Spike–wave discharges in WAG/Rij rats are preceded by delta and theta precursor activity in cortex and thalamus

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ABSTRACT

Objective: In order to unravel the mechanisms underlying the “sudden” onset of spontaneous absences in genetically prone subjects, we investigated the immediate precursors of spike–wave discharges (SWDs) produced in cortico-thalamo-cortical neuronal networks.

Methods: A time–frequency analysis of the cortical and thalamic ECoG of WAG/Rij rats was accomplished with a continuous wavelet decomposition of SWDs, 3 s prior to the onset of SWDs (pre-SWD), and in control periods devoid of SWDs.

Results: The pre-SWD ECoG consisted of delta and theta components in 80–90% of all SWDs simultaneously in cortex and thalamus, the co-occurrence of delta and theta was rare (7%) during control periods. The occurrence of delta and theta events in pre-SWDs in the cortex preceded that in the thalamus. The frequency of theta component in cortex correlated positively with that in thalamus, this correlation was less strong for delta.

Conclusion: Precursors of SWDs comprise of delta and theta, their co-occurrence is typical for non-epileptic periods. Thalamic and cortical theta are strongly related. Rhythmic precursors appear earlier in cortex than in thalamus, and this is in line with the cortical origin of SWD.

Significance: Simultaneous presence of delta and theta events in EEG is a condition for the occurrence of SWDs.

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1. Introduction

Visual analyzes of the scalp electroencephalogram (EEG) reveal that the electroencephalographic hallmark of absence seizures, spike–wave discharges (SWDs), appears abruptly from a normal background activity. Forerunners of SWDs cannot be easily detected in EEG, there are also no clinical signs from which absence seizures could be predicted. However, non-linear time series analyzes such as the instantaneous power spectrum, superior to traditional linear method such as Fourier transforms and power spectral analysis (Inouye et al., 1990; Rabinovich et al., 2006), revealed some unspecific disturbances in the EEG immediately prior to the onset of SWDs in patients (Inouye et al., 1990). The EEG in ~10 s period before the onset of SWDs showed a gradual increase in power in the low frequencies at frontal locations toward the beginning of the SWD (Inouye et al., 1994, 1995). These findings suggest that SWD-like events occurred in the EEG as poorly developed epileptiformic discharges buried in the background activity prior to the visual recognized epileptic activity.

Specific epileptogenic network interactions that prerequisite absence epilepsy were studied by measuring linear and non-linear synchronization in scalp EEG in patients (Aarabi et al., 2008). The authors characterized the spatiotemporal synchronization pattern before the occurrence of absence seizures, in particular, they found that two-third of the absence seizures were preceded by a decrease in synchronization, and about one-third – by an increase in synchronization. It was concluded that the transition from interictal state to ictal state is associated with an increased rhythmic activity with pronounced synchronization between several structures (Aarabi et al., 2008). However, it was not possible to establish an ultimate spatiotemporal synchronization pattern that would be solely associated with absences, mostly due to large individual differences. Moreover, different patient populations all endowed with spike and slow wave discharges might vary in the way in which and where SWDs are triggered (Rodin and Ancheta, 1987; Rodin and Cornellier, 1989). There are also technical difficulties that limit trustworthiness of traditional methods of EEG/MEG signal analysis.


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(those methods are often not suited for short, non-stationary and noisy biological signals). New signal analytical tools as developed by mathematicians and physicists might give clues how to extract relevant information from EEG or MEG signals from epileptic patients. In the present paper, we take advantages of the continuous wavelet transform in order to extract and specify seizure–precursor activity in the full length EEG.

SWDs are known to be produced in an interconnected cortico-thalamo-cortical neuronal network, in which the cortex interacts with an intact thalamus. SWDs are initiated in the cortex and the thalamus is only secondarily involved (Niedermeyer et al., 1979; Meeren et al., 2002, 2005; van Luijtelaar and Sitnikova, 2006). The feline penicillin model showed that SWDs are closely related to sleep spindle activity (Kostopoulos, 2000) and ova, 2006). The feline penicillin model showed that SWDs are closely related to sleep spindle activity (Kostopoulos, 2000) and has been assumed that both are initiated in the thalamus (Buzsáki, 1991; Avanzini et al., 1999; Huguenard and McCormick, 2007).

The thalamic region is not easily accessible. It could be reached with intracranial electrodes, but patients with absence epilepsy almost never undergo this operation. In human absence epilepsy, virtually no direct information about electrical activity in thalamus is available at the moment. Genetic animal models for absence epilepsy, such as WAG/Rij rats and GAERS (Coenen and van Luijtelaar, 2003; Depaulis and van Luijtelaar, 2006), can be easily supplied with intracranial depth and epidural electrodes and this obviously benefits our understanding of cortico-thalamo-cortical neuronal network mechanisms of absence epilepsy.

SWDs result from complex bidirectional (feed-forward and feedback) interactions within the cortico-thalamo-cortical network. Directionality of functional coupling in this network has been studied during time frames before the onset of SWDs in WAG/Rij rats, by means of non-linear association analyses (Meeren et al., 2002) and Granger causality (Sitnikova et al., 2008). In particular, the onset of SWDs was characterized by a gradual increase in association strength between nearby electrodes in the focal epileptic zone in the peri-oral area of the somatosensory cortex (Meeren et al., 2002). However, the coupling strength between frontal cortex and thalamus (in terms of Granger causality) remained unchanged in the few seconds preceding the onset of SWDs (Sitnikova et al., 2008), it only changed at the onset of SWDs. It was found in GAERS that pre-seizure EEG epochs exhibit a higher degree of determinism than seizure-free EEG epochs, but lower than those during SWDs (Ouyang et al., 2008). This suggests that seizure–precursor epochs differ from non-epileptic EEG and could be regarded as a transient between non-epileptic and epileptic activity. A neurophysiological approach was followed by Pinault et al. (2001, 2003), they recorded local field potentials LFP, intracellular and extracellular activity in GAERS. These authors noticed that medium-voltage 4–12 Hz oscillations were predominant in both the LFP and intraburst frequencies in intracellular recordings prior to SWDs in neuralt anesthetised and free moving animals. The Pinault et al. observations might agree with the finding, also done in GAERS, that intensive burst firing of cortical pyramidal cells already occurs before the onset of the EEG response (Polack et al., 2007).

An early incidental observation of Bosnyakova et al (2007) in LFP from WAG/Rij rats revealed that large amplitude slow wave activity (in delta range) may appear before the beginning of epileptic discharges, as revealed with a wavelet analyzer. An increase in cortical delta and high theta in the 2 s epoch before the onset of SWDs was noticed in GAERS (Gurbana et al., 2008). The general aim of the present study is to access characteristic time–frequency profile of the precursor activity of SWDs in free moving animals in cortical and thalamic recordings in WAG/Rij rats by means of continuous wavelet analysis and specifically focused on the low (from delta to high theta) frequency bands.

### 2.1. Animals

Six male 1-year-old (body weigh 320–360 g) WAG/Rij rats were used. Animals were born and raised at the laboratory of the Center for Cognition, Donders Institute of Brain, Cognition and Behavior of Radboud University Nijmegen (The Netherlands). They were kept in pairs in standard cages with food and water available ad libitum under a 12–12 h light–dark cycle, with white lights on at 18:00 at a constant environment temperature of 21 °C. After surgery, housing conditions were the same except that rats were housed individually. Distress and suffering of animals was kept to a minimum. The experiments were conducted in accordance with the legislation and regulations for animal care and were approved by The Ethical Committee on Animal Experimentation of the Radboud University Nijmegen.

### 2.2. EEG recording design and equipment

Animals were equipped with six stainless steel electrodes (two pairs of MS 333/2A, Plastic One Inc., Roanoke, VI, USA) for monopolar recordings. All electrodes were identical, were insulated with a non-insulated tip (diameter 0.2 mm). Two EEG electrodes were placed epidurally over the cortex in the frontal (AP 2; L 2.5) and occipital (AP –7; L 6) cortical areas, skull flat. Two depth electrodes were implanted into the ventroposteromedial nucleus of the thalamus (VPM, AP –3.5; L 2.5; H 7.2). All coordinates are given in mm relative to bregma (Paxinos and Watson, 1998). Ground and reference electrodes were placed symmetrically over both sides of the cerebellum. Electrodes were permanently attached to the rat’s skull with dental cement.

Stereotactic surgery was performed under isoflurane anesthesia. For post-surgery analgesia rats received a single i.m. injection of buprenorphinehydrochloride (Temgesic®, Reckitt Colman Products Ltd., Kingston-Upon Hull, UK) in a dose of 0.1 mg/kg. After surgery animals were allowed to recover for at least 10 days.

Positioning of electrodes was verified with a post-mortem histological control using a standard protocol of Nissl staining (0.1% cresyl violet staining of serial coronal 60 μm brain slices). Location of electrodes was determined according to the stereotaxic atlas of the rat’s brain (Paxinos and Watson, 1998).

The EEG was recorded in freely moving rats in a noise-isolated Faraday cage during 5–7 h in the dark period of the light–dark cycle. One day before the EEG recording session, rats were allowed to habituate to the recording procedure during the 2 h, they were moved to custom-made Plexiglas recording cages (25 × 30 cm width, 35 cm high). EEG signals were fed into a multi-channel differential amplifier via a swivel contact, band-pass filtered between 1 and 500 Hz, digitized with 1024 samples/s/channel (Data Acquisition Hardware and Software, DATAQ Instruments Inc., Akron, OH) and stored on hard disk.

### 2.3. EEG analysis

SWDs were first identified with custom-made software based on the threshold value of the EEG slope in the frontal channel with an efficiency of 97–100% in the artifact-free EEG. The algorithm and original software were developed by Dr. Philip van den Broek, NCI, Radboud University Nijmegen, The Netherlands, evaluated by Westerhuis et al. (1996) and visually checked against criteria described earlier (van Luijtelaar and Coenen, 1986; Midzianovskaia et al., 2001). SWDs were recognized by a train of surface negative 7–10 Hz sharp and asymmetric spikes with amplitude at least 2–3 times the background, duration of the discharge was more than 1 s. We examined cortical and thalamic records during SWDs, pre-SWD

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epochs (for about 3 s immediately before the onset of SWDs, as defined in the frontal EEG) and non-epileptic control EEG epochs. Control periods devoid of SWDs (6–12 10 s epochs per animal) were periods with active wakefulness (low amplitude high frequencies ECoG, mixture of theta and beta), passive wakefulness, light-slow wave sleep (immobile behavior, combined with the presence of intermediate amplitude, slow (<6 Hz) activity and the presence of low amplitude sleep spindles), and deep-slow wave sleep (immobile behavior, large amplitude slow waves).

Time–frequency analysis was carried out using a continuous wavelet decomposition (Chui, 1992; Torresani, 1995; Koronovskii and Hramov, 2003) with a mother Morlet-wavelet basis ($\omega_0 = 2\pi$).

The continuous wavelet transform (CWT), $W(s,\tau)$, was calculated as a result of convolving of EEG signal, $x(t)$,

$$W(s,\tau) = \int_{-\infty}^{\infty} x(t)\phi^*_s(t)dt$$

(1) \\

where $s$ is the time scale and $\tau$ is the time shift of the wavelet transform. The parameter $s$ in this formula is substituted by a reciprocal parameter $f_s$ so that $f_s = 1/s$.

Time ($t$) and time scales ($s$) (or corresponding frequencies, $f_s$) in the wavelet basis function are related through the uncertainty principle, which states that it is impossible to extract the exact frequency at the exact time when this frequency occurs in a signal.

The complex Morlet wavelet (Kaiser, 1994)

$$\psi(\eta) = \frac{1}{\sqrt{\pi}}e^{i\omega_0\eta}e^{-\eta^2}$$

(3)

has been used as the mother-wavelet function (with parameter $\omega_0 = 2\pi$, Sitnikova et al., 2009). We have selected the Morlet-wavelet basis function because it provides an optimal (for our purposes) time–frequency resolution of the ECoG signal and, allows to localize precisely SWDs in the ECoG (Torresani, 1995; Koronovskii and Hramov, 2003; Sitnikova et al., 2009).

We examined the matrix of wavelet coefficients at each time point and frequency in the ECoG epochs preceding and during SWDs. Precursor activity was defined by a gradual increase of the wavelet energy compared to baseline for minimally 100 ms noticed in the 3 s period preceding the onset of SWDs, it should reach a minimal threshold (more than two times higher than the background) and this should exceed the background energy for a minimum duration of 0.3 s. The background level is determined in the period preceding the precursor activity. It was found that delta and theta precursor activity were most dominant, their frequency of occurrence was measured, averaged per animal and statistically analyzed. The following time–frequency parameters of SWD–precursor activity were extracted independently in cortex and thalamus:

1. Mean frequency of SWD–precursors [$f$, Hz] in their characteristic frequency band (i.e., delta or theta). The frequency of each precursor type was determined by measuring the peak frequency in the wavelet spectrum (we visualized the peak frequency by the darkest spot in the wavelet spectrum in Fig. 1).

2. Mean duration of SWD–precursors [MD, s]. In order to localize the precursor in time, we defined the onset and offset of the precursor activity. The onset of SWD–precursor was determined when the wavelet energy for a specific frequency exceeded the mean background level and kept increasing for minimally 100 ms. Precursor offset was regarded as the moment when the wavelet energy in the selected frequency range returned to the mean background level for this frequency.

3. Difference between the frequency of the beginning of SWD ($f_b$) and its middle part ($f_m$). $\Delta$ (Hz): $\Delta = f_b - f_m$.

4. Time delay between SWD–precursors and the onset (the first spike) of the subsequent SWD [$T_{ss}$, s].

5. Percentage of two SWD–precursor types (3–5 Hz (delta) and 7–11 Hz (theta)) from the total number of SWDs: ($N_d/N_{SWD}) \times 100\%$, where $N_d$ is the number of each type of SWD–precursor as assessed in cortex and thalamus, $N_{SWD}$ is the total number of SWDs.

![Wavelet spectra of SWD in the cortex and thalamus](image)

Fig. 1. The figure illustrates the cortical (upper) and thalamic (lower) ECoG with different SWDs (A, B, C, note the different time scales). The onset of the SWD in (A) is preceded by 3.9 and 6.4 Hz in cortex, in the thalamus by 4.1 Hz, in (B) – by 7.1 and 4.2 Hz in cortex and by 4.0 and 6.0 Hz – in thalamus, in (C) clear precursor activity (3.7 Hz) is present in the cortex. SWD outlasts the X-axis in (A) and (B).

For the control periods the following parameters were extracted:

1. The number of observed delta events in control periods, Nd.
2. The mean number of delta events per second (=Nd/T, where T is the time duration of analyzed EEG in s).
3. The mean frequency of delta events [fb, Hz] was measured by averaging the instantaneous frequencies in every sampling point during the full duration of the corresponding delta event.
4. The number of observed theta events in the EEG of the control periods, Nt.
5. The mean number of theta events per second (=Nt/T, where T is the duration of analyzed periods, s).
6. The mean frequency of theta events [ft, Hz] was measured similar to fb.
7. The number (G) of events for which the co-occurrence of theta and delta was observed: a measure of the simultaneity of occurrence of theta and delta.
8. The relative number of events with simultaneous theta and delta to the full number of observed delta and theta events (K=G/[Nd+Nt−G]) [in %].

Pearson’s correlation coefficients were used to estimate the relation between the characteristics (fb, ft and corresponding MD) of SWD–precursor activity and fb. The means per subject were used. Students paired t-tests were done to test differences of characteristics of SWD and precursors activity in cortex and thalamus. Differences between states were analyzed with an ANOVA followed by post-hoc tests according to Duncan.

3. Results

SWDs were detected automatically from the EEG recordings of all rats. Next the detected SWDs were verified by one of us (EYS). Wavelet analysis was performed on all SWDs detected during the 5–7 h of recording. The number of SWDs in subject #23 was 107 seizures (6 h), #24 – 278 (6 h), #25 – 59 (7 h), #26 – 120 (6 h), #28 – 54 (5 h), #29 – 124 (6 h). Control periods were 6–12 unambiguous artifact-free 10 s periods of active wakefulness, passive wakefulness, light-slow wave sleep and deep-slow wave sleep.

3.1. Frequency characteristics of cortical and thalamic SWDs

Typically, the wavelet spectrum of SWD in the cortex showed a local maximum of about 10–11 Hz at the beginning and subsequently its frequency slowed down from 11 to 7–8 Hz in the middle part and even lower, to 5–6 Hz, at the end. Descriptive statistics of the frequency modulation of SWDs as recorded in cortex and thalamus can be found in Table 1.

The drop in frequency from the beginning to the middle part of the cortical SWDs was statistically evaluated and significant (t = 8.6, df = 5, p < .01), as well as the frequency drop in the thalamus (t = 8.0, df = 5, p < .01). The frequency at the beginning of the SWDs was higher in cortex than in the thalamus (t = 2.54, df = 5, p < .05).

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>10.1 ± 1.5</td>
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<tr>
<td>Thalamus</td>
<td>8.5 ± 2.5</td>
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<thead>
<tr>
<th>Parameter</th>
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<tbody>
<tr>
<td>Cortex</td>
<td>7.4 ± 0.8</td>
</tr>
<tr>
<td>Thalamus</td>
<td>6.4 ± 2.4</td>
</tr>
</tbody>
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3.2. Delta and theta precursors of SWD as measured in cortex and in thalamus

SWD–precursors consisted of several frequency components in the range from 2 to 12 Hz. The two most powerful components immediately preceding the onset of SWD in frequencies were 3–5 and 7–11 Hz considering their high prevalence and close proximity in time to the onset of SWDs. Some illustrative examples of delta and theta precursor activity in cortex and thalamus can be found in Fig. 1. Descriptive statistics of frequency and duration of delta and theta precursors in cortex and thalamus are given in Table 2.

The frequency of delta (fb) and theta (ft) precursor activity in the thalamus was similar to that in the cortex, however, the duration (MD) of theta precursor activity was slightly longer (t = 4.35, df = 5, p < .01) longer in the thalamus than in the cortex and also the duration of cortical delta precursor activity was longer than the cortical theta precursor activity (t = 2.71, df = 5, p < .05).

Delta precursor activity in the frontal cortex was found in 90% of all SWDs, and theta precursor activity – in 92%. High percentages were also found in the VPM: 82% and 83%, respectively, for delta and theta precursors. There were no differences in the percentages of SWD that were preceded by theta and delta in cortex and thalamus.

It was further examined whether delta precursor activity was simultaneously present in cortex and thalamus. It was found that in 79% of the cases, SWDs were preceded by simultaneous 3–5 Hz precursors in cortex and thalamus; in 11% – only in cortex, but not in thalamus; in 5% – only in thalamus, but not in the cortex; in 5% delta precursors were completely absent both in cortical and the thalamic EEG, an example of this exceptional situation is illustrated in Fig. 1C.

3.3. Correspondence between characteristics of delta and theta SWD–precursors and subsequent SWD

The time delay between delta precursor and subsequent SWD was longer for the cortex (0.48 ± 0.04) than for the thalamus (0.38 ± 0.04), and this difference was highly significant (paired t-test: t = 37.3, df = 5, p < .001).

Correlations between frequencies and durations of precursor activity were accessed in cortex and thalamus in order to determine how strong the correspondence was between cortical and thalamic counterparts of the two types of seizure precursors. The frequency of theta precursors (ft) in cortex and thalamus showed a strong and significant correlation (.98, p < .01): this might imply a strong coupling in oscillatory activity between cortex and thalamus during theta precursors. In contrast, the frequency of delta precursors (fd) in cortex and thalamus did not show a significant correlation (.60), suggesting that cortical and thalamic counterparts might work more independently during delta precursors. The correlation between the mean frequency of delta and theta precursor (fd and ft) in cortex was positive, high and significant (.84, p < .05); for the thalamus a lower value was found (.63, n.s.).

The mean duration (MD) of the delta precursors in cortex and thalamus was correlated .83 (p < .05) as well as the MD of theta precursor .85 (p < .01). Finally, the correlations between frequency and mean duration of the delta and theta precursor activity were all inversely related and varied between (−.11 and −.93). The correlation between duration and frequency of precursor activity was significant for delta in the cortex (−.89, p < .05) and theta in the thalamus (−.87, p < .05).

Correlations between time–frequency parameters of precursor activity and subsequent SWDs were calculated in order to investigate whether there were relations between time–frequency parameters of preictal and ictal EEG. Correlations between frequency of cortical delta and theta precursor activity and
subsequent SWDs were generally positive and also quite high (delta .63, theta .49), for the thalamus the coefficients for delta and theta were .78 (p < .1) and .41, respectively, but failed to reach significance with only six animals. Only the correlation between the frequency of delta precursor activity in the thalamus correlated significantly with the frequency of the subsequent SWD (.87, p < .05).

3.4. Control periods

The number of delta and theta events as recorded in cortex and thalamus in control periods are presented in Table 3.

The 2 factor ANOVA did not show differences between the cortex and thalamus for characteristics of delta and theta, nor an interaction with vigilance states. The subsequent one factor ANOVA showed significant clear differences between the four vigilance states for Nd, Nd/T, Nt, and Nt/T (all Fs > 20.45, df = 3.44, p's < .001). The outcomes of the post-hoc tests showed that Nd and Nd/T were highest in deep-slow wave sleep, followed by light-slow wave sleep, passive wakefulness and active wakefulness (all differences were significant). The post-hoc tests for Nt and Nt/T showed the highest theta scores (both Nt and Nt/T for active wakefulness, followed by passive wakefulness (p < .05), followed by light and deep-slow wave sleep (p's < .05). Light and deep-slow wave sleep did not differ from each other. The relative number (K) of co-occurrence of theta and delta activity was 8.1% for the cortex and 6.0% for the thalamus. This difference was not significant, however, an effect of vigilance state was found (F = 5.48, df = 3.44, p < .01): the co-occurrence was more rare during deep-slow wave sleep than during the other states (all p's < .05), yet these three states were not different.

The duration of the control epochs was 10 s, the duration of the preictal period 2–3 s. The chance for co-occurrence of two events is higher in longer episodes than in shorter episodes and, therefore, we do not perform a formal statistical test. However, it is obvious that, in case we would have chosen shorter control periods, the chance for co-occurrence would be only smaller. We found that the percentage co-occurrence during the control periods (7.7%) in the cortex was much lower than seen for the precursor activity (79%). Descriptive statistics on the probability of co-occurrence of theta and delta precursor activity are presented in Table 4.

4. Discussion

Analyses of the ECoG before the onset of SWDs with continuous wavelet transform have several advantages over traditional FFT. Non-periodic and non-stationary phasic events can be reliably detected. It adds also information in the time domain, i.e., information regarding the dynamic changes of spectral components. Here, we found several interesting phenomena with this method for the SWD–precursor activity in the cortical and thalamic ECoG, suggesting that seizure–precursor epochs differs from non-epileptic EEG and could be regarded as a transient between non-epileptic and SWD activity. The main finding is that both delta and theta precursor activity preceded the onset of SWDs and this combination rarely occurred in control periods. Other findings were the strong association between cortical and thalamic theta precursor activity and the earlier presence of cortical precursors in comparison with thalamic precursors.
The frequency modulation, as described with the continuous wavelet transform, within a train of SWD was earlier described by us in LFP in WAG/Rij rats (Midzianovskaia et al., 2001), as well as in EEG from epilepsy patients (Bosnyakova et al., 2007). We have focused on the 3–9 Hz frequency range considering that previous studies have shown that the activity in these frequency bands dominates the power spectrum in the second preceding the onset of SWDs (Sitnikova and van Luijtelaar, 2009).

### 4.1. Delta precursors

Delta events were rare during active wakefulness, and abundantly present during drowsiness and non-REM sleep. About 80% of the SWDs were preceded by delta activity in cortex and thalamus, and only rarely (about 5%) the delta precursor activity was missing in cortex and thalamus. Clear preceding delta could not be identified in cortical recordings before all SWDs, however, this is not a big surprise considering that we used a standard EEG recording protocol, and our electrodes were not aimed at the peri-orbital region of the somatosensory cortex, which is known as the initiating site of SWDs (Meeren et al., 2002; Klein et al., 2004; Gurbanova et al., 2006; Polack et al., 2007). Delta activity was also often present in the VPM, i.e., the somatosensory thalamic relay nucleus. SWD are assumed to be predominantly expressed in the somatosensory part of a cortico-thalamo-cortical network (Pinault, 2003; Pinault et al., 2006), under feedback control of GABAergic neurons in the reticular thalamic nucleus (RTN) (Huguenard and McCormick, 2007). The intensity of the delta precursor appeared small in less than 20% of the SWDs, the peak on the wavelet surfaces was, consequently, also small in these cases and delta precursor was poorly allocated in the background of the wavelet spectrum. This occurred more often in the thalamus than in the cortex and this is well understandable since the amplitude of the thalamic local field potentials is much smaller in the thalamus than in the cortex, due to differences in the aligning and orientation of neuronal cells (Lopes da Silva and van Rotterdam, 1987). The cortex with its layer structure and parallel orientation of dendrites facilitates large dipoles, in contrast to the thalamus, where the cells are more scattered and less stereotypically oriented.

SWDs have a tendency to occur when the patients or rats are quite, not engaged in serious physical or mental activity, at idle periods, at periods of drowsiness, or at transitional states (van Luijtelaar et al., 1991; Coenen et al., 1991; Drinkenburg et al., 1991; Luttjohann et al., submitted for publication). These periods are accompanied by a slowing of the EEG frequencies. Therefore, it is not surprising that SWDs are preceded by some activity in the delta range.

There are also some neuronal mechanisms that could account for a pro-epileptic nature of brief episodes of delta. The physiological characteristic firing mode of thalamo-cortical cells (TC in the thalamus) and cortico-thalamic neurons (CT in the cortex) during the cortically recorded SWDs is the burst mode. The burst mode is activated during drowsiness. The frequency of the burst firing mode is determined by the amount of hyperpolarization of TC and CT cells. The intrinsic oscillator properties of TC neurons, which form the constituent agent for the burst firing pattern, are well described: they cycle between a depolarization induced by a T-type Ca\(^{2+}\) current that triggers a burst of fast Na\(^+\)/K\(^+\) action potentials and a subsequent hyperpolarization that activates the hyperpolarization-activated cation I\(_h\) channel (McCormick and Pape, 1990; Pape, 1996). It is proposed here that hyperpolarization of a sufficient number of CT cells (a critical mass) in the peri-orbital region of the somatosensory cortex by the activity of cortical GABAergic neurons activate I\(_h\), and this result in burst firing. We assume that the preceding delta, as recorded with cortical LFP, is a manifestation of this hyperpolarization. Next and slightly later in time, TC cells in the ventral basal complex are hyperpolarized through the activation of the RTN, which receives excitatory projections from CT cells and sends inhibitory projections to the ventral basal complex. This might explain why thalamic precursors are slightly delayed in comparison to cortical ones.

There are strong links between the occurrence of seizures and I\(_h\) channels. They consist of moment to moment voltage changes induced by various synaptic and intrinsic currents, and of short- and long-term modulatory and plasticity processes (Chen et al., 2002). Various authors have established a role of I\(_h\) channels in particular absence epilepsy in WAG/Rij rats (Ludwig et al., 2003; Strauss et al., 2004; Schridde et al., 2006; Budde et al., 2005). A recent finding is that a pharmacological treatment that prevents SWDs also prevents the age dependent upregulation of h-channels (Blumenfeld et al., 2008). Other direct evidence for a crucial role of h-channels in the pathogenesis of absence seizures comes from systemic injection studies with h-channel blockers. Systemic injections in vivo studies with a new h-channel blocker in WAG/Rij rats resulted in a dose-dependent decrease in SWDs (van Luijtelaar et al., in preparation). In all, we suggest that the preceding delta activity is a reflection of the hyperpolarization of a substantial number of neurons, initiated in the cortex, followed in time by hyperpolarization of TC in the thalamus. Further experiments and other techniques (i.e., patch clamp, single-unit recordings and pharmaco-EEG) are needed in order to test this suggestion.

### 4.2. Theta precursors

Theta events were abundantly present during active wakefulness, one of the control periods. A high percentage (82.5 for the thalamus and 92 for the cortex) of SWDs was preceded by an additional elevation of 7–10 Hz. This pre-SWD activity in WAG/Rij might be similar to the 4–12 Hz middle-voltage oscillations (also described as 5–9 Hz seizure–precursor rhythm) that precede SWD in GAERS and appeared to be similar to wake-related normal 4–12 Hz oscillations (Pinault et al., 2001). The neuronal mechanisms of these oscillations were extensively studied intra- and extracellularly after barbiturate administration in neuroleptanalgesied rats (Pinault et al., 2006). It was shown that the 4–12 Hz was produced by neuronal networks comprising the somatosensory part of the thalamo-cortical system, i.e., somatosensory thalamus and fronto-parietal (somatosensory and motor) cortex (Pinault et al., 2006). It was further established that neurons in deep layers of the somatosensory cortex began to fire at 5–9 Hz a few milliseconds earlier than neurons in the corresponding specific (VPM) and non-specific RTN nuclei (Polack et al., 2007). Finally, it was found that cortical neurons are able to modulate membrane potentials of thalamic neurons throughout dense cortico-thalamic synaptic interactions. This suggests that cortical neurons in layer VI, and most likely also those in layer V (Jones, 2009) largely affect neuronal activity in the functionally related thalamic nuclei. In such a way, the cortex initiates a pro-epileptic state, in which the

### Table 4

Overview of co-occurrence of % delta and theta activity in cortex in 2.5–3 s preictally (Pre-SWD) and in control periods (Active and passive wakefulness, light and deep-slow wave sleep).

<table>
<thead>
<tr>
<th></th>
<th>Frontal cortex (%)</th>
<th>Thalamus, VPM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-SWD</td>
<td>79.0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Active wakefulness</td>
<td>8.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Passive wakefulness</td>
<td>12.9</td>
<td>11.7</td>
</tr>
<tr>
<td>Light-slow wave sleep</td>
<td>9.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Deep-slow wave sleep</td>
<td>2.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

n.a., not assessed.

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cortico–thalamo–cortical network produces 5–9 Hz seizure–precursor rhythm followed by SWD. According to Pinault et al. (2006), thalamic theta might be a fingerprint of cortical theta (or vice versa). It is hypothesized that impairment in the cortico-thalamo-cortical system in epileptic animals results in seizure–precursor rhythm (5–9 Hz) both in cortex and thalamus that could subsequently be transformed in absence seizures, if a sufficient number of neurons start firing synchronously.

The role of the somatosensory cortex in rodent absence epilepsy has gained an increased interest when it was established that the SWDs are initiated in the peri-oral region of the somatosensory cortex and quickly spread to the thalamus, including the RTN (Meeren et al., 2002; Klein et al., 2004; van Luijtelaar and Sitnikova, 2006; Polack et al., 2007, 2009). Here, we found that the latency between delta precursor activity and subsequent SWDs was longer in the cortex than in the thalamus, and this might agree with the Meeren et al. (2002) finding of the earlier onset of SWDs in the cortex (somatosensory area). Various neuroactive compounds increased and decreased SWD when injected locally in the cortex, in agreement with outcomes as predicted by the cortical focus theory (Citro et al., 2006; Gurbanova et al., 2006). Using in vivo intracortical recordings, it was found that SWDs are initiated in layer V/VI neurons of facial region of the somatosensory cortex (Polack et al., 2007). These neurons, which show a distinctive hyperactivity associated with a membrane depolarization, lead the firing of distant cortical cells during the epileptic discharge (Polack et al., 2007). They form the substrate for synchronization of widespread populations of cortical and thalamic neurons (Jones, 2009). The cortical neurons have icotogenic properties: a specific elevated and regular firing rate between, before and during the presence of SWD as established with multisite LFP and surface ECoG (electrocorticogram). Neurons in the deep cortical layers start to fire in the burst mode with a high frequency (15 Hz), while cortical cells in layer II–IV fire interictally with 3–4 Hz (Polack et al., 2007). It is, therefore, proposed that the 3–4 Hz precursor activity as found in the supragranular cortical layers with the aid of ECoG recordings, represent synchronized firing of ensembles of cortical neurons at 3–4 Hz. Laminar analyzes of precursor activity of local field potentials might provide the missing link between the Polack et al. (2007) results and the present data.

4.3. Correspondence between parameters of theta and delta precursors

The correlation analysis showed rather high and positive correlation between the frequency of theta precursor in cortex and thalamus, the correlation of cortical and thalamic delta was not significant. These correlations in mainly the theta frequencies suggest that the occurrence of theta (a possible role of delta in this cannot be excluded definitely) preictal activity of SWD occurs in parallel and perhaps even in a coordinated fashion. The role of preictal theta was recently discussed by Pinault et al. (2006). The correspondence between frequency parameters of thalamic and cortical delta is less strong, suggesting a certain independence between thalamic and cortical delta.

The negative correlation between duration and frequency of precursors is significant in cortex (delta precursor activity) and thalamus (theta precursor activity). This implies that precursors, whose frequency is higher, appear to be shorter. This could be explained by a hypothetical neurophysiological mechanism that might stop precursor activity when its frequency is too fast, therefore, precursors with a higher frequency will become shorter and in case this does not happen, SWDs might not emerge. Recently, we used fast Fourier transform (FFT) to analyze SWD–precursor activity in fixed time epochs (1 s) that immediately preceded SWDs in WAG/Rij rats, and we identified powerful delta and theta components (Sitnikova and van Luijtelaar, 2009). Here, we applied continuous wavelet transform in order to provide more accurate and detailed description of preictal activity in the time and frequency domains. In the present paper, we make step forward in understanding thalamo-cortical mechanisms of seizure–precursor activity by demonstrating that delta activity is present in the thalamus before the start of an SWD, that the time–frequency characteristics of delta and theta precursor activity in thalamus and cortex are not the same and that there is a certain time delay between delta precursor activity in cortex and thalamus.

4.4. Practical notes concerning the application of continuous wavelet transform in time–frequency EEG analysis

Continuous wavelet transform has several advantages over traditional FFT for representing non-periodic and non–stationary signals that have sharp peaks or other kinds of fast events. A limitation of FFT is that it characterizes EEG signal only in the frequency and not in the time domain, i.e., information regarding the dynamic changes of spectral components. In wavelet space, EEG signal power is represented as a function of time and frequency simultaneously. The wavelet coefficients represent the degree of correlation of a prototype wavelet function with the EEG signal. The wavelet prototypes (mother wavelet) are wave–like scalable function, which are well localized in both time and frequency domains (Kaiser, 1994; Koronovskii and Hramov, 2003). Some mother wavelets provide better resolution in time domain, and others in frequency domain. The choice of the mother wavelet is of ultimate importance and it is crucial for the accurate representation of the EEG signal into the wavelet space.

Important characteristics that were taken into account before making a choice of mother wavelet were complex/real, width and shape of the candidate wavelets.

(1) Complex or real. A real wavelet returns information about either amplitude or phase of the EEG signal and it may be sufficient for isolating EEG spikes or other discontinuities, but not sufficient for detecting sustained oscillatory processes. A complex wavelet returns information about both amplitude and phase and this is more suitable for representing oscillatory EEG phenomena in wavelet space (Kaiser, 1994).

(2) Shape. Accurate results of wavelet decomposition are dependent on the shape of the chosen wavelet function and it should be similar to the shape of the analyzed EEG signal (Latka et al., 2003). Rectangular functions, such as the Harr, are better in representing EEG spikes (and other sharp events in EEG), the Mexican hat is particularly suitable for the detection of epileptic spikes (Latka et al., 2003). Meanwhile, oscillatory EEG patterns can be more accurately represented with cosine wavelet functions, in particular, the Morlet wavelet was more suited for the detection of sleep spindles, because it mimics the characteristic spindle waveform (Latka et al., 2003; Sitnikova et al., 2009). Although the Morlet wavelet has no sharp elements equivalent to spikes in SWDs and its shape does not mimic SWDs, it provided the best time–frequency resolution in comparison to the ‘sharp–looking’ Mexican Hat.

(3) Width. If a candidate wavelet is too narrow, the frequency resolution will be poor, and if it is wide, the time localization will be less precise. It is also important to take into account the reverse relationship between time and frequency resolution. The higher the frequency resolution, the lower the time resolution and vice versa. The time–frequency resolution can be defined by the shape and by the width of wavelet function in the frequency domain. Parameter $\omega_0$ determines in the complex Morlet wavelet family the shape and the width of the wavelet function in the frequency domain. When $\omega_0 < \pi$, $\omega_0 \approx 0$ determines in the complex Morlet wavelet family the shape and the width of the wavelet function in the frequency domain.
the temporal resolution is high, but little information is available about the frequency content of EEG events (frequency resolution is low). When $\omega_0 > 4\pi$, the frequency resolution is high, but the time resolution is low. We chose the complex Morlet wavelet with $\omega_0 = 2\pi$ as the most appropriate basis which provided the optimal time–frequency resolution for both investigated EEG phenomena. This basis function was particularly good in localizing the abrupt onset of SWD as well as gradual amplitude changes during the seizure train.

4.5. Relevance and conclusion

The prediction of absence seizures by detectable dynamic changes in the EEG is still debated (Suffczynski et al., 2004) and computational models were developed to study the transient between normal and pathological oscillatory activity (Suffczynski et al., 2004; Sargsyan et al., 2007). None of these recent models included characteristics of h channels, nor its interaction with delta precursors, or the occurrence of theta precursor activity. The successful detection of a pre-seizure state before clinical onset, in principle, gives a chance for a therapist to prevent seizure, and also allows new treatment techniques, such as the delivery of an electric impulse to avoid an oncoming seizure. In addition, the exploration of pre-seizure state could be helpful in understanding the mechanisms underlying the generation of seizures and in particular, seizure onset (Litt and Lehnerz, 2002; Mormann et al., 2007).

Therefore, understanding the transition of brain activity towards an absence seizure and to look for some precursor activity might have promises for the future.

It can be concluded that SWD activity is preceded by short lasting delta and theta precursor activity in cortex and thalamus and that the combination rarely occurs during control periods. Most likely, the co-occurrence of the two types of precursor activity in cortex and thalamus is imperative for the occurrence of SWDs. It is proposed that the delta precursor activity represents the hyperpolarization of CT and TC cells.

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References


