Sleep in Disorders of Consciousness

Sleep in severe disorders of consciousness: 24-h behavioral and polysomnographic recording

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Abstract

Objectives: To quantify, on a reliable evaluation basis, the distribution of behavioral and neurophysiological sleep patterns in Disorders of Consciousness (DOC) over a 24h period while controlling for environmental factors (by recruiting a group of conscious tetraplegic patients who resided in the same hospital).

Methods: We evaluated the distribution of sleep and wakefulness by means of polysomnography (EEG, EOG, EMG) and video recordings in 32 DOC patients (16 Unresponsive Wakefulness Syndrome [UWS], 16 Minimally Conscious State [MCS]) and 10 clinical control patients with severe tetraplegia (CC). Three independent raters scored the patients’ polysomnographic recordings.

Results: All but one patient (UWS) showed behavioral and electrophysiological signs of sleep. CC and MCS patients spent significantly more time in sleep during the night than during daytime, a pattern that was not evident in UWS. DOC patients (particularly UWS) exhibited less REM sleep than control patients. 44% of UWS patients and 12% of MCS patients did not have any REM sleep, while all control patients (100%) showed signs of all sleep stages and sleep spindles. Furthermore, no sleep spindles were found in 62% of UWS patients and 21% of MCS patients. In the remaining DOC patients who had spindles, their number and amplitude were significantly lower than in controls.

Interpretation: The distribution of sleep signs in DOC over 24 hours differs significantly from the normal sleep-wakefulness pattern. These abnormalities of sleep in DOC are independent of external factors such as severe immobility and hospital environment.
Introduction

Acquired brain injury can result in a chronic state of severe disturbance or even the lack of awareness, referred to as severe disorders of consciousness (DOC). DOC differ from coma by the presence of signs of arousal, including alternation of behavioral sleep and wakefulness (i.e., open/closed-eyes episodes) (The Multi-Society Task Force on PVS, 1994). Two subgroups of DOC are Unresponsive Wakefulness Syndrome (UWS), where patients have reflexive responses but no sign of awareness, and Minimally Conscious State (MCS), in which patients show unstable conscious behavior but cannot communicate or intentionally use objects. The differential diagnosis between the two is extremely challenging and error prone (Schnakers et al., 2009), which is additionally complicated by strong fluctuations of the arousal level and the associated level of consciousness, particularly in MCS (Giacino et al., 2002), but also in UWS (Kotchoubey, Vogel, Lang, & Müller, 2014), leading to inconsistent results of repeated behavioral evaluations (Wannez, Heine, Thonnard, Gosseries, & Laureys, 2017).

Polysomnographic recordings could contribute to the diagnostics of DOC by improving the coordination of task-based diagnostic measurements (Bekinschtein, Golombek, Simonetta, Coleman, & Manes, 2009). Additionally, sleep parameters such as the presence of slow wave sleep (SWS), rapid eye movements sleep (REM) and sleep spindles may serve as independent markers of the severity of consciousness impairment (de Biase et al., 2014; Forgacs et al., 2014; Rossi Sebastiano et al., 2018). Some studies even suggest that these sleep parameters may predict the clinical outcome of DOC, i.e. whether or not patients will regain consciousness (Kang et al., 2014; Kotchoubey & Pavlov, 2018a).

Although the body of literature about sleep in DOC has substantially increased over the last years, a number of serious issues remain (Kotchoubey & Pavlov, 2018c). First, there is no consensus on systematic sleep stage classification in DOC patients (Cologan et al., 2013; Pavlov et al., 2017; Rossi Sebastiano et al., 2015, 2018; Wielek et al., 2018). The standard sleep criteria used in healthy individuals (Iber, 2007; Rechtschaffen & Kales, 1968) cannot be applied directly, but have to be adjusted to DOC sleep patterns (Pavlov et al., 2017). To our best knowledge, DOC sleep data are either scored by a single rater, whose blinding is rarely warranted, or a pure automatic analysis is performed. The former substantially decreases the reliability of sleep evaluation, and the latter
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reduces its validity because the results of the automatic EEG assessment cannot be directly projected onto sleep stages.

Second, many polysomnographic recordings in DOC have been performed during the night (8-10 hours) or an “extended night” interval (16 hours) (Landsness et al., 2011; Rossi Sebastiano et al., 2018; Wislowska et al., 2017). Thus, sleep of patients who slept during the day might have been incorrectly assessed.

Third, the diagnostic core feature of DOC, differentiating it from coma, is the presence of sleep-wake cycle as measured by the presence of episodes with opened and closed eyes, which are defined as ‘behavioral wakefulness and sleep’, respectively. It remains unclear, however, whether the behavioral sleep corresponds to ‘electrophysiological sleep’ (derived from polysomnography).

Fourth, none of the studies so far included clinical control groups but only healthy controls. Yet, the living conditions of severely handicapped DOC patients radically differ from that of healthy individuals, regarding many parameters that can affect sleep quality, such as the ward room, immobility, the lack of social pressure and unsolicited disturbance by the hospital personnel.

To solve these issues, in this study we carried out reliable expert evaluation of behavioral and electrophysiological sleep over a 24h period in two groups of DOC patients (UWS and MSC) and a group of tetraplegic control patients.
Methods

Patients

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine at the University of Tübingen. The patients and their legal representatives were informed about the study content before study enrollment and gave their written consent. They were informed that participation in the study can be terminated at any time without negative consequences. It was emphasized that participation in the study had no effect on further medical treatment. The study was registered in the German Clinical Study Register (DRKS00009326).

Patients were included according to the following criteria: age between 18 to 69 years (to minimize age-dependent effects on sleep quality (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004)); time elapsed since injury from 1 to 24 months; stable circulation and respiration. Exclusion criteria were: extremely slow basic EEG activity (below 3 Hz); a history of depression, schizophrenia, drug abuse or sleep disorders; or epileptic signs in premorbid EEG recordings. The diagnoses of DOC patients were either UWS or MCS. The diagnoses of Clinical Control group patients (CC) were Guillain-Barré syndrome or severe high-level spinal cord injury with tetraplegia. An additional criterion for CC patients was that they spend most of their time in bed (maximum time out of bed: 4 h per day).

All patients admitted to the Schön Clinics Bad Aibling (Bavaria, Germany) who fulfilled the above criteria were initially enrolled in the study, resulting in a total of fifty patients (19 UWS, 19 MCS, 12 CC). However, two control patients withdrew their consent. Further six DOC patients were excluded, because their condition worsened (n = 3) or improved (n = 3) and they did not meet the inclusion criteria anymore. Therefore, data from 42 patients (16 UWS, 16 MCS, 10 CC) were recorded and analyzed (see Table 1).

A trained and experienced neurologist repeatedly performed clinical assessment of DOC patients using the Coma Recovery Scale – revised (CRS-r: (Giacino, Kalmar, & Whyte, 2004)). The last CRS-r evaluation was done on the day before the polysomnographic recording (see Table 1). As can be seen in Table 1, UWS and MCS groups did not differ in terms of age (UWS: 46.8 ± 14.6
years; MCS: 48.8 ± 14.8 years), gender (UWS: m/f = 7/9; MCS: m/f = 11/5), time since injury (127 ± 68.6 days and 114 ± 46.6 days, for UWS and MCS, respectively), as well as the type of injury (traumatic/non-traumatic ratio 6/10 and 3/13, for UWS and MCS, respectively). In respect of age and gender, DOC patients also did not significantly differ from clinical control group patients (m/f = 7/3, age 43.7 ± 18.5 years).

Table 1 – Clinical details of patients

| id   | Age (y) | Range | Time (since injury (d)) | Etiology | CRS-r (A,V,M,OV,C,Ar) |
|------|---------|-------|-------------------------|----------|------------------------|
| UWS1 | 45-60   | 62    | NTBI                    |          | 5 (1,0,2,1,0,1)        |
| UWS2 | 45-60   | 151   | TBI                     |          | 5 (1,0,1,1,0,2)        |
| UWS3 | 60-70   | 273   | NTBI                    |          | 7 (2,1,2,1,0,1)        |
| UWS4 | 18-30   | 68    | TBI                     |          | 7 (2,1,1,1,0,2)        |
| UWS5 | 45-60   | 134   | NTBI                    |          | 5 (1,0,1,1,0,2)        |
| UWS6 | 30-45   | 69    | NTBI                    |          | 4 (1,0,0,1,0,2)        |
| UWS7 | 45-60   | 174   | TBI                     |          | 8 (2,1,2,1,0,2)        |
| UWS8 | 18-30   | 233   | TBI                     |          | 4 (1,0,1,1,0,1)        |
| UWS9 | 45-60   | 46    | NTBI                    |          | 7 (2,1,2,1,0,1)        |
| UWS10| 60-70   | 197   | NTBI                    |          | 6 (1,0,2,1,0,2)        |
| UWS11| 18-30   | 134   | TBI                     |          | 2 (0,0,0,1,0,1)        |
| UWS12| 30-45   | 52    | NTBI                    |          | 5 (1,0,1,2,0,1)        |
| UWS13| 60-70   | 98    | NTBI                    |          | 6 (2,0,2,1,0,1)        |
| UWS14| 45-60   | 116   | NTBI                    |          | 3 (0,0,2,1,0,0)        |
| UWS15| 18-30   | 169   | TBI                     |          | 6 (2,0,1,1,0,2)        |
| UWS16| 45-60   | 61    | NTBI                    |          | 7 (2,0,2,1,0,2)        |
| MCS1 | 60-70   | 69    | NTBI                    |          | 10 (2,2,2,1,1,2)       |
| MCS2 | 45-60   | 155   | NTBI                    |          | 12 (2,1,5,2,1,1)       |
| MCS3 | 45-60   | 84    | NTBI                    |          | 9 (2,0,2,2,1,2)        |
| MCS4 | 45-60   | 109   | TBI                     |          | 10 (2,3,2,1,0,2)       |
| MCS5 | 45-60   | 60    | TBI                     |          | 14 (3,3,5,1,1,1)       |
| MCS6 | 45-60   | 158   | NTBI                    |          | 12 (2,3,2,2,1,2)       |
| MCS7 | 60-70   | 66    | NTBI                    |          | 6 (2,2,0,1,0,1)        |
| MCS8 | 60-70   | 133   | NTBI                    |          | 9 (2,3,2,1,0,1)        |
| MCS9 | 18-30   | 150   | NTBI                    |          | 9 (1,0,5,1,0,2)        |
| MCS10| 60-70   | 140   | TBI                     |          | 16 (2,3,5,3,1,2)       |
| MCS11| 18-30   | 234   | NTBI                    |          | 8 (0,1,4,1,0,2)        |
| MCS12| 45-60   | 92    | NTBI                    |          | 11 (2,3,2,2,0,2)       |
| MCS13| 18-30   | 131   | NTBI                    |          | 8 (1,0,5,1,0,1)        |
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| MCS14 | 30-45 | 78 | NTBI | 8 (1,2,2,1,0,2) |
|-------|-------|----|------|----------------|
| MCS15 | 30-45 | 96 | NTBI | 9 (2,2,2,1,0,2) |
| MCS16 | 45-60 | 74 | NTBI | 14 (3,1,5,2,1,2) |
| CC1   | 45-60 | -  | -    | -              |
| CC2   | 18-30 | -  | -    | -              |
| CC3   | 30-45 | -  | -    | -              |
| CC4   | 18-30 | -  | -    | -              |
| CC5   | 60-70 | -  | -    | -              |
| CC6   | 60-70 | -  | -    | -              |
| CC7   | 45-60 | -  | -    | -              |
| CC8   | 60-70 | -  | -    | -              |
| CC9   | 18-30 | -  | -    | -              |
| CC10  | 18-30 | -  | -    | -              |

**Notes:** CRS-r: total score of Coma Recovery Scale – revised, A auditory, V visual, M motor, OV oromotor/verbal, C communication, Ar arousal subscores; Etiology: TBI – traumatic brain injury, NTBI – non-traumatic brain injury

**Recording**

A continuous 24h polysomnographic recording was carried out (including EEG, 2 channels of electrooculography (EOG), with the electrodes positioned 1 cm lateral and below and above to the outer canthi of both eyes; 3 channels of electromyography (EMG), with the electrodes placed on the chin; and video recording). The EEG electrodes were attached at F3, F4, Fz, C3, C4, Cz, P3, P4, Pz and both mastoids, referenced to Cz with the ground electrode at Fpz, according to the standards of the American Academy of Sleep Medicine (Iber, 2007).

The data were recorded using a mobile polysomnography device (SomnoScreenplus, Somnomedics, Randersacker, Germany) with 256 Hz discretization rate with 0.2 Hz high-pass and 35 Hz low-pass filters.

During the polysomnographic recording, all patients (including control patients) had to be moved by nurse personnel to prevent decubitus. After each such intervention, the experimenter checked electrode impedance and renewed electrodes whenever the impedance exceeded 5 kΩ.

All recordings were performed in the patients’ wards on weekends when no therapies took place. Since the patients were well acquainted to the sleep environment, there was no adaptation night.
The individual medication intake had remained stable during at least one week before recording. Recordings started between around 10 am and 12 noon and ended at the same time on the next day.

**Data analysis**

Based on video recordings, the behavioral sleep-wakefulness state of each patient was defined in 30-second intervals. An interval was classified as behavioral sleep if the eyes were closed at the onset of the interval, and as wakefulness, if they were open.

Before the evaluation of sleep stages for the electrophysiological sleep-wakefulness state, EEG data were re-referenced to averaged mastoids and pre-processed and notch filter (50 Hz) was applied using the program Brain Vision Analyzer (Version 2.1.2, Brain Products, Gilching, Germany). The program SchlafAus (developed by Steffen Gais, unpublished) was used for sleep scoring in 30-second intervals. Overall, there are 2880 30-s intervals during 24 hours, but the number of assessments per recording was different because sometimes more than 24 hours were recorded, and some epochs were lost due to the amplifier’s battery replacement (number of 30-sec epochs: mean = 2868, SD = 30.5, min = 2740, max = 2946).

To perform reliable sleep scoring, three independent raters (IM, YGP, CB) scored the data under the supervision of the most experienced fourth person (SD). All raters had previous experience in the classification of sleep stages and were familiar with the essential characteristics of the patient groups. Owing to the randomization of patient numbers, all raters were blind to clinical and demographic patient data.

The first ten recordings were scored by each of the three raters, who then met and discussed their agreements and disagreements. The aim of the meetings, led by the supervisor, was to adapt the scoring criteria and to reach a pairwise concordance of at least 80% in each of the three pairs of raters.

The following 32 recordings were randomly distributed among the three pairs of raters. Each of the raters worked independently, and no further discussions were allowed. The percentage agreement between the raters for the first ten cases was $83.53 \pm 3.84\%$, and for subsequent cases $82.23 \pm 8.06\%$. On average, the agreement was above the critical threshold of 80%, indicating a good match of scoring (Danker-Hopfe et al., 2004). In case of conflicting scorings, the raters agreed.
on the final version where all scoring conflicts were resolved by mutual consensus on an epoch-by-epoch basis.

The scoring criteria were based on those of Rechtschaffen and Kales (1968). By building on existing criteria, it was possible to better compare the results between DOC patients and the control patients within this study as well as between other studies.

DOC patients may show local pathological activity, such as intermittent rhythmic delta activity that might be confused with sleep-related delta waves. For this reason, and contrary to Rechtschaffen & Kales (1968), nine rather than two EEG electrodes were used for sleep scoring. This permitted us to detect deviating channels and exclude them from sleep stage evaluation.

Since the EEG amplitude in patients can be influenced by many extracerebral factors, the amplitude criterion for slow waves (>75 µV) was not followed. Some patients did not show typical markers for sleep stage S2 (i.e., sleep spindles, K complexes). In such cases, other EEG criteria for S2 (slowing of EEG and the appearance of slow wave activity up to 20% of the epoch) as well as further reduction of muscle tone were used for scoring S2.

To quantify spindle activity, sleep spindles can be visually evaluated, automatically detected or deducted from EEG spectrum (as power in the sigma frequency band) (De Gennaro & Ferrara, 2003; O’Reilly & Nielsen, 2015). In sleep research, there is no agreement on which approach for sleep spindle detection is optimal (Huupponen et al., 2007; Lacourse, Delfrate, Beaudry, Peppard, & Warby, 2018; Warby et al., 2014). In the present study, semi-automatic detection was preferred over manual detection, as it represents an objective and reproducible method. First, two scorers independently identified patients with at least one clearly present sleep spindle in the recording. The visual analysis permitted to exclude a number of patients who did not have any spindle activity in S2. Then, in patients with visually confirmed sleep spindles, we applied a recently published algorithm for sleep spindle identification and quantification to artifact-free sleep stage S2 epochs at Cz channel (Muehlroth & Werkle-Bergner, 2020). We used the default settings that, among others, included the frequency of spindles in the range of 12.5-16 Hz and the duration of spindles between 0.5 and 3 seconds. The following parameters of sleep spindles then entered the statistical analysis: total number, density (the number divided by the duration of S2 in min), and mean amplitude.
For statistical analyses of sleep data, R v.3.6.0 was used. The 24h recording was subdivided into day (08.00 - 20.00) and night (20.00 - 08.00), according to the time of intensive rehabilitation procedures and lights on in the patient’s room versus the time of rest and lights off. A mixed analysis of variance included a repeated measures factor DayTime (i.e., day/night) and a between-subject factor Group (UWS, MCS, CC) in the analysis of behavioral and electrophysiological sleep, and the factor Group in the analyses of sleep stage distribution. When the factor Group or the Group x DayTime interaction was significant, the effect was further examined by means of pair-wise comparisons with t-tests. Kruskal-Wallis and Wilcoxon Rank Sum tests were used for comparing proportions between the groups. When appropriate, we tested the lack of difference between the groups by calculating Bayes Factors (BF) using a Bayesian ANOVA or t-test. For this analysis, we used BayesFactor package for R with default priors.

Results

Behavioral and electrophysiological sleep

The overall proportion of eyes-closed and eyes-opened epochs in the 24h period was close to 50/50% (UWS: 49%, MSC: 51%, CC: 47% eyes-closed epochs) and did not significantly differ between all three patient groups (p = .89, BF₀₁ = 200). Interestingly, the groups differed in the proportion of eyes closed and eyes open when differentiating between night and daytime (DayTime by Group interaction: F(2, 39) = 8.11, p = .001, η² = .29). While CC (t(9) = 4.21, p = .0023, d = 1.40) and MCS patients (t(15) = 2.41, p = .03, d = 0.62) spent a greater amount of time with closed eyes during the night than during the daytime, this pattern was not evident in UWS patients (t(15) = 0.35, p = .73, d = 0.09, BF₀₁ = 3.7; see Figure 1A).
Figure 1 – Behavioral and electrophysiological sleep. UWS, Unresponsive Wakefulness Syndrome; MCS, Minimally Conscious State; CC, Clinical Control. (A) The amount of behavioral sleep (number of epochs with eyes closed) during the night and at daytime, for each group. (B) The amount of electrophysiological sleep evaluated by polysomnographic recording during the night and at daytime, for each group. (C) The distribution of sleep probability (percentage of patients who slept during the respective epoch) across all 2880 epochs. Solid lines show the results of smoothing according to the LOESS algorithm with the smoothing span of 0.2 by means of ggplot2 R package. (D) Scatterplots relating the probabilities (in %) that a particular epoch was a sleep epoch as scored with electrophysiological and behavioral measures (each dot represents one epoch). Note that the whole graph is “larger” for CC than for UWS and MCS, indicating a higher behavioral-electrophysiological correspondence among CC patients than DOC patients. There were epochs when all CC patients slept, and epochs when all of them were awake, but there were no such epochs in the two DOC groups. (E) Behavioral-electrophysiological sleep Kendall correlations calculated within each subject (dots) across epochs and averaged for each group (columns). * p < .05, ** p < .01, *** p < .001, ns not significant. Error bars are 95% confidence intervals.

To repeat the same analysis using electrophysiological sleep data, these data were dichotomized (i.e. 1 – any stage of sleep, 0 – wakefulness). In full agreement with the behavioral sleep data, CC (t(9) = 6.21, p = .0002, d = 2.07) and MCS (t(15) = 3.04, p = .008, d = 0.79) but not UWS (t(15) = 0.76, p = .46, d = 0.20, BF₀₁ = 3) patients showed a larger amount of sleep during the night than during the day (DayTime by Group interaction: F(2, 39) = 8.19, p = .001, η² = .30, see Figure 1B).
Most CC patients took a nap during the daytime (8 of 10). One DOC patient (UWS) did not show any signs of electrophysiological sleep despite having preserved eyes-open/closed behavior. Except for this patient, all DOC patients had at least a short interval of sleep during daytime. One UWS patient slept only during daytime. Figure 2 shows examples of hypnograms, illustrating individual differences in sleep distribution over the 24h period.

Figure 2 - Exemplary hypnograms of four patients. (A) an UWS patient who remained awake at night but slept during the day; (B) an MCS patient with close-to-normal sleep distribution; (C) an UWS patient with uniformly distributed sleep over the 24h period; (D) a CC patient with a pattern of well-structured sleep during the night and an afternoon nap. Notes: Sleep – electrophysiological sleep, Eyes – behavioral sleep.
Next, we tested whether the DOC patients’ sleep is characterized by a pattern of frequent changes between sleep and wakefulness. We calculated the number of transitions between sleep and wakefulness (i.e., the intervals that were classified as different from the immediately preceding intervals). The number of transitions did not significantly differ among groups, neither on the basis of behavioral sleep data ($F(2, 39) = 1.09, p = .35, \eta^2 = .05$) nor when the electrophysiological data were used ($F(2, 38) = 1.46, p = .24, \eta^2 = .24$). The number of transitions in the electrophysiological sleep data analysis was corrected for the number of sleep epochs because of unequal sleep duration between the patients. One UWS patient who did not sleep at all was excluded from this analysis.

We then examined the correspondence between sleep based on behavioral and electrophysiological data. First, we calculated the percentage of patients who slept at the same time during each of the 2880 epochs (sleep probability) based on behavioral and electrophysiological data separately (see Figure 1C). Second, we correlated behavioral and electrophysiological sleep probability across all the epochs. As shown in Figure 1D, behavioral sleep corresponded significantly with electrophysiological sleep in all three groups, with the strongest correspondence for CC and the weakest correspondence for UWS patients (Spearman’s $\rho = 0.87, 0.58, \text{and } 0.37$ for CC, MCS, and UWS, respectively; all $p$s < 0.001; pairwise comparisons between groups indicate significant differences, all $p$s < 0.001 in). The correspondence between behavioral and electrophysiological sleep likewise differed between groups when it was analyzed on the basis of single epoch data within each patient ($F(2, 38) = 4.27, p = .021, \eta^2 = .18$, see Figure 1E). The correlation was stronger in the CC group than in the MCS ($t(24) = 3.29, p = .006, d = 1.22$) and UWS groups ($t(23), = 2.60, p = .016, d = 1.06$), with no difference between the MCS and UWS groups ($t(29) = 0.62, p = .541, d = 0.22$).

Sleep stage distribution

The total amount of sleep as measured with polysomnographic recordings did not significantly differ between the groups ($F(2, 39) = 2.76, p = .08, \eta^2 = .12$; mean ± SD = 311 ± 184, 405 ± 185, 464 ± 98 min in UWS, MCS and CC groups respectively), although post-hoc tests revealed shorter overall sleep duration in UWS patients compared to the CC group (see Figure 3A).

With regard to single sleep stages, there were significant group differences concerning the time spent in sleep stage S1 ($F(2, 39) = 3.36, p = .045, \eta^2 = .15, \text{BF}_{10} = 1.8$) and REM sleep ($F(2, 39) =$
7.41, $p = .002$, $\eta^2 = .28$, $BF_{10} = 19.4$), but not in S2 ($F(2, 39) = 1.1$, $p = .34$, $\eta^2 = .05$, $BF_{01} = 2.73$) and SWS ($F(2, 39) = 0.28$, $p = .75$, $\eta^2 = .01$, $BF_{01} = 4.71$). As can be seen in Figure 3E and Table 2, MCS patients spent more time in S1 than UWS patients ($t(30) = 2.49$, $p = .019$, $d = 0.88$). Further, UWS patients spent less time in REM sleep than CC patients ($t(24) = 4.76$, $p < .001$, $d = 1.92$) and MCS ($t(30) = 2.15$, $p = .040$, $d = 0.76$).

Table 2 – Sleep characteristics

| id  | S1, min | S2, min | SWS, min | REM, min | Spindles present | N spindles | Density, sp/min | Amplitude, $\mu V$ |
|-----|---------|---------|----------|----------|------------------|------------|-----------------|-------------------|
| UWS1| 88      | 389.5   | 22       | 4.5      | 0                | 19         | 0.06            | 19.57             |
| UWS2| 33.5    | 434.5   | 31       | 5.5      | 0                | 31         | 0.08            | 14.28             |
| UWS3| 68.5    | 194     | 86       | 0        | 0                | 0          | 0               | 7.79              |
| UWS4| 15.5    | 77.5    | 72.5     | 7.5      | 0                | 0          | 0               | 0                 |
| UWS5| 171     | 189     | 27       | 28.5     | 0                | 10         | 0.06            | 6.86              |
| UWS6| 0       | 0       | 0        | 0        | 0                | 0          | 0               | 0                 |
| UWS7| 72.5    | 77.5    | 2        | 0        | 0                | 20         | 0.26            | 11.16             |
| UWS8| 31      | 154     | 242      | 30.5     | 1                | 14         | 0.10            | 9.22              |
| UWS9| 64      | 258     | 102.5    | 30.5     | 1                | 2          | 0.01            | 7.79              |
| UWS10| 71     | 3       | 0        | 0        | 0                | 0          | 0               | 0                 |
| UWS11| 2      | 479.5   | 200.5    | 0        | 1                | 89         | 0.21            | 38.29             |
| UWS12| 46.5   | 190.5   | 52       | 0        | 1                | 125        | 0.68            | 7.13              |
| UWS13| 49     | 69.5    | 2        | 1.5      | 1                | 7          | 0.15            | 6.99              |
| UWS14| 81     | 154     | 24.5     | 14       | 0                | 3          | 0.02            | 6.99              |
| UWS15| 24.5   | 64.5    | 68       | 67.5     | 1                | 65         | 1.13            | 13.62             |
| UWS16| 13.5   | 78.5    | 204.5    | 0        | 0                | 31         | 0.43            | 15.25             |
| UWS (M±SD)| 52±42.5 | 175.8±147.5 | 71±78.8 | 11.9±18.8 |
| MCS1| 83.5    | 163.5   | 33       | 0        | 0                | 1          | 0.01            | 7.80              |
| MCS2| 159.5   | 63      | 0        | 86.5     | 0                | 15         | 0.39            | 29.10             |
| MCS3| 162     | 119.5   | 47       | 7.5      | 0                | 2          | 0.02            | 7.80              |
| MCS4| 46.5    | 187     | 57       | 19.5     | 0                | 6          | 0.04            | 10.20             |
| MCS5| 119     | 88.5    | 4        | 24.5     | 1                | 143        | 1.71            | 14.01             |
| MCS6| 147.5   | 504.5   | 173      | 126      | 1                | 3          | 0.01            | 10.45             |
| MCS7| 216.5   | 110.5   | 11.5     | 28       | 1                | 8          | 0.08            | 11.71             |
| MCS8| 67.5    | 373.5   | 63.5     | 10.5     | 1                | 4          | 0.01            | 10.71             |
| MCS9| 93      | 262     | 75       | 38       | 1                | 120        | 0.48            | 18.64             |
| MCS10| 75     | 323.5   | 47.5     | 24       | 0                | 5          | 0.03            | 8.32              |
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| MCS11 | 4.5  | 295.5 | 253.5 | 90  | 1   | 121  | 0.61 | 8.48 |
|-------|------|-------|-------|-----|-----|------|------|------|
| MCS12 | 169  | 104   | 15    | 22  | 1   | 2    | 0.03 | 15.71|
| MCS13 | 25.5 | 206   | 119.5 | 0   | 1   | 19   | 0.10 | 12.21|
| MCS14 | 21.5 | 177   | 19.5  | 6   | 1   | 1    | 0.01 | 4.80 |
| MCS15 | 197.5| 225   | 6.5   | 31.5| 1   | 323  | 1.48 | 16.48|
| MCS16 | 34.5 | 194.5 | 0     | 25  | 1   | 11   | 0.06 | 6.60 |

| MCS (M±SD) | 101.4±67.2 | 212.3±117.3 | 57.8±70.3 | 33.7±36 |

| CC1 | 60  | 231  | 162.5 | 21  | 1   | 209  | 0.99 | 25.37|
| CC2 | 40  | 191.5| 144   | 91.5| 1   | 50   | 0.27 | 25.48|
| CC3 | 64.5| 244.5| 102   | 83  | 1   | 146  | 0.70 | 20.47|
| CC4 | 152 | 446.5| 41    | 49.5| 1   | 264  | 0.65 | 16.86|
| CC5 | 111 | 231  | 8.5   | 41  | 1   | 89   | 0.47 | 15.67|
| CC6 | 56.5| 209  | 30.5  | 33  | 1   | 20   | 0.11 | 11.42|
| CC7 | 68.5| 282.5| 56    | 17  | 1   | 276  | 1.08 | 15.48|
| CC8 | 56  | 286  | 24.5  | 84  | 1   | 130  | 0.52 | 15.77|
| CC9 | 170.5| 170.5| 101   | 88.5| 1   | 82   | 0.59 | 20.17|
| CC10| 36.5| 193.5| 110.5 | 51  | 1   | 281  | 1.53 | 20.50|

| CC (M±SD) | 81.5±46.8 | 248.6±79.1 | 78±53.2 | 56±28.7 |

Notes: UWS, unresponsive Wakefulness Syndrome; MCS, Minimally Conscious State; CC, Clinical Control; S1, S2, sleep stages 1 and 2, resp; SWS, Slow-Wave Sleep; REM, Rapid Eye Movement Sleep; Spindles present (defined by visual screening): 0 – no, 1 – yes; N Spindles, overall number of spindles; Density, sleep spindle density defined as overall number of spindles divided by the duration of artifact free S2 in minutes; Amplitude, the average amplitude of sleep spindles in µV.

As depicted in Figure 3D sleep stages S1, S2, SWS, and REM sleep were present in all CC patients, but not in all UWS and MCS patients. Statistically, the REM stage was less frequently found in UWS than in MCS ($\chi^2(1) = 3.74, p = .053$) and in CC ($\chi^2(1) = 5.76, p = .016$) but MCS did not differ from CC ($\chi^2(1) = 1.3, p = .25$), resulting in a generally significant Group effect ($\chi^2(2) = 8.02, p = .018$). All other sleep stages did not differentiate the groups significantly.

As it is known that the proportion of SWS and REM sleep decrease with age (Ohayon et al., 2004), we tested the possibility that our results were affected by age-related changes in sleep architecture. We confirmed general SWS decrement with age in our sample (Spearman correlation between the amount of SWS and age in years: $\rho = -0.44, p = .004$). A correlation of smaller magnitude with age was found for S1 ($\rho = 0.34, p = .027$) but not for REM sleep ($\rho = -0.18, p = .25$) or S2 ($\rho = -0.1, p = .53$). When introducing age as a covariate in our overall sleep stage analysis, the effect of Age was only significant for SWS ($F(1, 38) = 10.75, p = .002, \eta^2 = .22$). Controlling for age...
made the effect of Group on SWS even weaker \((F(2, 38) = 0.1, p = .90, \eta^2 = .005, \text{BF}_{01} = 5.29)\), while producing negligible effects on REM sleep \((F(2, 38) = 7.33, p = .002, \eta^2 = .28)\), S1 \((F(2, 38) = 3.34, p = .05, \eta^2 = .15)\), and S2 \((F(2, 38) = 1.02, p = .37, \eta^2 = .05)\), suggesting that the observed differences between patient groups were not considerably affected by age.

Figure 3 – Sleep stage distribution. (A) Total amount of sleep by group, (B) Number of sleep spindles by group. Only patients with present spindles (defined by visual screening) included (see Table 2), (C) Amplitude of sleep spindles by group. Only patients with present spindles (defined by visual screening) included (see Table 2), (D) The percentage of patients in each group, showing signs of the respective sleep stage during the 24h recording period. (E) Time spent in single sleep stages by group. UWS, unresponsive Wakefulness Syndrome; MCS, Minimally Conscious State; CC, Clinical Control; SWS, Slow-Wave Sleep; REM, Rapid Eye Movement Sleep; S1, S2, sleep stages 1 and 2, resp.; * \(p < .05\), ** \(p < .01\), *** \(p < .001\), \(n_s\) not significant. Error bars are 95% confidence intervals.

**Sleep spindles**

Some patients showed no or hardly any characteristic sleep spindles in sleep stage S2 (see Table 2). The presence of sleep spindles was significantly different between groups \((\chi^2(2) = 10.44, p = .005)\). Specifically, fewer UWS patients (38%) showed any sleep spindles than CC patients (100%).
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\( \chi^2(1) = 9.76, p = .002 \); the proportion of MCS patients with identified spindles (69%) only marginally differed from CC patients \( \chi^2(1) = 3.04, p = .08 \) and UWS patients \( \chi^2(1) = 3.72, p = .054 \).

To further explore the quality of sleep spindles, only patients with sleep spindles were included, i.e. \( n(UWS) = 6, n(MCS) = 11, n(CC) = 10 \). Although the average number of spindles significantly differed between the groups \( \chi^2(2) = 6.95, p = .03 \), the difference in sleep spindle density did not reach significance \( \chi^2(2) = 4.6, p = .1 \). CC patients showed a significantly higher number of spindles in sleep stage S2 \((155 \pm 97.2)\) than UWS patients \((50.3 \pm 50.6, U = 87, p = .024)\) and MCS patients \((68.6 \pm 101, U = 50.5, p = .029)\), whereas the MCS and UWS groups did not differ \((U = 31.5, p = .919)\). Similarly, UWS and MCS patients had a lower average spindle amplitude \((13.8 \pm 12.2 \mu V \text{ and } 11.8 \pm 4.23 \mu V, \text{ respectively})\) than CC patients \((18.7 \pm 4.52 \mu V; U = 49, p = .042 \text{ and } U = 95, p = .004 \text{ respectively})\), yielding a significant overall group effect \( \chi^2(2) = 8.85, p = .012 \).

**Discussion**

Previous studies indicated that sleep evaluations are a promising tool in the assessment of DOC patients as the presence of sleep stages seems to be related to the severity of consciousness impairment (Avantaggiato et al., 2015; de Biase et al., 2014; Forgacs et al., 2014; Kang et al., 2014). Sleep markers can have a higher prognostic value in DOC than many other neurophysiological tests, including event related brain potentials (ERP) and functional magnetic resonance imaging (fMRI) (Arnaldi et al., 2015; Kotchoubey & Pavlov, 2018a). Other authors propose a combination of ERP and polysomnography as a predictive tool in DOC (Rossi Sebastiano et al., 2015). Compared with fMRI and ERP, polysomnographic recordings are cost-effective and do not require an immediate response to a stimulus and are, therefore, independent of patient comorbidities (e.g. sensory deficits) and abnormal latency of brain responses.

A number of factors decrease the reliability of previous sleep studies in DOC. Due to numerous abnormalities of EEG and sleep patterns, sleep scoring in DOC is highly challenging. Oscillations in the same frequency range (e.g., 2-3 Hz) can indicate both normal SWS and severe brain pathology of DOC patients. Therefore, the mere description of spectral properties and spectrum-based automatic analyzes provide limited insights. The problem is further worsened by the lack of
concordant data from independent, blinded raters. In the present study, a group of experienced sleep raters was explicitly trained to adjust Rechtschaffen & Kales’ (1968) scoring criteria to the particular features of EEG in DOC. Each dataset was independently scored by two raters, who were unaware of the patient’s clinical data. The between-rater agreement above 80% can be regarded as high even for normal sleep.

In agreement with previous studies (Bekinschtein et al., 2009; Blume et al., 2017; Cruse et al., 2013; Wislowska et al., 2017), severe abnormalities of circadian rhythms were observed in virtually all DOC patients. Most obvious, the very cyclicity of sleep and wakefulness, i.e. the typical distribution of sleep over the day and night, was severely disturbed, suggesting a damage or severe dysfunction of the brain stem (Isono, Wakabayashi, Kamida, & Kobayashi, 2002). Even MCS patients slept less in the night and more during daytime than control patients. Much stronger was this anomaly in UWS patients in which the distribution of sleep and wakefulness did not differ between day and night. Some of the UWS patients did not show night sleep at all, but slept only during the day. This observation underlines the necessity of polysomnographic recordings for at least 24 hours, because the data of only night sleep can be strongly misleading. Given that a recording day might occasionally be atypical, data collection for a still longer period (48 or 72 hours) would be useful (Angerer, Blume, Schabus, & Wislowska, 2018; Blume et al., 2019), but not at the cost of the decrease of sample size (Kotchoubey & Pavlov, 2018b).

The presence of numerous sleep episodes during the daytime may account for strong fluctuations observed in the diagnostic behavioral assessment of both MCS (Giacino et al., 2002) and UWS (Wannez et al., 2017). It is expected that by determining the distribution of periods of decreased arousal level, the risk of diagnostic misjudgment can be reduced.

Although the correlation between behavioral sleep (eyes open/-closed) and electrophysiological (polysomnographic) signs of sleep was highly significant, it strongly varied among the groups. The correlation was close to 1.0 in control patients, and significantly decreased with severity of the DOC, indicating the possibility of behavioral/electrophysiological sleep dissociation in DOC patients. This finding supports the claim that from the appearance of eyes-open/-closed periods in DOC patients, it cannot be directly concluded that the patient is awake or asleep (Landsness et al., 2011).
Whereas all sleep stages were present in all control patients, some sleep stages were entirely lacking in DOC patients. Particularly, significantly fewer UWS patients showed any signs of REM sleep than MCS and control patients. This finding is in line with previous studies, observing greater anomalies in UWS patients than in MCS patients regarding REM sleep (Aricò et al., 2015; Cologan et al., 2013; de Biase et al., 2014; Isono et al., 2002). Other studies reported lower amounts of REM sleep in DOC compared with healthy participants (Oksenberg, Gordon, Arons, & Sazbon, 2001).

It has been suggested that the abnormalities in REM sleep indicate brain stem damage and could thus improve the identification of lesions in neuronal tissues and inform more targeted treatment of individual patients (Cologan et al., 2010). It could even be speculated that there is a specific relation between REM sleep and consciousness: REM sleep deficits might be associated with a lack of dreams, with dreams in turn being peculiar sleep states of consciousness. Furthermore, REM sleep has been proposed to serve the function of preparing the brain for the following state of wakefulness (evidence for this point was reviewed by (Peever & Fuller, 2017)). It is, however, much too early to speculate about possible causal relationships.

In the present study, DOC patients also showed marked abnormalities with regard to sleep spindles. A total of 15 patients did not show any signs of sleep spindles in S2. Moreover, even the DOC patients who did show spindles, displayed an overall smaller number of spindles and a lower spindle amplitude than control patients. This finding is in line with previous studies that reported sleep spindle deficits in DOC patients (Isono et al., 2002; Pavlov et al., 2017) and greater abnormalities in UWS than in MCS in terms of sleep spindles (Aricò et al., 2015). Spindle density is generally reduced in severe impairments of cognition and consciousness such as dementia (Ktonas et al., 2009; Scullin & Bliwise, 2015) and schizophrenia (Manoach, Pan, Purcell, & Stickgold, 2016; Wamsley et al., 2012). But to the best of our knowledge, there are no clinical conditions where sleep spindles are completely absent. This absence may indicate a loss of thalamic circuits integrity (Fernandez & Lüthi, 2020), which presumably plays a role in the emergence of DOC (Giacino, Fins, Laureys, & Schiff, 2014).

On the background of these multiple abnormalities, there are a number of sleep features for which we did not observe differences between DOC and control patients. The total amount of sleep in control patients was similar to that reported in completely healthy individuals. The total amount of sleep in DOC patients was slightly decreased, but this decrease did not reach significance. It could
be speculated, though, that this difference would reach significance in a larger sample. The average amount of S2 likewise appears to differ between patient groups (CC > MCS > UWS), but the intragroup variance in S2 was so large (see Table 2) that the intergroup effect did not become significant. The amount of SWS was virtually identical in all three groups and, moreover, identical to the amount of SWS in normal populations of the corresponding age (Ohayon et al., 2004). In this case, the F-ratio of 0.1 and BF of 5.29 indicate that the lack of significance may be a real null effect and not just a product of insufficient power. If this is true, this finding may indicate that SWS is so important as to maintain its overall stability, even under conditions of severe distortions of consciousness and notwithstanding strong fluctuations in the distribution between day and night.

While our findings are largely in line with previous data, there are some differences. In contrast with our results, a large study by Rossi Sebastiano et al. (2018) found a significant difference between UWS and MCS in terms of the presence of SWS but not of REM sleep. In a smaller study, Bedini et al. (2015) did not observe any differences between UWS and MCS neither in SWS nor in REM sleep. There are at least two important differences between our study and these previous studies. First, it is not quite clear how sleep stages were scored in these previous studies and whether the raters were blind with regard to the patients’ diagnosis. Perhaps even more important, Bedini et al. and Rossi Sebastiano et al. performed polysomnographic recordings for less than 24h. As we have seen that DOC patients, and particularly UWS patients, can show any sleep stage at any time of day/night, it is quite possible that some important sleep epochs were missed during those 5 to 8 hours when the recording was not performed.

A big difficulty in the interpretation of sleep abnormalities in DOC is that the life conditions of such patients differ drastically from those of healthy individuals with respect to many factors, each of which can potentially disturb sleep. DOC patients spend most of their time in bed; during the night they are regularly disturbed by the light and sounds that cannot be completely avoided in a hospital setting; they are periodically awaked and moved by the personnel to avoid decubitus. Finally, they do not experience the usual social pressure to stay awake during daytime, and the fact that they can sleep almost whenever they want naturally decreases their need in night sleep. These are only a few of numerous external factors of disturbed sleep besides the internal factors related to brain lesion.
Independent studies demonstrated considerable effects of such environmental factors on REM sleep in intensive care unit patients (Huang et al., 2015; Lewis, Schofield-Robinson, Alderson, & Smith, 2018), but similar studies in DOC patients are entirely lacking. The present study was the first to include a clinical control group that was exposed to the same external environmental factors as the examined DOC patients, but did not suffer from conditions of brain damage. Even though this group contained only ten patients, their sleep pattern was radically different from that of DOC patients. Therefore, we conclude that the external factors mentioned above only play a minor, if any, role in the development of massive sleep abnormalities characteristic for DOC.

Potential Conflicts of Interest

Nothing to report.

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Author Contributions

BK, YGP, FM, and SD contributed to the conception and design of the study. IM collected the data. YGP, IM and CB scored the sleep data, supervised by SD. YGP analyzed the data and prepared the figures. IM and YGP drafted the manuscript. BK, FM, CB, and SD brought major revisions in significant proportions of the manuscript.
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