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Rhythmic activity in EEG and sleep in rats with absence epilepsy

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ABSTRACT

This study examines the hypothesis that absence epilepsy is accompanied by disturbances of rhythmic activity in EEG during sleep. Sleep–wake architecture and time-frequency parameters of EEG were analyzed during drowsiness and sleep in WAG/Rij rats with genetic predisposition to absence epilepsy. The incidence of seizures varied in a group of 10 rats, in which 5 individuals did not develop epileptic discharges in their EEG (asymptomatic rats). In contrast to asymptomatic, symptomatic subjects (1) displayed less percentage of wakefulness EEG pattern and more non-REM sleep, (2) showed higher beta and less delta EEG power in frontal cortex during non-REM sleep. Mid-frequency oscillations, such as sleep spindles and 5–9 Hz oscillations, were detected in EEG automatically and underwent time-frequency analysis by means of skeletons of wavelet surfaces. Some mid-frequency oscillations showed "complex" frequency structure, consisting of the dominant and subdominant components. "Complex" sleep spindles more frequently appeared in asymptomatic rats than in symptomatic, whereas the dominant frequency of these spindles in symptomatic rats was higher than in asymptomatic (12.7 vs 11.9 Hz). In general, low-frequency components were readily integrated in sleep spindles in asymptomatic WAG/Rij rats, and decrease in number of "complex" sleep spindles may be associated with epileptic phenotype.

1. Introduction

The relationship between mechanisms of sleep and absence epilepsy is well recognized (Dinner and Lüders, 2001; Steriade, 2003). In human patients, absence epilepsy usually appears during somnolence and in transition periods between wakefulness and sleep. It manifests as brief episodes of unresponsiveness, loss of consciousness and confusion that can easily be mistaken for daydreaming (Halász et al., 2002; Kellaway 1985). High voltage spike-wave discharges (SWD) are the electroencephalographic hallmark of absence epilepsy in human patients and in genetic animal models, e.g., WAG/Rij rats and GAERS (Genetic Rats with Absence Epilepsy) (Coenen and Van Luijtelaar, 2003; Marescaux et al., 1992; Panayiotopoulos, 2005).

In WAG/Rij rats and GAERS, absence seizures appear spontaneously during passive wakefulness, drowsiness and light slow-wave sleep (Coenen and Van Luijtelaar, 2003; Drinkenburg et al., 1991; Lannes et al., 1988). The sleep architecture in WAG/Rij rats is known to be altered in comparison to non-epileptic rat strains; the percentage of REM sleep is lower, and the intermediate stage of sleep is longer (Gandolfo et al., 1990; van Luijtelaar and Bikbaev, 2007). *"The sleep cycle and the non-REM sleep duration is substantially shortened in genetic epileptic rats by the presence of SWD but only during periods when light sleep dominates, not during deep non-REM sleep"* (van Luijtelaar and Bikbaev, 2007; p. 264). Considering all these facts, we studied sleep–wake architecture in a group of WAG/Rij rats and focused specifically in the intermediate stage of sleep.

"*Absence epilepsy may have a common substrate with non-REM sleep*" (Steriade, 2003). Indeed, SWD and physiologic sleep spindles (EEG hallmarks of non-REM sleep in humans and animals) are generated by the same thalamo-cortical circuitry (De Gennaro and Ferrara, 2003; Kostopoulos, 2000; Steriade and Amzica, 2003). It is known that the number and duration of absence seizures in WAG/Rij rats are increased with age (Coenen and Van Luijtelaar, 1987, 2003), as well as the number of anterior sleep spindles (van Luijtelaar and Bikbaev, 2007). We found that age-related increase of absence epilepsy in WAG/Rij rats associated with changes in distri-

Abbreviations: GAERS, genetic rats with absence epilepsy; IS of sleep, intermediate stage of sleep; SWD, spike-wave discharges; WAG/Rij rats, wistar albino glaxo rats from rijswijk (genetic model of absence epilepsy).

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bution of seizure activity across wake-sleep cycle (Sitnikova, 2011) and with changes in intra-spindle frequency dynamics (Sitnikova et al., 2014a). Our experiments in WAG/Rij rats indicated that despite genetic predisposition to absence epilepsy, some subjects develop very few seizures or did not develop seizures at all (so-called 'asymptomatic' phenotype) (Sitnikova et al., 2012a, 2014c). The environmental factors during the first weeks of life are known to influence the incidence of genetically predetermined absence epilepsy (Blumenfeld et al., 2008). For instance, early-life manipulations in WAG/Rij rats, such as removal of vibrissae (Sitnikova, 2011) or variation in maternal care (Schridde et al., 2006; Sitnikova et al., 2015) significantly affected development of absence seizures in adulthood. Considering substantial variability in the incidence of absence epilepsy in WAG/Rij rats, we investigate particularities of EEG in WAG/Rij rats with more and less severe seizures.

Similar to that in human patients, there is a strong individual variation in duration and intensity of seizure activity in WAG/Rij rats, and severity of absence seizures correlated with morphological changes in the nigrostriatal dopamine system (Sitnikova et al., 2012a,b). There is a growing body of evidences indicating that absence epilepsy is accompanied by disturbances of sleep and rhythmic activity in cerebral cortex (reviewed by Halász, 2013). For a better understanding of this issue, here we study time-frequency parameters of sleep EEG in WAG/Rij rats with different intensity of seizure activity.

It has been acknowledged that absence seizures may result from impairment of wake-related processes (Halász and Kelemen, 2009; Pinault and O'Brien, 2005). It is known that SWD in GAERS are preceded by wake-related 5–9 Hz oscillations, which are clearly distinguished from sleep spindles and triggered by corticothalamic neurons (Pinault et al., 2003, 2006). Noteworthy is that non-epileptic rat strains also exhibit spontaneous 5–9 Hz oscillations, which are never followed by SWD (Pinault et al., 2001). Therefore, epileptic rats develop two kinds of 5–9 Hz oscillations: physiological oscillations during interictal periods and absence-related oscillations that appear immediately prior to SWD. We examined the assumption that time-frequency features of physiological 5–9 Hz oscillations during interictal periods (non-REM sleep) correlate with the intensity of absence epilepsy.

2. Material and methods

2.1. Animals and EEG recording procedure

Experiments were performed in 10 adult male WAG/Rij rats at Institute of Higher Nervous Activity and Neurophysiology RAS (IHNA, Moscow, Russia) in accordance with the EU Directive 2010/63/EU for animal experiments and approved by our institution's animal ethics committee. Animals were born and raised at IHNA. Prior to surgery rats were housed in small groups with free access to food and water and were kept at natural lighting conditions. After surgery rats were housed individually. Distress and suffering of animals were minimal.

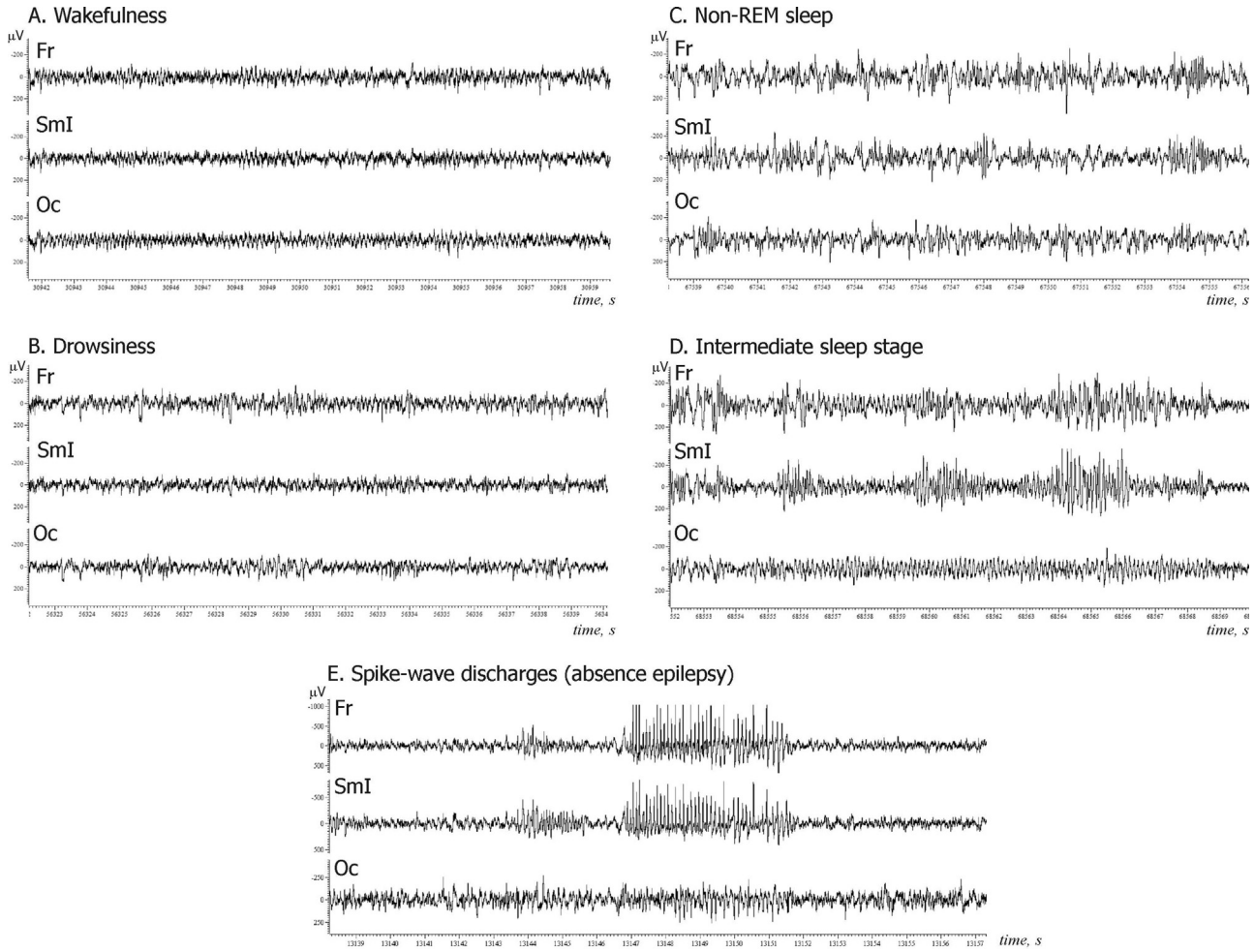

Fig. 1. Examples of multi-channel EEG recordings in a symptomatic WAG/Rij rat during different stages of vigilance (A–D) and SWD (E, note EEG amplitude difference). Fr—frontal cortex, SmI—somatosensory cortex (parietal area), Oc—occipital cortex.

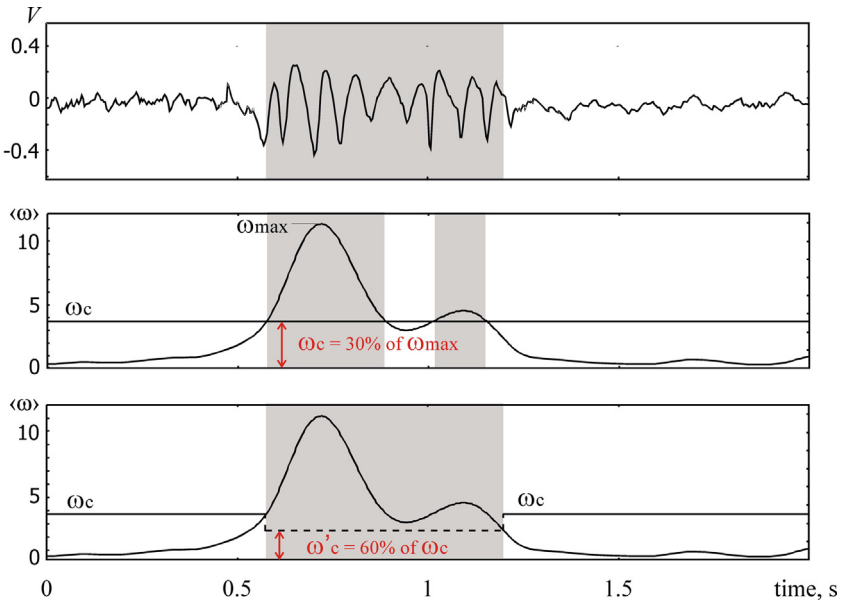

Fig. 2. Application of adjustable levels for detecting mid-frequency oscillations (here, sleep spindle) in EEG. <ω>—time-dependent distribution of wavelet energy as measured in two bands: 5–9 Hz and 10–15 Hz, ωc—the high threshold and ω'c—the low threshold of detection.

At the age of 8 months animals were equipped with screw electrodes for EEG recording. Stereotactic surgery was performed under chloralhydrate 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). Active electrodes were placed epidurally in the frontal cortex (AP 2; L 2.5), parietal area (somatosensory cortex, AP -2; L 6) and occipital cortex (AP -5; L 4). All coordinates are given in millimeter relative to the bregma. EEG recordings were monopolar with respect to reference electrode over the cerebellum. Electrodes were permanently fixed to the skull with methyl methacrylate monomer together with two additional anchoring screws. After the surgery, animals received *i.m.* injections of metamizol (FSSCI Microgen, Russia, 25 mg/kg) for pain relief, and then housed in individual cages in order to prevent damages of electrode connectors.

After the surgery, rats were allowed to recover minimum ten days before EEG recording session started (at the age of 9 months old). EEG signals were recorded in freely moving rats continuously during a period of 24 h and started between 4 and 5 p.m., fed into a multi-channel amplifier (PowerLab 4/35, ADInstruments) via a swivel contact, band-pass filtered between 0.5–200 Hz, digitized with 400 samples/second/per channel and stored in hard disk.

Time-frequency EEG analysis was performed in 1 h episodes as recorded from 8 to 9 p.m., i.e., during the first half of light–dark period. Stages of sleep–wake cycle were visually identified in EEG in accordance with 'R & K' rules (Rechtschaffen and Kales, 1968) and descriptive criteria for rat EEG (Gottesman, 1992; Kirov and Moyanova, 2002; Sitnikova, 2010). Waking stage (Fig. 1A) was determined by desynchronized EEG with occasional movement artifacts and sustained theta activity in occipital channel (Fig. 1A). The stage of drowsiness was characterized by EEG synchronization in frontal and parietal channels, when occipital theta disappeared (Fig. 1B). The stage of non-REM sleep was determined by the presence of slow waves and sleep spindles without occipital theta (Fig. 1C). During the intermediate stage (IS) of sleep, high-voltage spindle activity appeared in the frontal and parietal channels simultaneously with high-voltage theta in occipital channel (Fig. 1D) (Gottesmann, 1996; Sitnikova, 2010). EEG was visually examined for the presence of epileptic spike-wave discharges (SWD). SWD appeared as highly synchronous sequence of spikes and waves with frequency of 7–10 Hz and minimal duration of 1 s (Fig. 1E) (van Luijtelaar and Coenen, 1986). The number and duration of SWD were computed in the period of 1 h (8–9 p.m.) and statistically analyzed.

2.2. Continuous wavelet analysis of EEG signals and automatic recognition of sleep spindles and 5–9 Hz oscillations

Analysis was performed in EEG during drowsiness, non-REM sleep and IS of sleep. During each state, 5 representative epochs were selected in different sleep cycles, each epoch lasted 20–40 s; in total, 120–150 s per rat/per state were analyzed. The continuous wavelet transform was used for time-frequency EEG analysis and automatic recognition of sleep spindles and 5–9 Hz oscillations as described earlier (Hramov et al., 2015; Sitnikova et al., 2012a,b, 2014a,b). Complex Morlet wavelet was used as basis function in continuous wavelet transform, $W(t, f_s)$, and time scales (s) of wavelet transform were converted into Fourier frequencies, f. The resulting wavelet spectrum, i.e., wavelet energy distribution over frequency, $E(f_S)$ (scalogram, similar to Fourier power spectrum), was computed as

$$E(f_S) = \frac{1}{T}\int_0^T |W(t, f_S)|^2 \mathrm{d}t.$$

Wavelet spectra were statistically analyzed in frontal and parietal channels using t-test and GLM ANOVA.

Time-frequency EEG structure of sleep spindles and 5–9 Hz oscillations was similar, since both of them had spindle-like envelope (Fig. 2), therefore, we introduced unbiased criteria in the automatic system for selective detecting of both types of mid-frequency oscillations in frontal EEG based on the previously developed algorithm (Sitnikova et al., 2012a,b; 2014a,b). Briefly, the value of wavelet energy using the continuous wavelet transform, $w(\tau)$, was computed in the characteristic frequency bands, F_s, which were chosen empirically: $F_S \in$ 5–9 and 10–15 Hz.

$$w(\tau) = \int_{Fs} |W_{s,\tau}| \mathrm{d}s$$

The value of $w(\tau)$ was compared with the threshold level w_c, and the oscillatory EEG pattern was automatically recognized under condition that $w(\tau) > w_{cr}$. The borders of sleep spindles and 5–9 Hz oscillations were defined and distinguished from adjacent slow waves or slow waves patterns (K complexes) based on two adjustable threshold levels of wavelet power in 5–9 Hz and 10–15 Hz.

The threshold values of w_c were determined in each rat individually based on the distribution of wavelet energy. Time-frequency dynamics of both oscillations was complex, and wavelet energy often exceeded the threshold level several times during one oscillation, and this led to multiple short-lasting detections in a single oscillatory pattern. In order to solve this problem, we applied two adjustable thresholds: high and low. The high threshold level, w_c, was set at 30% of the maximum value of wavelet energy of visually detected sleep spindles and 5–9 Hz oscillations, and the low level, w'_c, was set at 60% of the higher level (Fig. 2). With the low threshold level, w'_c, wavelet energy exceeded the threshold level only ones—at the end of detected oscillation. When the end of oscillatory pattern has been detected, the threshold value was returned to the high value and detection continued. Average threshold values for detecting sleep spindles were: high (w_c) 4.1 and low (w'_c) 2.4; and for detecting 5–9 Hz oscillations were: high (w_c) 4 and low (w'_c) 2.4.

The quality of the wavelet-based detection method was evaluated by computing the percentage of true positive/negative detections, false positive/negative, sensitivity and specificity using 1 h EEG epochs in which sleep spindles and 5–9 Hz oscillations were detected visually beforehand. The true positive (TP) was computed as percentage of correct detections of sleep spindles (or 5–9 Hz oscillations). The true negative (TN) – the percentage of correct rejections of sleep spindles (or 5–9 Hz oscillations). False positive (FP) represented the percentage of incorrect automatic detections of sleep spindles (or 5–9 Hz oscillations). False negative (FN) – percentage of events missed by the automatic wavelet-based method.

The accuracy of automatic recognition was computed as

$$\rho = \left(\frac{\mathrm{TP}}{\mathrm{Ne}}\right) \times 100\%$$

where TP is the number of true positive detections and Ne is the number of expert selections. The sensitivity and specificity were assessed using the formulae:

$$\text{Sensitivity} = \frac{\mathrm{TP}}{(\mathrm{TP}+\mathrm{FN})} \times 100\%$$

$$\text{Specificity} = \frac{\mathrm{TN}}{(\mathrm{TN}+\mathrm{FP})} \times 100\%$$

It appeared that the most sleep spindles and 5–9 Hz oscillations in all animals were identified correctly by having high wavelet power in the two frequency bands: 5–9 Hz and 10–15 Hz. Table 1 shows statistical results of the accuracy, selectivity and specificity that were sufficiently high (83–95 %). Incorrect detections (false positive, FP or error type 1) were at the level of ~10%. The percentage of correct rejections of oscillatory patterns (sleep spindles or 5–9 Hz oscillations) was also high (~95%). All this suggests that sleep spindles and 5–9 Hz oscillations were reliably distinguished from non-epileptic background EEG and from each other.

2.3. Instantaneous frequency analysis: skeletons of wavelet surfaces, dominant and subdominant frequency components

Skeletons of wavelet surfaces were analyzed in order to determine the principle frequency components in sleep spindles and 5–9 Hz oscillations that were automatically determined in EEG Skeletons were constructed in 4 s time intervals in frontal EEG (Fig. 3). The instantaneous wavelet energy, $E_i(f_S, t_0) = |W(f_S, t_0)|^2$, was calculated for each time moment t_0, and distribution of wavelet energy E_i was examined for the presence of local maxima. Usually this function had several maxima, and the highest one corresponded to the dominant EEG frequency at time moment t_0, and all others—to the subdominant frequencies. The presence of noise and other artifacts in raw EEG resulted to numerous local maxima in E_i, therefore, an additional condition was applied in order to minimize the number of false definitions: each local maximum must exceed the value of instantaneous wavelet energy E_i into the predetermined frequency range (empirically chosen equal to 1 Hz).

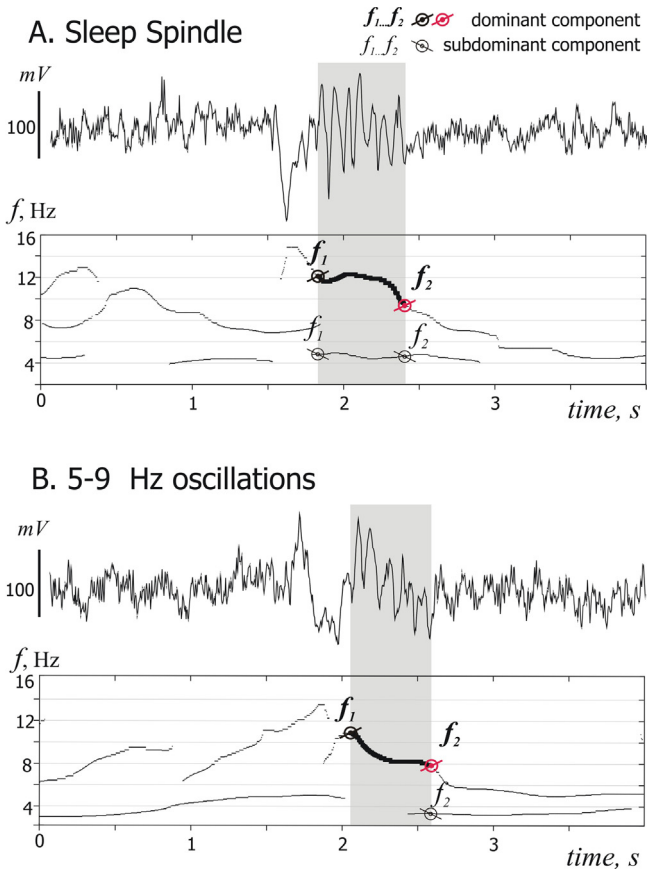

Fig. 3. Examples of a sleep spindle (A) and 5–9 Hz oscillations (B) as recorded in frontal EEG in a symptomatic WAG/Rij rat and their wavelet-based 'skeletons'. During each event, the highest extreme values of amplitude were recognized as the dominant frequency component (thick line) and their amplitude exceeded the detection threshold for automatic recognition of EEG events (see (Sitnikova et al., 2012a,b) for details); the second extreme values in wavelet skeleton represented subdominant low-frequency component. Frequencies of dominant and subdominant components were measured at the beginning (f_1) and at the end (f_2) of automatically recognized events.

In wavelet skeletons, the most powerful component was referred to as dominant frequency and the less powerful component—as subdominant (Fig. 3). Information about the dominant frequency component was employed in wavelet-based algorithm for automatic detection (Sitnikova et al., 2012a,b; Hramov et al., 2015); therefore, the mean frequency and the length of EEG oscillations were almost equal to that in the dominant component. The frequency of dominant and the largest subdominant were measured at the beginning and at the end of each EEG event (f_1 and f_2 correspondingly). The mean frequency, f_{mean}, was computed as: $f_{mean} = \frac{1}{h}\int_{t_1}^{t_1+h} f_b(t)dt$, where h is duration of oscillatory event; t_1 is time of beginning of oscillatory event, unless the subdominant component was not detected at the beginning (Fig 3B) $f_{mean} = \frac{1}{h}\int_{t_2}^{t_2-h} f_b(t)dt$ and the value of t_2 was used.

Table 1
Results of automatic detection of sleep spindles and 5–9 Hz oscillations using the continuous wavelet transform (mean ± s.d).

	Visual detections, N	Automatic detections				Accuracy, %	Sensitivity, %	Specificity, %
		TP	FP	FN	TN			
Sleep spindles	138 ± 51	129 ± 38	12 ± 5	28 ± 12	92 ± 18	93 ± 4	82 ± 4	89 ± 4
5–9 Hz oscillations	98 ± 21	92 ± 19	11 ± 6	19 ± 13	131 ± 26	94 ± 5	83 ± 5	95 ± 4

Table 2
Distribution of wavelet power in frequency bands (mean × 10^3 ± s.d.).

		Frequency bands			
		2–4.8 Hz	5–8.8 Hz	9–13.8 Hz	14–20 Hz
A. Frontal cortex					
Drowsiness	Asymptomatic rats	24.7 ± 12.2	35.2 ± 15.9	37.6 ± 16.4	34.7 ± 13.2
	Symptomatic rats	18.8 ± 6.7	30.3 ± 5.3	33.2 ± 11.0	37.8 ± 11.4
Non-REM sleep	Asymptomatic rats	11.0 ± 6.5	19.1 ± 7.4	16.5 ± 8.0	16.2 ± 5.4
	Symptomatic rats	11.0 ± 3.0	27.5 ± 8.9	25.5 ± 3.5	31.8 ± 7.7
B. Somatosensory cortex					
Drowsiness	Asymptomatic rats	23.3 ± 11.2	37.4 ± 23.5	46.8 ± 37.7	40.4 ± 21.0
	Symptomatic rats	16.0 ± 10.0	23.9 ± 8.6	24.4 ± 5.6	31.0 ± 6.7
Non-REM sleep	Asymptomatic rats	10.8 ± 5.4	23.8 ± 13.4	27.6 ± 28.5	22.1 ± 13.6
	Symptomatic rats	9.6 ± 4.4	22.7 ± 9.1	21.6 ± 6.4	29.0 ± 9.6

Statistical analysis was performed by means of Generalized Linear Model (GLM), nonparametric Mann–Whitney *U* test, parametric *t*-test and chi-square test.

3. Results

Among 10 WAG/Rij rats, five individuals did not show any SWD in their EEG during 24 h, and they were referred to as "asymptomatic" rats. The other five rats exhibited numerous SWD and called as "symptomatic" (Fig. 1E). Fig. 4A presents results of statistical analysis of duration and number of SWD as measured at the beginning of the dark period between 8 and 9 p.m. Distribution of sleep and wakefulness EEG patterns were analyzed during this period, and four states of vigilance were determined: waking state, drowsiness, non-REM sleep and IS of sleep (Fig. 1A–D). Fig. 4B displays the percentage of each state. Episodes of REM sleep were too short and infrequent, and they were not analyzed.

The structure of sleep–wake cycle in symptomatic and asymptomatic rats was significantly different ($F_{3;39} = 7.6$, $p < 0.001$). In contrast to asymptomatic rats, symptomatic subjects displayed lower percentage of wakefulness EEG pattern and higher percentage of non-REM (post-hoc test, both *p*'s < 0.05). Noteworthy is that the IS of sleep more frequently occurred in symptomatic rats: in 3 out of 5 symptomatic rats (3–4 episodes per hour, each episode lasted about 19–21 s) and in 1 out of 5 asymptomatic rats (1 episode per hour, 9.5 s duration). It seems that the IS of sleep was aggravated in symptomatic rats in comparison to asymptomatic.

3.1. Wavelet-based spectral analysis of EEG

Wavelet spectra were calculated in 20–30 s EEG intervals as recorded in frontal and parietal cortical areas during drowsiness and non-REM sleep. Spectral analysis of the IS of sleep was incomplete, because it was found only in 1 asymptomatic and 3 symptomatic rats.

Quantitative analysis of the wavelet power spectra was performed in frequencies 2–20 Hz, including the following bands of interests (2–4.8), (5–8.8), (9–13.8) and (14–20) Hz, using GLM ANOVA with 3 factors: 'epileptic state' (symptomatic and asymptomatic), 'waking state' (drowsiness and non-REM sleep) and 'frequency band'.

In frontal EEG (Table 2, A), symptomatic rats demonstrated the higher power than asymptomatic rats ($F_{1;63} = 7.3$, $p < 0.01$). Significant were effects of 'waking state' ($F_{1;63} = 35.9$, $p < 0.0001$) and 'band' ($F_{3;63} = 10.3$, $p < 0.0001$). Significant interactions between 'epileptic status' and 'waking state' ($F_{1;63} = 8.3$, $p < 0.01$) indicated that asymptomatic rats during non-REM sleep exhibited lower EEG wavelet power than symptomatic rats.

In parietal EEG (Table 2, B), no significant differences were found between symptomatic and asymptomatic rats, although effects of 'waking state' ($F_{1;63} = 9.2$, $p < 0.005$) and 'band' ($F_{3;63} = 5.9$, $p < 0.005$) were significant. Significant interactions between 'epileptic status' and 'waking state' ($F_{1;63} = 4.5$, $p < 0.05$) indicated that in asymptomatic rats EEG power during drowsiness was higher than that during non-REM sleep, but in symptomatic rats EEG wavelet power during non-REM sleep and during drowsiness were the same.

Wavelet power spectra were normalized in order to eliminate individual variations. In normalized wavelet spectra (Fig. 5), significant differences between symptomatic and asymptomatic rats were found only during non-REM sleep (Fig. 5B). In contrast to asymptomatic rats, symptomatic rats in frontal EEG showed significantly less power in 3.2–5.2 Hz and higher power in 12.6–15.6 Hz. In parietal (somatosensory) cortex, symptomatic rats exhibited less wavelet power in 5.2–6.2 Hz and more power in 12.4–17.2 Hz.

3.2. *Time-frequency analysis of sleep spindles and 5–9 Hz oscillations*

The analysis was carried out in 205 sleep spindles (20–21 events per rat) and 75 5–9 Hz oscillations (7–10 events per rat) that were automatically determined in frontal EEG during non-REM sleep. Time-frequency analysis was performed in frontal EEG, therefore, only anterior oscillations were examined. It was found that duration and mean frequency of sleep spindles and 5–9 Hz oscillations in symptomatic rats did not differ from that in asymptomatic rats. Basic characteristics of sleep spindles and 5–9 Hz oscillations (duration and mean frequency of dominant component) were measured in wavelet-based skeletons (Section 2.3, Fig. 3) and summed up in Table 3.

It was found that sleep spindles were more numerous than 5–9 Hz oscillations. The ratio between sleep spindles and 5–9 Hz oscillations was about 3:1, and it was the same in symptomatic and asymptomatic rats.

Analysis of wavelet-based skeletons of sleep spindles and 5–9 Hz oscillations indicated that the instantaneous frequency of dominant components changed from beginning to end of each

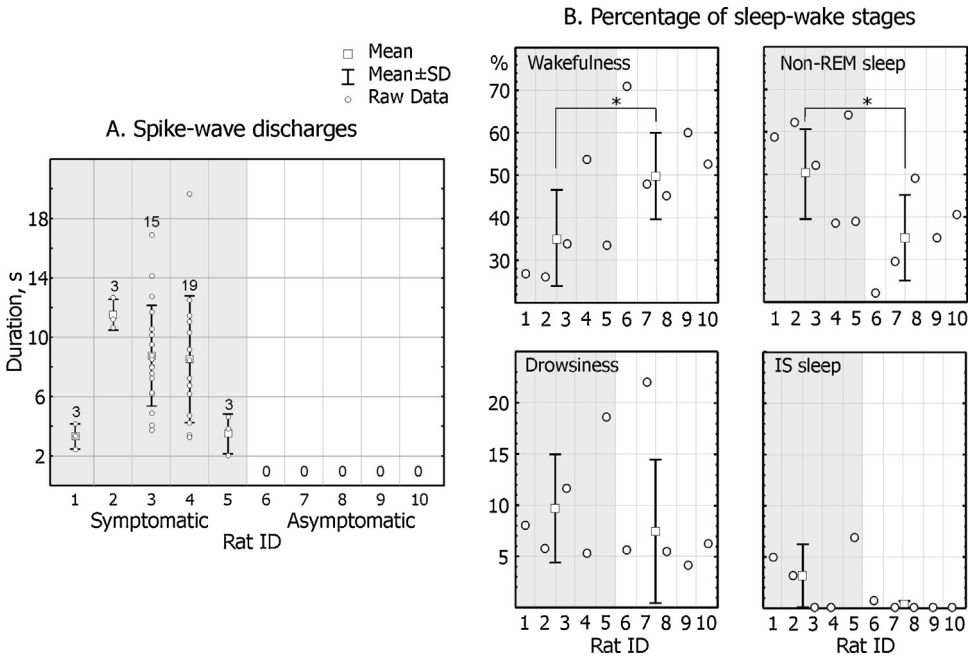

Fig. 4. Parameters of spike-wave discharges (A) and sleep–wake cycle (B) in WAG/Rij rats as measured during 1 h. In chart (A) labels indicate the number of seizures in each rat. Note that rats No 6–10 did not display any seizures (e.g., 'asymptomatic' rats). In chart (B) asterisked are significant differences between symptomatic and asymptomatic rats (post-hoc analysis, $p < 0.05$).

Table 3
Basic parameters of EEG oscillatory patterns in WAG/Rij rats.

	Sleep spindles		5–9 Hz oscillations	
	Symptomatic rats	Asymptomatic rats	Symptomatic rats	Asymptomatic rats
Duration, ms	676 ± 278	640 ± 226	676 ± 245	740 ± 250
Frequency, f_{mean}[a],Hz	12.1 ± 1.7	11.8 ± 1.7	8.3 ± 1.2	8.2 ± 1.8

[a] mean frequency of dominant component.

oscillatory event (Fig. 3). However, differences between starting and ending values of instantaneous frequencies (Fig. 3, f_1 and f_2 respectively) were not significant in both oscillatory events. No differences were found between symptomatic and asymptomatic rats in regard to the measured parameters of wavelet-based skeletons of sleep spindles and 5–9 Hz oscillations.

At it was mentioned above, EEG structure of sleep spindles and 5–9 Hz oscillations was similar due to the similar spindle-like envelope and mixed frequencies ranging from 5 to 15 Hz, however, these two patterns were clearly distinguished from each other by the value of wavelet energy in 5–9 Hz and in 10–15 Hz (these features were applied in wavelet-based algorithm for selective automatic recognition, Section 2.2). Frequency structure of sleep spindles and 5–9 Hz oscillations was either "simple" or "complex" (Fig. 6). "Simple" oscillatory events consisted of one dominant frequency component (Table 4), and "complex" events contained subdominant frequency components in addition to the dominant (Table 5).

In sleep spindles, the frequency of subdominant components varied between 2 and 9 Hz, while subdominants >9 Hz were found in two exceptional cases out of 207 sleep spindles, and these two spindles were excluded from analysis. Subdominant frequencies in "complex" sleep spindles were further analyzed. Analysis of frequency distribution indicated that subdominant frequencies were centered around 3–4 Hz (Fig. 6A). Table 5 shows results of statistical analysis, indicating that almost two thirds of "complex" sleep spindles comprised delta-frequency subdominant (2–5 Hz, mean 3.6 Hz) and one third—theta frequency subdominant (5–9 Hz, mean 6.7 Hz). No differences were found between symptomatic and asymptomatic rats.

Table 4
Time-frequency parameters of 'simple' (without subdominant frequency components) EEG oscillatory patterns in WAG/Rij rats as measured in wavelet skeletons (mean ± s.d.).

	Length, ms	Mean frequency, Hz
Sleep spindles		
Symptomatic rats	763 ± 331	11.3 ± 1.3
Asymptomatic rats	707 ± 238	11.8 ± 1.5
Total	742 ± 298	11.4 ± 1.4
5–9 Hz oscillations		
Symptomatic rats	766 ± 285	8.2 ± 0.8
Asymptomatic rats	761 ± 207	7.9 ± 1.1
Total	764 ± 250	8.1 ± 0.9

3.2.1. Sleep spindles

The mean frequency of the dominant component in "complex" sleep spindles in symptomatic rats was higher than in asymptomatic ($F_{1,200} = 6.8$, $p < 0.01$, Table 5). In both groups of rats, duration of "simple" sleep spindles (Table 4) was higher as compared to that in "complex" spindles ($F_{1,200} = 4.2$, $p < 0.05$, Table 5). In contrast to symptomatic rats, asymptomatic rats showed less number of "simple" spindles and higher number of "complex" spindles (Table 6, both p's < 0.05, chi-square test).

3.2.2. Five-9 Hz oscillations

In both groups of rats, "simple" 5–9 Hz oscillations lasted longer than "complex" ones ($F_{1,69} = 4.5$, $p < 0.05$, Table 5). The ratio between "simple" and "complex" 5–9 Hz oscillations was almost 1:1 (Table 6), and it did not differ in symptomatic and asymptomatic rats. Analysis of frequency distribution displayed (Fig. 7B)

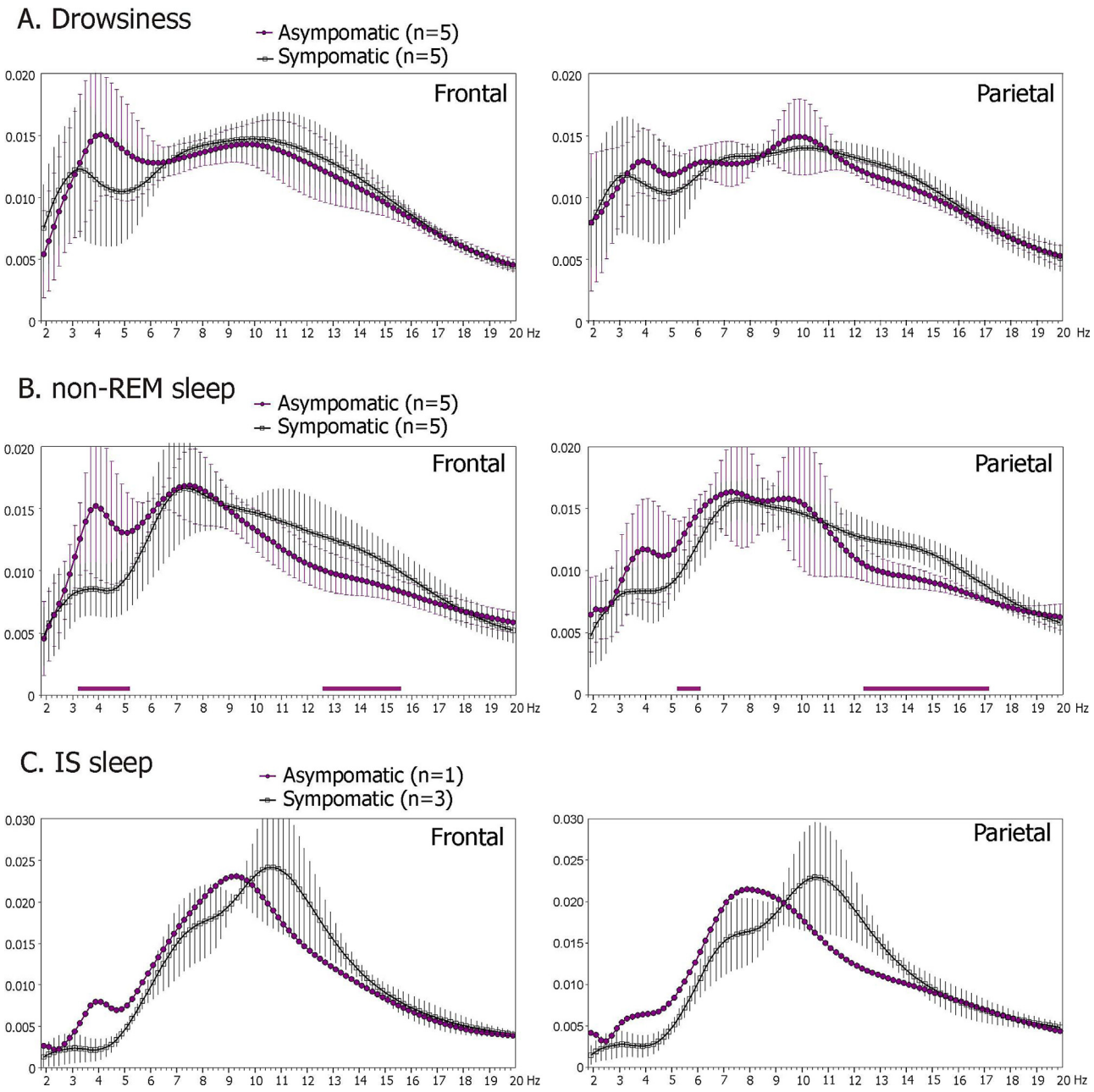

Fig. 5. Normalized wavelet power spectra in WAG/Rij rats (mean ± s.d.). Horizontal lines along the *x*-axis indicate significant differences between symptomatic and asymptomatic rats (*t*-test, $p < 0.05$).

Table 5
Time-frequency parameters of 'complex' (with subdominant frequency components) EEG oscillatory patterns in WAG/Rij rats as measured in wavelet skeletons (mean ± s.d.). In complex events, subdominants were examined at the beginning of EEG events.

	Length, ms	Mean frequency, Hz	<5 Hz subdominant component		>5 Hz subdominant component	
			%	Frequency, Hz	%	Frequency, Hz
Sleep spindles						
Symptomatic rats	614 ± 214	12.7 ± 1.8*	73.9	3.6 ± 0.9	26.1	6.8 ± 1.2
Asymptomatic rats	613 ± 217	11.9 ± 1.8	61.9	3.6 ± 0.7	38.1	6.5 ± 1.3
Total	614 ± 215**	12.3 ± 1.8	68.2	3.6 ± 0.8	31.8	6.7 ± 1.2
5–9 Hz oscillations						
Symptomatic rats	543 ± 84	8.2 ± 0.9	60	3.4 ± 1.5	40	14.3 ± 1.9
Asymptomatic rats	720 ± 285	8.3 ± 0.9	55	4.1 ± 1.4	45	12.8 ± 3.5
Total	644 ± 283***	8.2 ± 0.9	57.1	3.8 ± 1.4	42.9	13.4 ± 3.0

* "Complex" sleep spindles: significant difference of intra-spindle frequency between asymptomatic and symptomatic rats (GLM ANOVA, $p < 0.01$).
** Sleep spindles: significant difference in duration of "simple" vs "complex" events (GLM ANOVA, $p < 0.05$).
*** 5–9 Hz oscillations: significant difference in duration of "simple" vs "complex" events (GLM ANOVA, $p < 0.05$).

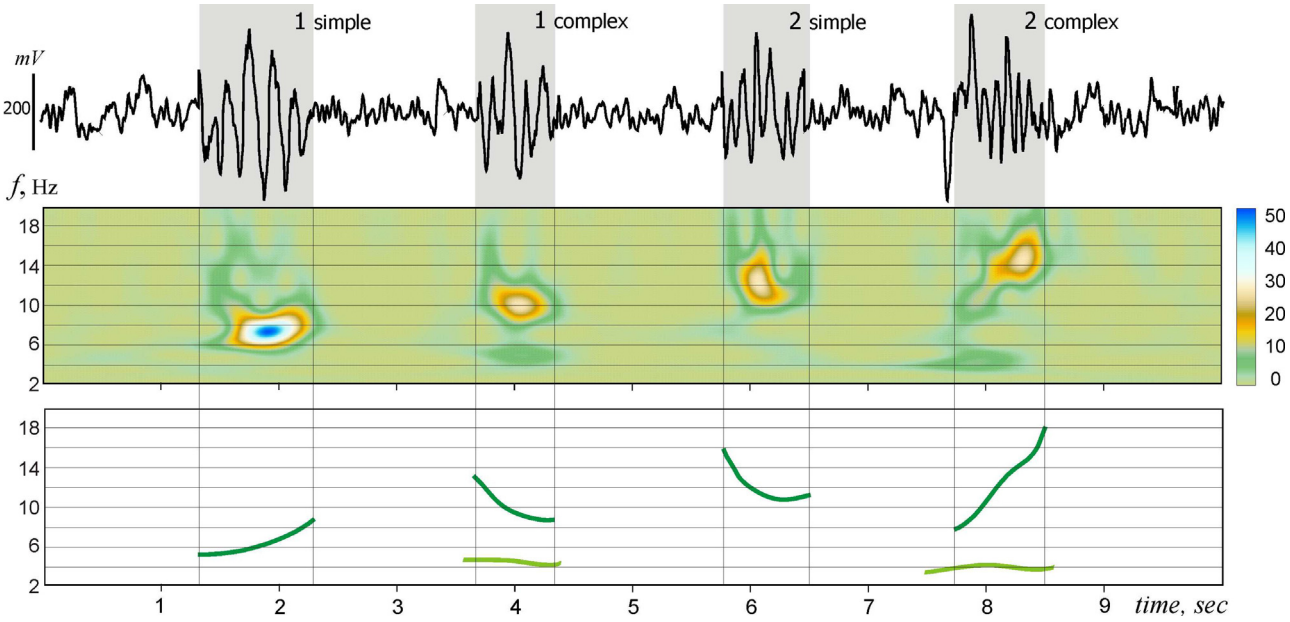

Fig. 6. Frontal EEG with sleep spindles (1) and 5–9 Hz oscillations (2) and corresponding wavelet surface. Both types of EEG events contained either one dominant frequency ("simple" events) or the complex of dominant and subdominant frequency components ("complex" events).

Table 6
Percentage of "simple" and "complex" EEG events.

	Simple	Complex
Sleep spindles		
Symptomatic rats	41.7	58.3
Asymptomatic rats	28.9*	71.1*
Total	35.6	64.4
5–9 Hz oscillations		
Symptomatic rats	59.5	40.5
Asymptomatic rats	45.9	54.1
Total	53.3	46.7

* Sleep spindles: significant difference between asymptomatic and symptomatic rats were (both p's < 0.05, chi-square test).

that subdominant frequencies in "complex" 5–9 Hz oscillations were located in two bands: <5 Hz (mean 3.8 Hz) and >5 Hz (mean 13.4 Hz). Symptomatic rats revealed noticeable bimodality in distribution of subdominant frequencies in 5–9 Hz oscillations.

4. Discussion

In the present study, strong individual variation in the incidence of absence seizures were found in Moscow's colony of WAG/Rij rats, suggesting that there is phenotypic heterogeneity in genetically predetermined absence seizures. We analyzed EEG in 9 months old rats and expected that all rats expressed fully matured SWD at this age (Coenen and Van Luijtelaar, 1987), however in 5 out of 10 animals SWD were absent during 24 h (asymptomatic rats) and the other 5 symptomatic rats expressed SWD (from 3 to 19/h). This difference in phenotypic expression of absence epilepsy in genetically prone subjects may be accounted for the environmental factors and sensory experience. It follows from our previous findings, indicating that early sensory deprivation through whisker trimming during the critical period of development resulted in increase of epileptic activity in adult WAG/Rij rats (Sitnikova, 2011).

Here we reported on differences in sleep–wake architecture between epileptic and non-epileptic phenotypes in WAG/Rij rats. In contrast to asymptomatic rats, symptomatic subjects displayed less wakefulness EEG pattern and more non-REM sleep. Inasmuch as non-REM sleep is a favorable state for SWD to occur (Drinkenburg et al., 1991), epileptic phenotype may be better expressed in subjects with longer and more frequent non-REM sleep. In contrary, Suntsova et al. (2009) found that the percentages of non-REM sleep, REM sleep and wakefulness in WAG/Rij rats did not differ from that in non-epileptic control (ACI and Wistar rats). These authors also found insufficiency of sleep-promoting neuronal mechanisms in WAG/Rij rats (Suntsova et al., 2009), suggesting imbalance in the ascending control of thalamocortical system that might facilitate the occurrence of SWD.

Absence epilepsy is known to associate with changes in REM sleep. In particular, in WAG/Rij rats, percentage of REM sleep is reduced and transition periods non-REM → REM sleep are prolonged in comparison to non-epileptic rats (Coenen and Van Luijtelaar, 2003; Gandolfo et al., 1990; van Luijtelaar and Bikbaev, 2007; Suntsova et al., 2009). In our subjects, episodes of REM sleep were very short and infrequent, so it was not possible to collect data sufficient for analyzed. We aimed to study the IS of sleep, which is characterized by theta rhythm in occipital EEG (similar to REM sleep) and by high-amplitude spindles in frontal and parietal EEG (similar to non-REM sleep) (Gottesmann, 1996). The IS of sleep in WAG/Rij rats is known to be almost three times longer than in control Wistar rats (Gandolfo et al., 1990), and it was easily recognized in some of our rats. The IS of sleep more often appeared in symptomatic rats than in asymptomatic, therefore, elevation of the IS of sleep may correlate with epileptic phenotype in WAG/Rij rats.

4.1. EEG spectrum during NREM sleep

Our data indicated that spectral features of EEG during non-REM sleep in symptomatic and asymptomatic rats were also different. The first difference referred to wavelet power in EEG during non-REM sleep and drowsiness. In contrast to asymptomatic rats, symptomatic rats in frontal EEG exhibited higher power during non-REM sleep.

The second difference related to the structure of wavelet power spectrum during non-REM sleep. In contrast to asymptomatic rats, symptomatic rats in frontal EEG showed significantly less power in 3.2–5.2 Hz and higher power in 12.6–15.6 Hz. In parietal (somatosensory) cortex, symptomatic rats exhibited less power in 5.2–6.2 Hz and more power in 12.4–17.2 Hz. In general, less power

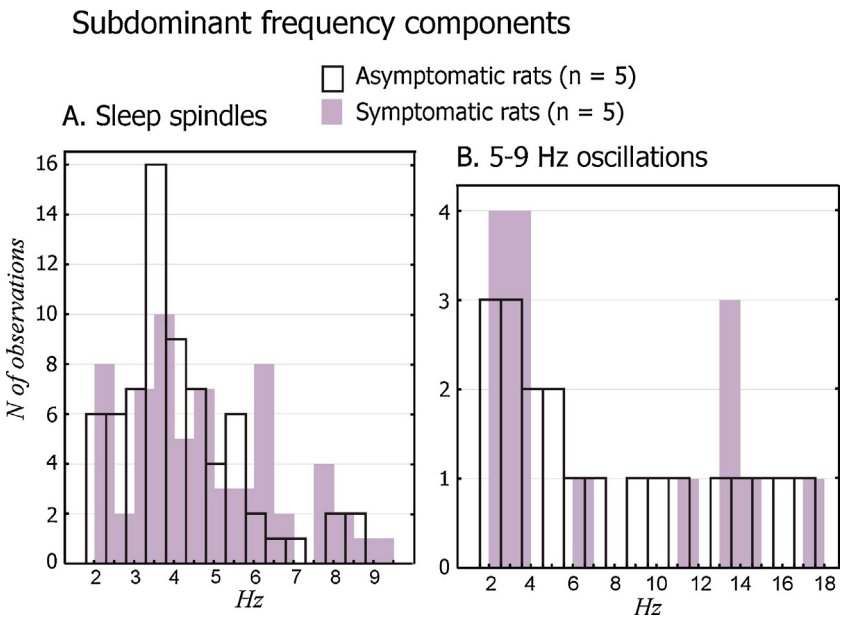

Fig. 7. Distribution histograms of subdominant frequencies in "complex" sleep spindles and 5–9 Hz oscillations.

in 3–6 Hz was found in fronto-parietal region in symptomatic WAG/Rij rats, suggesting that reduction of 3–6 Hz oscillatory activity during non-REM sleep might correlate with epileptic phenotype. High power in beta frequencies range (12–17 Hz) that was found in symptomatic rats might also associate with more intensive seizure activity. Noteworthy is that significant differences were found in frequencies that just slightly superimposed with dominant frequencies of sleep spindles (8–14 Hz) and 5–9 Hz oscillations.

4.2. Time-frequency structure of mid frequency oscillations (sleep spindles and 5–9 Hz oscillations)

The accurate time-frequency representation of sleep spindles and 5–9 Hz oscillations was achieved with the aid of skeletons of wavelet surfaces. This method was recently developed in order to determine instantaneous frequency and examine frequency dynamics during short-lasting events, such as sleep spindles (Hramov et al., 2015; Sitnikova et al., 2014a,b). Previously we (Sitnikova et al., 2014a) found an increase of the instantaneous frequency from beginning to the end of sleep spindles in 5 months old WAG/Rij rats, in which epileptic activity was immature, but this effect was no longer present at the age of 7 and 9 months, when animals expressed fully developed SWD (symptomatic stage). In addition to that, intrinsic frequency dynamics of sleep spindles in WAG/Rij rats at the younger age (5-months, preclinical state) was similar to that in non-epileptic control Wistar rats (Sitnikova et al., 2014b). In the current study, 9 months old WAG/Rij rats (both symptomatic and asymptomatic), did not display significant changes in instantaneous frequency during sleep spindles and 5–9 Hz oscillations. Therefore, intrinsic frequency dynamics of sleep spindles in WAG/Rij rats may be genetically predetermined.

In the present study, we analyzed frontal EEG, because the mid frequency rhythmic oscillations are better defined in frontal EEG than in occipital. It was demonstrated that (anterior) sleep spindles were often superimposed with slow-wave activity. In particular, about 35% of sleep spindles contained subdominant frequency components in delta (mean 3.6 Hz) and theta bands (mean 6.7 Hz) and were referred to as "complex". It is essential that sleep spindles in WAG/Rij rats are coincided in time with low-frequency rhythmic components in delta and theta bands. This contradicts to fact that sleep spindles and slow-wave activity are incompatible, because they arise at different hyperpolarization levels of individual thalamic neurons (Nuñez et al., 1992). Reciprocal relation between delta and sigma (spindle) activity are widely accepted (reviewed by De Gennaro and Ferrara, 2003). The presence of delta and theta subdominant components in sleep spindles in WAG/Rij rats might be an epiphenomenon of absence epilepsy or resulted from abnormal rhythmic activity in thalamocortical system. Short-lasting episodes of delta and theta activity are known to precede SWD in genetically prone rats (Pinault et al., 2001; van Luijtelaar et al., 2011). Mechanisms responsible for temporal integration of sleep spindle and slow-wave activity should be established in the future.

Here we demonstrated that time-frequency structure of sleep spindles in symptomatic rats differed from that in asymptomatic rats. First, the mean frequency of the dominant component in "complex" sleep spindles in symptomatic rats was higher than in asymptomatic (12.7 vs 11.3 Hz correspondingly). This corresponds well to the human data: the mean frequency of sleep spindles in patients with the untreated primary generalized epilepsy was higher than in control subjects (12.87 vs 12.21 Hz correspondingly) (Myatchin and Lagae 2007). Second, asymptomatic rats more often expressed "complex" sleep spindles in comparison to symptomatic rats (71.1 vs 58.3 % from the total amount of sleep spindles, correspondingly). In other words, low-frequency components (i.e., delta and theta) were readily integrated in sleep spindles in asymptomatic WAG/Rij rats, and decrease in number of "complex" sleep spindles associates with severe absence epilepsy.

We analyzed time-frequency structure of 5–9 Hz oscillations that were automatically recognized during interictal periods in frontal EEG. 5–9 Hz oscillations were about three times less frequent than sleep spindles and characterized by the dominant frequency of ~8 Hz in both symptomatic and asymptomatic rats. "Complex" 5–9 Hz oscillations contained either delta (mean 3.8 Hz) or beta (mean 13.4 Hz) subdominants. As known, the source of SWD in rats with absence epilepsy are physiological 5–9 Hz oscillations that suddenly become hypersynchronized (Pinault et al., 2001, 2006; Pinault, 2003), however, the correlation between of 5–9 Hz oscillations and EEG manifestation of absence epilepsy is not obvious. Physiological 5–9 Hz oscillations can be recorded in genetic absence epilepsy rats (GAERS) and in non-epileptic rat strains and not always lead to seizures (Pinault et al., 2001). We found that time-frequency features of 5–9 Hz oscillations did not

differ in symptomatic and asymptomatic WAG/Rij rats and did not correlate with the intensity of seizure activity.

5. Conclusions

Characteristics of sleep–wake cycle, EEG parameters sleep and mid-frequency oscillations (sleep spindles and 5–9 Hz oscillations) were examined in a group of WAG/Rij rats with different intensity of absence seizures. Considering strong individual variation in the incidence of absence seizures, we defined two epileptic phenotypes: asymptomatic and symptomatic. Differences between these phenotypes were found in sleep–wake cycle (i.e., symptomatic rats displayed less percentage of wakefulness EEG pattern and more non-REM sleep than asymptomatic subjects) and in spectral features of EEG during non-REM sleep (i.e., higher power in frontal EEG in symptomatic rats). Both types of mid-frequency oscillations were often superimposed with slow-wave activity (<5 Hz). The presence of delta (mean 3.6 Hz) and theta (mean 6.7 Hz) subdominant components in sleep spindles has not been reported before, and it may be an epiphenomenon of absence epilepsy in WAG/Rij rats or may result from abnormal rhythmic activity in thalamocortical system. It was found that delta or theta components more often appeared in sleep spindles in symptomatic WAG/Rij rats than in asymptomatic subjects, therefore, high percent of such "complex" sleep spindles may associate with negative prognosis of absence epilepsy. Time-frequency parameters of 5–9 Hz oscillations did not correlate with the intensity of absence epilepsy.

Conflict of interest

This paper does not pose a conflict of interest—neither actual nor potential.

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