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Slow-wave activity preceding the onset of 10–15-Hz sleep spindles and 5–9-Hz oscillations in electroencephalograms in rats with and without absence seizures

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Abstract

Cortico-thalamocortical networks generate sleep spindles and slow waves during non-rapid eye movement sleep, as well as paroxysmal spike-wave discharges (i.e. electroencephalogram manifestation of absence epilepsy) and 5–9-Hz oscillations in genetic rat models (i.e. pro-epileptic activity). Absence epilepsy is a disorder of the thalamocortical network. We tested a hypothesis that absence epilepsy associates with changes in the slow-wave activity before the onset of sleep spindles and pro-epileptic 5–9-Hz oscillations. The study was performed in the WAG/Rij genetic rat model of absence epilepsy and Wistar rats at the age of 9–12 months. Electroencephalograms were recorded with epidural electrodes from the anterior cortex. Sleep spindles (10–15 Hz), 5–9-Hz oscillations and their slow-wave (2–7 Hz) precursors were automatically detected and analysed using continuous wavelet transform. Subjects with electroencephalogram seizures (the "epileptic" phenotype) and without seizure activity (the "non-epileptic" phenotype) were identified in both strains. It was found that time–amplitude features of sleep spindles and 5–9-Hz oscillations were similar in both rat strains and in both phenotypes. Sleep spindles in "epileptic" rats were more often preceded by the slow-wave (~4 Hz) activity than in "non-epileptic" rats. The intrinsic frequency of slow-wave precursors of sleep spindles and 5–9-Hz oscillations in "epileptic" rats was 1–1.5 Hz higher than in "non-epileptic" rats. In general, our results indicated that absence epilepsy associated with: (a) the reinforcement of slow waves immediately prior to normal sleep spindles; and (b) weakening of amplitude growth in transition "slow wave → spindle/5–9-Hz oscillation".

KEYWORDS
absence epilepsy, anterior electroencephalogram oscillations, continuous wavelet transform, rat model, time–frequency electroencephalogram analysis

1 | INTRODUCTION

Non-rapid eye movement (NREM) sleep is characterized by the presence of sleep spindles (with frequency about 10–15 Hz) and delta waves (1–4 Hz) in electroencephalograms (EEGs) in humans and all mammal species (De Gennaro & Ferrara, 2003; Lüthi, 2013). Both sleep spindles and delta waves are generated by the thalamocortical circuitry, and are heavily controlled by the neocortex (Lüthi, 2013; McCormick & Bal, 1997; Steriade, McCormick, & Sejnowski, 1993; Steriade, 2006). The slow (< 1 Hz) rhythm and K-complexes

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are considered as precursors of sleep spindles and delta waves during NREM (Crunelli & Hughes, 2010). Slow oscillations (0.1–4 Hz) are generated in the widespread neocortical neuronal networks (Crunelli & Hughes, 2010; Niethard, Ngo, Ehrlich, & Born, 2018). In vivo studies in animals indicated that slow-wave oscillations are predominantly originated from the infragranular cortical layers (Chauvette, Volgushev, & Timofeev, 2010; Fiáth et al., 2016). In rats with a genetic predisposition to absence epilepsy, infragranular neurons in the somatosensory cortex appeared to be hyperexcitable (D'Antuono et al., 2006; Polack et al., 2007) and they are involved in initiating epileptic spike-wave discharges (D'Antuono et al., 2006; Lüttjohann & van Luijtelaar, 2012). In Genetic Absence Epilepsy Rats from Strasbourg (GAERS), epileptic spike-wave discharges are known to develop from the medium-voltage 5–9-Hz oscillations (Pinault, Slezia, & Acsady, 2006; Pinault, Vergnes, & Marescaux, 2001); a possible synaptic pacemaker of 5–9-Hz oscillations appeared in the somatosensory cortex (layer VI) (Pinault, 2003). In contrast, spindle-like oscillations functionally associated with delta waves (1–4 Hz), but not with spike-wave discharges (Pinault et al., 2001). This implies that: (a) absence epilepsy might link with modifications in < 10 Hz rhythmic activity in EEG caused by cortical abnormalities; (b) the presumed modifications of < 10 Hz activity might associate with the occurrence of 5–9-Hz oscillations, but not with the occurrence of sleep spindles.

Absence seizures in genetic rat models usually occur during rest immobility and during NREM sleep. They are not accompanied by gross changes in behaviour, and EEG monitoring is the only way to detect absence seizures in the form of spike-wave discharges (Coenen & van Luijtelaar, 2003; Vergnes, Marescaux, Depaulis, Micheletti, & Warter, 1986). In the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rat model of absence epilepsy, the low-frequency band of cortical EEG was found to differ from that in healthy control. First, symptomatic WAG/Rij rats showed lower spectral EEG power in 3–6 Hz in the frontal cortex during NREM sleep than asymptomatic WAG/Rij rats (Sitnikova, Hramov, Grubov, & Koronovsky, 2016). Second, epileptic WAG/Rij rats more often expressed < 10 Hz sleep spindles than non-epileptic Wistar rats (Sitnikova, Hramov, Grubov, & Koronovsky, 2014). Moreover, an abnormal co-occurrence of < 10 Hz components in delta and theta frequency bands was identified a few seconds prior to the onset of spike-wave discharges in the frontal cortex and in the thalamus in WAG/Rij rats, so-called slow-wave precursors (van Luijtelaar, Hramov, Sitnikova, & Koronovskii, 2011). Anterior sleep spindles in WAG/Rij rats also contained subdominant components in delta (mean 3.6 Hz) and theta (mean 6.7 Hz) bands (Sitnikova et al., 2016), whereas "epileptic" subjects more often showed sleep spindles with the low-frequency subdominants as compared with "non-epileptic". These findings altogether might suggest that absence epilepsy is related to a certain deceleration of thalamocortical rhythms.

Several theoretical concepts were developed to understand mechanisms underlying epileptic transformation of physiological oscillations during NREM sleep in patients with absence epilepsy (Halász, Bódizs, Ujma, Fabó, & Szűcs, 2019; Halász, Kelemen, & Szűcs, 2013; Kellaway, Frost, & Crawley, 1990); however, surprisingly few studies examined time–frequency features of NREM sleep oscillations. Two reports indicated that the number of sleep spindles in patients with generalized absence seizures was lower than in the control group (Kellaway et al., 1990; Myatchin & Lagae, 2007). In their case report, Kokkinos, Koupparis, Stavrinou, and Garganis (2011) showed that pharmacological treatment of childhood absence epilepsy with valproate caused abolishment of sleep spindles. However, a functional relationship between sleep-related slow oscillations and absence seizures is still uncertain. We hypothesize that slow-wave activity prior to the onset of sleep spindles in subjects with absence epilepsy differs from that in healthy controls. This hypothesis is tested here in freely moving WAG/Rij and Wistar rats using epidural EEG recordings from the anterior cortex. We are focused specifically on cortical activity, because: (a) sleep spindles are better expressed in the cortex than in the thalamus; and (b) scalp EEG in patients with absence epilepsy is easily available and therefore our results might have direct translational value.

Absence epilepsy has a strong genetic predisposition in human patients (for review, see Crunelli & Leresche, 2002), as well as in the WAG/Rij genetic rat model (for review, see Coenen & van Luijtelaar, 2003). Spontaneous spike-wave discharges are known to appear in the original Wistar rat strain (Vergnes et al., 1986) which is often used as a control rat strain. At our laboratory in Moscow, we started breeding the "non-epileptic" substrain of WAG/Rij rats that did not show seizure activity in EEG throughout their entire life (Sitnikova et al., 2016). In addition, EEG properties of 5–9-Hz oscillations in "epileptic" and "non-epileptic" WAG/Rij rats appeared to be the same (Sitnikova et al., 2016). We assumed that cortico-thalamocortical network pathology affects slow-wave activity preceding the onset of 5–9-Hz oscillations, but not oscillations themselves. Currently we report on the phenomenology of the slow-wave precursors of sleep spindles and 5–9-Hz oscillations in respect to the presence of absence seizures.

2 | METHODS

2.1 | Animals and electroencephalogram recording procedure

The study was performed in 10 WAG/Rij and 11 Wistar adult male rats aged between 9 and 12 months. Animals were born and raised at the Institute of Higher Nervous Activity and Neurophysiology RAS (Moscow, Russian Federation). The experiments were conducted in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Ethical Committee of Institute of Higher Nervous Activity. Prior to the surgery, rats were housed in small groups with free access to food and water, and were kept under natural lighting conditions. After the surgery, rats were housed individually. Distress and suffering of animals were minimal.

Animals were equipped with screw electrodes for EEG recording. Stereotactic surgery was performed under chloralhydrate anaesthesia (i.p. injections 325 mg kg^{-1}, 4% solution in 0.9% NaCl).

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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). WAG/Rij rats were implanted with three active epidural electrodes in the right hemisphere in the frontal cortex (AP 2; L 2.5), parietal area (somatosensory cortex, AP −2; L 6) and occipital cortex (AP −5; L 4). The amplitude and persistency of the examined anterior oscillations in the parietal cortex were much lower than in the frontal cortex, and this hampered the process of automatic analysis of parietal EEG. In order to minimize cranial injury, Wistar rats received only two active electrodes over the frontal and occipital cortical areas (same coordinates). All coordinates are given in mm relative to bregma. The reference and ground electrodes in all rats were located over the cerebellum. Electrodes were permanently fixed to the skull with methyl methacrylate monomer together with two additional anchoring screws. Immediately after the surgery, animals received i.m. injections of metamizol (FSSCI Microgen, 25 mg kg^{-1}) for pain relief, and were then housed in individual cages in order to prevent damage of electrode connectors. Rats were allowed to recover for a minimum of 10 days before EEG recording. EEG signals were recorded in freely moving rats continuously during a period of 24 hr (started at about 16:00 hours–17:00 hours), fed into a multi-channel amplifier (PowerLab 8/35, ADInstruments) via a swivel contact, band-pass filtered between 0.5 Hz and 200 Hz, digitized with 400 samples per s per channel, and stored on a hard disk.

2.2 | Electroencephalogram patterns

Epileptic spike-wave discharges appeared in the frontal and parietal EEG channels as a sequence of high-voltage surface negative spikes and negative waves with frequency 7–10 Hz and lasted longer than 1.5 s (van Luijtelaar & Coenen, 1986; Figure 1a). The first spike in the spike-wave sequence was high and sharp, and spike-wave discharges had rectangular envelope, in contrast to the waxing-waning envelope of sleep spindles.

Sleep spindles appeared during behavioural sleep as groups of 10–15-Hz waves with the predefined criterion for the minimal duration of 500 ms based on the recommendation of the American Academy of Sleep Medicine (Iber, Ancoli-Israel, Chesson, & Quan, 2007). Sleep spindles had a characteristic waxing-waning morphology and symmetrical waveform, and did not contain sharp elements (e.g. spikes). The amplitude of sleep spindles was at least 1.5 times higher than the background amplitude (Figure 1a).

Five–nine-Hertz oscillations appeared in the frontal and parietal EEG as sharp and negatively oriented oscillations during behavioural immobility and sleep, and met the descriptive criteria provided by Pinault et al. (2006). They might contain occasional spikes or spike-wave complexes, and sometimes occurred in the form of embryonic spike-wave discharges and lasted longer than 500 ms (Figure 1a).

Slow-wave activity (2–7 Hz) often occurred prior to the onset of sleep spindles, 5–9-Hz oscillations and spike-wave discharges (Figure 1a). The stepwise signal processing was used for the automatic identification of all oscillatory patterns, which allowed selective identification of EEG patterns with the overlapped frequencies, such as 2–7-Hz precursors and 5–9-Hz oscillations (Sections S2–S4 in Appendix S1). Initially, 5–9-Hz oscillations were selected as indicated in Figure S2. Next, 2–7-Hz precursors were selected based on the previously extracted 5–9-Hz oscillations (Section S4 in Appendix S1).

2.3 | Wavelet-based automatic recognition of electroencephalogram patterns

The procedure employed the continuous wavelet transform of EEG signal (Hramov, Koronovskii, Makarov, Pavlov, & Sitnikova, 2015). Sleep spindles and 5–9-Hz oscillations were automatically detected in EEG using a previously described method (Sitnikova et al., 2014, 2016). Figure 1(b) demonstrates the principle of wavelet-based detection; more details are given in Supporting information (Sections S1 and S2 in Appendix S1). Figure S2 shows the step-by-step progress of the algorithm for selective recognition of sleep spindles and 5–9-Hz oscillations. Sensitivity and specificity of the automatic detection can be found in Section S3 in Appendix S1. The procedure for detecting slow-wave precursors of spindles and 5–9-Hz oscillations is described in Section S4 in Appendix S1.

The intrinsic frequency and duration of the automatically recognized sleep spindles, 5–9-Hz oscillations and their precursors were computed and analysed. Amplitude changes in transition from precursor to consequent oscillations were also examined (see Section S5 in Appendix S1).

2.4 | Statistical analysis

Statistical analysis was performed in "STATISTICA 8.0" (StatSoft). Descriptive data were presented as mean ± *SD*. The full-length EEG records (22–24 hr) were first visually inspected for the presence of epileptic spike-wave seizures. Five out of 10 WAG/Rij rats showed less than five seizures during 22–24 hr and were considered as "non-epileptic" rats. The rest five "epileptic" WAG/Rij rats showed on average 46 spike-wave discharges per 6 hr (minimum 19 and maximum 64 as scored between 19:00 hours and 01:00 hours). Nine out of 11 Wistar rats were "non-epileptic", and the remaining two were "epileptic" and showed 10 and 42 seizures during 6 hr (between 19:00 hours and 01:00 hours). We performed statistical analysis of the strain effect and the effect of the "epileptic" phenotype.

In two exemplary WAG/Rij rats, analysis of sleep spindles, 5–9-Hz oscillations and their slow-wave precursors was performed in 24-hr EEG with respect to vigilance states (Sections S4 and S6.1 in Appendix S1). Sleep spindles were detected during NREM sleep EEG about five–six times more often than in the other states of vigilance (Section S6.2 in Appendix S1; Table S1). Also, the number of 5–9-Hz oscillations during NREM sleep was four–six times higher than during the other states of vigilance (Section S6.2 in Appendix S1; Table S1). Therefore, the automatic algorithm detected sleep spindles and 5–9-Hz oscillations preferably during NREM sleep.

(a) *Spike-wave discharges*   *Sleep spindle*   *5–9 Hz oscillation*

time [hh:ss]

(b)
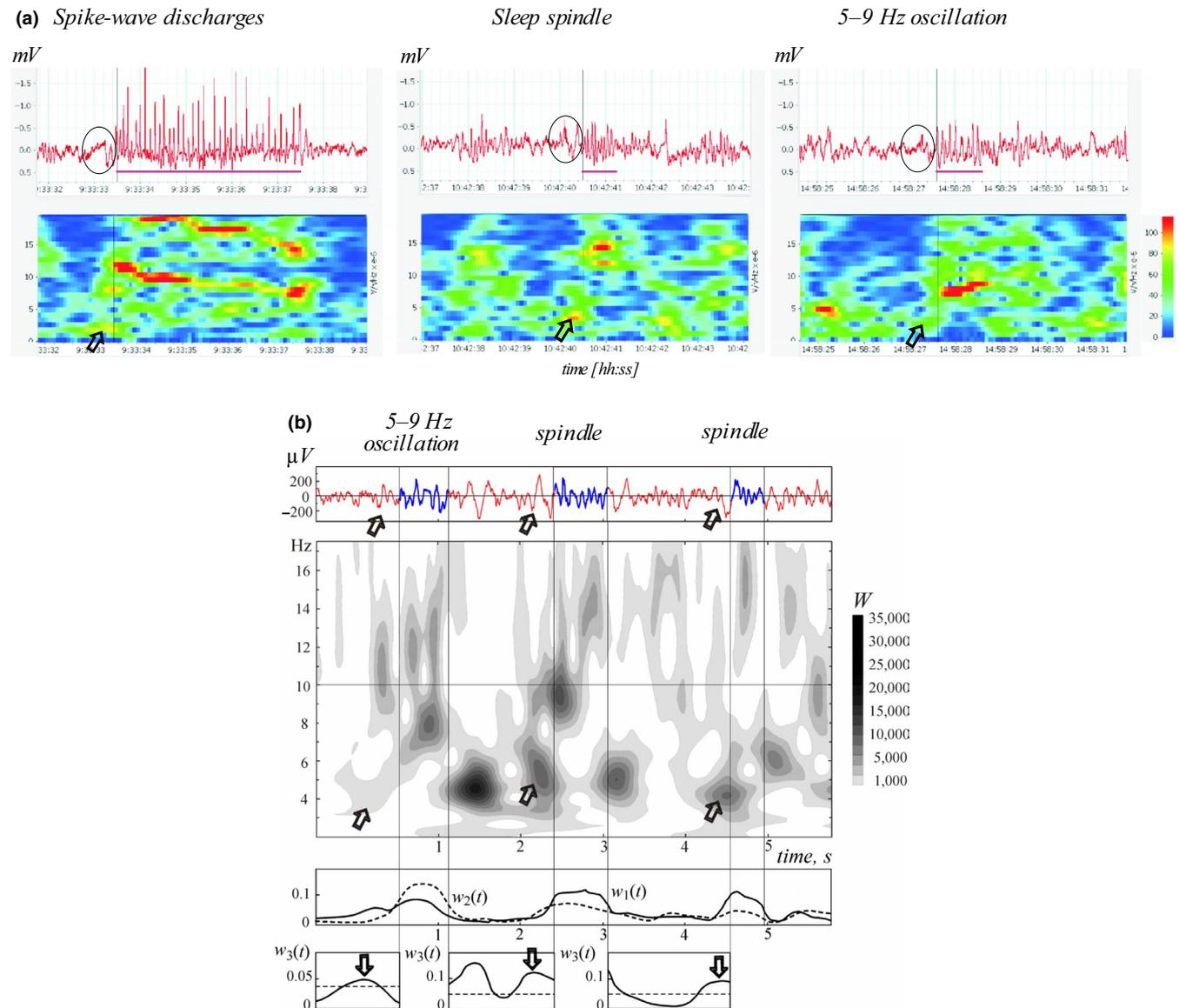
FIGURE 1 Examples of electroencephalogram (EEG) patterns and principles of their detection. (a) Frontal EEG as recorded in 11-month-old male WAG/Rij rats and corresponding spectrograms in which EEG patterns are marked by pink bars. The spectrogram represents amplitude spectral density (FFT size = 512, window overlap = 93.75%). Slow-wave components preceding the onset of EEG events are marked by black arrows. (b) An illustrative scheme of wavelet-based detection of sleep spindles, 5–9-Hz oscillations and their slow-wave precursors in frontal EEG in WAG/Rij rats (for details, see Section S1 in Appendix S1). Wavelet spectrum was computed using continuous wavelet transform with the complex Morlet basis function. Arrows indicate slow-wave precursors of sleep spindles and 5–9-Hz oscillations. The bottom plots demonstrate distribution of wavelet power in the following frequency bands: 5–9 Hz—$w_1(t)$, 10–15 Hz—$w_2(t)$ and 2–7 Hz—$w_3(t)$. See details in Sections S1 and S4 in Appendix S1

In WAG/Rij rats (n = 10), oscillations were analysed in the frontal and parietal EEG channels separately during 1 hr between 20:00 hours and 21:00 hours (without regard to the vigilance state). The differences between EEG features of frontally and parietally detected oscillations were analysed using non-parametric Wilcoxon matched-pairs test at the 5% level of significance.

In WAG/Rij (n = 10) and Wistar rats (n = 11), both types of oscillations were automatically detected in 1-hr frontal EEG as recorded from 20:00 hours and 21:00 hours (without regard to the vigilance

state). Time–frequency properties of oscillations and their slow-wave precursors were statistically analysed using GLM ANOVA with "the strain" and "the epileptic phenotype" as between factors and animal ID as a covariance. In all GLM ANOVA tests, the effect of animal ID was not significant (p > .05), and it was not indicated. Non-parametric Mann–Whitney test was used to evaluate differences between groups with respect to the presence/absence of slow-wave precursors of spindles and 5–9-Hz oscillations. Spearman rank test was used to analyse associations between the number of spike-wave seizures and characteristics of sleep spindles and 5–9-Hz oscillations.
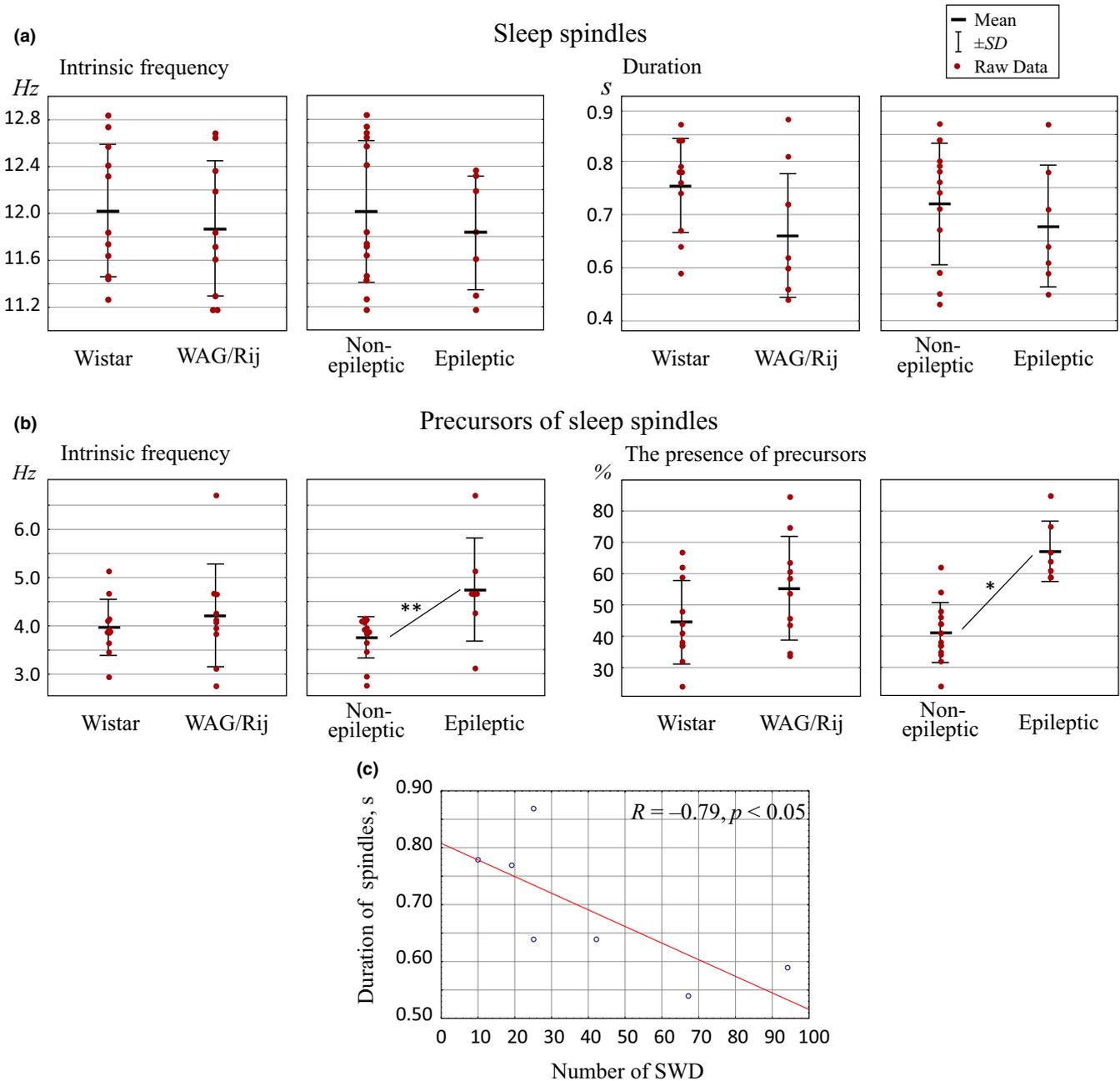
FIGURE 2 Basic parameters of the frontal sleep spindles and their slow-wave precursors. *Significant effect of the "epileptic" phenotype according to Mann–Whitney test ($p < .001$); **significant effect of the "epileptic" phenotype according to GLM ANOVA ($p < .05$)

3 | RESULTS

3.1 | WAG/Rij rats: Area-specific differences of sleep spindles, 5–9-Hz oscillations and their slow-wave precursors

In WAG/Rij rats ($n = 10$), sleep spindles and 5–9-Hz oscillations were detected in the frontal and parietal EEG channels separately during a 1-hr interval. The number of spindles as detected in the frontal and parietal EEG channels was the same (in both channels, 156 spindles on average; see individual data in Section S4.1 in Appendix S1). The intrinsic frequency of frontally and parietally detected spindles

was almost equal (11.87 ± 0.57 and 11.55 ± 0.61 Hz correspondingly, Wilcoxon matched pairs test $p > .05$). The mean duration of frontal and parietal spindles was also the same (678 ± 130 and 672 ± 124 ms correspondingly). The percentage of slow-wave precursors of frontal and parietal spindles was similar, as well as their intrinsic frequency. In general, no differences between frontal and parietal spindles and their slow-wave precursors were found in WAG/Rij rats.

The number of 5–9-Hz oscillations as measured in the parietal channel showed a strong tendency to be higher than in the frontal channel (88.4 ± 44.7 versus 50.0 ± 26.0, $p = .05$ Wilcoxon matched pairs test). The intrinsic frequency of 5–9-Hz oscillations was about 8.5 Hz in both frontally and parietally detected

oscillations. The mean duration of frontal and parietal 5–9-Hz oscillations was similar (677 ± 106 and 650 ± 125 ms correspondingly). In the parietal area, 5–9-Hz oscillations showed a higher percentage of slow-wave precursors than in the frontal area (36.0 ± 16.2 versus 17.9 ± 6.2, $p < .05$, Wilcoxon matched pairs test). The intrinsic frequency of slow-wave precursors of 5–9-Hz oscillations was about 4.1 Hz in both cortical areas. In general, 5–9-Hz oscillations in WAG/Rij rats tended to be enhanced in the parietal (somatosensory) area, which is known to be an epileptic focal zone (Meeren, Pijn, Van Luijtelaar, Coenen, & Lopes da Silva, 2002). Slow-wave (~4 Hz) precursors of 5–9-Hz oscillations in this area were found more often (almost twice) compared with that in the frontal cortex.

3.2 | WAG/Rij and Wistar rats: Frontal sleep spindles and their slow-wave precursors with respect to epileptic phenotype

The analysis was performed in Wistar ($n = 11$) and WAG/Rij rats ($n = 10$) in 1 hr frontal EEG as recorded between 20:00 and 21:00 hours. The average number of 10–15-Hz spindles was 110 per subject, with a minimum of 78 and maximum of 237 (Section S4.1 in

Appendix S1; Table S0). The intrinsic frequency of spindles was about 12 Hz in both rat strains ($F_{1,16} = 0.05$, $p = .83$) and in both phenotypes ($F_{1,16} = 0.009$, $p = .93$; Figure 2a). The mean duration of spindles varied between 636 and 764 ms, and it was neither influenced by the factor of "strain" ($F_{1,16} = 1.52$, $p = .23$) nor by the "epileptic phenotype" ($F_{1,16} = 0.33$, $p = .57$; Figure 2a). In "epileptic" subjects ($n = 14$), the number of spike-wave discharges negatively correlated with the duration of spindles (Spearman rank order correlations, $R = -0.79$, $n = 7$, $p < .05$; Figure 2c).

Slow-wave activity that appeared in association with sleep spindle and 5–9-Hz oscillations, so-called slow-wave precursors, was automatically detected and analysed (Section S4.1 in Appendix S1; Table S0). WAG/Rij and Wistar rats showed the same portion of spindles with slow-wave precursors (56% and 44% correspondingly; Figure 2b). The higher percentage of spindles with slow-wave precursors (67%) was detected in "epileptic" subjects compared with "non-epileptic" (41%, Mann–Whitney test, $p < .001$; Figure 2b). In "epileptic" subjects, no correlations were found between the number of spike-wave discharges and the percentage of spindles with slow-wave precursors (Spearman's rank test, $R = -0.08$, $p = .86$). The intrinsic frequency of spindle precursors in "epileptic" rats (4.8 Hz) was higher than in "non-epileptic" (3.8 Hz, $F_{1,16} = 7.8$, $p < .05$; Figure 2b).
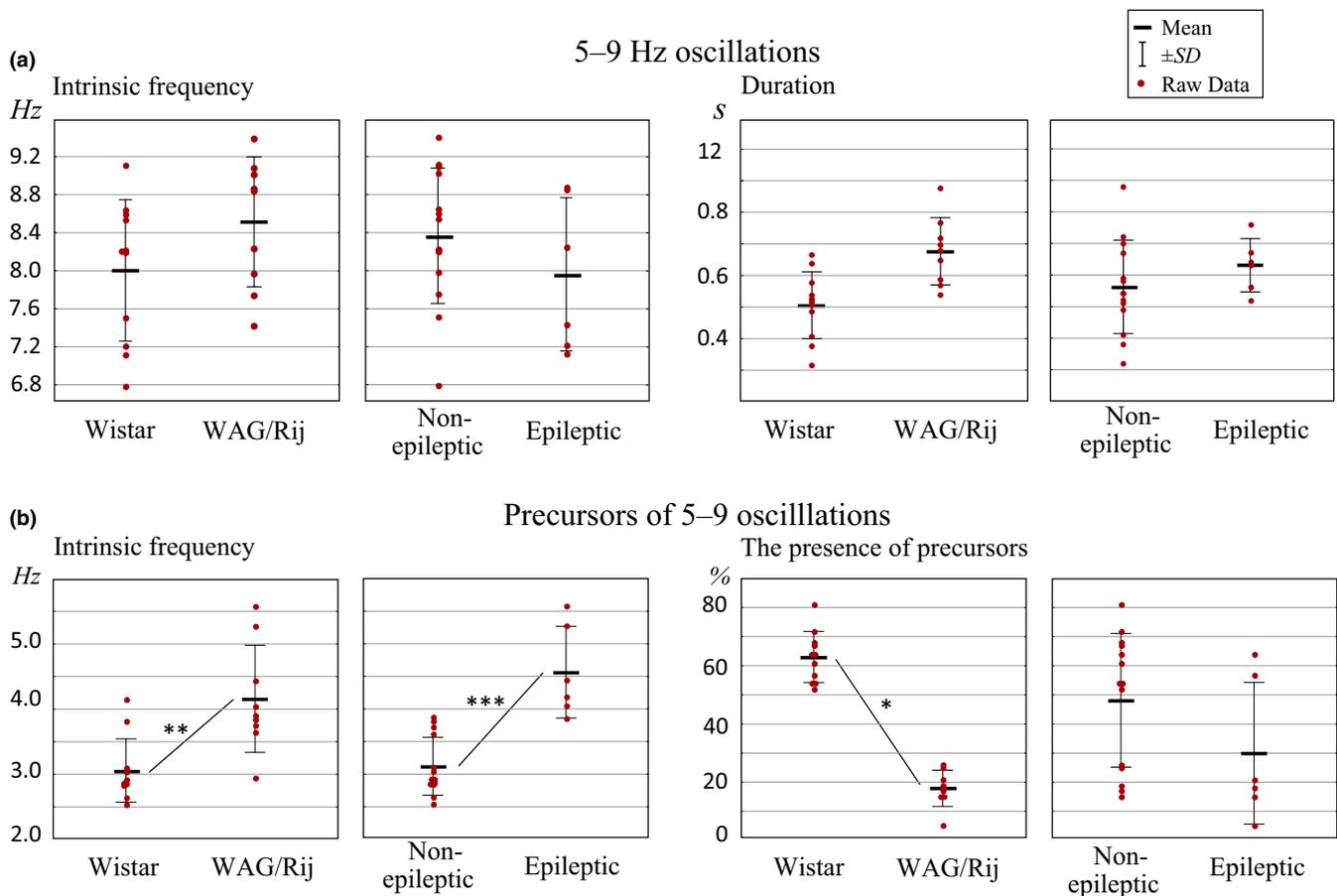
FIGURE 3 Basic parameters of the frontal 5–9-Hz oscillations and their slow-wave precursors. *Significant strain effect, according to Mann–Whitney test ($p < .0001$); **significant strain effect, according to GLM ANOVA (.00005); **significant effect of the "epileptic" phenotype, according to GLM ANOVA ($p < .00001$)

3.3 | WAG/Rij and Wistar rats: Frontal 5–9-Hz oscillations and their slow-wave precursors with respect to epileptic phenotype

The analysis was performed in Wistar ($n = 11$) and WAG/Rij rats ($n = 10$) in 1 hr frontal EEG. The average number of the automatically detected 5–9-Hz oscillations was 41 per subject (minimum = 24 and maximum = 88; see Section S4.1 in Appendix S1; Table S0). One "epileptic" WAG/Rij rat was excluded from the analysis, because it did not express 5–9-Hz oscillations during the examined period. The mean frequency and mean duration of 5–9-Hz oscillations in WAG/Rij rats did not differ from that in Wistar rats (Figure 3a). In subjects with the "epileptic" phenotype, the intrinsic frequency of 5–9-Hz oscillations did not significantly differ from that in "non-epileptic" subjects ($F_{1,15} = 3.72$, $p = .07$; Figure 3a). In "epileptic" subjects, parameters of 5–9-Hz oscillations and their precursors did not correlate with the number of spike-wave discharges.

Slow-wave precursors were found in 18% of 5–9-Hz oscillations in WAG/Rij rats, which was significantly lower than in Wistar rats (63%, $p < .0001$; Figure 3b). The intrinsic frequency of slow-wave precursors of 5–9-Hz oscillations in WAG/Rij rats was higher than in Wistar rats (4.2 and 3.1 Hz correspondingly, $F_{1,15} = 11.8$, $p < .005$; Figure 3b). In "epileptic" subjects, the percentage of slow-wave precursors of 5–9-Hz oscillations did not differ from that in the "non-epileptic" subjects (Figure 3b), but the intrinsic frequency of precursors in "epileptic" rats was higher than in "non-epileptic" rats (4.6 and 3.1 Hz correspondingly, $F_{1,15} = 31.8$, $p < .00005$; Figure 3b).

3.4 | WAG/Rij and Wistar rats: Amplitude changes in transition from precursors to oscillations

Details of wavelet-based analysis of amplitude changes are demonstrated in Figure 4(a). In "epileptic" subjects, the transition "precursor → sleep spindle" was accompanied by less noticeable growth in amplitude in comparison to that in "non-epileptic" subjects ($F_{1,16} = 7.17$, $p = .016$; Figure 4b). In the transition "precursor → 5–9-Hz oscillation", "epileptic" subjects showed a strong tendency for having a smaller growth in amplitude than "non-epileptic" subjects ($F_{1,15} = 4.39$, $p = .052$; Figure 4c). As to the transition "precursor → 5–9-Hz oscillation", the "strain" effect was significant ($F_{1,15} = 42.6$,
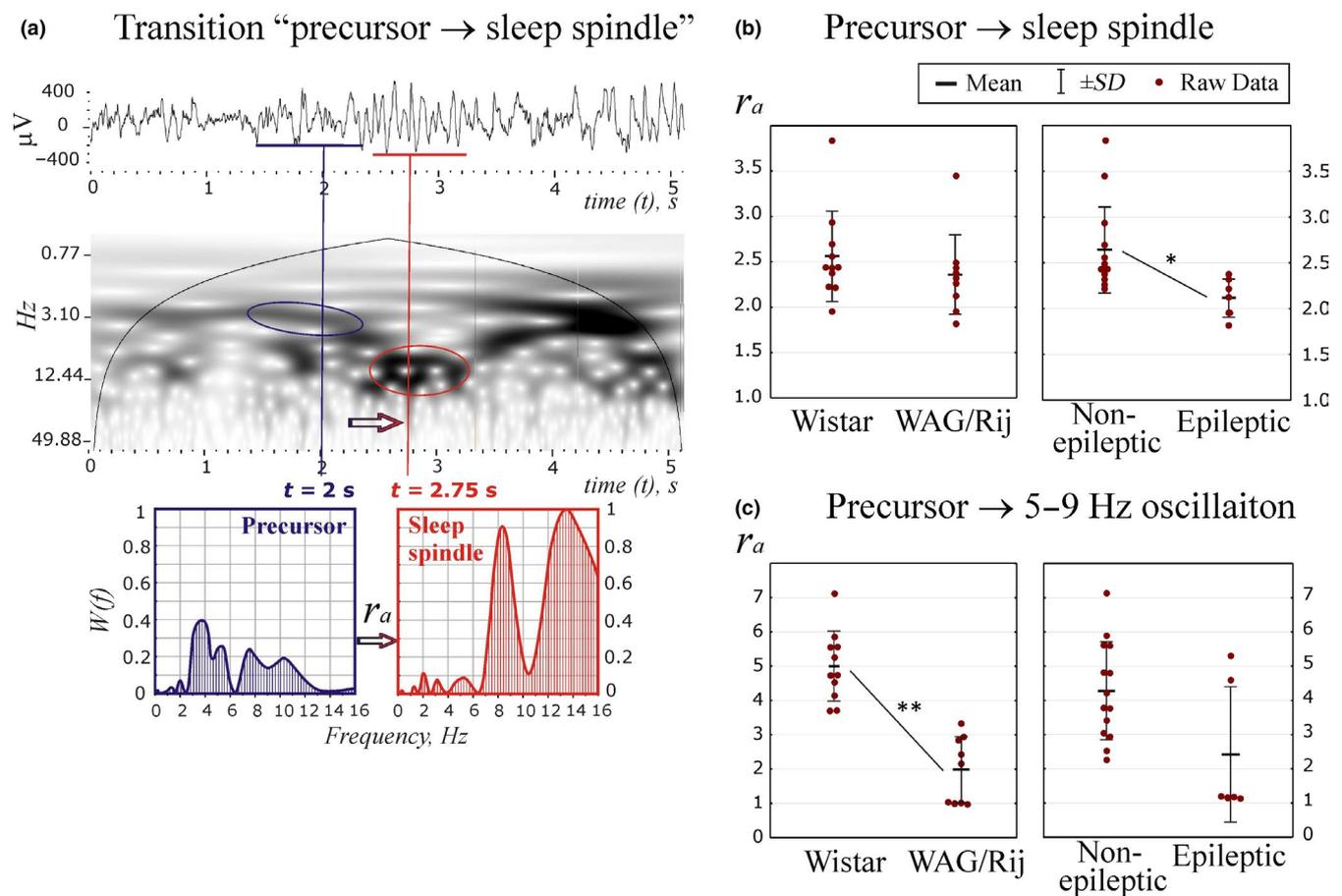
FIGURE 4 Wavelet-based analysis of amplitude changes in transition between slow-wave precursors and consequent oscillations. (a) Illustrative scheme of wavelet analysis of raw electroencephalogram (EEG). Wavelet power spectrum was obtained using continuous wavelet transform with complex Morlet wavelet basis function (see Section S1 in Appendix S1), and two inserted plots display wavelet power spectra as computed at the time moment $t = 2.25$ s (precursor) and $t = 2.75$ s (sleep spindle). (b, c) Relative changes in amplitude as measured in the frontal EEG (r_a, Section S5, Eq. 11 in Appendix S1). *Significant strain effect (GLM ANOVA, $p < .05$); **significant effect of the "epileptic phenotype" (GLM ANOVA, $p < .001$)

p = .0001; Figure 4c): a twofold increase in amplitude was found in WAG/Rij rats, and fivefold increase in Wistar rats. In general, "epileptic" rats showed a weaker amplitude growth during transition from precursor to oscillations (both types). The transition "precursor → 5–9-Hz oscillation" was accompanied by a greater increase in amplitude than the transition "precursor → sleep spindle".

4 | DISCUSSION

In the current study, we detected 3–4-Hz slow-wave activity 2 s prior to the onset of 41%–67% of 10–15-Hz sleep spindles and 18%–63% of 5–9-Hz oscillations in Wistar and WAG/Rij rats. The presence of the slow-wave activity immediately prior to sleep spindles was not surprising, but it is usually neglected, because spindle detection is often based on band-pass filtering with cut-off frequencies below 9(12) Hz (for review, see Liu, Huang, & Huang, 2017).

Our data indicate that in both rat strains and in both phenotypes, the mean intrinsic frequency of 10–15-Hz frontal spindles was about 12 Hz. This did not fit to our previous results indicating that the mean frequency of anterior sleep spindles in WAG/Rij rats (11.2 Hz) was lower than in Wistar rats (13.2 Hz; Sitnikova et al., 2014). In the abovementioned study, we used a broader frequency band for detecting sleep spindles, and showed that the "slow" (8–10.4 Hz) spindles constituted 43% from the total amount of sleep spindles in 9-month-old WAG/Rij rats, and only 11% in the age-matched Wistar rats. In the current research, we detected sleep spindles in the regular spindle range 10–15 Hz, and the "slow" spindles were excluded. No differences were found between the "epileptic" and "non-epileptic" phenotypes, suggesting that the intrinsic frequency and duration of 10–15-Hz sleep spindles did not have diagnostic value.

Although the frontal spindles were characterized by similar time–amplitude features in both rat strains, "epileptic" rats showed a negative correlation between spindle duration and the number of spike-wave discharges. This may suggest that the thalamocortical loop in "epileptic" subjects is prone to sustain shorter rhythmic spindle activity than that in "non-epileptic" subjects. In other words, more intensive seizures in symptomatic subjects might be associated with shortening of sleep spindle sequences.

To the best of our knowledge, the potential diagnostic value of slow-wave precursors of spindle-like oscillations has never been systematically addressed (neither in pre-symptomatic animals, nor in human patients). It is surprising, because idiopathic generalized epilepsies (in particular, absence epilepsy) are associated with pathological changes of spindle-generating circuits that give rise to the spike-wave discharges (for review, see Crunelli & Leresche, 2002; Leresche, Lambert, Errington, & Crunelli, 2012). In the present study, we defined some characteristics of slow-wave activity that might be considered as pathology-related biomarkers (i.e. the higher proportion of slow-wave precursors of frontal spindles, the weakening of amplitude growth in transition "slow wave → spindle/5–9-Hz oscillation"). However, it should be studied in the future whether these characteristics change as the disease progresses from pre-symptomatic to symptomatic stages. The clinical implication of our study is that shortening of sleep spindle sequences may be a sign for bad prognosis. In humans, a strong genetic influence on the spectral features of NREM sleep has recently been reported in several twin studies (Adamczyk, Genzel, Dresler, Steiger, & Friess, 2015; De Gennaro et al., 2008; Gorgoni et al., 2019). For instance, a heritability estimate of 96% was found in the 8–16-Hz frequency range during NREM sleep (De Gennaro et al., 2008). Adamczyk et al. (2015) showed that genetic factors affected parameters of slow spindles (11–12.9 Hz) and, much weaker, parameters of fast spindles (13.1–16 Hz). Gorgoni et al. (2019) reported on the similar morphology and amplitude of K-complexes in human twins. In the current study, we used genetically homogenous inbred WAG/Rij rats and Wistar rats, and did not find a significant genetic effect on the features of sleep spindles and their slow-wave precursors (i.e. "the strain" factor was not significant). Considering the fact that WAG/Rij rats derive from Wistar rats, our findings support the notion of Gorgoni et al. (2019) that "EEG during NREM sleep represents one of the most heritable traits of the human beings".

The vast amount of work has been done on the association between < 1 Hz slow oscillations and sleep spindles in animals (Crunelli & Hughes, 2010; Kim, Hwang, Lee, Sung, & Choi, 2015; Steriade & Amzica, 1998), as well as in humans (Andrillon et al., 2011; Klinzing et al., 2016; Mölle, Marshall, Gais, & Born, 2002; Neske, 2015; Yordanova, Kirov, Verleger, & Kolev, 2017). Our study shows that about half of the spindles preceded by ~4-Hz slow-wave activity. According to Steriade (2006), "Unlike 'pure' rhythms within distinct frequency bands, generated in restricted neuronal circuits of extremely simplified experimental preparations, the living brain does not generally display separate oscillations during slow-wave sleep, but a coalescence of the slow oscillation with other sleep rhythms (spindles and delta) as well as with faster (beta and gamma) rhythms that are superimposed on the depolarizing phase of the slow oscillation" (p. 1,090). Temporal associations between slow-wave and spindle activity were also reported in mice, in which 72.8% of anterior spindles (9–12 Hz) and 87.3% of posterior spindles (13–15 Hz) were associated with 0.5–4-Hz slow waves (Kim et al., 2015). This fits well to our results.

Here, we found a remarkably higher portion of the frontal spindles with slow-wave precursors in "epileptic" subjects than in "non-epileptic" (67% versus 41%). At the same time, the number of absence seizures in "epileptic" subjects did not correlate with the presence of slow-wave activity prior to the frontal spindles. Therefore, intensifying of ~4-Hz precursors of spindles in subjects with absence epilepsy might be considered as an additional diagnostic EEG marker, but it could not be related to disease severity.

Our research indicated that the properties of 5–9-Hz oscillations in rats in the "epileptic" and "non-epileptic" phenotypes were the same. In "epileptic" subjects, time–frequency parameters of the frontal 5–9-Hz oscillations did not correlate with the number of spike-wave discharges. However, amplitude–frequency properties of the frontal 5–9-Hz oscillations in "epileptic" rats showed ~1.5 Hz higher intrinsic frequency than in "non-epileptic" rats, and a weaker

increase in amplitude in transition "precursor → 5–9-Hz oscillation". Therefore, amplitude–frequency features of slow-wave precursors of 5–9-Hz oscillations were affected by absence epilepsy.

A probable EEG marker of absence epilepsy may refer to weakening of amplitude growth near the onset of spindles and 5–9-Hz oscillations as measured in the frontal EEG. In "epileptic" subjects, the intrinsic frequency of slow-wave precursors of spindles and 5–9-Hz oscillations was higher than in "non-epileptic". For precursors of spindles, this difference was 1 Hz (4.8 Hz versus 3.8 Hz), and for precursors of 5–9-Hz oscillations it was 0.5 Hz (4.6 Hz versus 3.1 Hz). An increased frequency of slow-wave activity prior to the onset of the frontal spindles and 5–9-Hz oscillations might have a diagnostic value and might have regard to human patients.

The following conclusions can be made based on our results.

1. Sleep spindles in "epileptic" rats were more often preceded by the slow-wave (~4 Hz) activity than in "non-epileptic" rats, but no correlations were found between the number of absence seizures and the percentage of sleep spindles with slow-wave precursors.
2. Slow-wave precursors of 5–9-Hz oscillations were influenced by the strain factor: they were detected in WAG/Rij rats three times less often than in Wistar, but their intrinsic frequency was ~1 Hz higher.
3. The transition "precursor → sleep spindle/5–9-Hz oscillation" in "epileptic" rats was characterized by a weaker amplitude growth than in "non-epileptic". Therefore, weakening of EEG amplitude growth at the onset of spindles and 5–9-Hz oscillations may be considered as a probable EEG marker of absence epilepsy.

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CONFLICT OF INTEREST

No conflicts of interest were declared.

AUTHOR CONTRIBUTIONS

ES planned and carried out the experiments; VG, AH and ES analysed the data; AH and ES wrote the article; ES supervised all aspects of the work.

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