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Are circadian rhythms in disarray in patients with chronic critical illness?

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A N N O T A T I O N (A B S T R A C T)

Aim: The aim of our study is to assess circadian rhythms in patients with chronic critical illness due to severe brain injury in intensive care unit by establishing the relation between melatonin and cortisol secretion, considering astronomical time and the sleep-wake cycle in chronic critical illness.
Materials and methods: The study included 54 adult patients with chronic critical illness who resided in the intensive care unit for at least 30 days. The level of consciousness was determined using the CRS-R scale. We did the continuous electroencephalographic (EEG) monitoring with polygraphic leads for 24 h. Also, we determined the serum levels of cortisol and melatonin using the tandem mass spectrometry method with ultra-performance liquid chromatography.
Results: 90.74 % of patients had one acrophase in melatonin secretion curve, which suggests the preservation of the rhythmic secretion of melatonin. These acrophases of the melatonin rhythm occurred during the night time in 91.8 % of patients. Most of the patients (69.3 %) slept during the period from 2:00 to 4:00 a.m. The evening levels of cortisol and melatonin had an inverse relation (r_s=*0.61*, $p < 0.05$), i.e., a decrease in the level of cortisol secretion accompanies an increase in melatonin.
Conclusions: We concluded from our study that the rhythmic secretion of melatonin and cortisol is preserved in patients with chronic critical illness that resulted from severe brain injury. No statistically significant discrepancy between melatonin and cortisol secretion, day-and-night time and the sleep-wake cycle are found. We may focus our future work on finding more reliable methods to stabilize the preservation of circadian rhythms to protect vital organ functions.

1. Introduction

Chronic critical illness (CCI) patients stay in the intensive care unit (ICU) for a longer period, which increases hospitalization costs and risk of complications at the post-hospital stage [1,2]. With the increasing age of the population and advances in intensive care, CCI is becoming more common [3].

Circadian rhythm and sleep disturbances are common in CCI [4,5]. These disturbances can have a negative impact on the brain arousal, sympathetic stability, cardiovascular functions, immune functions, and metabolic homeostasis [6,7], which extends the length of ICU stay.

The aim of our study was to evaluate circadian rhythms in CCI patients, who were in ICU for a long period under the influence of undesirable external factors. Little attention has been paid to circadian rhythms in the late period of critical illness. Whereas circadian rhythms during an acute period of the critical illness are a well-studied subject, our study focuses on improving our understanding of circadian processes in dynamics in post-acute period.

Among CCI patients, severe brain injury with concomitant disorders of the qualitative component of consciousness is most challenging to understand. We also had known this qualitative component disorder of consciousness as the prolonged disorders of consciousness (pDoC). Based on cognitive functions, pDoC is further divided into a vegetative state (VS), or in newer terminology, unresponsive wakefulness syndrome (UWS) and minimally conscious state (MCS). Along with the absence of intentional behavior, patients with vegetative state (VS) do not exhibit any indications of purposeful response to external stimuli, speech understanding, and maintenance of attention during irregular alternating states of sleep and wakefulness. They do not control functions of the pelvic organs and have partially or completely intact brainstem and spinal reflexes. A minimally conscious state (MCS) is a clinical state, accompanied by severe impairment of consciousness, in which, nevertheless, there are distinct, albeit minimal and often unstable, signs of intentional behavior. Patients in MCS can fix their gaze on a

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significant object and exhibit emotional responses, such as smiling or crying to stimuli that are meaningful to them in case of MCS–form, or even follow basic instructions and produce a simple answers, like yes/no with gestures in MCS + form.

For our study, we selected CCI patients out of coma with complete or partial reduction of higher mental functions in UWS or MCS, as well as patients out of coma or DoC with preserved functional communicative status (conscious) at the beginning of the study.

2. Materials and methods

2.1. Study participants

The study included 54 adult patients from the ICU unit of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology.

Inclusion criteria was CCI due to severe brain injury because of traumatic brain injury (TBI), global ischemia, and vascular incidents. At the time of inclusion, a minimum time frame of over 30 days was required after severe brain injury.

The exclusion criteria were hemodynamic instability, low-grade body temperatures of above 37.5 C, age over 70 years, and vasopressor support, as it has a substantial effect on the rhythms of melatonin secretion [7]. We also excluded patients with cervical spine and hypothalamic injuries confirmed by MRI.

The flowchart is shown in Fig. 1. It can be seen that a number of patients meeting the inclusion criteria were excluded from the study, such as: patients who required emergency surgery during the study (3 patients), patients who developed epileptic seizures (1 patient), delirium requiring large doses of sedatives (1 patient), hyperthermia at the moment of the study (2 patients), hemodynamic instability leading to death (1 patient). We also excluded patients who did not come from primary health care, i.e. patients who stayed at home or in palliative care before admission because this can imply the restoration of circadian rhythms outside of the ICU.

It must be noted that our research and clinical center specializes in the rehabilitation of patients with severe brain injuries, including prolonged impairment of consciousness in intensive care settings in patients with unexplored rehabilitation potential. Such patients stay at the center for a long time, and we studied a medical history of patients carefully to ensure their continuous prior ICU stay.

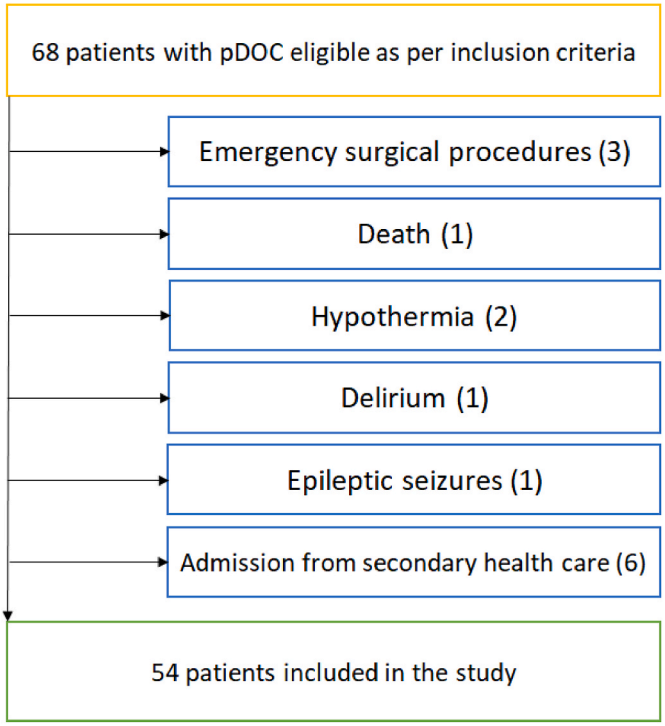

Fig. 1. Flowchart of the study.

2.2. Data collection

On the first day of the study, two neurologists independently assessed the level of consciousness of participants using the CRS-R scale.

We performed an electroencephalogram (EEG) using gold-plated cup electrode for long-term EEG recording, applied to adhesive paste under the standard 10/20 system. The number of channels included two electrooculogram (EOG) channels, one electromyogram (EMG) channel, one electrocardiogram (ECG) channel, and 19 EEG channels: Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, Pz using head reference and ground electrode. We fixed electrodes using patches, a mesh or headwear worn on top, or bandaging with a cohesive bandage. We used NicoletOne™ EEG system for EEG in ICU under the supervision of the attending physician and neurophysiologist and to record, we used a bipolar montage "sleep" with additional channels. The impedance between the electrode and the scalp was <5 kOhm. The frequency of the high-cutoff was 0.5 Hz, whereas the low-cutoff was 35 Hz. Gain - 7 μV/mm.

We collected the blood samples every 2 h from a central venous catheter for 24 h. Then the collected samples were immediately centrifuged for 10 min. It was then frozen for temperature around −60 °C and later stored at −80 °C.

We assessed the level of illumination in the intensive care unit during the daytime using a GL5528 photo resistor placed next to the patient's head position.

3. Data analysis

3.1. EEG

We calculated the total sleep time with no division by phases and stages because, in most cases, accurate staging and phasing of the sleep-wake cycle was impractical to achieve. Cortical rhythm in severe brain injuries has traits that make it difficult to apply the standard criteria for sleep analysis, such as a slowdown of wakefulness rhythms to the delta-theta range, a decrease in their amplitude up to a flattening of the curve (<15 μV), a decrease in amplitudes and frequencies of sleep spindles, the presence of periodic and rhythmic patterns, etc.

However, the wakefulness phase is most often distinguished accurately by blinking and movement artifacts. Thus, the first step in analyzing the diurnal recordings was to find a valid waking episode. For this purpose, we evaluated the epoch of reliable wakefulness, where blink artifacts, the presence of muscle artifacts, alpha-rhythm in occipital regions, and data from the video camera served as a marker of reliability. In case an epoch did not meet valid criteria for wakefulness, it was graded to one of the sleep phases according to the following criteria.

In the second stage, we searched for graph elements normally characteristic of sleep.

1. Floating eye movements in surface slow-wave sleep;
2. changing (deceleration and growth of amplitude) of cortical activity relative to the epoch of reliable wakefulness;
3. Absence of blink artifacts;
4. reduction of artifacts from muscle activity;
5. Appearance of K-complexes and spindles, including in a reduced form;
6 presence of artifacts from rapid eye movements;
7 presence of sawtooth waves in the central leads;
8. Flattening of cortical activity with complete absence of muscle artifacts

Each recording was analyzed by two independent qualified neurophysiologists, who had the necessary experience in EEG analysis in patients with severe brain injury.

3.2. Melatonin

We measured the melatonin content in blood-serum samples by using tandem mass spectrometry in conjunction with ultra-performance liquid chromatography. The method included the determination of melatonin in the extract on an AB SCIEX QTRAP 5500 (AB SCIEX, Concord, ON, Canada) tandem mass spectrometer with triple quadrupole and ion trap, equipped with an atmospheric pressure chemical ionization (APCI) source controlled by Analyst software, version 1.6.2 (AB Sciex Pte. Ltd., Woodlands, Singapore). Nebulizer current was 2 mA, and source temperature was 450 °C. We carried melatonin monitoring out in a positive mode, using optimized parameters of ion transitions (multiple reaction monitoring). We used deuterium-labeled melatonin (melatonin-d6) as an internal standard and processed the measurement results using the MultiQuant 3.0.1 program (AB Sciex LLC, Framingham, MA, USA).

3.3. General approach to circadian rhythms analysis

To comprehensively analyze the collected data, we divided the 24 h period into 12 2-h intervals (see appendix A). The time from 20:00 to 08:00 was considered as astronomical night, the time from 8:00 to 20:00 (24 h format) – as an astronomical day. This corresponds to the daylight hours in the present part of the world.

Based on the melatonin level in each time interval, we divided the entire period into two phases of equal duration (6 2-h intervals in each, forming a 12-h continuous phase): melatonin (biological) night and melatonin (biological) day. The biological night phase included an interval containing the acrophase of the melatonin cycle, two intervals to the right of the acrophase with decreasing melatonin concentration) and three intervals towards the beginning of melatonin rise. The flatter nature of the increment in melatonin concentration compared with that of decrement [8,9] explained this explains the choice of intervals on either side of the acrophase. The biological day phase was constituted of the remaining intervals, so that biological day and night phases had an equal lengths.

Therefore, we characterized each of 2-h intervals for each patient by the following indicators (Fig. 1): value of melatonin (pg/ml); sleep time for this interval (minutes); relation of the interval to the day-night time; relation of the interval melatonin phase. Dissociation between day-night time, melatonin phase, and the sleep-wake cycle can characterize based on the above mentioned indicators the presence or absence of circadian synchronization in each patient.

3.4. Statistical analysis

We accumulated, corrected, and systematized the initial information collected by using the STATISTICA 10 (developer - StatSoft, Inc, Tulsa, OK, USA).

We present quantitative data as medians and quartiles (25–75 % of the interquartile range), whereas nominal data with absolute values and percentages. We used the nonparametric Kolmogorov-Smirnov test to check the nature of the distribution of interval variable. The statistical significance of differences in quantitative traits was assessed by using the Wilcoxon W-test. Differences were considered statistically significant at $p < 0.05$.

To study the relationship between phenomena represented by quantitative data, the distribution of which differed from the normal one, a nonparametric method - Spearman rank correlation coefficient was used. We interpreted the values of the correlation coefficient under the Chaddock scale to assess the direction and strength of the correlation.

When comparing several samples of non-normally distributed quantitative data, the Kruskal-Wallis test was used, which is a nonparametric alternative to one-way analysis of variance. If the calculated value of the Kruskal-Wallis criterion exceeded the critical value, the differences in the indicators were considered statistically significant. Otherwise, the null hypothesis was accepted as true.

3.5. Legal issues

Written consent was obtained from the first-degree relative of the patient, as patients could not give their consents themselves. We provided detailed clarification on the study matter to the concerned subjects.

The research is carried out under the Helsinki Declaration adopted at the 18th General Assembly of the world medical Association (WMA) (Helsinki, Finland, June 1964), 59th WMA General Assembly, Seoul, Republic of Korea, October 2008; Article 21 of the Constitution of the Russian Federation, Principles of Public Health Legislation, orders, and instructions issued by the Ministry of the health of the Russian Federation. The research was approved by the Ethics Committee of the Federal State Budgetary Institution of the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology (No. 08/19/22).

There is no conflict of interest.

4. Results

4.1. Demographic and clinical data

Demographic and clinical data of the 54 patients included in the study are presented in Table 1.

The median age of patients at the time of the event that led to impaired consciousness was 46.5 years [35, 58]. There were 27 men and 27 women in the study group. The minimum days of stay in intensive care were 34 days, the maximum achieved 648 days. The median time from the event, which lead to pDoC was 77 days [53, 143]. Based on the etiology, 15 patients had TBI, 23 - anoxic brain injury, and 16 had vascular incidents.

According to the level of consciousness at the beginning of the study 23 patients were in VS/UWS and 24 were in MCS. Out of 24 patients in MCS, 15 were in MCS- and 9 were in MCS+, whereas 7 patients were conscious. In VS/UWS, the median score on the CRS-R scale was 4 [3,5], in MCS- median score was 8 [7,9], in MCS + median score constituted 15 [14,17].

4.2. Exogenous factors

During the study, 12 patients (22.2 %) were on mechanical

Table 1
Demographic and clinical data of patients included in the study.

	Anoxic brain injury, n = 23	Traumatic Brain injury, n = 15	Vascular incidents, n = 16	All Etiologies n = 54
Sex	13 f/10 m	7 f/8 m	7 f/9 m	27 f/27 m
Age	41 [36; 53]	32 [22; 47]	59 [48.5; 65]	46.5 [35; 58]
CRS-R	7 [4; 13]	8 [6; 15]	6.5 [4; 12]	7 [4; 14]
Time from incident	77 [52; 143]	81 [58; 234]	64.5 [49.5; 97]	77 [53; 143]
Level of consciousness:				
VS/UWS	11	8	8	27
MCS-	6	1	4	11
MCS+	3	5	1	9
Consciousness	3	1	3	7

[Note: VS/UWS- Vegetative state/Unresponsive wakefulness syndrome; MCS – Minimally conscious state; CRS-R- Coma recovery scale revised.].

ventilation (MV), whereas the rest of the patients were under no ventilator support.

Pharmacological therapy included β-blockers, muscle relaxants, anticonvulsants (Table 2).

Pharmacological intervention which included β-blockers ($p = 0.85$), muscle relaxants ($p = 0.15$), anticonvulsants ($p = 0.37$), did not have a significant effect on secretion of melatonin (measured by mesor and amplitude of the secretion rhythm).

The average level of illumination at night (from 20:00 to 08:00) was 8 lux, the minimum was 0.15 lux. The highest level of illumination recorded during the day was about 200 lux.

The patients received no nutrition from 10 p.m. to 6 a.m. During the day, patients received specialized enteral feeding formulas through a gastrostomy tubes. An interval between each meal was 2–4 h.

4.3. Melatonin secretion

We consider the secretion of the melatonin cycle to be a 24 h period. The melatonin secretion levels for each patient at 12 intervals are given in Appendix A.

The melatonin rhythm curve has one acrophase in 90.74 % of patients (49 patients out of 54) (Fig. 2, a). Three patients have several peaks during the period (Fig. 2, b), due to this it was difficult to define acrophase in the melatonin rhythm in these patients. Two patients did not have significant peaks over the entire period (Fig. 2, c).

We calculated the mesor of the melatonin biorhythm as an average daily value. In the study group, the median value of the average daily indicator was 13.2 [7.5; 27.6].

The amplitude of the rhythm, defined as the largest deviation of the signal from the mesor, in the study group was 51.1 [26.9; 101].

4.4. Cortisol secretion

In 31 patients, we analyzed the level of cortisol secretion 4 times in 24 h: at 08:00 (morning level), at 14:00 (daytime level), at 20:00 (evening level) and at 02:00 (night level).

Differences between morning and evening cortisol levels are statistically significant ($p < 0.05$).

We found a statistically significant inverse relationship ($r_s = 0.61$, $p < 0.05$) between evening cortisol and melatonin levels, i.e. an increase in melatonin is accompanied by a decrease in the level of cortisol secretion (Fig. 3).

4.5. The sleep-wake cycle

The sleep time for each patient at 12 intervals are given in Appendix A. The median total sleep time during day-and-night time was 342.5 [194, 436] minutes, median sleep time during night was 207 [83, 358] minutes, median sleep time during day was 54 [19, 145]. 3 people out of 54 did not sleep during day-and-night time.

Median sleep time, when we considered melatonin night, was 186 [83, 317] minutes and 59 [17, 171] during melatonin day.

Table 2
Pharmacological treatment given to the patients included in the study.

Pharmacological group	Medications	Number of patients receiving treatment
β-blockers	atenolol, metoprolol, bisoprolol, sotalol	38 (70.3 %)
muscle relaxants	tizanidine, baclofen, tolperisone	9 (11.1 %)
anticonvulsants	carbamazepine, topiramate, valproic acid, clonazepam, gabapentin, pregabalin	22 (40.7 %)

4.6. Synchronization of day-and-night time, circadian rhythm and sleep-wake cycle

We demonstrated a radar chart (Fig. 4) indicating the number of patients with melatonin rhythm acrophase (green curve), melatonin night phase (red curve) and over 15 min of sleep (black curve) in each of the intervals. We excluded 5 patients mentioned above from this chart because of the difficulty of tracing the acrophase in these patients (see section 3.3).

Thus, the “Acrophase” curve (green) measures the fraction of patients (relative to the 49 patients included in the study) who have acrophase within an indicated time frame. We observed that in 46.9 % of patients, the acrophase fell on the interval from 2:00 to 4:00, which corresponds to the indicators of a healthy population. In 18.3 % of patients, the acrophase fell on the interval from 4:00 to 6:00, in 16.3 % - from 0:00 to 2:00 (that are also night times).

The “Sleep over 15 min” (black) curve indicates the fraction of patients (relative to the 49 patients included in the study) who slept for over 15 min in a 2-h interval. We did not consider intervals in which the duration of sleep was less than 15 min in this computation. The largest number of sleeping patients (69.3 %) fell on the period from 2:00 to 4:00.

The “Melatonin night” (red) curve expresses the fraction of patients (relative to the 49 patients included in the study) who have a phase of melatonin night during this interval. The share of intervals of melatonin night falling on the astronomical night was calculated as $\frac{n}{m} * 100\%$, where n is the number of intervals attributed in each particular patient to melatonin night, m is the number of intervals of the astronomical night, and in this study it is assumed that $m = 6$. These numbers are given directly in Appendix A. The average share of intervals of melatonin night falling on the astronomical night across all patients is 84.8 %, on astronomical day – 15 %. The statistically significant difference between shares of intervals of melatonin night and day falling on the astronomical nights at the level of $p = 0.018$.

To characterize the synchronization of sleep and melatonin night, we used the interval shift in the onset of sleep relative to the beginning of the melatonin night phase. Among all the subjects, this shift was 1 [0, 3] interval, i.e. from 120 to 240 min. Sleep was completely absent during the melatonin night phase in 6 patients, of which 3 people did not sleep at all, and the sleep of three others falls on the melatonin day phase. There is a significant difference between the time of sleep at a melatonin night and at a melatonin day with $p = 0.0002$.

We found a direct weak correlation between the time of the patient’s stay in the intensive care unit and the magnitude of the shift in the onset of sleep relative to the beginning of the melatonin night phase ($r_s = 0.31$ and $p < 0.05$).

4.7. Etiological differences

We analyzed the study group for possible differences related to the etiology of brain injury. Three subgroups - patients with the consequences of anoxic brain damage, traumatic brain injury and vascular accidents - were compared according to 4 parameters.

1. The presence of one acrophase per 24-h cycle;
2. The mesor of the melatonin biorhythm;
3. The degree of synchronization between the rhythm of melatonin secretion and the sleep-wake cycle, measured in the number of 2-h intervals by which one rhythm lags relative to the other;
4. The degree of synchronization between the onset of biological night and astronomical night, measured in the number of 2-h intervals by which one rhythm lags relative to the other.

The Kruskell-Wallace test was used to compare subgroups.
One acrophase per 24-h cycle was observed in 12 out of 15 patients

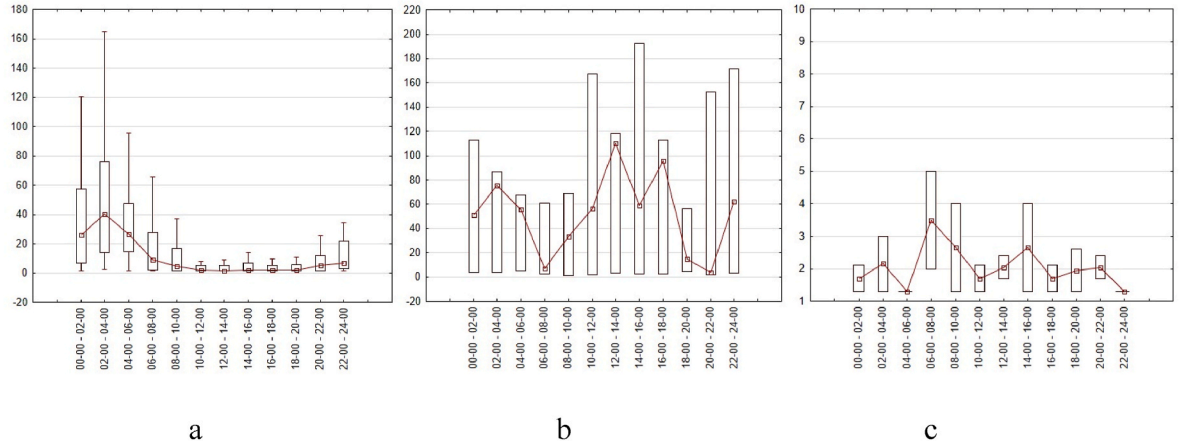

Fig. 2. Melatonin rhythm curve in patients with one acrophase per period (a), with several acrophases per period (b) and without significant peaks (c, scaled up).

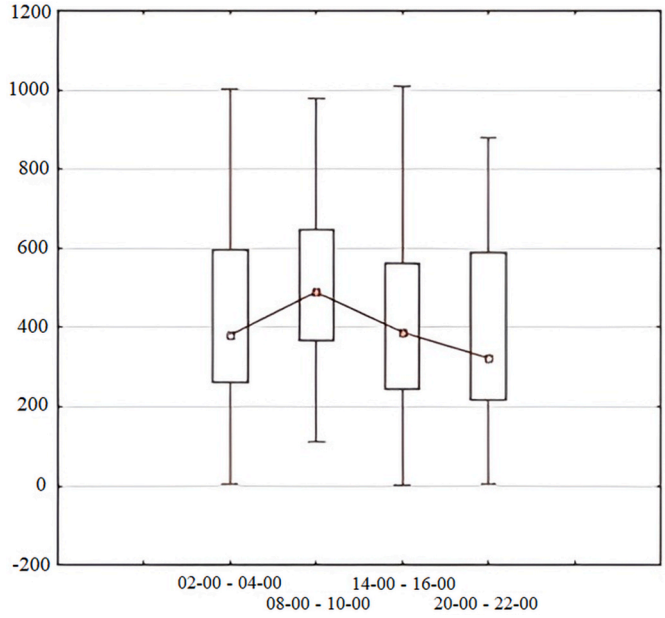

Fig. 3. Cortisol secretion rhythm curve in 31 patients.

with TBI, 14 out of 16 patients with vascular lesions, and 23 patients with anoxic brain injury (ABI). In comparison of groups TBI-ABI, TBI-VL and VL-ABI statistically significant differences between groups are nonexistent ($p=0.9$, $p=1.0$, $p=1.0$ respectively).

The median values of mesor of the melatonin biorhythm in TBI, ABI, and VL groups are 15.18, 15.96, and 12.54. No statistically significant differences between groups are found ($p=1$ for all groups).

In the group of ABI patients 2 patients did not sleep at all during 24 h period. In the group of TBI patients 1 patient did not sleep. In the group of VL patients all patients slept during 24 h period.

2 patients in the group of VL patients and 1 patient in the group of ABI patients had absolute desynchronization of circadian rhythms: they did not sleep during biological night. In TBI group there was no one with absolute desynchronization of circadian rhythms.

The median value of the number of 2-h intervals by which melatonon rhythm lags relative to the sleep-wake cycle for TBI, ABI, and VL are 1[0; 1], 1[0; 3], and 1.5[0.5; 2] respectively. No statistically significant differences between groups are found (with $p=0.9$ for TBI-ABI, and $p=1$ for TBI-VL and VL-ABI groups).

The median value of the number of 2-h intervals between the onset of biological night and astronomical night for TBI, ABI, and VL are 0[−1; 0], 0[−1; 0.5], and 0[0; 1] respectively. Statistically significant differences between groups are absent ($p=1$ for all groups).

5. Discussion

The study aimed to identify the presence and establish a discrepancy between the rhythm of melatonin and cortisol secretion, day-night time and the sleep-wake cycle.

Our research revealed.

1. In patients who have been in the intensive care unit for a long time (over 1 month) because of severe brain injury with various cognitive dysfunction the circadian rhythm is preserved, and the melatonin secretion rhythm has one acrophase in 90.74 % of patients. 3.7 % of patients did not have significant peaks of melatonin secretion rhythm. Multiple peaks were observed in 5.5 % patients, which may be related to the traumatic etiology of the disease. However, cases of melatonin curve deviation from the norm require further analysis and search for the causes of this phenomenon.
2. In the vast majority of patients, the acrophase falls on the interval from 2:00 to 4:00 o'clock in the night. There is no significant mismatch between the rhythm of melatonin secretion and the "day-night" cycle;
3. The daily average values of melatonin were at the lower limit of the norm or slightly derailed relative to healthy patients;
4. The total sleep time during the day in pDoC patients was shortened compared to that of the average values in the healthy population;
5. Patients slept in the intensive care unit mainly in the phase of melatonin night, which corresponds to the night time;
6. Patients with pDoC had an increase in cortisol levels in the morning and a decrease in the evening, which is inverse to that with melatonin secretion.

We demonstrated the features of the course of circadian rhythms in patients who are in the intensive care unit for a long time under conditions of light deprivation for the first time.

To date, there has been no agreement on circadian rhythms during the acute phase of a critical condition. Several studies [10–14] demonstrate an infringement in the normal cyclicity of melatonin secretion in acute cases, including the absence of a nocturnal peak [15]. Several other works provide information on the preservation of circadian secretion of melatonin in the presence of a large variability in the peak time between study participants [16–18]. A mismatch in the work of central and peripheral tissue oscillators during the most acute period was shown in Ref. [19] using the clock gene expression transcriptome obtained by RNA sequencing.

We concluded that the factors such as the volume and nature of the

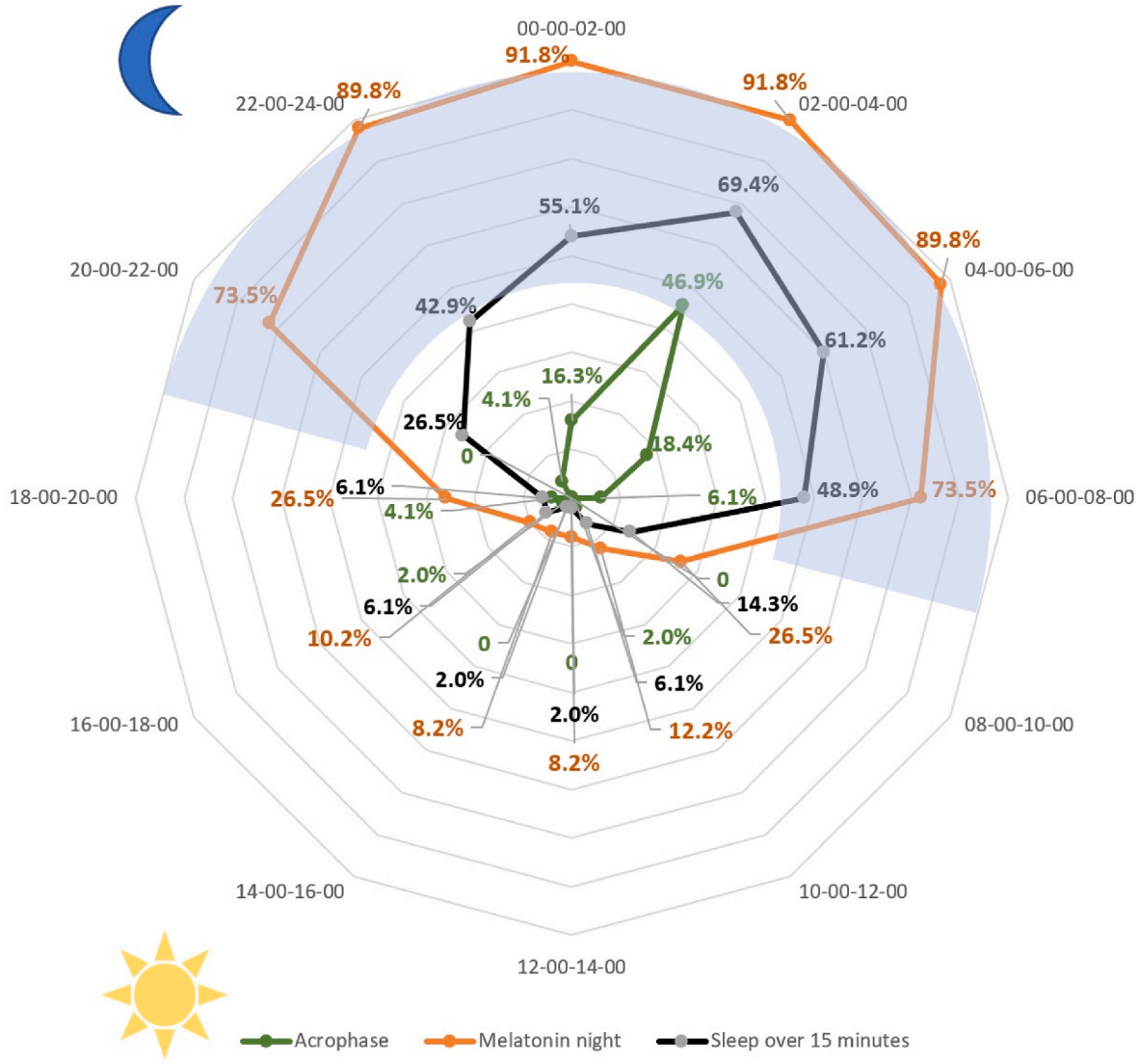

Fig. 4. Radar chart of the number of patients per interval for various criteria.

brain injury have no effect on the secretion of melatonin in chronic critical condition because we did not find statistically significant correlations between the level of consciousness, CRS-R score, the etiology of the disease and the level of melatonin. Prior research of *Maas* et al. demonstrated a significant relation between such factors in the acute phase of critical illness [7,19,20]. It is possible that the stabilization of the somatic status of patients leads to and accompanied by the restoration of the circadian rhythm of melatonin secretion. This is also consistent with the data presented in the literature [21].

Despite low contrast and intensity of light, the central circadian oscillator continues to obey the "daylight-dark" cycle in ICU, which is an artificial reflection of the day-and-night cycle outdoors. It is the intraday artificially created change in the day and night time, which determines the absence of a significant phase shift in our patients. There are also known reports in the literature on the favorable effect of additional contrasting day and night illumination on circadian rhythms [22].

However, the decrease in amplitude of the melatonin secretion may occur because of the absence or low illumination contrast between night and day, expressed in lux. Light intensity in the intensive care unit during the daytime did not exceed 200 lux, which is significantly less than the intensity of light outside during the daytime. These results comply with the other previous research data [7,16,18].

Patients in chronically critical condition have more nighttime sleep than daytime sleep. Patients sleep during the melatonin rise phase, which again illustrates the absence of serious desynchronization between the sleep-wake cycle and the main pacemaker in the suprachiasmatic nucleus (SCN). We found the significant correlation between the duration of the patient's stay in the ICU and the shift in the onset of sleep relative to the onset of melatonin secretion. This correlation suggests that the impact of destructive exogenous factors, such as low light intensity, noises, dietary traits, and others, can over time lead to dissynchronization among circadian rhythms.

We have also pointed out that the cortisol secretion is maximum in the morning, whereas secretion decreases during the evening, which corresponds to the rise in melatonin. This allows us to assume with caution that the synchronization between the central and peripheral oscillators is preserved in our cohort.

The study's limitation and the prospects for further work include the following factors.

1. 24-hour period can artificially limit circadian rhythms which, under light deprivation, can go beyond the specified time frames. However, the presence of a single acrophase in most patients minimizes the potential error and allows for a 24-h study.
2. Insufficient amount of biomaterial sampling points to check the cortisol level may have resulted in poor judgment about the

circadian rhythm. Although we analyzed the level of cortisol in the most representative time intervals for patients in chronic critical condition, however, a study with many biomaterial sampling points is necessary.

3. In our study, the median duration of ICU stay was approximately 2 months. Further, we must carry studies to find the dynamics of circadian rhythm at more distant stages. The purpose of this long-term study may be to plan recommendations to prevent dis-synchronization of circadian rhythm, which may adversely affect the somatic status.

6. Conclusion

We found no gross disturbances in the circadian rhythmicity of melatonin and cortisol in patients in chronic critical illness as a result of a severe acquired brain injury. The severity of dis-synchronization of circadian rhythm between melatonin-cortisol secretion and the day-night cycle is low. We will focus our future work on the study of circadian processes at more distant stages to search for new methods to stabilize and preserve circadian rhythm to protect vital organ functions.

CRediT authorship contribution statement

Mikhail Kanarskii: Conceptualization, Methodology, Writing – original draft. **Julia Nekrasova:** Writing – original draft, Writing – review & editing. **Ekaterina Kondratieva:** Writing – review & editing. **Ilya Borisov:** Formal analysis, Software. **Elena Simenel:** Investigation. **Yurii Sviryaev:** Investigation. **Pranil Pradhan:** Investigation, Visualization. **Kirill Gorshkov:** Investigation. **Alexander Shestopalov:** Investigation. **Marina Petrova:** Supervision, Writing – review & editing.

Declaration of competing interest

We, the authors of this paper, declare that there is no conflict of interest associated with the publication of this paper. We declare that we have no financial or personal relationship with any other organization or individual that could inappropriately influence, or be perceived to influence, our work.

We also declare that all authors contributed significantly to the work and that all authors have approved the final version of the paper. Furthermore, we declare that the paper has not been published elsewhere and is not under consideration for publication in any other journal.

We are aware of the importance of maintaining ethical standards and we will strive to adhere to the guidelines set out by our institution and/or journal.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleepx.2023.100101.

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