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ORIGINAL PAPER

EEG Microstate Analysis in Patients with Disorders of Consciousness and Its Clinical Significance

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Abstract

Disorders of Consciousness are divided into two major categories such as vegetative and minimally conscious states. Objective measures that allow correct identification of patients with vegetative and minimally conscious state are needed. EEG microstate analysis is a promising approach that we believe has the potential to be effective in examining the resting state activities of the brain in different stages of consciousness by allowing the proper identification of vegetative and minimally conscious patients. As a result, we try to identify clinical evaluation scales and microstate characteristics with resting state EEGs from individuals with disorders of consciousness. Our prospective observational study included 28 individuals with a disorder of consciousness. Control group included 18 healthy subjects with proper EEG data. We made clinical evaluations using patient behavior scales. We also analyzed the EEGs using microstate analysis. In our study, microstate D coverage differed substantially between vegetative and minimally conscious state patients. Also, there was a strong connection between microstate D characteristics and clinical scale scores. Consequently, we have demonstrated that the most accurate parameter for representing consciousness level is microstate D. Microstate analysis appears to be a strong option for future use in the diagnosis, follow-up, and treatment response of patients with Disorders of Consciousness.

Keywords Disorders of consciousness · EEG microstate analysis · Minimally conscious state · Vegetative state · Microstate D

Introduction

Neurological intensive care and regular medical treatment have greatly improved in recent years. As a result, most of patients are recovering from significant brain injuries (Wutzl et al. 2021). However, not everyone recovers entirely. Many patients stay in a prolonged coma, defined as the lack of alertness and awareness after the acute condition, with a considerable number of survivors having impaired consciousness (Xie et al. 2017). Patients suffering from Disorders of Consciousness (DOC); divided into three states: coma, vegetative state (VS), and minimally conscious state (MCS) (Giacino et al. 2002). Patients with VS voluntarily open their eyes but do not respond to external stimuli. Patients with MCS show signs of awareness of themselves or their surroundings, albeit this awareness fluctuates (Giacino et al. 2002).

Various measures have been developed throughout the years to classify DOC patients. The Coma Recovery Scale-Revised (CRS-R) is the most recent and preferable scale for evaluating consciousness in the post-acute period (Giacino et al. 2004). Simplified Evaluation of CONsciousness Disorders (SECONDs) is introduced as a novel quick assessment method that may be used in daily practice to identify the state of consciousness in patients with severe brain injury. Its primary advantages are that it is substantially quicker and simpler to deploy than CRS-R (Aubinet et al. 2021).

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Table 1 Characteristics of the groups included in the study

Features	VS	MCS	DOC (total)	Healthy controls
Number of subject	12	16	28	18
Male:female	10:2	12:4	22:6	12:6
Age	57.0 ± 17.3	48.9 ± 19.3	52.4 ± 18.6	51.7 ± 15.3
CRS-R	5.67 ± 1.83	12.3 ± 3.84	9.46 ± 4.56	
SECONDs	1 ± 0	4.69 ± 1.74	3.11 ± 2.27	
Etiology				
Hypoxic brain ınjury	10	8	18	
Cerebrovascular disease	2	4	6	
Traumatic brain ınjury	0	4	4	
Time of onset				
Acute	4	4	8	
Prolonged	2	5	7	
Chronic	6	7	13	

Values are «mean ± SD» and «number of subjects»

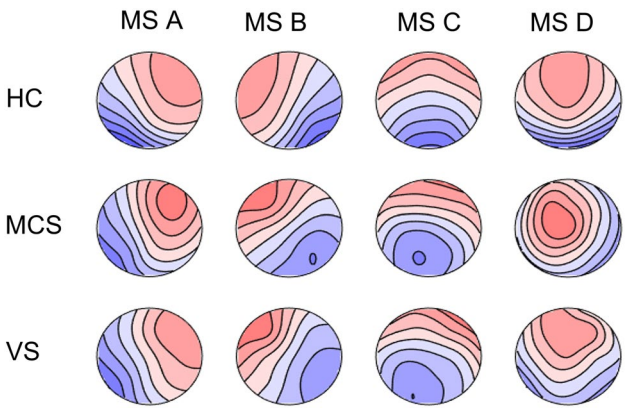


Fig 1. Mean microstate topographies of the groups included in the study

The first step in developing a clinical strategy and selecting appropriate treatment is to make a correct diagnosis. The appropriate therapy can only be identified if the correct diagnosis is available. Although it has limitations, behavioral testing is the gold standard for diagnosis and prognosis (Giacino et al. 2009). However, bedside clinical evaluation has challenges; it necessitates up to date knowledge and may be susceptible to the practitioner's subjectivity. As a result, objective metrics that allow correct diagnosis of VS and MCS patients are required (Luauté et al. 2010). The use of various neuroimaging and neurophysiological methods for this objective is a research area.

Quantitative electroencephalography (EEG) uses algorithms to ease the visual evaluation of EEG traces and add objective data to them. Resting-state EEG, when examined quantitatively, eliminates subjective inaccuracies in diagnosis and prognosis, allowing objective clinical evaluation and tracking of therapy response. Visual examination of raw EEG data is less objective than quantitative EEG, and quantitative EEG has been found to give better validation than visual grading (Nuwer 1997). Quantitative EEG, besides alternative evaluation approaches such as functional magnetic resonance imaging or positron emission tomography, has the advantages of being non-invasive, less costly, generally applicable, and measuring at the bedside.

Microstate analysis is a spatial–temporal quantitative EEG analysis approach that examines topographic maps of electrical potentials on an electrode array and their temporal evolution. Multi-channel EEG data is fundamentally considered and processed as a series of electrical field topography in this technique (Lehmann et al. 1987; Pascual-Marqui et al. 1995).

Microstate analysis is a promising tool that we believe can be effective in exploring the brain's resting state activity in different stages of awareness, allowing for the accurate diagnosis of VS and MCS patients. Furthermore, such analyses can help us comprehend the significant disparities in the resting state activities of patients with varying degrees of impaired consciousness and identify levels of awareness that behavioral tests cannot detect. As a result, we attempted to compare clinical evaluation scales and microstate characteristics in DOC patients' resting state EEGs using microstate analysis.

Materials and Methods

Participants

The Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University approved this study with decision number 711 dated 17.09.2020.

Thirty patients between 18 and 80 who met the criteria for DOC were enrolled in our prospective observational

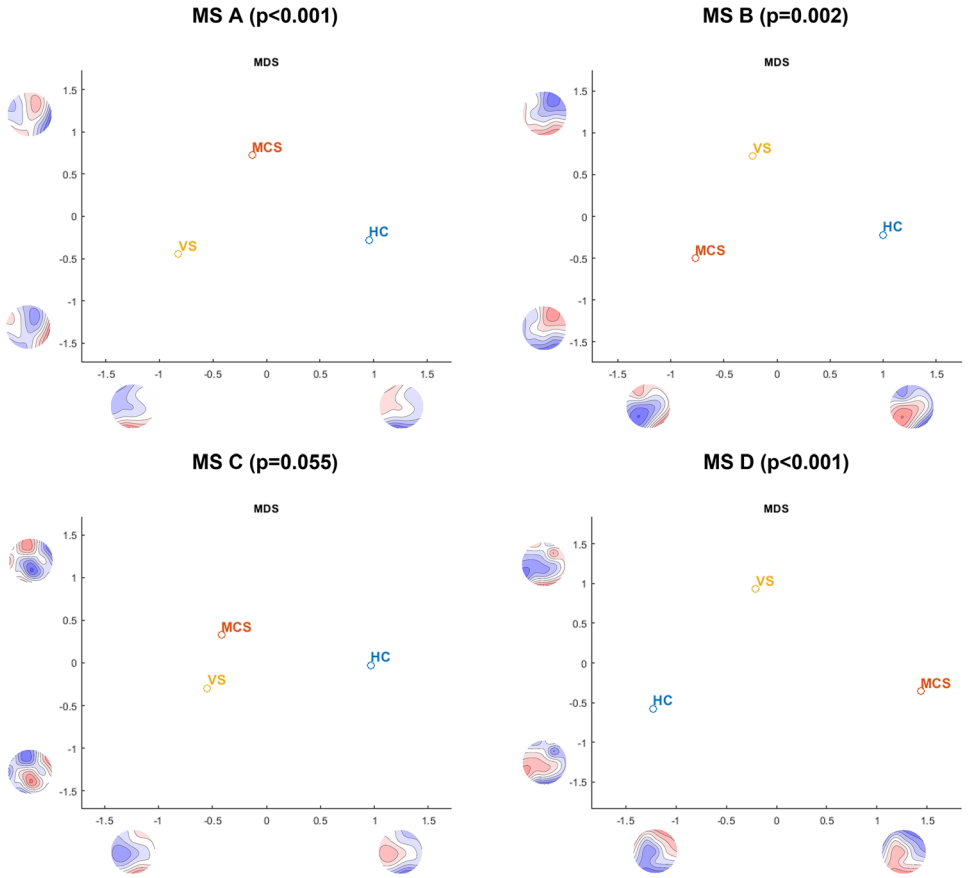


Fig 2. Examination of topographical variations in microstate maps. TANOVA of the individual microstate topographies for MS A, MS B, MS C, and MS D compared to HC, MCS, and VS. Each microstate class that was used as input for a multidimensional scaling (MDS) analysis is shown in the figure with between-group spatial comparison. By applying the spatial principal component analysis (PCA) to all mean group maps, a method used in MDS analysis, high-dimensional result spaces are downscaled into lower-dimensional ones, enabling the visualization of the data. PCA eigenvector maps are depicted on the x- and y-axes. As a result, each group point on the graph is depicted so that groups with similar topographies can be located closer together while groups with different topographies can be located at a greater mutual distance.

Table 2 Topographical group-wise comparison of microstate topographies using post-hoc TANOVA. For maps A, B, and D, the overall TANOVA was significant; the dash denotes that the overall TANOVA was not significant and that no post-hoc analyses were performed

Group pair	MS A	MS B	MS C	MS D
HC vs MCS	0.007*	0.004*	–	< 0.001*
HC vs VS	0.004*	0.028*	–	0.003*
MCS vs VS	0.068	0.112	–	0.011*

*Denotes p values less than 0.05. All values corrected for multiple comparisons

research (Giacino et al. 2018). The relatives of the patients were informed about the study in advance, and their formal agreement was acquired. Prior to examination patient's history regrading underlying chronic condidition and medications (drug history) were obtained. Unstable medical conditions, use of sedative/hypnotic drugs, severe heart, lung, liver, or kidney failure, history of primary psychiatric, developmental, or neurological disease that may cause known functional disability, uncontrolled epileptic seizures, coma, and patients with a brain death diagnosis were excluded from the study. As a control group, we used EEG data from 18 healthy controls (HC) of similar ages and gender to the patient group.

The CRS-R and SECONDs scales were administered to the study participants multiple times during the day at various intervals, and the neurologist provided clinical assessments. To assure the patients' wakefulness prior to EEG recording, we administered verbal and tactile stimulation as recommended on the CRS-R scale (Giacino et al. 2004).

EEG Recordings

At the bedside, EEG was recorded digitally for 20 min at a sample rate of 256 Hz from 19 channels positioned according to the 10–20 system using the Neurosoft NEURONSPECTRUM 5 equipment and Neurosoft neuron-spectrum.net software. For recording, Ag–AgCl ring electrodes were utilized. For a clean EEG recording, electrode impedances were maintained below 10 kΩ.

EEG Preprocessing

We collected the resting state EEG data from the control and patient groups for processing. To preprocess the EEG data, we used the EEGLAB (2021.0) toolbox, that runs on the MATLAB (2021b) software. We set the data sampling

Table 3 Microstate parameters values of groups (GEV: global explained variance)

Microstate parameters	VS	MCS	DOC (total)	Healthy controls
GEV (%)	62.55 ± 12.077	65.66 ± 4.493	64.33 ± 8.550	79.91 ± 3.409
Mean duration (ms)	15.301 ± 17.601	79.93 ± 17.381	79.53 ± 17.154	66.36 ± 8.568
Mean occurrence(hz)	13.32 ± 3.346	13.10 ± 2.949	13.20 ± 3.067	15.3 ± 1.924
Microstate A				
Duration (ms)	81.28 ± 22.833	81.36 ± 19.706	81.33 ± 20.691	68.66 ± 9.002
Occurrence (hz)	3.61 ± 0.952	3.28 ± 0.906	3.42 ± 0.924	4.07 ± 0.864
Coverage (%)	28.28 ± 7.396	25.86 ± 7.193	26.89 ± 7.247	28.08 ± 7.166
Microstate B				
Duration (ms)	88.80 ± 24.114	77.95 ± 16.549	82.60 ± 20.468	58.99 ± 9.427
Occurrence (hz)	3.99 ± 1.146	3.32 ± 1.131	3.61 ± 1.166	3.34 ± 0.681
Coverage (%)	34.63 ± 10.581	25.76 ± 9.911	29.56 ± 10.964	19.77 ± 5.371
Microstate C				
Duration (ms)	72.38 ± 18.157	82.40 ± 24.995	78.10 ± 22.515	59.21 ± 9.203
Occurrence (hz)	3.33 ± 1.260	3.56 ± 0.780	3.46 ± 0.999	3.67 ± 0.763
Coverage (%)	23.62 ± 8.751	28.41 ± 6.958	26.36 ± 7.996	21.83 ± 6.139
Microstate D				
Duration (ms)	58.62 ± 16.624	70.27 ± 18.829	65.27 ± 18.547	71.93 ± 18.914
Occurrence (hz)	2.39 ± 0.685	2.94 ± 0.871	2.70 ± 0.830	4.23 ± 0.647
Coverage (%)	13.46 ± 4.115	19.98 ± 6.033	17.18 ± 6.155	30.32 ± 8.626

Values are «mean ± SD»

rate to 128 Hz (EEGLAB function resample). We used a 50 Hz notch filter (EEGLAB function eegfiltnew), a 2–20 Hz band-pass Finite Impulse Response (FIR) filter (EEGLAB function eegfiltnew), and recomputed to the average reference (EEGLAB function reref). We used Artifact Subspace Reconstruction (max acceptable 0.5-s window std dev 10) to remove artifacts (EEGLAB function clean rawdata) automatically (Chang et al. 2018, Plechawska-Wojcik et al. 2018). Eye movement and muscular artifacts were automatically rejected from the data using independent component analysis (ICA) (EEGLAB function runica) (Delorme & Makeig 2004). After all artifact removal methods, if they remained, we discarded manually less than two-second intervals, body movements, muscle activity, and technical artifacts. Some DOC patients had shorter EEG data due to artifacts. To balance the remaining data, we kept the clean 120 s of data for microstate analysis.

Microstate Analyses

We performed microstate analysis using additional compatible software (Version 1.2; http://www.thomaskoenig.ch/Download/EEGLAB_Microstates/), microstate analysis for the EEGLAB toolbox through MATLAB (2021b). We first identified the topographic maps in the momentary peak of Global field power (GFP, at the time points of the optimum signal-to-noise ratio, the maximum voltage values on all electrodes) by the standard procedure and calculated the individual microstate maps for each participant using the k-means clustering method. We have already adjusted the number of clusters to four since previously published four microstate classes described a high amount of EEG data variation in patient groups and healthy subjects and terms of comparability to earlier studies. As a result, the clustering method identified each participant's four most prominent cluster maps. We used a permutation approach to minimize the common variance across patients to compute the group model maps individually for each group (VS, MCS, and HC). According to the norm model maps published by Koenig et al., we classified the classes as microstates (MS) A-B-C-D (Koenig et al. 2002). We assigned individual microstate maps using the map template for HC from the sorted group model maps. We extracted the following parameters from the microstate data: The total global variation rate explained by microstates, coverage (percentage of total time covered by a microstate class), duration (the average time when a microstate class remains temporarily stable in milliseconds), occurrence (frequency of occurrence of one microstate class), transitions (comparison of differences between observed and expected transition probabilities) calculated to reveal communication between networks (Murphy et al. 2020). A non-parametric randomization topographic analysis of variance (TANOVA) (Strik et al. 1998) was performed using the Ragu software to compare the microstate topographies of each microstate class between the HC and patient groups (Koenig et al. 2011). The three groups (HC, MCS, and VS/UWS) were compared using the four unique microstate maps for each participant that were obtained from

the cluster analysis and canonical sorting procedure in order to find any appreciable reference-independent topographical differences. If there was a significant TANOVA, post-hoc electrode-wise t-maps were created between all possible group pairs (HC vs MCS, MCS vs VS/UWS, HC vs VS/UWS).

Statistical Analysis

We used the JAMOVI 1.8.4 application to analyze the data statistically. Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean ± standard deviation or median (min–max) depending on the distribution of normality. We used visual and analytical approaches to check that the variables had normal dispersion (Kolmogorov—Smirnov/Shapiro—Wilk tests). Using Spearman correlation analysis, we determined microstate characteristics' connection, statistical implications, and clinical evaluation scales. In comparing the independent variables between the two groups, we utilized the Student t test for parameters with normal distribution and the Mann–Whitney U test for parameters with non-normal distribution. When we classified the patient group by clinical evaluation scales and compared different groups, we used ANOVA for normally distributed parameters and kruskal wallis test for parameters with non normal distribution. We performed cross-group comparisons in significant parameters using post-hoc Games-Howell testing. The statistical significance p-value is 0.05.

Results

Demographic Data

Two patients were excluded from the study because the EEG data contained heavy artifacts. 12 of the 28 DOC patients had VS/UWS, 16 had MCS (Table 1).

Of the 18 HC we included in the study, 12 were male, and 6 were female. Of 12 VS/UWS patients, 10 were male, and 2 were female. Of 16 MCS patients, 12 were male, and 4 were female. There was no gender difference between the groups ($p = 0.591$). The average age; was 57.0 ± 17.3 years in VS/UWS patients, 48.9 ± 19.3 in MCS patients, and 51.7 ± 15.3 in HC. There was no age difference between the groups ($p = 0.477$). According to the duration of the disease, 4 of the VS/UWS patients were acute, 2 were prolonged, and 6 were chronic. We had 4 acute, 5 prolonged, and 7 chronic MCS patients. According to etiologies, 10 VS/UWS patients had hypoxic brain injury (HBI), and 2 had cerebrovascular disease (CVD). MCS patients had 8 HBI, 4 CVD, and 4 traumatic brain injuries (TBI). The mean CRS-R scores of the patients were 5.67 ± 1.83 in the VS/UWS group, 12.3 ± 3.84 in the MCS group. The mean SECONDs scores of the patients were 1 ± 0 in the VS/UWS group, 4.69 ± 1.74 in the MCS group (Table 1).

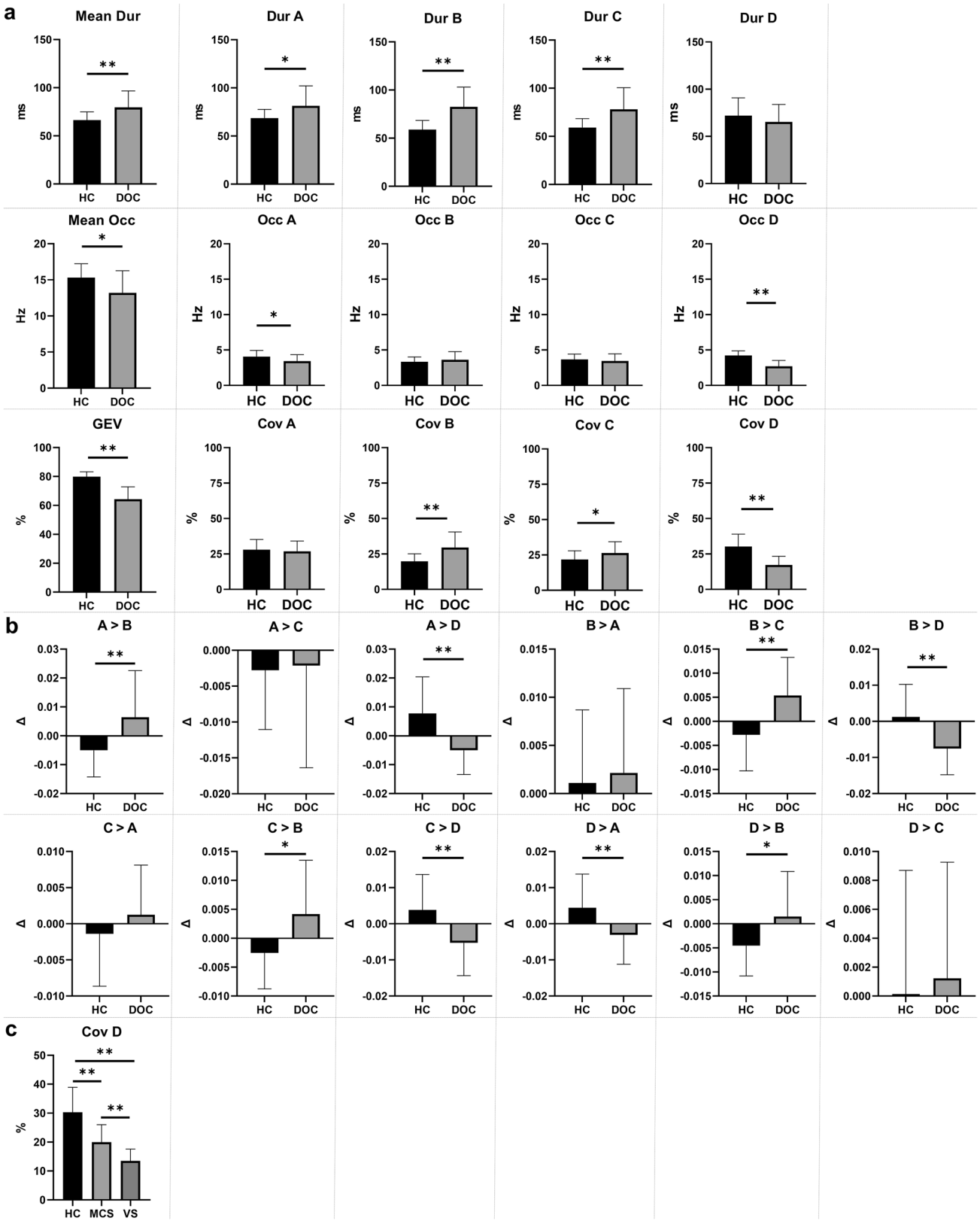
Microstate Topographies

Microstate topographies of HC were similar to those in the literature (Koenig et al. 2002) (Fig. 1). We performed TANOVA of the individual microstate topographies for MS A, MS B, MS C, and MS D compared to HC, MCS, and VS. For maps A, B, and D, the overall TANOVA was significant (< 0.001, 0.002, < 0.001 respectively) (Fig. 2). We discovered that HC distinguished in all maps with both MCS and VS when we compared each group to one another using post-hoc TANOVA analysis. MCS and VS, however, were only distinguished on the MS D map (Table 2).

Microstate Parameters

We comparatively examined the microstate parameters between groups (Table 3). The explained variance was significantly higher in the HC than in the DOC ($p < 0.001$). There was no significant difference in the variance explained between the patient groups. The mean duration of microstates was higher in DOC patients ($p = 0.004$). The frequency of occurrence of microstates was lower ($p = 0.008$). There was no significant difference between the patient groups regarding the mean duration and frequency of microstates (Fig. 3a).

When we compared DOC patients with HC, the mean duration of Microstate A was longer ($p = 0.014$), and MS A occurrence was lower ($p = 0.022$). There was no significant difference in microstate A parameters between the patient groups. In the DOC group, the mean duration of microstate B was long ($p < 0.001$), and MS B coverage was high ($p = 0.001$). There was no significant difference in microstate B parameters between the patient groups. DOC patients had longer mean microstate C duration ($p = 0.002$) and greater MS C coverage ($p = 0.047$). There was also no significant difference in microstate C parameters between the patient groups. The occurrence ($p < 0.001$) and coverage of D microstate was less in the DOC group ($p < 0.001$) (Fig. 3a). Among the groups, HC had greater microstate D coverage than both MCS patients and VS/UWS patients ($p < 0.001$), and MCS patients had greater MS D coverage than VS/UWS patients ($p = 0.013$) (Fig. 3c).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◂**Fig 3.** **a** Comparison of the microstate parameters (Duration, Occurrence, Coverage) between the HC group and the DOC group. **b** Comparison of the microstate transition probabilities (In seconds scale, the observed transition probabilities given the known distribution of microstate labels minus the expected transition probabilities) between the HC group and the DOC group. **c** Bar plot of microstate D coverage with significant difference between MCS group and VS/UWS group. Significant differences between groups were marked with "*" (*p<0.05, **p<0.005, *Dur* duration, *Occ* occurrenge, *Cov* coverage, *GEV* global explained variance)

Transition Probabilities

We also compared the temporal transitions of the intergroup microstate topographies (Fig. 4). In DOC patients, transition rates were greater from Microstate A to B ($p = 0.004$), from Microstate B to C ($p < 0.001$), from C to B ($p = 0.010$) and from D to B (0.021). HC group transition rates were greater from MS A to D ($p < 0.001$) from D to A ($p = 0.004$), from B to D ($p < 0.001$) and from C to D ($p = 0.001$) (Fig. 3b and 4). There was no significant difference between patient groups.

Correlation with Clinical Scales

CRS-R and SECONDs scores were positively correlated with the occurrence and coverage of microstate D (Fig. 5).

Discussion

Our study aimed to investigate the microstate parameters in patients with unconsciousness and to evaluate the relationship of these parameters with the level of consciousness and clinical assessment scales. For this purpose, we compared the microstate analyses of the resting state EEGs of VS, MCS patients and HC. This study is one of the few studies examining the microstate parameters of patients with DOC. In the results of our study, we determined that patients with DOC differed from HC with changes in microstate parameters. In particular, we found that the microstate D parameters correlated with consciousness level and clinical assessment scales. We believe that our findings will contribute to the consciousness disorders and microstate analysis literature.

We analyzed the resting-state EEG data of VS, MCS, and HC by separating them into four microstate maps. Topographic maps of HC group were consistent with the literature (Khanna et al. 2015; Koenig et al. 2002; Michel & Koenig 2018). This situation is essential in terms of comparability with the literature. We found that the mean microstate maps of both the MCS group and the VS group diverged topographically with the HC group. However, the MCS group and the VS group were only differentiated in the MS D map.

While examining the microstate parameters, we used the mean topographic maps of our HC data, which formed our normals, as a template in all groups. According to this, In the DOC group, the total explained variance of the four microstate maps was significantly lower, as we expected. The literature has repeatedly reported that the total explained variance rate of the four microstate maps is above 70% in studies performed with HC (Khanna et al. 2015; Koenig et al. 2002; Michel & Koenig 2018). The decrease in the variance explained in the patient group shows that brain oscillations differ from HC. However, we could not find a significant difference between the MCS and VS groups. Therefore, we think that the explained variance rate is a general parameter in showing consciousness disorders but not a sensitive enough indicator.

In DOC patients, the mean duration of microstates was significantly longer, and their frequency was significantly less. The literature has reported that the mean microstate durations increase and the frequencies decrease with age (Koenig et al. 2002). In a study conducted in 2012, it was determined that the average microstate durations were prolonged during deep sleep (NREM 3) (Brodbeck et al. 2012). In addition, in a recent study, it was reported that the mean microstate durations were prolonged in dementia with Lewy bodies, and this prolongation was negatively correlated with the functional connectivity of the basal ganglia network and the thalamic network; It was emphasized that this situation might be related to the slowdown in mental activities (Schumacher et al. 2019). However, studies conducted in recent years have shown that the average microstate parameters are prolonged in Alzheimer's patients (Tait et al. 2020). The increased mean duration was suggested as a finding reflecting cognitive decline, indicating that the conversion of microstate parameters to each other slowed down (Tait et al. 2020). There was no age difference between our patient and healthy groups. Mean duration and total frequency values did not successfully differentiate VS and MCS. Similar to the variance rate explained, we think these parameters are a general finding showing that the transition between microstates in patients with cognitive impairment slows down. Therefore brain oscillations slow down, but they are not sensitive enough to show the severity of the disease. In recent studies, it has been determined that the durations of all microstates are prolonged and their occurrences are reduced under sedation (Artoni et al. 2022). Being in deep sedation, which is another representation of decreased consciousness, coincides with the unconsciousness in our study in this sense. However, mean duration and mean occurrence are not sensitive enough to differentiate DOC patients.

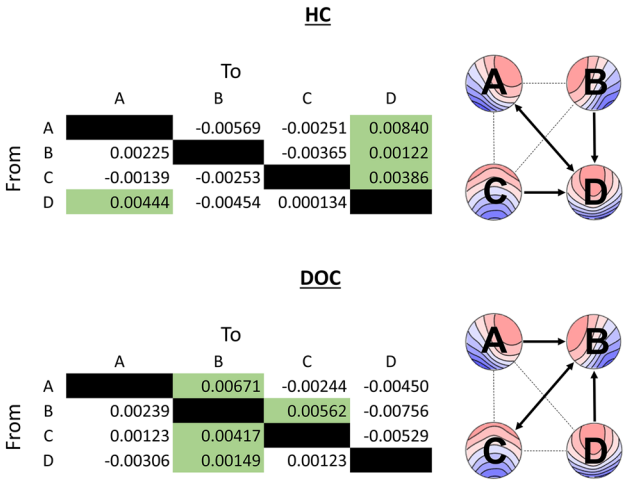

Fig 4. In seconds scale, this matrix shows the observed transition probabilities given the known distribution of microstate labels minus the expected transition probabilities. Positive values indicate that there are more transitions in the listed group compared to other, negative values indicate that there are less transitions in the listed group compared to the other. Colored boxes indicate statistically significant more transitions compared to the other group ($p < 0.05$). The illustrations on the right side, the paths that the groups prefer statistically significantly more are shown with arrows

In DOC patients, the mean duration of Microstate A was prolonged, and its occurrence decreased. A study conducted in 2018 suggested that the incidence of Microstate A was a finding reflecting the prognosis of DOC patients (Stefan et al. 2018). Microstate A is defined as a network associated with the auditory network (Custo et al. 2017). The auditory network is associated with the bilateral superior and middle temporal gyrus, and the left middle frontal gyrus regions show functional connectivity (Britz et al. 2010). The changes we see in the parameters of microstate A may result from these regions being affected in DOC patients. In addition, Microstate A parameters did not distinguish VS-MCS sensitively, and it was not a sensitive parameter in showing the severity of the disease.

We found that the mean duration of microstate B was prolonged, and its coverage increased in DOC patients. Studies report that the duration or coverage of B is increased in patients with Parkinson's and dementia with Lewy bodies, and its duration is decreased in patients with schizophrenia (Chu et al. 2020; Nishida et al. 2013; Schumacher et al. 2019). Microstate B is a network location associated with the visual network (Custo et al. 2017). In this network, bilateral occipital areas and related subcortical structures show functional connectivity (Britz et al. 2010). Changes in MS

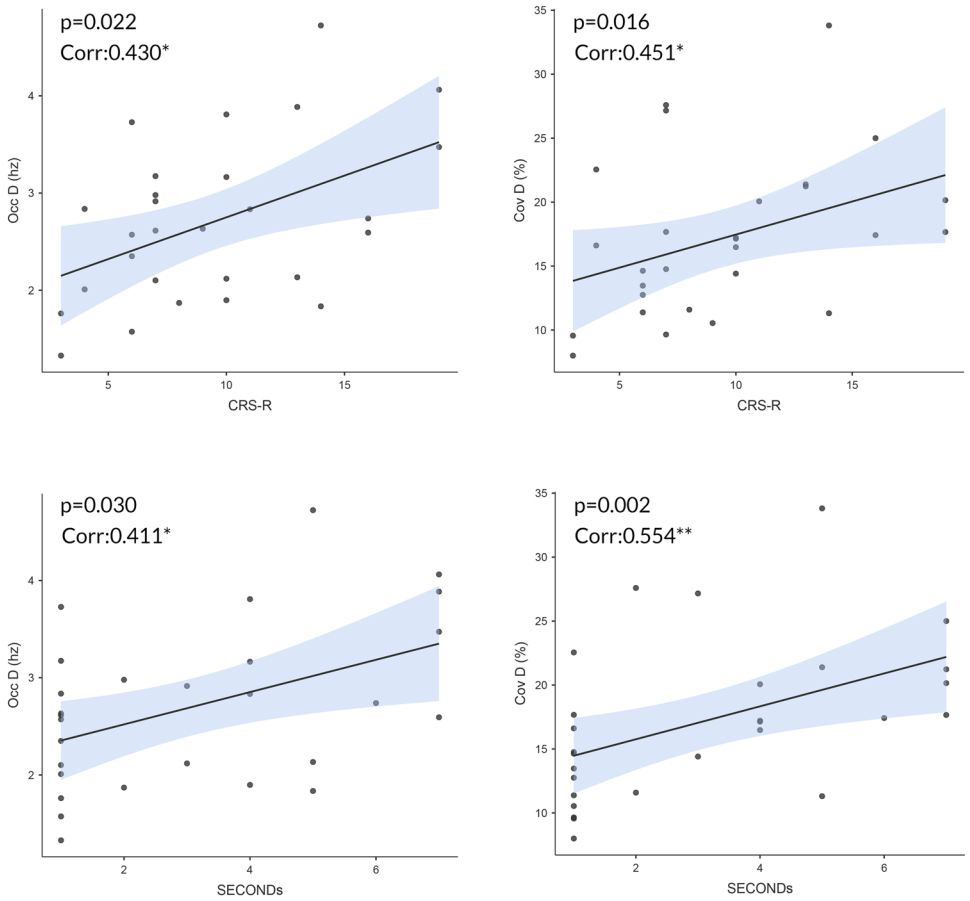

Fig 5. Correlation graphs showing the relationship between microstate D parameters and clinical assessment scales

B may indicate that these areas are affected in DOC patients. This parameter was also not found sensitive enough to show the severity of the disease.

In microstate C, similar to MS B, the mean duration was prolonged, and the coverage increased in DOC patients. Microstate C is a map associated with the saliency network. The anterior cingulate cortex, medial cingulate gyrus, left inferior frontal gyrus, left claustrum, right inferior frontal gyrus, and right amygdala show functional connectivity in this defined network (Britz et al. 2010). However, in recent studies with a larger number of microstate maps, it has been shown that MS C is differentiated and the networks it represents may be different (Custo et al. 2017). While MS A and B maps are more prominently associated with audio and visual networks, this is not the case for the remaining maps. Custo et al. found that MS C was most associated with activity in the posterior cingulate cortex (PCC) and precuneus regions. These regions mostly reflect the default mode network. Although it was known that there was decreased activity in all networks in the DOC group, it was reported that the most affected region was PCC (Vanhaudenhuyse et al. 2010). In this sense, although our results may indicate decreased activity in these networks, MS C parameters were unfortunately not found sensitive enough to differentiate patient groups. In a recent study showing the sleep microstate relationship, it was reported that the coverage of microstate 3 and 4 increased significantly during deep sleep (Bréchet et al. 2020). There were more than 4 microstate maps in this study, and it can be assumed that both microstate 3 and microstate 4 maps correspond to MS C in our study. If so, Cov C increased in the sleep state, which we consider as one of the states of decrease in the level of consciousness, as in the DOC group.

When we examined the parameters related to the microstate D maps, we found that the coverage and occurrence decreased. These parameters also differed significantly between MCS and VS and correlated with clinical assessment scales. A study conducted in 2018 reported that the coverage of microstate D is the most sensitive parameter in differentiating VS and MCS patients (Stefan et al. 2018). The increasing coverage of microstate D was presented as a parameter indicating the severity of the disease. Surprisingly, in our study, the coverage in microstate D was associated with higher clinical scores, namely increased awareness. This difference may be due to methodological changes, we examined the 2–20 Hz frequency band, but the result found in the literature was only in the alpha frequency band. In a very recent study, the occurrence of microstate D was also found to correlate with clinical assessment scores in DOC patients. They also reported that they improved these parameters with noninvasive brain stimulation methods (Guo et al. 2022). There seems to be increasing evidence that MS D may be a sensitive parameter in the diagnosis and follow-up of the DOC group. In a study conducted in 2012 examining the relationship between sleep stage microstates, it was reported that the incidence and frequency of Microstate D decreased as sleep deepened (Brodbeck et al. 2012). Publications show that microstate D's topography and parameters are also affected in Parkinson's disease and Alzheimer's disease (Chu et al. 2020; Ignacio Serrano et al. 2018; Pal et al. 2021; Tait et al. 2020). Microstate D is a map associated with the frontoparietal network, working memory network, and attention network (Britz et al. 2010). In particular, areas lateralized to the right, covering the right upper and middle frontal gyrus and the right upper and lower parietal lobules, show functional connectivity in this network (Custo et al. 2017). We think that the frequency and ratio of this map, which is associated with the frontoparietal network, are the most sensitive parameters in distinguishing DOC patients and showing the severity of the disease. Results of previous resting state network studies in DOC patients highlight the coexistence of a common disorder involving the associative cortices such as the midline of the frontoparietal network (i.e., anterior cingulate cortex-mesiofrontal and posterior cingulate cortex associated with internal awareness or self-related processes) and lateral network (i.e., prefrontal and posterior parietal area associated with environmental awareness) (Beuthien-baumann et al. 2003; Juengling et al. 2005; Laureys et al. 1999a, b; Laureys et al. 1999a, b; Lull et al. 2010; Nakao et al. 2010; Silva et al. 2010). It has been shown that the connection in the midline frontoparietal cortex, also called the default mode network (DMN), reflects the consciousness level of DOC patients (Vanhaudenhuyse et al. 2010). We speculate that the most affected network in DOC, which is known to affect a large network structure, is the frontoparietal network associated with MS D.

Examining the transition probabilities between microstate maps, we found that DOC patients are more likely to prefer transitions over Microstate B and less likely to transition over Microstate D. It is thought that transitions between microstates are not random (Khanna et al. 2015). More work is needed to link these transition possibilities with functional networks.

Our study has certain limitations. First, we did not use a structural neuroimaging method in our study. We had a heterogenous group of patient populations with diverse conditions. Different brain regions may be affected due to hypoxia, trauma, or cerebrovascular disease. In subsequent studies, structural brain imaging can be used to improve group homogeneity. We selected the four maps a priori by comparing them to other articles. However, determining the number of optimum maps would be a more data-driven procedure. As previously stated, our study examined four microstate topographies in the frequency range of 2–20 Hz. In order to have more precise measurements, analysis of different frequencies (delta, theta, alpha, and

beta) and examination of more than four topographies can be done. Finally, we did not conduct a long-term follow-up in our study. In future studies, longitudinal follow-up can be performed to compare the prognosis of the patients and to know the sensitivity of microstate parameters in determining the prognosis of patients.

Conclusion

In this study, where we analyzed the resting state EEGs of patients with consciousness disorders, we successfully determined the relationship between the microstate parameters (especially Microstate D) and the severity of the disease. Among the microstates presented by Lehmann as atoms of thought, microstate D was identified as the best parameter reflecting consciousness. Our study is one of the few studies in this field and sheds light on the literature in this respect. Evaluation of parameter changes of Microstate D with repeated EEGs can be a clinical sign that can be used in the patient's follow-up. Microstate analysis seems to be a strong candidate for the future identification, follow-up, and treatment response of patients with Consciousness Disorders. Future studies with longitudinal follow-ups and confirmation of our data are needed.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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