

Electrophysiological correlates of behavioural changes in vigilance in vegetative state and minimally conscious state

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The existence of normal sleep in patients in a vegetative state is still a matter of debate. Previous electrophysiological sleep studies in patients with disorders of consciousness did not differentiate patients in a vegetative state from patients in a minimally conscious state. Using high-density electroencephalographic sleep recordings, 11 patients with disorders of consciousness (six in a minimally conscious state, five in a vegetative state) were studied to correlate the electrophysiological changes associated with sleep to behavioural changes in vigilance (sustained eye closure and muscle inactivity). All minimally conscious patients showed clear electroencephalographic changes associated with decreases in behavioural vigilance. In the five minimally conscious patients showing sustained behavioural sleep periods, we identified several electrophysiological characteristics typical of normal sleep. In particular, all minimally conscious patients showed an alternating non-rapid eye movement/rapid eye movement sleep pattern and a homeostatic decline of electroencephalographic slow wave activity through the night. In contrast, for most patients in a vegetative state, while preserved behavioural sleep was observed, the electroencephalographic patterns remained virtually unchanged during periods with the eyes closed compared to periods of behavioural wakefulness (eyes open and muscle activity). No slow wave sleep or rapid eye movement sleep stages could be identified and no homeostatic regulation of sleep-related slow wave activity was observed over the night-time period. In conclusion, we observed behavioural, but no electrophysiological, sleep wake patterns in patients in a vegetative state, while there were near-to-normal patterns of sleep in patients in a minimally conscious state. These results shed light on the relationship between sleep electrophysiology and the level of consciousness in severely brain-damaged patients. We suggest that the study of sleep and

homeostatic regulation of slow wave activity may provide a complementary tool for the assessment of brain function in minimally conscious state and vegetative state patients.

Keywords: sleep; vegetative state; minimally conscious state; consciousness; EEG

Abbreviations: REM = rapid eye movement

Introduction

Traditionally, coma is clinically defined by a state of total lack of arousal and behavioural unresponsiveness. When patients in comas start to open their eyes and show a behavioural sleep–wake cycle (extended periods of eye closure and muscle inactivity), they usually evolve towards a vegetative state, which can be transient or persistent. Vegetative state is defined by periods of preserved behavioural arousal (eyes open with decreased arousal threshold), but an absence in signs of self-awareness or the environment (Laureys and Boly, 2007, 2008). Patients in a minimally conscious state additionally show non-reflexive or purposeful behaviours, but are unable to communicate effectively (Giacino *et al.*, 2002). Clinically, the differential diagnosis between these two patient populations is extremely challenging, leading to a high rate of misdiagnosis in the absence of a proper clinical scale (Schnakers *et al.*, 2009). Several studies have however shown important differences in brain function (Boly *et al.*, 2008a; Vanhaudenhuyse *et al.*, 2010) and prognosis (Laureys and Boly, 2007; Luaute *et al.*, 2010) between patients in vegetative or minimally conscious states.

Sleep is a universal phenomenon that is present in all animal species and throughout life (Cirelli and Tononi, 2008). In addition to behavioural decreases in vigilance (assessed clinically by the closing of eyes and muscle inactivity), mammalian sleep has a number of highly reliable electrophysiological features such as slow waves, spindles and rapid eye movements (REMs). The sequential alternation of sleep stages, the presence of sleep cycles and the homeostatic regulation of slow waves are characteristics of sleep that are found universally in healthy human volunteers (Dijk *et al.*, 1993; Riedner *et al.*, 2007). The presence of standard sleep elements could also reflect global brain integrity as they have been shown to be altered in several pathological states such as stroke (Bassetti and Aldrich, 2001; Gottselig *et al.*, 2002) and Alzheimer's disease (Crowley *et al.*, 2005).

There remains a debate about the presence of sleep electrophysiological correlates to changes in behavioural vigilance observed in patients in vegetative or minimally conscious states (Bekinschtein *et al.*, 2009a; Cologan *et al.*, 2010). Indeed, behavioural fluctuations in arousal are classically related mainly to brainstem and basal forebrain function, i.e. to a differential activity of the ascending reticular activating system (Boly *et al.*, 2008b). Consequently, closing of the eyes and inactivity does not necessarily imply related changes in thalamocortical function, which would more likely be reflected by detectable scalp EEG changes, particularly in patients presenting widespread thalamocortical disconnections (Vanhaudenhuyse *et al.*, 2010). Previous studies have suggested that patients in a vegetative state may show both

preserved behavioural and electrophysiological sleep (Oksenberg *et al.*, 2001; Isono *et al.*, 2002). However, these studies did not explicitly differentiate patients in a vegetative state from patients in a minimally conscious state when assessing the patients' residual brain function. Furthermore, only a restricted set of sleep parameters were reported and therefore the interpretation of these findings in brain-damaged patients is limited. Hence, we performed sleep recordings in 11 patients with severe brain damage using high density EEG and investigated the presence of different stages of sleep, sleep cycle architecture and the regulation of homeostatic slow-wave activity (the EEG power density between 0.5 and 4.5 Hz during non-REM sleep) in each individual patient. The use of high density EEG allowed us to maximize our chances of detecting electrophysiological features of sleep that correlate to changes in behavioural vigilance, despite the presence of extensive focal lesions commonly found in these patients. We hypothesized that the presence of normal sleep patterns, likely reflecting underlying functional brain integrity, could potentially differentiate patients in a vegetative state from those in a minimally conscious state.

Materials and methods

Patients

We recorded nocturnal high-density EEG recordings in 11 brain-injured patients (six patients in a minimally conscious state, age 39 ± 15 years (mean \pm standard error of the mean), range 19–55; five patients in a vegetative state, age 54 ± 18 years, range 35–75). Further two patients were acquired but had to be excluded from our analyses due to excessive movement artefacts and ocular contamination of the recordings, resulting in poorly interpretable traces. Tables 1 and 2 report the demographic and clinical characteristics of the patients. Clinical examinations were performed repeatedly by trained neuropsychologists/assessors using the standardized Coma Recovery Scale-Revised (Giacino *et al.*, 2004) and the Glasgow Liege Scale (Born, 1988) on the day of EEG recordings, in the week before and the week after. Patients were recorded in a non-sedated condition. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège and written informed consent was obtained from the patients' legal representatives.

Data acquisition and analysis

In all subjects, 12 h recordings were acquired using a 256 electrode high-density EEG system (Electrical Geodesics) sampled at 500 Hz and referenced for acquisition purposes to Cz [the EEG acquisition system default setting; (Landsness *et al.*, 2009)]. Recordings were performed from 8 a.m. to 8 p.m.; room lights were turned off at 10 p.m. and

Table 1 Clinical, electrophysiological and structural imaging data of patients in a minimally conscious state

	MCS1	MCS2	MCS3	MCS4	MCS5	MCS6
Clinical Features						
Gender (age, years)	F (22)	M (46)	F (45)	M (46)	M (19)	M (55)
Cause	Traumatic brain injury	Sub-arachnoidal haemorrhage	Embolism, anoxia	Traumatic brain injury and cardiac arrest	Traumatic brain injury	Cardiac arrest
Days after insult	2 years	1.5 year	5 years	25 years	7 months	25 days
Outcome at 12 months	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3
Breathing	Tracheotomized	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Spontaneous
Paralysis/paresis	Right paresis, left paralysis	Right paresis, left paralysis	Tetraparesis	Tetraparesis	Tetraparesis	Tetraparesis
Coma Recovery Scale -Revised						
Diagnosis	Minimally conscious state	Minimally conscious state	Minimally conscious state	Minimally conscious state	Minimally conscious state	Minimally conscious state
Auditory function	Auditory startle	Consistent movement to command	Localization to sound	Reproducible movement to command	Reproducible movement to command	Reproducible movement to command
Visual function	Visual pursuit	Object recognition	Visual pursuit	Visual pursuit	Visual pursuit	Object localization
Motor function	Flexion withdrawal	Automatic motor response	Flexion withdrawal	Localization to noxious stimulation	Flexion withdrawal	Localization to noxious stimulation
Oromotor/Verbal function	Vocalization/oral movement	Intelligible verbalization	Vocalization/oral movement	Vocalization/oral movement	Vocalization/oral movement	Intelligible verbalization
Communicate	None	Non-functional	None	None	None	Non-functional
Arousal	Eyes open without stimulation	Attention	Eyes open without stimulation	Eyes open without stimulation	Eyes open without stimulation	Attention
Total score	10	21	11	13	12	17
Medication						
Daily dosage	Phenobarbital 100 mg Baclofen 10 mg Scopolamine 1 patch	Levetiracetam 3*500 mg Valproic acid 3*287 mg Clonazepam 3*1 mg Esomeprazole 20 mg Perindopril 4 mg Amlodipine 10 mg Acetylcysteine 600 mg Bisoprol 10 mg	Baclofen 25 mg Cetirizine 10 mg Tramadol 25 mg Duloxetine 60 mg Omeprazole 10 mg Domperidone 3*15 mg	Amantadine 200 mg Baclofen 2*10 mg Esomeprazole 20 mg Tizanidine 4 mg	Baclofen 3*15 mg Levetiracetam 3*500 mg Paracetamol 3*500 mg Caffeine 3*50 mg Atenolol 25 mg Enoxaparine 40 µg	Lorazepam 2.5 mg Esomeprazole 20 mg Clonidogrel 75 mg Acetylsalicylic acid 500 mg Simvastatine 40 mg Perindopril 4 mg Bisoprolol 2.5 mg Olanzapine 10 mg Enoxaparine 40 µg Glucophage 850 mg Amoxiclav 3*2 g Ketoconazole 200 mg
EEG background activity	Fundamental rhythm: delta of low voltage	Fundamental alpha rhythm with right and left lateralized theta	desynchronized beta activity	symmetrical theta activity	Fundamental delta rhythm and irregular theta activity.	Theta activity
MRI/CT	Mesio-frontal and right brainstem lesions. Diffuse frontal and left cerebellum axonal injury. Diffuse cerebral atrophy.	Sub-arachnoidal hemorrhage due to aneurysm disruption, with lesions predominant in the left hemisphere.	Left frontal-temporal ischemic lesions; diffuse left cerebral and left cerebellar peduncle atrophy, bilateral periventricular white matter damage. Semi-oval centre and centroprotuberantial sequelae.	Quadri-ventricular hydrocephalus. Periventricular leucoencephalopathy, bilateral fronto-parietal lesions. Diffuse cerebral atrophy, maximum in frontal and temporal lobes; hippocampus, and thalami.	Diffuse post traumatic axonopathy. Cerebellar and right middle peduncular lesions. Post traumatic micro hemorrhagic lesions in frontal lobes predominant on the right side. Diffuse cerebral and hippocampal atrophy.	No detectable focal lesion.

Table 2 Clinical, electrophysiological and structural imaging data of patients in a vegetative state

	VS1	VS2	VS3	VS4	VS5
Clinical features					
Gender (age, years)	F (45)	F (70)	F (75)	M (62)	F (35)
Cause	Hypoglycaemia	Meningo-encephalitis	Traumatic brain injury	Pontine haemorrhage	Anoxia
Days after insult	108 days	31 days	38 days	49 days	1 ½ year
Outcome at 12 months	Glasgow Outcome Scale 1	Glasgow Outcome Scale 3	Glasgow Outcome Scale 3	Glasgow Outcome Scale 1	Glasgow Outcome Scale 2
Breathing	Tracheotomized	Tracheotomized	Tracheotomized	Tracheotomized	Tracheotomized
Paralysis/paresis	Tetraparesis	Tetraparesis	Tetraparesis	Tetraparesis	Tetraparesis
Coma Recovery Scale-Revised					
Diagnosis	Vegetative state	Vegetative state	Vegetative state	Vegetative state	Vegetative state
Auditory function	Auditory startle	Auditory startle	Auditory startle	None	Auditory startle
Visual function	None	Visual startle	Visual startle	None	None
Motor function	Abnormal posturing	Flexion withdrawal	None	None	Flexion withdrawal
Oromotor/Verbal function	Vocalization/oral movement	Oral reflexive movement	Oral reflexive movement	None	Oral reflexive movement
Communicate	None	None	None	None	None
Arousal	Eyes open without stimulation	Eyes open with stimulation	Eyes open with stimulation	Eyes open with stimulation	Eyes open with stimulation
Total score	6	7	4	1	5
Medication					
Daily dosage	Valproic acid 3* 287 mg Tetrazepam 3*25 mg Enoxoparine 40 µg	Lisinopril 10 mg Esomeprazole 20 mg Methylprednisolone 16 mg Furosemide 40 mg Amlodipine 5 mg	Amlodipine 5 mg Esomeprazole 40 mg Acetylsalicylic acid 100 mg Acetylcysteine 600 mg Enoxoparine 40 µg	Furosemide 2*40 mg Aldactazine 50 mg Acetylcysteine 600 mg Enoxoparine 40 µg	Levetiracetam 1000 mg Phenytoin 750 mg Valproate 4*650 mg Ranitidine 300 mg Aldactazine 50 mg Lorazepam 4 mg Levofloxacin 2*500 mg Paracetamol 4*1 g Vancomycin 1 g Enoxoparine 40 µg
EEG background Activity	Iso-electric	Symmetrical theta	Symmetrical theta	Irregular diffuse delta theta	Disorganized status epilepticus
MRI/CT	Diffuse white matter and basal ganglia lesions.	Frontal, temporal, periventricular, semi-oval centre, basal ganglia, thalamus and brainstem lesions.	Right temporal contusion & oedema. Bilateral frontal petechias. Left subdural hygroma.	Pontine, cerebellar peduncle and 4th ventricle haemorrhage.	No detectable focal lesion.

turned on again at 7.30 a.m. Video-taped recordings were acquired simultaneously and synchronously to the EEG data to confirm the patients' behaviour.

For each patient, sleep staging was based on EEG electrodes C3 and C4 referenced to mastoid electrodes A1 and A2 in accordance with Landsness *et al.* (2009). Sleep was scored in 20 s epochs according to standard schemes (Rechtschaffen and Kales, 1968). All EEG signals were down-sampled to 128 Hz and band-passed (two-way least-squares finite impulse response) filtered between 0.5 and 45 Hz (35 Hz for one patient, due to abundant muscular artefacts). Electromyogram recordings were band-passed filtered between 5 and 40 Hz, whereas electro-oculogram recordings were filtered between 0.5 and 40 Hz.

As in previous studies (Riedner *et al.*, 2007; Landsness *et al.*, 2009), EEG power spectral analysis was performed on consecutive 4 s epochs (fast Fourier transform routine, Hanning window) using EEGLab (Delorme and Makeig, 2004). All artefacts were excluded both semi-automatically based on a threshold criteria and visually; then signals were average-referenced (Huber *et al.*, 2004, 2006; Riedner *et al.*, 2007; Landsness *et al.*, 2009; Murphy *et al.*, 2009, 2011; Kurth *et al.*, 2010). For subjects in a minimally conscious state, we used 3.44 ± 0.95 h of artefact free data (mean \pm standard error of the mean) and 5.79 ± 1.20 for the patients in a vegetative state. Scalp topographies of non-REM slow-wave activity (0.5–4.5 Hz) were obtained averaging EEG power across all artefact-free epochs over the entire night (Figs 1 and 3). To compute differences in slow-wave activity between behavioural states, EEG power was averaged across all electrodes and compared using a paired *t*-test. While we have developed a sophisticated automatic algorithm for detecting spindles in healthy controls (Ferrarelli *et al.*, 2010), it has not been validated in brain damaged patients. Given the challenges posed by data obtained from brain damaged patients, we chose to count spindles visually using the criteria of ~ 20 – 40 μ V activity with a waxing–waning appearance in the 12–16 Hz range with 0.5–2 s duration. Finally, in Figs 2 and 4, the homeostatic decline of slow-wave activity is plotted over a single channel near Fz (Riedner *et al.*, 2007). Values were obtained by averaging slow-wave activity power in each 20 s window; then each segment of slow-wave activity was displayed sequentially over time. Subject-by-subject topographic changes in the homeostatic decline in slow-wave activity were computed using statistical non-parametric mapping (simple threshold corrected *P*-value = 0.05 permutation test; Nichols and Holmes, 2002).

Results

For patients in a minimally conscious state, behavioural sleep (sustained periods of eye closure and muscle inactivity) was accompanied by clearly detectable changes in EEG features, which looked similar to normal sleep (Fig. 1, *left*; Table 3). In the five patients in a minimally conscious state who showed consistent behavioural sleep over the night, we could detect periods corresponding to non-REM sleep (Stages 2–3) and periods of REM sleep, which occurred predominantly at the end of the night. In all minimally conscious state patients, we could also observe spindles during non-REM sleep. Within a given 30 min segment of data there was on average 16.3 ± 8.0 spindles per patient (mean \pm standard error of the mean). Compared to periods of wakefulness, non-REM sleep showed a global increase in slow-wave activity ($636 \pm 119\%$ of average waking power, mean \pm standard

error of the mean; $P = 0.004$, $T = 4.8$ paired *t*-test, $n = 5$). The topographic distribution of high frontal slow-wave activity (Fig. 1, *right*) was also near to normal for patients in a minimally conscious state, though some showed lateralization due to massive hemispheric brain lesions. The polysomnography of patients in a minimally conscious state also resembled that of healthy controls with alternating cycles of non-REM and REM sleep, with REM sleep periods progressively increasing in duration at the end of the night (Fig. 2). Finally, the five patients in a minimally conscious state who showed consolidated behavioural sleep showed a statistically significant ($P < 0.05$) homeostatic decline of slow-wave activity over the night (Fig. 2).

In contrast, no pattern of normal electrophysiological sleep could be observed in the five patients in a vegetative state (Fig. 3, Table 3). Consistent with the clinical definition of vegetative state, prior to, during and after the study, four out of five vegetative state patients showed a clear behavioural sleep wake cycle, i.e. the alternating of sustained periods of eyes opening, followed by long-lasting eyes-closed periods during the night. One patient (VS4) clinically presented with only intermittent eye opening and very low arousal prior to and during most of the recording. In all patients in a vegetative state, behavioural transition to sleep was not accompanied by clear changes in the EEG (Fig. 3, *left*). No stages 2–3 of non-REM sleep or REM sleep could be identified and therefore we could only classify the EEG as either 'eyes open' or 'eyes closed.' While most of the patients in a vegetative state did have the characteristic slowing of EEG rhythms in the delta and theta frequency ranges, there was no significant change in slow-wave activity when patients closed their eyes ($97 \pm 19\%$ of the global average eyes open power, mean \pm standard error of the mean; $P = 0.45$, $T = -0.15$ paired *t*-test, $n = 5$). We did not observe sleep spindles in any of the patients in a vegetative state. Polysomnography did not reveal any pattern of sleep cycles and we could not observe a homeostatic decline of sleep-related slow-wave activity ($P > 0.05$) during the night in any of the patients in a vegetative state. Figure 4 reports Patients VS1–4. Although they did not correspond to normal sleep, Patient VS5's results were atypical and are described in the Supplementary material.

Discussion

Clinical and neuroscientific relevance of a link between the presence or absence of normal sleep patterns and the level of consciousness

In contrast to patients in a minimally conscious state, none of the patients in a vegetative state showed any electrophysiological features of normal sleep, even if preserved behavioural sleep–wake cycles could be observed in most patients. These results suggest that electrophysiological sleep features observed during the night could possibly be a reliable indicator of the patient's level of consciousness, differentiating patients in a vegetative state from those in a minimally conscious state. Such studies could potentially be a

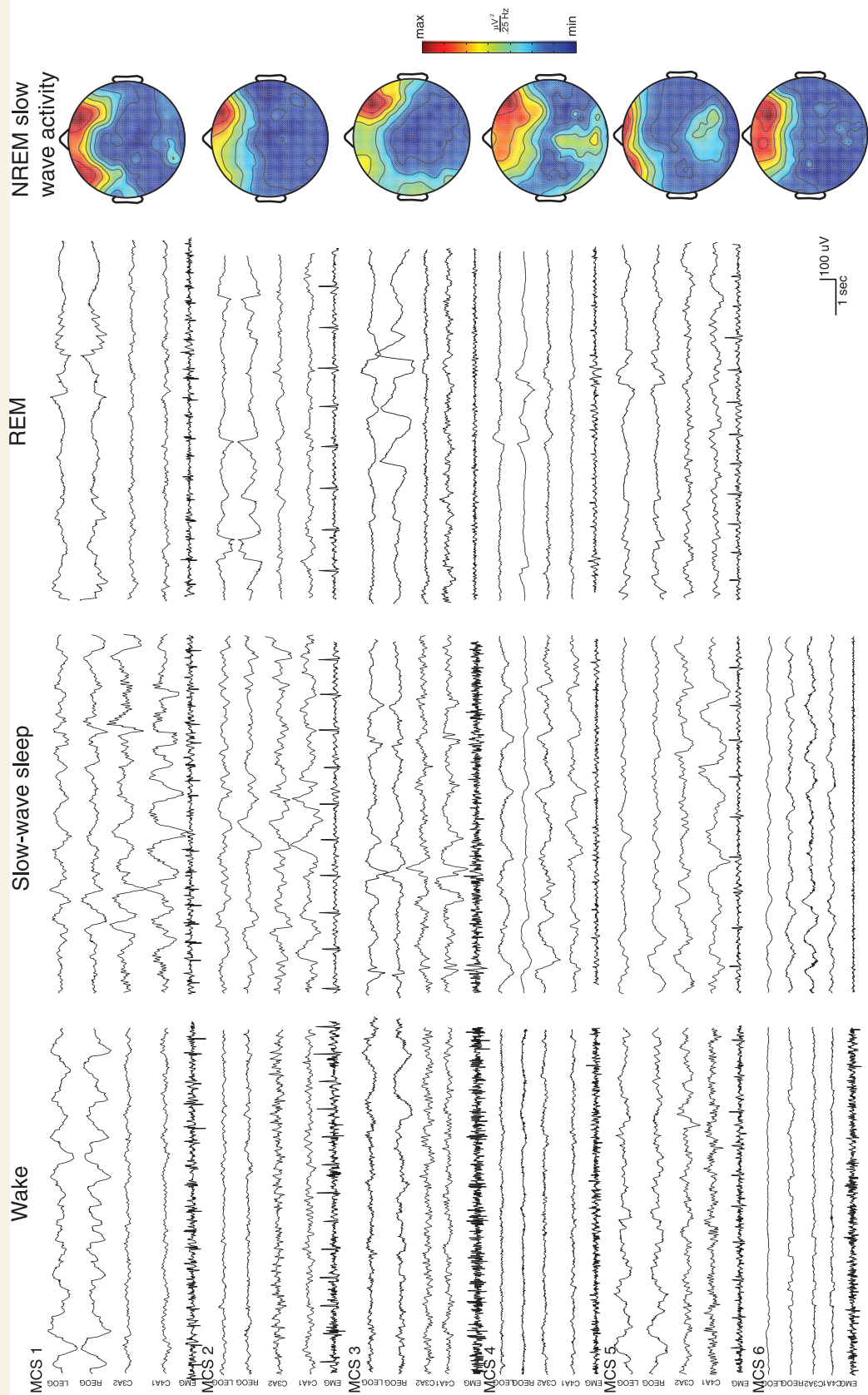


Figure 1 Sleep in six patients in a minimally conscious state. Left: electrooculogram, EEG and EMG 10 s traces for three states: Wake, slow-wave sleep and REM sleep. One subject, MCS6, did not have any REM during the recording. Right: Topographic distribution of slow wave activity during non-rapid eye movement (NREM) sleep for the entire night. Colour bars represent the average absolute electrographic power (uV/0.25 Hz frequency bin for the 0.5–4.5 Hz range) expressed as the maximum and minimum power for each individual.

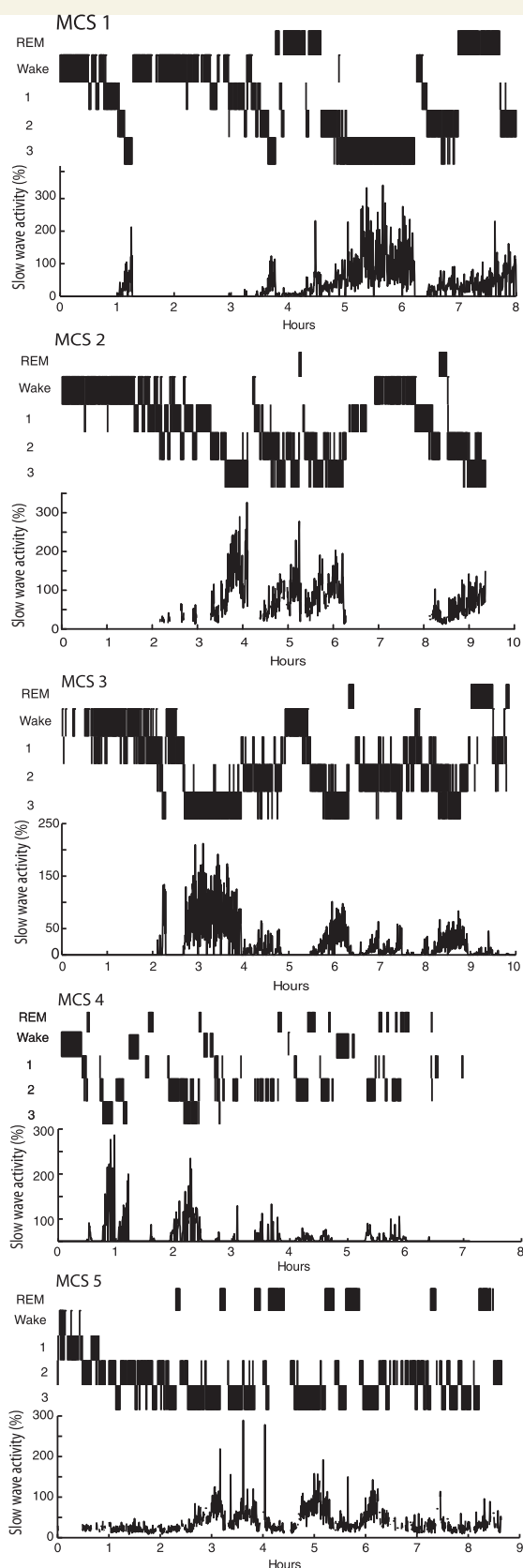


Figure 2 Homoeostatic decline in slow wave activity of five patients in a minimally conscious state. Hypnogram and slow-wave activity time course. Sleep stages represented as REM, Wake, Stages 1, 2 and 3. The time course of slow-wave

Table 3 Presence or absence of sleep patterns

Subject	Eyes open	Eyes closed	Spindles	Slow waves	REM
MCS 1	+	+	+	+	+
MCS 2	+	+	+	+	+
MCS 3	+	+	+	+	+
MCS 4	+	+	+	+	+
MCS 5	+	+	+	+	+
MCS 6	+	+	+	+	—
VS 1	+	+	—	—	—
VS 2	+	+	—	—	—
VS 3	+	+	—	—	—
VS 4	+	+	—	—	—
VS 5	+	+	—	—	—

useful complement to bedside behavioural assessment in the evaluation of brain function in non-communicative brain-damaged patients.

Failure to show EEG correlates of behavioural changes in vigilance for patients in a vegetative state could have at least two different origins. First, they could be due to insufficient modulation of corticothalamic activity by a deficient ascending reticular activating system. This explanation could prevail in Patient VS4, where the altered state of consciousness was caused by a focal pontine haemorrhage, and in which behavioural sleep-wake cycles were also relatively deficient. Even if transient periods of behavioural wakefulness (increased arousal, eyes open) were observed, no related EEG change could be detected in this patient. On the other hand, the absence of electrophysiological sleep could also be explained by a preserved modulation of activity in the ascending reticular system, but a failure in corticothalamic function to propagate signals from the ascending reticular activating system. Patients in a vegetative state typically show a pattern of widespread thalamocortical dysfunction and metabolic impairment, in particular in frontoparietal cortices (Laureys *et al.*, 2002, 2004). Frontoparietal cortices, including default network areas, have also been involved in the generation of sleep patterns such as slow waves (Dang-Vu *et al.*, 2008; Murphy *et al.*, 2009). Though spindles are thought to involve primarily a thalamic generator, frontoparietal circuits are involved in their propagation (Schabus *et al.*, 2007) and synchronization (Evans, 2003). More generally, widespread functional impairment of thalamocortical circuitry could potentially explain both the impairment of consciousness and the absence of normal sleep electrophysiological features of patients in a vegetative state. These two different mechanisms could lead to a differential prognosis and different therapeutic approaches in individual patients. For example, at the population level, patients with isolated subcortical arousal system function impairment could

Figure 2 Continued
activity for a single channel near Fz is expressed as a percentage of the mean slow-wave activity reading for the entire night. MCS = patients in a minimally conscious state.

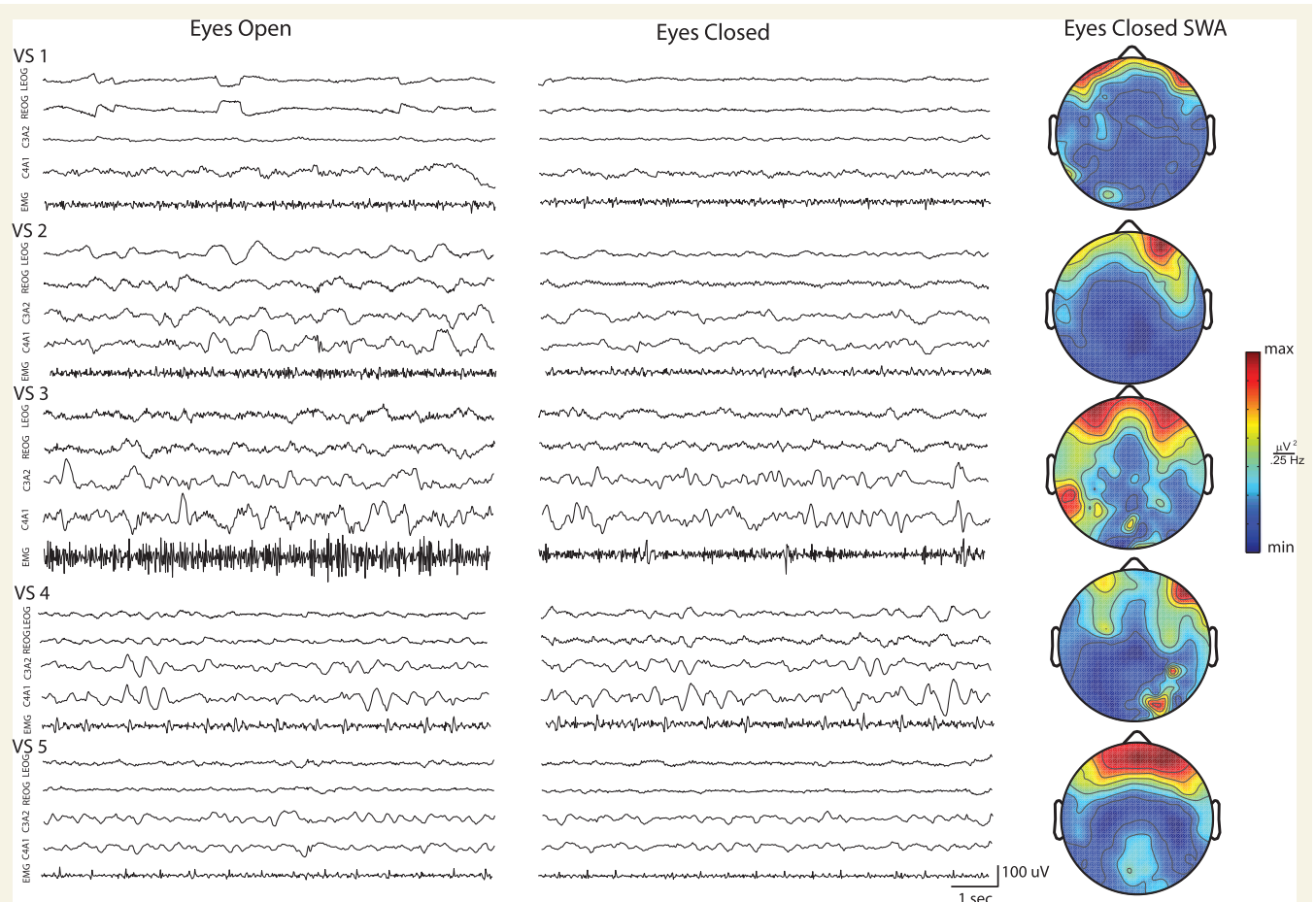


Figure 3 Behavioral sleep patterns for patients in a vegetative state and slow-wave activity topography. (Left) electrooculogram, EEG and EMG 10 s traces for eyes open and eyes closed. (Right) Topographic distribution of the average slow wave activity during the eyes closed condition for the entire night. Colour bars represent the absolute electrographic power ($\mu\text{V}^2/0.25\text{ Hz}$ frequency bin for the 0.5–4.5 Hz range) expressed as the maximum and minimum power for each individual. SWA = slow-wave activity; VS = patients in a vegetative state.

have a better prognosis and would be more likely to respond to central thalamic stimulation or arousal-promoting drugs than patients with more widespread thalamocortical damage. Further studies are needed before a link between polysomnographic EEG patterns and differences in prognosis and response to treatment can be assessed in populations of patients in a vegetative state.

No REM sleep patterns, as defined by wakefulness-like EEG associated with rapid ocular saccades and decreased muscle tone, were observed for patients in a vegetative state. As shown in early experiments in cats, REM sleep generating mechanisms located in the dorsal pontine tegmentum are sufficient to generate periods of atonia accompanied by rapid eye movements (Jouvet, 1972). Brainstem function is typically preserved in patients in a vegetative state (Boly *et al.*, 2008b). Thus, it is possible that brainstem-generated features of REM sleep not involving changes in cortical activity may be present in at least some vegetative patients. A recent study (Bekinschtein *et al.*, 2009b) suggests that circadian rhythmicity may be impaired in patients in a vegetative state, depending on the type of injury and the level of consciousness. In the present study, while we could not rigorously establish

to what extent circadian rhythmicity was preserved, we did observe extended periods of behavioural sleep that occurred more at night than during the day.

In contrast to patients in a vegetative state, subjects in a minimally conscious state showed all stages of sleep. These results suggest a global preservation of brain function in these patients. This observation is in line with previous studies showing preserved response to external stimuli (Boly *et al.*, 2004, 2008a) and large scale brain network connectivity (Vanhaudenhuyse *et al.*, 2010) in minimally conscious patients. The presence of spindles also suggests that patients in a minimally conscious state show a relative preservation of thalamocortical connectivity compared to patients in a vegetative state (Vanhaudenhuyse *et al.*, 2010). The preservation of REM sleep patterns in minimally conscious patients also has some potential clinical relevance. REM sleep has been linked in healthy volunteers to emotional content and vivid visual imagery (Nir and Tononi, 2010) that could also be present in these patients. Further investigations should study residual cognitive function of patients in a minimally conscious state, which, though unable to communicate, seem to have relatively preserved brain function and likely present some capacity for perception and cognition.

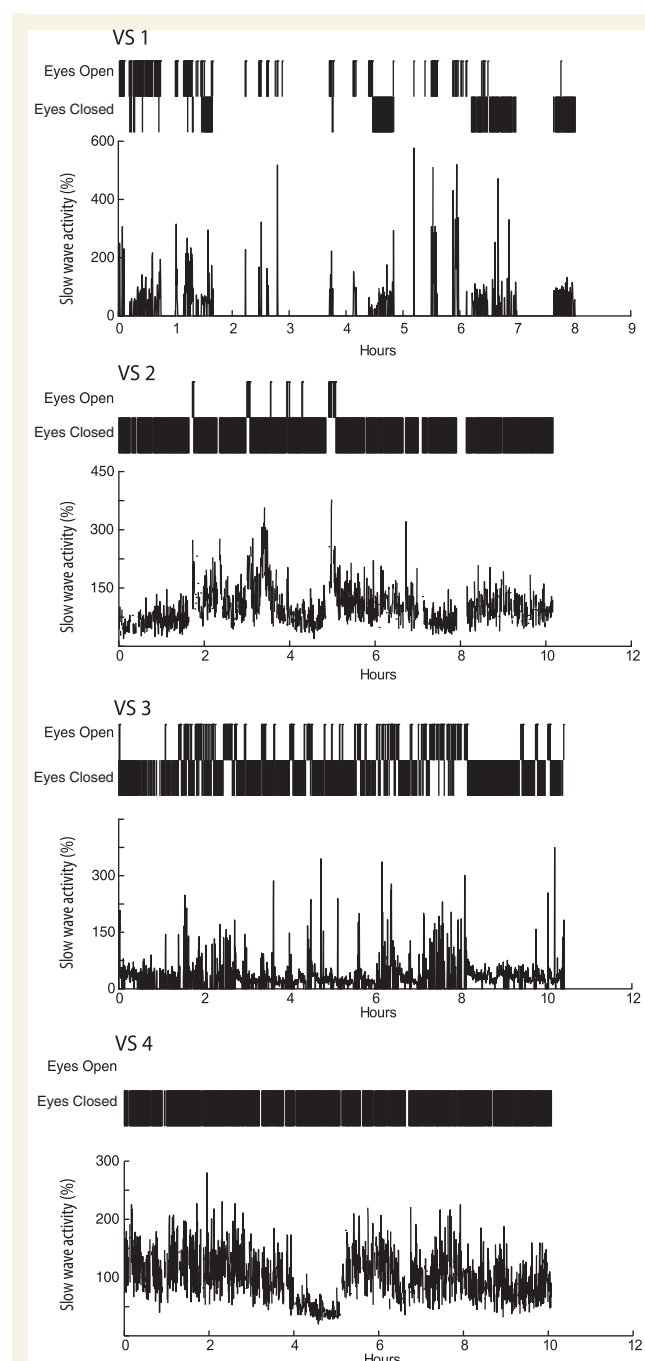


Figure 4 Hypnogram and slow-wave activity time course in four patients in a vegetative state. Behavioural sleep patterns represented as eyes open and eyes closed. The time course of slow-wave activity for a single channel near Fz is expressed as a percentage of the mean slow-wave activity reading for the entire night.

We also observed that in contrast to patients in a minimally conscious state, patients in a vegetative state did not show detectable sleep cycle architecture and homeostatic regulation of slow-wave activity. As slow-wave activity has been suggested to be linked to plasticity (Huber *et al.*, 2004; Landsness *et al.*, 2009), our findings could also partially explain the better prognosis observed

in large cohort studies comparing patients in a minimally conscious state to those in a vegetative state (Luaute *et al.*, 2010). However, further studies on larger numbers of patients are required before a link between prognosis and different sleep patterns can be asserted.

It should be stressed that one should remain cautious when interpreting the functional significance of sleep measurements in terms of level of consciousness in brain damaged patients. For now, one cannot exclude that individual patients in a vegetative state could present some partial preservation of sleep electrophysiological features. More investigations during pharmacological manipulations, such as general anaesthesia, in patients with status epilepticus and a larger cohort of patients with pathological alterations of consciousness are also needed before a consensus can be reached on the generalizability of our findings.

Technical issues in the study of EEG sleep recordings analyses in severely brain damaged patients

Studying polysomnographic recordings in patients with altered states of consciousness represents a major technical and neuroscientific challenge. Special care should be taken while examining EEG traces and analyses should be performed only on artefact free segments. Two patients in a minimally conscious state had to be discarded from our analyses because of excessive noise and artefacts in the recordings. In addition, special care should be taken in the differentiation of patients in minimally conscious and vegetative states and in the use of appropriate behavioural scales. Videotaped recordings are also very helpful as these patients can present fragmented sleep, leading to more difficult interpretation of the findings in the absence of behavioural status. Automatic sleep scoring should be only used with caution, due to the presence of slower baseline EEG in a number of brain damaged patients, as compared to healthy controls. The assessment of alternating sleep stages, as well as quantification of spindles and slow-wave activity topography and homeostasis were used to illustrate differences between-patient populations. A comparison of slow-wave activity between wake and sleep was performed to provide an additional, quantitative assessment of EEG changes in relation to changes in vigilance. We used a two electrodes system for the sleep scoring due to the well-established tradition of using C3 and C4 (Rechtschaffen and Kales, 1968). All other analysis (homeostatic decline and topography) took advantage of the high spatial resolution and the larger amount of data provided by high-density EEG. It is likely that most of the findings reported here could have been identified with a smaller number of electrodes, as used in clinical routine. However, high-density EEG recordings allowed us to perform a detailed topographic analysis of sleep patterns in individual subjects, despite the presence of extensive focal brain lesions that are common in these patients. In our case, the use of a large number of electrodes minimized the risk of false negative findings by covering a larger surface of the brain than conventional recordings and by allowing the sampling of isolated functioning brain regions, which are not uncommon in these patients (Schiff *et al.*, 2002). In addition, this technique is

ideally suited to the fine-grained topographic analysis of sleep-related EEG features such as slow-waves and spindles. Future studies should also investigate scalp propagation and cortical generators of these sleep-related rhythms in individual brain damaged patients. Further studies investigating changes in brain responses to external stimulation of patients in minimally conscious and vegetative states in different behavioural vigilance stages would also provide a useful complement to studies investigating changes in spontaneous EEG.

A remaining question is the relationship between structural and functional brain connectivity preservation and sleep patterns in non-communicative brain-damaged patients. Further multi-modal studies should combine EEG recordings to resting state functional MRI data and structural MRI (e.g. diffusion tensor imaging data) in these patients to better address this question. Further studies should also investigate the relationship between prognosis and fine-grained sleep EEG characteristics of individual patients in minimally conscious or vegetative states.

Conclusion

We have identified a relationship between the presence of normal sleep electrophysiological features and the level of consciousness in severely brain damaged patients. These results suggest that after further validation, the present methodology could potentially be rapidly translated into a routine clinical setting and bring relevant complementary information on the patients' residual brain function to bear on their clinical evaluation. In addition, assessment of sleep homeostatic measures such as slow-wave activity could be a useful paraclinical marker of the potential for plasticity in individual patients in a minimally conscious or vegetative state, complementing their bedside and standard prognostic assessment. Future studies on larger samples of patients will aim at correlating electrophysiological sleep features with prognosis and white matter anatomical damage (as assessed for example by diffusion tensor imaging) in these severely brain damaged patients.

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Supplementary material

Supplementary material is available at *Brain* online.

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