2]	4 [2]
Visual	2 [2]	5 [3]
Motor	2 [2]	6 [0]
Oro-motor	1 [0]	3 [3]
Communication	0 [1]	2 [0]
Arousal	2 [1]	3 [0]
ERBI, points	−275 [0]	−275 [38]

Note: Median and interquartile ranges (in square brackets) are reported for numerical variables, whereas counts and percentages (in parenthesis) are reported for categorical variables.
Abbreviations: CRS-R, Coma Recovery Scale-Revised; eMCS, emergence from minimally conscious state; ERBI, Early Rehabilitation Barthel Index; F, females; pDoC, prolonged disorders of consciousness; TBI, traumatic brain injury; TPO: time post-onset.

3.2 | HEP spectral analysis

EEG power spectrum was computed with the Welch method *Before* and *After* within the low-frequency bandwidth (δ and θ) and the high-frequency bandwidth (α and β). No significant differences were found between eMCS and pDoC patients in both bandwidths and windows (<8 Hz/*Before*: $p = 0.351$; <8 Hz/*After*: $p = 0.964$; 8–30 Hz/*Before*: $p = 0.570$; 8–30 Hz/*After*: $p = 0.651$, Figure 5).

However, patients were found to have a higher absolute power in the *During* window both in the <8 Hz band ($p < 0.001$, $U = 29978.000$) and in the 8–30 Hz band ($p < 0.001$, $U = 27944.000$). Via a two-way mixed ANOVA, the interaction between windows (within factor) and consciousness (between factor) was found to be not significant at low ($p = 0.192$, $F = 1.706$) and at high ($p = 0.471$, $F = 0.520$) frequencies. Also combining the frequency range (1–30 Hz) resulted in a non-significant interaction ($p = 0.217$, $F = 1.528$). Therefore, despite the decrease in absolute power *After* the HEP, no significant difference was detected between eMCS and pDoC patients.

3.3 | HEP morphological descriptors

The averaged evoked recordings were grouped (and averaged) across the two groups (Figure 6, eMCS in panel a

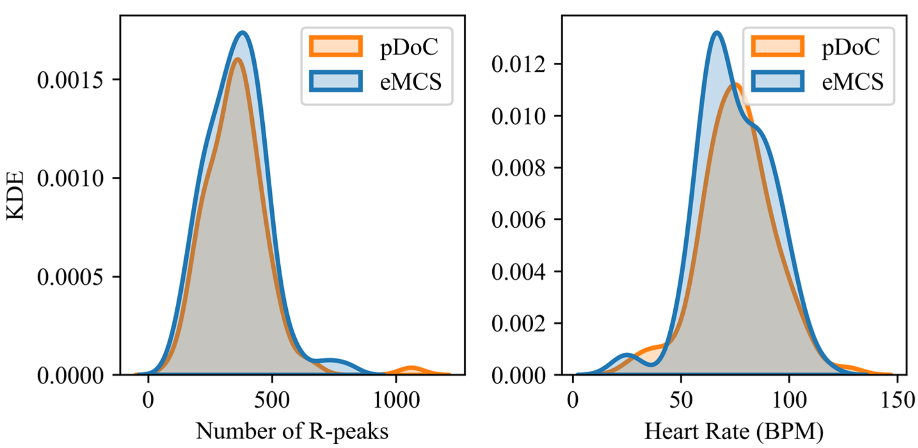

FIGURE 3 Kernel density estimate of the number of peaks (upper panel) and HR (lower panel) after R-peaks detection for all included patients. BPM, beats per minute; eMCS, emergence from minimally conscious state; KDE, kernel density estimate; pDoC, prolonged disorder of consciousness.

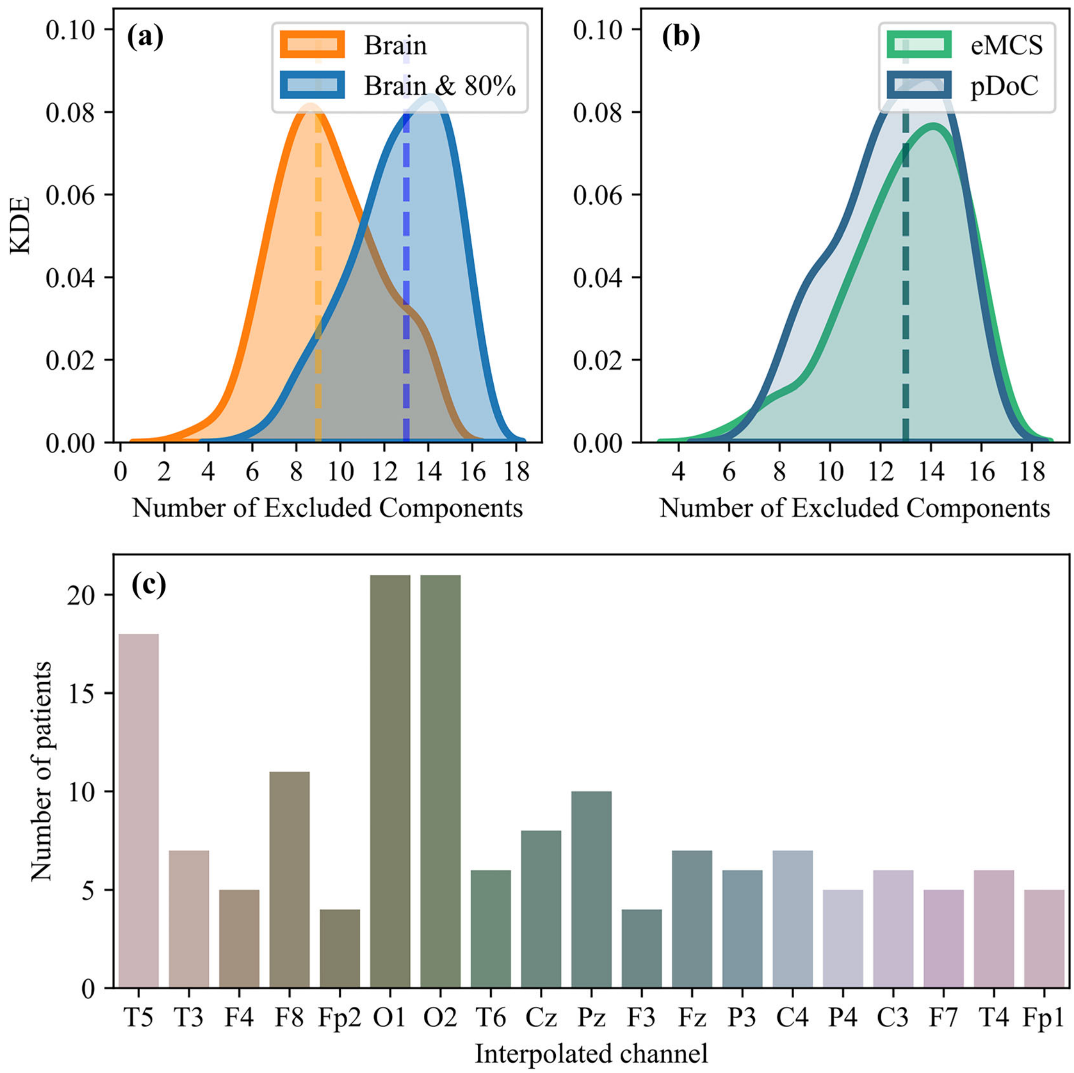

FIGURE 4 Number of excluded components (panel a) when retaining all the *brain*-labelled components (orange) and all the *brain*-labelled components with confidence of >80% (blue). Panel (b) reports the division of excluded components of the adopted technique in the manuscript. The number of times each channel was interpolated is reported in panel (c). eMCS, emergence from minimally conscious state; KDE, kernel density estimate; pDoC, prolonged disorder of consciousness.

and pDoC in panel b). Grouping the HEP in two regions of interest (fronto-central and posterior) resulted in a significantly delayed frontal peak in both cohorts, ~0.15 s after the posterior one. After computing the GFP of each patient, and the related peak/latencies (Figure 7, panels B/C), the peak value (absolute maxima in the interval 0–0.5 s) was found to be significantly higher ($p < 0.001$, $U = 3104$) in eMCS patients (median of 30.36 μV [IQR 13.89 μV]) than in pDoC (median of 26.78 μV [IQR = 9.24 μV]), with no significant difference in latencies ($p = 0.615$, median eMCS of 0.21 s [IQR = 0.16 s], median pDoC of 0.21 s [IQR = 0.13 s]).

Deriving the peak/latency values of the mean group-level GFP (Figure 7a) resulted in a peak of 28.78 μV (latency = 0.19 s) and 23.36 μV (latency = 0.20s) for eMCS and pDoC patients, respectively.

3.4 | PCI

PCI values were reported in Figure 8. PCI was found to be significantly higher ($p = 0.005$, $U = 4470.000$) in eMCS (median 0.25 [IQR = 0.21]) than in pDoC patients (median 0.21 [IQR = 0.20]).

3.5 | Graph metrics

Node betweenness centrality and closeness centrality (Figure 9a,b) were found to increase *After* the HEP, for both eMCS ($p < 0.001$, $U = 4373.500$ and $p < 0.001$, $U = 4087.000$, respectively) and pDoC patients ($p < 0.001$, $U = 2600.500$ and $p < 0.001$, $U = 2570.000$, respectively). Conversely, weighted clustering coefficient

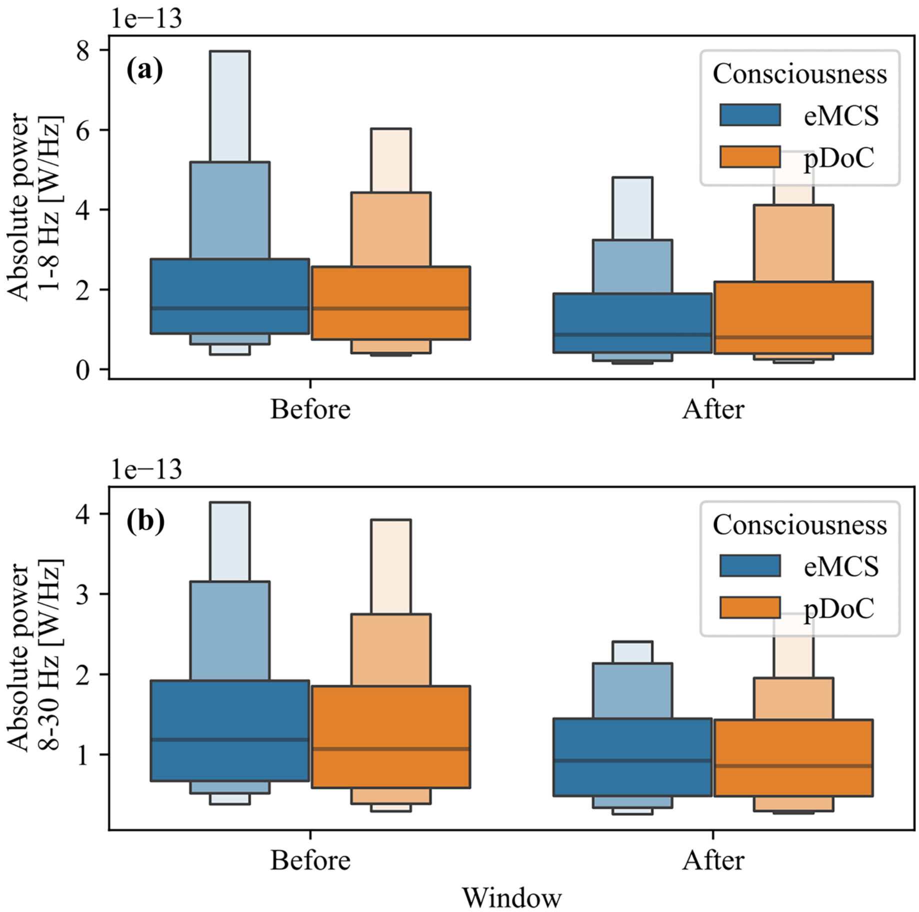

FIGURE 5 EEG absolute power for eMCS and pDoC patients both in the low-frequencies (upper plot) and high-frequencies (lower plots), *Before* and *After* the R-peak. eMCS, emergence from minimally conscious state; pDoC, prolonged disorder of consciousness.

(Figure 9c) was found to decrease *After* the HEP ($p < 0.001$ for both groups). Coherently with their definition, the correlation between node betweenness centrality, closeness centrality and clustering coefficient was found to be strong (Spearman's R: Node-Closeness = 0.611, Node-Clustering = −0.738, Closeness-Clustering = −0.659, all $p < 0.001$). Assortativity (Figure 9d) was found to be significantly lower *After* (median = 0.83, IQR = [0.14]) than *Before* (median = 0.87, IQR = [0.13]) for eMCS patients ($p = 0.008$, $U = 8460.000$), opposite to pDoC (*Before* 0.89 [IQR = 0.11], *After* 0.93 [IQR = 0.08], $p = 0.001$, $U = 3449.000$). When performing two-way mixed ANOVA, consciousness (between factor) was found to be significantly influencing assortativity ($p_{ADJ} < 0.001$, $F[1] = 30.484$, $\eta^2 = 0.125$) as well as the interaction consciousness × window ($p_{ADJ} = 0.001$, $F[1] = 12.812$, $\eta^2 = 0.056$).

Global efficiency (Figure 9e) was found to be significantly higher ($p < 0.001$, $U = 9167.000$) in eMCS patients *Before* (median 0.488 [IQR = 0.022]) compared with *After* (median 0.479 [IQR = 0.027]), whereas non-significant in pDoC patients ($p = 0.158$). Focusing only on global efficiency *Before*, a receiver operating characteristic (ROC) analysis resulted in an AuRoC = 0.79 (with trapezoidal rule, Figure 9F). Importantly, when performing two-way mixed ANOVA, besides consciousness ($p_{ADJ} < 0.001$) and window ($p_{ADJ} < 0.001$), also the interaction consciousness × window was found to be significant ($p_{ADJ} < 0.001$, $F[1] = 55.716$, $\eta^2 = 0.206$).

4 | DISCUSSION

In this work, we proposed and tested a preliminary pipeline that uses the evoked cortical response of peripheral rhythms (i.e., heart cycle) to evaluate whether such measures can be considered correlated with (un)consciousness. To achieve this, we compared spectral estimates, topological descriptors, the PCI and graph metrics of the HEP (*Before* and *After*) between pDoC and eMCS patients.

Initially, we validated previous results on HEP timing (Luft & Bhattacharya, 2015; Park & Thayer, 2014) in healthy controls (i.e., latency of the GFP) with the HEP reaching its maximum peak approximately 0.2 s after the R-peak, with a potential reaching the left side of the brain first (Figure 6a/b). The higher peak of the GFP is coherent with the fact that the evoked response of a more complex (*healthy*) brain results in a more integrated (and differentiated) response (Hudson & Pryor, 2016; Stein &

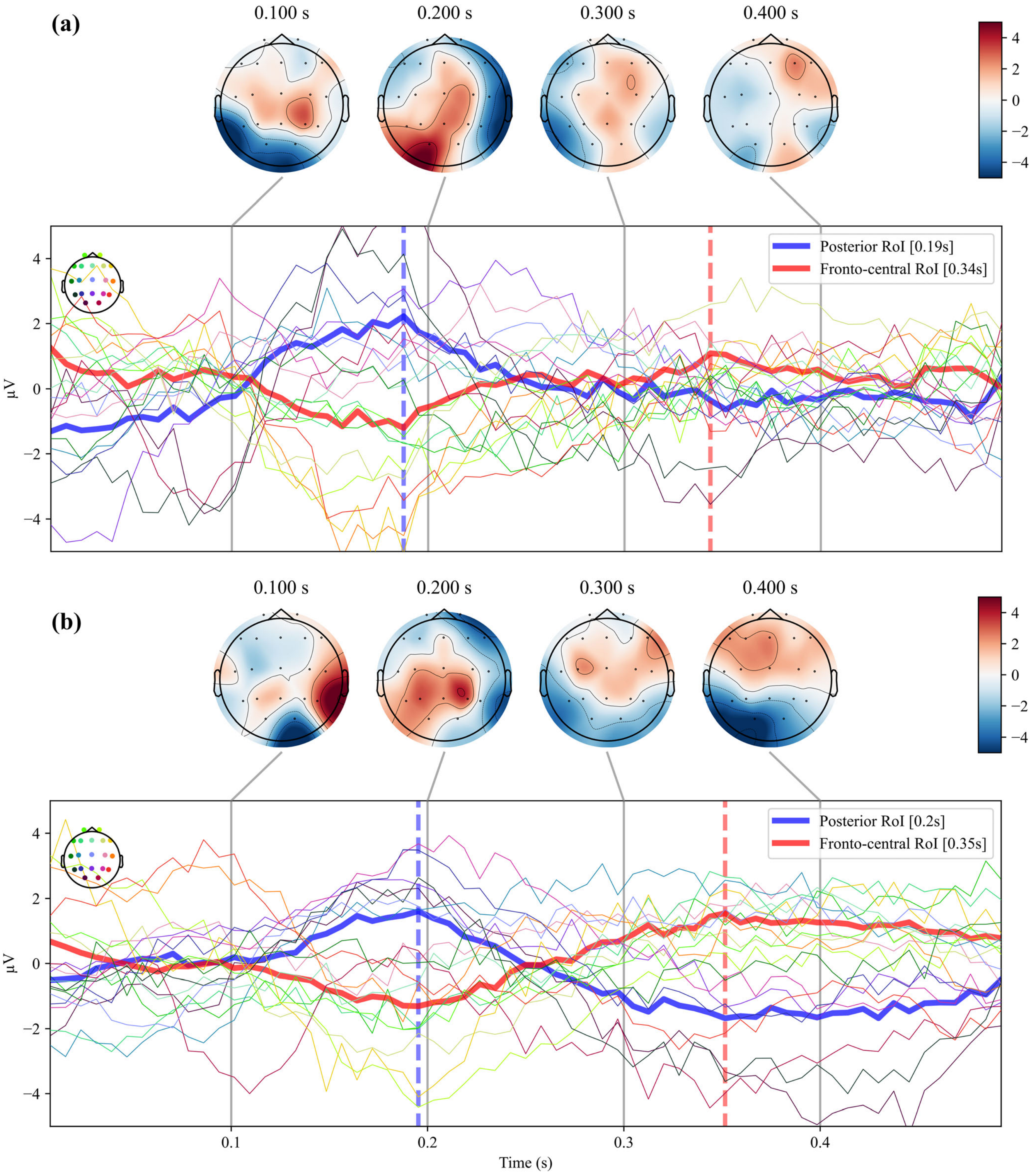

FIGURE 6 HEP representation for eMCS (panel a) and pDoC (panel b) patients. In each panel, a topographic interpolation at 0.1 s steps is reported together with the individual channel's activity. With thicker blue and red lines, the average of posterior and anterior channels is shown. eMCS, emergence from minimally conscious state; pDoC, prolonged disorder of consciousness; RoI, region of interest.

Peelen, 2021; Tononi, 2005), thus generating higher *variability* across channels (i.e., the GFP), enforcing previous results on pDoC patients on HEP (Candia-Rivera et al., 2023) or other evoked potentials (Piarulli et al., 2015; Tzovara et al., 2015). However, the ascendant pathways to the CNS conducting and delivering the heart depolarization to the brain are not damaged in sABI patients as when the structural impairment affects also

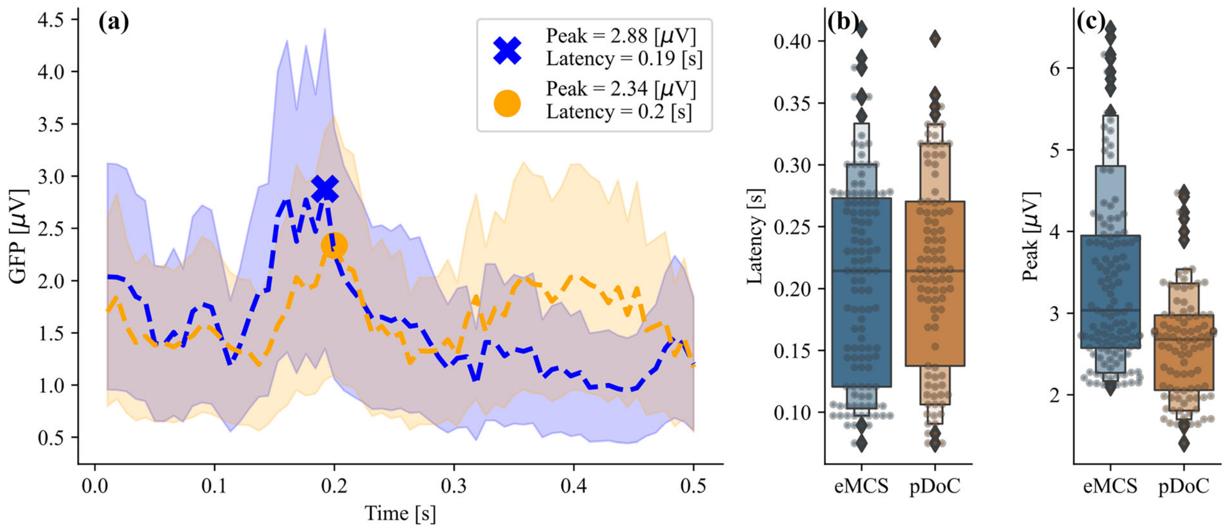

FIGURE 7 GFP estimate (panel a) in pDoC (blue) and eMCS (orange) patients. Box plot of latency and peak values in μV are reported respectively in panels (b) and (c). eMCS, emergence from minimally conscious state; GFP, global field power; pDoC, prolonged disorder of consciousness.

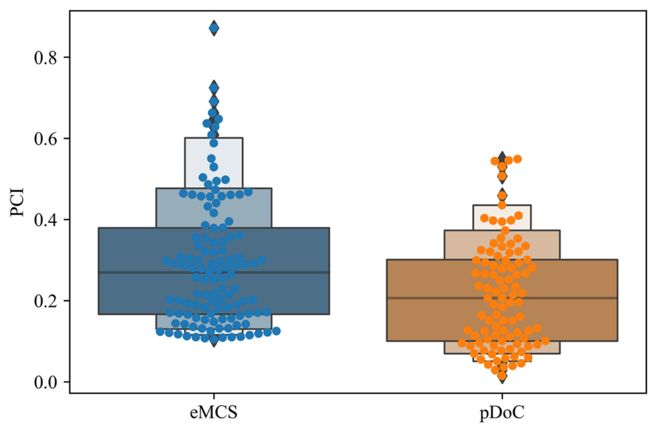

FIGURE 8 PCI values of pDoC (orange) and eMCS (blue) patients. eMCS, emergence from minimally conscious state; PCI, erturbational Complexity Index; pDoC, prolonged disorder of consciousness.

the brainstem. This is confirmed by the fact the HEP mean latency does not differ between pDoC and eMCS. But, the variability of the HEP peaks' latency across patients (from ~0.15 to 0.4 s after the R-peak) introduces a fundamental constraint to the choice of the window, combined with the necessity of using equally long windows to avoid metric dependences from the number of samples. Therefore, most HEP-related depolarization occurs in the *After* window (0.25 to 0.5 s, after the maximal HEP peak (~0.2 s), whereas the baseline signal can be found in the *Before* (or *Much After*) window. Nevertheless, even if *much after*, it is physiologically impossible to obtain a window where the effect of the HEP is completely absent since it would imply an EEG recording during no cardiac activity.

The complexity of the evoked response (PCI) could distinguish the two cohorts at the group level, with eMCS having higher values. Nevertheless, comparing TEP and HEP shows a significant difference in amplitude and in the number of phasic modes, both higher when the stimuli are the TMS (Edlow et al., 2023; Ort et al., 2023). Furthermore, in most PCI studies, the *baseline* window is notably longer than the *response* window and does not include any transitory effects of the evoked potential thanks to the proper stimulus trigger (Comolatti et al., 2019). All this makes impossible to compare HEP- and TEP-derived PCI values in absolute value.

Confirmation of previous findings (i.e., higher integration and efficiency in eMCS patients; Chennu et al., 2017; Rizkallah et al., 2019) were found with graph theory metrics. However, node and closeness centrality, proxies of the importance of node removal, increased *After* the HEP peak, coherent with the focal nature of an evoked potential. Furthermore, the HEP acts as a soft desynchronization stimulus disrupting local clusters and resting-state thalamocortical networks (i.e., ABCD model; Forgacs et al., 2017; Forgacs et al., 2022). Therefore, in a more efficient network at baseline (eMCS *Before*), the HEP desynchronization and resynchronization (from *After* to *Before*) are significant, while leaving unchanged a less efficient one (pDoC). Assortativity is the preference for a network's nodes to connect to similar ones, with connections' similarity determined by the connectivity function (Oujamaa et al., 2023). Thus, the instability of brain hubs at unconsciousness operated via more homogeneous and more structurally constrained local dynamics (Achard et al., 2012; Chennu et al., 2014; Forgacs et al., 2017;

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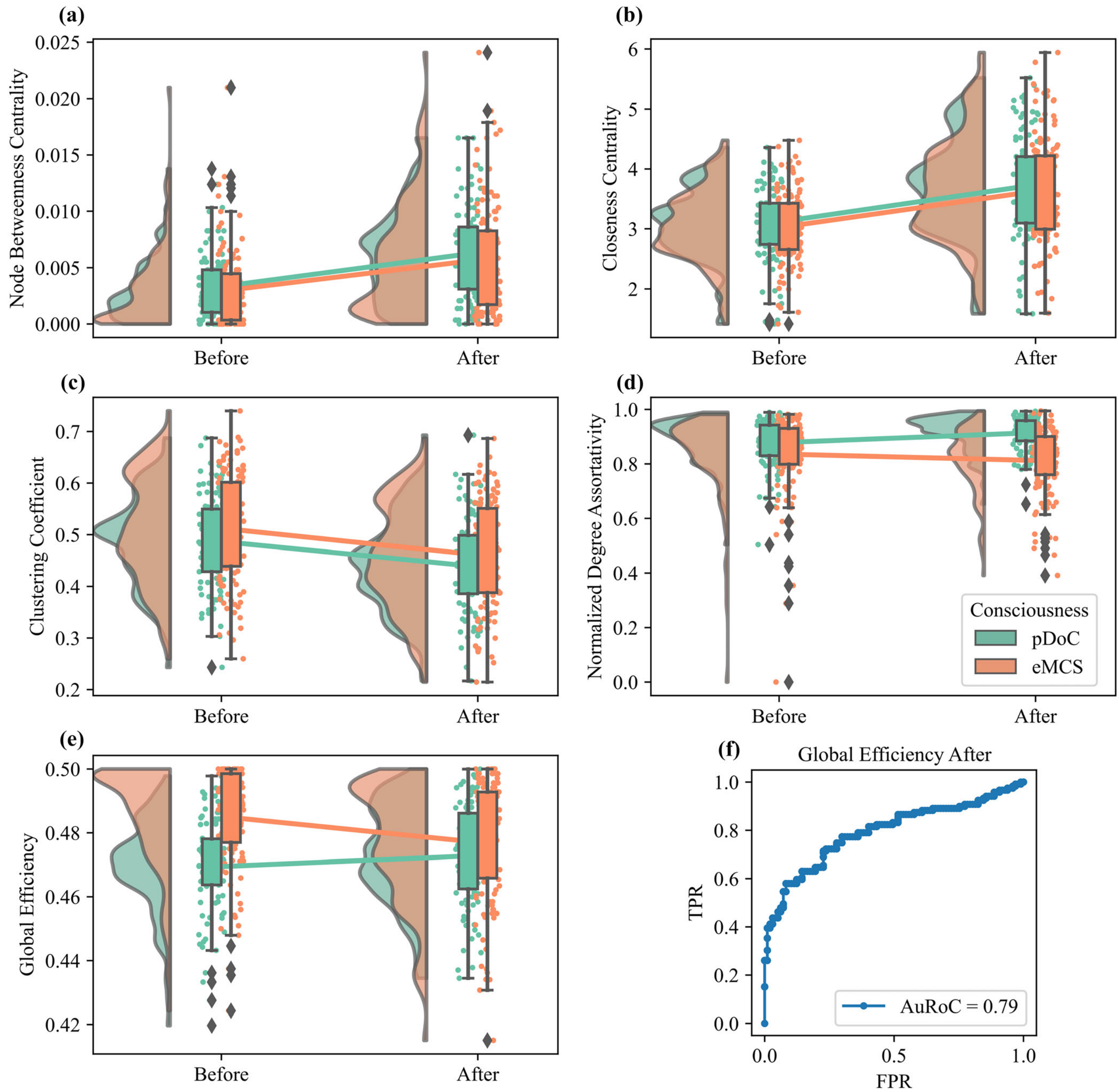

FIGURE 9 RainCloud plots (kernel density estimate on the left and box plot with superimposed swarm plot on the right) of graph metrics (panels a to e). AuRoC considering the global efficiency *After* capability of distinguishing pDoC and eMCS patients is reported in panel (f). AuRoC, area under the receiver operating characteristic; eMCS, emergence from minimally conscious state; KDE, kernel density estimate; pDoC, prolonged disorder of consciousness.

Forgacs et al., 2022) are coherent with the stronger tendency of pDoC patients to form connections following *similarity* instead than *functionality*.

Nevertheless, any of the measures computed in the manuscript are yet ready to be used at the single-subject level and may not be viewed as a diagnostic criterion of consciousness but should be used as an additional support to the behavioural/neuroimaging assessment. The derived pipeline, differently from the TMS-EEG paradigms, suffers from a moderate inter-subject variability, which may be due to a number of (bidirectional) factors influencing the HEP. In particular, attention and arousal deficits (Wang et al., 2020), impairments in the sensory-motor pathways (Thibaut et al., 2014) and a number of dysautonomic behaviours in sABI patients (Riganello et al., 2015) may influence in a patient-specific manner of the analysis of evoked potentials, which are not centrally *induced* as done by the TMS.

However, compared with the TMS, even if the HEP does not specifically aim at desynchronizing brain networks, it has a similar effect of the insertion of a magnetic dipole within an electrical network overcoming some of the disadvantages of the TMS. Seizures (very common in sABI patients; Pease et al., 2022), the presence of pacemakers or implanted metal objects, craniectomies (Hakiki et al., 2021) and overall clinical complexity often limit its use in the everyday clinical practice.

Among the limitations of this work, the monocentric nature of the study must be acknowledged, which inherently calls for external validations. However, the large sample size used and the evidence obtained from the analysis allow us to moderately infer results to the general population. Also, whether high-density EEG set-ups may increase the diagnostic performances of ECG-locked graph metrics has to be tackled in further studies, despite 19-channel set-ups have been proven to be fully acceptable for quantitative EEG analysis and graph theory (Miraglia et al., 2021). Concerning the choice of the connectivity metric, we need to acknowledge that MI may suffer from volume conduction effects, and further work is required using more refined metrics that are less affected by volume conduction. However, the large sample size allows us to consider the results valid and the fact that already a sub-optimal set-up provides valuable insights into the phenomenon corroborates the stability of the approach. The moderate sampling frequency of the EEG recording (128 Hz) allowed us only to estimate the *Before/After* condition, without performing analysis in smaller bins (three or more) and impaired the computation of frequency-specific connectivity patterns, specifically at low-frequencies. Higher sampling rates may allow for the use of event-related desynchronization technique (i.e., the study of time-dependent power changes) to confirm such findings. Lastly, the parameters adopted to calculate PCI_{st} followed the ones used in literature for TMS-EEG paradigms. However, given the dependence of the PCIst values on the parameters used and on the noise levels of the response/baseline windows, further exploration should be done concerning the best parametrization needed to compute PCI_{st} after the HEP.

5 | CONCLUSION

Our work poses preliminary steps into the use of the HEP as a complementary information guiding behavioural diagnosis. Such an approach opens a new perspective on the diagnosis of consciousness levels by offering a safe, low-cost and bedside support, based on innate and causally related proxies of brain efficiency.

The developed algorithms, without making use of less accessible techniques as fMRI/PET or invasive stimulations such as TMS/tDCS, may easily be integrated into daily clinical and neurophysiological assessments. Finally, we show a profitable way to complement consciousness assessment by including the ANS-CNS interaction within the loop.

AUTHOR CONTRIBUTIONS

Piergiuseppe Liuzzi: Conceptualization; formal analysis; methodology; software; writing—original draft. **Tiziano Cassioli:** Formal analysis; investigation; software. **Sara Secci:** Formal analysis; writing—original draft. **Bahia Hakiki:** Conceptualization; investigation; supervision; writing—review and editing. **Maenia Scarpino:** Data curation; investigation. **Rachele Burali:** Data curation; investigation. **Azzurra Di Palma:** Validation; writing—original draft. **Tanita Toci:** Data curation; investigation. **Antonello Grippo:** Conceptualization; project administration; resources; writing—review and editing. **Francesca Cecchi:** Funding acquisition; project administration; writing—review and editing. **Andrea Frosini:** Conceptualization; supervision; writing—review and editing. **Andrea Mannini:** Project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have to be declared.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data and code required to reproduce the results are provided together with the manuscript. Raw EEG recordings can be provided for research purposes upon reasonable request to the corresponding author.

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REFERENCES

Achard, S., Delon-Martin, C., Vértes, P. E., Renard, F., Schenck, M., Schneider, F., Heinrich, C., Kremer, S., & Bullmore, E. T. (2012). Hubs of brain functional networks are radically reorganized in comatose patients. *Proceedings of the National Academy of Sciences*, *109*(50), 20608–20613. https://doi.org/10.1073/pnas.1208933109

Bayne, T., Hohwy, J., & Owen, A. M. (2017). Reforming the taxonomy in disorders of consciousness. *Annals of Neurology*, *82*(6), 866–872. https://doi.org/10.1002/ana.25088

Bigdely-Shamlo, N., Mullen, T., Kothe, C., Su, K.-M., & Robbins, K. A. (2015). The PREP pipeline: Standardized preprocessing for large-scale EEG analysis. *Frontiers in Neuroinformatics*, *9*(16). https://doi.org/10.3389/fninf.2015.00016

Bredart, S., Delchambre, M., & Laureys, S. (2006). One's own face is hard to ignore. *Quarterly Journal of Experimental Psychology*, *59*, 46–52. https://doi.org/10.1080/17470210500343678

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*(7), 1373–1384. https://doi.org/10.1007/s00415-011-6114-x

Candia-Rivera, D., Annen, J., Gosseries, O., Martial, C., Thibaut, A., Laureys, S., & Tallon-Baudry, C. (2021). Neural responses to heartbeats detect residual signs of consciousness during resting state in postcomatose patients. *The Journal of Neuroscience*, *41*, 5251–5262. https://doi.org/10.1523/JNEUROSCI.1740-20.2021

Candia-Rivera, D., Raimondo, F., Pérez, P., Naccache, L., Tallon-Baudry, C., & Sitt, J. D. (2023). Conscious processing of global and local auditory irregularities causes differentiated heartbeat-evoked responses. *eLife*, *12*, e75352. https://doi.org/10.7554/eLife.75352

Casarotto, S., Comanducci, A., Rosanova, M., Sarasso, S., Fecchio, M., Napolitani, M., Pigorini, A., G. Casali, A., 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

Chennu, S., Annen, J., Wannez, S., Thibaut, A., Chatelle, C., Cassol, H., Martens, G., Schnakers, C., Gosseries, O., Menon, D., & Laureys, S. (2017). Brain networks predict metabolism, diagnosis and prognosis at the bedside in disorders of consciousness. *Brain: a Journal of Neurology*, *140*, 2120–2132. https://doi.org/10.1093/brain/awx163

Chennu, S., Finoia, P., Kamau, E., Allanson, J., Williams, G. B., Monti, M. M., Noreika, V., Arnatkeviciute, A., Canales-Johnson, A., Olivares, F., Cabezas-Soto, D., Menon, D. K., Pickard, J. D., Owen, A. M., & Bekinschtein, T. A. (2014). Spectral signatures of reorganised brain networks in disorders of consciousness. *PLoS Computational Biology*, *10*, e1003887. https://doi.org/10.1371/journal.pcbi.1003887

Comolatti, R., Pigorini, A., Casarotto, S., Fecchio, M., Faria, G., Sarasso, S., Rosanova, M., Gosseries, O., Boly, M., Bodart, O., Ledoux, D., Brichant, J. F., Nobili, L., Laureys, S., Tononi, G., Massimini, M., & Casali, A. G. (2019). A fast and general method to empirically estimate the complexity of brain responses to transcranial and intracranial stimulations. *Brain Stimulation.*, *12*(5), 1280–1289. https://doi.org/10.1016/j.brs.2019.05.013

Cruse, D., Fattizzo, M., Owen, A., & Fernández-Espejo, D. (2017). Why use a mirror to assess visual pursuit in prolonged disorders of consciousness? Evidence from healthy control participants. *BMC Neurology*, *17*, 14. https://doi.org/10.1186/s12883-017-0798-1

Demertzi, A., Antonopoulos, G., Heine, L., Voss, H. U., Crone, J. S., de Los Angeles, C., Bahri, M. A., di Perri, C., Vanhaudenhuyse, A., Charland-Verville, V., Kronbichler, M., Trinka, E., Phillips, C., Gomez, F., Tshibanda, L., Soddu, A., Schiff, N. D., Whitfield-Gabrieli, S., & Laureys, S. (2015). Intrinsic functional connectivity differentiates minimally conscious from unresponsive patients. *Brain: a Journal of Neurology*, *138*, 2619–2631. https://doi.org/10.1093/brain/awv169

Edlow, B. L., Fecchio, M., Bodien, Y. G., Comanducci, A., Rosanova, M., Casarotto, S., Young, M. J., Li, J., Dougherty, D. D., Koch, C., Tononi, G., Massimini, M., & Boly, M. (2023). Measuring consciousness in the intensive care unit. *Neurocritical Care*, *38*(3), 584–590. https://doi.org/10.1007/s12028-023-01706-4

Engemann, D., 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: a Journal of Neurology*, *141*, 3179–3192. https://doi.org/10.1093/brain/awy251

Forgacs, P. B., Allen, B. B., Wu, X., Gerber, L. M., Boddu, S., Fakhar, M., Stieg, P. E., Schiff, N. D., & Mangat, H. S. (2022). Corticothalamic connectivity in aneurysmal subarachnoid hemorrhage: Relationship with disordered consciousness and clinical outcomes. *Neurocritical Care*, *36*(3), 760–771. https://doi.org/10.1007/s12028-021-01354-6

Forgacs, P. B., Frey, H. P., Velazquez, A., Thompson, S., Brodie, D., Moitra, V., Rabani, L., Park, S., Agarwal, S., Falo, M. C., Schiff, N. D., & Claassen, J. (2017). Dynamic regimes of neocortical activity linked to corticothalamic integrity correlate with outcomes in acute anoxic brain injury after cardiac arrest. *Annals of Clinical Translational Neurology*, *4*(2), 119–129. https://doi.org/10.1002/acn3.385

Formisano, R., Contrada, M., Aloisi, M., Ferri, G., Schiattone, S., Iosa, M., & Buzzi, M. G. (2019). Nociception Coma Scale with personalized painful stimulation versus standard stimulus in non-communicative patients with disorders of consciousness. *Neuropsychological Rehabilitation*, *30*, 1–12. https://doi.org/10.1080/09602011.2019.1614464

Formisano, R., Contrada, M., Iosa, M., Ferri, G., Schiattone, S., & Aloisi, M. (2019). Coma recovery scale-revised with and without the emotional stimulation of caregivers. *Canadian Journal of Neurological Sciences*, *46*, 1–5. https://doi.org/10.1017/cjn.2019.227

Freeden, W. (1984). Spherical spline interpolation—Basic theory and computational aspects. *Journal of Computational and Applied Mathematics*, *11*(3), 367–375. https://doi.org/10.1016/0377-0427(84)90011-6

Giacino, J., Kalmar, K., & Whyte, J. (2005). 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

Gramfort, A., Luessi, M., Larson, E., Engemann, D. A., Strohmeier, D., Brodbeck, C., Goj, R., Jas, M., Brooks, T., Parkkonen, L., & Hämäläinen, M. (2013). MEG and EEG data analysis with MNE-python. *Frontiers in Neuroscience*, *7*, 267. https://doi.org/10.3389/fnins.2013.00267

Hagberg, A. A., Schult, D. A., & Swart, P. J. (2008). Exploring network structure, dynamics, and function using NetworkX. In G. Varoquaux, T. Vaught, & J. Millman (Eds.), *Proceedings of the 7th python in science conference* (pp. 11–15). Pasadena.

Hakiki, B., Cecchi, F., Pancani, S., Romoli, A., Draghi, F., Scarpino, M., Sterpu, R., Mannini, A., Macchi, C., & Grippo, A. (2022). Critical illness polyneuropathy and myopathy and clinical detection of the recovery of consciousness in severe acquired brain injury patients with disorders of consciousness after rehabilitation. *Diagnostics*, *12*(2), 516. https://doi.org/10.3390/diagnostics12020516

Hakiki, B., Donnini, I., Romoli, A. M., Draghi, F., Maccanti, D., Grippo, A., Scarpino, M., Maiorelli, A., Sterpu, R., Atzori, T., Mannini, A., Campagnini, S., Bagnoli, S., Ingannato, A., Nacmias, B., de Bellis, F., Estraneo, A., Carli, V., Pasqualone, E., … Cecchi, F. (2022). Clinical, neurophysiological, and genetic predictors of recovery in patients with severe acquired brain injuries (PRABI): A study protocol for a longitudinal observational study. *Frontiers in Neurology*, *13*, 711312. https://doi.org/10.3389/fneur.2022.711312

Hakiki, B., Liuzzi, P., Pansini, G., Pancani, S., Romoli, A., Draghi, F., Orlandini, S., Mannini, A., Della Puppa, A., Macchi, C., & Cecchi, F. (2021). Impact of decompressive craniectomy on functional outcome of severe acquired brain injuries patients, at discharge from intensive inpatient rehabilitation. *Disability and Rehabilitation*, *44*, 1–7. https://doi.org/10.1080/09638288.2021.2015461

Hermann, B., Goudard, G., Courcoux, K., Valente, M., Labat, S., Despois, L., Bourmaleau, J., Richard-Gilis, L., Faugeras, F., Demeret, S., Sitt, J. D., Naccache, L., Rohaut, B., & Pitié-Salpêtrière hospital Neuro-ICU. (2019). Wisdom of the caregivers: Pooling individual subjective reports to diagnose states of consciousness in brain-injured patients, a monocentric prospective study. *BMJ Open*, *9*, e026211. https://doi.org/10.1136/bmjopen-2018-026211

Hudson, A. E., & Pryor, K. O. (2016). Integration and information: Anesthetic unconsciousness finds a new bandwidth. *Anesthesiology*, *125*(5), 832–834. https://doi.org/10.1097/ALN.0000000000001344

Laureys, S., Celesia, G. G., Cohadon, F., Lavrijsen, J., León-Carrión, J., Sannita, W. G., Sazbon, L., Schmutzhard, E., von Wild, K., Zeman, A., Dolce, G., & European Task Force on Disorders of Consciousness. (2010). Unresponsive wakefulness syndrome: A new name for the vegetative state or apallic syndrome. *BMC Medicine*, *8*, 68. https://doi.org/10.1186/1741-7015-8-68

Laureys, S., Perrin, F., & Bredart, S. (2007). Self-consciousness in non-communicative patients. *Consciousness and Cognition*, *16*, 722–741; discussion 742. https://doi.org/10.1016/j.concog.2007.04.004

Li, A., Feitelberg, J., Saini, A., Höchenberger, R., & Scheltienne, M. (2022). MNE-ICALabel: Automatically annotating ICA components with ICLabel in Python. *Journal of Open Source Software*, *7*, 4484. https://doi.org/10.21105/joss.04484

Liuzzi, P., Campagnini, S., Hakiki, B., Burali, R., Scarpino, M., Macchi, C., Cecchi, F., Mannini, A., & Grippo, A. (2023). Heart rate variability for the evaluation of patients with disorders of consciousness. *Clinical Neurophysiology*, *150*, 31–39. https://doi.org/10.1016/j.clinph.2023.03.007

Liuzzi, P., Grippo, A., Campagnini, S., Scarpino, M., Draghi, F., Romoli, A., Hakiki, B., Sterpu, R., Maiorelli, A., Macchi, C., Cecchi, F., Carrozza, M. C., & Mannini, A. (2022). Merging clinical and EEG biomarkers in an elastic-net regression for disorder of consciousness prognosis prediction. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, *30*, 1504–1513. https://doi.org/10.1109/TNSRE.2022.3178801

Liuzzi, P., Grippo, A., Hakiki, B., Macchi, C., Cecchi, F., & Mannini, A. (2022). Can respiration complexity help the diagnosis of disorders of consciousness in rehabilitation? *Accept Acta Neurologica Scandinavica*, *13*, 507. https://doi.org/10.3390/diagnostics13030507

Liuzzi, P., Hakiki, B., Draghi, F., Romoli, A. M., Burali, R., Scarpino, M., Cecchi, F., Grippo, A., & Mannini, A. (2023). EEG fractal dimensions predict high-level behavioral responses in minimally conscious patients. *Journal of Neural Engineering*, *20*, 046038. https://doi.org/10.1088/1741-2552/aceaac

Liuzzi, P., Hakiki, B., Scarpino, M., Burali, R., Maiorelli, A., Draghi, F., Romoli, A. M., Grippo, A., Cecchi, F., & Mannini, A. (2023). Neural coding of autonomic functions in different states of consciousness. *Journal of NeuroEngineering and Rehabilitation*, *20*, 96. https://doi.org/10.1186/s12984-023-01216-6

Luft, C. D. B., & Bhattacharya, J. (2015). Aroused with heart: Modulation of heartbeat evoked potential by arousal induction and its oscillatory correlates. *Scientific Reports*, *5*(1), 15717. https://doi.org/10.1038/srep15717

Magri, C., Whittingstall, K., Singh, V., Logothetis, N. K., & Panzeri, S. (2009). A toolbox for the fast information analysis of multiple-site LFP, EEG and spike train recordings. *BMC Neuroscience*, *10*, 81. https://doi.org/10.1186/1471-2202-10-81

Makowski, D., Pham, T., Lau, Z. J., Brammer, J. C., Lespinasse, F., Pham, H., Schölzel, C., & Chen, S. H. A. (2021). NeuroKit2: A Python toolbox for neurophysiological signal processing. *Behavior Research Methods*, *53*(4), 1689–1696. https://doi.org/10.3758/s13428-020-01516-y

McDonnell, E., Giacino, J., & Kolakowsky-Hayner, S. (2015). A brief overview of the coma recovery scale–revised. *The Journal of Head Trauma Rehabilitation*, *30*, 143–145. https://doi.org/10.1097/HTR.0000000000000133

Menon, D., Owen, A., Boniface, S., & Pickard, J. (1998). Cortical processing in persistent vegetative state. *The Lancet*, *352*, 1148–1149. https://doi.org/10.1016/S0140-6736(05)79795-6

Miraglia, F., Tomino, C., Vecchio, F., Alù, F., Orticoni, A., Judica, E., Cotelli, M., & Rossini, P. M. (2021). Assessing the dependence of the number of EEG channels in the brain networks' modulations. *Brain Research Bulletin*, *167*, 33–36. https://doi.org/10.1016/j.brainresbull.2020.11.014

Ort, A., Smallridge, J. W., Sarasso, S., Casarotto, S., von Rotz, R., Casanova, A., Seifritz, E., Preller, K. H., Tononi, G., & Vollenweider, F. X. (2023). TMS-EEG and resting-state EEG applied to altered states of consciousness: Oscillations, complexity, and phenomenology. *iScience*, *26*(5), 106589. https://doi.org/10.1016/j.isci.2023.106589

Oujamaa, L., Delon-Martin, C., Jaroszynski, C., Termenon, M., Silva, S., Payen, J. F., & Achard, S. (2023). Functional hub disruption emphasizes consciousness recovery in severe traumatic brain injury. *Brain Communications*, *5*(6), fcad319. https://doi.org/10.1093/braincomms/fcad319

Owen, A., Coleman, M., Menon, D., Johnsrude, I., Rodd, J., Davis, M., Taylor, K., & Pickard, J. (2005). Residual auditory function in persistent vegetative state: A combined PET and fMRI study. *Neuropsychological Rehabilitation*, *15*, 290–306. https://doi.org/10.1080/09602010443000579

Park, G., & Thayer, J. (2014). From the heart to the mind: Cardiac vagal tone modulates top-down and bottom-up visual perception and attention to emotional stimuli. *Frontiers in Psychology*, *5*, 278. https://doi.org/10.3389/fpsyg.2014.00278

Park, H.-D., Bernasconi, F., Salomon, R., Tallon-Baudry, C., Spinelli, L., Seeck, M., Schaller, K., & Blanke, O. (2018). Neural sources and underlying mechanisms of neural responses to heartbeats, and their role in bodily self-consciousness: An intracranial EEG study. *Cerebral Cortex N. Y. N 1991*, *28*(7), 2351–2364. https://doi.org/10.1093/cercor/bhx136

Park, H.-D., & Tallon-Baudry, C. (2014). The neural subjective frame: From bodily signals to perceptual consciousness. *Philosophical Transactions of the Royal Society Series B, Biological Sciences*, *369*(1641), 20130208. https://doi.org/10.1098/rstb.2013.0208

Pease, M., Gonzalez-Martinez, J., Puccio, A., Nwachuku, E., Castellano, J. F., Okonkwo, D. O., & Elmer, J. (2022). Risk factors and incidence of epilepsy after severe traumatic brain injury. *Annals of Neurology*, *92*(4), 663–669. https://doi.org/10.1002/ana.26443

Pernice, R., Zanetti, M., Nollo, G., Cecco, M., Busacca, A., & Faes, L. (2019). Mutual information analysis of brain-body interactions during different levels of mental stress*. In *Conference proceedings: … annual international conference of the IEEE engineering in medicine and biology society* (pp. 6176–6179). IEEE Engineering in Medicine and Biology Society. Conference. https://doi.org/10.1109/EMBC.2019.8856711

Piarulli, A., Charland-Verville, V., & Laureys, S. (2015). Cognitive auditory evoked potentials in coma: Can you hear me? *Brain*, *138*(5), 1129–1137. https://doi.org/10.1093/brain/awv069

Raimondo, F., Rohaut, B., Demertzi, A., Valente, M., Engemann, D. A., Salti, M., Fernandez Slezak, D., Naccache, L., & Sitt, J. D. (2017). Brain-heart interactions reveal consciousness in non-communicating patients. *Annals of Neurology*, *82*, 578–591. https://doi.org/10.1002/ana.25045

Riganello, F., Cortese, M. D., Dolce, G., Lucca, L. F., & Sannita, W. G. (2015). The autonomic system functional state predicts responsiveness in disorder of consciousness. *Journal of Neurotrauma*, *32*(14), 1071–1077. https://doi.org/10.1089/neu.2014.3539

Riganello, F., Larroque, S. K., Bahri, M. A., Heine, L., Martial, C., Carrière, M., Charland-Verville, V., Aubinet, C., Vanhaudenhuyse, A., Chatelle, C., Laureys, S., & di Perri, C. (2018). A heartbeat away from consciousness: Heart rate variability entropy can discriminate disorders of consciousness and is correlated with resting-state fMRI brain connectivity of the central autonomic network. *Frontiers in Neurology*, *9*, 769. https://doi.org/10.3389/fneur.2018.00769

Riganello, F., Zubler, F., Haenggi, M., & De Lucia, M. (2021). Heart rate complexity: An early prognostic marker of patient outcome after cardiac arrest. *Clinical Neurophysiology*, *134*, 27–33. https://doi.org/10.1016/j.clinph.2021.10.019

Rizkallah, J., Annen, J., Modolo, J., Gosseries, O., Benquet, P., Mortaheb, S., Amoud, H., Cassol, H., Mheich, A., Thibaut, A., Chatelle, C., Hassan, M., Panda, R., Wendling, F., & Laureys, S. (2019). Decreased integration of EEG source-space networks in disorders of consciousness. *NeuroImage: Clinical*, *23*, 101841. https://doi.org/10.1016/j.nicl.2019.101841

Scarpino, M., Lolli, F., Hakiki, B., Lanzo, G., Sterpu, R., Atzori, T., Portaccio, E., Draghi, F., Amantini, A., Grippo, A., & for the Intensive Rehabilitation Unit Study Group of the IRCCS Don Gnocchi Foundation, Italy. (2020). EEG and coma recovery scale-revised prediction of neurological outcome in disorder of consciousness patients. *Acta Neurologica Scandinavica*, *142*, 221–228. https://doi.org/10.1111/ane.13247

Scott, D. W. (2009). Sturges' rule. *WIREs Computational Statistics*, *1*(3), 303–306. https://doi.org/10.1002/wics.35

Seel, R. T., American Congress of Rehabilitation Medicine, Brain Injury-Interdisciplinary Special Interest Group, Disorders of Consciousness Task Force, Sherer, M., Whyte, J., Katz, D. I., Giacino, J. T., Rosenbaum, A. M., Hammond, F. M., Kalmar, K., Pape, T. L., Zafonte, R., Biester, R. C., Kaelin, D., Kean, J., & Zasler, N. (2010). Assessment scales for disorders of consciousness: Evidence-based recommendations for clinical practice and research. *Archives of Physical Medicine and Rehabilitation*, *91*(12), 1795–1813. https://doi.org/10.1016/j.apmr.2010.07.218

Sitt, J., 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: a Journal of Neurology*, *137*, 2258–2270. https://doi.org/10.1093/brain/awu141

Soddu, A., Vanhaudenhuyse, A., Bahri, M. A., Bruno, M. A., Boly, M., Demertzi, A., Tshibanda, J. F., Phillips, C., Stanziano, M., Ovadia-Caro, S., Nir, Y., Maquet, P., Papa, M., Malach, R., Laureys, S., & Noirhomme, Q. (2012). Identifying the default-mode component in spatial IC analyses of patients with disorders of consciousness. *Human Brain Mapping*, *33*, 778–796. https://doi.org/10.1002/hbm.21249

Stein, T., & Peelen, M. V. (2021). Dissociating conscious and unconscious influences on visual detection effects. *Nature Human Behaviour*, *5*(5), 612–624. https://doi.org/10.1038/s41562-020-01004-5

Thibaut, A., et al. (2014). Spasticity in disorders of consciousness: A behavioral study. *European Journal of Physical and Rehabilitation Medicine*, *51*, 389–397.

Tononi, G. (2005). Consciousness, information integration, and the brain. In S. Laureys (Ed.), *Progress in brain research* (Vol. 150). The Boundaries of Consciousness: Neurobiology and Neuropathology, vol. 150. (pp. 109–126). Elsevier. https://doi.org/10.1016/S0079-6123(05)50009-8

Tononi, G., Boly, M., Massimini, M., & Koch, C. (2016). Integrated information theory: From consciousness to its physical substrate. *Nature Reviews. Neuroscience*, *17*(7), 450–461. https://doi.org/10.1038/nrn.2016.44

Tzovara, A., Simonin, A., Oddo, M., Rossetti, A. O., & De Lucia, M. (2015). Neural detection of complex sound sequences in the absence of consciousness. *Brain: a Journal of Neurology*, *138*(Pt 5), 1160–1166. https://doi.org/10.1093/brain/awv041

Vanhaudenhuyse, A., Schnakers, C., Bredart, S., & Laureys, S. (2008). Assessment of visual pursuit in post-comatose states: Use a mirror. *Journal of Neurology, Neurosurgery, and Psychiatry*, *79*, 223. https://doi.org/10.1136/jnnp.2007.121624

Wang, J., Hu, X., Hu, Z., Sun, Z., Laureys, S., & Di, H. (2020). The misdiagnosis of prolonged disorders of consciousness by a clinical consensus compared with repeated coma-recovery scale-revised assessment. *BMC Neurology*, *20*, 343. https://doi.org/10.1186/s12883-020-01924-9

Wannez, S., Heine, L., Thonnard, M., Gosseries, O., & Laureys, S. (2017). The repetition of behavioral assessments in diagnosis of disorders of consciousness. *Annals of Neurology*, *81*, 883–889. https://doi.org/10.1002/ana.24962

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