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Dissociation of vegetative and minimally conscious patients based on brain operational architectonics: factor of etiology

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Abstract

At present, the discrimination between patients in vegetative (VS) and minimally conscious state (MCS) is based upon the behavioral gold standard. At the same time, such behavioral assessment remains equivocal and difficult to interpret as evidence for the presence or absence of consciousness, resulting in possible clinical misdiagnosis in such patients. Application of operational architectonics strategy to EEG analysis in patients in VS and MCS reveals that absence of consciousness in patients in VS is paralleled by significant impairment in overall EEG operational architecture compared to patients in MCS: neuronal assemblies got smaller, their life span shortened and they became highly unstable and functionally disconnected (de-synchronized). However, in that study, patients with different brain damage etiologies were intermixed. Therefore, the goal of the present study was to investigate whether the application of operational architectonics methodology on EEG could reliably dissociate patients in VS and MCS independent of brain damage etiology. It was found that it is indeed the case. Therefore, we could conclude that the observed EEG operational architectonics structure impairment in patients in VS and partial preservation in patients in MCS is a marker of (un)consciousness rather than the physiological damage. Results of this study may have neuroscientific, clinical and ethical implications.

Key words: EEG alpha and beta rhythms, brain operations, neuronal assemblies, minimally conscious state (MCS), vegetative state (VS), functional connectivity, structural synchrony, neurophysiological pattern, synchronization, consciousness/unconsciousness.

1. Introduction

Disorders of consciousness (DOC), commonly divided into coma, vegetative state (VS) and minimally conscious state (MCS), remain among the least understood, treatable, and most ethically troublesome conditions of modern neurology.[1,2] At present, the discrimination between VS and MCS, and generally the discrimination between any patients considered to be either unconscious or conscious, is based upon the (in)ability of such patients to signal their awareness by a definite, reproducible, and voluntary behavioral response.[3] At the same time, such behavioral responses remain equivocal and difficult to interpret as evidence for the presence or absence of consciousness,[4] thus resulting in considerable percentage of clinical misdiagnosis[5] and serious ethical problems[6] in such patients. Therefore, researchers try to find reliable and objective brain activity markers of (un)consciousness that could be applicable to DOC patients.

Functional magnetic resonance imaging (fMRI) was used to detect awareness in patients in VS[1] as well as to distinguish patients in MCS from patients in VS.[7-9] Although such metabolic studies certainly provide valuable information,[10] they have several important limitations. For example, both, resting-state and task-related fMRI approaches are prone to noise contamination (such as movement and physiological artifacts), which are difficult to avoid in patients with DOC.[11,12] Furthermore, fMRI temporal resolution is much slower than the temporal scale where conscious scenes are likely to arise (for a review see ref[13]). Additionally, these techniques cannot be used routinely because they are complex, expensive and available only in a limited number of centers. Thus in practice, application of fMRI techniques to patients with DOC is often difficult and ambiguous.[10]

In this context, electroencephalogram (EEG) that permits bedside assessment could be particularly suitable since it directly and objectively records spontaneous brain activity without requiring any behavioral response by the patient and has a temporal resolution compatible with conscious events. Although there is a clear indication that certain EEG features reliably correlate with brain damage severity[14-18] and whose absence is associated with brain death,[19] there is a marked controversy about the value of EEG in unambiguous distinguishing of VS and MCS[20-26] and even locked-in syndrome.[27,28] The equivocal results may be caused by methodological difficulties encountered in EEG analysis strategies that utilize EEG parameters/indexes that relate to the physiological rather than conscious processes/states/contents (for similar view see[4,6,29,30]). In other words, these parameters are independent of consciousness theory. This presents a serious limitation since, as it is correctly pointed out by Schnakers and Zasler,[31] "in absence of a full understanding of the neural correlates of conscious perception, it remains difficult to interpret functional imaging data in brain-damaged patients as proof or disproof of their conscious experience" as well as reliably predicting its presence or absence in noncommunicative patients with brain damag.[32] For these reasons, it is essential to resort to theory-based EEG indexes of (un)consciousness in order to guide clinicians in the choice of meaningful diagnostic criteria (for similar argumentation see also refs[32-35]).

Consciousness is a concept that remains in flux, and objective assessment of it is methodologically difficult due to its first-person nature. In search of an adequate model, several theoretical frameworks about consciousness have been proposed over the last two decades.[36-46] However, practically all of them do not take phenomenal consciousness (subjective experience) of a mind as important variable and, at best, try to explain consciousness through its neurophysiological correlates,[47-49] despite the fact that correlation is too weak relation to be definitive in any explanation.[50] Even when phenomenology aspects are considered, most theories either do not take the dynamic and compositional nature of the phenomenal content seriously or disagree about the relevant for the consciousness level of the brain organization.[13] They are particularly problematic in respect to the lack of methodological tools/indexes that are theory motivated: they postulate many entities that cannot be easily measured in practice, and their experimental exploration stands as an important challenge.[51] Therefore, there is a need for a theoretical and methodological framework that gives researchers an idea how consciousness phenomenon, along with its measurable physical counterparts, fits into a unified brain–mind architecture, whilst still paying attention to the philosophical problems of how these physical processes relate to mental processes.

The Operational Architectonics (OA) theory of brain and mind functioning[13,52-57] is an example of such a theoretical framework. Besides the fact that it is mathematically simple, neurophysiologicaly accurate and compatible with the cognitive and phenomenal perspective,[58,59] OA offers a range of methodological tools which allow direct and practical measurement of the entities it postulates.[55] For a direct comparison of OA theory with other theoretical frameworks about consciousness currently dominant in the research field, readers are advised to consult references.[55,60]

In short, OA theory posits[57] that local fields of transient functional neuronal assemblies are equivalent to operations which can be conscious (phenomenal). Indeed, it has been shown that distinct neuronal assemblies display preferential processing for certain features (a preference for color, shape, motion, smell, etc[61]). Such simple operations responsible for qualia are reflected in the electrical brain field (EEG) in the form of local quasi-stationary segments, which can be conceptualized as standing waves within a 3D volume (for a review see ref[57]), and could be measured using adaptive segmentation procedure.[55] It has been shown experimentally that parameters of EEG segments (number, duration, amplitude and others) are reliably and consistently correlated with changes in the phenomenal (subjective) content during both spontaneous (stimulus independent) and induced (stimulus dependent) experimental conditions.[57] Moreover, it has been documented that the local fields of various neuronal assemblies correlate with different conscious percepts[62,63] and if cognitive processing does not take place, such transient neuronal assemblies do not form.[64] At the same time, to have a phenomenal experience of any complex object/concept or scene, several simple features of that object/concept/scene should be spatially and temporally integrated. According to the OA theory, this complexity requires temporally coordinated operations (equivalent of synchrony of local bioelectrical fields) of many neural assemblies, which selectively emerge from the entire brain (for a detail discussion see ref[57]). This process could be measured directly by the EEG operational synchrony index.[55] Several synchronized complexes of neuronal assemblies (so called operational modules, OM) could be further synchronized forming even more complex spatial-temporal structures, thus constituting a clear nested functional hierarchy[65] that could serve as the needed ingredient of brain organization and allow conscious mind to be expressed.[57] This OA strategy of EEG analysis has been validated in a number of electrophysiological, cognitive and clinical studies, and is proven to be robust, consistent and statistically reliable.[55,66,67]

The OA theory predicts that both low and high levels of operational synchrony among neuronal assemblies can result in a dramatic fading of consciousness.[57] In the first case, consciousness is likely to vanish in the presence of many small, short-lived and highly unstable neuronal assemblies that perform their operations totally independently from one another (functional disconnection), while in the second case a state of hypersynchrony of operations of large, long-lived, and hyper-stable neuronal assemblies is also likely to lead to the vanishing of consciousness. Application of OA strategy to EEG analysis in patients in VS and MCS[68] favor the first option: it has been found that the absence of consciousness in patients in VS is paralleled by impairment in overall EEG operational architecture (Fig. 1). Specifically, neuronal assemblies got smaller, their life span shortened and they became highly unstable and functionally disconnected (de-synchronized). At the same time, fluctuating (minimal) awareness in patients in MCS was paralleled by partial restoration of EEG operational architecture (increased size, life span and stability of neuronal assemblies, together with increased number and strength of functional connections among them; Fig. 1), approaching the level found in healthy fully-conscious subjects.[68]

In light of these results it is reasonable to assume that EEG operational architecture can be useful for distinguishing patients in VS and MCS. In context of the present article though, the previous study[68] had an important limitation: patients in both (VS and MCS) subgroups had mixed etiologies of brain damage. However, if OA methodology is to reveal true signs of conscious awareness in patients with DOC, these signs should be independent from the etiology of brain injury because they should not be sensitive to physiological parameters of brain damage, but rather to the consciousness phenomenon itself.

Therefore, the *goal of the present study* was to investigate whether the OA methodology could reliably discriminate patients in VS and MCS independent of brain damage etiology, when applied to EEGs of patients with DOC.

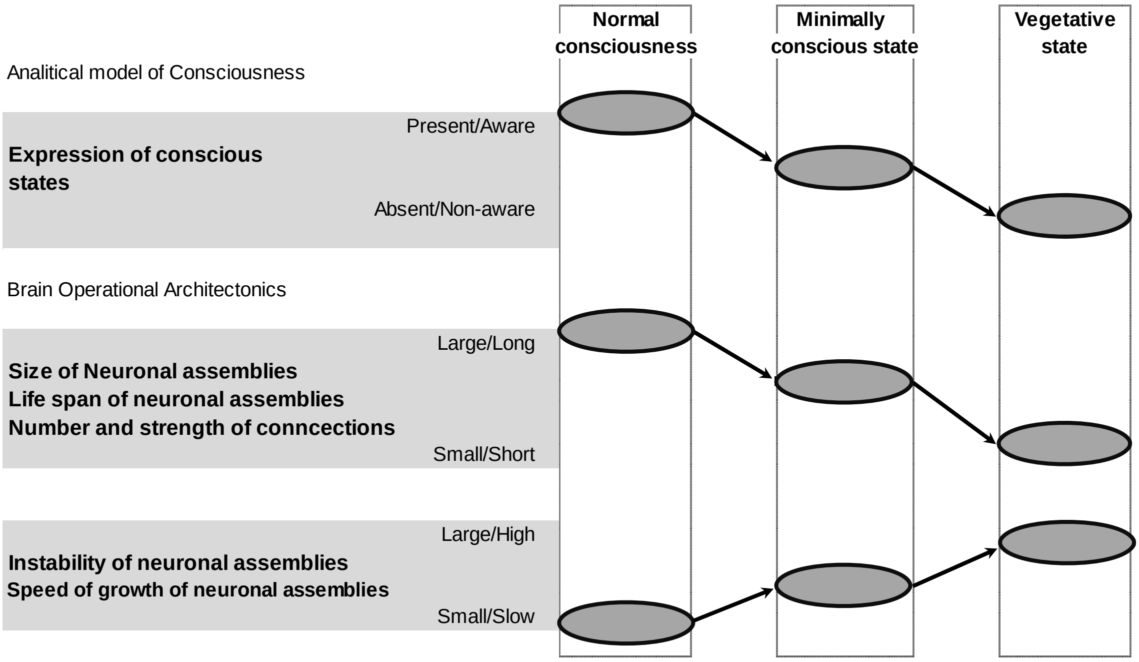

Figure 1. Relation of analytical model of consciousness to brain operational architectonics. Explanations are in the text.

2. Methods

2.1. Participants

The EEG data of the present study were taken from the clinical archive of patients from the Neurorehabilitation Unit at the Fondazione Istituto "San Raffaele – G. Giglio" (Cefalù, Italy), who participated in our previous studies.[68,69] Therefore, this is a retrospective study. At the same time, this present study is original, since these EEG data had not yet been used for the evaluation of OA indexes of resting EEG as a function of brain damage etiologies; in previous studies the factor of etiology was not considered.

The EEG was recorded from 19 DOC patients with severe brain injuries, admitted to the Neurorehabilitation Unit at the Fondazione Istituto "San Raffaele – G. Giglio" to carry out an intensive neurorehabilitation program. Upon admission, all patients were submitted to a thorough and repeated clinical neurological examination. The diagnosis of VS and MCS was made in accordance to the currently accepted diagnostic criteria based on the standard behavioral assessment.[3,70-72] Additionally, the Levels of Cognitive Functioning (LCF) score[73] was assessed on the day of admission and three days later when the EEG was registered. We choose to use the LCF scale instead of the Glasgow Outcome Scale (GOS),[74] the Glasgow Coma Scale[75] or the JFK Coma Recovery Scale,[76] because LCF evaluates not only behavioral patterns but cognitive functions as well, which are closely related to consciousness in contrast to behavioral patterns. Previous studies have also found that LCF is very well correlated with the presence of EEG abnormalities in patients with DOC.[77,78] The LCF has a linearly graded scale ranging from 1 to 8 (1 – patient does not respond to external stimuli and/or command; 8 – patient is self-oriented and responds to the environment, but abstract reasoning abilities are decreased relative to pre-morbid levels). This feature of LCF scale is important for the aim of this study, because in contrast to other scales (e.g. CRS-R), it is not possible to have a total score higher for VS than for MCS using the LCF scale.

Based on the strict adherence to the aforementioned diagnostic criteria, 12 patients (mean age 42.9 ± 19 years) were classified as being in VS (6 traumatic and 6 vascular) and the remaining 7 patients (mean age 48.7 ± 19.8 years) were classified as being in MCS (3 traumatic and 4 vascular). Patients in VS had a LCF score of 1 or 2, while patients in MCS had a LCF score of 3. In order to reduce the variability of clinical evaluation, LCF scores were assigned to all patients only if LCF scores were unchanged between the day of admission and the day of the EEG registration (3 days later); otherwise, patients were excluded from the study. Other exclusion criteria for the patients comprised: (a) any acute comorbidity or unstable vital signs; (b) obvious communicating or obstructive hydrocephalus; (c) a history of neurological disease before admission; and (d) severe spasticity (causing constant EMG artifacts). Inclusion criteria for the patients were: (a) less than 3 months after the acute brain event onset; (b) first-ever acute brain event; and (c) the available EEG record with unchanged vigilance level during recording (see below).

The study was approved by the local institutional Ethics Committee, and complies with Good Medical Practice. Informed and overt consent of subjects' legal representatives, in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and standards established by the Fondazione Istituto "San Raffaele – G. Giglio" Review Board were acquired. The use of the data was authorized by means of written informed consent of caregivers (VS and MCS patients).

2.2. The EEG recording

Spontaneous electrical brain activity was recorded with a 21-channel EEG data acquisition system (Neuropack electroencephalograph; Nihon Kohden, Tokyo, Japan). The EEG data were collected (cephalic reference – mean of the signals from C_3 and C_4 electrodes; 0.5–70 Hz bandpass; 200 Hz sampling rate; around 30 min) in patients during a waking resting state (eyes-closed) from 19 electrodes positioned according to the International 10–20 system (i.e. O_1, O_2, P_3, P_4, P_z, T_5, T_6, C_3, C_4, C_z, T_3, T_4, F_3, F_4, F_z, F_7, F_8, Fp_1, Fp_2). The impedance of recording electrodes was monitored for each patient and was always below 5 kΩ. To monitor eye movements, an electrooculogram (0.5–70 Hz bandpass) was also collected.

The EEG recordings were performed late morning in all patients. The EEG recordings were started in all cases only if patients had their eyes open spontaneously; the eyelids were then closed by hand until the end of EEG registration. At the end of the recordings all patients opened their eyes spontaneously thus suggesting that the level of vigilance (i.e. capability to open eyes) remained unchanged compared to the onset of EEG recording. In order to control a constant level of vigilance, an experimenter monitored patient's EEG traces in real time, looking for signs of drowsiness and sleep onset (increase of "tonic" theta rhythms, K complexes and sleep spindles). The presence of an adequate EEG signal was determined by online visual inspection of the raw signal on the computer screen. Although it may be difficult to precisely assess the level of vigilance in patients in VS, preserved sleep patterns may be observed in the majority of patients in VS (for review see ref[79]).

2.3. The EEG-signal data preprocessing

The presence of an adequate EEG signal was determined off-line by visually checking each raw signal. Epochs containing artifacts due to eye movement, eyes opening, significant muscle activity, and movements on EEG channels were marked and then automatically excluded from further analysis.

For each patient a full EEG stream, free from any artifacts, was fragmented into consecutive one-minute epochs. Therefore, the "MCS-T" group (patients in MCS of traumatic etiology) has 40 one-min EEGs, "MCS-V" group (patients in MCS of vascular etiology) has 47 one-min EEGs, and "VEG-T" group (patients in VS of traumatic etiology) has 70 one-min EEGs, while "VEG-V" group (patients in VS of vascular etiology) has 67 one-min EEGs. Within each group further data processing was performed for each separate one-minute portion of the signal. Due to the technical requirements of the tools used to process the data, EEGs were re-sampled to 128 Hz.

After re-sampling and prior to further processing procedures, each EEG signal was bandpass-filtered (Butterworth filter of the sixth order) in three frequency bands: alpha (7–13 Hz), beta1 (15–25 Hz) and beta2 (25–30 Hz). Phase shifts were eliminated by forward and backward filtering. The alpha and beta frequency bands were chosen because in our previous study,[68] among five EEG frequency bands (delta, theta, alpha, beta1 and beta2) only alpha, beta1 and beta2 rhythms have shown behavior consistent with the analytical consciousness model proposed in Fingelkurts et al.[69] According to this model (Fig. 1) the features of EEG operational architectonics that are responsible for the subjective (un)awareness of self and environment should satisfy one of the following rules: (a) NORM $\geq$ MCS $>$ VS (for awareness) or (b) NORM $\leq$ MCS $<$ VS (for unawareness).

2.4. Adaptive-level EEG segmentation:Estimation of the local functional interrelations

According to the OA framework each homogeneous segment in the EEG signal corresponds to a temporary stable microstate – an *operation* executed by a neuronal assembly.[52,54] The transition from one segment to another then reflects the moment of *abrupt switching* from one neuronal assembly operation to another (see examples in ref[55]). *Rapid transitional processes* (RTPs) occurring in the amplitude of a continuous EEG activity mark the boundaries between quasi-stationary segments for this activity. The RTP is defined as an abrupt change in the analytical amplitude of the signal above a particular threshold. This threshold is determined for each local EEG by using a statistical procedure developed in earlier modeling and empirical studies (see ref[55]). To uncover these quasi-stationary segments from the complex non-stationary structure of local EEG signals, adaptive segmentation procedures should be used. The aim of the segmentation is to divide the EEG signal into *naturally existing* quasi-stationary segments by estimating the intrinsic points of "gluing" — RTPs.

The general statistical principles of the *microstate segmentation* have been described in detail elsewhere.[52,54,55,66] Therefore, here we provide only a brief overview of this approach. An adaptive segmentation procedure[55] was used for automatic segmentation of local EEG signals within the multichannel EEG record. This method is based on an automated algorithm that moves a double window screening along each separate EEG signal/channel. The following steps were taken to estimate RTPs: (1) Comparisons were made between ongoing EEG amplitude absolute values averaged in two windows – 'test' and 'level' (duration of test window << duration of level window), both starting from the first data point. The durations of test and level windows were identical across different subjects and EEG channels for each frequency band (but different between the bands) to guarantee the best conditions for RTP evaluation. (2) If the absolute maximum of the averaged amplitude values in the test window is less or equal to the averaged amplitude values in the level window, then the hypothesis of EEG homogeneity is accepted. (3) If the absolute maximum of the averaged amplitude values in the test window exceeds the averaged amplitude values in the level window, according to the threshold of "false alerts"—first condition (the Student criteria)—its time instant becomes the preliminary estimate of the RTP. (4) A second condition must be fulfilled to eliminate "false alerts" associated with possible anomalous peaks in the amplitude; the five points of the digitized EEG following this preliminary RTP must have a statistically significant difference between averaged amplitude values in the test and level windows (Student's *t*-test). (5) If these two criteria are met then the preliminary RTP is considered real. (6) Thereafter, both windows are shifted from this RTP on one time-point, and the procedure is repeated. With this technique (*phase one*) the sequence of RTPs with statistically proven ($p < 0.05$, Student's *t*-test) time coordinates is determined for each EEG channel and for each one-min epoch individually.

After the acquisition of quasi-stationary segments (indexed by RTPs) for each EEG channel, several EEG segment attributes[80] were calculated (*phase two*). The attributes are: (1) Average amplitude within each segment (microvolts) – as generally agreed, this index mainly indicates the volume or *size of neuronal assembly*, because the number of synchronized neurons recruited into assembly is reflected in the EEG amplitude.[81,82] (2) Average length of segments (milliseconds) –

illustrates the functional *life-span of neuronal assembly* or the *duration of operation* produced by this assembly. Because the transient neuronal assembly functions during a particular time interval, this period is reflected in EEG as a stabilized interval of quasi-stationary activity.[80] (3) Coefficient of amplitude variability within segments (%) – shows the *stability of local neuronal synchronization* within neuronal assembly.[80,83] (4) Average amplitude relation among adjacent segments (%) – indicates *neuronal assembly growth* (recruitment of new neurons) or *disassembling* (functional elimination of neurons).[80] (5) Average steepness among adjacent segments estimated in the close area of RTP (%) – shows the *speed of neuronal assembly growth or disassembling*.[80] These attributes reflect different aspects of local processes in the cortex and thus permit assessment of the *mesolevel* description of cortex interactions (interactions within *transient neuronal assemblies*) through large-scale EEG estimates.[84]

2.5. The EEG Structural Synchrony Index:Estimation of the remote operational (functional) connectivity

The synchronization of operations (i.e. *operational synchrony*) produced by different neuronal assemblies, which are located in different cortex regions, serve to bind *spatially* dispersed phenomenal features (bases of sensations) of a multimodal stimulus or objects into integrated and unified patterns of qualities and further into phenomenal objects or complex scenes.[44,57,85,86] At the EEG level, the operational synchrony phenomenon is expressed through *synchronization of the EEG quasi-stationary segments* (indexed by *Structural Synchrony*, ISS) obtained from different brain locations.[55]

The details of this technique are beyond the scope of this article; therefore, we will concentrate only on some essential aspects. In brief, each RTP in the reference EEG channel (channel with the minimal number of RTPs from any pair of EEG channels) was surrounded by a short “window” (ms). Any RTP from another (test) EEG channel was considered to coincide if it fell within this window. It can be noted that such coincidence of RTPs is related to a specific type of signal coupling – the structural synchronization of discrete events – which completely ignores the level of signal synchronization in the intervals (segments) between the coinciding RTPs.[55] To arrive at a direct estimate at the 5% level of statistical significance ($p < 0.05$) of the ISS, computer simulation of RTPs synchronization was undertaken based on random shuffling of time segments marked by RTPs (500 independent trials). As a result of this procedure, the stochastic levels of RTP coupling (ISS_{stoch}), together with the upper and lower thresholds of ISS_{stoch} significance (5%) were calculated. The ISS tends towards zero if there is no synchronization between EEG segments derived from different EEG channels and has positive or negative values whenever such synchronization occurs. Positive values (higher than upper stochastic level) indicate ‘active’ coupling of EEG segments (synchronization of

EEG segments is observed significantly more often than expected by chance as a result of random shuffling during a computer simulation), whereas negative values (lower than low stochastic level) mark ‘active’ decoupling of segments (synchronization of EEG segments is observed significantly less than expected by chance as a result of random shuffling during a computer simulation). The strength of EEG structural synchrony is proportional to the actual value of ISS: the higher this value, the greater the strength of functional connection.

Using pair-wise analysis, structural synchrony was identified in several (more than two) channels – synchrocomplexes (SC), the stable sequence of which is described as an operational module – OM. The criterion for defining an OM is a consistent sequence of the same synchrocomplexes (SC) during each 1-min epoch, whereas a SC is a set of EEG channels in which each channel forms a paired combination with valid values of ISS with all other EEG channels in the same SC; meaning that all pairs of channels in an SC have to have statistically significant ISS linking them together.[13,55]

Although it is often claimed that volume conduction is the main obstacle in interpreting EEG data in terms of brain connectivity, it has been shown in previous experimental and modeling studies that the values of the ISS are sensitive to the morpho-functional organization of the cortex than to volume conduction (for relevant details, we refer the reader to refs[55,66]; substantial discussion on local brain activity and volume conduction is presented in Section 3.2 in ref[87]).

2.6. Statistics

For each analyzed condition (MCS-T vs VEG-T and MCS-V vs VEG-V), group EEG segment-attribute averages and respective standard deviations were calculated for the whole pull of correspondent one-minute EEGs. As in previous work,[68] comparison of the same segment attributes between different group conditions (considering each EEG electrode) was performed using Wilcoxon matched pairs *t*-test.

The differences in number and strength of ISS patterns between different groups (MCS-T vs VEG-T and MCS-V vs VEG-V) were also assessed using Wilcoxon matched pairs *t*-test, considering nine categories of functional connectivity. At first, all valid EEG functional connections were averaged within each analyzed condition for the whole pull of corresponding one-minute EEGs within nine categories of functional connectivity ($short_{left/right}$, $short_{anterior/posterior}$, $long_{left/right}$, $long_{anterior/posterior}$, and $long_{interhemispheric}$), separately for the number of functional connections and for the strength of these connections. Since the absolute number of possible functional connections within each category was different, their per-category percentage was calculated. During the final stage an average of all the categories was calculated.

3. Results

3.1. Features and dynamics of neuronal assemblies (measured by EEG) as a function of brain damage etiology in VS and MCS

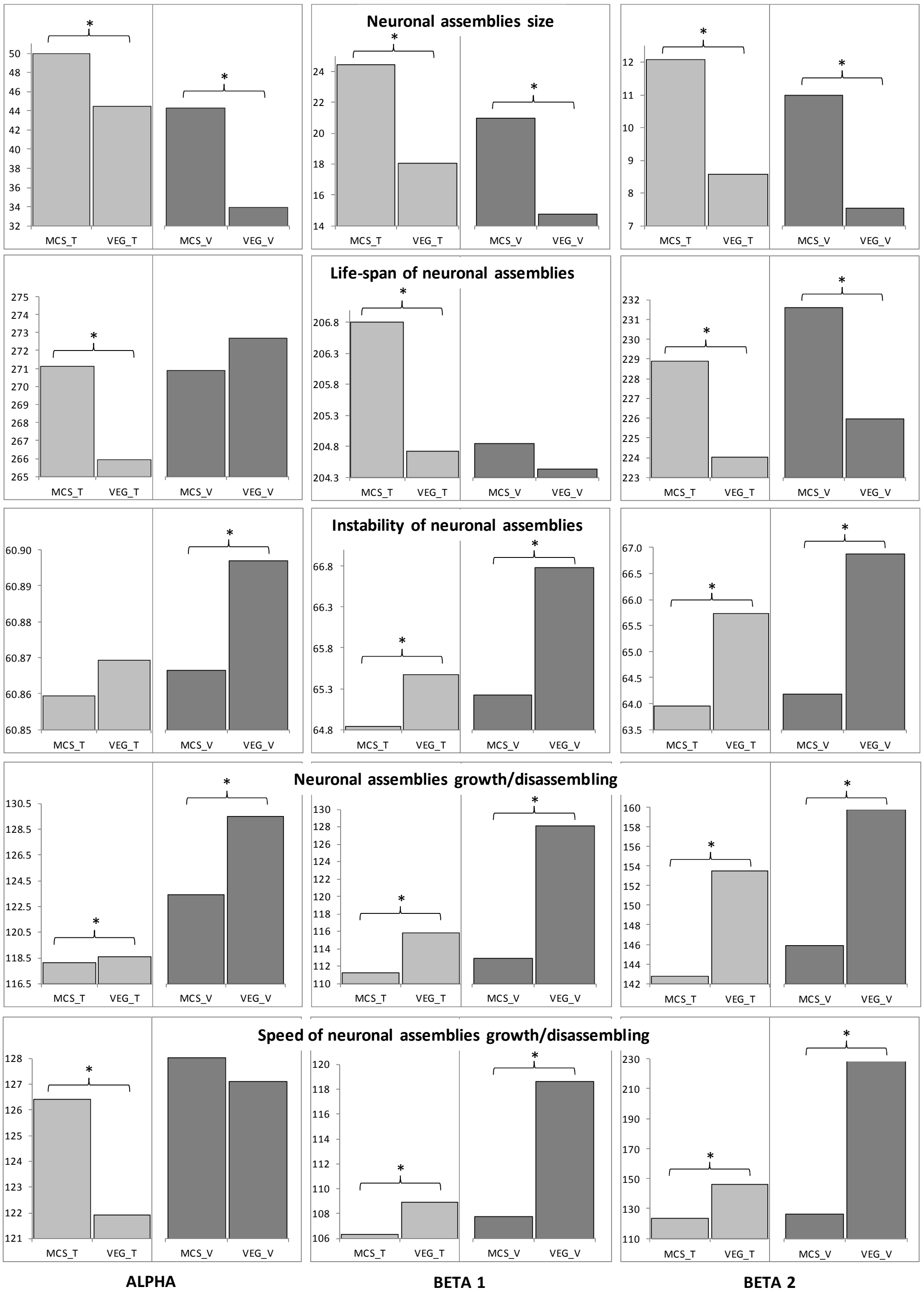

Figure 2. Features and dynamics of neuronal assemblies (indexed by EEG segment attributes) as a function of brain damage etiology. Data are averaged across all EEG channels and all patients within each state/condition (MCS, VS). The values of attributes of neuronal assemblies indicated by the *Y*-axis: size of neuronal assemblies — amplitude within each segment (microvolt); life-span of neuronal assemblies — length of segments (milliseconds); instability of neuronal assemblies — coefficient of amplitude variability within segments (%); growth/disassembling of neuronal assemblies — amplitude relation among adjacent segments (%); the growth/disassembling speed of neuronal assemblies — steepness among adjacent segments estimated in close vicinity of the boundary between segments (%). MCS-T / VEG-T – patients in minimally conscious or vegetative state after traumatic brain damage; MCS-V / VEG-V – patients in minimally conscious or vegetative state after vascular brain damage. * – indicates statistically significant difference in the range $p < 0.05$ – $p < 0.00004$ (see Table 1 for concrete values).

Figure 2 presents the mean values of EEG segment attributes that characterize different features of neuronal assemblies for all EEG locations and patients in MCS and VS for traumatic and vascular brain damage etiology. Corresponding data are presented separately for five features of neuronal assemblies (see Subsection 2.4). Other than the "life-span" for alpha frequency band, one can see that all studied parameters followed identical relations between MCS and VS conditions for both traumatic and vascular etiologies: a decrease in "size" and "life-span" and increase in "instability", "growth" and "speed" in VS when compared with MCS. The vast majority of MCS-VS comparisons got statistically significant differences (Table 1). Despite the "speed" attribute for the alpha frequency band, similar direction of differences was observed in all three (alpha, beta1 and beta2) frequency bands (Fig. 2).

Table 1. Statistically significant differences between MCS and VS conditions.

Attribute of neuronal assembly	MCS-T x VEG-T (p value)	MCS-V x VEG-V (p value)	Rhythm
Size	0.005	0.0001	ALPHA
Life-span	0.006	0.002	
Instability	0.84 / n.s.	0.0002	
Growth / disassembling	0.0006	0.0003	
Speed of growth / disassembling	0.0001	0.34 / n.s.	
Size	0.0001	0.0001	BETA 1
Life-span	0.05	0.98 / n.s.	
Instability	0.0004	0.0001	
Growth / disassembling	0.0001	0.0001	
Speed of growth / disassembling	0.00004	0.0001	
Size	0.002	0.0001	BETA 2
Life-span	0.0008	0.0007	
Instability	0.0001	0.0001	
Growth / disassembling	0.0002	0.0001	
Speed of growth / disassembling	0.0006	0.0002	

MCS-T – minimally conscious state of traumatic etiology; VEG-T – vegetative state of traumatic etiology; MCS-V – minimally conscious state of vascular etiology; VEG-V – vegetative state of vascular etiology; n.s. – not significant.

3.2. Operational synchrony of neuronal assemblies (measured by EEG structural synchrony) as a function of brain damage etiology in VS and MCS

Figure 3 presents the mean values of number and strength of functional connections for all EEG pair combinations that characterize remote functional connectivity between neuronal assemblies (see Subsection 2.5). Corresponding data are organized the same way as in Fig. 2 and are presented separately for traumatic and vascular brain damage etiology. We observed a decrease in the average number and strength of functional connectivity between neuronal assemblies in patients in VS compared to patients in MCS, though only some comparisons reach a statistically significant level (Fig. 3). At the same time, if all three frequency bands were included in the analysis, the observed decrease reached a statistically significant level ($p < 0.05$) for both parameters (number and strength) of functional connectivity. A similar direction of differences was observed in all three (alpha, beta1 and beta2) frequency bands (Fig. 3).

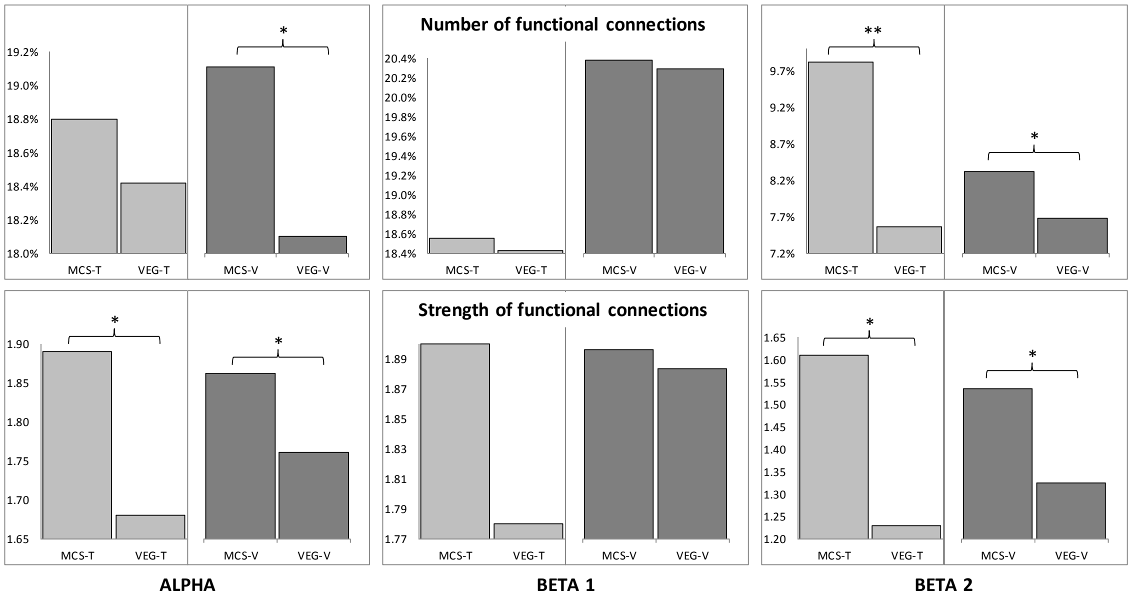

Figure 3. Operational connectivity among neuronal assemblies (indexed by EEG structural synchrony) as a function of brain damage etiology. Data averaged across all pairs of EEG channels and all subjects within each state/condition (MCS, VS). The *Y*-axis presents values of either number or strength of functional connections. * – $p < 0.05$, ** – $p < 0.01$.

4. Discussion

In the present retrospective study, we tested the hypothesis that OA methodology could reliably discriminate patients in VS and MCS (at the group level) independent of brain damage etiology when applied to the EEG of patients with DOC. Results showed that it is indeed the case (Fig. 2 and 3): patients in VS showed decreased size and life span of neuronal assemblies with a simultaneous increase in their instability, tendency to grow, and fast speed of such growth compared to patients in MCS irrespective of brain damage etiology. Additionally, patients in VS had a significantly decreased number and strength of functional connections between neuronal assemblies compared to patients in MCS, again, irrespective of brain damage etiology. Moreover, the observed in the present study decreases and increases coincide precisely with our previous results,[68] where patients with different brain damage etiologies were intermixed (see Fig. 1).

Therefore we could conclude that the observed impairment in the structure of EEG operational architectonics reflects rather the signs of unconsciousness than the physical brain damage. In other words, consciousness is likely to vanish in the presence of many *small*, *short-lived* and highly *unstable neuronal assemblies* that perform their operations totally independently from one another (*functional disconnection*) and are not capable to support any content to be experienced subjectively (for a detailed discussion of contents of consciousness as opposed of levels of consciousness see refs[6,29,69]). An independent line of support for this conclusion comes from the study of non-REM sleep[88] and pharmacologically induced loss of consciousness,[89] where it has been demonstrated that during unconsciousness brain cortical activity breaks down into causally independent, small, and short-lived modules producing local responses. According to the integrated information theory of Tononi,[46] such state of cortical functional architecture significantly reduces available information in addition to inhibiting the capacity for information integration, leading to a lack of subjective content – conditions suggested to be associated with unconsciousness.[6,33]

Since observed in the present study impairment in EEG operational architectonics structure (due to its independence from brain damage etiology) reflects functional and thus *potentially reversible* damage as opposed to irreversible structural neuronal loss, we could speculate that in those cases where patients in VS recover awareness of self and environment, EEG operational architecture should also show a functional recovery. Such data are not yet available; however, in a recent prognosis study it has been found that patients in persistent VS who had EEG operational architecture parameters closer to patients in MCS recovered some level of consciousness in future compared to those patients in VS who had significantly impaired EEG operational architecture parameters – they continued to stay in persistent VS.[90] Further support comes from a recent independent TMS-EEG study which demonstrated recovery of effective connectivity in the brain of noncommunicating patients that paralleled the recovery of consciousness from VS.[91]

Although patients in MCS demonstrated evidence of EEG operational architecture closer to normal fully-conscious subjects, significant differences are still present in patients in MCS when compared to normal subjects.[68] The fMRI studies have corroborated this finding: resting cerebral metabolism in patients in MCS also showed significant differences from normal subjects.[92,93] Any consciousness that might occur in patients in MCS is likely to be extremely short lasting and fragmented as observed in the clinical practice; thus, it is designated as "fluctuating".[3] This has been confirmed using the EEG operational architecture methodology.[68,69]

The obtained results were similarly found in all three frequency bands – alpha, beta1 and beta2 (Fig. 1 and 2). This is in line with the modern view that conscious awareness might depend on the formation and disassembling of synchronized neuronal assemblies characterized by various frequency bands.[44,94-98]

5. Conclusions, clinical and ethical significance

5.1. Concluding discussion

Taking the results of this study together with our previous findings,[68] we could conclude that EEG operational architectonics profoundly shapes awareness and other conscious states, including their contents: it is the sufficient level of *intact coordinated activity* among *relatively large*, *long-lived* and *stable neuronal assemblies* that is important for enabling routine representational processes to be integrated within a coherent phenomenal world from the first-person perspective. At the same time, impairment in the characteristics of such EEG operational architectonics may underlie the fading of consciousness until its complete absence, if such impairment reaches a critical level as in the patients in VS (Fig. 2, 3), who by definition have complete unawareness of self and the environment[70-72]. Apart from their clinical relevance, these data contribute to the current debate among neuroscientists concerning the relationship between neuronal activity in the brain and human consciousness[57,99-101] and hopefully will lead to important new insights in the mechanisms that underlie consciousness in the human brain during further studies (though see Overgaard's argument[6] that there is always an open possibility that the registered signal in some cases may fail as an indicator of consciousness, thus at best it can only indicate consciousness but not measure it).

5.2. Clinical relevance

This new theoretically based methodological approach has a clear potential to objectify the patients' clinical state and improve inter-rater (observer-dependent) variability if proven to be effective at the individual level. Therefore, if confirmed upon further studies, it may help to differentiate between patients in VS and MCS and eventually locked-in syndrome (locked-in syndrome patients may also be mistakenly considered unconscious[102]), as well as reduce the current level of misdiagnosis.[5] Finally, if prove to be successful upon further validation, the ability to evaluate the most probable prognosis/outcome would satisfy a major request of rehabilitation centers – planning and implementing a rational and adequate rehabilitation intervention.

5.3. Relevance for medical ethics

The present study could be relevant to the field of medical ethics, because it may trigger an ethical reframing due to the shifting models of consciousness phenomenon, diagnosis, recovery and prognosis.[6,103,104] Indeed, patients in VS condition and the uncertainty about their state of awareness along with decisions about life-sustaining treatment in such cases have often been the focus of media attention[105] and ethical debates.[106] As it is stated by Mappes,[107] "recognizing prospective autonomy in the context of a living will or establishing a substituted judgment is always necessary when a patient is no longer able to declare his or her current wishes". In this context, if one considers "personal identity" and a "living will" existing only when certain states of consciousness are present (as the findings of the present study seem to point to), then establishing a substituted judgment (the will of the patient is determined by a family member or a doctor) on this basis could make sense.[101] Therefore, knowledge of the state of awareness beyond clinical assessment in patients with DOC is absolutely imperative for making a substituted judgment and can be extremely helpful when there is a need to establish or even justify particular treatment options. Furthermore, knowledge of possible awareness can influence the contact behavior of family members: they may, for example, feel more compelled to directly address their noncomunicative relative, if signs of awareness could be demonstrated through some neuroimaging technology where clinical signs are absent.[106]

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