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Automatic wavelet-based assessment of behavioral sleep using multichannel electrocorticography in rats

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Abstract

Purpose During the last decade, the reported prevalence of sleep-disordered breathing in adults has been rapidly increasing. Therefore, automatic methods of sleep assessment are of particular interest. In a framework of translational neuroscience, this study introduces a reliable automatic detection system of behavioral sleep in laboratory rats based on the signal recorded at the cortical surface without requiring electromyography.

Methods Experimental data were obtained in 16 adult male WAG/Rij rats at the age of 9 months. Electrocorticographic signals (ECoG) were recorded in freely moving rats during the entire day (22.5 ± 2.2 h). Automatic wavelet-based assessment of behavioral sleep (BS) was proposed. The performance of this wavelet-based method was validated in a group of rats with genetic predisposition to absence epilepsy (n=16) based on visual analysis of their behavior in simultaneously recorded video.

Results The accuracy of automatic sleep detection was 98% over a 24-h period. An automatic BS assessment method can be adjusted for detecting short arousals during sleep (microarousals) with various duration.

Conclusions These findings suggest that automatic wavelet-based assessment of behavioral sleep can be used for assessment of sleep quality. Current analysis indicates a temporal relationship between microarousals, sleep, and epileptic discharges in genetically prone subjects.

Keywords ECoG · Wavelet transform · Automatic detection · Sleep-states · Microarousal

Introduction

Accurate assessment of the physiological state of the nervous system is a challenging task of experimental neurophysiology, especially during long-term monitoring of laboratory animals. Three states of vigilance can be recognized based on physiological parameters: wakefulness, rapid eye movement sleep (REM) (paradoxical or active sleep), and non-REM sleep (passive sleep). The level of vigilance is controlled by neuromodulatory systems which are involved in modulation of breathing [1]. During the last decade, the reported prevalence of sleep-disordered breathing in adults has been rapidly increasing. PubMed indexed 4261 original research articles on "sleep-disordered breathing" between 2010 and 2020. Therefore, automatic methods of sleep assessment have become of particular interest, especially in the framework of translational neuroscience.

Vigilance state can be influenced by experimental conditions [2, 3], by the chronic development of diseases especially in case of progressive development of neurological disorders [4, 5], by normal/pathological aging [6, 7], etc. In contrast to wakefulness, sleep is subjectively perceived as a reduced responsiveness to environmental stimuli. Non-REM sleep characterized by "synchronization" of cortical electrical activity, i.e., by the presence of high-amplitude low-frequency activity in the encephalogram. Identification of exact moments of falling asleep gains a better understanding as shown in various physiological states [8, 9]. Furthermore, automatic detection

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of reduced vigilance or sleepiness can be adapted for devices which control waking level in human operators.

Vice versa, discontinuous sleep, sleep fragmentation, and brief arousals even in healthy subjects often negatively impact health. The nature of arousals during sleep has been reviewed [10], and the relationship between arousals and epilepsy has been thoroughly examined [11–13]. As to the methodology of sleep research, there is a high demand for reliable, precise, and accurate measurement of sleep parameters. A huge number of methods have been developed to detect normal sleep oscillations and pathological patterns in encephalograms, such as spike-wave discharges and sleep spindles (e.g., [14, 15]). At the same time, the problem of automatic recognition of the physiological states in laboratory animals (i.e., sleep, wakefulness) has not been solved yet, and researchers often detect sleep/waking states visually in electroencephalograms using video recordings [16]. Methods of accurate sleep detection rely upon information about muscle activity obtained with electromyography and/or oculomotor activity obtained with oculography [17–19]. The need for synchronous recording of additional signals (video) significantly complicates experiments and increases their cost. In this paper, we introduce a reliable automatic detection system of behavioral sleep in laboratory rats based on the signal recorded at the cortical surface without requiring electromyography. This method has been validated using video-ECoG and it can be adjusted for detecting short arousals during sleep (microarousals). The proposed method can easily be implemented for the automatic detection of decreases in vigilance and it may occupy a free niche in biotechnology. This method can also be adapted for real-time use, for example, based on the CUDA technology [20].

Material and methods

Experimental data were obtained in 16 adult male WAG/Rij rats at the age of 9 months. Experiments were performed at Institute of Higher Nervous Activity and Neurophysiology RAS (Moscow, Russia) and approved by the animal ethics committee of this Institute. All animals were implanted with screw electrodes under chloral hydrate anesthesia (i.p. injections 325 mg/kg, 4% solution in 0.9% NaCl). Electrodes were secured to the skull using stainless steel screws (shaft length = 2.0 mm, head diameter = 2.0 mm, shaft diameter = 0.8 mm) located at the cortical surface over the frontal area (symmetrically in the left and right hemispheres, AP +2; L ±2) and occipital area (right, AP −6; L 3). Reference electrode was places at over the right cerebellum. Coordinates are given in mm relative to the bregma. After the surgery, animals were housed individually under 12:12 h light:dark cycle (light on 8 a.m.) with free access to food and tap water. Recovery period lasted 10–14 days.

Electrocorticographic signals (ECoG) were recorded in freely moving rats placed in Plexiglas cages (25x60x60 cm) under 12:12 h light:dark cycle (light on at 8 a.m.). ECoG were recorded continuously during the entire day (22.5 ± 2.2 h), fed into a multi-channel amplifier (PowerLab 4/35, ADInstruments) via a swivel contact, band-pass filtered between 0.5 and 200 Hz, digitized with 400 samples/second/per channel, and stored in hard disk. In addition to ECoG, rat's behavior was video recorded during 1–2 h using high resolution video camera Genius eFace 1325R. Video-ECoG in all 16 rats was visually inspected to detect behavioral sleep and waking states. Behavioral sleep (BS) was determined when an animal took relaxed sleeping with closed or semi-closed eyes, and these periods were accompanied by ECoG synchronization in all channels. Waking state (AW) included passive and active wakefulness when an animal was in standing position, moved around the cage, or was immobile. The period of AW was accompanied by theta in occipital ECoG and desynchronization.

Majority of WAG/Rij rats (13 out of 16 subjects) showed typical for absence epilepsy spike-wave discharges in ECoG. SWDs occurred spontaneously during the state of passive wakefulness and sleep, and were not associated with changes in behavior (typical "absence" state). SWD during passive wakefulness were associated with an increased breathing rate [21]. Accelerated breathing rate during SWD might be used as an additional behavioral hallmark of absence epilepsy and relates to physiological changes during absence seizures. Immediately after SWD, an animal often flinched or scratched. In the present study, we used previously described method for SWD detection [22–24]. Briefly, increase of wavelet power measured two narrow frequency bands, 8–10 and 17–20 Hz. SWDs were automatically detected during the entire ECoG record (22.5 ± 2.2 h) in all 16 rats. The outcomes of the automatic detection were visually corrected so the accuracy of SWD detection reached 100%. Figure 1 displays an example of 3-channel ECoG with typical SWDs.

Results

Results of the manual detection of BS in 1–2 h video-ECoG in all animals were used to define criteria for the automatic system of sleep detection. They were also used to access the quality of the developed method. Below we describe the automatic detection method.

M registered ECoG signals were denoted as $x_1,\ldots,x_M$, the duration of ECoG signals as T, and the total number of samples in each ECoG signal is 512 T. A continuous wavelet transform (CWT) $W_i(f,t)$ was calculated for each ECoG signal x_i based on the Morlet wavelet with the parameter $\Omega_0 = 2\pi$. With $\Omega_0 = 2\pi$, time scale in CWT approximated to the classical representation of Fourier frequency f, Hz, [25, 26]. For automatic detection of BS, it was empirically found that the maximum quality and detection speed were achieved when the signal was set to the frequency range Δf = [5;10] Hz. For

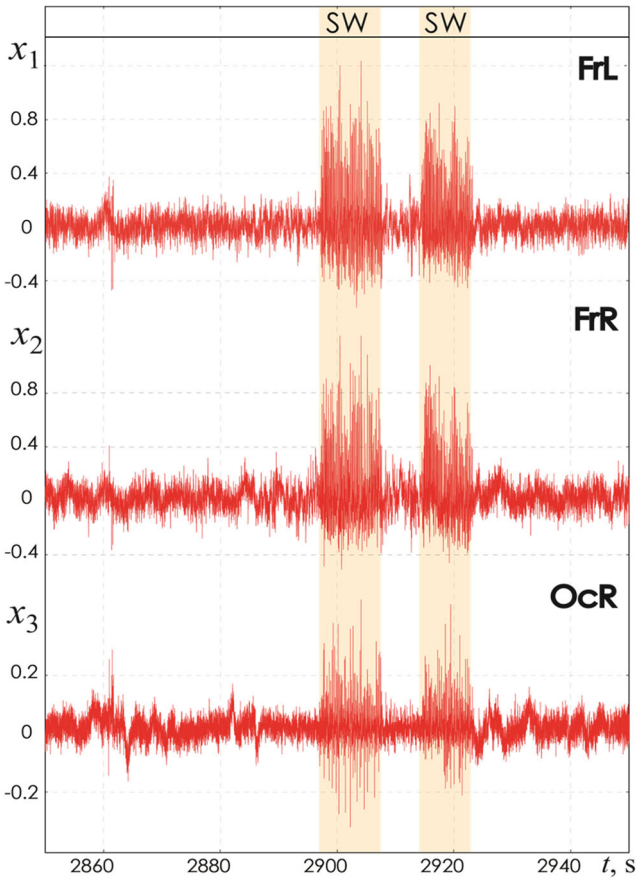

Fig. 1 Typical spike-wave discharges (SW, orange) detected in 3-channel ECoG record in WAG/Rij rat#1. ECoG tracks are abbreviated as FrL (frontal left), FrR (frontal right), and OcR (occipital right)

each ECoG channel, we computed instantaneous CWT energy $E_i(f,t)$ as:

$$E_i(f,t) = W_i(f,t)^2. \quad (1)$$

The total instantaneous CWT energy $E_{\Delta f}(t)$ was calculated at each time point t in frequency interval Δf as:

$$E^i_{\Delta f}(t) = \sum_{f \in \Delta f} E_i(f,t). \quad (2)$$

Note that calculation of the instantaneous energy was limited to the frequency range Δf; therefore, the number of operations and machine time required for the analysis of experimental data was substantially reduced. The integral value of the total energy $E_{\Delta f}(t)$ (2) takes the form:

$$\varepsilon^i(t_0) = N \cdot \Delta t \cdot \sum_{t_1}^{t_2} E^i_{\Delta f}(t), \quad (3)$$

where t_0 is current time moment, $\Delta t = 0.5$ s, $t_1 = t_0 - 0.5 \cdot \Delta t$, and $t_2 = t_0 + 0.5 \cdot \Delta t$. For all M frontal ECoG-channels, we assessed the multichannel energy characteristic $\widetilde{\varepsilon}_{\Delta f4}(t_0)$ as:

$$\widetilde{\varepsilon}(t_0) = \frac{\sum_{i=1}^{M} \varepsilon^i_{\Delta f}(t_0)}{M}. \quad (4)$$

Detection of BS was carried out on the basis of dependence (4) analysis. Some principles of this analysis are shown in Fig. 2. In particular, we considered two threshold values $T\uparrow$ and $T\downarrow$ in the form as:

$$T\uparrow = 1.3 \cdot N \cdot T \cdot \sum_0^T \widetilde{\varepsilon}_{\Delta f}(t_0), \quad (5)$$

$$T\downarrow = 0.45 \cdot N \cdot T \cdot \sum_0^T \widetilde{\varepsilon}_{\Delta f}(t_0). \quad (6)$$

The thresholds $T\uparrow$ (5) and $T\downarrow$ (6) are individual characteristics of ECoG activity assessed in each animal. Note substantial variation of threshold values across 16 rats as shown in Table 1.

Next, the dependence (4) and found time moments t_1, in which the value of $\widetilde{\varepsilon}(t)$ exceeds the threshold $T\uparrow$ (5), i.e. $\widetilde{\varepsilon}(t_1) \geq T\uparrow$ were analyzed. Next, we went back to the time $t \uparrow \leq t_1$, for which the value of $\widetilde{\varepsilon}(t_0)$ exceeded the threshold $T\downarrow$ (6), i. e. $\widetilde{\varepsilon}(t\uparrow) \geq T\downarrow$). We considered this time moment $t\uparrow \leq t_1$ as the moment when the animal fell to sleep, i. e., beginning of BS.

Next, in order to detect the awakening moment, we compared the current value of the dependence (4) with the threshold value $T\downarrow$ (6). The time moment $t\downarrow$ was considered the beginning of wakefulness stage if $\widetilde{\varepsilon}(t_1)$ has become less than $T\uparrow$. However, states with duration less than 3 s were considered incorrectly defined and were excluded (Fig. 2).

Results of manual (Fig. 2a) and automatic (Fig. 2b) detection of sleep and waking states were matched closely. The

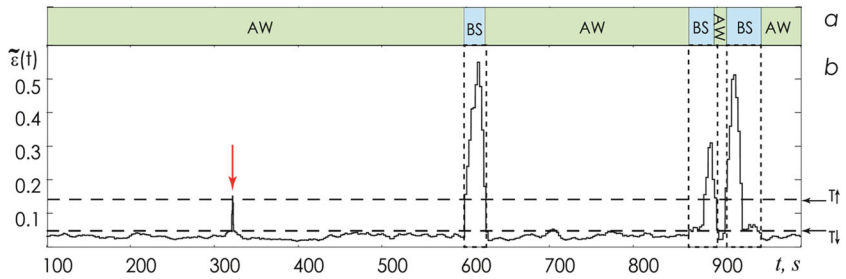

Fig. 2 The results of manual and automatic detection of sleep-wake stages: (**a**) a fragment of manual detection done by neurophysiologist using video-ECoG in rat #4; (**b**) the corresponding fragment of timing dependence $\widetilde{\varepsilon}(t)$ computed in ECoG signals $x_1(t)$ and $x_2(t)$. The colored areas show manually detected states of sleep (BS, blue) and wakefulness (AW, green). The horizontal thick dashed lines mark the threshold values $T\uparrow$ and $T\downarrow$, and the dashed vertical lines show the results of automatic recognition of the same states. Red arrowhead marks the artifact in which the energy characteristic $\widetilde{\varepsilon}(t)$ reaches the threshold for less than 5 s.

Table 1 The four thresholds value in individual rats.

#	$T\uparrow$	$T\downarrow$	#	$T\uparrow$	$T\downarrow$	#	$T\uparrow$	$T\downarrow$	#	$T\uparrow$	$T\downarrow$
1	0.18	0.06	5	0.25	0.09	9	0.23	0.08	13	0.13	0.05
2	0.21	0.07	6	0.17	0.06	10	0.22	0.08	14	0.13	0.05
3	0.20	0.07	7	0.32	0.11	11	0.25	0.09	15	0.23	0.08
4	0.15	0.05	8	0.19	0.07	12	0.17	0.06	16	0.17	0.06

statistical accuracy of the presented method was evaluated by measuring the quality of automatic detection (see below). The arrow in Fig. 2b points to the brief increase in characteristic energy $\widetilde{\varepsilon}(t_0)$ (4), but was not detected as sleep because of short duration. An advantage of the automatic detection method is its high sensitivity. This method was particularly useful to identify short microarousal states interrupting continuous sleep and to assess sleep fragmentation. This issue is illustrated in Fig. 3.

For the entire duration of ECoG, the relative errors in detection of the start (δ_s) and end (δ_e) moments were estimated in each BS episode. Then, the quality of automatic detection Q↕ was calculated as:

Table 2 Values of the relative errors δ_s, δ_e, and the quality Q↕ of automatic as compared to manual detection in all rats (# denotes ID number)

#	δ_s, %	δ_e, %	Q↕, %	#	δ_s, %	δ_e, %	Q↕, %
1	96.53	94.7	95.62	9	83.46	90.33	86.9
2	94.55	97.29	95.92	10	92.32	93.84	93.08
3	88.41	96.12	92.27	11	94.94	96.31	95.63
4	90.35	86.22	88.29	12	99.23	97.34	98.29
5	86.75	89.43	88.09	13	82.52	87.36	84.94
6	83.88	91.39	87.64	14	87.15	90.14	88.65
7	99.09	91.59	95.34	15	92.38	89.65	91.02
8	76.13	81.85	78.99	16	91.97	95.98	93.98

Group medians for relative errors δ_s, δ_e, and quality Q↕ are 91.16, 91.49, and 91.64, respectively

$$Q\updownarrow = \sum_{i=1}^{S} \frac{(\delta_s + \delta_e)_i}{2}. \quad (7)$$

Table 2 demonstrates the results of quantitative assessment of the quality Q↕ of automatic detection compared to the manual detection in all animals.

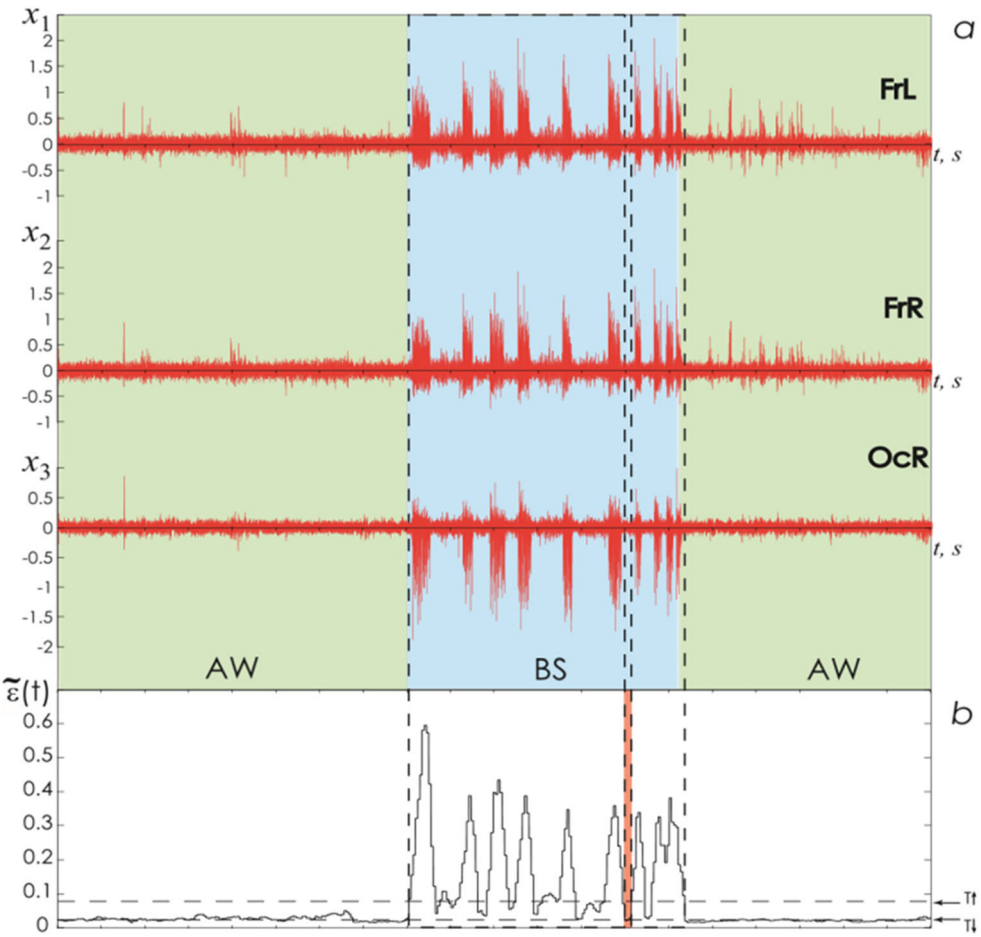

Fig. 3 Detection of the state of microarousal. (**a**) Three-channel ECoG recorded at the frontal left cortex (FrL), frontal right cortex (FrR) and occipital right cortex (OcR) in rat #7. Behavioral sleep (BS) and waking state (AW) were manually assigned using video recorded behavior. (**b**) A corresponding fragment of timing dependence $\widetilde{\varepsilon}(t_0)$ calculated from ECoG signals $x_1(t)$ and $x_2(t)$. The colored areas show manually detected sleep (blue), wakefulness (green), and microarousal (red). The horizontal thick dashed lines mark the threshold values $T\uparrow$ and $T\downarrow$, and the dashed vertical lines show the results of automatic diagnostics of the state of sleep and wakefulness.

Special attention was paid to brief episodes of awaking during sleep, so-called microarousals. Microarousals were referred to as "phasic EEG events which were not associated with awakenings regardless of their desynchronizational or synchronizational (sleep response-like) morphology and regardless of their connection with autonomic or some sort of behavioral arousal" [10]. In accordance to the criteria developed by the Sleep Disorders Atlas Task Force of the American Academy of Sleep Medicine [27], we detected microarousals as 3–15 s waking state preceded by at least 10 s of non-interrupted sleep. Wakening episodes longer than 15 s were recognized as wakefulness (Fig. 3a and b).

Noteworthy is that vigilance state in WAG/Rij rats is affected by the occurrence of spontaneous spike-wave discharges (two examples of SWD are shown in Fig. 1). The number of SWD in 16 subjects varied from the maximum of 302 SWDs in rat #1 to single SWD in rats #11–13 and no SWD in rats #14–16. Figure 4a displays the number of SWDs (N_{sw}) and microarousals (N_{ma}) in all 16 rats as detected in 24h ECoG. The number of microarousals in non-epileptic rats #11-16 was in average 62.0±21.7 (mean±s.d.) demonstrated a strong tendency to be lower than in epileptic rats # 1–10 (45.2±17.5, p=0.056, Mann-Whitney U test).

In order to examine distribution of sleep episodes, microarousals, and SWD over 24-h time interval, we performed additional analysis. Duration of sleep was measured in each hour, Δh, as well as the number of sleep episodes, N_S; relative duration of sleep per min was computed as $T = \sum_{S \in \Delta h} T_s/60$. The relative number of microarousals and SWDs were computed as:

$$\langle N_{ma} \rangle = \frac{N_{ma}}{T \cdot 60}, \quad \langle N_{sw} \rangle = \frac{N_{sw}}{60}. \tag{8}$$

Figure 4b–d demonstrates the results of 24-h dynamics of SWDs, microarousals, and sleep episodes in three epileptic rats (#1, #3, and #5). These data indicate that, first, the number of SWD, N_{sw}, was higher during the 1st half of light period that fits well to the literature [29–31], suggesting the presence of circadian of absence seizures in WAG/Rij rats. Second, distribution of sleep episodes, N_S, and microarousals, N_{ma}, over 24-h period varied across three rats, therefore, circadian dynamics of sleep and microarousals was not obvious. This contradicts to the classical studies in rats indicating that slow-wave sleep readily occurred at the beginning of the light-phase and then exhibited a decreasing trend [31]. This discrepancy might be caused by differences in methodology. Here we used ECoG synchronization as an objective marker of sleep state and sometimes included the state of drowsiness (if it meets the inclusion criteria). No earlier studies have examined the temporal relationship between microarousals, sleep, and SWD during 24-h period, and we will study this relationship in the future. In addition, we expect age-related changes in the system sleep-microarousals-SWD that will also be examined.

Discussion

Our paper addressed methodological issues, which were crucially important for the automatic analysis of multi-channel EEG data. First, we proposed wavelet-based method of automatic sleep recognition in rats. We found that an increased wavelet power in 5–10 Hz frequency band measured in frontal and occipital cortical areas could be used as reliable marker of behavioral sleep. An increase of 5–10 Hz rhythmic brain activity simultaneously in three ECoG channels seems to be a reliable biomarker of behavioral sleep in rats. This can be accounted for neuronal synchronization and the presence of

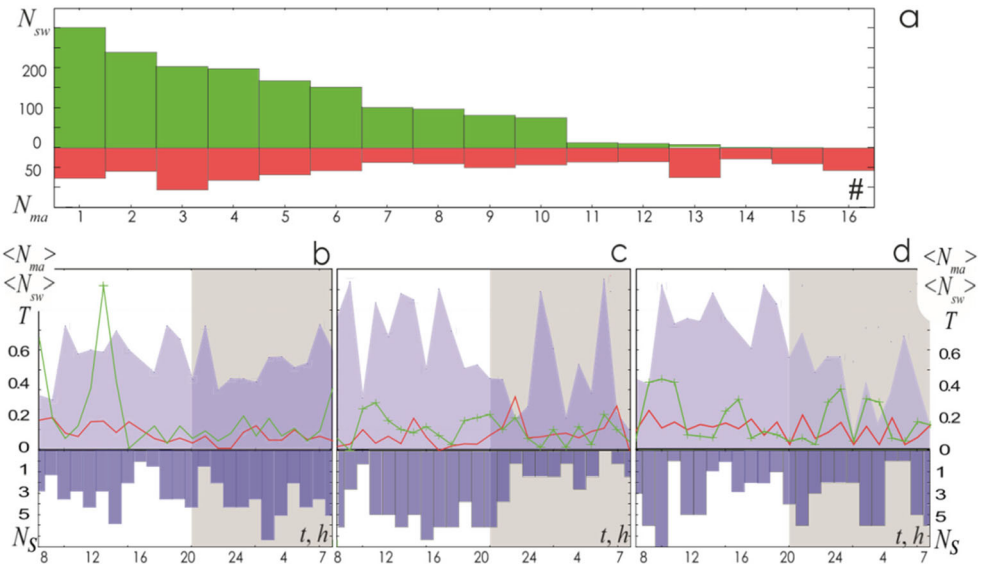

Fig. 4 Individual statistics of spike-wave discharges and microarousals automatically detected in WAG/Rij rats. (**a**) The number of spike-wave discharges N_{sw} (green scale) and microarousals N_{ma} (red scale) detected in 24-h ECoG record in 16 rats 16 rats (# denotes rat's ID). The bottom plots show distribution of the number of spike-wave discharges N_{sw} (green line) and microarousals N_{ma} (red line) over the time in 24h ECoG with the dark period highlighted in gray: (**b**) rat #1 (N_{sw} = 302), (**c**) rat #3 (N_{sw} = 168), (**d**) rat #5 (N_{sw} = 152). The total number of sleep stages, N_S, is shown on the bottom panel

so-called mid-frequency oscillations such as sleep spindles and 5–9 Hz oscillations detected in WAG/Rij rats [32, 33]. Further analysis in non-epileptic rats is needed to evaluate an increase of 5–10 Hz rhythmic brain activity as a hallmark of behavioral sleep.

Second, we detected microarousals in ECoG as 3–15 s periods of wakening during sleep using criteria which satisfy the rules of the American Academy of Sleep Medicine [27]. We found that microarousals interrupted continuous sleep in all individuals, and they were missed by visual inspection, but were accurately detected using the proposed method. Our analysis was done in rats with genetic predisposition to absence epilepsy (WAG/Rij), in which epileptic activity varied from 0 (non-epileptic rats) to 302 seizures per 24 h. Statistical results demonstrated that the number of microarousals in non-epileptic rats showed a strong tendency to be lower than in epileptic rats; therefore, absence epilepsy seems to promote microarousals. Sleep microstructure in WAG/Rij rats is known to differ from that in healthy rats. In as much as SWDs predominantly occur during drowsiness and light slow-wave sleep, during transition from wakefulness to sleep [21], slow-wave sleep in WAG/Rij rats was often interrupted by the occurrence of SWDs. In addition, WAG/Rij rats show longer intermediate sleep state [28].

The nature of microarousals in epileptic patients has been studied by prof. Peter Halász and his coauthors [10–13] suggesting antagonistic relationship between sleep promoting system and the arousal-promoting systems. Standard sleep scoring systems identify states (i.e., wake, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep), but not transient event (i.e., microarousals). However, reliable identification of transient microarousals might be useful for both clinical and theoretical purposes as it was stressed by [27]. Statistical analysis of microarousals may expand our understanding of the nature of sleep and its disturbances. The interest to the microarousal state has recently been increased, and studies of microarousals were done in patients with epilepsy [12, 34], apnea [35], primary insomnia [36], chronic pain syndromes such as migraine [37, 38], and cardiovascular vascular diseases [39].

One of the key problems in automatic detecting microarousals - assessment of the moments of falling asleep. A number of studies have considered the possibility of automatic diagnostics of micro-awakenings in patients based on the analysis of EEG together with ECG and/or EMG data [40–42]. The proposed method has at least two advantages. First, it is rather simple and requires solely electrocorticographic data. Second, it implements a fully automatic signal processing cycle by taken into account individual characteristics of EEG dynamics such as the threshold values $T\uparrow$ (5) and $T\downarrow$ (5). The limitation of the proposed method relates to its restricted application to WAG/Rij rats. Further development of the proposed method in rodent models and in human patients may facilitate automatic analysis of sleep and better understand phenomenology of arousals during sleep in physiologic and pathologic conditions.

Conclusion

Our study is the first to propose an automatic wavelet-based method of sleep recognition in rats that relies upon multichannel electrocorticographic data. We defined a reliable marker of behavioral sleep—an increased wavelet power in 5–10 Hz frequency band as measured in frontal and occipital cortical areas. Second, we introduced an approach for the automatic detection of microarousals using criteria which satisfy the rules of the American Academy of Sleep Medicine [27]. Transient interruptions, such as microarousals during sleep, are often overlooked, because they could not be recognized by standard sleep stage scoring systems. According to our results, the number of microarousals in non-epileptic rats showed a strong tendency to be lower than in epileptic rats; therefore, absence epilepsy seems to promote microarousals.

Epilepsy could successfully be modeled in vivo, more specifically, in genetic rat models. The proposed method was developed in WAG/Rij rats, and it could be directly applied to other rodent models. With regard to differences between rat and human brains, we hope that the proposed approach can be adjusted to the analysis of humans EEG data.

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Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare no competing interests.

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