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Methods of automated absence seizure detection, interference by stimulation, and possibilities for prediction in genetic absence models

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HIGHLIGHTS

- The detection of spike-wave discharges in the rodents ECoG can be automated with high sensitivity and specificity.
- The real-time early detection of spike-wave discharges with continuous wavelet transform of rodents ECoG is feasible.
- Spike-wave discharges can be aborted with various forms of stimulation.
- Spike-wave discharges are preceded by precursor activity, allowing SWD prediction, and control.

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ABSTRACT

Background: Genetic rat models for childhood absence epilepsy have become instrumental in developing theories on the origin of absence epilepsy, the evaluation of new and experimental treatments, as well as in developing new methods for automatic seizure detection, prediction, and/or interference of seizures. **Method:** Various methods for automated off and on-line analyses of ECoG in rodent models are reviewed, as well as data on how to interfere with the spike-wave discharges by different types of invasive and non-invasive electrical, magnetic, and optical brain stimulation. Also a new method for seizure prediction is proposed.

Results: Many selective and specific methods for off- and on-line spike-wave discharge detection seem excellent, with possibilities to overcome the issue of individual differences. Moreover, electrical deep brain stimulation is rather effective in interrupting ongoing spike-wave discharges with low stimulation intensity. A network based method is proposed for absence seizures prediction with a high sensitivity but a low selectivity. Solutions that prevent false alarms, integrated in a closed loop brain stimulation system open the ways for experimental seizure control.

Conclusions: The presence of preictal cursor activity detected with state of the art time frequency and network analyses shows that spike-wave discharges are not caused by sudden and abrupt transitions but that there are detectable dynamic events. Their changes in time-space-frequency characteristics might yield new options for seizure prediction and seizure control.

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1. Introduction

The possibility to record the electroencephalographic activity over a prolonged period in chronically implanted well accepted genetic models such as WAG/Rij and GAERS (Depaulis and van Luijtelaar, 2006), but also in other rats such as some Wistars, Fischer 344, Brown Norway, and their F1 and intercrosses, and transgenic mice endowed with spontaneous occurring spike-wave discharges (SWDs) (Willoughby and Mackenzie, 1992; Burgess, 2006; Noebels, 2006) have yielded large data sets, e.g., for genetic analyses of absence epilepsy (Jandó et al., 1993; Vadász et al., 1995; Gauguier et al., 2004). These long lasting recordings are also necessary for the study of long term drug effects and epileptogenesis (van Luijtelaar et al., 2013), for chronic drug studies (D'Amore et al., 2014), for the study of circadian rhythms of SWDs and the consequences of their shifts (Smyk et al., 2012). The analyses of these large data sets have led to new insights on the characteristics of absence epilepsy, but also to refinement of the experiments by having larger and more solid data sets. These large data sets prompted the need for reliable and automated methods to detect and describe these pathological phenomena and their difference with sleep spindles (Sitnikova et al., 2009). Thanks to the availability of new signal analytical methods it is now possible to analyse in larger details first off line but now also on-line SWDs in the genetic rodent models. The new methods describe seizures in the time and frequency domain without violating assumptions on stationarity, but also across space as done in network analyses (Meeren et al., 2002; Lüttjohann and van Luijtelaar, 2012). They offer new possibilities for understanding the (absence) epileptic brain and their typical SWDs but the methods might have also some value for describing other seizure types, the consequences of seizures, and whether new and different interventions, dependent on real-time seizure detection might be putative effective treatment options. Examples are neuro feedback training in WAG/Rij rats (Osterhagen et al., 2010), deep brain stimulation (Lüttjohann and van Luijtelaar, 2013; Blik, 2015), or stimulation with laser light; these SWD aborting stimuli can be given contingent upon the real-time detection of SWDs (Paz et al., 2013). The application of new signal analytical tools have demonstrated the presence of preictal activity in cortex and thalamus, while different types of network analyses have, in principle, paved the way for SWD prediction. In the following paragraph the different systems and principles used for the reliable detection of SWD in the ECoG of rat/mice strains endowed with mainly spontaneous SWDs are reviewed. This is followed by a short review of the various DBS techniques in order to interrupt ongoing seizure activity (Vercueil et al., 1998; Feddersen et al., 2007; Lüttjohann and van Luijtelaar, 2013) and other stimulation techniques. In the third paragraph activity preceding the onset of SWDs (precursor activity) based on time frequency analyses and on networks will be discussed (van Luijtelaar et al., 2011; Li et al., 2007; Lüttjohann et al., 2013), followed by a paragraph on possibilities for seizure prediction in genetic models based on network precursor activity.

2. Detection of SWDs

2.1. Off line methods

An overview of the different methods that have been described is presented in Table 1. The first modern approach to develop a reliable off line SWD detection system for the analyses of previously obtained data sets was in fact a High Voltage Spindle (HVS) detection system (Jandó et al., 1993). HVS, large amplitude sharp spindles, mimic SWDs, although not all included HVS would unambiguously classify as SWDs, since the spikes of the HVS might be less sharp and the waves were sometimes missing. 16 6–8 month old rats (Fischer 344 and Brown Norway strains) were used to develop and train the system, as well as their F1 and F2 descendents, and back-crosses with the parental strains for its evaluation and subsequent quantitative genetic analyses of the HVS (Vadász et al., 1995). An artificial neural network was used, which should be properly trained first before the desired output of the test data set is generated. The input of this three layer (input, hidden, and output) back propagation neural network was either a raw single channel frontal ECoG with visually marked HVS and ECoG's from 16 animals from the same strains but without HVS, or Fast Fourier Transformed (FFT) ECoG's from the same rats. A sliding window of 10 ms was used. It was found that feeding the network with the outcomes of the FFT significantly shortened the training time compared to having the raw ECoG as input and that the network performed better if it was fed by data of several animals. It was mentioned also that search for the most optimal effective neural network configuration, given the variable number of cells within each of the three layers of the neural network, is rather time consuming; if done, than the network could be used for large data sets. The performance of the network was evaluated in a test data set of 137 animals against visual scoring: the number of HVS which were detected properly by the network reached 93–99% of the manually marked HVS. HVS with small spike components or small amplitude were sometimes missed, while falsely detected events (non-HVS, body scratching, grooming, typical sleep spindles, artifacts) varied between 18% and 40%. The authors discussed extensively that there is always a trade-off between number of correct detections, number of missed detections, and number of false positives. Despite its good sensitivity this back propagation neural network was not used by others, its number of false positives, the low learning speed and the time consuming procedure to optimize the network configuration might have been a major bottleneck for its broad and widespread application.

A second automated off line detection system was developed by Westerhuis et al. (1996) and it was used in many subsequent ECoG studies in WAG/Rij rats. WAG/Rij and GAERS are currently the most used and best characterized and established genetic rat models of absence epilepsy. The cortical frontal–parietal differential ECoG of adult male WAG/Rij rats was used, it was filtered between 1 and 100 Hz, sample rate 200 Hz. It was based on a moving window and it subsequently calculated the absolute difference of two consecutive

Table 1
Comparison of off-line spike-wave discharges detection methods in rodent models.

Species	Strain	Age	Electrode, Position channels	EEG preprocessing and sample rate	Methods	Peculiarities	Evaluation	Application in humans
Rats a <i>n</i> = 16, <i>n</i> = 137	F344, BN F1, F2	6–8 m	L2.5–AP2.5 L5.0–AP0.0	1–50 Hz, 100 Hz	Backpropagation three layer neural network	determination of network configuration is time consuming	93–99% correct detection; 18–40% FP	N.A.
Rats b <i>n</i> = 9	WAG/Rij	>6 m	L2.0–AP2.0; L4.0–AP–5.0 1 Ch	1–100 Hz 200 Hz	First derivate, steepness in 0.25 s for 1 s	Fast enough for on-line detection	>98% correct detection, 7.9% FP	N.A.
Rats c <i>n</i> = 4	F344	4 m	2 F, 2 C, 2 P; 6 Ch	Not given	Wavelet energy at 7 Hz		Sensitivity rates >90% FP rate 2–4 per hour	Adapted from detection method in humans
Rats d <i>n</i> = 5	WAG/Rij	11–12 m	L2.5–AP2.0 1ch, quasi monopolar	0.5–100 Hz, 1024 Hz	Morlet wavelet, energy in 30–50 Hz band	Sleep spindles and SWD are fundamental different	Sensitivity 98.8%, Specificity 98.7%	N.A.
Rats e <i>n</i> = 13	GAERS	Not given	Frontoparietal	Not given	Reservoir computing, first derivate, harmonics of 8 Hz	Threshold is automatically determined; Fast enough for on-line detection	Performance index of 0.99. Westerhuis et al., 0.91 Fanselow et al. i 0.96,	N.A.
Rats f <i>n</i> = 23	GAERS	>4 m	Three epidural bilateral implanted electrodes over the frontoparietal cortex, reference lambda	Not given 200 Hz	Harmonic analysis with continuity analysis to estimate the fundamental frequency	Individual amplitude differences tackled	Sensitivity and selectivity of 96% and 97%	N.A.
Rats g <i>n</i> = 6	WAG/Rij	>6 m	Layer V/VI facial area of SS cortex and Posterior thalamus	1–100 Hz, 1024 Hz	A model base d on radial basis functions of SWD and non-SWD are used as a template	Individual amplitude differences tackled; Performance is better on 2 vs 1 channel	Accuracy. 94.8%; two artifacts were absence-like sleep spindles	N.A.
Mice h <i>n</i> = 5, <i>n</i> = 6 <i>n</i> = 8	Gria4, Gabrg2, Scn8a		4 electrodes all L: ±2.0 AP + 1. 6 ch: three bipolar differential and three referenced recordings	0.3–50 Hz 200 Hz	Morlet wavelet transform: spectral power and spectral phase values; four harmonics	Quantification of morphological variants of SWDs.	Accuracy*: Gria4: 91.8% Gabrg: 98.9% Scn8a: 94.9% FP: Gria4: 8.4% Gabrg: 4% Scn8a: 3.7%	N.A.

(a) Jandó et al. (1993); (b) Westerhuis et al. (1996); (c) Xanthopoulos et al. (2009); (d) Sitnikova et al. (2009); (d) Buteneers et al. (2009); (f) van Hese et al. (2009); (g) Startceva et al. (2015); (h) Richard et al. (2015); (i) Fanselow et al. (2000).

* Inferred from their Table 1.

digitized samples. This value is called the steepness of the ECoG, and this parameter reliably detects sharp peaks in the ECoG signal; basically it uses the first derivate which is large for sharp events to detect sharp events. This variable was more sensitive and robust than amplitude of the spikes and the asymmetric pattern of SWD. The maximum of the steepness over a time period of 0.25 s was obtained. If its average value over a time period of 0.25 s exceeded a certain arbitrary chosen threshold level for 1 s (this is a temporal constraint which was adopted in many different systems, since short SWDs might have no or less clinical relevance), an SWD was detected. The threshold can be automatically estimated on the basis of the steepness of the interictal ECoG during wakefulness. This system was evaluated by comparing its performance in a data set of nine WAG/Rij rats (2 h each), to the consensus of two experts. The consensus detected in total 405 SWDs. The automatic system detected 392 phenomena correctly, 97%. 36 incorrect detections were made, mainly consisting of movement artifacts (Westerhuis et al., 1996).

At the beginning of the century, the continuous wavelet transform (CWT) was introduced for the analyses of time frequency dynamics of SWD e.g., in WAG/Rij rats (Bosnyakova et al., 2006),

and for the analyses of large ECoG data sets. Time frequency analysis was used first in off-line recording of ECoG data, later for real-time analyses. A major advantage of CWT over other time-frequency analyses techniques is that CWT does not require stationarity of the time series under investigation (EEG or ECoG signal). The electrographic recordings of humans and mammals contain non-stationary phasic events such as sleep spindles and K-complexes, as well as interictal and ictal activity. The frequency changes abruptly over time and this restricts applicability of traditional frequency-domain measures (such as FFT). In contrast, in wavelet decomposition the ECoG signal can be represented in a two-dimensional time–frequency-domain, in which signal power changes as a function of time and frequency simultaneously (Daubechies, 1993, Koronovskii and Hramov, 2003).

Xanthopoulos et al. (2009) used skull electrodes in four 4 month old Fischer344 rats. A time frequency decomposition of the ECoG, as collected during 8 h employing the Morlet mother wavelet function was used for off-line analyses. Subsequently, the variance profile of the wavelet energy around 7 Hz of all six channels was computed in a moving window of 200 samples (1 s) and seizures were detected by a thresholding process. The algorithm was found to

have a high sensitivity (>95%) along with a low false positive rate (~2–4 false positive epochs per hour). The nature of the likely false positives (sleep spindles, grooming, movement artifacts) were not mentioned.

Sitnikova et al. (2009) developed a specific complex Morlet wavelet-based algorithm for the automatic off line identification of SWD and sleep spindles in the ECoG of WAG/Rij rats. A single channel frontal cortical recording was used, sample rate 1024 Hz. None of standard wavelet templates could precisely identify all sleep spindles and SWD and different wavelet templates were imperative in order to detect reliably both types of phasic events. It was found that SWD were identified with high probability using the standard Morlet wavelet, but sleep spindles were only identified using two types of customized adoptive ‘spindle wavelets’. Another observation was that SWD and sleep spindles were detected due to the elevation of wavelet energy in different frequencies: SWD in the 30–50 Hz band, sleep spindles in 7–14 Hz. It was concluded that SWD can be accurately detected based on energy in the 30–50 Hz range and that this energy was several times higher than the energy in the mean interspike frequency of SWD (8–12 Hz). Interestingly, the lack of difference for sleep spindles and SWDs in the fundamental frequencies and the large differences in the harmonic spectral components (high in SWDs and low in sleep spindles, see also Drinkenburg et al., 1993, vanHese et al., 2009) might be very well suited for automated distinction between sleep spindles and (short) SWDs and contribute to a reduction of false positives.

Buteneers et al. (2009) reduced the issue of individual differences (different threshold values were necessary for individual rats) to some extent by taking first the mean of the absolute value of the ECoG data set. This value is then used as a scaling factor by normalizing the amplitude of the ECoG data set. A single channel ECoG channel was used from existing data sets, selected by an expert based on the cleanness of the recordings and the quality of SWDs. The first derivative and the power of the frequency bands, obtained via time frequency analyses around 8 Hz, and two harmonics of the interspike frequency (16 and 24 Hz) were used to “feed” or better to train a simple linear readout function of a randomly created neural network. This method is called “Reservoir Computing (RC)”. It greatly simplified the training of recurrent neural networks since only the weights of the output function are trained. RC was successfully applied to perform fast detection SWDs in the ECoG in GAERS. SWD were detected with an average detection delay of 0.3 s. The method has an estimated 95% sensitivity and specificity. Moreover, a comparison with two other SWD detection methods (e.g., Fanselow et al., 2000; Westerhuis et al., 1996) with the same GAERS data set showed that specifically the speed of detection of SWD was higher compared to the other methods, suggesting that it might be suitable for on-line SWD detection.

vanHese et al. (2009) used the cortical ECoG of adult GAERS, sample frequency of 200 Hz. Gabor transform was used to obtain time frequency plots to overcome the issue of a non-stationary ECoG signal. Also these authors estimated the background spectrum as a reference value to cope with the variability of the amplitude of the ECoG signal amplitudes among the different animals. They dealt also with the inharmonicity of SWDs, and used a pitch continuity analyses in order to obtain a more robust algorithm. Their detection of SWD was also based on the presence of harmonics in the signals during SWDs. Moreover, they compared their proposed method also on two data sets from GAERS with other SWD methods developed to detect SWD in rodents (Fanselow et al., 2000; Westerhuis et al., 1996). All methods were sensitive, but large differences in selectivity were found. The method developed by the authors was superior (>showing a sensitivity and selectivity of 96% and 97%), respectively, on a first large marked data set, and a sensitivity and selectivity of 94% and 92%, respectively, on a second large test set. In general, methods that used energy levels of ECoG

during SWD relative to the background level or that used peaks in the spectrum relative to estimates of the background spectral activity were better in terms of selectivity. Artifacts (in this case stimulation artifacts) were successfully removed by making a template and if above a threshold for a certain duration, the pieces of ECoG were disregarded.

An approach based on nonlinear predictive (autoregressive) model construction from experimental ECoG data was recently proposed for off-line SWD detection in WAG/Rij rats (Startceva et al., 2015). The method suggests the construction of two empirical models: one for SWD and the other for interictal (=baseline) activity. These models are nonlinear multidimensional maps with approximation of nonlinearity by radial basis functions. The number and type of radial basis functions are adjusted to data based on statistical criteria of SWD and interictal episodes. The main idea is that the prediction error of the model, constructed from a fragment of the SWD signal, is significantly smaller for all SWD than for other, non-absence fragments of series, as the prediction error of the baseline mode is significantly smaller for baseline prediction than for SWD prediction. Using two models prevented the detection of artifacts as SWD patterns and increases the specificity of the method.

It turned out that for all six studied WAG/Rij rats, the same optimal model parameters from their somatosensory cortex ECoG signals could be obtained (as well as for and ventroposteromedial thalamic nucleus), and that the optimal model parameters do not differ for different seizures and different subjects. The model seems to be robust since being applied to clear SWD fragments of other rats it gave the same 100% amount of detected SWDs and only about 5% more false alarms than for rats used for model construction. This might imply that the issue of inter individual differences in amplitude, and differences in time frequency characteristics of SWDs within the same animal and between SWD of different animals are tackled by this approach. The time resolution necessary to obtain and classify data from a sliding window is close to the sampling interval of the original data (in this case 2048 Hz), and therefore real time detection seems possible. The proposed approach demonstrates a high accuracy in detection of the moment in time of SWD onset, with a mean error being close to zero. Finally the authors noted that the method can be readily applied to similar ECoG data and only boundaries (threshold values) for the SWD model error and for the non-SWD model error should be chosen.

A SWD reader for mice was recently described (Richard et al., 2015). The cortical ECoG's (three bipolar differential and three referenced recordings) of mice carrying the Gria4, Gabrg2, or Scn8a mutations showing SWDs were analysed with a Morlet wavelet transform and both spectral power and spectral phase values were calculated, the latter in order to quantify morphological variants of the SWDs, a feature that has not been quantified before. Their analysis method relies heavily on the analyses of the harmonics of the fundamental frequency of SWDs. A metric was obtained for the strength of four harmonics of the fundamental SWD frequency, independent of the fundamental frequencies of the tested mouse strains. Morphological different variants, but still SWDs, were reliably identified. Moreover, their method was able to accurately detect SWDs that fell within the parameter values found to be characteristic of SWD morphology. The performance of their SWD detection was very satisfying, see Table 1.

2.2. On line methods

The first real on-line SWD detection system was proposed by Fanselow et al. (2000). Their method was based on thresholding the amplitude of the 30 Hz low pass filtered multichannel thalamic and cortical ECoG in Long-Evans hooded rats, treated with a low dose of PTZ, inducing SWD-like seizures. Sample frequency of the ECoG was 512 Hz. The step size and the window length were equal to 0.5

and 1 s, respectively. No performance evaluation of the method has been reported in their original paper.

Ovchinnikov et al. (2010) developed and evaluated an on-line SWD detection system based on a single frontal ECoG channel in WAG/Rij rats, sample rate 500 Hz. They used the energy in the 30–80 Hz characteristic frequency band during SWDs as was proposed by Sitnikova et al. (2009). The Morlet wavelet power for this frequency range at each moment in time showed a drastic increase in power in that specific band at the very beginning of a SWD and a rapid decrease of the power at the end of a SWD. The wavelet transform on a total of 15 scales proportional to 15 frequencies equally distributed between 30 and 80 Hz was used and the sum of the calculated wavelet power values for each frequency is the absolute wavelet power over the frequency domain. Processing of additional (more than 15 scales) time scales did not improve the accuracy of the detection. An optimal threshold value was chosen for each animal on the basis of previous recorded data. The system was evaluated in 5 h recordings in eight rats. All SWDs were detected, with only 2–3% false positives. These errors were identified as intermediate state sleep spindles (Gottesmann, 1996). The system has been applied in a closed loop DBS paradigm (Lüttjohann and van Luijtelaar, 2013).

A combination of approximate entropy and the power of two selected frequency bands (intraspike frequency and first harmonic) of the ECoG of the frontal barrel cortex filtered between 0.32 and 80 Hz, sampled at 200 Hz was used by Liang et al. (2011) to detect seizure activity in an epilepsy model (Long-Evans rats with spontaneous non-convulsive SWDs) and in a seizure model (the proconvulsant PTZ (20 mg/kg i.p.) was injected into Long-Evans rats to provoke SWD). A five hour training set was used of the epilepsy data from three rats, 2 × 2 h ECoG in case of the PTZ seizures, also in three rats. The accuracy rate on the spontaneous SWDs varied between 92.0 and 99.1, the false detection rate about 2–3%, and the detection delay was about 0.5 s. Also the performance rates of the detection of the PTZ induced seizures were excellent and comparable with the performance of the spontaneous occurring SWDs. The authors mentioned that the combination of entropy and spectral powers is essential for the high performance of classification. The false detections induced by grooming and slow wave sleep were reduced by conditional enhancing the detection threshold. The system was used in closed loop DBS experiments.

Line length obtained from a single cortical EEG signal of rats, obtained from skull screws, band-pass filtered (1–40 Hz was allowed to pass) in a moving window of 2 s, was used for on-line seizure detection. The seizures were detected within 1 s of initiation. The by focal cortical strokes induced seizures mimicking SWD, contained peak frequencies of 4–5 and 8–10 Hz. These spectral properties are different from what is commonly seen in rats with spontaneous occurring SWDs. Although no quantitative data were presented regarding the sensitivity and specificity of the method applied to the detection of the seizures elicited here, the authors have used the method up to one year post-stroke in two injured rats with chronic implants (Paz et al., 2013). Line-length has been as a validated as an efficient parameter for seizure detection in a population of presurgical patients (Esteller et al., 2001). Whether this system is also able to detect SWD as occur spontaneously in the genetic rodent models with a high sensitivity and specificity, seems likely, although is awaiting experimental verification.

A genuine SWD on-line detection system was developed and evaluated by Aghazadeh et al. (2015). Once more a single ECoG channel was chosen (frontal-occipital) with a sampling rate 1000 Hz. The authors also used the CWT approach and now the SWD pattern adapted mother wavelet was chosen in two frequency bands: the 10–11 and 30–80 Hz. The first is the frequency band of the onset of SWD activity, the latter band has been proposed by Ovchinnikov et al. (2010). The reason for their choice is that sleep

spindles and intermediate state on the one hand and SWDs on the other differ in the absence-presence of these high frequencies and in this way the false detection rate might be reduced. The authors claim to have developed a system that is independent of the individual properties of the ECoG signal of each rat. The system was first evaluated in six, later in eight 6–8 months old WAG/Rij rats, its sensitivity was 100%, its specificity 97.8% with on average 1 false positive during the whole recording session (length not specified). A tradeoff between precision and detection delay can be obtained since the number of false positives was found to decrease with an increase in window size at the cost of a small increase in SWD detection delay. The authors propose that their system can also be used for clinical use.

Considering that reliable on line SWD systems have been applied it seems that there is no longer a computer resource limitation for on-line processing of the ECoG. Analyzing the results of the developing of the different mathematical methods and software for on-line ECoG application and perhaps diagnostics, we conclude that nowadays the computing power of personal computers or laptops and the effectiveness of developed modern algorithms of data processing allows the analysis of multi-channel EEG in real time. For example, Ovchinnikov et al. (2010, 2011) noted that the developed wavelet-based method of on-line SWD detection can be effectively used for simultaneous processing of about 20 channels on a single middle-power standard PC (hardware processor Intel Core 2 Quad Q6600 Kentsfield (2400 MHz, LGA775, L2 8192 Kb, 1066 MHz), RAM 2 GB). In a recent monography (Hramov et al., 2015) the fast algorithms of wavelet transform are described and discussed with an eye to the application of on-line EEG/MEG digital processing.

In all, SWDs can be detected with a high sensitivity in a single ECoG channel in the genetic rodent models, nowadays also on-line. Although there are clear differences among the various systems regarding the used technology, there does not seem to be a single unique and best method. Methods that use energy in the high frequency bands as captured with adapted Morlet wavelet, combined with either steepness and or entropy, while controlling for interindividual differences in ECoG amplitude interictally offer currently excellent possibilities for sensitive and selective real time early SWD detection.

3. Brain-stimulation as a new treatment for epilepsy investigated in genetic absence models

Clinicians and epileptologists are faced with the situation that up to 30% of epilepsy patients do not respond to any of the currently available anti-epileptics drugs (Remy & Beck, 2006). Not all of those patients classify as candidates for the highly invasive treatment option of epilepsy surgery, where parts of the brain, containing the epileptic onset zone is resected: patients where the epileptic onset zone is located in a functionally relevant brain area, or patients with multiple epileptic-foci remain insufficiently treated. This prompted the need for the development of new alternative treatment strategies including various types of brain stimulation like transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), deep brain stimulation (DBS) and very recently optogenetic stimulation, all aimed to modulate or abort aberrant seizure activity, get experimental control over seizure generation and termination and by this learn about both seizure generation and termination processes and mechanisms of stimulation.

Although absence epilepsy is a rather mild form of epilepsy, which is well controlled by medications, the genetic absence rat models are frequently used in this search for optimal brain-stimulation paradigms yielding maximal therapeutic efficacy while reducing clinical side-effects. They are regarded as model systems

for 'prototypical secondary generalized seizures' and it is hoped that effects of stimulation might be generalizable to more severe, refractory types of epilepsy. Indeed GAERS and WAG/Rij rats can be regarded as rather suitable research models considering that all individuals show up to several hundred spontaneous occurring SWDs per day (Depaulis and van Luijtelaar, 2006). This implies that the effects of stimulation can directly be tested in a diseased brain. The high SWD rate in these animals allows the evaluation of stimulation paradigms in short time intervals with a sufficient number of seizure events that can be influenced by stimulation. Also a reliable description of the dynamics of stimulation effects can be obtained in chronically equipped rats and questions such as how long does it take until maximal seizure suppression is achieved, is there habituation toward stimulation at a given point in time or does a rebound of SWD activity occur with longer lasting recording and stimulation intervals, can be answered. Indeed several stimulation techniques have been tested in these models within the last years with the aim to reduce the probability of the occurrence of seizures either by modulating the epileptogenic zone via procedures such as long term depression, or transcranial DC stimulation, or by stimulating a circuit or a more remote brain region affecting the epileptic circuitry. An example of the latter procedure is the work of Godlevsky et al. (2006). They investigated in WAG/Rij rats whether it might be possible to influence SWD occurrence in a non-invasive manner via TMS. Low frequent magnetic pulses (0, 5 Hz, three pulses were applied in total) were delivered to the brain via an insulated copper coil placed above the surface of the head. Comparison of SWD parameters (number and mean duration) between pre-stimulation and post stimulation revealed a stronger reduction of SWD duration by 31% for the stimulation as compared to the sham stimulation group in the first 30 min post stimulation, while the number of SWD was not differently affected in the experimental and sham stimulated control group. It seems that TMS has the potential to reduce SWD activity on short term.

Non-invasive bilateral tDCS was also performed in WAG/Rij rats (Zobeiri and van Luijtelaar, 2013; van Luijtelaar and Zobeiri, 2014). A small electrical current (100–150 μ A) was administered four times per day for 15 min via electrodes placed on top of the skull above the left and right epileptic focal onset-zone (located in the deep layers of the perioral somatosensory cortex) and at the frontal cortex. Only cathodal tDCS at the focal area caused a stimulation intensity dependent inhibitory effect on SWD occurrence, while anodal stimulation was not effective. The behavior of the rats was not changed except at the highest stimulation intensity. The authors also reported an increase in the power of delta and subdelta frequencies in the ECoG, confirming the idea that cathodal tDCS reduced the excitability of the brain via hyperpolarizing cortical cell assemblies in or near the focal region. Importantly, post experimental histology did not reveal any morphological changes/lesions induced by repeated tDCS. The efficacy and safety of longer lasting tDCS remains to be investigated.

Sadighi et al. (2013) applied low frequent electrical stimulation (3 Hz monophasic square pulses of 200 μ s and 400 μ A for 25 min on five consecutive days) to the deep perioral somatosensory cortex of WAG/Rij rats to reduce the excitability of the hyperexcitable epileptic focus (van Luijtelaar and Smitnikova, 2006). Low frequency electrical stimulation has been applied classically in long term hippocampal depression paradigms, where it has been shown to reduce local brain excitability over an extended period of time (Dudek and Bear, 1992). The number and duration of SWD between sham stimulated rats and unilaterally and bilaterally stimulated rats were compared. Only unilaterally stimulated rats showed significantly fewer SWD than sham stimulated rat during the 30 min post stimulation period on day 1, while this effect did not persist till the next day, suggesting that this type of stimulation induced only short lasting effects. The temporal diminishment of SWD at

day 1 can perhaps be ascribed to stimulation induced changes in cortical arousal.

Similar results were also obtained by our group (unpublished data) investigating both the short term (2 h) effects of a single session of low frequency electrical stimulation to the focal region in the deep somatosensory cortex of WAG/Rij rats, the long term (24 h) effects of a single session of low frequency stimulation, the effects of repeated 10 day period of stimulation and its evaluation after a seven day interval without any stimulation (see Fig. 1 for stimulation schedule and results). The stimulation paradigm was a classically used LTD paradigm (Dudek and Bear, 1992): a train of 1 Hz, 1 mA, 1 ms biphasic square pulses for 15 min. A small reduction of SWD number was also only seen on the short term (a significant pre-post difference in number of SWDs was repeatedly found), both for a single as well as after multiple stimulation sessions. Such a short term effects however might also be explained by a change in vigilance (rat is more active or more asleep) after the stimulation session since it is well established both behavioural states reduce the occurrence of SWDs (Coenen et al., review 1991). There were no long term effects, suggesting that LTD did not occur.

Low frequency, closed loop electrical stimulation was used by Berenyi et al. (2012) in a rat model. SWDs were detected by a seizure/spike detection algorithm, stimulation was applied through many different electrodes placed on the skull surfacing the somatosensory cortex. They reported that closed loop low frequency stimulation (1 Hz), starting either 0, 10, or 40 ms following the detected spike component, successfully disrupted and shortened ongoing SWD. They argued that stimulation might need to be applied during the wave component of the SWD in order to induce a refractory period during which the neurons are in their duty phase to create the next spike of the SWD, demonstrating a delicate timing requirement for low frequent closed loop stimulation to be successful. This might be very true since low frequency stimulation of the somatosensory cortex has also the potential to induce SWD, if the network is in a for SWD occurrence appropriate state. The latter has been found in both WAG/Rij rats and in GAERS (Lüttjohann et al., 2011; Zheng et al., 2012).

Vercueil et al. (1998) investigated in GAERS the effects of continuous and closed loop high frequency stimulation (130 Hz) of the sub-thalamic nucleus projecting to the substantia nigra, a structure thought to be involved in the inhibitory control of convulsive and non-convulsive generalized seizures (Depaulis et al., 1994). They determined for each rat an optimal intensity threshold, needed to disrupt ongoing SWD and demonstrated that this threshold needed to effectively disrupt SWD is significantly (65%) lower than the threshold intensity to induce motor side effects. Furthermore they reported that continuous high frequency stimulation at the seizure disruption threshold intensity only temporarily (within the first 2 min) suppressed SWD, while thereafter habituation to stimulation occurred and SWDs reappeared.

Closed loop high frequency stimulation of the substantia nigra was performed by Feddersen et al. (2007). In their detailed and elaborated study they compared the effect of referential vs. bipolar stimulation, monophasic vs biphasic pulses, different frequencies of stimulation as well as the effect of different stimulation pulse widths. They described a significantly lower intensity threshold needed to successfully disrupt SWD for bipolar as compared to referential stimulation, monophasic stimulation having a three-fold lower intensity threshold compared to biphasic stimulation, and a reduction of the intensity threshold with increasing widths and frequencies in a hyperbolic fashion (Fig. 2).

In a 40 min closed loop stimulation session (bipolar, monophasic, bilateral stimulation at 60 Hz) Feddersen et al. (2007), however, only achieved a total percentage of successfully disrupted SWD of 50%. Further analysis of the not successfully interrupted SWD revealed that those most often occurred shortly following a

Day1	2h baseline	15min 1Hz stim	2h post
Day 2	2 hours recoding	15min 1Hz stim	
Day 3 & 4		15min 1Hz stim	
Day 5	2 hours recoding	15min 1Hz stim	
Day 6 to 9		15min 1Hz stim	
Day 10	2 hours recoding	15min 1Hz stim	2h post
Day 11 to 16	free period		
Day 17	2 hours recoding	15min 1Hz stim	2h post

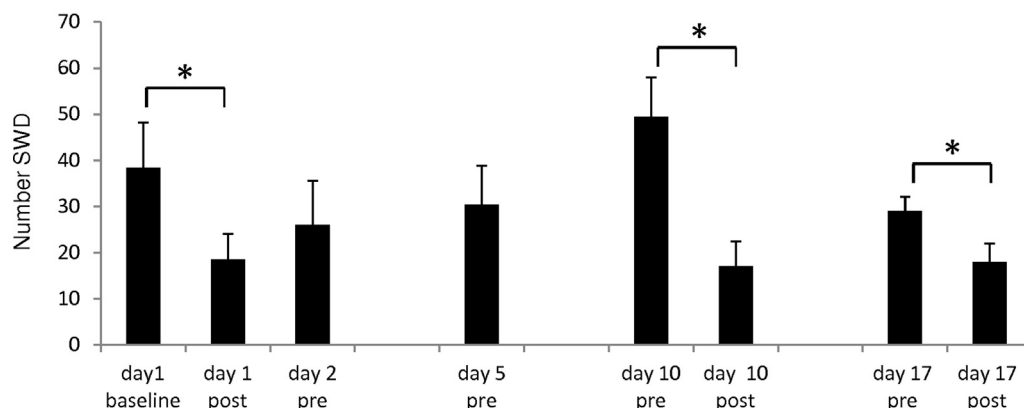


Fig. 1. Recording and stimulation schedule of 15 min low frequency (1 Hz) electrical stimulation in the deep somatosensory cortex of WAG/Rij rats (upper panel) and effects of stimulation on number of SWD before (baseline) and after 15 min stimulation on days 1, 10, and 17. The figure shows only within day effects, but a clear lack of long lasting effects of stimulation on days 2, 5, and 10 (pre).

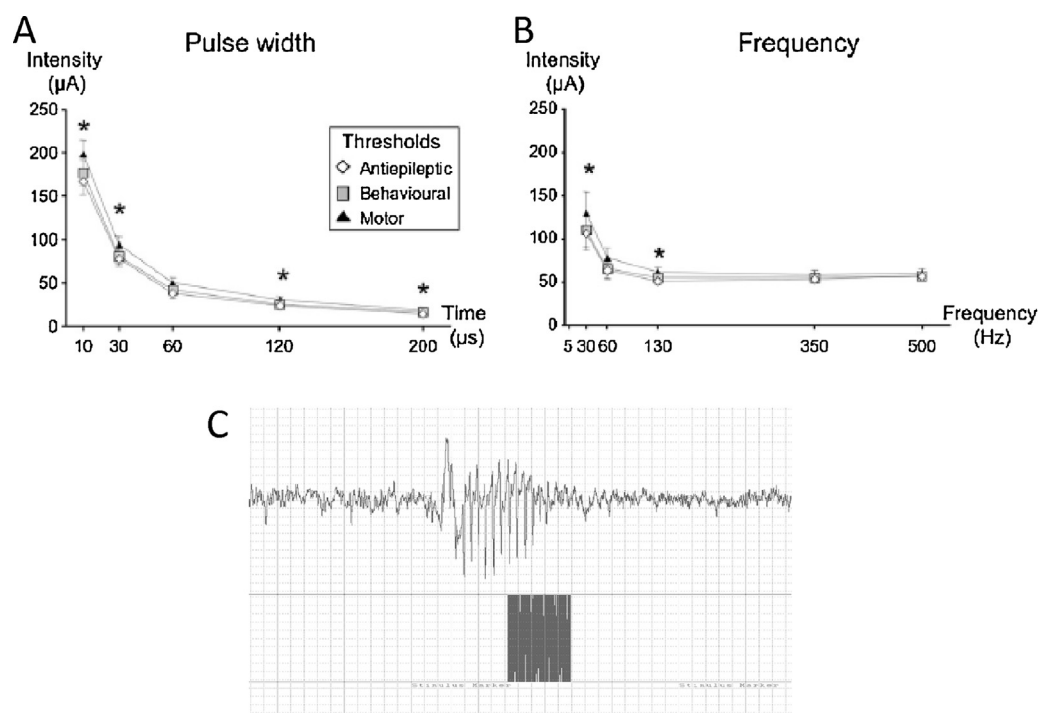


Fig. 2. Efficacy of single bipolar monophasic unilateral stimulation parameters: pulse width (left panels) or frequency (right panels). (A) Effect of pulse width on intensity thresholds ($p < 0.05$, Wilcoxon test, compared to antiepileptic threshold at 60 μ s) and on differences between antiepileptic, behavioural and motor thresholds ($\#p < 0.05$, Wilcoxon test) using 130 Hz stimulation. (B) Effect of frequency on intensity thresholds (upper panel, $p < 0.05$, Wilcoxon test, compared to antiepileptic threshold at 60 Hz) and on differences between antiepileptic and motor thresholds ($\#p < 0.05$, Wilcoxon test) using 60- μ s pulse width. (After Feddersen et al., 2007). (C) A spontaneous occurring SWD is aborted by a 1 s train of 130 Hz stimulation.

disrupted SWD, which might indicate the existence of a refractory period in which SWD disruption is not possible or at least more difficult. Furthermore, they noticed a significant increase in the number of SWD during the stimulation session. Such a rebound of

SWD activity was also reported by Blik (2015) who performed also successfully (SWD were aborted) high frequency (100 Hz) closed loop stimulation of the tubero-mamillary nucleus in WAG/Rij rats.

Saillet et al. (2013) used the SWD detection system described by Li et al. (2007) and elaborated on the refractory period as was reported by Feddersen et al. They determined, both for high frequency electrical stimulation as well as for auditory stimulation, an optimal inter stimulus interval (the minimal time interval in which closed loop stimulation is not effective in disrupting SWD), which lasted on average for 40 s. With such an inter stimulus interval, where no stimulation is delivered irrespective whether an SWD occurs, they reported stable SWD disruption of on average 97% during a 5 h stimulation session (60 Hz) and 72% during a 24 h electrical stimulation session, while closed loop auditory stimulation was found to be much less effective (52% of SWD were disrupted). Interestingly, these authors also reported a significant increase in the number of SWD in their 5 h stimulation session but an overall decrease in SWD number in their 24 h stimulation session. They argued that the presence of the experimenter during the 5 h session, which was often observed to increase SWD occurrence, might explain that difference.

High frequency (130 Hz) closed loop stimulation in two thalamic nuclei (VPM and ATN) in WAG/Rij rats for 8 h did not induce an increase in SWD number (Lüttjohann and van Luijtelaar, 2013). They interrupted 89% of SWD at stimulation intensities of 71.6 μ A (VPM) and 113.6 μ A, without changing the activity level of the animals (see Fig. 2C for an example of a disrupted SWD by closed loop thalamic DBS of this study).

A closed loop seizure control system consisting of a neural interface for ECoG acquisition and stimulator to provide responsive electrical stimulation and a microcontroller for e.g., for executing a seizure detection algorithm and generating pulse trains and wireless transmission on the animals head was used to evaluate the efficacy of the system in interrupting SWDs. The animal model in which the system was evaluated was Long–Evans rats, either with spontaneous SWDs or after PTZ-administration (Liang et al., 2011). Stimulation parameters were 800 Hz, 0.5 s pulse train, pulse duration 0.5 ms, 30–40 microA, the zone inserta was stimulated. About 95% of the spontaneous SWDs and about 70% of the PTZ induced were terminated by a single train of electrical stimulation in a 4 h ECoG recording and stimulation session.

Another recently developed type of stimulation that could also be implemented in a closed loop paradigm to disrupt ongoing SWD is optogenetic stimulation. In this method neuronal populations can be transfected with light sensitive opsins with the aid of viral vectors. Depending on the type of opsin, light stimulation via e.g., a locally implanted glass-fiber, can either activate or inhibit neuronal firing in the transfected cells. This method, given the availability of cell specific promoters not only allows for a high temporal precision of stimulation, as in the case of DBS, can achieve also a higher spatial (i.e. cell-type specific) precision, while in the case of electrical stimulation, even 'local' stimulation might activate a heterogeneous cell population. Dissection of seizure generating networks components become feasible, as has been demonstrated elegantly by Paz et al. (2011). These authors noticed a reduced feed forward inhibition from cortex to RTN in the Gria4(-/-) mouse absence epilepsy model, while other elements of the thalamo-cortical system (T-C, C-T and T-RTN) were unchanged. Restoration of the feed forward inhibition, using ontogenetic stimulation, might be regarded as an interesting treatment strategy. Given the fact that optogenetic stimulation is a very recent developed technique, the therapeutic potential of it for seizure intervention has only been investigated in a few studies: In a post cortical stroke model, for example, with seizure focus in the somatosensory cortex, transfection of thalamocortical neurons, passing from the VPM to the somatosensory cortex, with a Halorhodopsin, an opsin which silences cellfiring upon stimulation with yellow light, ipsilateral stimulation was reported to successfully disrupt seizures within 1 s after detection and start of stimulation, while seizure duration in untreated animals and sham

stimulated animals (where cells were only transfected reporter gene but no with the light sensitive opsin) varied between 10 and 120 s (Paz et al., 2013). Likewise, Kros et al. (2015) transfected neurons of the cerebellar nuclei with channel rhodopsin 2, a opsin that activates cell firing upon stimulation with blue light in two mouse models of absence epilepsy, and reported successful disruption of SWD within 500 ms. (For a more detailed review on the current employment of optogenetics within the field of epilepsy research the reader is referred to Paz and Huguenard, 2015).

It can be concluded that the different paradigms of open loop stimulation (including TMS, tDCS and continuous electrical stimulation) seem to have only an acute or short term effects and sometimes lead to a habituation of stimulation. Closed loop stimulation, especially high frequency stimulation, seems to be somewhat dependent on timing and location of stimulation, stable SWD disruptive effects. Together with the steadily growing development of smaller stimulators, electrodes with good tissue tolerance and longer lasting batteries and detections systems (see above) closed loop DBS classifies as the promising new treatment approach for epilepsy. In addition, the steadily growing knowledge on seizure precursor for both absence epilepsy (see Section 4) as well as other types of epilepsy (see for example Le Van Quyen et al., 1999; Kalitzin and Lopes da Silva, 2014) even the development of a closed loop BCI system triggering DBS based on on-line precursor detection might be thinkable. Whether also less invasive techniques like high or low frequent TMS can be effectively implemented in a closed loop system remains to be investigated.

4. The existence of precursors of SWDs in cortex and thalamus

4.1. Time frequency analyses: Delta- and theta-precursors of SWDs

The classical description of the SWD in the multi-channel ECoG is that the SWD appear abruptly from a normal background activity (Panayiotopoulos, 1997; Sitnikova and van Luijtelaar, 2007). However, a gradual increase in power in the low frequencies at frontal but also at other cortical locations toward the beginning of the SWD has been found in patients in both EEG and MEG studies (Inouye et al., 1994; Gupta et al., 2011). Whether precursor activity could also be reliably found in frontal cortex and thalamus (VPM) in the genetic absence models was investigated in WAG/Rij rats as well as in GAERS. It is obvious that the reliable detection of precursor activity, in combination with different forms of electrical stimulation, may not only open new research tools that may help to explain the cause of the origin of the seizure, but also the possibility to investigate whether the prevention of SWD might be possible.

A first study towards precursor activity in WAG/Rij rats was based on a frontal and thalamic ECoG recording (van Luijtelaar et al., 2010). Cortical and thalamic ECoG records were examined 3 s before the onset of SWD and during SWD. Non-epileptic 10 s control ECoG epochs of background activity were also analyzed: these control ECoG epochs did not contain SWD and included periods of active and passive wakefulness, light and deep slow-wave sleep including sleep spindles. The continuous wavelet transform (CWT) with complex Morlet mother wavelet was used to analyze time-frequency structure of 'pre-ictal $\rightarrow$ ictal transition' epochs, which would provide an optimal time–frequency resolution and allows to localize in time precisely different oscillatory patterns in the ECoG signal from our rats (Sitnikova et al., 2009, 2014; Hramov et al., 2015). 'Skeletons' of wavelet surfaces were also constructed in order to extract dominant ECoG rhythms and determine the evolution of the instantaneous frequency in the each ECoG channel before SWD onset.

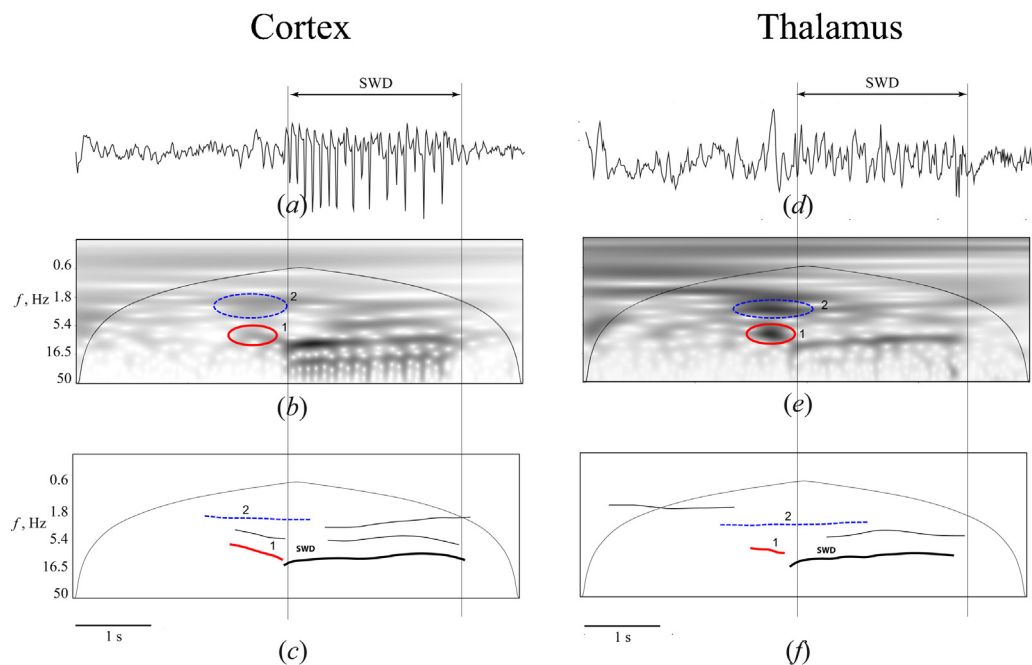


Fig. 3. The time series (a, d), wavelet spectra (b, e) and skeletons (c, f) of EEG signals containing SWD simultaneously recorded at frontal cortex (left panel) and thalamus, VPM (right panel). Wavelet spectra were calculated using a mother Morlet wavelet with central frequency $\omega_0 = 2\pi$. The onset of the SWD was preceded by 8.6 and 2.2 Hz in cortex and by 7.9 and 3.3 Hz (mean frequencies)—in thalamus (shown by ellipses). $\cap$ -shaped curve on the wavelet surfaces marked the area of boundary effects (Hramov et al., 2015). The vertical dotted lines indicate the beginning and end of SWD. Ellipses and solid lines 1 correspond to theta/alpha-precursors, ellipses and dotted line 2—delta precursors. Line 3 indicates dynamics of main rhythm during SWD.

The matrix of wavelet coefficients at each time point and frequency in ECoG epochs prior and during SWD was examined, and it was found that SWD-precursors consisted of several frequency components in the range from 2 to 12 Hz. The typical ECoG recordings and corresponding wavelet spectra and skeletons are shown in Fig. 3 for cortical and thalamic channels, respectively. The two most powerful rhythmic components in the frequencies 3–5 and 7–12 Hz immediately preceded the onset of SWD, considering their predominance and close proximity in time to the onset of SWD (see Fig. 3b–e). Theta/alpha precursor activity was found in the frontal cortex and thalamus at the same time. However, the delta events appeared first in the cortex and it was followed by a small but significant delay by delta precursor activity in the thalamus. The time until the onset of SWD for the cortex was 0.48 ± 0.04 s, and 0.38 ± 0.04 s for the VPM (for details see van Luijtelaar et al., 2010).

Descriptive statistics of frequency and duration of delta and theta precursors in cortex and thalamus are given in Table 1. The dominant frequency of delta and theta/alpha precursor activity in the thalamus was similar to that in the cortex. However, the duration of theta precursor activity was slightly 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. Delta precursor activity in the frontal cortex was found in 90% of all SWD, and theta/alpha precursor activity in 92%. High percentages were also found in the thalamus (VPM): 82% and 83%, respectively, for delta and theta/alpha precursors. There were no differences in the percentages of SWD that were preceded by delta and theta/alpha precursors in cortex and thalamus. The average duration of precursor activity was approximately equal to 0.5 s.

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; and in 5% delta precursors were completely absent both in cortical and the thalamic ECoG. The percentage of co-occurrence during the control

periods (8.1% for the cortex and 6.0% for the thalamus) in the cortex was much lower than that during the precursor activity (79%).

Spectral properties preictal were also accessed via a time frequency analysis (TFA) using Hanning tapering on the Lüttjohann and van Luijtelaar data set (2012) using FieldTrip, an open-source Matlab-based toolbox for advanced analysis of e.g. electrophysiological data (Oostenveld et al., 2011). This data set consists of eight channel ECoG data set of 16 adult male WAG/Rij rats (for details of the electrode locations see Lüttjohann and van Luijtelaar, 2012; van Luijtelaar et al., 2011). Briefly electrodes were positioned in layer V and VI of the somatosensory cortex (the assumed cortical focus) (Meeren et al., 2002; Polack et al., 2007), layer IV of the somatosensory cortex (the major input layer of signals from the thalamus), the VPM, sending information to cortical layer IV and receiving information from layer VI (Deschênes et al., 1998), the posterior thalamic nucleus, which receives input of layer V (Deschênes et al., 1994), the rostral and caudal RTN, as potential synchronizers of the system (Huguenard and McCormick, 2007), and the anterior thalamic nucleus, as an outside somatosensory loop thalamic control site. The 2.5 s preceding the occurrence of the first epileptic cortico-thalamic spike (FCTS) was analyzed in 500 ms time frames shifting along the pre-ictal-to-ictal transition period in steps of 50 ms. FCTS was defined as a first sharp spike of at least twice the background LFPs, visible in all cortical and thalamic recordings, which is followed by rhythmic SWD activity. The FCTS is considered to be the moment of ‘generalization’ of epileptic activity from cortex to thalamus or equivalently the start of ‘full blown’ cortico-thalamo-cortical SWD (Lüttjohann and van Luijtelaar, 2012). Given the size of the analysis window, frequency resolution was restricted to an accuracy of 2 Hz. TFA was performed for in the frequency-range between 2 and 60 Hz. All channels showed a significant increase in power in the delta (2–4 Hz) and theta (6–12 Hz) frequency range as compared to an interictal control period. Channels however differed regarding their pattern of maximal pre-ictal power values (increases in power larger than two times the background power): Earliest and most pronounced preictal power was noticed for the

deep layers of the somatosensory cortex, with Ctx layer V showing simultaneously delta and theta precursor activity in 75% of preictal periods. These started on average 2 s prior to SWD onset. In thalamic recordings, on the other hand, maximal power values were found around 0.75 s (range 0.25–1 s) prior to SWD onset (Lüttjohann et al., 2013).

The Bandt and Pompe (2002) permutation entropy (PE) method, that determines the probability distribution of subsequent points in time series (complexity index for dynamical systems) was used and compared with sample entropy (SE) to investigate whether there is preictal activity in the cortical frontal ECoG signal in GAERS (Li et al., 2007). Both entropy methods have certain advantages above the more classical ApEn (approximate entropy) measure: a fast calculation time, and largely independent of record length. PE can be applied to any type of time series (regular, chaotic, noisy, or reality based), with a weak stationary assumption, and it exhibits important advantages such as its robustness to noise, and its invariance with respect to nonlinear monotonous transformations. Fixed (1.2 s) preictal time blocks with 1.1 s overlap of frontal bipolar ECoG records from 314 SWD from 28 GAERS were analyzed. The values of PE and SE were higher during the interictal state compared to the SWD state, however, the values of PE were more constant than the SE values. Moreover, both PE and SE began to decrease prior to SWD onset: this illustrates the reduction of the dynamical complexity of the oscillatory activity of the thalamo-cortical neural network changing from noise-like complex oscillations to simple regular ones (hyper-synchronization) prior to absence seizure onset. PE successfully detected the pre-ictal state prior to SWD onset seizure in 54% of the SWD, SE in 21%. The mean anticipation times were 4.9 and 3.7 s respectively. PE seems more suitable (more sensitive and more robust) to describe the nonlinear activity of ECoG data, or better extract the pattern of ECoG data for the prediction of absence seizures. Considering that the electrodes were not in the focal region, one may wonder whether this might further improve the sensitivity of the method. The authors mentioned that, considering the calculation time, the system can be applied real-time and offers possibilities for its application in patients (Li et al., 2007).

In all, three different data sets from two genetic rodent models analyzed with different methods for establishing preictal activity showed that there is preictal activity, and that it can be detected with the analyses of a single ECoG channel. There is room for optimization of the sensitivity and specificity of the methods, also regarding the location of the ECoG electrode(s). In all, the presence of precursor activity shows that, in principle, a certain percentage of SWDs can be reliably predicted. The appearance of delta alone and or in combination with theta activity in the cortex immediately prior to SWD might be regarded as seizure-related trigger activity instantiating hypersynchronous discharges in the cortico-thalamo-cortical network.

4.2. Network analyses of seizure-precursors at the frequency band of SWDs

The discovered delta- and theta/alpha-precursors might be difficult to detect considering the low spatial resolution of having only single cortical and single thalamic ECoG recording electrodes. Moreover, there is some variation in the exact location of the focal region. The thalamus is heterogeneous regarding projections to and from the cortex. Therefore, analysis of multi-channel ECoG might be more promising for efficient and accurate diagnostics of the ECoG activity preceding SWDs. In addition, it allows a better spatial resolution for mainly the contribution of the various thalamic nuclei in SWD generation and maintenance. Moreover, absence epilepsy is a network type of epilepsy, in which already before SWD onset coupling among different parts of the SWD circuit emerges.

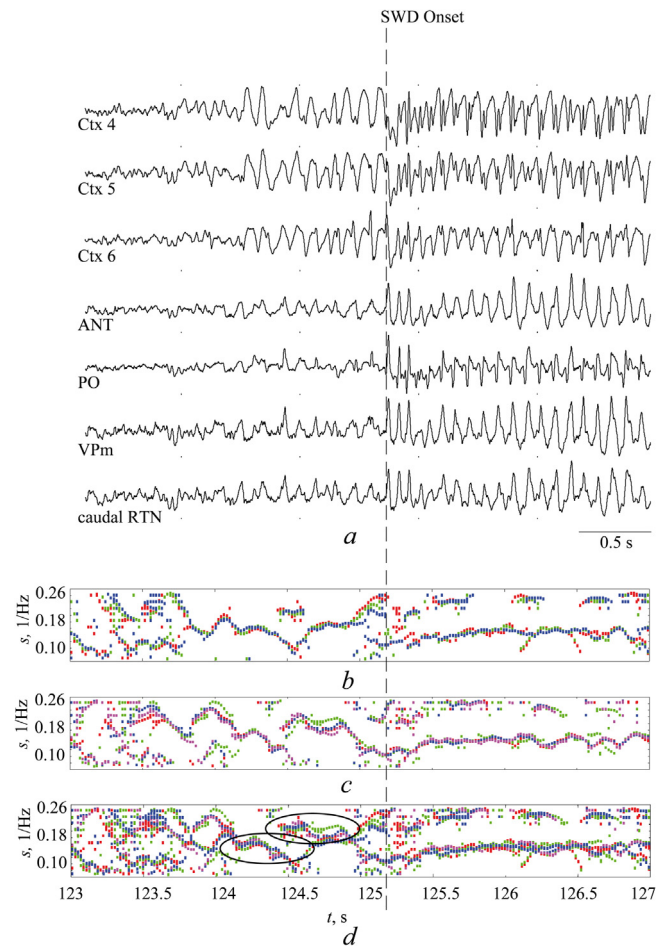


Fig. 4. Electrophysiological and spectral wavelet-based characteristics of SWD: (a): Exemplary multi-site local field potentials recording showing the transition from pre-ictal to ictal LFP recordings; the black vertical dotted line indicates the time-point of the first cortico-thalamic spike, or onset of a 'full blown' SWD. (b,c): skeletons of wavelet spectra of the three cortical (b) and thalamic (five) channels (c) (the different colors correspond to the different EEG channels). Panel d presents the merge of cortex and thalamic skeletons where ellipses denote the delta (top) and alpha/theta (bottom) precursor activity before SWD onset. Note that delta SWD precursor activity is more pronounced in the thalamic nuclei than in the somatosensory cortex. Conversely, the alpha/theta precursors are more clearly expressed in the somatosensory cortex channels. The same figures are observed for all analyzed WAG/Rij rats.

Methods aimed at detecting preictal changes in coupling in the network might have some promises for SWD prediction.

Under the assumption that SWD represent synchronous activity in the cortico-thalamo-cortical network, it was proposed to establish a measure of synchronization along the frequency domain. All 'pre-ictal → ictal transition' periods of 6 s duration as selected for each rat in the Lüttjohann and van Luijtelaar data set (12 WAG/Rij rats) were analyzed. Fig. 4a illustrates the typical multi-site local field potentials recording showing the transition from pre-ictal to ictal ECoG, where the dotted vertical line indicates the time-point of the first cortico-thalamo-cortical spike (FCTS, the SWD onset). Epochs of non-epileptic activity were used as control data: for each rat 30 epoch of 10 s duration were randomly selected during passive wakefulness distant (by at least 5 min) to SWD. A major proportion of SWD tends to emerge during this state of vigilance (Drinkenburg et al., 1991).

Fig. 4b and c show the local maxima of wavelet surfaces in the 'pre-ictal → ictal transition' period obtained for the cortical channels (Fig. 4b) and thalamic (Fig. 4c) ones in the frequency range (3.5–12.5 Hz). Fig. 4d presents a merge of Fig. 4b and c

illustrating the complete time-frequency dynamics of the cortico-thalamo-cortical network oscillations registered with the help of the multi-channel ECoG. If the maxima of wavelet spectra (points of skeletons) of the different channels are observed in one time-point and in one place on frequency f axis, then it can be concluded that these ECoG oscillations corresponding to these channels demonstrate synchronous dynamics because their fundamental frequencies coincide (Hramov and Koronovskii, 2004; Hramov and Koronovskii, 2005). Pre-ictally, at the 123–124 s time interval, a set of unordered points are observed that indicates that oscillations in each channel are characterized by their own frequencies which are not correlated with the frequencies in the other channels. In other words, there is no inter-channel synchronization.

The time-frequency characteristics of multi-channel ECoG dramatically change in the time period before SWD (124–125 s in Fig. 4). The maxima of wavelet surfaces begin to cluster in this time interval, demonstrating the presence of synchronization among ECoG channels. Two clusters were found: one with typical frequency about 8–10 Hz (corresponding to theta precursor as was described in Section 4.1) and a second one 3–4 Hz (delta precursor) before SWD. Analysis of the sets of SWDs showed that delta precursor activity was more pronounced in the thalamus than in the somatosensory cortex. Conversely, the alpha/theta precursors were more clearly expressed in the channels from the somatosensory cortex. Next, a well-pronounced inter-channel synchronization in the frequency band 8–11 Hz (main rhythm of SWD oscillations in WAG/Rij rats) and occasionally in delta-range can be appreciated. A larger data ($n = 16$) set was also used to establish directed connectivity analysis (Nonlinear association analysis and Granger Causality) to investigate preictal activity and to establish whether the thalamus drives the cortex or vice versa (Lüttjohann and van Luijtelaar, 2012; Lüttjohann et al., 2013). Lüttjohann and van Luijtelaar found a leading cortex at SWD onset. Preictal increases in cortex and thalamus communication were bidirectional in nature. Sitnikova et al. (2008), who, using Granger causality analysis, did not find a preictal leading role of the frontal cortex preceding SWD onset when analyzing connectivity and coupling changes preictally and ictally between I cortex and VPM, while David et al. (2008) applying Granger Causality on fMRI data collected in GAERS, did find a leading role for the somatosensory cortex during SWD, in support of the selectivity for the somatosensory cortex driving capacities.

Significant increases in the communication between cortex and thalamus were seen preictally in WAG/Rij rats: the deep layers of the somatosensory cortex and the strong, reciprocally connected the Posterior thalamic nucleus, start to gradually increase their communication in a bidirectional fashion as early as 1.25 s prior to FCTS, until reaching a maximal value at around 375 ms following FCTS. The directional non-linear association analysis demonstrated that the cRTN reduces its communication to the Po at about 1 s prior to FCTS. Since these increases are only detected by the non-linear association analysis, but not by linear GC analysis, it is likely that these increases in communication, take place in a non-linear fashion. Indeed epileptic seizures are often regarded to be of non-linear nature and signal analyses derived from the theory/mathematics of non-linear dynamics are proposed to be of particular value for the understanding of seizure generation mechanism (Le Van Quyen et al., 1999; Lehnertz et al., 1999; Litt and Echaz, 2002; Lopes da Silva et al., 2003b; Le Van Quyen, 2005; Stefan and Lopes da Silva, 2013). Linear coupling changes were also detected pre-ictally: at 1.2 s prior to FCTS a phasic decoupling between the caudal RTN and layer 4 of the somatosensory cortex and rostral RTN and layer 5 of the somatosensory cortex, was observed, shortly followed by a phasic decoupling between cRTN and Po and cRTN and VPM. It can be noted that in all cases these linear connectivity changes described decreases in coupling.

Based on the above reported dynamics of pre-ictal changes it can be concluded that, in contrast to a long lasting view that SWD are sudden and unpredictable events (Panayiotopoulos, 1997), SWD generation is a gradual process which already starts more than a second prior to the onset of a full blown SWD. Such early changes might open the possibility for SWD prediction. The obtained results obtained with both time frequency and network analyses demonstrate the possibility to detect SWD preceding activity. It is by no means clear which of the methods is preferable, and most robust. The well-pronounced synchronous cluster at the frequency band of SWDs (8–11 Hz) suggests that synchronization in the cortico-thalamo-cortical network at the 8–11 Hz range might be a possible candidate to act as a biomarker of the subsequent formation of SWD in cortex and thalamus. In Section 5 some possibilities for the creation of an effective system for on-line seizure prediction based on these findings will be discussed.

5. On the feasibility of seizure prediction

An important feature of the SWD dynamics is the generalized activity across an extended area of cortex and thalamus. The high amplitude SWD pattern activity is observed in all ECoG channels, where the main frequencies of the SWD oscillations in the different channels coincide (see Fig. 4d). Additionally, the high amplitude oscillations in the same frequency range are observed preictally. Therefore, the detection of the precursors seems to be possible with the help of the monitoring of the complete energy of wavelet spectrum, $W(f, t)$,

$$E_i(t) = \int_{f_{\text{SWD}}} W_i^2(f, t) df \quad (5.1)$$

in the $f_{\text{SWD}} = (7–12 \text{ Hz})$ frequency band corresponding to the SWD activity for the most typical channels. Since the shape of ECoG signals during the precursor differs from ones containing the SWD, the types of wavelets used for the prediction have to be specified according to the features of preictal ECoG recordings. Sitnikova et al. (2009) showed that the Morlet wavelet is well-suited in this respect.

In case of on-line processing of ECoG data there are two more difficulties added on top of the obstacles, which are already present for the analysis of already recorded data sets (the off-line detection mode). Firstly, the «future» data samples are not available, therefore, the standard methods used in the case of the off-line processing (like the methods described in the previous Section) cannot be used directly and, as a consequence, an adaptation of the wavelet transform is required. Secondly, real time calculations impose limitations on the algorithm complexity. It must be fast enough to produce all necessary calculations within the time window between two consecutive data samples.

Both problems may be solved (or, at least, reduced) with the help of reducing the length of time interval to be analyzed to characterize the system state at the fixed moment of time, t . Different approaches may be used for this purpose, such as the modification of the width of the mother wavelet, the reduction of the number of cycles of wavelet function, the use of the fast Fourier transform with the proper taper size, etc. The difference between these approaches as well as their particularities may be found, e.g., in (Hramov et al., 2015). In this work we propose to use one of this methods based on the modification of the standard Morlet function: to decrease its width.

$$\psi_M(\eta) = \frac{1}{\pi^{1/4}} e^{i\omega_0\eta} e^{-10\eta^4/2} \quad (\omega_0 = 2\pi), \quad (5.2)$$

The core criterion of the precursor appearance is the simultaneous excess of the threshold energy level E_{ith} in all channels of ECoG recording being analyzed on-line. One of the possible way to

reveal the simultaneous growth of the wavelet energy in all ECoG channels is the examination of the product

$$G(t) = \prod_i E_i(t), \tag{5.3}$$

where, $E_i(t)$ is the wavelet energy calculated in the considered frequency band for i -th channel. If and only if for all considered ECoG channels the wavelet energy values $E_i(t)$ within the examined frequency range are high, the resulting product $G(t)$ exceeds some threshold value $G_{\text{threshold}}$ that may be considered as the marker of the precursor.

The algorithm described above may be implemented with the help of the interface for the automatic seizure prediction. Typically, there is no need to analyze a lot of channels of ECoG to detect the precursor, especially, in the real-time regime. ECoG channels with different skeletons (see Section 4, Fig. 4) should be chosen. In Fig. 4b it is illustrated that all cortical channels show the same frequency dynamics, therefore, only one cortical channel (Layer 5) was used. Frequency dynamics of thalamic recordings is significantly different from the cortex dynamics (compare Fig. 4b and c). Among thalamic channels the largest difference in frequency dynamics holds for the anterior nucleus (ATN) and posterior nucleus of thalamus (PO). Therefore, these three channels were used to consider the possibility of SWD prediction. In this case the expression for the resulting product of the wavelet energy values of the different ECoG channels Eq. (5.3) may be written as

$$G(t) = E_{\text{ctx5}}(t) \times E_{\text{ATN}}(t) \times E_{\text{PO}}(t) \tag{5.4}$$

where, $E_{\text{ctx5}}(t)$ is the wavelet energy calculated in the considered frequency band for Cortex 5 channel, $E_{\text{ATN}}(t)$ and $E_{\text{PO}}(t)$ are the energy values obtained for ATN, and PO, respectively.

The typical dependence of the quantity Eq. (5.4) on time before and during the onset of the seizure is given in Fig. 5b. It can be seen that before the onset of SWD the value of $G(t)$ starts to increase; it witnesses the simultaneous increase of the wavelet energy within the frequency range corresponding to SWD oscillations for all examined ECoG channels. The time intervals when the value of the energy product exceeds the threshold are the markers of the precursor activity. Note, that these markers are also observed after SWD starts, but in this case the method of SWD detection

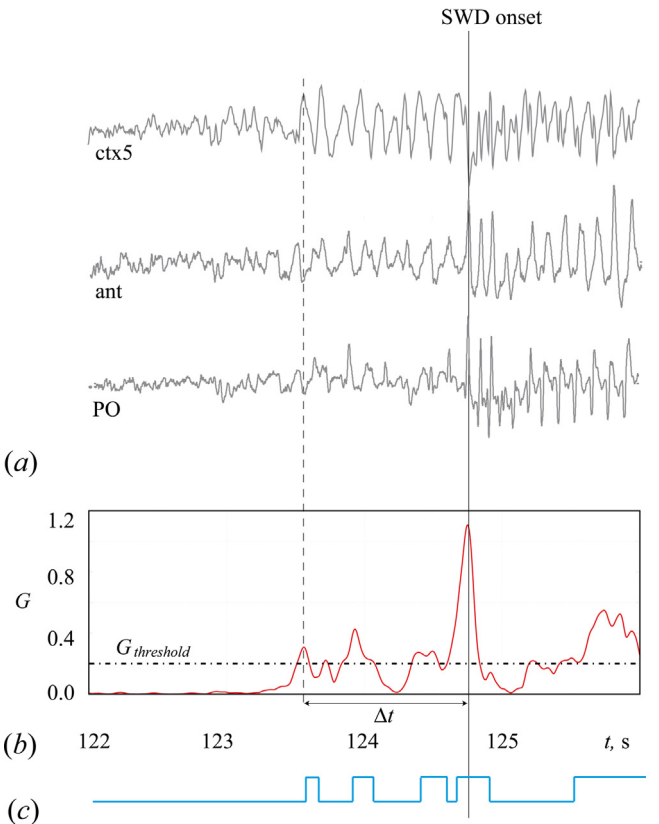


Fig. 5. The ECoG recordings corresponded to different ECoG channels. (a) the dependency of the considered characteristics G on time. (b) The moments of time, considered as the precursor and the onset of seizure (the point of clinical attack is marked as “SWD onset”) are shown by the dashed and solid lines, respectively). (c) The result of prediction, based on the use of threshold value $G_{\text{threshold}} = 0.2$.

described above in Section 2 allows to recognize also the epileptic dynamics. So, the analysis of the wavelet energy in the frequency range corresponding to SWD dynamics allows not only to recognize automatically the seizure event but also to predict it.

Table 2
Time–frequency characteristics of two types (delta and theta/alpha activity) of SWD-precursor activity averaged for six WAG/Rij rats.

	Delta (3–5 Hz) SWD–precursors			Theta/alpha (7–11 Hz) SWD–precursors		
	Nd, %	Dominant frequency, Hz	Duration, s	Nt, %	Dominant frequency, Hz	Duration, s
Frontal cortex	89.7 ± 2.3	4.1 ± 0.6	0.48 ± 0.04	92 ± 1.4	8.6 ± 1.0	0.46 ± 0.05
Thalamus (VPM)	81.7 ± 9.9	4.3 ± 0.8	0.50 ± 0.07	82.5 ± 6.2	8.5 ± 0.8	0.50 ± 0.06

$nk = N_k / N_{\text{SWD}} \times 100\%$ is the percentage of two types of SWD-precursor: 3–5 Hz (delta) and 7–11 Hz (theta) from the total number of SWDs, N_k is the number of each type (d, t) of SWD-precursor as assessed in cortex and thalamus, N_{SWD} is the total number of SWD.

Table 3
The characteristics of the precursor of SWD (such as threshold value, $G_{\text{threshold}}$, or the average time interval between precursor event and SWD, Δt) are varied from rat to rat and, having chosen threshold value, $W_{\text{threshold}}$, properly for each rat, an excellent precision in the precursor of SWD detection can be obtained (see data in Table 2).

Rat #	Spike-wave discharges			Control periods (Number of events in five control intervals with each duration 50 s)			
	Precision of precursor event detection, %	Average time interval between precursor event and SWD, second	Threshold of method	Active wakefulness	Passive wakefulness	Light-slow wave sleep	Deep-slow wave sleep
1	92	0.82	0.2	6	4	108	26
2	100	0.78	0.17	4	4	136	1
3	85	0.94	0.17	2	1	54	4
4	80	1.02	0.27	2	2	94	2
5	90	0.5	0.12	3	2	116	12
6	80	0.75	0.27	3	4	52	9
Mean ± S.D.	87.8 ± 7.08	0.8 ± 0.16	0.2 ± 0.054	3.3 ± 1.5	2.8 ± 1.3	93.3 ± 34.1	9.0 ± 9.3

According to the preliminary results concerning the online SWD prediction, based on the considered wavelet-based method (Table 2), one can find the high (up to 90%) predicted seizures (high sensitivity of the algorithm). But at the same time it comes with the large amount of false detections, which are caused by the appearance of any other oscillatory patterns on ECoG during light-slow wave sleep. Whether these precursors are only present in these genetic epileptic rats, or whether these precursors should be considered as a marker of a process of synchronization of cortical brain activity in general during the process of the transition from passive wakefulness to drowsiness and light slow wave sleep, favorable for SWD occurrence, needs further analyses. Moreover, it cannot be excluded that our precursors only reflect the risk of possible epileptic seizures, expressing necessary but not sufficient conditions for a possible transition to the ictal state (Kalitzin and Lopes da Silva, 2014). Table 3.

6. Concluding remarks

While nowadays multiple reliable offline but also online SWD detection system for rodents are available and take into account wavelet energy, and most of them can be easily adapted for murine models, the development of (on-line) SWD prediction systems have never been considered, probably cause of the long lasting view that generalized seizures are per definition unpredictable events.

Time-frequency and network analyses have shown that absence seizures are preceded by short lasting delta and theta precursor oscillatory activity in cortex and thalamus and that the combination rarely occurs during control periods. The presence of precursor activity, as detected in off-line analyses of ECoG data sets, however, shows that in contrast to this long lasting view, SWD are not sudden events, but develop out of pre-ictal seizure preparation processes. A new effective wavelet-based method of identification of precursor synchronous cluster in the cortico-thalamo-cortical network by means of multi-channel ECoG recording was proposed. Our studies on this network based CWT approach revealed the presence of a well-pronounced synchronous cluster at the frequency band of SWDs (the 8–11 Hz range) in the ‘pre-ictal → ictal transition’ period in the cortico-thalamo-cortical network. It can be considered as a possible candidate to act as a biomarker of the subsequent formation of SWD in cortex and thalamus. This method has been shown to be suited for precursor detection (a sensitivity of >90% percent), although at this moment with the trade off of a high false alarm rate, mainly during light-slow wave sleep. Research efforts should be aimed in finding out what is the optimal channel or channel combination for the detection of precursor activity, and recordings with a high spatial resolution are imperative to find the (sub) optimal recording site(s). Another future direction is to fine-tune algorithm to achieve optimal balance between sensitivity and specificity. Further, one should analyze the circumstances (sleep spindles, grooming, movement artifacts, non-REM sleep) in which false alarms occur. These circumstances can be taken into account to reduce the false detection rate, e.g., by conditional enhancing the detection threshold, and by detection of the artifacts causing false alarms. A good balance between sensitivity and sensitivity has been found for SWD detection systems, it might also be possible to improve mainly the false alarm rate for SWD precursor activity detection systems. The current state of the art of nonlinear mathematics does allow on-line and fast detection of precursors, so from a computational view the on-line analysis of ECoG, including the extraction of SWD precursors, and SWD prediction becomes feasible.

The successful detection of a pre-seizure state before clinical onset gives the possibility to deliver electric impulses series in cortical or subcortical regions or transcranial stimulation to avoid an

oncoming seizure. This is a likely possibility since indeed progress has been made in electrical stimulation studies in the rodent models and SWD interruption with low intensity stimulation seems possible (in contrast to seizures in mesial temporal lobe epilepsy, where seizures are difficult to abort), although rebound effects have been reported. This potential relevant effect is poorly understood, it might point towards an endogenous SWD promoting system. It can be stated that depending on location and timing of stimulation, high frequency unilateral closed loop electrical stimulation in different brain regions has been proven as an efficient and stable treatment strategy to abort SWD in short (1 s) stimulation trains at intensities below 150 microA. It also seems now well possible to control seizures for a longer time period and to study its long term effects. One wonders, whether the same type of electrical stimulation in cortex, thalamus, SN, brain stem, zona incerta, but now preceding SWD onset, might prevent the occurrence of SWD and what would be its effects.

The improvement on on-line SWD prediction systems would then even open the door for closed loop SWD prevention systems, where high frequency DBS is applied to the brain as soon as a SWD precursor is detected. This approach might also become of therapeutic interest for different types of intractable epilepsies, as it spares cortical function between seizures, in contrast with existing treatments, such as open loop stimulation, surgical lesioning, or drugs. It would allow for closed-loop, feedback control that could prevent seizures, and if seizures are not predicted, they can be quickly detected by a second algorithm in order to abort seizure episodes without inducing detrimental side effects of continuous stimulation in refractory patients (Morrell, 2011).

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