Night sleep in patients with vegetative state

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SUMMARY

Polysomnographic recording of night sleep was carried out in 15 patients with the diagnosis vegetative state (syn. unresponsive wakefulness syndrome). Sleep scoring was performed by three raters, and confirmed by means of a spectral power analysis of the electroencephalogram, electrooculogram and electromyogram. All patients but one exhibited at least some signs of sleep. In particular, sleep stage N1 was found in 13 patients, N2 in 14 patients, N3 in nine patients, and rapid eye movement sleep in 10 patients. Three patients exhibited all phenomena characteristic for normal sleep, including spindles and rapid eye movements. However, in all but one patient, sleep patterns were severely disturbed as compared with normative data. All patients had frequent and long periods of wakefulness during the night. In some apparent rapid eye movement sleep episodes, no eye movements were recorded. Sleep spindles were detected in five patients only, and their density was very low. We conclude that the majority of vegetative state patients retain some important circadian changes. Further studies are necessary to disentangle multiple factors potentially affecting sleep pattern of vegetative state patients.

INTRODUCTION

The notion of disorders of consciousness (DoC) encompasses several conditions in which individual awareness is either completely lost or severely disturbed as a consequence of an acquired brain damage. Two most important chronic forms of DoC are vegetative state [VS; syn. unresponsive wakefulness syndrome (UWS); Laureys *et al*., 2010] and minimally conscious state (MCS; Giacino *et al*., 2002).

The examination of neurophysiological functions in VS and MCS pursues two aims. On the one hand, the lack of consistent behaviour in both conditions makes the diagnosis highly error-prone (Andrews *et al*., 1996). We do not really know what happens in the mind of these patients (Kotchoubey *et al*., 2002). An analysis of their brain functions is expected to provide us with additional information about their condition.

Among the possible neurophysiological markers of disordered consciousness in VS and MCS patients, sleep pattern is attracting increasing attention during the last years. Neurophysiological changes in sleep are well studied in healthy humans (Hobson and Pace-Schott, 2002). The presence of standard sleep markers is related to normal course of cognitive and emotional processes in wake state (Walker, 2009), plays a key role in memory consolidation (Diekelmann and Born, 2010), in hormonal regulation (Van Cauter *et al*., 2008) and immune functions (Besedovsky *et al*., 2011). Close relationships between the quality of neurophysiological sleep patterns and clinical symptoms are demonstrated in a number of neurological diseases.

Only eight studies of sleep in DoC had been published before 2010 (D'Aleo *et al*., 1994a,b; Giubilei *et al*., 1995; Gordon and Oksenberg, 1993; Isono *et al*., 2002; Oksenberg *et al*., 2000, 2001; Valente *et al*., 2002). These studies revealed various patterns of sleep in patients with DoC, particularly rapid eye movement (REM). In some experiments, REM sleep was even found in every patient included in the study (D'Aleo *et al*., 1994a; Gordon and Oksenberg, 1993; Oksenberg *et al*., 2000, 2001). Markers of non-(N) REM sleep were also found (D'Aleo *et al*., 1994a,b). Unfortunately, these studies did not distinguish between VS and MCS.

Table 1 Recent data about sleep phenomena in adult patients with the diagnosis VS (syn. UWS)

	N	*Cyclicity*	*Spindles*	*K-complexes*	*SWS*	*REM*	*All sleep signs*
Alekseeva *et al.* (2010)	64	38	n.r.	n.r.	n.r.	n.r.	n.r.
Aricò *et al.* (2016)	8	5	2	2	–	1	–
Arnaldi *et al.* (2015)	20	17	17‡	17‡	–	3	2
Bedini *et al.* (2015)	27†	27	27‡	27‡	21	9	n.r.
Cologan *et al.* (2013)	10	3	4	n.r.	4	3	n.r.
de Biase *et al.* (2014)	27	22	15	22	–	4	2
Forgacs *et al.* (2014)	8	5	4	5	2	2	n.r.
Landsness *et al.* (2011)	5	–	–	–	–	–	–
Kang *et al.* (2014)	56	24	24	n.r.	n.r.	n.r.	n.r.
Rossi Sebastiano *et al.* (2015)	85	65	n.r.	n.r.	n.r.	n.r.	3*

N, number of patients; REM, rapid eye movement sleep; SWS, slow-wave sleep.
'Cyclicity' means any clear neurophysiological indication of sleep/wakefulness cycle, even if it is not strictly defined according to the usual sleep criteria.
(–) Indicates that the corresponding pattern was not found in any patient.
'n.r.' means 'not reported'.
*The quantity was not reported but could be extracted from graphical data.
†A total of 42 patients were examined but, in 15 of them whose EEG activity was below 20 μV, polysomnograms were not analysed.
‡The authors did not report K-complexes and spindles separately.

The interest in this issue increased significantly with a fundamental publication from Cologan *et al.* (2013), after which 12 studies (some with large patient samples) were published (Table 1). These studies indicate that sleep data may be among the best predictors of the outcome of VS or MCS (Alekseeva *et al.*, 2010; Aricò *et al.*, 2016).

One broadly cited study (Landsness *et al.*, 2011) did not find any markers of sleep in VS patients at all, although sleep spindles, slow-wave sleep and REM sleep patterns were observed in MCS. The authors concluded that VS patients exhibit only behavioural signs of sleep–wakefulness cycle but no physiological features of sleep. The issue is particularly controversial because sleep is a part of the very definition of VS. Whereas patients in a coma remain in the same condition all the time, the transition from coma to VS is characterized by alternation of apparent sleep and wakefulness periods. This alternation may not follow the normal circadian pattern (i.e. a long period with closed eyes during the night), but at least it must be present. From a purely applied point of view, a practitioner can simply use a diagnostic rule 'episodes of open eyes indicate VS' without worrying about the real functional meaning of open-eyes and closed-eyes episodes. However, a good medical symptom is not just a sign, it should have theoretical significance. If the claim of Landsness *et al.* (2011) was correct, and if there are no neurophysiological changes underlying the alternation of behavioural sleep–wakefulness changes, then the differential diagnostics between coma and VS would lose theoretical foundation.

These results, however, were not supported by later observations. The data of the recent studies, summarized in Table 1, suggest that at least some elements of NREM sleep can be found in most VS patients, and patterns of REM sleep in about 15–20% patients. Rossi Sebastiano *et al.* (2015) reported in the largest study to date that only 20 of 85 VS patients did not show any sign of sleep; moreover, 10 of these 20 patients had an isoelectrical electroencephalogram (EEG), which is rather atypical for VS. Unfortunately, these authors did not report specific sleep signs.

All studies converge on the fact that sleep patterns in patients in a MCS are substantially better than in VS. Rossi Sebastiano *et al.* (2015) observed only one patient in a MCS who was 57 years old who did not show any physiological sign of sleep.

The interpretation of sleep data in DoC is further complicated by the necessity to find a right balance between objective sleep measures and expert ratings. As emphasized by Cologan *et al.*, 2013); many patients show various atypical patterns of activity, whose scoring in classical terms of sleep stages (Iber *et al.*, 2007; Rechtschaffen and Kales, 1968) is very difficult. Thus, many authors preferred to abandon the classical sleep scoring in patients with DoC. However, the alternative strategies have their own traps. Sometimes the subjective scoring criteria are replaced by other kinds of expert classification (Arnaldi *et al.*, 2015; Rossi Sebastiano *et al.*, 2015). Such classifications can be useful in DoC, but they are not less subjective, and definitely less standardized than Rechtschaffen and Kales' (1968) or AASM criteria. To our best knowledge, none of the recent sleep studies in DoC used more than one rater, and only de Biase *et al.* (2014) explicitly mention that the rater was blinded regarding the clinical aspects of patients.

The subjectivity can, of course, be avoided if we decide for purely automatic methods (Kang *et al.*, 2014; Malinowska *et al.*, 2013). This strategy, however, is related to the risk to obtain quantitative data whose functional meaning may be difficult to interpret. Furthermore, recent analyses raise

considerable doubt that the existing automatic methods of sleep evaluation can replace the consent expert judgement (O'Reilly and Nielsen, 2015; Warby *et al*., 2014). This is particularly true for scoring of sleep spindles, which plays an important role in the evaluation of sleep in general. Also ratings of single (even quite experienced) experts may be insufficient as compared with consent scores (Wendt *et al*., 2015). To summarize, reliability of sleep assessment in patients with DoC remains a major issue. Therefore, in the present study each polygram was independently assessed by three raters and, additionally, an automatic analysis of EEG, electromyogram (EMG) and electrooculogram (EOG) signals was performed.

The aim of this study was to investigate night sleep patterns in a group of VS patients, using a combination of both blind expert ratings and objective methods. The hypothesis that there are no physiological sleep–wakefulness changes underlying behavioural episodes of open versus closed eyes was taken as a zero hypothesis. Because there is no controversy in the literature about sleep in MCS, patients in a MCS were not included in the current study.

MATERIALS AND METHODS

Patients

Seventeen VS patients were investigated in this study. One of them was excluded from the analysis because of excessive amount of muscle artefacts. Another patient was excluded because immediately after sleep recording the diagnosis was changed to MCS. After exclusion we analysed polysomnography data of 11 male and four female patients aged 45.5 $\pm$ 15.7 years (range 21–72 years). All patients were spontaneously breathing. None of them received tranquilizers, barbiturates, neuroleptics or antidepressive drugs. None had had any neurological or mental disease prior to the current brain lesion.

Coma Recovery Scale-Revised (CRS-R) was applied for clinical assessment at most 2 days before the study enrolment (Giacino *et al*., 2009). CRS-R examinations were performed by two experienced and trained neurologists. Maximal possible care was given to the clinical assessment. The frequency of assessments depended on the length of the previous stay. At the beginning after the admittance from an ICU CRS-R was evaluated every 2 days, after the stabilization of the patient's state the frequency was reduced to once per week or once per 2 weeks. CRS-R was applied only once in 2–3 months in patients who were in VS/UWS for 5 years and longer. In none of the examined patients was the diagnosis supported by only one single CRS-R evaluation. The diagnosis for each patient was stable and reliable. Clinical, demographic and sleep data are summarized in Table 2. The study was approved by the ethical committee of the University of Tübingen. Informed consent was obtained from the patients' legal representatives.

Data acquisition and analysis

Data were collected with a BrainAmp amplifier (Brain Products). We recorded the EEG from F3, F4, C3, C4, P3 and P4 sites (10-20 system) with linked mastoid reference. Additionally, two channels of chin EMG and two EOG channels (positioned 1 cm lateral to the outer canthi of both eyes, and 2 cm below and above the left eye) were recorded. All signals were recorded using Ag-AgCl cup electrodes and Grass electrode paste with 500 Hz sampling frequency, 0.3 Hz high-pass filter, 70 Hz low-pass filter and 50 Hz notch filter. During the recording, patients were in their usual environment on the wards. The light was turned off at 22:00 hours.

Visual scoring

Sleep scoring was performed visually on 30-s epochs according to the criteria of AASM (Iber *et al*., 2007). Three scorers performed this analysis independently, two of which (SG and MS) did not know any characteristics of the patients except the diagnosis VS. The agreement between these two 'blind' raters in terms of Cronbach's α was 0.84 (SE = 0.08). All scoring conflicts were resolved via a final discussion among the scorers. Criteria of the waking state were a higher EEG frequency than in other periods of the recording, high muscular tone and eye blinks. Stage N1 was scored if EEG frequency became slower than during wakefulness, eye blinks disappeared and muscle activity decreased. The presence of rolling eye movements was an additional optional criterion. Stage N2 was indicated by the presence of K-complexes and (sometimes) sleep spindles. A sleep spindle was defined as a 0.5–3 s EEG pattern with the amplitude between 20 and 100 μV, and a frequency between 11 and 16 Hz. N3 was scored when an epoch included >20% of high-amplitude delta waves, which had to occur synchronously in different but not injured cortical areas. REM sleep was defined as a combination of fast, low-voltage EEG activity with minimal muscular tone. As mentioned in the literature (Forgacs *et al*., 2014), eye movements of patients with DoC in REM often have abnormalities. Therefore, the presence of muscle atonia and the EEG activity faster than in other conditions was given a higher weight in the definition of REM sleep than the EOG.

Automatic data analyses

In patients in whom sleep spindles were observed, their density per minute was calculated by each rater. Additionally, this number was checked automatically using SPD Toolbox (Molle *et al*., 2002). This analysis starts with building a power spectrum for stage 2 episodes, in which a distinct peak between 11 and 16 Hz is usually seen. In the present study, the range for the search of spindles was defined as the spectral peak frequency $\pm$ 2 Hz. In those patients, in whom no spectral peak was detected, this range was 12–16 Hz. The amplitude threshold for spindle activity was set at 1.5

Table 2 Clinical, demographical and sleep data of the patients

Pat.	*Age (years)*	*Gender*	*Time*[†]	*CRS-R*[‡]	*Duration*	*Aetiology*	*Main lesion(s)*	*N1*	*N2*	*N3*	*REM*	*Spindles*
1	50	m	8	5 (1,0,2,1,1)	9.2	multiple infarcts*	Midbrain, thalamus, basal ganglia	–	–	–	–	–
2	51	f	3	3 (0,0,1,1,1)	7.8	tumour resection	Inferior frontal lobe L, midbrain, thalamus L	+	+	–	–	0.4
3	21	m	6	8 (2,1,2,1,2)	4.9	TBI	DAI	+	+	+	+	0.5
4	72	m	3	5 (1,0,1,1,2)	7.5	anoxia	Diffuse atrophy both sides	+	+		–	–
5	40	m	12	8 (2,1,2,1,2)	9.9	TBI, traumatic SAB, anoxia	Frontal + occipital lobes L, DAI	–	+	+	+	–
6	44	m	11	6 (1,0,2,1,2)	4.2	anoxia	Frontal atrophy	+	+	+	+	0.53
7	33	m	2	2 (0,0,1,0,1)	9.9	SAB	Temporal, parietal, frontal R	+	+	+	+	–
8	21	m	45	6 (1,0,2,1,2)	9	anoxia	Frontotemporal atrophy	+	+	+	+	0.88
9	59	m	3	6 (1,0,2,1,2)	9.9	anoxia	Diffuse atrophy both sides	+	+	–	+	–
10	64	f	3	7 (2,1,2,1,1)	10	TBI + SAB	Frontal, parafalxial, occipital R	+	+	–	–	–
11	47	m	4	5 (1,0,1,1,2)	8.6	anoxia	Cortical atrophy R > L	–	+	+	–	0.52
12	54	f	80	6 (1,1,2,1,1)	10.7	anoxia	Diffuse atrophy both sides	+	+	–	+	–
13	65	m	182	4 (1,1,1,0,1)	8.3	status epilepticus	Diffuse atrophy both sides	+	–	–	+	–
14	33	f	39	9 (2,1,2,2,2)	8.8	SAB	Frontal, temporal R > L	+	+	+	+	–
15	57	m	30	8 (2,1,1,2,2)	10.8	TBI	Temporal R	+	+	+	+	–

CRS-R, Coma Recovery Scale-Revised; DAI, diffuse axonal injury; REM, rapid eye movement; SAB, subarachnoidal bleeding; TBI, traumatic brain injury.
The sign '+' means 'present'.
Duration – duration of sleep recording, h.
Spindle density (last column) was calculated per minute of stage N2, because very few spindles (if any) were observed during N3.
*Probably thrombosis of a. basilaris.
[†]Time elapsed since the injury, in months.
[‡]CRS-R, total score (numbers in parentheses: scores in the auditory, visual, motor, verbal and arousal scales; the score of communication scale was zero in all patients).

standard deviation of the mean EEG amplitude within the selected frequency range.

Further, a frequency analysis using a Fast Fourier Transformation was performed for the EEG, EOG and EMG signals on the same 30-s epochs. The power of EEG activity was calculated within the frequency ranges of delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta1 (13–20 Hz). The EMG was analysed within the range of 20–30 Hz. Because of local brain lesions, channels for spectral analysis differ among patients. We used F3, F4 or C4 channels for delta and theta activity analysis, P3, P4, C3 or C4 for alpha activity, C3, C4, P3 or P4 for beta1 activity.

To compare the objective spectral power data with expert scoring, these data underwent a one-way analysis of variance (ANOVA) with sleep stages (1–4 and REM) as a factor, followed by the Dunnett's *post hoc* test for multiple comparisons. Single 30-s epochs were used as units of the analysis. Spectral power data were log transformed to normalize their distribution. The analysis was carried out for each patient separately, because individual patients widely differed in respect to the number of sleep stages (Table 2) and power values. The epochs of wakefulness were excluded from the analysis.

RESULTS

As can be seen in Table 2, sleep signs were observed in 14 of the 15 examined VS patients. They were also found in those patients who had a very long history of the disease and very severe brain lesion. More specifically, stage N1 was observed in 13 patients, N2 in 14 patients, N3 in nine patients and REM sleep in 10 patients. Sleep spindles were found in five patients. Three patients exhibited all phenomena listed above, also including REM during REM phases. Data of two patients are

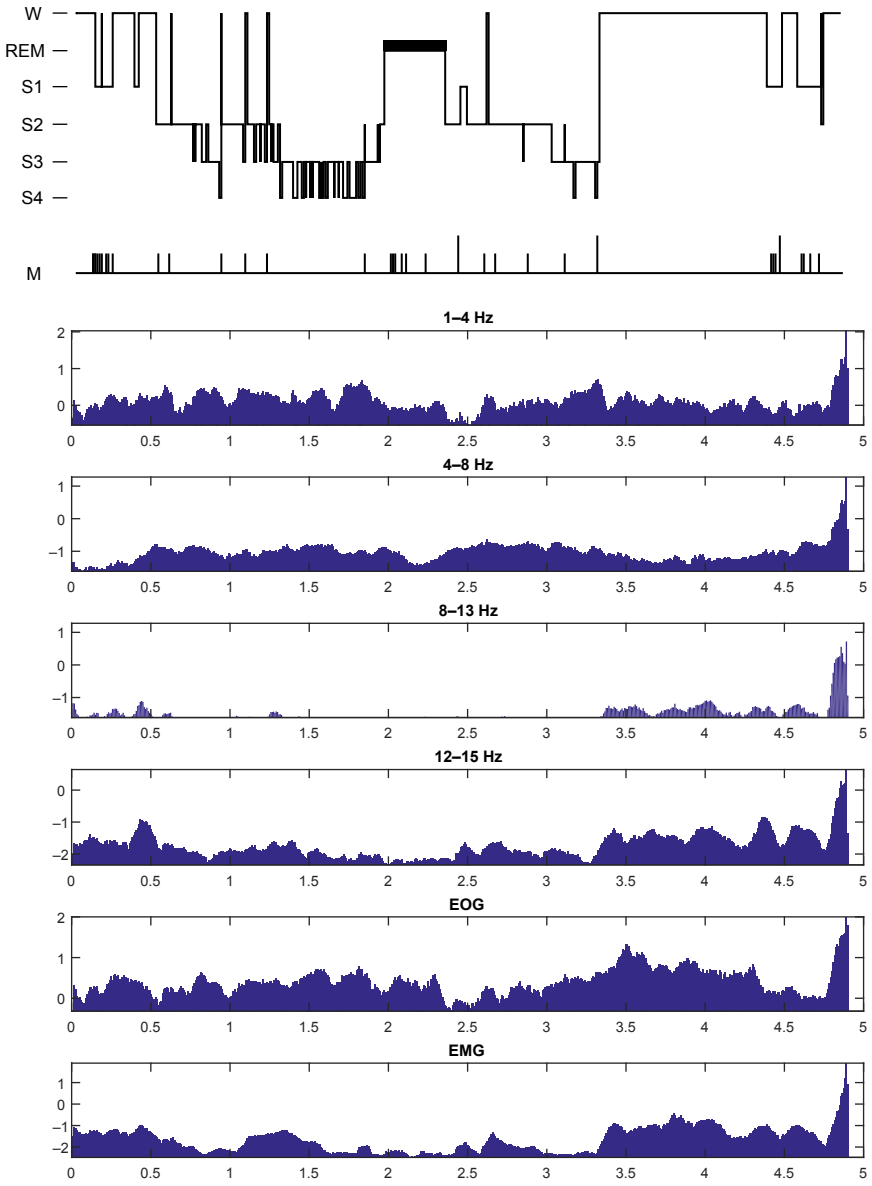

Figure 1. The hypnogram of patient 3, and the correspondence between the scoring data and the power of electroencephalogram (EEG) frequency bands, the electrooculogram (EOG) and the electromyogram (EMG). M, large body movements; W, wakefulness.

illustrated in Figs 1–4, showing their somnograms, power spectra and sleep stages that were identified in these patients.

The only patient without a definite differentiation between sleep and wakefulness was a patient with diffuse delta activity at all EEG leads. This activity did not change during 9.2 h of recording. The data of Table 2 as well as the somnograms demonstrate that also in other patients sleep patterns substantially deviated from the pattern typical for normal sleep. Only one patient (No. 3) demonstrated a cyclic pattern approximating the normal one, i.e. regular alternation of sleep stages with the usual prevalence of slow-wave sleep in the first half of the night and REM sleep in the second half. All recordings were characterized by long and frequent epochs of wakefulness during the night; some patients even remained awake most of the night. Stage N2 was mostly characterized by typical K-complexes, but spindles were observed only in five patients, and their density was low (Table 2). Some REM sleep episodes, characterized by fast low-amplitude EEG activity and a very low muscle tone, did not contain REM (Figs 5–7).

The results of the spectral analysis agree with the data of visual scoring. As shown in Table 3, the power of the EEG oscillations in delta, theta, alpha and beta ranges, as well as EMG power follows in each patient the dynamics expected on the basis of expert ratings. The correspondence between subjective sleep ratings and the results of the spectral analysis is illustrated by Figs 1 and 3.

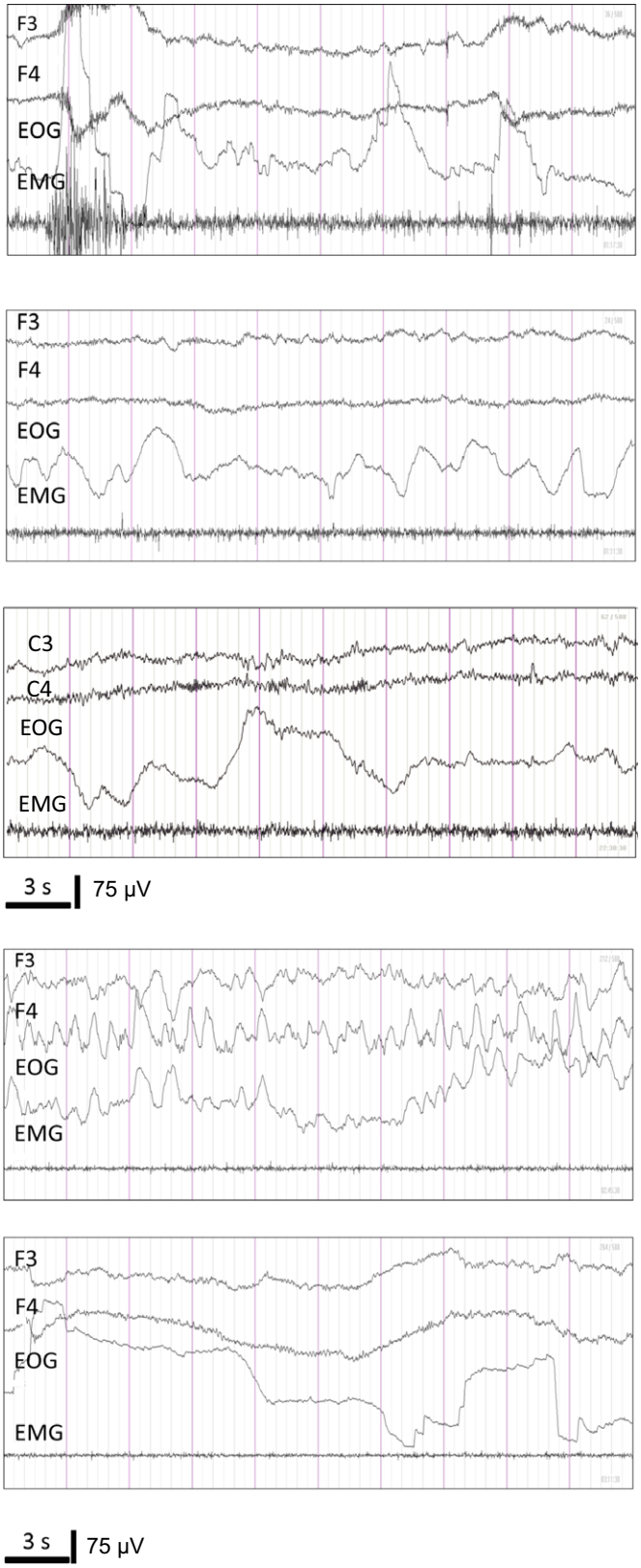

Figure 2. Patterns of activity obtained in patient 3, whose hypnogram is presented in Fig. 1. First panel from the top: wakefulness. Mixed, mainly low-amplitude electroencephalogram (EEG), high electromyogram (EMG) activity, saccades and blinks. Second panel: N1 stage. High-frequency EEG, rolling eye movements, no body movements, no alpha and theta activity. Third panel: N2 stage. Regular sleep spindles and K-complexes. Right top corner: the number of the presented 30-s epoch. Fourth panel: N3 stage, abundant slow waves. Fifth (bottom) panel: rapid eye movement (REM) sleep, REM and very low muscle tone.

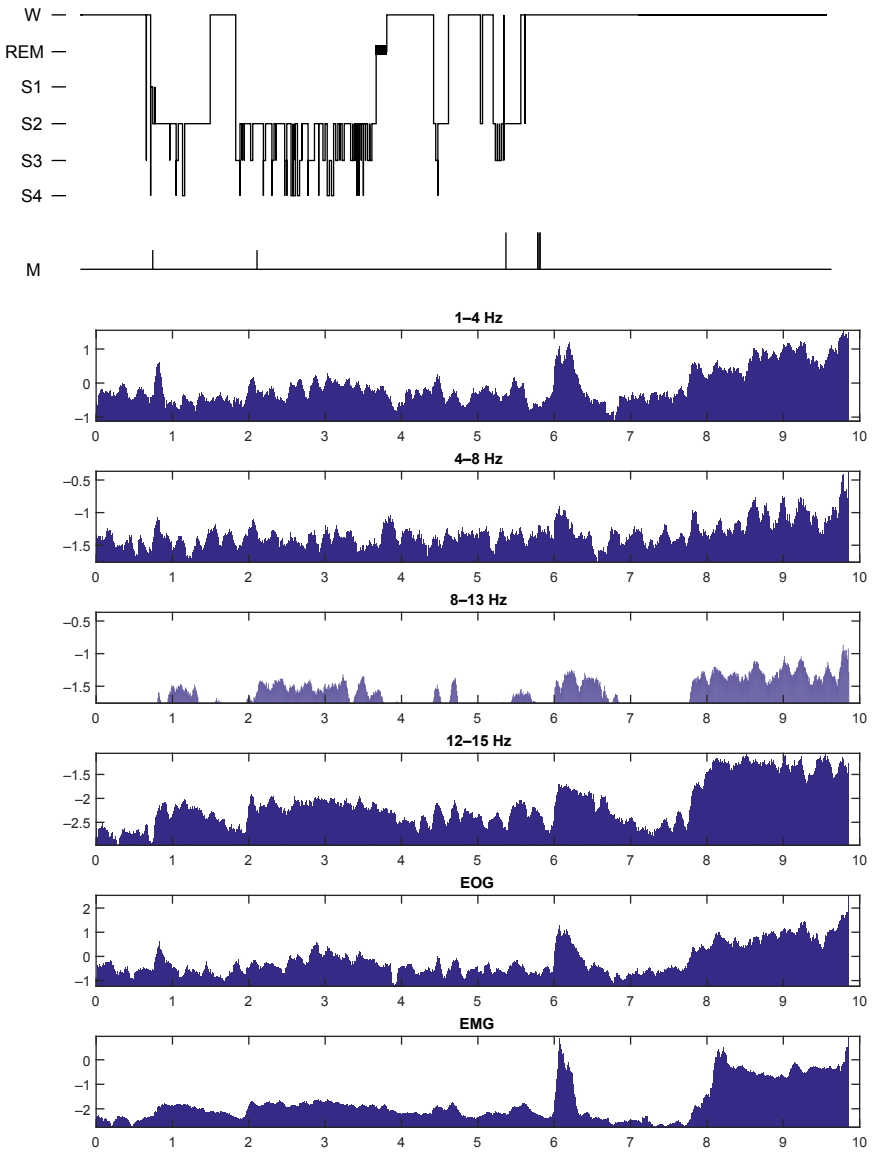

Figure 3. The hypnogram of patient 7, and the correspondence between the scoring data and the power of electroencephalogram (EEG) frequency bands, the electrooculogram (EOG) and the electromyogram (EMG). M, large body movements; W, wakefulness.

DISCUSSION

Abnormal sleep in VS

The results of the present study are in line with the data of several recent investigations (Arnaldi *et al.*, 2015; Bedini *et al.*, 2015; de Biase *et al.*, 2014) and strongly contradict those of Landsness *et al.* (2011). Only one of the 15 examined VS patients exhibited a uniform pattern of brain activity all the time. At least some sleep components were unequivocally present in the other patients, and most sleep components were present in some patients. These findings were obtained in the agreement of three independent sleep scorers, and confirmed by the spectral analysis of EEG and EMG signals. This mutual confirmation is not trivial, because the agreement both among human raters and between raters and automatically selected patterns of activity is known to be generally low even as concerns sleep stages of normal human individuals (Wendt *et al.*, 2015). This agreement decreases further when sleep of patients with anxiety or depression is analyzed. Thus, one might not be surprised if the agreement in the present population with very atypical sleep patterns was particularly poor. The results presented in Table 3 indicate satisfactory reliability of the classification performed in this study. Note that we do not claim that we can clearly and unequivocally classify each 30-s epoch in each patient according to the AASM scale. However, the agreement is possible for most epochs, which yields at least an approximate evaluation of the representation of sleep stages. The combination of several scorers with objective 'blind' techniques such as the spectral analysis substantially increases the reliability of results as compared with using

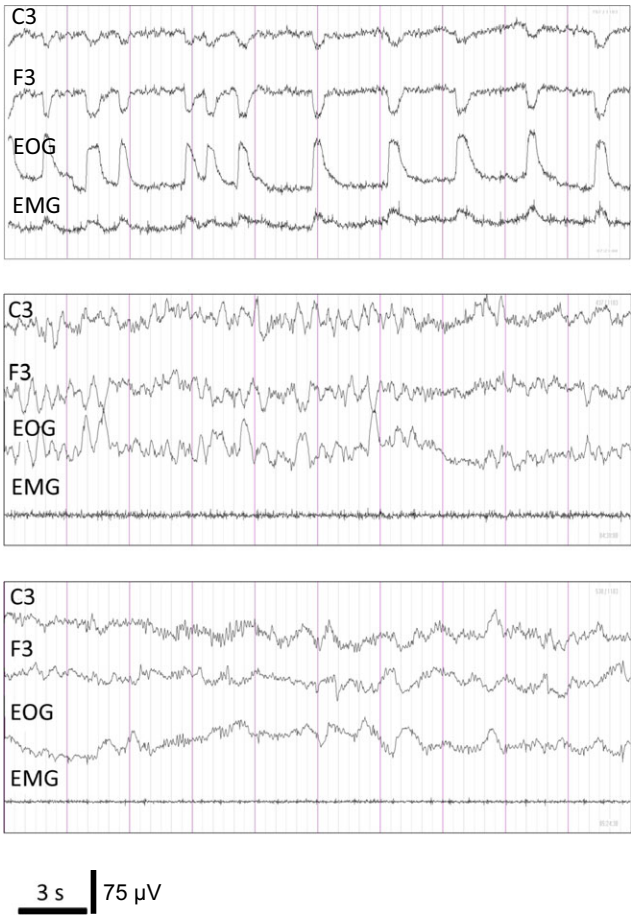

Figure 4. Patterns of activity obtained in patient 7, whose hypnogram is presented in Fig. 3. Top panel: possible wakefulness, recorded in the evening and morning. Continuous activity <1 Hz, slower and of different shape than typical delta waves. Middle panel: N3 stage. Clear 10–20 s bouts of delta activity alternating with low-amplitude electroencephalogram (EEG). Bottom panel: rapid eye movement (REM) sleep. Disappearance of slow EEG waves, strong theta activity, minimal muscle tone. No eye movements. Right top corner: the number of the presented 30-s epoch.

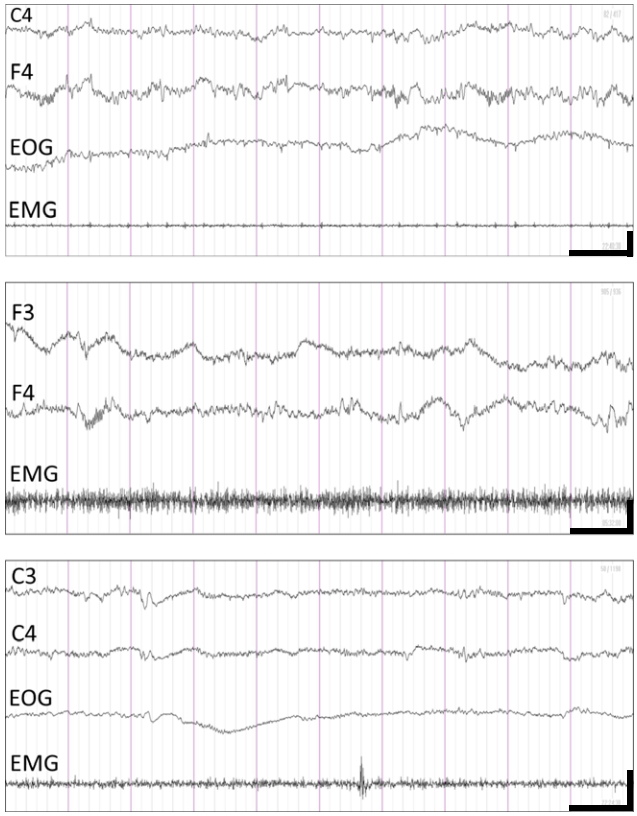

Figure 5. Examples of N2 sleep stage with (top and middle panels) and without spindles (bottom panel). Top panel: patient 8; middle panel: patient 2; bottom panel: patient 10. Right top corner: the number of the presented 30-s epoch. The scales on this and the following figures are: horizontal axis, 3 s; vertical axis, 75 μV.

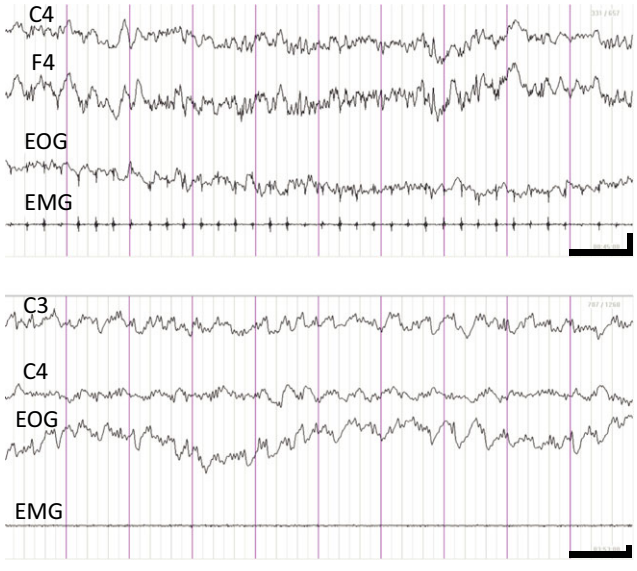

Figure 6. Examples of N3 sleep stage. Top panel: patient 8; bottom panel: patient 8. Right top corner: the number of the presented 30-s epoch.

only one rater or simply running an automatic classification procedure over the data.

Several patients reported here belong to the most severe VS patients. One of them only attained a total CRS-R score of 2, indicating almost complete behavioural unresponsiveness, and about half of his brain was destroyed. Nevertheless, he exhibited a clear alternation of episodes with <10% to >50% slow waves over the healthy hemisphere, phases of REM sleep, and K-complexes during stage 2 (Figs 4 and 5). Another patient had been in VS for more than 15 years before the sleep examination, had a quite low total CRS-R score of 4 and an extremely severe hypoxic brain lesion. His sleep was severely disturbed, but even in this patient all scorers detected five REM episodes.

While we can maintain that most VS patients sleep in the night, their sleep is abnormal. Most patients remained awake for long time periods and repeatedly awaked after short sleep episodes that did not constitute a complete cycle. Two phasic sleep phenomena were severely disturbed, namely

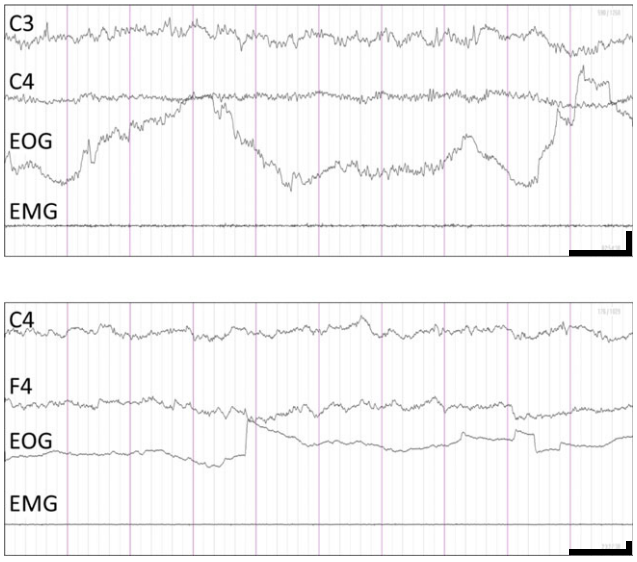

Figure 7. Examples of rapid eye movement (REM) sleep. Top panel: patient 14; bottom panel: patient 11. Right top corner: the number of the presented 30-s epoch.

spindles and REM. No sleep spindles at all were found in 10 patients, and in those few who had spindles, their density was several times lower than in the normal population (Gais *et al.*, 2002). This fact is of particular significance because sleep spindles have been shown to play a highly important role in memory consolidation during sleep (Diekelmann and Born, 2010).

Factors of sleep abnormalities

The possible causes of these abnormalities can be subdivided into two categories: related and unrelated to the specific brain damage underlying the VS. The latter factors may be common for many groups of patients, including fully conscious, and their effect should not be underestimated. To avoid decubitus, patients are regularly turned by the personnel during the night. While healthy individuals and mobile patients move during sleep, they do this at physiologically suitable moments (e.g. at the end of a sleep cycle), while VS patients are moved (and, therefore, frequently awakened) independently of their actual state, sometimes even in the middle of N3, which may strongly disturb all the course of sleep (Figs 8 and 9).

Furthermore, these patients can be subjected to both external and internal stimulation. As regards the former, there is no complete darkness in the hospitals. There is noise from the lobby, beeps of therapeutic equipment and other auditory stimuli related to behaviour of other patients.

The amount of internal stimulation can hardly be estimated given the lack of subjective reports. Despite extensive physical therapy, chronic patients always have spastic phenomena, particularly in low extremities. The spasticity can result in increased activation of somatosensory cortical regions. Other sources of stimulation may be pathological changes, even relatively minor ones such as dryness of mucous membranes. According to Markl *et al.* (2013), more than 50% of patients carefully diagnosed as VS respond to weak pain stimuli with activations of several parts of the pain matrix of the brain. One can only speculate how strong these activations may be with natural pain stimuli such as headache or toothache. The effect of pain on sleep should not be confused with the issue of conscious experience of pain as a subjective phenomenon. Even if the brain activity induced by pain and discomfort does not reach consciousness, it can nevertheless affect the fundamental circadian rhythms and, therefore, sleep.

We believe that future studies of sleep in chronic DoC, and specifically VS, should first of all be aimed at the control of the numerous factors potentially affecting sleep pattern and sleep quality. Previous 24-h recordings (Table 1) have been quite useful in revealing sleep episodes during the daytime, but future studies with 24 h registration should more specifically aim at the analysis of the distribution of different stages and phases within a circadian cycle. In this way, night sleep abnormalities caused by naps during daytime could be controlled. Other factors might be checked by selection of appropriate control groups. Thus, the examination of patients with high-level spinal cord injury (i.e. patients with an intact brain) would permit to control the effects of immobility and enforced turning in the night. The effect of the heightened muscle tone can be controlled using other (conscious) patients who have severe spastic phenomena. Only a careful control of such additional factors will finally tell us which abnormalities are really the results of the underlying brain lesion.

However, already at the present stage we can conclude that VS patients do have circadian changes in brain state, even if these changes frequently differ from those typical for normal sleep and are sometimes difficult to classify. They do not just periodically open and close their eyes, but exhibit neurophysiological components of sleep. This indicates that sleep–wakefulness cycles belong to very basic phenomena of brain functioning, which are present in most (maybe even in all) VS patients.

Limitations

First, the sample size in the present study was small, which did not allow us to draw conclusions based on a statistical analysis. The study is mainly descriptive, and the performed statistical analysis was carried out for each patient separately. Second, the recording time was limited. As said above, night sleep may partially be disturbed simply because patients have slept on the day before. Although several studies performed 24-h recordings in VS patients (Aricò *et al.*, 2016; de Biase *et al.*, 2014; Rossi Sebastiano *et al.*, 2015), they did not report the distribution of sleep components between the daytime (when patients are largely busy with therapy, but can

Table 3 Relationships between sleep scores and spectral power data in individual patients

Pat. no.	*Delta*	*Theta*	*Alpha*	*Beta*	*EMG*
1					
2	N1 < N2 $F_{1,211} = 6.58$; $\eta^2 = 0.03$				
3	(R = N1) < N2 < N3 $F_{4,371} = 34.3$; $\eta^2 = 0.27$	R < (N2 = N3) $F_{4,371} = 8.72$; $\eta^2 = 0.09$	R < N3 < (N2 = N1) $F_{4,371} = 13.7$; $\eta^2 = 0.13$	N1 < (N2 = N3) < R $F_{4,371} = 61.2$; $\eta^2 = 0.40$	R < N1 < (N2 = N3) $F_{4,371} = 23.3$; $\eta^2 = 0.20$
4	N2 < (N1 = N3) $F_{3,266} = 6.6$; $\eta^2 = 0.07$	N2 < N1 $F_{3,266} = 3.3$; $\eta^2 = 0.04$	N3 < N2 < N1 $F_{3,266} = 11.0$; $\eta^2 = 0.11$	N3 < N1 $F_{3,266} = 4.9$; $\eta^2 = 0.05$	N2 < (N1 = N3) $F_{3,266} = 33.5$; $\eta^2 = 0.27$
5	R < N3 $F_{1,70} = 28.2$; $\eta^2 = 0.29$	R < N3 $F_{1,70} = 6.98$; $\eta^2 = 0.09$	R > N3 $F_{1,70} = 10.4$; $\eta^2 = 0.13$	R > N3 $F_{1,70} = 4.62$; $\eta^2 = 0.06$	R < N3 $F_{1,70} = 12.9$; $\eta^2 = 0.16$
6	R < N2 < N1 $F_{2,188} = 20.1$; $\eta^2 = 0.18$	R < N2 < N1 $F_{2,188} = 26.7$; $\eta^2 = 0.22$	R < (N1 = N2) $F_{2,188} = 4.93$; $\eta^2 = 0.05$	R < N2 < N1 $F_{2,188} = 17.8$; $\eta^2 = 0.16$	R < N2 < N1 $F_{2,188} = 36.3$; $\eta^2 = 0.28$
7	R < N2 < N3 $F_{4,401} = 24.1$; $\eta^2 = 0.19$	(N1 = N2) < N3; R < N3 $F_{4,401} = 13.5$; $\eta^2 = 0.12$	R < (N2 = N3) $F_{4,401} = 8.14$; $\eta^2 = 0.08$	R < (N2 = N3) $F_{4,401} = 8.08$; $\eta^2 = 0.08$	R < (N2 = N3); N2 < N3 $F_{4,401} = 8.91$; $\eta^2 = 0.08$
8	(R = N1) < N2 $F_{2,729} = 51.8$; $\eta^2 = 0.12$	(R = N1) < N2 $F_{2,729} = 153$; $\eta^2 = 0.30$	(R = N1) < N2 $F_{2,729} = 56.3$; $\eta^2 = 0.13$	R < N2 < N1 $F_{2,729} = 23.9$; $\eta^2 = 0.06$	(R = N1) < N2 $F_{2,729} = 21.7$; $\eta^2 = 0.06$
9	(N2 = R) < N1 $F_{2,411} = 8.67$; $\eta^2 = 0.04$	(N2 = R) < N1 $F_{2,411} = 11.1$; $\eta^2 = 0.05$	(N2 = R) < N1 $F_{2,411} = 12.6$; $\eta^2 = 0.06$	(N2 = R) < N1 $F_{2,411} = 20.6$; $\eta^2 = 0.09$	(N2 = R) < N1 $F_{2,411} = 12.3$; $\eta^2 = 0.06$
10	N2 < N1 $F_{1,290} = 17.0$; $\eta^2 = 0.06$				N1 < N2 $F_{1,290} = 7.54$; $\eta^2 = 0.03$
11	R < (N2 = N3) $F_{3,719} = 6.55$; $\eta^2 = 0.03$		R < (N2 = N3) $F_{3,719} = 9.95$; $\eta^2 = 0.04$	N2 < N3 < R $F_{3,719} = 9.80$; $\eta^2 = 0.04$	R < (N2 = N3) $F_{3,719} = 383$; $\eta^2 = 0.62$
12	R < N1 $F_{2,426} = 21.7$; $\eta^2 = 0.09$	R < N1 $F_{2,426} = 64.8$; $\eta^2 = 0.23$	R < N2 < N1 $F_{2,426} = 331$; $\eta^2 = 0.61$	N2 < N1 < R $F_{2,426} = 399$; $\eta^2 = 0.65$	R < N2 < N1 $F_{2,426} = 748$; $\eta^2 = 0.78$
13		R < N1 $F_{1,363} = 41.4$; $\eta^2 = 0.10$	R < N1 $F_{1,363} = 42.7$; $\eta^2 = 0.11$	R < N1 $F_{1,363} = 43.2$; $\eta^2 = 0.11$	R < N1 $F_{1,363} = 21.6$; $\eta^2 = 0.06$
14	R < N1 < (N2 = N3) $F_{3,569} = 2.63$; $\eta^2 = 0.01$	R < N1 < (N2 = N3) $F_{3,569} = 4.68$; $\eta^2 = 0.02$	(N2 = N3 = R) < N1 $F_{3,569} = 5.79$; $\eta^2 = 0.03$	N3 < N1 < R $F_{3,569} = 18.6$; $\eta^2 = 0.09$	(R = N3) < N2 < N1 $F_{3,569} = 3.36$; $\eta^2 = 0.02$
15	N1 < N3 $F_{3,495} = 5.14$; $\eta^2 = 0.03$	N1 > N2 $F_{3,495} = 4.34$; $\eta^2 = 0.03$	N3 < R < N2 < N1 $F_{3,495} = 27.6$; $\eta^2 = 0.14$	N3 < R $F_{3,495} = 15.7$; $\eta^2 = 0.09$	R < N2 $F_{3,495} = 3.36$; $\eta^2 = 0.07$

X < Y means a significantly ($P < 0.05$) smaller power in a condition X than Y in a pairwise comparison following a significant ($P < 0.05$) *F*-test. An omnibus *F* and the effects size are indicated below each line of pairwise data. When the *F*-test did not reach significance, the cell remains empty. N1, N2 and N3 designate the corresponding sleep stages according to Iber *et al.* (2007). R means REM sleep.

nevertheless use breaks for naps) and the night-time. Third, video control, which was lacking in this and most other studies, would help to separate sleep from wakefulness when EEG and other polygraphic measures are not sufficient. Finally, although the number of EEG electrodes used in this study was minimally sufficient for stage scoring, much more data might be obtained using a dense electrode net that would permit to perform a source analysis of EEG phenomena.

CONCLUSIONS

In most VS/UWS patients several distinct electrophysiological patterns can be distinguished and qualified as

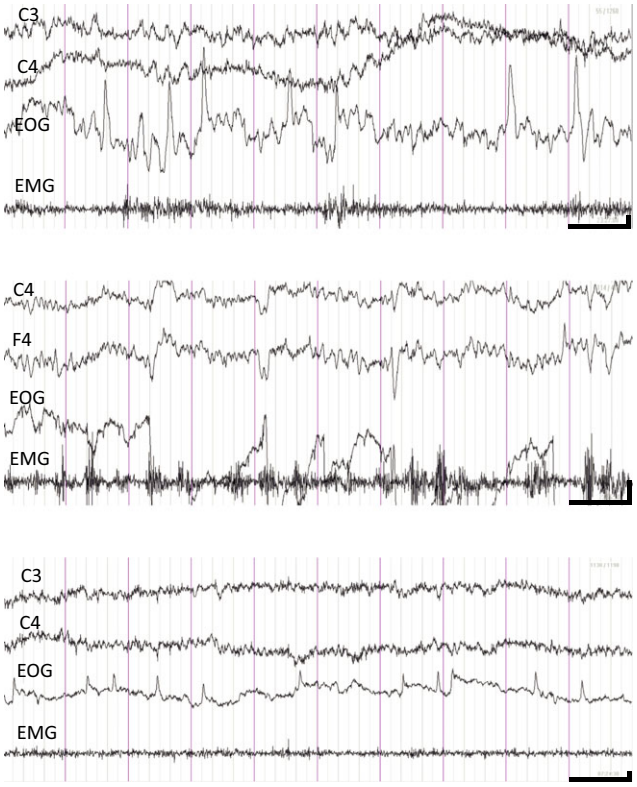

Figure 8. Examples of wakefulness. Top panel: patient 14; middle panel: patient 8; bottom panel: patient 10. Right top corner: the number of the presented 30-s epoch.

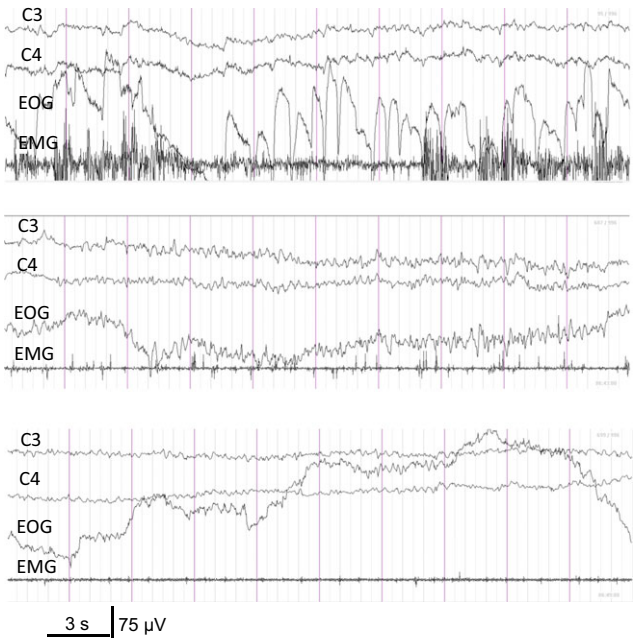

Figure 9. Patterns of activity obtained in patient 13, >15 years with the diagnosis vegetative state (VS)/unresponsive wakefulness syndrome (UWS). Top panel: wakefulness. Blinks, bouts of muscle activity, low-amplitude electroencephalogram (EEG). Middle panel: non-rapid eye movement (NREM) sleep (possibly N2 stage). Slower EEG with higher amplitude. No eye movements. Bottom panel: REM sleep. EEG similar to NREM sleep. REM. Minimal muscle tone. Right top corner: the number of the presented 30-s epoch.

wakefulness, NREM sleep and REM sleep. Nevertheless, the sleep patterns were abnormal in all examined patients. These abnormalities may be in part attributed to non-specific factors, such as the opportunity of naps during daytime. Only after such unspecific influences are ruled out, the exact role of specific factors (i.e. brain lesions) can be investigated.

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