Neuroscience and Behavioral Physiology, Vol. 44, No. 3, March, 2014

Developmental Changes in the Frequency-Time Structure of Sleep Spindles on the EEG in Rats with a Genetic Predisposition to Absence Epilepsy (WAG/Rij)

E. Yu. Sitnikova,[1] V. V. Grubov,[2] A. E. Khramov,[2,3] and A. A. Koronovskii[2]

UDC 612.81+51-76+57.089

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 62, No. 6, pp. 733–744, November–December, 2012. Original article submitted March 27, 2012. Accepted October 19, 2012.

Sleep spindles are known to form spontaneously in the thalamocortical system during slow-wave sleep; pathological processes in the thalamocortical network may be the cause of absence epilepsy. The aim of the present work was to study developed changes in the frequency-time structure of sleep spindles during the progressive development of absence epilepsy in WAG/Rij rats. EEG recordings were made at age 5, 7, and 9 months using epidural electrodes implanted into the frontal cortex. Automatic recognition and subsequent analysis of sleep spindles on the EEG were performed using continuous wavelet transformation analysis. The duration of epileptic discharges and the total duration of epileptic activity were found to increase with age, while the duration of sleep spindles, conversely, decreased. In terms of mean frequency, sleep spindles could be divided into three classes: "slow" (mean frequency 9.3 Hz), "transitional" (11.4 Hz), and "fast" (13.5 Hz). Slow and transitional spindles in five-month-old animals showed increases in frequency from the beginning of the spindle to the end. The more intense the epilepsy, the shorter the durations of spindles of all types. The mean frequencies of "transitional" and "fast" spindles were higher in rats with more intense signs of epilepsy. Overall, high epileptic activity in WAG/Rij rats was linked with significant changes in spindles of the transitional type, with less marked changes in the two traditionally identified types of spindle, slow and fast.

Keywords: sleep spindles, genetic model of absence epilepsy, developmental changes in the EEG, rhythmic brain activity, frequency-time analysis of EEG, wavelet analysis, continuous wavelet transformation.

Sleep spindles represent one of the most typical patterns of rhythmic brain activity and appear on EEG traces during slow-wave sleep. Sleep spindles are surprisingly conserved in evolution: they are seen not only in the human EEG, but also in mammals and some reptiles [17]. It is well known that sleep spindles result from the rhythmic activity of the thalamocortical network in the state of sleep, when the thalamus temporarily ceases to pass external information on to the higher sectors of the analyzers in the cortex and starts to generate an autonomous rhythm with a frequency of 10–12 Hz. This rhythm propagates through the thalamocortical network and acquires the form of sleep spindles. As sleep spindles reflect the spontaneous rhythmic activity of the thalamocortical network, their frequency-time parameters can serve as an important criterion for the functional properties of the thalamocortical system and can be used for the early diagnosis of neurological diseases such as absence epilepsy, sensorimotor disorders, and sleep disturbances.

Functional impairments to the thalomocortical system (and, according to our recent data, changes in the microstructure of its cortical part) [4] are the main reason for the formation of absence epilepsy [14, 18, 22]. The pathogenesis of this disease is linked with neuron hyperexcitability in the neocortex, with the result that sleep spindles are trans-

[1] Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow; e-mail: eu.sitnikova@gmail.com.
[2] N. G. Chernyshevskii Saratov State University.
[3] Yu. A. Gagarin Saratov State Technical University.

0097-0549/14/4403-0301

formed into the peak-wave discharges typical of absence epilepsy [14]. This concept is widely recognized, despite weighty counterarguments [18, 22]. Direct studies of sleep spindles in patients with absence epilepsy have not produced unambiguous conclusions regarding changes in the structure of sleep spindles on development of this disease. The main focus of our study was on changes in the properties of sleep spindles as absence epilepsy progressed in WAG/Rij rats, which have a genetic predisposition to this disease [6, 7]. Epileptic discharges appear at age 3–5 months in most individuals of this strain and increase significantly with age [6], though convulsive activity does not develop in some individuals [19]. Our task was to follow age-related changes in sleep spindles during individual development in WAG/Rij rats allowing for the presence (or absence) of convulsive activity.

METHODS

Experiments were performed on six male WAG/Rij rats kept in standard animal-house conditions at the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences. Experiments were performed in compliance with the "Regulations for Studies Using Experimental Animals," No. 755 of August 12, 1977, and the "Regulations for Laboratory Practice in the Russian Federation," approved by Russian Federation Ministry of Health decree No. 267 of June 19, 2003.

EEG studies. EEG recordings were made with implanted electrodes. Electrodes were implanted at age 4.5 months under general anesthesia with chloral hydrate (4% chloral hydrate in 0.9% NaCl, i.p., 325 mg/kg). The recording electrode was placed epidurally in the right hemisphere in the region of the frontal cortex (AP 2, L 2.5 relative to the bregma) and the indifferent electrode was placed over the right hemisphere of the cerebellum.

The first EEG trace was made in animals aged five months after a two-week post-operative recovery period. Animals were then kept singly in cages. EEG traces were recorded again at ages seven months and nine months. EEG recordings were made using a Sagura multichannel computerized electroencephalograph and the Leonardo program (MKE Medizintechnik GmbH, Germany). EEG signals were recorded in the frequency band 0.5–200 Hz with a sampling frequency of 576 Hz/channel. Trace duration was 20 h.

The epileptic status on each animal was established in terms of the presence of peak-wave discharges, which serve as EEG markers of absence epilepsy [16]. Peak-wave discharges on EEG traces were identified using the standard criteria described for WAG/Rij rats [5, 6]. The number and duration of peak-wave discharges were determined and the total duration of convulsive activity was measured for 6 h during the dark period of the day, when epileptic activity in WAG/Rij rats is maximal [10].

EEG wavelet analysis. Frequency-time analysis was performed by continuous wavelet transformation $W(s, \tau)$ by convolution of the experimental EEG signal $x(t)$ with a time-limiting (local) basic function $\varphi_{s,\tau}(t)$ [3].

$$W(s, \tau) = \int_{-\infty}^{+\infty} x(t)\varphi_{s,\tau}{}^{*}(t)dt, \quad (1)$$

where * indicates a complex conjugation. The basic function $\varphi_{s,\tau}(t)$ is formed by the mother wavelet with the following transformation:

$$\varphi_{s,\tau}(t) = \frac{1}{\sqrt{s}}\varphi_0\left(\frac{t-\tau}{s}\right), \quad (2)$$

where s is the time scale determining expansion or compression of the maternal function, τ is the time shift of the wavelet transformation, and φ_0 is the mother wavelet. Instead of the time scale s, it is convenient to use frequency $f_s = 1/f$, by analogy with the frequencies of the Fourier spectrum.

The characteristics of the frequency dynamics of the signal at a given point in time t_{fix} were identified using the instantaneous distribution of signal amplitude on the wavelet spectrum, which is an analog of the spectrum obtained using a Fourier window transformation

$$E(f_s) = \left| W(f_s, t = t_{\text{fix}}) \right|. \quad (3)$$

We have previously shown [21] that complex Morley wavelets are optimal for frequency-time analysis of sleep spindles

$$\phi(\eta) = \frac{1}{\sqrt[4]{\pi}} e^{j\omega_0\eta} e^{\eta^2/2} \quad (4)$$

with a central frequency $\omega_0 = 2\pi$, which provides a maximally balanced frequency-time signal representation. Thus, sleep spindles on the wavelet surface are well localized in the time and frequency areas and automatic analysis of changes in the frequency and amplitude of EEG oscillations during sleep spindles is possible.

The method for automatic extraction of sleep spindles on the EEG was based on analysis of the distribution of energy in the wavelet spectrum, as described in [2, 20]. The time series studied (the EEG) was subjected to wavelet transformation followed by calculation of the instantaneous energy of transformation $w(t)$ in the characteristic frequency range Fs.

$$w(\tau) = \int_{Fs} \left| W_{s,\tau} \right| ds. \quad (5)$$

Allowing for the characteristics of the frequency composition of sleep spindles, they were identified using two frequency ranges $F_{s1} \in$ [5–9] Hz and $F_{s2} \in$ [10–16] Hz. The conclusion that the EEG included sleep spindles was reached on the basis of analysis of energies $w_1(\tau)$ and $w_2(\tau)$ in each of the ranges F_{s1} and F_{s2}, where the magnitudes of $w_1(\tau)$ and $w_2(\tau)$ were greater than threshold values $w_{1\text{thr}}$ and

w_{2thr} (for the first and second ranges, respectively) and the integral value for energy in the second frequency range was greater than the energy in the first frequency range.

$$w_1(\tau) > w_{1thr} \wedge w_2(\tau) > w_{2thr} \wedge w_2(\tau) > w_1(\tau). \quad (6)$$

A distinguishing features of wavelet transformation of EEG signals containing sleep spindles is the strong discontinuity of the energy function of the wavelet transformation $w(\tau)$ (5) with respect to time, resulting in oscillations in amplitude and frequency within the sleep spindle. Increases in the accuracy of automatic recognition of spindles were therefore obtained in Equations (5) and (6), comparisons of the magnitudes of w_1 and w_2 with threshold values w_{1thr} and w_{2thr} were made using not the instantaneous values of energy transformation (5), but values averaged for some period of time

$$\langle w(t) \rangle = \frac{1}{T} \int_T w(t)\, dt. \quad (7)$$

Numerical analysis showed that the optimum width of the window for decreasing the effects of short artifacts on spindle recognition quality was $T = 0.5$ sec.

The moment at which sleep spindles ceased was identified by comparing the wavelet energy with the energy of the baseline EEG

$$w_1(\tau) < w_0 \wedge w_2(\tau) < w_0, \quad (8)$$

where w_0 is the mean power of the wavelet spectrum calculated for the baseline EEG in the frequency range of interest. Use of condition (8) avoids the erroneous detection of multiple "short" sleep spindles instead of a single long spindle.

Automatic recognition and further analysis of sleep spindles were performed using EEG intervals of duration 3–10 min recorded during slow-wave sleep with sleep spindles. The frequency-time parameters of 20 sleep spindles were studied in each of six experimental animals, at ages five, seven, and nine months.

Data were analyzed statistically using nonparametric analysis methods: the paired Wilcoxon test (for linked sets) and the Mann–Whitney test for unlinked sets, along with factor analysis based on a general linear model (GLM), allowing the existence of individual trends in the development of sleep spindles to be followed, and the Shapiro–Wilk test for normality.

RESULTS

This study used EEG traces recorded from the frontal cortex, as this area is known to have the highest-amplitude sleep spindles in rats [8, 11]. The instantaneous frequency of sleep spindles varied over the range 7.5–17 Hz. Figure 1 shows an EEG fragment and a "skeleton" wavelet spectrum (1) constructed using the baseline Morley wavelet. The skeleton was constructed be seeking the local extremes of the function $E(f)$ (3) for fixed values of t at varying frequency f, i.e., by seeking the local maxima on the wavelet surface $|W(t, f)|$. This Figure also allows tracking of the dynamics of local maxima of the wavelet spectrum corresponding to sleep spindles. This shows that the frequency changed during each spindle, in most cases increasing from the beginning to the end of the spindle (Fig. 1, left). At the same time, there were some spindles for which frequency decreased over time (Fig. 1, right).

Statistical analysis of the frequency composition of sleep spindles was performed by identifying instantaneous frequencies at the beginning and end of the spindle (f_1 and f_2) and the mean frequency (f). Frequencies f_1 and f_2 corresponded to the most intense frequencies in the wavelet spectrum (shown on the skeleton in Fig. 1), at the beginning and end respectively of the sleep spindle being analyzed. Sleep spindle beginnings were defined in accordance with the energy criteria (6) and the end in accordance with criterion (8), allowing formal definition of the sleep spindles parameters of interest.

Developmental changes in the frequency-time parameters of sleep spindles. The distributions of the mean frequency of sleep spindles f at ages five and nine months were significantly different from normal (Shapiro–Wilk test, in both cases $p < 0.01$). Considering the variability of the mean frequency and the nature of its distribution, sleep spindles were arbitrarily divided into three groups, with mean frequencies located in the frequency ranges 8–10 Hz, 10–12 Hz, and 12–14 Hz (Fig. 2). An analogous criterion, i.e., the frequency of the main spectral component, was used in classical studies [12, 13, and others] to separate sleep spindles in the human EEG into two classes of 12(11) Hz and 14(13) Hz and in rats, where sleep spindles were divided into two types – with frequencies of 9–10 Hz and ~12 Hz [11]. In our case, we identified a third, "transitional," type of spindle, with a frequency of 10–12 Hz which, in terms of the frequency criterion, was significantly different from the other two traditionally identified types of sleep spindles. This group of sleep spindles has the most interesting frequency dynamics, with changes during ontogeny and a high level of dependence on the intensity of epileptic manifestations (see below).

As shown in Table 1, the three types of sleep spindle differed not only in terms of the mean frequency (f), but also in terms of the values of the instantaneous frequencies at the beginning and end of the spindle (f_1 and f_2). Differences between "slow," "transitional," and "fast" spindles in terms of f, f_1, and f_2 were statistically significant (Wilcoxon test, $p < 0.01$) in all age groups.

Half of the sleep spindles analyzed (~50%) were of the "slow" type. "Transitional" and "fast" spindles were present in essentially equal proportions of about a quarter of the total number of spindles. The ration of the three types of sleep spindle showed virtually no change with age.

Developmental changes also affected the internal parameters of spindles. Firstly, there was a significant reduction

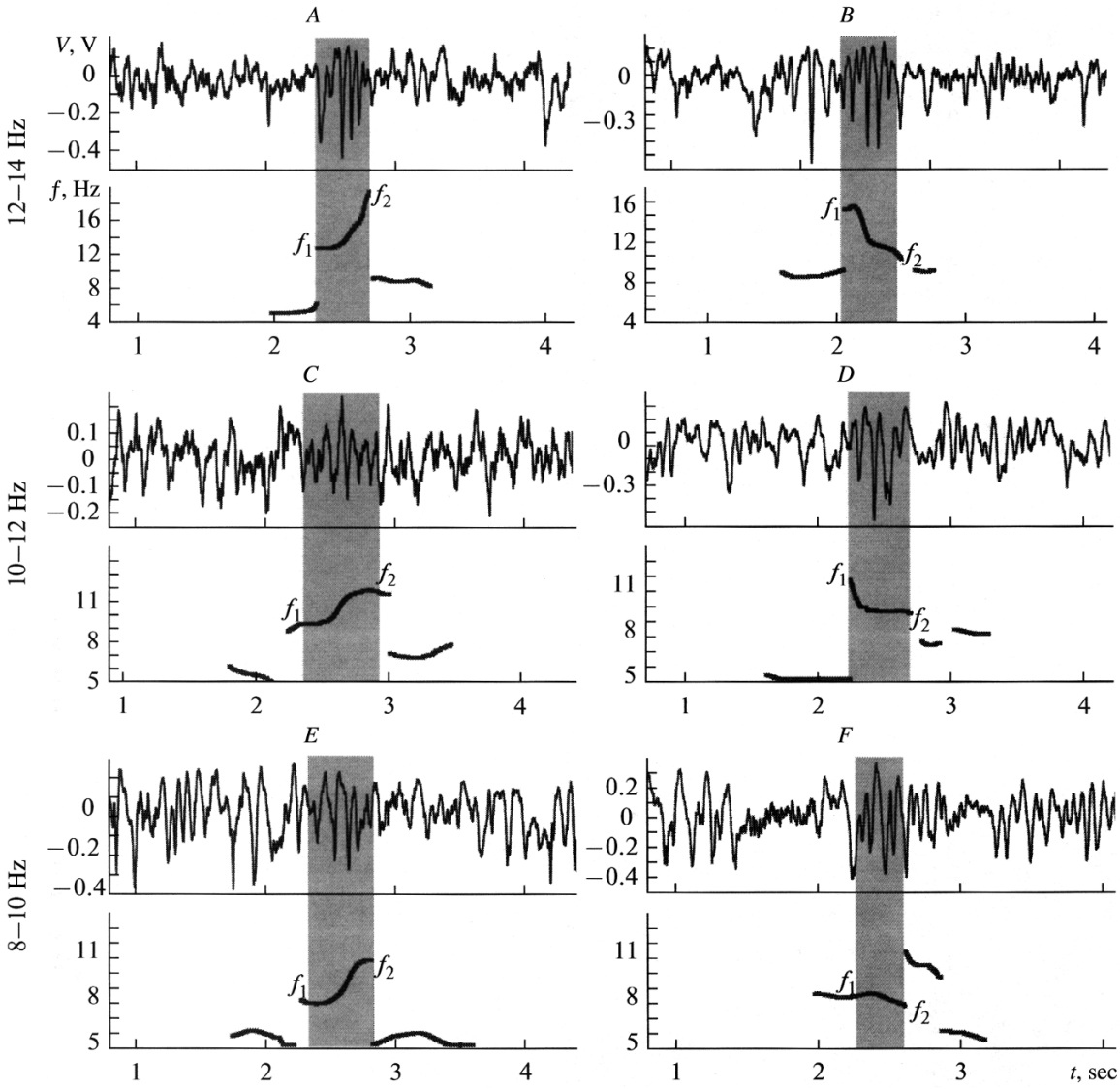

Fig. 1. EEG sleep spindles and corresponding "skeletons" of the wavelet surface: *A*, *B*) "fast" spindles (12–14 Hz), showing increases (*A*) and decreases (*B*) in frequency during the spindle; *C*, *D*) "transitional" spindles (10–12 Hz); *E*, *F*) "slow" spindles (8–10 Hz). Each point on the "skeleton" of the wavelet surface corresponds to a peak on the wavelet spectrum.

in the total duration of sleep spindles (GLM, $F_{2,340} = 24.5$, $p < 0.0001$). In animals aged five months, spindle duration averaged 597 ± 398 msec (here and henceforth, ± standard deviation) and decreased significantly to 382 ± 116 msec by age seven months (post hoc Fisher test, $p < 0.0001$) and remained unaltered to age nine months at 366 ± 86 msec. The durations of the three types of sleep spindles were significantly different (GLM, $F_{2,340} = 7.1$, $p < 0.001$), in accordance with the following rule (at all ages): the duration of "slow" spindles was significantly greater than that of "transitional" (post hoc $p < 0.0001$) and "fast" spindles ($p < 0.002$). The latter two types of spindle showed no difference in duration.

Secondly, "slow" and "transitional" spindles in animals aged five months showed increases in the frequency from the beginning to the end of the spindle: in "slow" spindles, frequency during the spindle increased by an average of 0.49 Hz, compared with an increase by 2.66 Hz in "transitional" spindles (Table 1, note). Rats aged seven and nine months did not show such clear frequency dynamics within sleep spindles, though the opposite tendency was seen in "transitional" spindles, with a decrease in frequency from the beginning to the end of the spindle. The absence of any statistically significant change in frequency during the spindle in animals aged seven and nine months is linked with

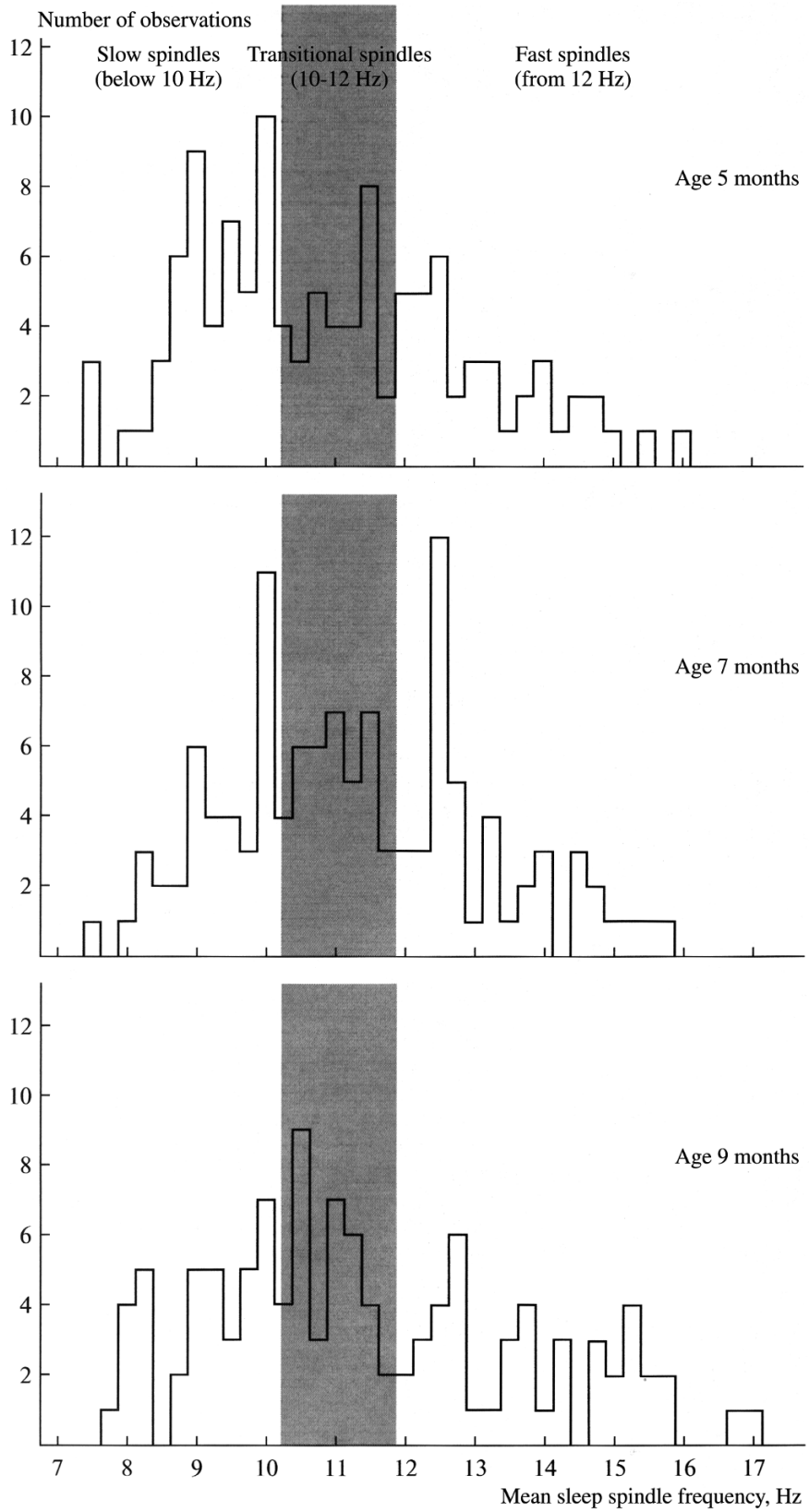

Fig. 2. Histograms showing the distribution of mean sleep spindle frequency in WAG/Rij rats plotted with a bin width of 0.25 Hz (n = 6 rats in each age group). In animals aged five and nine months, the distributions of mean frequencies were significantly different from normal.

TABLE 1. Main Characteristics of the Three Types of Sleep Spindles in WAG/Rij Rats (n = 6 rats in each age group)

Type of sleep spindle	Proportions of the three spindle types, %	Spindle duration, msec	Sleep spindle frequency, Hz		
			mean, f	at beginning of spindle, f_1	at end of spindle, f_2
Age 5 months					
Slow (8–10 Hz)	50.1 ± 13.4	645 ± 432	9.3 ± 0.8	9.1 ± 0.9	9.6 ± 0.9*
Transitional (10–12 Hz)	26.5 ± 7.2	627 ± 476	11.4 ± 0.7	10.0 ±1.4	12.7 ± 1.5*
Fast (12–14 Hz)	23.3 ± 13.0	441 ± 142	13.5 ± 1.0	13.4 ±1.2	13.6 ± 1.4
Age 7 months					
Slow (8–10 Hz)	50.0 ± 19.0	403 ± 120	9.7 ± 0.9	9.7 ±1.1	9.7 ± 0.9
Transitional (10–12 Hz)	17.5 ± 9.4	395 ± 107	11.4 ± 0.5	11.5 ± 1.4	11.2 ± 1.5
Fast (12–14 Hz)	32.9 ± 11.1	338 ± 102	13.3 ± 1.0	13.3 ±1.2	13.3 ± 1.1
Age 9 months					
Slow (8–10 Hz)	50.4 ± 15.5	396 ± 81	9.5 ± 1.0	9.4 ±1.1	9.6 ± 1.2
Transitional (10–12 Hz)	20.2 ± 5.8	350 ± 80	11.5 ± 0.6	11.7 ±1.0	11.2 ± 1.0
Fast (12–14 Hz)	28.3 ± 16.0	320 ± 68	14.1 ± 1.2	13.9 ±1.5	14.3 ± 1.2

Notes. At age five months the difference between f_2 and f_1 was statistically significant for slow spindles and transitional spindles (Wilcoxon test, $*p < 0.01$).

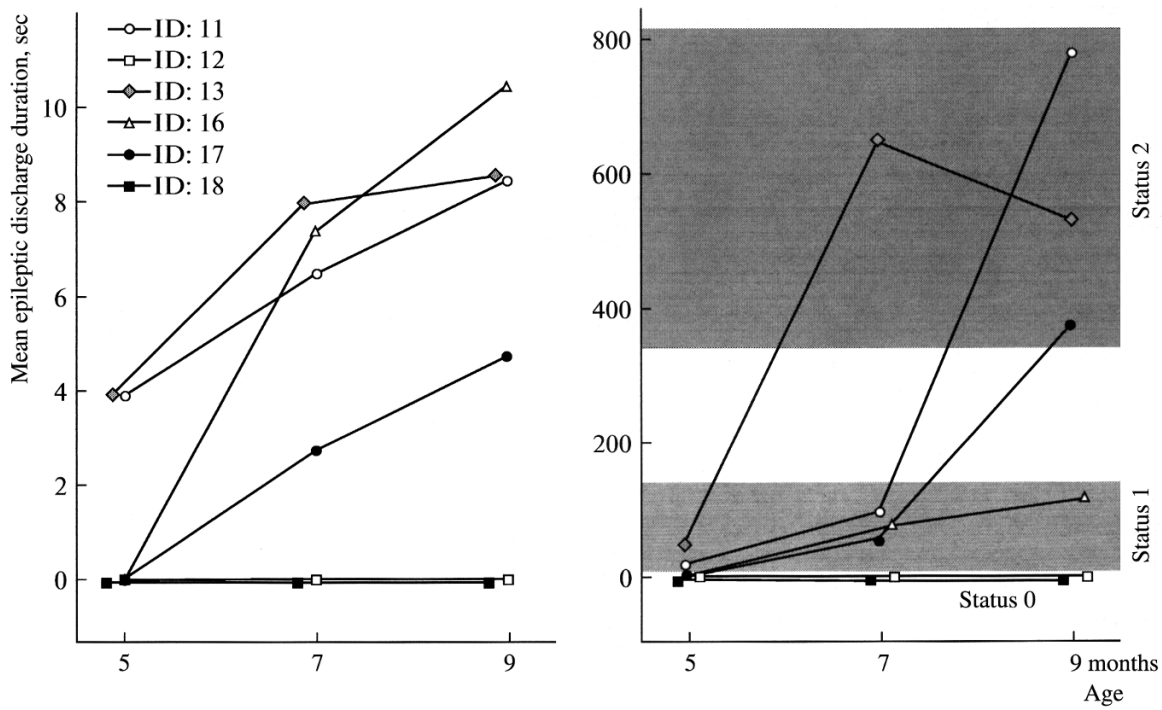

Fig. 3. Individual data on the developmental dynamics of the duration of epileptic discharges on the EEG and total duration of epileptic activity (ID – animal identification No.). The duration of peak-wave discharges increases progressively with age. In the shaded areas, darkness illustrates the animals' epileptic "status," determined in terms of the total duration of epileptic activity and the number of peak-wave discharges. For explanation see text.

the decreases in total spindle duration at this age, such that the changes in frequency during such a short period of time were less significant.

Relationship between the frequency-time parameters of sleep spindles and the intensity of absence epilepsy. At age five months, peak-wave discharges were seen in two of the six experimental animals, while at ages seven and nine months they were seen in four animals. Convulsive activity was not seen in the other two animals throughout the whole of the study period. Quantitative parameters of

TABLE 2. Main Characteristics of Sleep Spindles in WAG/Rij Rats with Different Episode Status

	Type of sleep spindle	Status 0	Status 1	Status 2
Percentage ratio	Slow (8–10 Hz)	45.4 ± 12.8	55.9 ± 13.0	51.1 ± 15.7
	Transitional (10–12 Hz)	22.8 ± 5.6	21.3 ± 12.3	16.9 ± 2.2 *
	Fast (12–14 Hz)	30.8 ± 11.4	22.8 ± 13.5	32.0 ± 16.3
Mean duration, sec	Slow (8–10 Hz)	599 ± 371	430 ± 157 [a]	369 ± 78 [a]
	Transitional (10–12 Hz)	555 ± 416	381 ± 93 [a]	330 ± 95 [a]
	Fast (12–14 Hz)	390 ± 125	354 ± 99 [a]	264 ± 50 [a]
Mean frequency, f, Hz	Slow (8–10 Hz)	9.5 ± 0.9	9.5 ± 0.9	9.6 ± 1.0
	Transitional (10–12 Hz)	11.4 ± 0.7	11.5 ± 0.4	11.1 ± 0.6 [b]
	Fast (12–14 Hz)	13.7 ± 1.1	13.2 ± 0.9	14.0 ± 1.37 [b]
Dynamics of frequency	Slow (8–10 Hz)	0.25 ± 1.15	0.15 ± 1.00	0.42 ± 1.09
within spindles, $f_2 - f_1$, Hz	Transitional (10–12 Hz)	1.58 ± 2.98	0.24 ± 2.43	−0.67 ± 1.5 [c]
	Fast (12–14 Hz)	0.19 ± 1.39	0.27 ± 1.48	0.34 ± 1.37

Notes. The relative numbers of transitional spindles with "status 2" was lower than that in non-epileptic animals with "status 0" (Mann–Whitney test, $*p < 0.05$). [a] Decrease in duration of all types of spindles with increase in epileptic status: differences between "status 0" and "1" were statistically significant ($p < 0.005$), as were those between "status 1" and "2" ($p < 0.001$), "status 0" and "2" ($p < 0.0001$). [b] The mean frequencies of transitional spindles in rats with "status 1" and "status 2" were significantly different ($p < 0.05$), as was the mean frequency of "fast" spindles ($p < 0.05$). [c] Changes in frequency $f_2 - f_1$ within transitional spindles in non-epileptic rats ("status 0" were significantly different from those in rats with "status 2" ($p < 0.01$).

epileptic activity on the EEG were used as formal criteria for identifying the following categories characterizing the intensities of epileptic discharges (Fig. 3): "status 0" – epileptic activity not detected, "status 1" – low intensity of epileptic activity, total duration 19–146 sec over 6 h, number of discharges 4–19 during this time; "status 2" – a high level of epileptic activity, with a total duration of 290–783 sec over the 6-h period and 59–93 discharges during this time. Animals of a given age could have epileptic "status" which varied depending on the intensity of convulsive activity; epileptic "status" changed with age, showing increases (except in the two individuals without signs of epilepsy).

Discharge duration increased significantly with age (GLM,* $F_{2,8} = 10.5$, $p < 0.006$), while the total duration of epileptic activity also increased significantly (GLM,* $F_{2,8} = 4.2$, $p < 0.05$).

Our studies showed that the more intense the epilepsy, i.e., the greater the epileptic status, the shorter the durations of spindles of all three types (Table 2, note, *a*). The relative number of "transitional" spindles in animals with null status was greater than that in rats with "status 2" (Table 2, note). The frequency-time characteristics of spindles showed the following features: 1) the mean frequencies of "transitional" and "fast" spindles in rats with "status 1" were greater than in rats with "status 2" (Table 2, note, *b*); 2) the dynamics of intraspindle frequency in spindles of the "transitional" type (value $f_2 - f_1$) changed sign when epileptic status increased: spindle frequency increased from the beginning to the end of the spindle in rats with null status and decreased in those with "status 2."

DISCUSSION

This report describes the frequency-time structure of sleep spindles and identified patterns determining changes in the internal dynamics of the frequencies of sleep spindles during ontogeny in rats with genetic predisposition to absence epilepsy.

Guided by the criterion of the frequency of the main component of sleep spindles, in the present study we discriminated "slow" (8–10 Hz) and "fast" (12–14 Hz) spindles, which belonged to the two types traditionally identified on EEG recordings from humans and animals [8, 11]. Spindles with a frequency range of 10–12 Hz formed a separate class of "transitional" spindles. The ratio of the three types of sleep spindle (slow:fast:transitional spindles) showed virtually no change with age and was 2:1:1. The duration of sleep spindles decreased significantly with age. The decrease in spindle duration appears to be associated with the progressive development of epilepsy in our animals. Our data indicate that the stronger the signs of epilepsy (the greater the epileptic "status"), the short the duration of spindles. It is possible that the thalamocortical system loses the ability to maintain the normal spindle rhythm, leading to a decrease in sleep spindle duration.

* Analysis was performed using data from animals with non-null epileptic status.

Despite the fact that the pathogenesis of absence epilepsy is linked with impairments to the thalamocortical system, the literature contains virtually no data on the possible influences of this pathology on the formation of the normal thalamocortical rhythm – sleep spindles. In patients with epilepsy with primary generalized peak-wave activity on the EEG (pediatric, juvenile, monoclonal absence epilepsy respectively, diagnoses CAE, JAE, MAE), the frequency of sleep spindles during phase II of slow sleep is 12.87 Hz, which is significantly higher than that in healthy subjects – 12.21 Hz [15]. This is consistent with our data on "fast" spindles: their frequency in rats with high epileptic status (14.0 Hz) was higher than in animals with low epileptic status (13.2 Hz).

Overall, our results make a significant addition to the pattern of qualitative changes to sleep-spindle activity in absence epilepsy. Thus, our data indicate that the clearest changes linked with the intensity of epilepsy are seen in spindles of the "transitional" type with a frequency of 10–12 Hz.

Firstly, in comparison with animals without signs of epilepsy, rats with high epileptic status had a smaller number of "transitional" spindles; their mean frequency was also lower – 11.1 Hz in rats with "status 2" versus 11.4 Hz in animals with "status 0." At high levels of epilepsy, the thalamocortical network appears to be less inclined to maintain spindle-shaped oscillations at the transitional frequency of 10–12 Hz, demonstrating a tendency to decreases in the frequency and total number of such oscillations.

Secondly, the dynamics of the frequency within "transitional" spindles changed to its opposite as the intensity of epilepsy increased: in rats with null status, the instantaneous frequency increased from the beginning to the end of the spindle, while the frequency decreased in rats with "status 2". This property, i.e., a decrease in frequency from the beginning to the end of the oscillations, is typical for epileptic peak-wave discharges, whose frequency was 12–16 Hz at the beginning of the discharge, decreasing to 5–7 Hz at the end [1, 21]). A similar pattern in sleep spindles of the "transitional" type may be associated with "epileptization" of sleep-spindle activity due to impairments in the thalamocortical system in the progressive development of absence epilepsy. It may be that some "transitional" (10–12 Hz) sleep spindles are replaced by peak-wave discharges, whose frequency is also 10–12 Hz at the beginning of the epileptic discharge, decreasing to 5–7 Hz at the end.

Studies of the internal dynamics of the frequency during sleep spindles constitute a complex technical task which cannot be solved using traditional EEG spectral analysis methods because of their low time resolution. Positive changes in the frequency during the sleep spindle, i.e., increases in the main frequency from its beginning to its end, were first observed by ourselves in the EEG of WAG/Rij rats aged five months using continuous wavelet transformation of the EEG, where the basic function was a complex Morley wavelet ($\Omega = 2\pi$) [2].

A topographical analysis of the distribution of the power levels of the spectral components of sleep spindles with frequencies of 12 and 14 Hz in humans was reported very recently [9]. In particular, the peak of the 12-Hz components was found to be displaced from the frontal to the central areas during the sleep spindle, while the 14-Hz component was displaced in the opposite direction; the power of the 12-Hz component increased from the beginning to the end of the spindle, while that of the 14-Hz component, conversely, decreased. Thus, the internal dynamics of sleep spindle frequency in humans was complex, due not so much to power changes as to topographical changes. The internal dynamics of sleep spindles in humans (displacement of peak power from 14 Hz at the beginning to 12 Hz at the end of the spindle) are opposite to those most clearly apparent at age five months in our animals – a decrease in frequency during the sleep spindle. A possible explanation for this divergence is that we did not investigate the topography of individual components, but only their dynamics in a single local area. Furthermore, the authors of [9], in contrast to ourselves, 1) worked with healthy subjects, without any neurological pathology; 2) did not employ wavelets for automatic extraction of sleep spindles and describing their frequency-time structure; 3) analyzed spindles of average duration 721 msec (427–1386 msec).

In our studies, use of complex Morley wavelets ($\Omega = 2\pi$) as the basic function yielded an accurate representation of the structure of sleep spindles in time. This allowed us to work with spindles using a minimum duration of 300 msec, i.e., 3–4 cycles of oscillations at a frequency of 9–12 Hz, which is sufficient for analysis of the frequency zone of interest here in wavelet space.

CONCLUSIONS

The studies reported here used continuous wavelet transformation for the automatic recognition and subsequent analysis of the frequency-time structure of sleep spindles on the EEG in WAG/Rij rats, which have an inherited predisposition to absence epilepsy. In terms of mean frequency, sleep spindles were divided into three groups: "slow" (mean frequency 9.3 Hz), "transitional" (11.4 Hz), and "fast" (13.5 Hz); the ratio between these groups was 2:1:1 and this showed virtually no change with age. "Slow" and "transitional" spindles in animals aged five months were characterized by an increase in frequency from the beginning to the end of the spindle.

The stronger the signs of epilepsy (the greater the "status"), the shorter-lived the sleep spindle. The parameters of "transitional" spindles with a frequency of 10–12 Hz showed the strongest dependence on the intensity of epileptic manifestations: in rats with high epileptic status, the relative number of spindles of this type was lower than that in

animals with low epileptic status and their mean frequencies were also lower.

Overall, high epileptic status in WAG/Rij rats had quite minor effects on the structure of sleep spindles belonging to the two traditionally defined types, with frequencies of 8–10 and 12–14 Hz, but had significant influences on the quantitative and qualitative parameters of spindles of the "transitional" type, with a frequency of 10–12 Hz.

The authors would like to thank E. M. Rutskova (Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences) for technical support during EEG recordings.

This study was supported by the Russian Foundation for Basic Research (Grant No. 12-02-00221) and the Federal Targeted Program on Scientific and Teaching Staff for an Innovative Russia in 2009–2013.

REFERENCES

1. A. V. Gabova, D. Yu. Bosnyakova, M. S. Bosnyakov, et al., "Frequency-time structure of peak-wave discharges in genetic absence epilepsy," *Dokl. Ros. Akad. Nauk.*, **396**, No. 4, 557–560 (2004).
2. V. V. Grubov, A. A. Ovchinnikov, E. Yu. Sitnikova, et al., "Wavelet analysis of sleep spindles on the EEG and development of a method for their automatic diagnosis" *Izv. Vuz. Prikl. Nelin. Dinamika*, **19**, No. 4, 91–108 (2011).
3. A. A. Koronovskii and A. E. Khramov, *Continuous Wavelet Analysis and Its Applications* Fizmalit, Moscow (2003).
4. S. N. Kulikova, E. Yu. Sitnikova, and V. V. Raevskii, "Cytomorphological characteristics of the cerebral cortex in rats with an inherited predisposition to absence epilepsy," *Zh. Vyssh. Nerv. Deyat.*, **59**, No. 4, 506–512 (2009).
5. I. S. Midzyanovskaya, "Two types of 'peak-wave'-discharges on the electrocorticogram of WAG/Rij rats, a genetic model of absence epilepsy," *Zh. Vyssh. Nerv. Deyat.*, **49**, No. 5, 855–859 (1999).
6. A. M. Coenen and E. L. van Luijtelaar, "The WAG/Rij rat model for absence epilepsy: age and sex factors," *Epilepsy Res.*, **1**, No. 5, 297–301 (1987).
7. A. M. L. Coenen and E. L. J. M. van Luijtelaar, "Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats," *Behav. Genet.*, **33**, 635–655 (2003).
8. L. De Gennaro and M. Ferrara, "Sleep spindles: an overview," *Sleep Med. Rev.*, **7**, No. 5, 423–440 (2003).
9. N. Dehghani, S. S. Cash, and E. Halgren, "Topographical frequency dynamics within EEG and MEG sleep spindles," *Clin. Neurophysiol.*, **122**, No. 2, 229–235 (2011).
10. W. H. Drinkenburg, A. M. Coenen, J. M. Vossen, and E. L. van Luijtelaar, "Spike-wave discharges and sleep-wake states in rats with absence epilepsy," *Epilepsy Res.*, **9**, No. 3, 218–224 (1991).
11. G. Gandolfo, L. Glin, and C. Gottesman, "Study of sleep spindles in the rat: a new improvement," *Acta Neurobiol. Exp. (Warsaw)*, **45**, No. 5–6, 151–162 (1985).
12. W. R. Jankel and E. Niedermeyer, "Sleep spindles," *J. Clin. Neurophysiol.*, **2**, No. 1, 1–35 (1985).
13. M. Jobert, E. Poiseau, P. Jähnig, et al., "Topographical analysis of sleep spindle activity," *Neuropsychobiology*, **26**, No. 4, 210–217 (1992).
14. G. K. Kostopoulos, "Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis," *Clin. Neurophysiol.*, **111**, Suppl. 2, S27–S38 (2000).
15. I. Myatchin and L. Lagae, "Sleep spindle abnormalities in children with generalized spike-wave discharges," *Pediatr. Neurol.*, **36**, No. 2, 106–111 (2007).
16. C. P. Panayiotopoulos, "Typical absence seizures and their treatment," *Arch. Dis. Child.*, **81**, 351–355 (1999).
17. R. V. Rial, M. Akaârir, A. Gamundí, et al., "Evolution of wakefulness, sleep and hibernation: from reptiles to mammals," *Neurosci. Biobehav. Rev.*, **34**, No. 8, 1144–1160 (2010).
18. E. Sitnikova, "Thalamo-cortical mechanisms of sleep spindles and spike-wave discharges in rat model of absence epilepsy (a review)," *Epilepsy Res.*, **89**, No. 1, 17–26 (2010).
19. E. Sitnikova, "Neonatal sensory deprivation promotes development of absence seizures in adult rats with genetic predisposition to epilepsy," *Brain Res.*, **1377**, 109–118 (2011).
20. E. Sitnikova, A. E. Hramov, V. V. Grubov, et al., "On-off intermittency of thalamo-cortical oscillations in the electroencephalogram of rats with genetic predisposition to absence epilepsy," *Brain Res.*, **1436**, 147–156 (2012).
21. E. Sitnikova, A. E. Hramov, A. A. Koronovskii, and G. van Luijtelaar, "Sleep spindles and spike-wave discharges in EEG: their generic features, similarities and distinctions disclosed with Fourier transform and continuous wavelet analysis," *J. Neurosci. Meth.*, **180**, 304–316 (2009).
22. G. van Luijtelaar and E. Sitnikova, "Global and focal aspects of absence epilepsy: the contribution of genetic models," *Neurosci. Biobehav. Res.*, **30**, No. 7, 983–1003 (2006).