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Research Report

Time-frequency characteristics and dynamics of sleep spindles in WAG/Rij rats with absence epilepsy

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ARTICLE INFO

Article history:
Accepted 3 November 2013
Available online 11 November 2013

Keywords:
Sleep spindles
Absence epilepsy
WAG/Rij rats
Continuous wavelet transform
Instantaneous frequency dynamics

ABSTRACT

In rat models of absence epilepsy, epileptic spike-wave discharges appeared in EEG spontaneously, and the incidence of epileptic activity increases with age. Spike-wave discharges and sleep spindles are known to share common thalamo-cortical mechanism, suggesting that absence seizures might affect some intrinsic properties of sleep spindles. This paper examines time–frequency EEG characteristics of anterior sleep spindles in non-epileptic Wistar and epileptic WAG/Rij rats at the age of 7 and 9 months. Considering non-stationary features of sleep spindles, EEG analysis was performed using Morlet-based continuous wavelet transform. It was found, first, that the average frequency of sleep spindles in non-epileptic Wistar rats was higher than in WAG/Rij (13.2 vs 11.2 Hz). Second, the instantaneous frequency ascended during a spindle event in Wistar rats, but it was constant in WAG/Rij. Third, in WAG/Rij rats, the number and duration of epileptic discharges increased in a period between 7 and 9 months of age, but duration and mean value of intra-spindle frequency did not change. In general, age-dependent aggravation of absence seizures in WAG/Rij rats did not affect EEG properties of sleep spindles; it was suggested that pro-epileptic changes in thalamo-cortical network in WAG/Rij rats might prevent dynamic changes of sleep spindles that were detected in Wistar.

1. Introduction

Sleep spindles are essential electroencephalographic (EEG) hallmarks of non-REM sleep (Steriade et al., 1993; De Gennaro and Ferrara, 2003; Destexhe, 2009). Sleep spindles are known to originate from the thalamus and spread to the cortex by ascending thalamo-cortical projections (refs in Destexhe and Sejnowski, 2001; Steriade, 2003). The American Academy of

Abbreviations: TC, thalamocortical; RTN, reticular thalamic nucleus; CWT, continuous wavelet transform; SWD, spike-wave discharges; GLM, general linear model

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0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.11.001

Sleep Medicine (AASM) determines sleep spindle as "*a train of distinct waves with frequency 11-16 Hz (most commonly 12–14 Hz) with a duration of >0.5 s*" (Iber et al., 2007). Considering the fact that sleep spindles are produced by thalamo-cortical network, and their frequency appeared to be sensitive to central nervous system disorders (e.g., Gibbs and Gibbs, 1950; Gandolfo et al., 1985; Himanen et al., 2003), it is assumed that intrinsic frequency of sleep spindles might conceal additional information about spatiotemporal organization of thalamo-cortical rhythmic activity. In humans, there is a clear predominance of the two spindle types, ~12 Hz in the frontal area and ~14 Hz in the centro-parietal region (Gibbs and Gibbs, 1950; Jankel and Niedermeyer, 1985; De Gennaro and Ferrara, 2003). Also in rats, the frequency of anterior sleep spindles 10–11 Hz is lower than in posterior spindles – 12.4 Hz (Terrier and Gottesmann, 1978; Gandolfo et al., 1985). The present paper examines variability of intra-spindle frequency of anterior sleep spindles in rats.

In 1950, Gibbs and Gibbs noted fluctuations of frequency of sleep spindle in healthy human EEG and described three spindle types, i.e., ~14 Hz spindles with amplitude maximum in central regions, ~12 Hz spindles that appeared in frontal areas during light sleep and ~10 Hz spindles that were more generalized during deep sleep. The existence of ~10 Hz spindle type was not confirmed by latter investigators, and ~10 Hz spindle oscillations were considered as a forerunner of 6–10 Hz rhythmic activity during transition between sleep stages 2 and 3 (Jankel and Niedermeyer, 1985). However, ~10 Hz sleep spindles were common in more than 50% of patients with different types of epileptic syndromes (Drake et al., 1991). Eventually, the frequency of sleep spindles is known to be lower in patients with neurological and sleep-related disorders. For instance, in patients with obstructive sleep apnea syndrome, the frequency of sleep spindles was 11.9 Hz versus 12.9 Hz in healthy control group (Himanen et al., 2003). It seems likely that neuronal mechanisms underlying epileptic and sleep-related disorders may influence time–frequency structure of sleep spindles.

In animals, sleep spindles are abundant during slow-wave sleep, and their frequency 7–14 Hz (Steriade, 2003) tends to be slightly lower than in humans. Previously we analyzed sleep spindles in EEG in adult WAG/Rij rats using Morlet-based wavelet transform and noted large between-subject variation of spindle frequencies: the averaged frequency of sleep spindle varied from 12.1 Hz to 14.1 Hz from subject to subject (Sitnikova et al., 2012). In addition to that, there were substantial frequency fluctuations within one spindle train (intra-spindle frequency variation) and between different sleep spindles in one subject (within-subject variation). In the present paper, we acknowledge the non-stationary features of sleep spindle, i.e. that frequency content and amplitude of sleep spindles change with time. Traditional methods of spectral analysis (fast Fourier transform, FFT) require the signal to be stationary or quasi-stationary. Serious limitation of FFT is that it characterizes EEG signals only in the frequency domain, but not in time domain, as a consequence, dynamic changes of spectral components are not present in Fourier spectrum. Therefore, temporal changes of frequency during sleep spindles are usually neglected. As a non-stationary signal, sleep spindle could be characterized by 'instantaneous frequency'. The latter is a time-varying parameter that defines the location of the signal's spectral peak as it varies with time (Boashash, 1992). Continuous wavelet transform has several advantages over traditional FFT that are crucial for time–frequency analysis of non-periodic and non-stationary signals, such as EEG (Pavlov et al., 2012). First, in wavelet space, EEG signal power is distributed throughout time and frequency domains that enables tracking frequency dynamics over time. Second, adjustable parameters in wavelet spectra provide the best time–frequency representation of signals that cannot be achieved with the other methods of time–frequency analyses (i.e. empirical mode analysis, windowed Fourier transform, Gabor–Wigner transform).

In the present paper we measured 'instantaneous frequency' at the endpoints of a spindle event (at the beginning and at the end) and also assessed the mean frequency of each sleep spindle that roughly corresponds to its Fourier frequency, which helped us to compare our data with the literature that applied spectral analysis.

Sleep spindles are in particular interest to specialists in clinical and basic neuroscience, because sleep spindle oscillations has long been known to associate with absence seizures (spike-wave discharges in EEG, reviewed in Kostopoulos, 2000; Leresche et al., 2012). Furthermore, pharmacological manipulations, such as injection of pentobarbital and penicillin in cats, may lead to a gradual transformation of sleep spindles into epileptic spike-wave discharges (SWD) (Gloor, 1968; Steriade et al., 1993; Kostopoulos, 2000). SWD are electroencephalograpic manifestation of absence seizures and other generalized idiopathic epilepsies in humans (Panayiotopoulos, 1997). Some inbreed rat strains are prone to develop SWD spontaneously (Inoue et al., 1990). For example, WAG/Rij rat strain has a genetic predisposition to absence seizures (Coenen and van Luijtelaar, 2003). The current paper uses WAG/Rij rats as a genetic model of absence epilepsy.

Considering the fact that SWD result from an impaired functioning of thalamo-cortical network (reviewed in Meeren et al., 2005; Sitnikova, 2010; Urakami et al., 2012; Leresche et al., 2012), we hypothesize that time–frequency structure of physiological sleep spindles might also be impaired due to disturbances in thalamo-cortical network as absence epilepsy progresses. In this paper we analyze putative changes of sleep spindle oscillations that might correlate with absence epilepsy.

Sleep spindles in rats display maximum amplitude in the frontal cortical area (Terrier and Gottesmann, 1978; van Luijtelaar, 1997; Gandolfo et al., 1985), and the present study is focused in frontal spindle spindles (anterior spindles). Previously we found that about 10% of anterior sleep spindles in WAG/Rij rats were characterized by abnormal features and considered as pro-epileptic (Sitnikova et al., 2009; Sitnikova, 2010; Pavlov et al., 2012). In the current paper we examined putative changes of sleep spindles in WAG/Rij rats during a short period of life (between 5 and 7 months of age) when seizure activity is rapidly aggravating in comparison with the age-match non-epileptic control Wistar rats (Coenen and van Luijtelaar, 1987; van Luijtelaar and Bikbaev, 2007). Continuous wavelet transform was used for time–frequency EEG analysis and automatic selection of sleep spindles in raw EEG data.

2. Results

Two kinds of oscillatory events were studied in frontal EEG: anterior sleep spindles and spike-wave discharges, SWD (Fig. 1). Sleep spindles represented groups of 8–14 Hz waves with characteristic waxing-waning morphology and symmetrical waveform that were not contaminated by sharp (spike) components and whose amplitude exceeded background level at least twice (van Luijtelaar, 1997). SWD appeared in EEG as a sequence of repetitive high-voltage negative spikes and negative waves that lasted longer than 1 s; amplitude of SWD exceeded background more than three times (van Luijtelaar and Coenen, 1986). The amplitude of the first spike in SWD was as high as the amplitude of the next spikes in SWD train, therefore amplitude envelope of SWD was rectangular in contrast to waxing–winning envelope of sleep spindles (Fig. 1).

2.1. Basic characteristics of sleep spindles

Sleep spindles were automatically selected in EEG using the wavelet-based algorithm that was applied off-line in 24-h EEG recordings. EEG processed automatically with the aid of the own original software (see Section 4.2 for details). Sleep spindles were detected in EEG according to time–frequency creteria both at day and night periods. In total, 318 sleep spindles were analyzed in six Wistar rats and in 232 sleep spindles in six WAG/Rij rats (the number of sleep spindles varied from 18 to 30 per animal of each age). Continuous wavelet transform was used for the time–frequency analysis of sleep spindles with respect to non-stationary properties of the signal (Section 4.2).

The instantaneous frequencies as measured at the beginning (f_1) and at the end (f_2) of each sleep spindle were averaged in order to assess the mean frequency, f_{mean} (Eq. (7)). Noteworthy is that f_{mean} was close to averaged value of instantaneous frequency at the beginning and at the end of a sleep spindle: $f_{mean}=\left|\frac{f_1-f_2}{2}\right|$. Fig. 2a–c demonstrates EEG epoch with typical sleep spindle, and its wavelet spectrum and 'skeleton' of wavelet surface (see Section 4.3), as well as Fourier spectrum of sleep spindle (Fig. 2D). 'Skeleton' (Fig. 2C) displays characteristic dynamics (i.e., an increase) of instantaneous frequency during spindle event. The mean frequency of a spindle, f_{mean} as computed by Eq. (7), was about 12 Hz that was almost equal to the maximum of its spectral power in Fourier power spectrum, ~12 Hz (Fig. 2D). Therefore, Fourier frequency corresponded well to the frequency of wavelet spectrum analysis, yet Fourier analysis did not provide information about temporal dynamics of EEG frequency. So, the mean frequency, f_{mean}, can be considered as the central frequency of sleep spindles that takes into consideration the non-stationary nature of sleep spindles and corresponds to Fourier frequency of traditional spectral analysis.

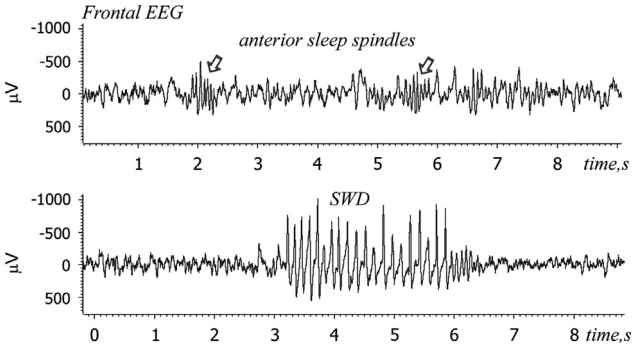

Fig. 1 – Examples of anterior sleep spindles (indicated by arrows) and spike-wave discharges, SWD, as recorded in frontal EEG in 9-months old WAG/Rij rat.

It was found that at the age of 7 months, the average frequency of sleep spindles (f_{mean}) in WAG/Rij rats was 11.2 Hz and it was about the same at the age of 9 months – 11.3 Hz. In Wistar rats, the average spindle frequency was about 13.2 Hz in both ages. In sum, the mean frequency of sleep spindles in WAG/Rij rats was lower than in non-epileptic Wistar rats (GLM test for the 'strain' effect, $F_{1;546}=50.7$, $p<0.0001$) and did not change with age (GLM test for the 'age' effect in each strain, $p>0.05$, Table 1).

Mean duration of sleep spindles in WAG/Rij and Wistar rats displayed different age-related dynamics (significant interaction between two factors 'age' and 'strain', $F_{1;546}=4.6$, $p<0.05$). In 7-months old Wistar rats, the average duration of sleep spindles was 0.406 ± 0.148 s (mean ± s.d. here and below) and increased to 0.437 ± 0.168 s at the age of 9 months ($p<0.05$, *post-hoc*). In WAG/Rij rats, duration of sleep spindles did not change with age (0.388 ± 0.107 s and 0.371 ± 0.075 s in 7- and 9-months old animals correspondingly).

The incidence of absence seizures in WAG/Rij rats increased in the period between 7 and 9 months of age: the number of SWD was doubled, from 19 ± 24 to 40 ± 41 ($p<0.05$, Wilcoxon matched pairs test). The total duration of seizure activity in EEG increased from 125 ± 202 s at the age of 7 months to 317 ± 327 s at the age of 9-months ($p<0.05$). Wistar rats did not develop any SWD. In WAG/Rij rats, age-dependent increase of absence seizures did not affect the basic parameters of sleep spindles (duration and frequency).

2.2. Frequency content of sleep spindles

In all groups of WAG/Rij and Wistar rats, statistical distribution of f_{mean} was not normal ($p<0.05$, Kolmogorov–Smirnov test) with noticeable multimodality (Fig. 3). Based on the whole distribution of f_{mean} (Fig. 3), sleep spindles were divided in several categories: 'slow' (8–10.4 Hz), 'medium' (10.5–12.4 Hz), 'fast' (12.5–14.4 Hz) and additional 'extra' spindle type (14.5–16 Hz, whose frequency was beyond the spindle rage).

As it was reported in Wistar rats (Terrier and Gottesmann, 1978; Gandolfo et al., 1985), the frequency of sleep spindles in anterior cortex was ~11.2 Hz, and in posterior – ~12.4 Hz. We accessed only anterior sleep spindles, and it was surprising that the mean frequency of sleep spindles in Wistar rats was ~13 Hz that was higher than reported in literature ~11.2 Hz (Gandolfo et al., 1985). However, in WAG/Rij rats the average frequency of sleep spindles was found to be 11.2–11.3 Hz (Table 1).

In 'slow' sleep spindles, the average frequency was 9.3–9.8 Hz (Table 1), in 'medium' spindles – 11.4–11.6 Hz, in 'fast' – 13.2–13.4 Hz and in 'extra' – 14.9–15.3 Hz. Fig. 4 shows the percentage of each spindle type relative to the total amount of sleep spindles

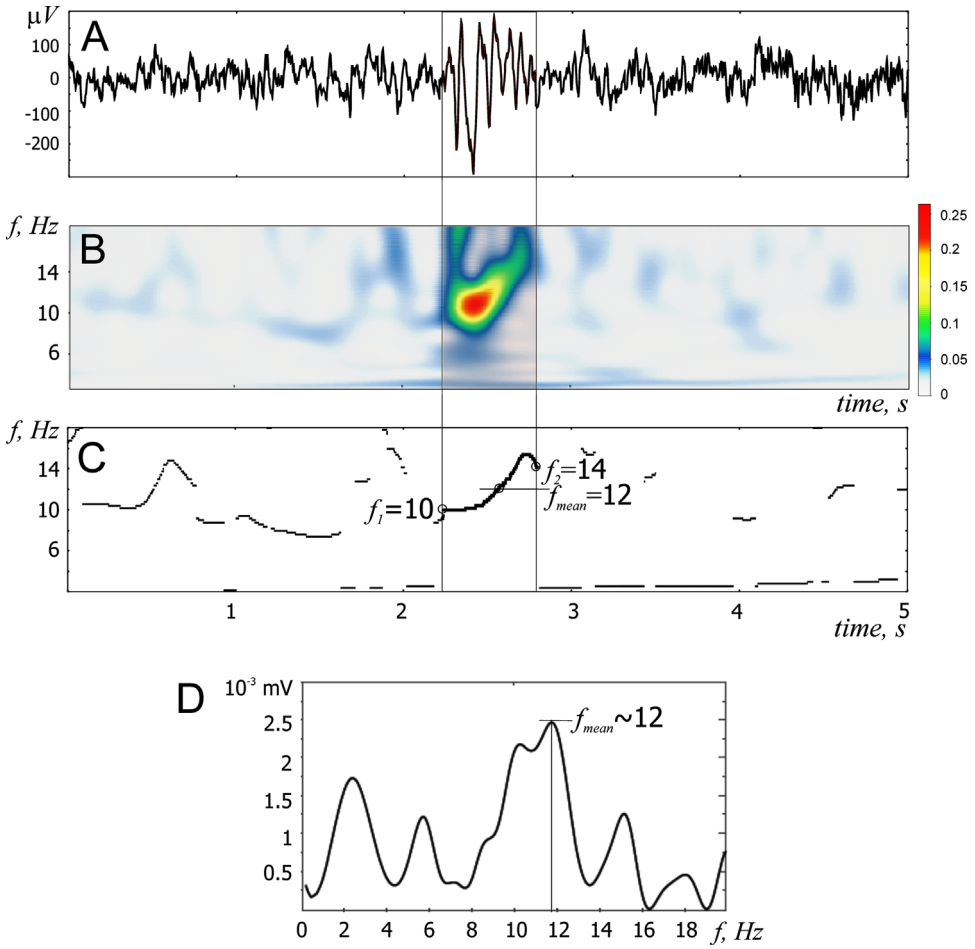

Fig. 2 – Anterior sleep spindle (marked by rectangle) as recorded in EEG in 7-months old Wistar rat (A), corresponding wavelet spectrum (B) and 'skeleton' of wavelet surface (C). 'Skeleton' illustrates dynamics of instantaneous intra-spindle frequency, where f_{mean}—mean frequency, and f_1 and f_2 - two values of the instantaneous frequency at the beginning and the end of a sleep spindle correspondingly. Note an ascending dynamics of intra-spindle frequency: $f_1 > f_2$ (f_{mean}=12 Hz). Fourier power spectrum of the marked sleep spindle (D) with central peak frequency ~12 Hz.

Table 1 – Average frequency of sleep spindles (Hz, mean ±s.d.; %–percent of each spindle type relative to the total amount of sleep spindles).

Spindle type	7 months		9 months	
	Wistar	WAG/Rij	Wistar	WAG/Rij
slow	9.8±0.6 (17%)	9.4±0.7 (38%)	9.3±0.8 (11%)	9.6±0.8 (43%)
medium	11.5±0.5 (21%)	11.5±0.7 (41%)	11.3±0.5 (22%)	11.6±0.6 (28%)
fast	13.3±0.4 (25%)	13.2±0.5 (13%)	13.2±0.5 (29%)	13.4±0.5 (14%)
extra	15.3±0.8 (37%)	14.9±0.5 (8%)	15.0±0.6 (38%)	15.1±0.7 (15%)
Mean	13.1±2.1	11.2±1.8*	13.2±1.2	11.3±2.2**

* Differences between Wistar and WAG/Rij rats were significant at the age of 7 months ($F_{1;267}$=22.9, p<0.0001).
** at the age of 9 months ($F_{1;278}$=27.8, p<0.0001).

in Wistar and WAG/Rij rats. In 7-months old WAG/Rij rats, the percent of 'slow' and 'medium' spindle types was higher than in Wistar rats, and the percent of 'extra' type was lower (all p's<0.05 in *post-hoc* test for the interaction 'strain' □ 'spindle type' $F_{3;42}$ =11.6, p<0.0001). At the age of 9 months, relative amount of 'slow' spindles in WAG/Rij rats was higher than in Wistar rats (p<0.05), but the amount of 'fast' and 'extra' spindles - lower (all p's<0.05 in *post-hoc* test for the interaction 'strain' □ 'spindle type' $F_{3;44}$ =10.7, p<0.0001, Fig. 4). About two-thirds of sleep spindles in Wistar rats characterized by a frequency higher than 12.5 Hz, and the sum percent of 'fast'+'extra' sleep spindles in 7-months old Wistar rats was 62%. This kind of sleep spindles was rare in WAG/Rij rats. In 7-months old WAG/Rij rats, only 21% of sleep spindles had a frequency

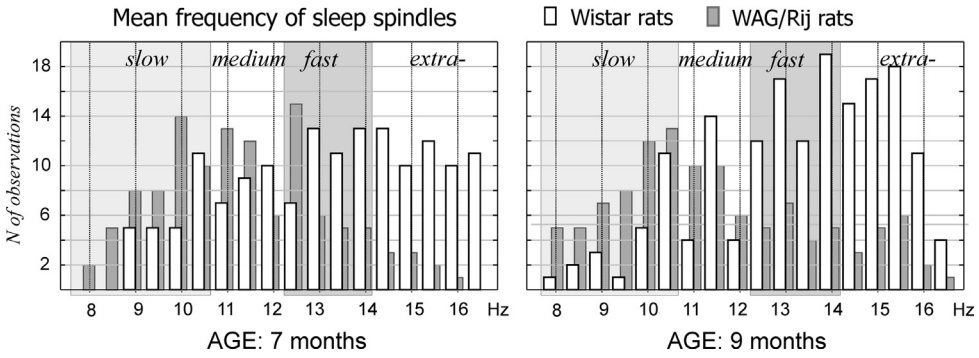

Fig. 3 – Distribution histograms of mean frequencies (f_{mean}) of sleep spindles as measured in skeletons of wavelet spectra in WAG/Rij and Wistar rats. Frequency-specific groups of sleep spindles were chosen empirically.

above 12.5 Hz, and only 8% corresponded to 'extra' spindle. According to the GLM analysis, percentage distribution of sleep spindles across spindle types did not change with age. However, there was a significant interaction 'strain' □ 'spindle type' ($F_{3;87}=21.0$, $p<0.0001$), suggesting that the percent of frequency-specific spindle types differed in two rat strains and was not influenced by the age. *Post-hoc* test showed that 'slow' and 'medium' spindles were more numerous in WAG/Rij rats than in Wistar, and 'fast' and 'extra' spindles—less numerous (both p's<0.05). Fig. 5

2.3. *Frequency dynamics of sleep spindles*

In order to disclose intra-spindle frequency dynamics, we examined 'skeletons' of wavelet surface of each spindle event (Fig. 2) and analyzed instantaneous frequency at the beginning (f_1) and at the end (f_2) of a spindle (Section 4.3). Statistical analysis in Wistar rats revealed that intra-spindle frequency of sleep spindles increased from the beginning to the end ($f_1<f_2$, $F_{1;629}=9.3$, $p<0.0001$). This effect became more pronounced with age (the effect of 'age' was significant, $F_{1;629}=11.9$, $p>0.001$), suggesting that the difference between f_1 and f_2 in 9-months Wistar rats was larger than in 7 months old). An increase of intra-spindle frequency differed in different spindle types ($F_{3;629}=9.3$, $p>0.001$): it was significant in 'medium' and 'fast' spindles (*post-hoc* test, both p's<0.05, Fig. 6), but was not found in 'slow' and 'extra' spindle types.

In opposite to Wistar rats, the instantaneous frequency of sleep spindles in WAG/Rij rats did not change during a spindle, i.e., $f_2=f_1$ (differences between two strains were significant $F_{1;1085}=66.7$, $p<0.0001$). The intra-spindle frequency in WAG/Rij rats remained unchanged during sleep spindle in both ages and in all spindle types (Fig. 5).

3. Discussion

Anterior sleep spindles in Wistar and WAG/Rij rats are non-stationary phenomena characterized by the smooth variation of amplitude and frequency (yet, the frequency stayed within a 'spindle'-frequency band, i.e. from 8 to 16 Hz). Considering non-stationary properties of sleep spindles, we performed EEG analysis with the aid of continuous wavelet transform, and studied instantaneous frequency in order to determine

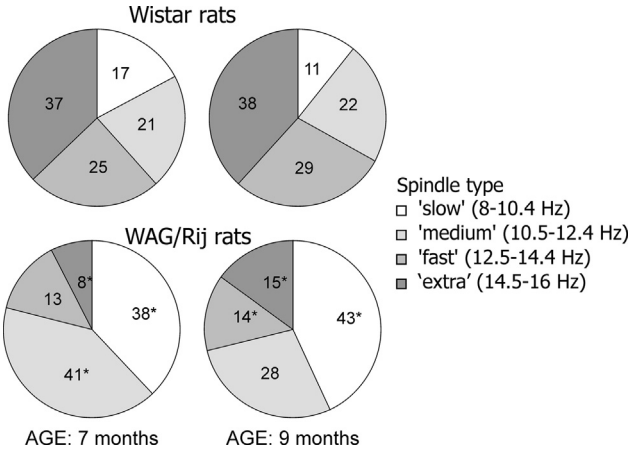

Fig. 4 – Percentage distribution of frequency-specific spindle types in WAG/Rij and Wistar rats. Asterisks indicate significant differences between age-matched WAG/Rij and Wistar rats (post-hoc test in the GLM, $p<0.05$).

the frequency content of sleep spindles and characterize dynamics of intra-spindle frequency over time.

It was found that the mean values of instantaneous frequencies in anterior sleep spindles were centered in the following bands: 'slow' (8–10.4 Hz), 'medium' (10.5–12.4 Hz), 'fast' (12.5–14.4 Hz) 'extreme' spindle-frequencies (14.5–16 Hz).

Three major conclusions can be drawn from our results. First, the mean frequency of sleep spindles in drug-naïve WAG/Rij rats with absence epilepsy was lower than in non-epileptic Wistar rats. In WAG/Rij rats, 38–43% out of the total amount of sleep spindles characterized by mean frequency 8–10 Hz, but in Wistar rats this percentage was lower (11–17%), suggesting that absence epilepsy may be associated with the presence of slow (<10 Hz) sleep spindles. The same conclusion was made by Drake et al. (1991). These authors detected ~10 Hz sleep spindles during stage 2 sleep in patients with partial seizures and also in patients with primary and secondarily generalized epilepsy, yet there was a strong effect of medication: polytherapy might significantly decrease the frequency of sleep spindles. These findings were not confirmed in children with primary generalized epilepsy (Myatchin and Lagae, 2007). In young patients, who received antiepileptic medication (monotherapy) and in previously

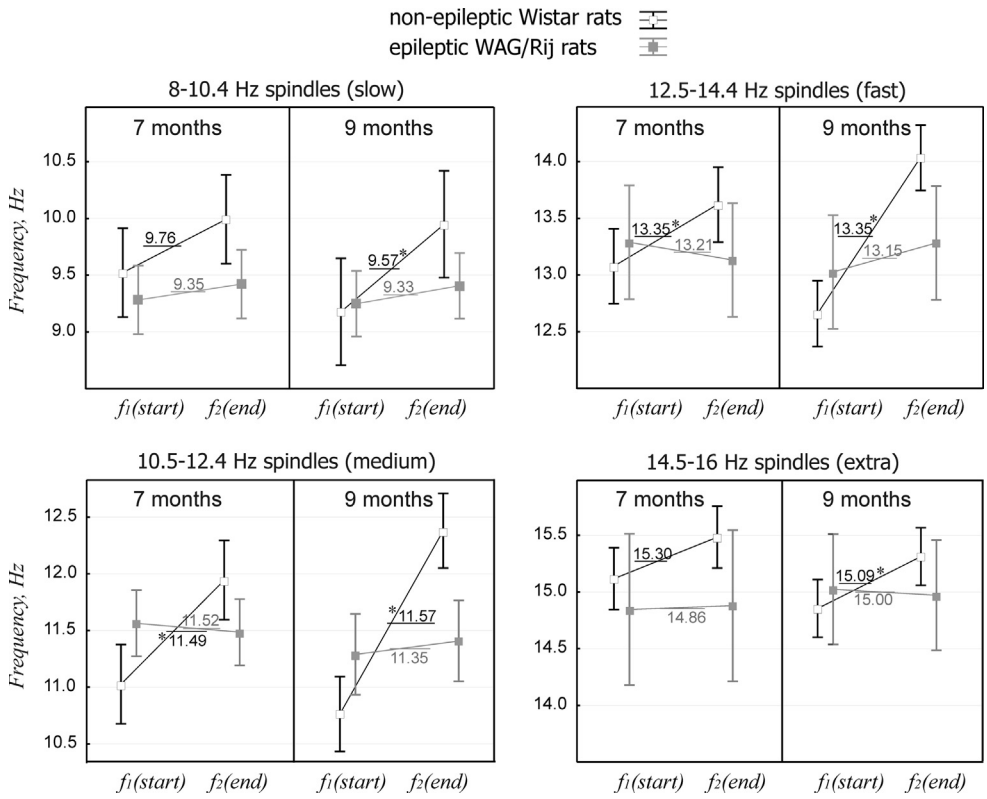

Fig. 5 – The instantaneous frequency of sleep spindles in two rat lines at the beginning (f_1) and at the end (f_2) of spindle events (mean ±0.95 confidential interval). Asterisks indicate significant differences between f_1 and f_2 (post-hoc test in the GLM, $p<0.05$).

untreated group, the mean frequency and duration of sleep spindles during stage 2 did not differ from the healthy control. This contradicts to our data, and the discrepancy may be caused by the differences in EEG recording procedure and analysis: (1) we selected sleep spindles automatically using wavelet-based algorithm, but Myatchin and Lagae (2007) selected sleep spindles manually, (2) we did not determine sleep stages (this is not appropriate for rats), but the cited authors studied sleep stage 2, (3) we recorded EEG epidurally at the frontal cortex, but the authors used scalp EEG recordings, (4) we applied continuous wavelet transform for the time–frequency analysis of sleep spindles, but the authors measured spindle frequency "*using the electroencephalographic software*".

The presence of slow (<10 Hz) sleep spindles in rats with absence epilepsy may have important implications, but it needs to be confirmed in other animal models and in epileptic patients. Unfortunately, the frequency content of sleep spindles seems to be underestimated, especially in human patients. The problem is that EEG frequency is traditionally determined by peak-to-peak calculations or by traditional spectral analysis (fast Fourier transform). Fourier-based frequency estimates have serious limitations when applied to the short-lasting transient events in EEG, such as sleep spindles (Sitnikova et al., 2009; Pavlov et al., 2012). Jankel and Niedermeyer (1985) emphasized that "*as "transient nonstationarities", spindles escape methods of EEG computer analysis*". The advantage of continuous wavelet transform is that it considers non-stationary properties of EEG signals, and application of continuous wavelet transform in this study enabled us to define the fine time–frequency structure of sleep spindles. We believe that development of appropriate and user-friendly methods for the analysis of non-stationary EEG data, e.g. sleep spindles in epileptic patients, may enhance our knowledge about pro-epileptic changes in EEG and might be useful for diagnostic and prognostic purposes.

Second, sleep spindles characterized by upward-sloping dynamics of instantaneous frequency during a spindle event in non-epileptic Wistar rats, in opposite to epileptic WAG/Rij rats, whose intra-spindle frequency remained constant during a spindle ($f_1 = f_2$). In Wistar rats, this effect was significant in 'medium' (10.5–12.4 Hz) and 'fast' (12.5–14.4 Hz) spindles and enhanced with age (from 7 to 9 months of age). This ascending frequency dynamics along a sleep spindle can be accounted for the properties of thalamo-cortical network activity. It is likely that thalamo-cortical mechanism of spindle maintenance and termination is affected by genetic factors (predisposition to absence epilepsy), but it does not associate with the severity of seizures.

Third, according to our data, duration of sleep spindles in non-epileptic Wistar rats increased from 7 to 9 months of age, but it was constant during this period of life in WAG/Rij rats. It is known that the length of sleep spindles is determined by membrane properties of TC cells, e.g., by the time constant of I_h current deactivation (Zygierewicz, 2000; Bonjean et al.,

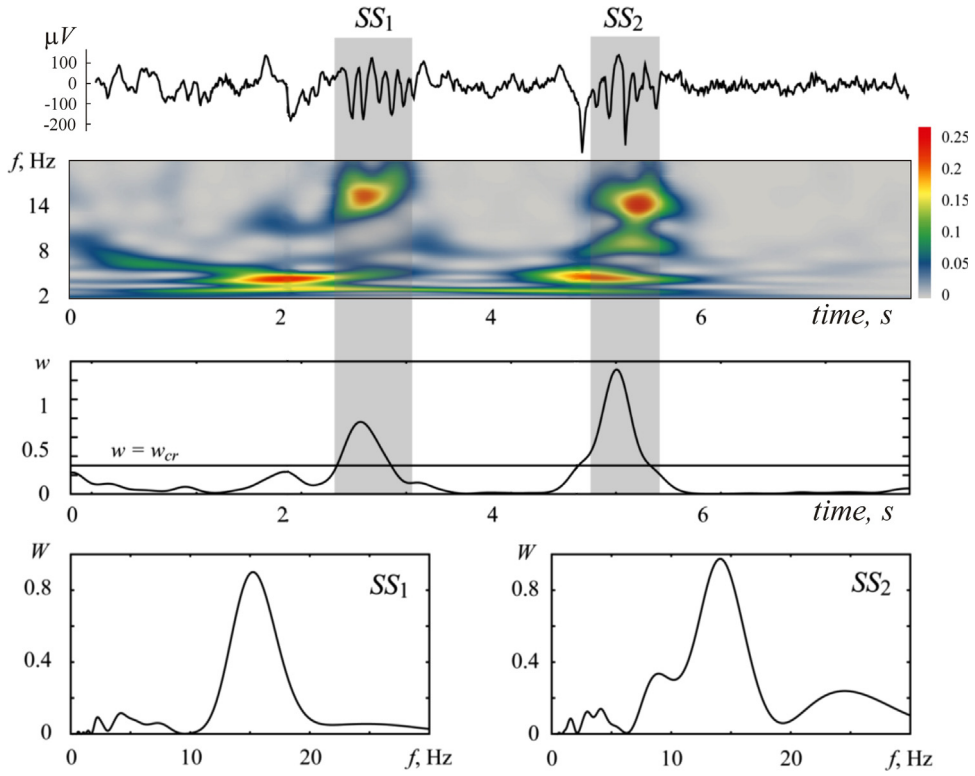

Fig. 6 – Illustration of the wavelet-based procedure for the automatic detection of sleep spindles in EEG. Raw EEG with two sleep spindles, SS_1 and SS_2 (shown by gray boxes), corresponding Morlet-based wavelet spectrum and distribution of wavelet energy $w(t)$ as measured in the frequency band 8–16 Hz. Color (gray) scale indicates the magnitude of wavelet coefficients. Sleep spindles were recognized under condition that wavelet power in 8–16 Hz exceeded the threshold, w_{cr} (horizontal line). The bottom graphs demonstrate distribution of wavelet energy of sleep spindles SS_1 and SS_2; note characteristic elevation of wavelet power $W(f)$ in frequencies 8–16 Hz.

2011), suggesting that properties of I_h channels in TC cells in Wistar rats (not in WAG/Rij) might change with age.

Time–frequency characteristics of sleep spindles in WAG/Rij rats did not change with age, in opposite to Wistar rats, therefore, it is hypothesized that age-related aggravation of absence seizures in WAG/Rij rats precluded normal developmental changes of sleep spindles. In general, epileptic processes in thalamo-cortical network underlying age-related aggravation of absence seizures might interfere with the mechanisms of normal development of sleep spindles.

4. Conclusion

This study examines time–frequency characteristics of sleep spindles in non-epileptic Wistar and epileptic WAG/Rij rats. The incidence of absence seizures in WAG/Rij rats was doubled from 7 to 9 months of age, however, neither duration, nor the average intra-spindle frequency were changed. In Wistar rats, the average duration of sleep spindles increased with age, but the average intra-spindle frequency did not. In general, the mean intra-spindle frequency in Wistar rats was higher than in WAG/Rij (11.2 vs 13.2 Hz) and did not change with age.

The mean frequency of sleep spindles centered in the following bands: 'slow' (8–10.4 Hz), 'medium' (10.5–12.4 Hz), 'fast' (12.5–14.4 Hz) and 'extra' (14.5–16 Hz). Relative amount of frequency-specific sleep spindles differed in the two rat strains. 'Slow' and 'medium' spindles (8–12.4 Hz) were more numerous in WAG/Rij rats, 'fast' and 'extra' spindles (12.5–16 Hz)—in Wistar rats.

In Wistar rats, instantaneous frequency of sleep spindles increased from the beginning to the end of a spindle event. Elevation of intra-spindle frequency was significant during 'medium' and 'fast' sleep spindles (10.5–14.4 Hz) and became greater with age (from 7 to 9 months). An increase of intra-spindle frequency during a spindle may represent a normal development of rhythmic mode in the thalamo-cortical neuronal network. In general, age-related changes of sleep spindles in Wistar rats were associated with the increased duration and ascending dynamics (elevation) of intra-spindle frequency.

In WAG/Rij rats, instantaneous frequency remained constant during a spindle event. There were no age-related changes in time–frequency profile of sleep spindles in WAG/Rij rats, despite progressive development of seizure activity. Even in younger animals (7 months old), in which absences were less frequent and severe, intra-spindle frequency was constant during a spindle event, and intra-spindle frequency was lower than in non-epileptic control Wistar rats. These data suggest that (1) the low value of intra-spindle frequency and (2) constancy of instantaneous frequency

during a spindle might be used as biomarkers for early (perhaps, preclinical) stage of absence epilepsy.

5. Experimental procedures

5.1. Animals and EEG recording

Experiments were performed in rats (WAG/Rij and Wistar strains) in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the animal ethics committee of the Institute of Higher Nervous Activity and Neurophysiology RAS. EEGs were recorded in six male WAG/Rij rats and six male Wistar rats in two successive sessions: at the age of 7 and 9 months. In order to record anterior sleep spindle spindles, one recording EEG electrode was implanted epidurally over the frontal cortex (AP +2 mm and L 2.5 mm relatively to the bregma). Ground and reference electrodes were placed over the cerebellum. EEG signals were recorded in freely moving rats continuously during a period of 24 h, fed into a multi-channel differential amplifier via a swivel contact, band-pass filtered between 0.5–200 Hz, digitized with 400 samples/second/per channel and stored in hard disk for the further off-line analysis.

5.2. Continuous wavelet transform of EEG and automatic detection of sleep spindles

Epochs of artifact-free EEG during slow-wave sleep (sleep EEG) were extracted from the raw EEG in order to set up inclusion/exclusion criteria for the automatic detection of sleep spindles. Continuous wavelet transform (CWT) was used for time–frequency EEG analysis (Koronovskii and Hramov, 2003). The CWT, $W(s,\tau)$, was obtained by convolving the EEG signal, $x(t)$, with basis function (complex Morlet wavelet) (Eqs. (1) and (2))

$$W(s,\tau)=\frac{1}{\sqrt{s}}\int_{-\infty}^{+\infty}x(t)\phi_0^*\left(\frac{t-\tau}{s}\right)dt,\ \text{'*' denotes complex conjugation,} \tag{1}$$

where parameter s is the time scale (that corresponds to the 'wavelet' frequency, see below); τ is the time shift, and $\phi_0(\eta)$ is the basic complex function ('mother' wavelet) of wavelet transform. We used complex Morlet wavelet as the basis function (Eq. (2))

$$\phi_0=\frac{1}{\sqrt[4]{\pi}}e^{j\Omega\eta}e^{-\eta^2/2}, \tag{2}$$

where Ω is a central frequency of Morlet wavelet. Previously we found that parameter $\Omega=2\pi$ in the CWT provides an optimal time–frequency resolution of EEG signal (Sitnikova et al., 2009; Ovchinnikov et al., 2010; Pavlov et al., 2012). Here we used $\Omega=2\pi$, considering that this value of Ω simplifies relation between time scales of CWT, s, and Fourier frequencies, f, to $f\approx 1/s$ (for other Ω values, this formula is more complex). Hereafter we used the frequency f_s instead of the time scale s. Fig. 6 demonstrates two typical sleep spindles in EEG of WAG/Rij rat and corresponding Morlet-based wavelet spectrum.

The modulus of the CWT, $|W(f_s,t_0)|$, represents wavelet energy of the frequency f_s at the time moment t_0. Energy distribution $E(f_s)$ of $|W(f_s,t_0)|$ in the time interval h (t_0–$h/2$, t_0+$h/2$) represents wavelet power spectrum, which is similar to Fourier power spectrum:

$$E(f_s)=\frac{1}{h}\int_{t-h/2}^{t+h/2}|W(f_s,t')|^2dt', \tag{3}$$

where h is the duration of time interval in EEG with sleep spindles.

For the automatic detection of sleep spindles in EEG, we modified the algorithm used in our earlier studies (Sitnikova et al., 2009; Ovchinnikov et al., 2010, Pavlov et al., 2012) in order to achieve high selectivity and detect sleep spindles separately from epileptic spindle-like oscillations.

First, wavelet energy $w(t)$ was measured in the fixed spindle frequency band, $F\in(8,16)$ Hz (Fig. 6):

$$w(t)=\int_F|W(f_s,t)|^2df_s. \tag{4}$$

Second, wavelet power, $w(t)$, was averaged in time window, T. This was necessary to increase the accuracy and selectivity of automatic sleep spindle detections in EEG. This automatic algorithm displayed the best performance when T was set to 0.5 s that roughly corresponded to the length a sleep spindle.

$$\langle w(t)\rangle=\int_{t-T}^{t+T}w(t')dt' \tag{5}$$

Third, threshold level of wavelet power, w_{cr}, was empirically defined (Fig. 6). The presence of sleep spindles was detected if the averaged value $\langle w(t)\rangle$ in frequencies $F\in(8,16)$ Hz (Eqs. (4) and (5)) exceeded the threshold w_c: $\langle w(t)\rangle > w_{cr}$. This method provided 90–95% of true positive detections of sleep spindles among manually selected sleep spindles both in WAG/Rij and Wistar rats.

In order to determine the end point of sleep spindles, wavelet power of background EEG was averaged in the frequency band $F\in(8,16)$ Hz over the time period of 10 s, w_0. The value of w_0 was compared with the averaged wavelet power in the same band $F\in(8,16)$ Hz, $\langle w(t)\rangle$. Termination of sleep spindle events was assigned when $\langle w(t)\rangle < w_0$.

In raw EEG, sleep spindles were often preceded by a slow-wave component or K-complex (note slow waves in EEG preceding sleep spindles in Fig. 1). In wavelet power spectra in Fig. 5, a remarkable increase of wavelet power can be seen in 2–4 Hz just prior to sleep spindles. As known, slow waves and K-complexes play a role in triggering and synchronization of sleep spindle oscillations (Amzica and Steriade, 2002), and may also be involved in initiation of sleep-related seizures (refs in Halász, 2005). We found that slow-wave (<4 Hz) components often associated with sleep spindles, slow-wave EEG activity preceding sleep spindles was beyond the scope of the current study and we concentrated in spindle-specific frequencies.

Sleep spindles were localized in wavelet spectrum (Fig. 5, SS_1 and SS_2) as clearly defined elevation of wavelet power in frequency band between 8 and 16 Hz (maximum in 14–15 Hz). Frequency was very unsteady and varied across sleep spindles and during a spindle (see below), therefore, we introduced a necessary criterion for sleep spindles identification: instantaneous frequency stayed within 8–16 Hz frequency band for at least 0.3 s. Shorter spindle-like busts of

8–16 Hz (whose duration was less than 0.3 s) were excluded. We further examined instantaneous frequency of sleep spindles and its dynamics.

5.3. *Time–frequency analysis of sleep spindles*

After the CWT of automatically detected sleep spindles (Section 4.2), 'skeletons' of wavelet surfaces were constructed in order to extract dominant EEG frequencies and determine the evolution of instantaneous frequency during each spindle event. For this purpose, a distribution of instantaneous wavelet energy $E_i(f_s,t_0)=|W(f_s,t_0)|^2$ was calculated for the time moments t_0, and then the function $E_i(f_s, t_0)$ was examined for the presence of local maxima, $E_{max,k}$:

$$E_{max,k} = \max(E_i(f_s, t_0)) \quad (6)$$

If this function had several maxima, the highest maximum was assessed, and its frequency was considered as the dominant frequency of EEG at time moment t_0. Assuming that there is only one dominant frequency in EEG at the same time, this frequency was the initial point in the 'skeleton' plot (Fig. 2). The abovementioned procedure was repeated for the next time point. Skeletons of wavelet surfaces were constructed in 5 s time intervals containing sleep spindles (Fig. 2). Further analysis was concentrated in wavelet-based 'skeletons' of sleep spindles, but not in wavelet spectra (the latter provided redundant information), and data were processed automatically. In skeletons, both extreme values of the frequency at the beginning (f_1) and the end (f_2) of a spindle event were defined, and mean frequency, f_{mean}, was computed as:

$$f_{mean} = \frac{1}{h}\int_{t_1}^{t_1+h} f_b(t)dt, \quad (7)$$

where h is the duration of the sleep spindle; t_1 is the time of the beginning of sleep spindle.

Statistical analysis was performed by means of Generalized Linear Model (GLM) and non-parametric Wilcoxon matched pairs tests.

Acknowledgments

This study was supported by Russian Foundation for Basic Research (RFBR, projects № 13-04-00084 and 12-02-31544) and by the Ministry of Education and Science of Russian Federation (project SGTU-79). We thank Dr. Elizaveta Rutskova for the technical support and valuable assistance and Dr. Ivan N. Pigarev for providing electronic equipment. A.A.K. thanks "Dynasty" Foundation for financial support.

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