

Stratification of Unresponsive Patients by an Independently Validated Index of Brain Complexity

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Objective: Validating objective, brain-based indices of consciousness in behaviorally unresponsive patients represents a challenge due to the impossibility of obtaining independent evidence through subjective reports. Here we address this problem by first validating a promising metric of consciousness—the Perturbational Complexity Index (PCI)—in a benchmark population who could confirm the presence or absence of consciousness through subjective reports, and then applying the same index to patients with disorders of consciousness (DOCs).

Methods: The benchmark population encompassed 150 healthy controls and communicative brain-injured subjects in various states of conscious wakefulness, disconnected consciousness, and unconsciousness. Receiver operating characteristic curve analysis was performed to define an optimal cutoff for discriminating between the conscious and unconscious conditions. This cutoff was then applied to a cohort of noncommunicative DOC patients (38 in a minimally conscious state [MCS] and 43 in a vegetative state [VS]).

Results: We found an empirical cutoff that discriminated with 100% sensitivity and specificity between the conscious and the unconscious conditions in the benchmark population. This cutoff resulted in a sensitivity of 94.7% in detecting MCS and allowed the identification of a number of unresponsive VS patients (9 of 43) with high values of PCI, overlapping with the distribution of the benchmark conscious condition.

Interpretation: Given its high sensitivity and specificity in the benchmark and MCS population, PCI offers a reliable, independently validated stratification of unresponsive patients that has important physiopathological and therapeutic implications. In particular, the high-PCI subgroup of VS patients may retain a capacity for consciousness that is not expressed in behavior.

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The clinical evaluation of disorders of consciousness (DOCs) in severely brain-injured patients relies on their ability to connect to the surrounding environment and

demonstrate their subjective experience through motor behavior.¹ However, some patients may become unable to respond to stimuli despite still having conscious experiences.^{2,3} This

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may happen because of motor or executive function impairments^{4,5} and/or because of sensory disconnection from the environment.⁶ States of disconnected consciousness can occur in healthy subjects during dreaming⁷ and some forms of anesthesia,⁸ and may result from severe brain injury.⁹ In the latter case, covertly conscious patients may be misdiagnosed as being in a vegetative state (VS).¹⁰

This discrepancy advocates the development of ancillary brain-based measures of consciousness that are independent of sensory processing, motor outputs, and subject participation. With this in mind, a novel metric—the Perturbational Complexity Index (PCI)¹¹—has recently been developed based on a quantification of the electroencephalographic (EEG) responses to transcranial magnetic stimulation (TMS). Inspired by theoretical considerations,^{12,13} PCI directly gauges the ability of many functionally specialized modules of the thalamocortical system (differentiation) to interact rapidly and effectively (integration), thus producing complex patterns of activity. In a reduced sample, PCI has been shown to distinguish between conscious and unconscious subjects,¹¹ prompting further validation and testing especially in VS patients, in whom unequivocal signs of consciousness are lacking by definition. This task, however, is not straightforward; because behavior-based clinical diagnosis may fail to recognize brain-injured patients who are conscious but disconnected and unresponsive, the true state of affairs necessary to define the accuracy and the optimal cutoff for a given brain-based measure of consciousness remains unknown.^{14,15}

Here, we apply PCI to a cohort of 38 minimally conscious state (MCS) and 43 VS patients while attempting to overcome this problem. Hence, we first validate this index on a large benchmark population of 150 subjects who could confirm the presence or absence of conscious experience through immediate or delayed reports.^{9,16} This population included: (1) healthy subjects of different age (range = 18–80 years) and conscious brain-injured patients who were awake and able to communicate; (2) unresponsive subjects who reported no conscious experience upon awakening from non-rapid eye movement (NREM) sleep or midazolam, xenon, or propofol anesthesia; and (3) subjects who were disconnected and unresponsive during rapid eye movement (REM) sleep and ketamine anesthesia but retrospectively reported having had vivid conscious experiences upon awakening. Considering subjects' reports as the provisional gold standard for assessing consciousness (no report = unconscious condition; immediate or delayed report = conscious condition), we perform receiver operating characteristic (ROC) curve analysis. Next, we identify an empirical PCI cutoff that discriminates with

100% accuracy between conscious and unconscious conditions, irrespectively of connectedness, responsiveness, and presence of brain lesions. Finally, we apply this independently validated cutoff to a large population of DOC patients (1) to assess the sensitivity of PCI in detecting MCS patients and (2) to objectively stratify VS patients based on the complexity of their brain responses to TMS.

Materials and Methods

Participants

BENCHMARK POPULATION. The benchmark population consisted of 102 healthy subjects and 48 conscious brain-injured patients. Healthy volunteers with history or presence of major medical/neurological disorders and of drug/alcohol abuse were excluded. All participants underwent neurological screening to exclude those at risk of potential adverse effects of TMS. TMS/EEG data were recorded from healthy subjects (female, $n = 63$; age range = 18–80 years) in the following conditions: (1) while they were unresponsive and did not provide any subjective report upon awakening (NREM sleep, $n = 18$; midazolam sedation at anesthetic concentrations, $n = 6$; anesthesia with xenon, $n = 6$; anesthesia with propofol, $n = 6$); (2) while they were unresponsive but able to provide a delayed subjective report upon awakening (dreaming during REM sleep, $n = 8$; and during ketamine anesthesia, $n = 6$); and (3) while they were awake and able to provide an immediate subjective report ($n = 102$, including 48 subjects also recorded in the previously described unresponsive conditions). The experimental protocols applied during sleep and anesthesia have been detailed elsewhere.^{11,17,18} Brain-injured conscious patients were always recorded during wakefulness and encompassed (1) individuals affected by locked-in syndrome (LIS; $n = 5$); (2) conscious individuals affected by ischemic or hemorrhagic stroke involving subcortical ($n = 16$) or cortical regions ($n = 18$); and (3) individuals who recovered functional communication after a previous DOC (emergence from minimally conscious state [EMCS], $n = 9$). Overall, this large benchmark population included subjects (47 of 150) reported in previous works.^{11,17}

TEST POPULATION. The test population consisted of 81 brain-injured patients (12 previously reported)¹¹ with severe DOC (Supplementary Table). Besides screening for potential adverse effects of TMS, exclusion criteria were medical instability, refractory generalized seizures, and history of neurodegenerative or psychiatric disease. All included patients had anoxic, traumatic, or vascular etiology. Each DOC patient was repeatedly evaluated with the Coma Recovery Scale-Revised (CRS-R)¹ for a period of 1 week (4 times, every other day). Patients showing only reflexive behavior across all evaluations were considered as being in a VS, whereas patients showing signs of nonreflexive behaviors in at least 1 evaluation were considered as minimally conscious (MCS⁺/MCS⁻). The best CRS-R score ensured the detection of minimal signs of consciousness even when behavioral responsiveness was fluctuating. One TMS/EEG recording session was scheduled in the same evaluation

week, at least 20 days after DOC onset and 3 days after withdrawal of sedation.

Experimental Protocol and Data Collection

The experimental protocols were approved by the local ethical committees of the following Institutions: Istituto di Ricovero e Cura a Carattere Scientifico Fondazione Don Gnocchi Onlus, Azienda Socio-Sanitaria Territoriale, Grande Ospedale Metropolitano Niguarda Cà Granda, and Fondazione Europea per la Ricerca Biomedica in Milan, Italy; Medical School of the University of Liège in Liège, Belgium. Written informed consent was obtained from healthy subjects, from communicative patients, and from legal surrogates of DOC patients. EEG responses to TMS were recorded from all participants to compute PCI. In addition, EEG data at rest were collected from DOC patients to characterize their background activity. DOC patients were recorded without sedation, and in case of behavioral signs of drowsiness (e.g., eye closure) recordings were momentarily interrupted to apply the CRS-R arousal facilitation protocols.¹ All experiments were conducted between 9.30 AM and 4.30 PM so as to maximize the chance of having a stable vigilance level. During the recordings, vigilance was assessed according to the arousal subscale of the CRS-R and continuously monitored so as to ascertain that the patients always had their eyes open either spontaneously or with stimulation.

Resting EEG and artifact-free EEG responses to TMS were recorded with a 60-channel TMS-compatible amplifier. EEG recordings were referenced to an additional electrode on the forehead, band-pass filtered between 0.1 and 350Hz, and sampled at 1,450Hz. Two extra sensors were used to record the electrooculogram. During TMS stimulation, participants wore inserted earplugs continuously playing a masking noise that abolishes the auditory potentials elicited by TMS-associated clicks. Structural magnetic resonance (MR) images were always recorded within 1 week prior to the TMS/EEG assessment, to provide reliable anatomical information to the navigated-TMS equipment. Using this navigation system, single TMS pulses were delivered with a focal biphasic stimulator. TMS targets were selected bilaterally within the middle-caudal portion of the superior frontal gyrus (BA6 and BA8) and within the superior parietal lobule (BA7), about 1cm lateral to the midline. These targets were chosen because they are part of a cortical network that has been suggested to be relevant for consciousness^{19–21} and because they are far from the insertion of head muscles that may induce TMS-related artifacts.²² In brain-injured patients, the stimulation of targets affected by cortical lesions identified on individual MR images was deliberately avoided because, in these cases, TMS is ineffective and does not evoke measurable responses.²³ Moreover, in 2 MCS patients, TMS pulses could not be delivered in 3 cortical targets that were close to skull breaches and internal drain placement for safety reasons. Each eligible cortical target was stimulated with an estimated electric field, orthogonal to the gyral crown, of about 120V/m. This intensity, applied to the selected cortical targets, has been shown to produce robust and reproducible EEG responses.^{24,25} If no large muscular or magnetic artifacts were

visible on single-pulse responses, a few tens of trials were averaged and displayed in average reference to check online the presence of an early (0–50 milliseconds) evoked potential with a minimum peak-to-peak amplitude of 10 μ V. In this case, at least 200 trials were recorded and subsequently analyzed for computing PCI offline. Otherwise, stimulation intensity was increased up to 160V/m and at least 200 trials were recorded, provided that muscular or magnetic artifacts were still absent. When these artifacts were unavoidable even after rotating and translating the coil within the same area, the stimulation session at that site was aborted. The same procedure was also applied in case of diffuse postanoxic damage, which was associated with diffuse cortical atrophy.

Data Analysis

PCI COMPUTATION AND ROC ANALYSIS. EEG responses to TMS were visually inspected to reject single trials and channels with bad signal quality. Recording sessions either with <80 good trials or with >10 bad channels were excluded from further analysis. Independent component analysis was applied to reduce ocular and muscular artifacts. The subsequent analysis was fully automatic and accurately complied with the procedure described in Casali et al,¹¹ including 0.1 to 45Hz band-pass filtering, downsampling at 362.5Hz, estimation of cortical current density, and related statistical analysis to extract the deterministic pattern of TMS-evoked responses at the source level. PCI was obtained as the Lempel–Ziv complexity of the matrix of significant cortical source activity, normalized by source entropy, resulting in a positive real number between 0 (minimally complex patterns) and 1 (maximally complex patterns).¹¹ If the percentage of spatiotemporal activations surviving statistical analysis was <1% (corresponding to the maximum rate of false positives), PCI was set to 0, indicating that cortical neurons failed to engage in any significant activation pattern in response to TMS perturbation. The absolute PCI values necessarily depend on the specific data acquisition/analysis protocol (e.g., sampling rate, type of source modeling, statistical analysis) applied in the present as well as in previous^{11,17} studies.

For each individual, the maximum value of PCI (PCI_{max}) was considered for ROC analysis. This choice aims at detecting the presence of cortical islands with high complexity, reflects the need for a univocal brain-based classification parameter as an input to ROC analysis, and parallels the diagnostic use of the best behavioral (CRS-R) score. PCI_{max} values computed in the benchmark population were subdivided into 2 clusters, respectively representing “unconscious condition” (unresponsive individuals who did not provide any subjective report) and “conscious condition” (responsive healthy and brain-injured individuals combined with unresponsive subjects able to provide a delayed subjective report). Based on this dichotomy, ROC curve analysis was applied to PCI_{max} values to identify an optimal empirical cutoff (PCI^*) that discriminates between the unconscious and the conscious conditions. Then, PCI^* was first used to assess the sensitivity of PCI_{max} in objectively detecting MCS patients. Finally, the same independently validated cutoff was employed to slice through and stratify the VS population.

TABLE 1. PCI values in the Benchmark Population

Responsiveness	Report	Condition	PCI				PCI _{max}				Subjects
			No.	Median	Min	Max	No.	Median	Min	Max	
Behavioral unresponsiveness	No report	NREM sleep	31	0.23	0.12	0.31	18	0.25	0.15	0.31	Healthy subjects
Behavioral unresponsiveness	No report	Midazolam	6	0.30	0.23	0.31	6	0.30	0.23	0.31	Healthy subjects
Behavioral unresponsiveness	No report	Xenon	6	0.23	0.11	0.31	6	0.23	0.11	0.31	Healthy subjects
Behavioral unresponsiveness	No report	Propofol	20	0.23	0.13	0.31	6	0.26	0.23	0.31	Healthy subjects
Behavioral unresponsiveness	Delayed report	REM sleep	10	0.48	0.35	0.56	8	0.48	0.36	0.56	Healthy subjects
Behavioral unresponsiveness	Delayed report	Ketamine	6	0.43	0.36	0.52	6	0.43	0.36	0.52	Healthy subjects
Behavioral responsiveness	Immediate report	Wakefulness	314	0.51	0.24	0.70	102	0.53	0.39	0.70	Healthy subjects
Behavioral responsiveness	Immediate report	LIS	14	0.44	0.32	0.60	5	0.47	0.44	0.60	Brain-injured patients
Behavioral responsiveness	Immediate report	Subcortical stroke	52	0.46	0.31	0.61	16	0.52	0.36	0.61	Brain-injured patients
Behavioral responsiveness	Immediate report	Cortical stroke	50	0.40	0.26	0.57	18	0.46	0.34	0.57	Brain-injured patients
Behavioral responsiveness	Immediate report	EMCS	31	0.43	0.30	0.61	9	0.52	0.39	0.61	Brain-injured patients

EMCS = emergence from minimally conscious state; LIS = locked-in syndrome; NREM = non-rapid-eye-movement; PCI = Perturbational Complexity Index; PCI_{max} = individual maximum value of Perturbational Complexity Index; REM = rapid-eye-movement.

QUALITATIVE EEG ASSESSMENT. Continuous resting EEG recordings, re-referenced to the standard double banana montage after 1 to 70Hz band-pass filtering and downsampling to 725Hz, were evaluated according to the clinical neurophysiological descriptors proposed by Forgacs et al,²⁶ namely predominant background EEG frequency, organization of the anteroposterior gradient, and presence of any diffuse/focal slowing. Accordingly, 4 EEG categories with increasing degree of abnormality were defined (i.e., normal, mildly abnormal, moderately abnormal, severely abnormal) and used to classify the resting EEG recording of each patient. This classification was then compared to behavioral diagnosis and PCI_{max} results.

Results

Validation of PCI_{max} in a Large Benchmark Population

Overall, 540 sets of TMS-evoked potentials were analyzed in the benchmark population during different conditions (Table 1) and the corresponding PCI values are displayed in Figure 1A. At the group level, in awake

healthy subjects PCI_{max} was not significantly different (Wilcoxon test, $p = 0.38$) between male ($n = 57$) and female ($n = 45$) and between younger (<50 years, $n = 80$) and older (≥ 50 years, $n = 22$) subjects (Wilcoxon test, $p = 0.25$). To evaluate a possible effect of the stimulation site, we selected a subgroup of healthy awake subjects ($n = 50$) who were stimulated at least once in both frontal and parietal sites. In this subset, we found that the likelihood of finding maximum complexity values (PCI_{max}) did not differ between frontal and parietal sites (z test for proportions, $p = 0.30$). Instead, we found a significant reduction of complexity (PCI_{max}) at the group level (Wilcoxon test, $p < 0.016$) in conscious brain-injured patients (LIS, stroke, and EMCS; median = 0.48, range = 0.34–0.61) as compared to healthy awake controls (median = 0.53, range = 0.39–0.70).

At the individual level, PCI_{max} was invariably higher in the conscious (as assessed through immediate or delayed reports) as compared to the unconscious (no

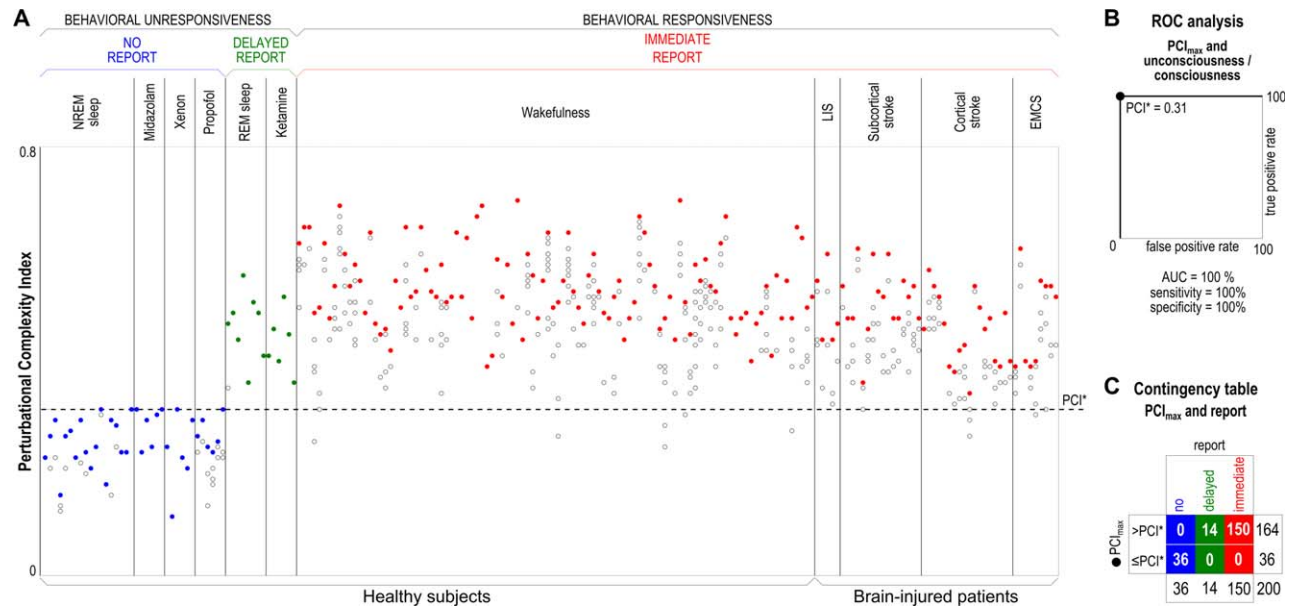


FIGURE 1: (A) Each circle represents the Perturbational Complexity Index (PCI) value computed from the cortical responses to transcranial magnetic stimulation (TMS) of one stimulation site. Several PCI values computed in each individual are aligned along vertical columns. PCI values are computed from TMS-evoked potentials recorded in healthy subjects and conscious brain-injured patients during different conditions. Individuals are grouped by condition, and within each condition are sorted by increasing age. For each individual, the maximum PCI value (PCI_{max}) is represented by a solid circle, whereas lower PCI values are represented by open circles. During non-rapid eye movement (NREM) sleep and anesthesia with midazolam, xenon, and propofol, subjects were behaviorally unresponsive and did not provide any report upon awakening. During dreaming and ketamine anesthesia, subjects were behaviorally unresponsive but provided delayed subjective reports upon awakening. During wakefulness, both healthy subjects and conscious brain-injured patients could immediately report their subjective experience. (B) Receiver operating characteristic (ROC) curve analysis applied to PCI_{max} values for computing the optimal cutoff (PCI* = 0.31) that discriminates between unconsciousness (as assessed through the absence of any subjective report) and consciousness (as assessed through the presence of either an immediate or a delayed subjective report). Area under the curve (AUC) is 100%; using PCI* as a cutoff, sensitivity and specificity both result in 100%. (C) Contingency table obtained by slicing through the PCI_{max} values with PCI*, also highlighted by a dashed horizontal line in panel A. EMCS = emergence from minimally conscious state; LIS = locked-in syndrome; REM = rapid eye movement. [Color figure can be viewed at wileyonlinelibrary.com]

report) conditions (see Fig 1A). Although a few PCI values computed during conscious wakefulness (13 of 461 measurements) fell within the distribution obtained during the unconscious condition, the corresponding distributions of PCI_{max} did not overlap. Thus, ROC curve analysis applied to PCI_{max} resulted in 100% area under the curve and yielded an empirical cutoff PCI* of 0.31, which discriminated between the unconscious and the conscious conditions with 100% sensitivity and 100% specificity (see Fig 1B, C). Crucially, this accuracy was obtained irrespectively of behavioral responsiveness, age, gender, stimulation site, and presence of brain lesions.

Clinical Assessment of DOC Patients

The DOC population consisted of 38 MCS and 43 VS patients diagnosed according to the best CRS-R score. The best total score was significantly lower in the VS as compared to the MCS cohort (Wilcoxon test, *p* < 0.0001) and in the MCS⁻ (*n* = 21) as compared to the MCS⁺ subgroup (*n* = 17; Wilcoxon test, *p* < 0.001). To rule out a possible

unbalance of vigilance between MCS and VS patients, we analyzed the arousal subscale of the CRS-R administered during the recordings. All patients were scored either 1 (i.e., eye opening with stimulation) or 2 (i.e., eye opening without stimulation); although the proportion of VS patients with vigilance score = 1 was slightly higher than that of MCS patients, the difference between these cohorts was not significant (*z* test for proportions, *p* = 0.07).

Etiology distribution was not significantly different in the VS and MCS cohorts as assessed by *z* test for proportions (postanoxic damage: 18 VS and 9 MCS patients, *p* = 0.07 with a slight prevalence in the VS cohort; traumatic insult: 14 VS and 12 MCS patients, *p* = 0.93; vascular lesion: 11 VS and 17 MCS, *p* = 0.084 with a slight prevalence in the MCS cohort). Splitting patients into subacute and chronic subgroups based on the time lag (shorter or longer than 3 months, respectively) between DOC onset and TMS/EEG recording, we did not observe a significantly different prevalence between VS (15 subacute and 28 chronic patients) and MCS (10 subacute and 28 chronic patients) cohorts (*z* test for proportions, *p* = 0.41).

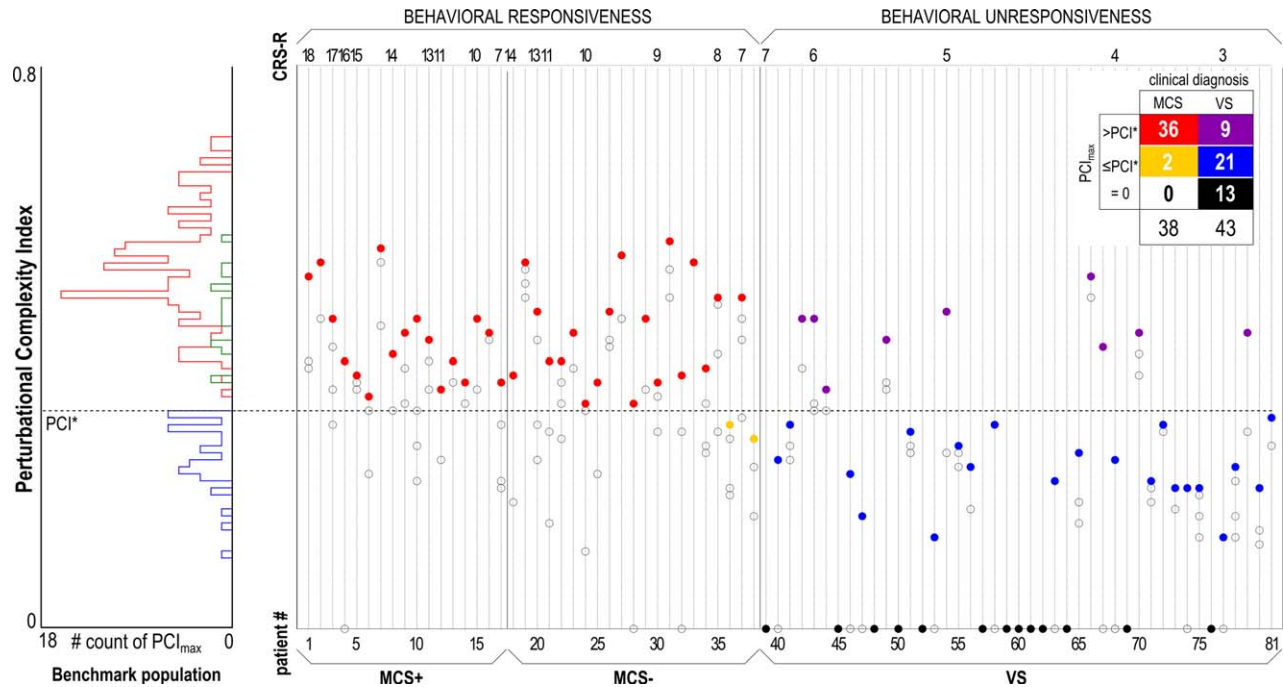


FIGURE 2: The histogram (left) summarizes the distribution of maximum Perturbational Complexity Index values (PCI_{max}) in the benchmark population, specifically obtained in the absence of subjective report (blue) and in the presence of subjective report (delayed, green; immediate, red) conditions. The dashed horizontal line highlights the optimal cutoff (PCI^*) computed from receiver operating characteristic curve analysis on the benchmark population. The scatter plot (right) shows all the PCI values obtained in minimally conscious state (MCS^+ / MCS^-) and vegetative state (VS) patients. The PCI values computed in each patient (2–4 values) are aligned along vertical columns. Within each diagnostic group, patients are sorted by the Coma Recovery Scale-Revised (CRS-R) total score in decreasing order. For each patient, the PCI_{max} is represented by a color-filled circle, whereas lower PCI values are represented by empty circles. The contingency table (right upper corner) is obtained by slicing through the PCI_{max} values with PCI^* and shows that 36 MCS patients resulted in $PCI_{max} > PCI^*$ (red), whereas in 2 MCS^- patients PCI_{max} was lower than PCI^* (yellow). In addition, VS patients could be divided into 3 subgroups according to PCI_{max} : 9 patients with $PCI_{max} > PCI^*$ (purple), 21 patients with $PCI_{max} \leq PCI^*$ (blue), and 13 patients with $PCI_{max} = 0$ (black).

Sensitivity of PCI_{max} in Detecting MCS Patients

Overall, 254 sets of TMS-evoked potentials were analyzed in the DOC population and the corresponding PCI values are displayed in Figure 2. In all patients, 4 TMS targets were selected from the superior frontal gyrus and superior parietal lobule bilaterally. Based on previous studies,²³ TMS was not targeted on cortical lesions because it would have failed to elicit any significant brain response. As a result, at least 2 PCI values were actually computed in each patient (except for Patient 33; see Supplementary Table); on average, the number of PCI measurements was 3.30 and 2.95 in the VS and MCS cohorts, respectively. At the group level, PCI_{max} was lower in MCS patients (median = 0.40, range = 0.27–0.55) as compared to conscious brain-injured patients (Wilcoxon test, $p < 0.0001$). However, PCI_{max} was not significantly different between MCS^+ and MCS^- patients (Wilcoxon test, $p = 0.92$).

To assess the sensitivity of PCI_{max} in detecting patients showing minimal but unequivocal behavioral signs of consciousness, we sliced through the PCI_{max} distribution of the MCS cohort with the empirical cutoff

derived from the benchmark population. Considering all measurements, we found that the stimulation of some cortical sites could result in PCI values lower than PCI^* (37 of 112 measurements). Crucially, however, PCI_{max} was higher than PCI^* in 36 of 38 patients, indicating that PCI_{max} had a sensitivity of 94.7% in detecting minimal signs of consciousness. In 2 MCS^- patients, TMS delivered at different cortical sites could only trigger a simple response, resulting in PCI_{max} lower than PCI^* .

According to conventional EEG assessment (Fig 3A), MCS patients were distributed across 3 categories: 18.4% in the severely abnormal, 44.7% in the moderately abnormal, and 36.9% in the mildly abnormal background. Of note, the 2 MCS^- patients with PCI_{max} lower than PCI^* showed a severely abnormal background. No patient showed a normal EEG pattern. Grouping MCS patients according to background EEG did not result in significantly different PCI_{max} values (Kruskal–Wallis test, $p = 0.41$; see Fig 3B). Thus, in MCS patients TMS typically triggered complex spatial–temporal dynamics, despite a heterogeneous EEG background, including 5 patients with a severely abnormal pattern. This finding is exemplified in

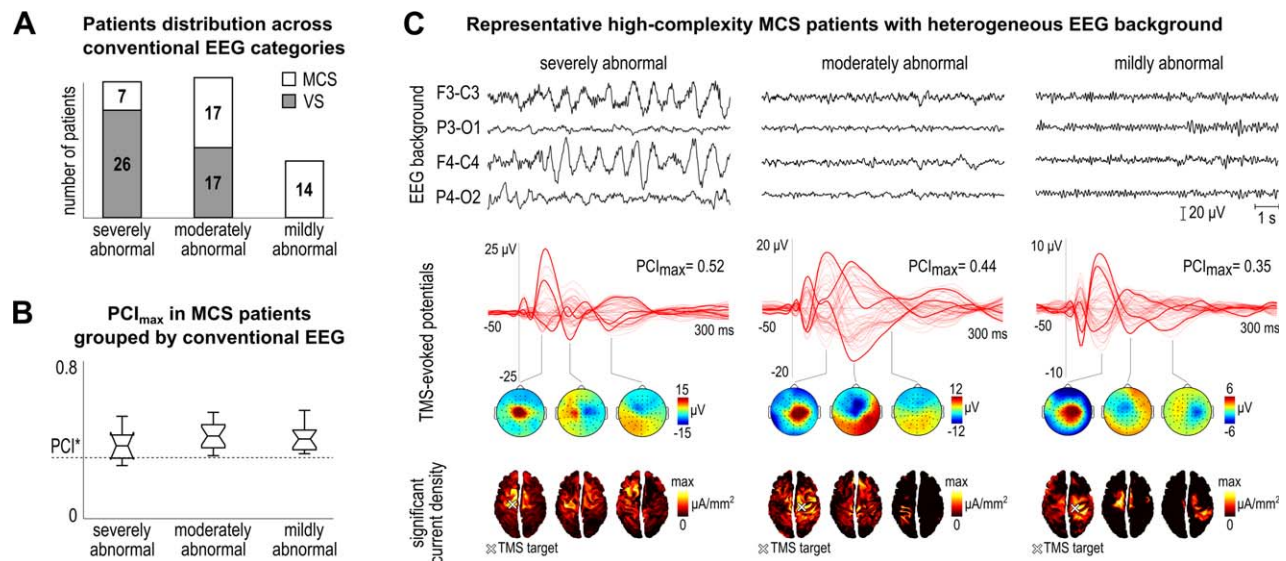


FIGURE 3: (A) Distribution of vegetative state (VS) and minimally conscious state (MCS) patients across conventional electroencephalographic (EEG) categories (i.e., severely abnormal, moderately abnormal, and mildly abnormal). The number of patients in each EEG category is explicitly indicated within the bars for VS and MCS patients. (B) Boxplot of the maximum individual Perturbational Complexity Index values (PCI_{max}) computed in MCS patients as a function of conventional EEG category. The dashed horizontal line highlights the optimal cutoff (PCI^*) obtained from the benchmark population. (C) The first row shows 10-second continuous EEG recordings from 4 bipolar channels (F3-C3, P3-O1, F4-C4, P4-O2) in 3 representative MCS patients with PCI_{max} higher than PCI^* (from left to right: Patients 19, 10, and 25), and respectively with a severely abnormal (left), a moderately abnormal (center), and a mildly abnormal (right) background. The second row shows the corresponding average transcranial magnetic stimulation (TMS)-evoked potentials (all channels superimposed, with 3 illustrative channels highlighted in bold) together with the PCI_{max} values. Three voltage scalp topographies (third row) and significant current density cortical maps (fourth row) are shown at selected time points for each patient. A white cross on the cortical map indicates the stimulation target. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3C, where the TMS-evoked cortical responses in 3 representative MCS patients are shown together with the corresponding background EEG traces.

Stratification of VS Patients

After ascertaining the sensitivity of PCI_{max} in detecting MCS patients who showed unequivocal behavioral signs of consciousness, we employed PCI_{max} to stratify unresponsive patients. We found that the VS population could be stratified in 3 different subgroups: a “no-response” subgroup ($PCI_{max} = 0$) of 13 patients (30%), a “low-complexity” subgroup ($PCI_{max} \leq PCI^*$) of 21 patients (49%), and finally a smaller “high-complexity” subgroup ($PCI_{max} > PCI^*$) of 9 patients (21%). The TMS-evoked responses together with selected structural images of 3 representative VS patients belonging to these subgroups are displayed in Figure 4A. In the no-response subgroup TMS targeted over different cortical areas failed to engage any significant cortical response, whereas in the low-complexity subgroup TMS triggered a local and stereotypical positive–negative response, similar to the one observed in healthy controls during unconscious NREM sleep and anesthesia. Notably, in the high-complexity subgroup, TMS engaged a rapidly changing

and spatially differentiated cortical response, similar to the one observed in MCS patients and in responsive (wakefulness) or unresponsive (REM sleep and ketamine anesthesia) conscious controls.

Comparing the 3 subgroups of VS patients, we did not find a significant effect of the best CRS-R total score (Kruskal–Wallis test, $p = 0.28$) or of the vigilance level (χ^2 test for proportions: no-response vs low-complexity, $p = 0.45$; no-response vs high-complexity, $p = 0.94$; low-complexity vs high-complexity, $p = 0.53$). Considering etiology and EEG category, the low-complexity subgroup was composed of patients with heterogeneous etiology (5 postanoxic, 6 traumatic, 10 vascular) showing 2 different EEG patterns (12 severely and 9 moderately abnormal background; see Fig 4B); patients with vascular or traumatic etiology equally showed either a moderately or a severely abnormal pattern, whereas 4 of 5 postanoxic patients showed a severely abnormal pattern. Accordingly, within the low-complexity subgroup, PCI_{max} did not significantly differ between patients with different EEG background (see Fig 4C). However, the 2 extreme subgroups identified by the PCI_{max} -based stratification were associated with a characteristic prevalence of etiology and EEG background organization (see Fig 4B); all except 1

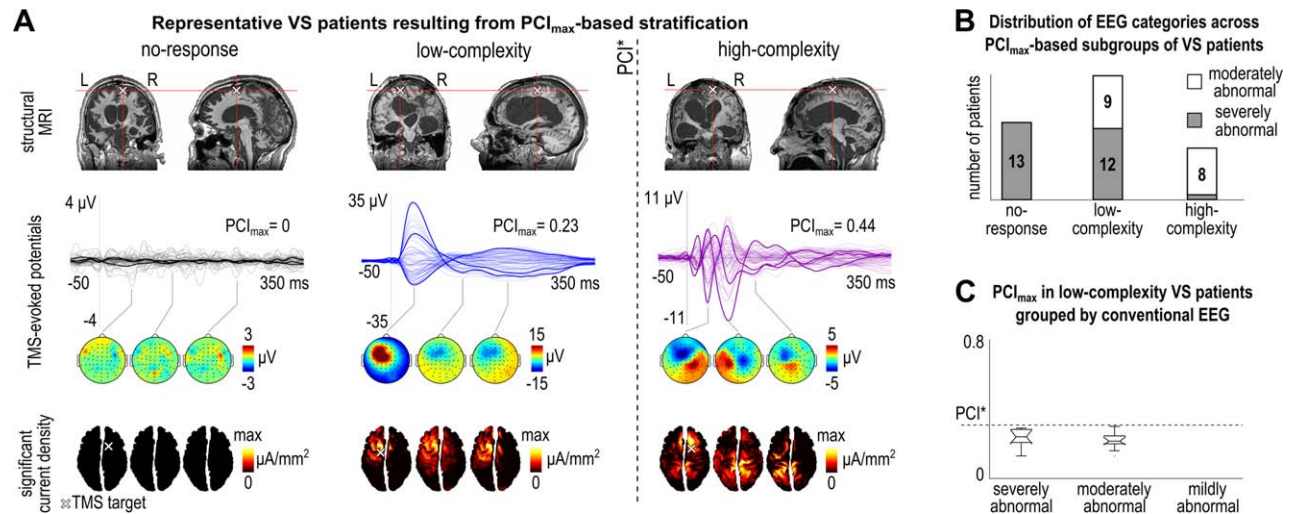


FIGURE 4: (A) The first row shows structural images of 3 representative vegetative state (VS) patients (from left to right: Patients 57, 78, and 42), with individual maximum value of Perturbational Complexity Index (PCI_{max}) respectively = 0 (left), PCI_{max} lower than the optimal empirical cutoff obtained from the benchmark population (PCI^* ; center), and PCI_{max} higher than PCI^* (right). One coronal and 1 sagittal view for each patient are displayed in correspondence with the stimulation site (white cross). The second row shows the corresponding average transcranial magnetic stimulation (TMS)-evoked potentials (all channels superimposed, with 3 illustrative channels highlighted in bold), together with the PCI_{max} values. Three voltage scalp topographies (third row) and significant current density cortical maps (fourth row) are shown at selected time points for each patient. A white cross on the cortical map indicates the stimulation target. The dashed vertical line highlights the PCI^* obtained from the benchmark population. (B) Distribution of the severely abnormal and moderately abnormal background patterns across the 3 subgroups of VS patients defined by PCI_{max} -based stratification, namely no-response, low-complexity, and high-complexity. (C) Boxplot of the PCI_{max} values computed in VS patients with PCI_{max} lower than PCI^* as a function of conventional electroencephalographic (EEG) category. The dashed horizontal line highlights the PCI^* obtained from the benchmark population. L = left; MRI = magnetic resonance imaging; R = right. [Color figure can be viewed at wileyonlinelibrary.com]

no-response VS patient had suffered severe postanoxic damage and were all characterized by a severely abnormal background, whereas the high-complexity subgroup had a prevalent traumatic etiology (7 of 9) and almost all showed a moderately abnormal EEG (8 of 9). Concerning the outcome at 6 months, we found that 6 of 9 (1 unknown) high-complexity VS patients transitioned to a behavioral MCS, whereas such transition was observed in 5 of 21 (2 unknown) low-complexity patients. None of the no-response subgroup showed any improvement.

Discussion

Towards a Calibration of PCI

Validating and calibrating an objective index of the brain's capacity for consciousness when behavioral signs of consciousness are unreliable or inconsistent represents a formidable challenge.^{14,15} Here we consider PCI, a measure that gauges the ability of thalamocortical circuits to integrate information irrespectively of the integrity of sensory processing, motor behavior, and subject participation. To validate PCI, we chose benchmark conditions¹⁵ in which subjects could provide a report about the absence or presence of conscious experience, including delayed reports upon awakening from sleep²⁷ and anesthesia.⁹ Retrospective reports, either immediate or

delayed, are the current gold standard for assessing the presence and content of subjective experience¹⁶ and therefore for validating objective measures of consciousness.⁹ Based on these premises, if a brain-based test of consciousness is positive in subjects who are fully unresponsive at the time of measurement but provide a delayed report of a vivid dream upon awakening, the result should be considered a true positive. The contingency table based on PCI^* cutoff (see Fig 1C) shows that in the delayed report conditions PCI_{max} always provides positive results. This finding is relevant, especially concerning ketamine anesthesia and REM sleep, conditions in which consciousness is present but is disconnected from the external environment.^{27,28} Importantly, the benchmark population included not only conscious subjects disconnected from the environment but also a large group of conscious brain-injured patients (LIS, subcortical and cortical stroke, EMCS) as a necessary requirement for the calibration of a test aimed at a target population of MCS and VS patients with severe neurological damage.

Overall, referring to subjective reports as ground truth and to PCI_{max} as a test, we find a PCI^* that optimally discriminates between the conscious and the unconscious conditions in the benchmark population,

irrespective of behavioral unresponsiveness and brain lesions (see Fig 1C). Although subjective reports are currently the gold standard for assessing the presence of consciousness, they are not necessarily always reliable unless the context is carefully considered. Thus, in certain situations people may be conscious but subsequently forget their experiences, or be unconscious and confabulate upon awakening. Accordingly, PCI* should not be interpreted as an absolute boundary between consciousness and unconsciousness, but rather should be used as an operational threshold to be applied to conditions in which no reliable behavioral reference is available.

PCI-Based Detection of MCS Patients

Although MCS patients are unable to provide a verbal report about their subjective experience, they show behavioral signs of consciousness.^{1,29} The presence of such signs, albeit fluctuating, can be considered as a minimal standard against which the sensitivity of any candidate index of consciousness should be tested. This check is important because the reliable detection of MCS patients represents a general challenge for bedside, brain-based indices of consciousness. For example, the P3b potential elicited by global violations of auditory regularities, a candidate signature of the presence of consciousness, can be found in only up to 31% of MCS patients,³⁰ measures of resting EEG connectivity such as weighted symbolic mutual information result in a 71% sensitivity, and the best combination of 92 quantitative measures derived from both resting and evoked EEG achieves a sensitivity of 78%.³¹ Besides quantitative analysis, the clinical qualitative assessment of the EEG background offers a practical tool at the bedside that, if properly interpreted, may outperform some of the above indices.²⁶ In the present data set, a cutoff between severely and moderately abnormal EEG patterns reached a remarkable sensitivity of 81.6% (see Fig 3A). Importantly, however, the highest sensitivity (94.7%) was obtained by quantifying with PCI_{max} the complexity of the EEG responses to a direct cortical perturbation (see Fig 2). Notably, TMS perturbations revealed complex cortical responses (PCI_{max} > PCI*) also in 5 MCS patients, who according to the clinical classification of the resting EEG were characterized by a severely abnormal pattern (see Fig 3B, C). This suggests that assessing the joint presence of integration and differentiation through direct cortical perturbations^{17,32} reveals additional information that is not immediately captured by conventional EEG descriptors and may direct further refinement of the classification of EEG resting activity.

PCI-Based Assessment of VS Patients

In VS patients, the absence of behavioral signs of consciousness *per se* cannot be considered a proof of the absence of consciousness.^{2,33} For this reason, the alternative term unresponsive wakefulness syndrome, which is more descriptive and more neutral regarding the patient's capacity for consciousness, has recently been recommended.³⁴ This widely acknowledged notion also implies that in principle, these patients should not be employed for calibrating measures of consciousness; rather, they ought to represent the endpoint for the application of brain-based measures that have been validated on an independent gold standard.^{14,15} Here, by applying an externally validated cutoff (PCI*), we find that 9 of 43 unresponsive patients had PCI_{max} > PCI* (see Fig 2). Are these false or true positives? Across 200 measurements in the benchmark population, PCI_{max} was always higher than PCI* when consciousness was present and never when consciousness was absent, as assessed through subjective report. Notably, the PCI_{max} values found in the high-complexity VS patients (0.34–0.50; see Supplementary Table) were well within the range (0.32–0.70) found in conscious controls (see Table 1), and did not depend on the level of arousal as assessed by the CRS-R vigilance subscale (see Supplementary Table). Hence, it is parsimonious to assume that these high-complexity unresponsive patients may retain a capacity for consciousness that is not expressed in behavior. A similar dissociation between consciousness and behavior is observed in ketamine-anesthetized subjects who lie eyes-open, completely unresponsive at the time of measurement, but provide a delayed report upon awakening^{8,17,28}; unlike these high-complexity anesthetized subjects, however, high-complexity VS patients might remain unable to report due to pathological disconnection/unresponsiveness.

From this perspective, we suggest that PCI should not be considered as a prognostic marker—although the outcome at 6 months was more favorable in the high-complexity subgroup—but rather as an index of the current capacity for consciousness that is independent of the patient's ability to process external inputs or to engage in motor behavior. Practically, by approximating an optimal tradeoff between sensitivity and specificity, the PCI test may represent an important step within a hierarchical diagnostic flow of DOCs. For example, one may apply PCI after a first screening with tests characterized by high sensitivity and low specificity, such as positron emission tomography assessment of cortical metabolic rates³⁵; then, patients with PCI_{max} > PCI* could be selected to confirm the presence of covert consciousness through more demanding tests characterized by maximal specificity, such as functional MR imaging active paradigms.³³

Physiopathological Implications

We observed a progressive group-level reduction of PCI_{max} from healthy controls to conscious patients with focal brain injury to the MCS cohort, suggesting that overall lesion load may eventually affect the upper bound of complexity. In parallel, and confirming the impact of lesions, we found a larger number of TMS/EEG sessions resulting in PCI measurements lower than PCI^* (empty circles in Fig 2) in MCS patients, suggesting that some cortical areas may fail to engage in complex activation patterns when stimulated. Despite this, the TMS mapping yielded PCI_{max} values higher than PCI^* in 84 of 86 (97.7%) brain-injured conscious patients, including LIS, stroke, EMCS, and MCS. Thus, instances of high perturbational complexity linked to behavioral signs of consciousness could be detected even in brains affected by multifocal, widespread lesions. Clearly, the cohort of brain-injured patients enrolled in the present study did not allow inferences regarding the functional role of specific brain networks in consciousness, because of their different etiology and because their anatomical lesions were characterized by heterogeneous extent and spatial location. Nonetheless, this finding highlights the sensitivity of PCI and raises the basic question of what are the minimal anatomical and functional requirements to sustain such complex interactions, a question that is even more relevant for those unresponsive patients in whom severe brain damage spares the function of large brain islands.³⁶

We describe 3 possible TMS/EEG patterns in clinically vegetative patients (see Fig 4A); when directly perturbed at multiple locations, the patients' cerebral cortex may (1) fail to engage in any significant response, (2) engage in a low-complexity response similar to the one observed in NREM sleep and anesthesia unconsciousness, or (3) engage in complex spatiotemporal dynamics similar to that observed in conscious awake or dreaming subjects. The no-response subgroup was mostly (12 of 13) composed of postanoxic patients who showed diffuse cortical necrosis (see Fig 4A) and a severely abnormal, voltage-suppressed EEG. The low- and the high-complexity patterns, conversely, could be found in the presence of similar lesion load as roughly inspected by structural imaging (see Fig 4A). Several factors, in addition to the extent of structural lesions, may affect the ability of the residual brain to engage in complex interactions. One possibility to be investigated is whether the integrity of some specific structures, such as the precuneus,^{20,37} the thalamus,^{5,38} and the claustrum,^{39,40} or a critical level of overall anatomical connectivity⁴¹ may be key in sustaining such interactions. Another non-mutually exclusive possibility is that the complexity of residual

thalamocortical networks may be reduced by functional imbalances leading to an excessive degree of neuronal bistability.⁴² This may happen, for example, following changes in the neuromodulatory milieu, when potassium currents are abnormally increased and when the balance between excitation and inhibition is disrupted.^{43,44} In this regard, it is worth recalling that low complexity responses to cortical stimulation are the rule during states such as anesthesia¹⁸ and NREM sleep,⁴⁵ when bistability is present but can be readily reversed.⁴⁶

The proposed physiopathological stratification has practical implications for patient management. For example, whereas no-response patients may be further investigated in search of preserved cortical and subcortical metabolic activations that may have escaped the TMS probing, the patients in whom TMS triggered a significant response with $PCI_{max} < PCI^*$ should be directed toward neuromodulation with medications or brain stimulation techniques⁴⁷ aimed at restoring complex patterns of activity. For example, although anatomical lesions and disconnections cannot be easily reversed, it may still be possible to reduce sleep-like bistability by deep brain stimulation⁴⁸ or by acting pharmacologically on intrinsic neuronal properties.⁴⁹ In this process, longitudinal TMS/EEG measurements also offer the fundamental readout to direct and titrate intervention toward the desired endpoint of $PCI_{max} > PCI^*$. Finally, unresponsive patients in whom TMS/EEG already documents a core of high complexity should be selected for intensive interventions aimed at restoring responsiveness to the external environment, such as by increasing behavioral output through thalamic stimulation⁵⁰ or by establishing communication through active paradigms or brain-machine interface.^{51,52}

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Author Contributions

Conception and design of the study: G.D., S.L., G.T., M.Mas. Acquisition and analysis of data: S.C., A.C., M.R., S.S., M.F., M.N., A.P., A.G.C., P.D.T., M.B., O.G., O.B., F.C., C.L., M.Mar. Drafting a significant portion of the manuscript or figures: S.C., A.C., M.Mas.

Potential Conflicts of Interest

Nothing to report.

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