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The role of the cortico-thalamo-cortical system in absence epilepsy

Unraveling brain networks using multi-site local field potential recordings, dynamical signal analysis and deep brain stimulation

Proefschrift

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Chapter 1

GENERAL INTRODUCTION
General Introduction

1.1 Childhood Absence Epilepsy, the clinical picture

Childhood Absence Epilepsy, formerly also called petit mal epilepsy, is a neurological disorder, which can be found in about 10% of children with epilepsy usually occurring around the ages of 4 to 12 years (Loiseau et al., 2002). It is categorized as a so called non-convulsive epilepsy, since patients do not express clonic (involuntary repetitive movements) or tonic (involuntary tension/spasm) behavior of arms, legs and or the rump of the body during an epileptic attack (also called a seizure), as is the case for convulsive forms of epilepsy, but instead show a sudden, short lasting (about 5-15 seconds) behavioral arrest. From an observer’s point of view, like a parent, it seems that during these seizures their child loses the ability to maintain contact with the environment (van Luijtelaar et al., 1991), just sitting there with a blank facial expression, his or her eyes drifting upwards, occasionally with some mild, myoclonic twitches of the facial muscles, and often not being able to respond to external stimuli like a question addressed to them by the parent or teacher (Gloor, 1986).

Therefore, absence seizures have long been considered as lapses of consciousness. More careful analysis on cognitive functions of patients during seizures, however, exploiting different experimental tasks, showed that consciousness is not completely disrupted during absences, but that some cognitive functions can remain functional depending on the complexity of the task but also on seizure characteristics like its duration (Blumenfeld, 2011; Mirsky and Vanburen, 1965).

The same is true for the time patients need to regain normal cognitive performance following the seizure: it ranges from immediate continuation of their pre-seizure activity seen for simple tasks; and short spike wave discharges (SWD) to some moments of disorientation, where patients need to sort/regain their thoughts and orient themselves, paired with a lack of knowledge on how much time has elapsed (Penry et al., 1975; van Luijtelaar et al., 1991). It has been proposed that the selective cognitive deficit may be related to a selective impairment of brain networks during seizures (Blumenfeld, 2011).

Careful behavioral (video) analysis revealed that there also seems to be a temporally, sequential pattern of the mild myoclonic activity starting from the ocular region (eyes drift upwards; mild, rhythmic eyelid-automatisms) to the oral region (lip smacking, lip licking, chewing, swallowing) up to extremities (stretching rubbing hands or fingers), which was said to be reminiscent of a march of seizure activity along several brain regions (Panayiotopoulos, 1999; Sadleir et al., 2009; Stefan, 1983). Again, however, the exact configuration of myoclonic activity can be said to be variable between patients and between seizures within the same patients depending on factors like the duration of the seizure as well as the prior
level of alertness or the means of seizure induction (spontaneously occurring vs. induced by hyperventilation) (Sadleir et al., 2008; Sadleir et al., 2009).

If untreated absences can occur up to several hundred times a day, summing up to quite some time in which children can miss important information presented to them, which can easily be regarded to be detrimental for children of young and school age (Loiseau et al., 2002).

The treatment of first choice is Ethosuximide or Valproic acid (Glauser et al., 2010; Wheless et al., 2005), a T-type calcium and sodium channel blocker, respectively, which prevent burst discharges of neurons in the brain of patients, and thereby effectively prevent the occurrence of absences (Coulter et al., 1989a, b; Goren and Onat, 2007; Loscher, 1999).

During puberty most patients (about 70%) show a remission of their disease, whereas in up to 30% of cases a progression towards more severe forms of epilepsy including tonic-clonic seizures can be seen (Wirrell et al., 1996).

Absences are accompanied by a highly characteristic pattern of brain activity, so called spike and wave discharges (SWD) or spike and slow wave seizures, which can be recorded in the encephalogram (EEG) of patients (Figure 1).

![Figure 1: Child with electrode cap for the recording of the electroencephalogram (right); EEG of a child with childhood absence epilepsy containing a for this pathology characteristic spike and wave discharge (SWD) (left). Sources: Picture on the left panel: http://buffaloepilepsy.org/evaluation.html; Picture on the right panel adapted from: http://absence-siezures.blogspot.nl/](image)

The presence of this EEG pattern is the major diagnostic criterion for childhood absence epilepsy, given the non-convulsive nature of this syndrome. It must be noted, however, that there are also some other epileptic syndromes, i.e. juvenile absence epilepsy, juvenile myoclonic epilepsy, myoclonic absence epilepsy and eyelid myoclonia with absences, in which SWD can be seen (Panayiotopoulos, 1999).

As the name already says, one can observe a highly regular and rhythmic pattern of spike and wave activity, which can be recorded all over the head although spike amplitude is
highest in mid-frontal regions and gradually decaying along the caudal-lateral direction. SWD in children with childhood absence epilepsy have an average frequency of 3 Hz with some frequency modulation along their time-course (Bosnyakova et al., 2007). They usually start with a slightly higher frequency of 4-5 Hz and gradually decrease to a frequency of 2.5 Hz at the end of SWD. For longer lasting SWD a repetition of this pattern of frequency modulation can sometimes be seen (Bosnyakova et al., 2007). In other syndromes with SWD like e.g. juvenile absence epilepsy, juvenile myoclonic epilepsy, frequency may be somewhat different (Duncan, 1997).

The most striking feature of SWD is its apparent bilateral synchrony. At ‘paper-speed’ SWD seem to start all over the head at exactly the same time. For this reason and since no structural abnormalities can be seen in the MRI of patients, SWD have long been regarded/categorized as so called ‘primary generalized’ as contrasting to simple partial seizures (defined as seizures that only have a local expression with intact consciousness of the patient), complex partial seizures (localized seizures accompanied by a loss of consciousness) and partial seizures with secondary generalization (defined as seizures starting at a clearly identifiable location but rapidly spread to other brain-areas, thereafter). With advances of modern techniques, however, it becomes more and more apparent that all kind of seizures can better be regarded as occurring in selective, rapidly interacting brain-networks. This new understanding of seizures has been recently been put forward under the name ‘system epilepsy’ by the International League Against Epilepsy. It guides the development of a new seizure and epilepsy classification system. Moreover this concept of system epilepsies is hoped to

“provide an interesting basis for testable hypotheses that may significantly advance our understanding of the pathogenesis of epilepsies” (Capovilla et al., 2009, p.1646).

In the case of childhood absence epilepsy there is a general agreement that SWD are generated within the cortico-thalamo-cortical system (Coenen and van Luijtelaar, 2003; Danober et al., 1998; Gloor et al., 1990; Huguenard and McCormick, 2007; Jasper and Fortuyn, 1947; McCormick and Contreras, 2001; Meeren et al., 2005; Seidenbecher et al., 1998; van Luijtelaar and Sitnikova, 2006). Much of our current knowledge about SWD is based on animal studies (see next paragraph), however, our understanding on the generation, generalization, maintenance and abortion of SWD is still not complete despite more than half a century of research. Especially the interactions between cortex and thalamus are poorly understood.
1.2 Animal models of absence epilepsy and absence seizures

As mentioned above much of our current knowledge about SWD is based on animal studies. A variety of models have been used including both so called seizure models, in which SWD are acutely induced by either a chemical substance or electrical stimulation, and so called epilepsy models, in which animals show spontaneous SWD (Pitkänen et al., 2006). The most famous seizure model to study SWD is the Feline Penicillin Generalized Epilepsy model (FGPE-model). In this model 2-3 Hz SWD are induced by an injection of the GABA<sub>A</sub> antagonist Penicillin into the carotid artery of cats. This artery supplies large parts of the cortex so that the excitability the entire cortex can be enhanced. The same phenomenon of SWD induction can be seen after large cortical application, or intramuscular injection of the same drug or of application of (low doses) of other GABA<sub>A</sub> antagonists such as Pentylenetetrazol (PTZ) (Cortez and Snead, 2006; Gloor and Testa, 1974). Another well known seizure model is the Gamma-hydroxybutyric acid GHB) model in which GHB is injected in the thalamus of animals (mostly rodents) (Budde et al., 2006; Cortez and Snead, 2006). GHB may act via excitatory GHB receptors as well as inhibitory GABA<sub>B</sub> receptors (Wu et al., 2004).

A major critique to these seizure models is that seizures are only induced in an otherwise ‘healthy’ brain, so that studying SWD in this condition might not resemble the exact pathological state. In addition, it might be the case that the role of a given structure, like the cortex in the FGPE model, might be overestimated by an artificial manipulation of excitability.

The availability of genetic absence epilepsy models has shifted the study on the origin of SWD towards these models. There are quite a number of epilepsy models which show spontaneous SWD, mostly either due to a spontaneous point-mutation of certain genes or a manipulation of genes (knock out models) followed by selective breeding. These include the Fisher 344 rats, Brown Norway and Long Evans rats, as well as the Gria4<sup>−/−</sup> mouse, tottering mouse, lethargic mouse and the ducky mouse. It must be noted, however, that some of these animals also show other neurological disturbances next to SWD (Budde et al., 2006; Crunelli and Lerêsche, 2002; Paz et al., 2011; Sarkisian, 2001).

The most commonly used epilepsy models for childhood absence epilepsy are the Genetic Absence Epileptic rats from Strasbourg (GAERS) and the Wistar Albino Glaxo rat from Rijswijk (WAG/Rij) (Coenen and van Luijtelaar, 2003; Depaulis, 2006; Marescaux et al., 1992) (Figure 2). GAERS were selected from a substrain of Wistar rats, now they are inbred, the WAG/Rij rat strain was already inbred before the SWD were discovered. The SWD in both strains have a polygenetic background, most likely a different one (Jones et al., 2011). Both strains show spontaneous SWD of 7-12 Hz following an age related onset (WAG/Rij rats: onset at about 4 month until after 6 month all rats show SWD; GAERS: onset at about 2
month until after 3-4 month all rats show SWD) (Coenen and Van Luijtelaar, 2003; Depaulis, 2006; Marescaux et al., 1992), with a somewhat higher discharge frequency for WAG/Rij rats but a somewhat higher number and mean duration found for GAERS rats (Akman et al., 2010).

Currently both are the best validated models of absence epilepsy with a high face- and predictive validity to the human condition; e.g. both human and rat SWD parallel the typical clinical picture of a reduced but not complete disruption of consciousness and unresponsiveness (Meeren et al., 1998; van Luijtelaar et al., 1991; van Luijtelaar et al., 2007). They show the same preferred vigilance state (drowsiness, light slow wave sleep) for SWD occurrence (Coenen et al., 1991; Smyk et al., 2011) and the same pharmacological profile, i.e. they are blocked by anti-absence drugs and aggravated by drugs that also aggravate SWD in patients, indicating similar cellular/molecular mechanisms (Depaulis and van Luijtelaar, 2006).

In this thesis the WAG/Rij rat will be used as the experimental subject.

1.3 Anatomy of the cortico-thalamo-cortical system

The study of the cortico-thalamo-cortical system requires a good understanding about its underlying structural/anatomical connectivity. This anatomy is complex but rather structured:

Cortex and thalamus are connected by mostly reciprocal, excitatory (glutamatergic) cells, which can be grouped into separate, but interacting, functional loops (e.g. somatosensory loop, visual loop, auditory loop, motor or limbic loop) (FitzGerald and Folan-Curran, 2002; Sherman, 2005). In the following the basic principles and classification concepts of anatomical cortico-thalamo-cortical network connectivity will be exemplified for the somatosensory cortico-thalamo-cortical loop (a supporting graphical illustration is presented in figure 3). It needs to be mentioned, however that the same classification and connectivity principles also hold for other functional loops (Sherman, 2005).
Next to their affiliation to a particular functional loop thalamic nuclei are classified as either ‘first order’ or ‘higher order’ thalamic nuclei (Guillery and Sherman, 2002). First order nuclei, formerly also called specific nuclei, are defined as receiving their primary or ‘driving’ input from subcortical structures, whereas higher order thalamic nuclei are defined as receiving their primary or driving input from cortical areas (Sherman and Guillery, 2002). An example of a first order nucleus is the Ventral-Postero-Medial thalamic nucleus (VPM), which belongs to the somatosensory cortico-thalamo-cortical loop. It receives sensory information picked up by e.g. the vibrissae, which reach the VPM via the trigeminal nerve (Kleinfeld et al., 1999), and relays this information to neurons in layer IV (the major input layer) of the primary somatosensory cortex via thalamo-cortical relay cells (T-C cells) (Deschenes et al., 1998; Lu and Lin, 1993; Oda et al., 2004).

An example of a higher order thalamic nucleus is the Posterior thalamic nucleus (Po), also belonging to the somatosensory cortic-thalamo-cortical loop. The Po receives its primary or driving input from the primary somatosensory cortex via cortico-thalamic-cells (C-T cells) originating in layer V and sends information via (T-C) cells both back to S1 as well as, via collaterals of the T-C cells, to other cortical areas, terminating in multiple, including superficial layers (II-III, V) (Deschenes et al., 1994; Deschenes et al., 1998; Killackey and Sherman, 2003; Sherman, 2005; Veinante et al., 2000). Therefore the Po but also other higher order thalamic nuclei for which the same principle (receiving information of one cortical structure and sending it to others) holds, have been suggested to play a role in cortico-cortical communication (Sherman, 2005; Sherman, 2007).
The role of the cortico-thalamo-cortical system in absence epilepsy – Chapter 1

Next to C-T cells projecting to the thalamic nucleus Po, the C-T neurons in the primary somatosensory cortex also project to its first order thalamic nucleus (VPM). These C-T cells depart from layer VI and can be said to be functionally different to the C-T cells projecting from layer V to higher order nucleus Po (Bourassa et al., 1995; Sherman and Guillery, 1998). Therefore, C-T cells from layer V to higher order nuclei are classified as ‘drivers’, whereas C-T cells from layer VI projecting back to first order nuclei are classified as ‘modulators’. Drivers are characterized by their action via fast ionotrophic receptors whereas modulators act via slower postsynaptic metabotropic receptors (Reichova and Sherman, 2004; Sherman and Guillery, 1998). This difference between the strong and fast acting drivers as compared to the weaker and slower action of modulators can best be visualized in their input and output firing correlograms (Figure 4) (Sherman and Guillery, 1998).

![Figure 4](image)

**Figure 4**: Response patterns of two classes of neurons. Left: Fast response pattern of a driver; Right: Slow, weak response pattern of a modulator (From Sherman & Guillery, 1998).

On top of the classification between drivers and modulators, Jones (2009) proposed an additional categorization for T-C cells as either being ‘core’ or ‘matrix’ cells. Core cells are defined as T-C cells projecting with a topographical specificity to only a single cortical area, thus including all T-C cells with their origin in the first order thalamic nuclei. T-C cells originating in the higher order nuclei, but also T-C cells originating in the intralaminar and some midline thalamic nuclei (these receive their input from subcortical, vigilance regulating, brainstem structures) cells by contrast, are examples of matrix cells. These cells project more diffusely to multiple cortical areas and have terminals in multiple cortical layers, highlighting the ability of higher order nuclei to be involved in cortico-cortical communication (Jones, 2009; Sherman and Guillery, 2002).

On their way to the cortex all T-C cells give collaterals to the reticular thalamic nucleus (RTN), which is positioned like a shield between thalamus and cortex and is the only GABAergic thalamic structure in the rodent brain (Pinault, 2004). In turn, the RTN sends back GABAergic neurons terminating on the cell-bodies and dendrites of T-C cells (Pinault and Deschenes, 1998).
On the other hand, not all C-T cells give collaterals to the RTN: whereas the C-T modulators projecting form layer VI to first order nuclei do send collateral the RTN, C-T drivers projecting form layer V to higher order nuclei do not. Instead C-T cells from layer V to Po have been found to be collaterals of corticofugal axons (Deschenes et al., 1994).

The RTN is subdivided into distinct functional sectors, it has a structured organization (Guillery et al., 1998). The somatosensory sector e.g. resides in the caudal part of the RTN: this part receives and sends input from/to VPM (Pinault and Deschenes, 1998; Stehberg et al., 2001). The rostral part of the RTN, on the other hand, is considered to be a component of the limbic loop in the sense that it e.g receives and sends projections from/to the anterior thalamic nucleus (ATN) (Gonzalo-Ruiz and Lieberman, 1995; Pinault and Deschenes, 1998). Communication between sectors of the RTN and between different RTN cells occurs via GABAergic interneurons but also via fast acting gap junctions (Pinault, 2004).

By projecting GABAergic neurons onto T-C cells the RTN is involved in switching between the two main firing patterns of the system: During active wakefulness cells are in a so called tonic or ‘relay’ firing mode. During this state the GABA-ergic influence of the RTN onto T-C cells is weak (lowest) and the (resting state) membrane potential of T-C cells stably lies at about -60 mV. Consequently excitatory post synaptic potentials (EPSPs), produced by incoming stimuli, can easily reach the action potential (AP) threshold (at about -55 mV), T-C cells show a sustained high firing rate and the transfer ratio, defined as the percentage of externally presented stimuli (e.g. a visual light flash projected on the retina or a tactile stimulus to a whisker) that reach the cortex (as measured by the amount of AP produced by the T-C cells) is high (Coenen, 1995, 1998; Coenen et al., 1972; Coenen and Vendrik, 1972). The GABAergic influence on T-C cells gradually increases upon entering drowsiness and slow wave sleep. This results in a situation where the membrane potential of T-C cells becomes more and more hyperpolarized reaching levels about -70 to -90 mV during deep slow wave sleep. As a consequence of the hyperpolarization not every incoming (external) stimulus can produce an EPSP which exceeds the AP threshold, the tonic firing pattern vanishes and the transfer ratio drastically drops. The firing pattern of T-C cells changes from a tonic firing pattern via an oscillatory firing pattern to a so called burst or burst-pause firing pattern, in which groups of neurons synchronously fire/produce high frequency burst of APs regularly occurring at a frequency around 2 Hz (Coenen, 1995, 1998). The strong hyperpolarization of T-C cells, brought about by the RTN, results in activation of hyperpolarization activated cation currents ($I_h$), which in turn activates low threshold calcium currents ($I_t$), which depolarizes the cell membrane towards the threshold for a burst of Na$^+$/K$^+$ dependent fast APs. Depolarization in turn inactivates $I_h$ and $I_t$ which supports the repolarization of the cell membrane. In addition, the burst of AP in turn activates, via the
collateral connections of T-C cells to the RTN, the GABAergic RTN cells, leading to rhythmic hyperpolarization of T-C cells and accompanied \( I_h \) and \( I_l \) dependent burst discharges (McCormick and Bal, 1997; Steriade, 2003).

Given the effect of the GABAergic RTN inhibition onto T-C cell firing behavior and transfer ratio, the RTN is often considered as a gate keeper and potential synchronizer of the cortico-thalamo-cortical system.

At the same time, however, it must be noted that also the cortex is able to control the activity of RTN cells, via the above described collaterals of the modulator C-T cells, projecting to the RTN. In this way the cortex has the potential to activate the thalamus via its direct C-T cells but also to provide a feed-forward synaptic inhibition of TC cells via activation of the RTN (Golshani and Jones, 1999; Huguenard and McCormick, 2007; Steriade, 1998).

1.4 Current prevailing view on the origin of SWD

During the last century much debate has taken place regarding the question whether the thalamus or the cortex can be held responsible for the generation of SWD and multiple theories on their origin have been proposed (Reviewed in detail by Meeren et al. (2005)).

In the early theories the responsibility for SWD generation, generalization and maintenance was solely attributed to a single structure. The centrencephalic theory, proposed by Penfield and Jasper in the late 1940s and early 1950s, attributed the responsibility to a central subcortical pacemaker in upper brainstem and midline-thalamus, which, with its diffuse projections to the cortex, might explain the apparent ‘primary’ generalization of SWD (Penfield and Jasper, 1954). Instead, findings in patients by Bancaud (1969), reporting that SWD tend to start in the cortex near an identified lesion and then spread via cortico-cortical connections shifted the attention and the complete responsibility for SWD generation and generalization to the cortex (the cortical theory).

Nowadays, both of these theories do not play much of a role anymore. Instead, two theories which highlight the importance of both structures (cortex and thalamus) that interact with each other, are prevailing although with a somewhat different weighting for the role of the cortex and thalamus respectively. Since in these ‘newer’ theories a functionally significant role for the generation of SWD is attributed to cortex and thalamus, which need to interact with each other, they can be regarded as ‘network theories’.

The first of these ‘network’ theories is the cortico-reticular theory. This theory was proposed by Piere Gloor in 1968 in an attempt to reconcile the earlier centrencephalic and cortical theory (Gloor, 1968). It proposes that a generally hyperexcitable cortex transforms normal sleep spindles into pathological SWD, since the application of the GABA\(_A\) antagonist penicillin to the cortex but not to the thalamus of cats led to a gradual transformation of sleep...
spindles into SWD with first morphological changes occurring in the cortex (Gloor et al., 1977; Kostopoulos et al., 1981). Sleep spindles are known to be generated in the intra-thalamic circuitry (RTN and T-C relay cells). Upon diminishing depolarizing inputs from vigilance regulating brainstem nuclei, signaling the transition towards sleep, the RTN starts to rhythmically fire (produces rhythmic $I_T$ currents or $Ca^{2+}$ currents) in the spindle frequency range of 7-14 Hz. This results in the rhythmic generation of IPSPs in the thalamo-cortical relay cells and thus in a rhythmic hyperpolarization of their membrane potential. This in turn leads to a rhythmic generation of activation of $I_T$ currents in the relay cells. The relay cells then transport the spindly oscillation to the cortex and, via their collaterals to the RTN, reactivate RTN cells to keep the rhythm going (Jahnsen and Llinas, 1984; Steriade, 2003). The switch from the higher spindle frequency to the SWD typical 3Hz rhythm was shown to be dependent on the degree of the relative activation of GABA$_A$ vs. GABA$_B$ receptor activation (a switch towards stronger GABA$_B$ receptor activation) within the thalamic relay nuclei (Destexhe, 1999; McCormick and Contreras, 2001). Therefore, the cortico-reticular theory assigns the thalamus with the function of the rhythm generator, whereas the cortex is seen as a generally hyperexcitable transformer from normal into pathological oscillations.

Over the last years the concept of the cortico-reticular theory has been challenged by several experimental findings (Leresche et al., 2012; Pinault and O'Brien, 2005; Sitnikova, 2010): Several observations in patient populations (Zarowski et al., 2011) and animal models (Drinkenburg et al., 1991) of absence epilepsy showed that SWD do not only occur during light slow wave sleep, the state of vigilance in which sleep spindles are most common, but can often be seen during quiet wakefulness. Moreover it was found that in GAERS rats SWD are always preceded by a 5-9Hz oscillation which, in terms of electrophysiological properties are more reminiscent of sensory-motor-rhythm oscillations than of sleep spindles (e.g. 5-9 Hz oscillations emerge from a somewhat more depolarized membrane potential than sleep spindles) (Pinault, 2003; Pinault and O'Brien, 2005; Pinault et al., 2006; Pinault et al., 2001). The sensory-motor rhythm is a cortically generated rhythm which, like SWD, is seen during passive wakefulness (Nicolelis et al., 1995). In WAG/Rij rats such 5-9Hz oscillations preceding SWD have not been studied, but also in these rats it has been reported that the pre-SWD LFP signal as well as the SWD LFP signal are clearly distinct from sleep spindles (Sitnikova, 2008, 2010) (see chapter 2 of this thesis for a more detailed discussion on these results).

Next to this several physiological studies in both cats displaying 3-4 Hz SWD and rats with 8-12 Hz SWD (reviewed in Leresche et al., 2012) show that there are no indications for a reduced function of GABA$_A$ receptors in T-C cells, which was held to be responsible for the slowing of the high (8-15 Hz) spindle frequency towards the 3-4 Hz SWD frequency.
Lastly, Meeren and colleagues (2009) reported that ibotenic acid lesions of the RTN including both RTN subparts led to a strong decrease or complete abolishment of both sleep-spindles and SWD whereas lesions sparing the rostral pole of the RTN still let to a strong decrease of sleep spindles but to a massive increase of SWD. This demonstrates that sleep spindle occurrence strongly depends on a completely intact RTN whereas the occurrence of SWD is not depended on an intact caudal RTN.

In 2002 a second ‘network’ theory on the origin of SWD was proposed. Like the cortico-reticular theory it also attributes an important functional role to both cortex and thalamus for the generation of SWD, but the distribution of tasks and therefore the contribution value of cortex and thalamus is somewhat different:

The cortical focus theory is based on experimental findings of Meeren and colleagues (2002). They obtained local field potential recordings form a wide cortical grid and the lateral dorsal (LD)-, ventral-postero-lateral (VPL)- and Ventral-Postero-Medial (VPM) thalamic nucleus in freely moving and conscious WAG/Rij rats and discovered with the aid of a nonlinear association analysis (Lopes da Silva et al., 1989; Pijn et al., 1990; Pijn, 1990) that SWD always started in a localized region of the perioral-somatosensory cortex whereas all other cortical and thalamic recordings lacked behind. During the first 500 ms of SWD this focal region was found to drive the other cortical and thalamic sites, whereas thereafter cortex and thalamus turned into an alternating resonance state (with cortex and thalamus taking turn in driving each other) (Meeren et al., 2002). Based on these observations the ‘cortical focus theory’ was formulated stating that the somatosensory cortex contains a ‘hot spot’ that initiates a cascade of events that ultimately leads to the occurrence of the bilateral and generalized SWDs, if the thalamo-cortical circuitry is in an appropriate state (Meeren et al., 2005; Meeren et al., 2002; van Luijtelaaar and Sitnikova, 2006).

By this the ‘cortical focus theory’ not only challenges the ‘cortico-reticular theory’ by disagreeing on the concept of a generally hyperexcitable cortex but it also puts much stronger weighting for the generation of SWD to the cortex by also attributing the role/function of the rhythm generator to the local intra-cortical circuit of the perioral-somatosensory cortex.

However despite the fact that there might be a local, cortical epileptic focus which is the generator of SWD this does not mean that the thalamus becomes trivial, as was for example the case in the former ‘cortical theory’. Several studies clearly show that the thalamus is involved in SWD as occurring in genetic absence models such as WAG/Rij and GAERS: Vergnes and colleagues demonstrated in a functional mapping study using bipolar local field potential recordings of several thalamic nuclei that some nuclei showed SWD activity while others were spared (Vergnes et al., 1987).
Multiple and single unit recordings in different thalamic nuclei combined with cortical local field potential recordings confirmed the participation of the thalamus (Gorji et al., 2011; Inoue et al., 1993; Seidenbecher and Pape, 2001; Seidenbecher et al., 1998; Staak and Pape, 2001), with some nuclei showing a spike concurrent firing pattern, some showing a spike concurrent silence in firing and some showing a wave concurrent firing. Interestingly, however, in GAERS rats increased precursor unit firing was reported in the layer IV/V of the somatosensory cortex concurrent to embryonic SWD oscillations, which preceded unit firing in the ventrobasal complex (Seidenbecher et al., 1998).

Lastly modern fMRI imaging reported bold signal increases in the medial dorsal, RTN, Po, VPM/VPL in awake WAG/Rij rats (Tenney et al., 2004) and in anaesthetized WAG/Rij rats bilateral synchronous increases in frontoparietal cortex, thalamus, and brainstem nuclei, whereas other cortical areas such as temporal and occipital regions did not show significant changes (Nersesyan et al., 2004). In anaesthetized GAERS fMRI changes (increases) in barrel field of the primary somatosensory cortex (S1BF), and various thalamic nuclei such as centromedial, mediodorsal, and ventrolateral parts of the thalamus including VL and Po, the retrosplenial cortex, and the reticular part of the substantia nigra (SNR), the cerebellum and nuclei of the pons (Mo5) were found (David et al., 2008).

These above mentioned studies demonstrated that the thalamus participated in SWD activity; thalamic lesion studies, leading to a strong decrease or abolishment of SWD (Avanzini et al., 1992; Avoli and Gloor, 1981; Meeren et al., 2009) clearly demonstrated that the proposed cortical focus of the ‘cortical focus theory’ might be a necessary but not sufficient condition for the generation of SWD, and that an intact cortico-thalamo-cortical system (including an epileptic focus) is also required. Paradoxically, however, in the case of Meeren’s lesion study (2009) it was the lesion of the rostral RTN that resulted in a reduction of SWD rather than the lesion of the caudal RTN, which is part of the somatosensory cortico-thalamo-cortical loop that also contains the supposed cortical epileptic focus.

Meeren and colleagues themselves acknowledge the importance of the thalamus by showing that following the first 500 ms the thalamus is not only purely driven by the cortex (thus is not purely a passive receiver), but engages in driving the cortex. They attribute the function of an important resonator to the thalamus to maintain the oscillation, whereas the role of the rhythm generator and initiator of SWD is given to the somatosensory cortex. Compared to the cortico-reticular theory in which the thalamus has the function of the rhythm generator and the cortex the function of a transformer (see above) resulting in an almost equal functional significance for cortex and thalamus, respectively, it can be noted that in the cortical focus theory a stronger weighting (greater functional significance) is given to the somatosensory cortex as compared to the thalamus. Based on this differential weighting the
The role of the cortico-thalamo-cortical system in absence epilepsy – Chapter 1

Cortical focus theory has even been proposed to be a ‘new cortical theory’, which was further manifested upon detection/verification of a cortical focus in GAERS rats. Via intracellular recordings in different layers of the somatosensory cortex Polack et al. (2007) showed that cells located in deep layers (layer V and VI) of the somatosensory cortex show a massive increase in firing shortly before SWD onset, leading to a location refinement of the supposed cortical focus to these layers.

As noted above, Meeren and colleagues state that “the cortical focus initiates a cascade of events that ultimately leads to the occurrence of the bilateral and generalized SWDs if the thalamo-cortical circuitry is in an ‘appropriate state’” (Meeren et al., 2005; Meeren et al., 2002; van Luijtingelaar and Sitnikova, 2006).

However the description of this ‘appropriate state’ of the cortico-thalamo-cortical system, remains on a preliminary level so far:

1) Dynamics of cortico-thalamo-cortical interactions, which might be necessary for the generation, generalization and maintenance of SWD so far remain poorly understood/described.

2) In addition, differences in functional contributions of the network (cortex and between different thalamic nuclei) which might be expected from several thalamic studies described above (e.g. a different role of rostral and caudal RTN revealed by thalamic lesion studies or VPM and ATN as indicated by functional mapping studies) remain to be clarified.

Earlier attempts to describe ‘network’ changes associated with SWD generation, reviewed in detail in chapter 2 either do not investigate interactions between the deep layers of the cortical focus and several thalamic nuclei and or do not investigate dynamical changes of cortico-thalamo-cortical interactions, so that the above stated lack of knowledge remains present.

Next to testing/evaluating the cortical-focus theory (chapter 3), this thesis aims to shed more light on the above stated unraveled topics using a variety of methods (see next paragraph) and refined network analysis, which overcomes the above mentioned shortcomings (no simultaneous investigation of multiple important brain structures; no investigation of dynamical changes).

1.5 Two approaches to study brain-networks

The aim to study the cortico-thalamo-cortical system confronts a researcher with the question about appropriate/suited methods to do so. In the following, two approaches which have been chosen/employed in this theses will be shortly introduced:
a) Studying the brain via electrical stimulation:

The first approach is an experimental approach. In this approach electrical pulses are either locally or globally applied to different parts of the brain and its reaction toward stimulation is investigated.

In fact brain stimulation is a quite old technique that has already been used at the beginning of the 20th century with different functional goals including the mapping of brain-functions (e.g. the detection and description of the motor and sensory homunculus by Penfield and Jasper (Penfield et al., 1954)); investigation of seizure propagation patterns (David Ferrier, John H. Jackson), test/measurement of brain excitability and treatment of diseases (e.g. electroconvulsive therapy for the treatment of psychiatric disorder (Meduna, 1937). Today brain-stimulation is still used although partially with refined stimulation paradigms.

A current method to measure brain excitability is the single or paired pulse paradigm in which either one pulse or two consecutive pulses (usually with a fixed interpulse-interval of 50-800 ms) are applied to a given brain structure. If applied to a single cell, combined with intracellular recordings the amplitude of the induced EPSP gives a good estimate of the excitability (Bliss and Lomo, 1973; Gazzaniga. et al., 2002).

In this thesis such a paired-pulse stimulation paradigm will be applied to specific structures within the cortico-thalamo-cortical system (see paragraph 1.6 for details) and the response to stimulation will be measured with the aid of local field potentials. By averaging the stimulus locked fluctuations of the LFP signal, the average or typical reaction of the brain to the stimulus, an event related potential, can be revealed (Gazzaniga. et al., 2002). Such an event related potential (ERP), or, specified for electrical stimulation, electrical evoked potential (EEP), is composed mostly of a series of bi-phasic waves or components which differ with respect to their amplitude and latency. It can be shown that the amplitude of ERPs is more pronounced if more cells fire synchronously (Coenen, 1995). Therefore the amplitude of an ERP can be/is often used as a measure of excitability. Whereas early components are predominantly sensitive to stimulus properties and thus, if recorded locally, are a good indicator local excitability, later components are also influenced by subsequent information processing and thus can be seen to reflect network-properties (Coenen, 1995).

Electrical stimulation has also been found to be able to elicit epileptic oscillations, termed afterdischarges (AD), which are often accompanied by epileptic motor symptoms (convulsions). The intensity of stimulation needed to induce an AD or the intensity of the seizure given a fixed stimulus intensity is equally used an indication of brain excitability (Adrian, 1936; Mares and Kubova, 2006; Racine, 1972 a, b). As is the case for the later components of an EEP, however, self sustained AD require the circulation of neuronal activity in a closed neuronal circuit which can either be local or more widespread.
Despite its ability to elicit AD the above mentioned goal of disease treatment has also, again, been coming up in recent years under the name of deep-brain-stimulation (DBS). Even for epileptic seizures DBS has been proposed as a new potential therapeutic treatment, but the search for optimal therapeutic stimulation protocol, as well as research on its exact mechanism of action is still in its infancy (Huang and van Luijtelaar, 2013; Pollo and Villemure, 2007; Theodore and Fisher, 2007; Theodore and Fisher, 2004). This can be partially attributed to a large number of stimulation parameters, like the frequency, intensity, interstimulus intervals, duration of stimulation and type of pulse, all of which can be varied. In addition, the optimal stimulation site(s) are also poorly characterized.

Two stimulation variants of DBS (both applied in this thesis) are the ‘open-loop’ and ‘closed loop’ stimulation paradigms. In an open loop paradigm electrical stimulation is prescheduled and delivered to the brain. This paradigm aims to modulate excitability, probably by inducing plasticity in particular brain structures or networks, and thereby to reduce the number or duration of seizures on the long run. In a closed loop, or responsive stimulation paradigm, an electrical pulse train is only delivered contingently upon detection of a seizure. In contrast to the ‘open loop’ stimulation this protocol does not aim to induce plasticity but rather to immediately and acutely disrupt ongoing seizures. Therefore this protocol mostly employs a high frequency pulse train, which is believed to have desynchronizing effects (Pollo and Villemure, 2007; Theodore and Fisher, 2007; Theodore and Fisher, 2004).

b) Studying the cortico-thalamo-cortical system with a mathematical signal analytical approach:

Next to the experimental approach of electrical stimulation it is also possible to investigate the cortico-thalamo-cortical system by signal analytical methods. In this approach different mathematical analyses are used to extract ‘hidden’ properties of an LFP signal and properties of multiple LFP signals in relation to each other.

In general signals can be investigated in the time domain (the raw fluctuations of the signal over time) or in the frequency domain, where the signal is split up into its composing sine components (each signal can be regarded as a summation of sine wave with different amplitude, phase and frequency) (Rosen and Howell, 1991).

This decomposition is commonly done with a Fourier analysis, or when done for multiple timepoints by a Time-Frequency-Analysis (TFA). Fourier analysis and TFA decompose a signal within a fixed length analysis window into its sine and cosine components and determines, per frequency, the amplitude and phase. A variant of the classical TFA is the Wavelet analysis, which does not apply a fixed-length analysis window but a variable length
analysis window depending on the frequency to analyze (Chui, 1992; Koronovskii and Hramov, 2003).

The most common output of the TFA and Wavelet analysis is a time frequency power-spectrum. The power spectrum gives information about the absolute power (squared amplitude of the amplitude) of a given frequency within the signal while ignoring the phase information.

Although a time-frequency power spectrum is done for a single signal (one brain structure), and does not reveal interactions between multiple signals (brain structures), comparing the time-point and degree of spectral power changes of one signal (brain structure) with the time-point and degree of spectral power changes of other signals (brain structures) has the potential to reveal relevant information about the relative contribution of brain structures in a given process like the generation of SWD.

In order to investigate interactions between brain structures so called connectivity analysis can be used. Generally speaking there are two classes of connectivity analysis: functional connectivity analyses and directed connectivity analyses. Directed connectivity analyses describes (statistical) dependencies between signals and try to reveal causality between signal events, whereas the latter is not performed by functional connectivity analysis (So et al., 2011).

An example of a functional connectivity analysis is the pairwise-phase-consistency analysis (PPC) (Vinck et al., 2010). Taking the phase information of the TFA as input, PPC estimates the stability of phase differences between two signals across trials, as a function of oscillation frequency. Its metric is normalized between 0 (signifying the lack of synchronization) and 1 (signifying perfect synchronization). Basically the following calculation steps are performed:

For each trial the difference in phase (given a particular frequency) between two LFP signals is calculated from the cross spectral density: 

\[ \text{csd} = A_1 \cdot A_2 \cdot e^{i(\varphi_1 - \varphi_2)} \]

with A being the amplitude of the signal and \( \varphi \) being the phase of it. The cross-spectral density can be visualized in a 2-dimensional Cartesian coordinate system as a vector from the origin, where the angle relative to the positive X-axis represents the phase difference (Figure 5).

**Figure 5** Schematic illustration of the PPC analysis. The angle between x-axis and a vector represents the phase difference between two signals for a single trial (csd). To estimate the stability of phase differences across trials (PPC) the angular distance between each vector pair is determined and averaged.
From these single trial cross spectral densities (CSDs), angular distance between all trial-pairs are calculated. The idea is that in case of high phase-stability the angular distance between trials should be small (For all trials the phase difference between two signals should be similar). PPC now averages the cosine of these angular distances. Since the cosine of the angular distances greater 90° result in negative dot-products, angular distances smaller than 90° result in positive dot-products and an angular distances of 90° (a cosine shifter by 90°) result in a dot-product of zero, the average dot-product across all trial-pairs is a valid estimation of phase consistency or synchrony (Vinck et al., 2010).

An example for a directed connectivity method is the non-linear association analysis by Pijn and Lopes da Silva: It is a time domain analysis and reveals information about the degree of coupling between brain structures, the directionality of coupling and the corresponding time-delay (signal transduction time) of signals, by calculating the nonlinear correlation coefficient $h^2$ as a function of timeshift ($\tau$) (Lopes da Silva et al., 1989; Pijn et al., 1990). The general principle is the following: the raw signal (amplitude) values of signal A are correlated with the signal (amplitude) values of signal B in a stationary data-window. To allow a non-linear relation between both signals, the window is subdivided into bins. For each bin the x value of the midpoint ($p_i$) and the average value of $y$ ($q_i$) are calculated, and the resulting points ($p_i,q_i$) are connected by segments of straight lines. This allows an approximation of the non-linear association curve by linear correlations within segments (Figure 6). By calculating the correlation ratio ($(total\ variance- unexplained\ variance)/total\ variance$) or

$$h^2 = \frac{\sum_{i=1}^{N}(y_i - \langle y \rangle)^2 - \sum_{i=1}^{N}(y_i - f(x_i))^2}{\sum_{i=1}^{N}(y_i - \langle y \rangle)^2}$$

one receives the non-linear association-coefficient $h^2$ at time point zero ($\tau=0$), which can vary between 0 (no association between signals) and 1 (perfect predictability of one signal based on the other).

Next, one of signals is shifted in time while the other remains stationary and $h^2$ is again calculated for each time shift ($\tau$). By comparing $h^2(0)$ with $h^2(\tau)$ it is established whether signal A can better predict the future of signal B or the other way around (Figure 6). The result is an estimation of the maximal degree of association (strength of coupling) that two signals can reach given a particular time shift $h^2(\tau)$, the directionality of coupling (either maximal association between signals is reached when signal A is shifted or maximal association between signals is reached when signal B is shifted) and a value for signal transduction time (the time shift or delay at which the maximal association between signals is reached).

As the name already says, one big advantage of the non-linear association analysis is that it does not presume a linear relationship between signals, which makes it especially suited for supposed nonlinear events like epileptic seizures. On the other hand, the non-linear
association analysis is an amplitude based method in the time domain, so that information about the phase and the frequency range where a change does occur is not revealed.

**Figure 6** Basic calculation steps of $h^2(\tau)$ analysis. Upper left panel: amplitude values of two signals are plotted against each other for a time-window (calculation window) of 500ms. The resulting plot is divided into equal sized bins and the midpoint coordinates of the bins (asterisk) are calculated; upper right panel: The midpoint coordinates are connected by linear line segments. These form a linear approximation the nonlinear regression curve; lower left panel: the calculation window of signal Y is shifted to the right and $h^2(\tau)$ is again calculated to see how well signal X can predict the future of signal Y; lower right panel: the calculation window of signal X is shifted to the right and $h^2(\tau)$ is again calculated to see how well signal Y can predict the future of signal X (Figure by Pijn, 1989).

Another directed (linear) connectivity analysis is Granger Causality (Dhamala et al., 2008a; Granger, 1969). It investigates whether the prediction of signal X based on its own past can be improved by also including information of the past of signal Y (Figure 7).

**Figure 7**: Schematic illustration of the concept of Granger Causality. See text below for details. Figure is taken form “MEG connectivity analysis” tutorial by J.M. Schoffelen.
This prediction can be done with the aid of autoregression models. Given a particular analysis window as well as particular model order (a given number of timeshifts to the past based on which a prediction shall be done) these models extract weighting factors for the prediction of future signal point, which keep the prediction errors as low as possible.

The comparative factor for the determination of Granger Causality is the variance of the prediction errors based on two auto-regression models.

The first model tries to predict a ‘future’ signal point \( X(t) \) only based on its own past. \( X(t) = \sum \beta_{\tau_1} \cdot X(t-\tau_1) + \eta_1 \), where \( \tau \) represents each timeshift to the past up to a specified maximal shift, \( \beta \) represent the weighting factors determined by the model and \( \eta \) represents the prediction error (the discrepancy between the predicted point and the real measured data point). The second model tries to predict a ‘future’ signal point \( X(t) \) based on its own past plus the past of the second signal \( Y \):

\[
X(t) = \sum \beta_{\tau_11} \cdot X(t-\tau) + \sum \beta_{\tau_21} \cdot Y(t-\tau) + \varepsilon_1 ,
\]

again with \( \tau \) representing the timeshifts, \( \beta \) representing the weighting factors and this time \( \varepsilon \) representing the prediction error.

In case the variance of the prediction errors \( \text{var}(\varepsilon) \) (average prediction error of all predicted points) of the second model is smaller than the variance of the prediction errors based on the first model \( \text{var}(\eta) \), it is assumed that signal \( Y \) granger causes signal \( X \). The degree of this causal influence is expressed in the ratio between both:

\[
F_{Y \rightarrow X} = \ln \left( \frac{\text{var}(\eta_1)}{\text{var}(\varepsilon_1)} \right)
\]

Although initially developed for analysis within the time domain, also a frequency domain version of this analysis is currently available, which tries to reveal whether a general influence of signal \( Y \) on signal \( X \) can be attributed to certain frequency bands. Moreover, recently a non-parametric version of Granger Causality has been introduced, which does not rely on autoregression models but extracts GC directly form Fourier or Wavelet transforms of the signals (Dhamala et al., 2008b).

Some of the above described methods (nonlinear association analysis, wavelet analysis, Granger causality, spectral power analysis as stationary version of the time frequency analysis) have already been applied for the analysis of SWD and have been proven to reveal relevant information:

- The nonlinear association analysis was used in the study by Meeren et al. (2002), which was able to detect the existence of a potential cortical focus by showing that a small area of the somatosensory cortex drives all other cortical and recorded thalamic nuclei at the onset of SWD.
- Nonlinear association analysis was also applied to MEG data of children with absence epilepsy. It was shown that the onset (first spike) of SWD was characterized by a local cluster of MEG sensors showing increased association strength positioned in frontal cortical regions, supporting the idea of a local cortical seizure onset zone, followed by an alternating pattern of globally increases of $h^2$ (between all MEG sensors) during the wave of SWD and only local increases in $h^2$ during each spike of the SWD (Westmijse et al., 2009).

- Wavelet analysis revealed the existence of SWD specific precursor activity, demonstrating that SWD generation is not as sudden as previously believed but seems to be a more gradual process (van Luijtelaar et al., 2011). It was only established for a single thalamic nucleus and the frontal cortex instead of the potentially more relevant deep somatosensory cortex.

- Spectral power analysis was applied to describe changes in power found for multiple cortical as well as thalamic recordings by Sitnikova and van Luijtelaar (2009). However, comparison was restricted to only two analysis windows (1 sec prior to as compared to 1 sec at the onset of SWD) and did not include recordings of the deep somatosensory cortex.

- Granger Causality was used to describe dynamics of coupling at SWD onset and SWD termination, leading to the hypothesis that the cortex might also be responsible for SWD termination, but again analysis was restricted to the frontal cortex and only one thalamic nucleus (Sitnikova et al., 2008).

A more detailed description and discussion of the previous results is given in chapter 2.

### 1.6 Global goals and outline of the thesis

In this thesis a refined network analysis will be applied: This includes, that,

- the anatomical connectivity profile of the cortico-thalamo cortical system, described in section 1.2, is kept in mind for the selection of appropriate recording and stimulation sites, as well as for the interpretation of results.

- measurements and stimulation studies are performed in the deep layers of the somatosensory cortex (the refined seizure focus) and multiple nuclei of the thalamus, selected based on anatomical and prior neurophysiological data, indicating a functionally significant role in SWD generation.

- signal analysis is performed in a dynamical fashion along extended SWD transition periods.

This refined network analysis is performed in order to:

1) Perform experimental test of the cortical focus theory

2) Describe differences in functional contribution between network structures for the generation, maintenance and termination of SWD. Such functional differences might
be expected from several thalamic studies described above (e.g. a different role of rostral and caudal RTN revealed by thalamic lesion studies or VPM and ATN as indicated by functional mapping studies).

3) Describe dynamics of cortico-thalamo-cortical interactions, which might be necessary for the generation, generalization, maintenance and termination of SWD.

The application of a mixture of above described methods is believed to be optimally suited for the realization of these goals: EEPs are suitable for the determination of excitability, a crucial characteristic of a seizure generating structures; High frequency closed-loop stimulation can be used to test the potential/involvement of given structures in SWD termination; Low frequency, open loop stimulation might induce plastic changes to particular structures and investigating the resulting changes of SWD characteristics might help to unravel a particular functional role of these structures in SWD generation, maintenance or termination; TFA is suited to investigate the degree and timepoint of SWD related power changes and by this might be suited to unravel differences in functional contribution of structures; and non-linear association analyses, PPC as well as Granger Causality, can be used to study functional and directed (causal) interactions between structures, which might be crucial for SWD generation, generalization, maintenance or termination.

In concrete terms, the following steps are taken to realize/approach the above listed goals:

In chapter 2 a review of current studies offering experimental support for the cortical focus theory as well as earlier network studies which also try to better describe ‘network changes’ occurring with SWD generation is given. One of the conclusions on all previous studies is that in none of these earlier network studies interactions between the assumed cortical focus in the deep somatosensory cortex to several thalamic nuclei were taken into account. In addition, a new multi-electrode system, suited for the simultaneous recording of LFP signals at multiple specific brain structures, and thus for optimized data acquisition relevant for a refined network analysis, is introduced.

Preliminary speculations/hypothesis upon the relative contribution of different structures of the cortico-thalamo-cortical system in the generation and disruption of SWD, based on exemplary/anecdotal observations in LFP signals of SWD obtained with this new electrode system are discussed.

Chapter 3 is aimed to experimentally test the cortical-focus theory: More concretely, the assumption that there is an increase in local excitability of the deep layers of the somatosensory cortex, as predicted by the cortical focus theory, is investigated. This is done with the aid of the above described (paragraph 1.5) stimulation paradigm. Two pulses
(interval 400 msec) were either applied to the deep somatosensory cortex or the deep motor cortex of absence epileptic WAG/Rij rats and the excitability of these structures, reflected in the amplitude of the locally (next to the stimulation) recorded EEPs, was compared. Furthermore, the same comparison was performed in healthy Wistar control rats in order to assess whether differences in excitability are simply be the result of a difference between sensory and motor cortices in general, or whether such a difference is specific for absence epileptic rats.

In addition, to stimulation induced AD in WAG/Rij rats and some signal properties of these AD are described. It is hypothesized that these AD reflect cortico-thalamo-cortical oscillation and can be considered as a marker of network excitability in WAG/Rij rats, although thalamic recordings were missing.

In chapter 4 an online SWD detection algorithm, which might be suited for the implementation of a closed loop DBS system for the rapid and automatic application of responsive/closed loop stimulation, was described and evaluated. This algorithm applies wavelet analysis (section 1.4) to estimate the power of LFP signals in the frequency range between 30-80Hz. The performance of this algorithm was tested on real-time recordings of 8 WAG/Rij rats recorded for either 3 or 24 hours. Performance was quantified by calculating the sensitivity of SWD detection and the specificity of SWD detection. In addition, the average detection-time was determined. The dependence of the degree of performance on analysis parameters is discussed.

The first part of chapter 5 tests the hypothesis put forward in chapter 3, that AD are indeed cortico-thalamo-cortical phenomena. It is tested whether these AD can be recorded simultaneously in cortex and thalamus and whether the same type of stimulation as used in chapter 3, applied to a thalamic nucleus (either VPM or ATN) is also able to induce the kind of AD seen after cortical stimulation. ATN and VPM stimulation is compared in the total and relative amount of induced AD, in order to reveal the loop specificity for the induction of epileptic oscillations.

In addition, the effect of ATN as compared to VPM stimulation on properties of SWD (number and duration) are described and discussed in relation to its functional role in SWD generation and maintenance.

Using the above (chapter 4) evaluated online SWD detection algorithm, the second part of chapter 5 investigates the potential and brain-site specificity (stimulation is either applied to ATN or VPM) of an opposite type of stimulation (high frequency, closed loop DBS) to disrupt SWD. Intensity needed to disrupt SWD as determined in an intensity threshold test as well as the percentage of disrupted SWD during a three hour lasting stimulation session are used as indicators for brain site specificity. The mean duration of SWD and the total
duration of SWD (mean duration timed average number), as compared between baseline and stimulation session is taken as an indicator for the effectiveness in SWD disruption. Results are discussed in terms of the functional role of brain structures in the disruption of SWD and the importance of timing of stimulation. In addition, based on the current results combined with additional observations from other DBS studies, reported in literature, some ideas on the underlying mechanisms of action of DBS are proposed.

In chapter 6 local field potentials of 16 freely moving WAG/Rij rats are obtained. Within a rat, recordings are simultaneously done/taken in layer IV, V and VI of the somatosensory cortex, rostral and caudal RTN as well as the VPM, Po and ATN, using the newly developed multiple electrode system introduced in chapter 2. The non-linear association analysis is used to assess the degree of coupling, the direction of coupling (which structure drives the other) and the signal transduction time between channels. In order to investigate the dynamics of network changes going along with SWD generation these parameters are assessed along a pre SWD -> SWD transition period lasting 6 seconds and are compared to values obtained during non-epileptic control periods. The timepoint and degree of changes, as well as the selectivity of changes for given channel pairs, are discussed in the framework of functional importance of given structures and pathways for SWD generation, generalization and maintenance.

Chapter 7 (re)analyses the same dataset as used in chapter 6. This time the dataset was not analyzed with a time domain analysis like non-linear association, but by two frequency domain analyses. In concrete terms, PPC analysis was used to assess dynamics in phase characteristics and TFA was used to describe dynamics in spectral power-characteristics seen with SWD generation and to search for/validate the existence SWD precursor activity. The application of these analyses to the same dataset as used in chapter 6 enables to explore potential underlying explanations for changes found within the time-domain and to search for (method independent) consistent, as well as (method dependent) replenishing results. Extracting from results presented in chapter 6 and 7 a SWD generation scenario is proposed.

Chapter 8 applies spectrally resolved Granger Causality (GC) to local field potential recordings (again obtained from layer IV, V and VI of the somatosensory cortex, rostral and caudal RTN as well as the VPM, Po and ATN) on 5 seconds lasting SWD -> post SWD transition periods. GC values are compared both to GC valued obtained in non-epileptic periods as well as stable SWD values obtained from the middle of a SWD.

Using the preliminary hypotheses put forward in chapter 2 as a starting point, it is sought to investigate whether the cortex or the thalamus and/or which thalamic nucleus “takes the
initiative" to abort an ongoing SWD and how this abortion is achieved. A preliminary SWD termination scenario is proposed in the discussion.

**Chapter 9** represents the general discussion of this thesis. It discusses all results in the light of the above (beginning of paragraph 1.6) global goals (questions) of this thesis. Next to this the generalizability of these findings to the human condition are discussed as well as the potential implications for the treatment of SWD.

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Chapter 2

On the Origin and Suddenness of Absences in Genetic Absence Models

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Abstract

The origin of spike-wave discharges (SWDs), typical for absences, has been debated for at least half a century. While most classical views adhere to a thalamic oscillatory machinery and an active role of the cortex in modifying normal oscillations into pathological SWDs, recent studies in genetic models such as WAG/Rij and GAERS rats have challenged this proposal. It seems now well established that SWDs originate from the deep layers of the somatosensory cortex, that the activity quickly spreads over the cortex and invades the thalamus. The reticular thalamic nucleus and other thalamic nuclei provide a resonance circuitry for the amplification, spreading and entrainment of the SWDs. Conclusive evidence has been found that the changed functionality of HCN1 channels is a causative factor for the changes in local excitability and age-dependent increase in SWD. Furthermore, upregulation of two subtypes of Na+ channels, reduction of GABAB and mGlu 2/3 receptors might also play a role in the local increased excitability in WAG/Rij rats. Signal analytical studies have also challenged the view that SWDs occur suddenly from a normal background EEG. SWDs are recruited cortical responses and they develop from increasing associations within and between cortical layers and subsequently subcortical regions, triggered by the simultaneous occurrence of theta and delta precursor activity in the cortex and thalamus in case both structures are in a favorable condition, and increased directional coupling between cortex and thalamus. It is hypothesized that the cortex is the driving force throughout the whole SWD and is also responsible for its end.

Keywords Cortical Focus; GAERS Rats; Networks; Spike-Wave Discharges; WAG/Rij Strain

Introduction

The genetic animal models such as GAERS and WAG/Rij rats are currently at the forefront of basic research towards mechanisms involved in the pathogenesis of absence epilepsy. Discoveries in these genetic absence models have seriously challenged the classical concept that the spike-wave discharges (SWDs) arise suddenly, that the rhythmic EEG pattern originates from a pacemaker in the lateral thalamus, that a diffuse hyperexcitable cortex modifies normal thalamic oscillations into pathological SWDs, the latter suggesting an intimate relationship between sleep spindles and SWDs [1]. Many of the classical views on absences were based on discoveries in the feline penicillin generalized epilepsy model, discovered by Prince and Farrell [2], who showed that large doses of intramuscular injection penicillin were modifying normal sleep spindles into bilaterally synchronized SWDs in the cat. Moreover, diffuse cortical application also produced SWDs, while thalamic penicillin injections did not [3] These SWDs in cats were accompanied by mild clinical signs that were reminiscent to what was seen in absences in children. In this overview we will review evidence that SWDs in genetic absence models have a cortical origin, that there is no general increase in cortical excitability and that SWDs do not arise suddenly. The emphasis on the cortex does not imply that extra cortical structures are not involved. It is acknowledged that SWDs need a resonant circuitry, consisting of the thalamus including the rostral pole of the reticular thalamic nucleus (RTN), for the occurrence of the full blown SWD.
GENETIC MODELS

The genetic absence models were discovered in the eighties of the last century, when two different groups of researchers discovered that their stock of albino rats contained many animals with spontaneously occurring SWDs, concomitant with behavioral immobility and mild facial clinical signs [4,5]. In Strasbourg, France, it concerned a selection line originating from a colony of Wistar rats (that were subsequently inbred as Genetic Absence Epileptic Rats from Strasbourg). At Radboud University Nijmegen, the Netherlands, it concerned an existing inbred strain (WAG/Rij, Wistar Albino Glaxo, originally raised in the city of Rijswijk, the Netherlands), also with a Wistar background, in which all animals of at least 6 months of age were affected. Younger animals 2-3 months of age are presymptomatic or have a much lower incidence [6]. Many efforts were aimed in the beginning years in validating the models as models for absence epilepsy, next the research was more aimed in discovering the underlying pathophysiology, mainly the presumed disturbances in thalamic GABA-ergic inhibition [7-11]. Now both strains are considered as rather similar and well validated models with excellent face and predictive (in our model, predictive is based on response to pharmacological treatment with different types of antiepileptic drugs) and some construct validity [12-14] and are used worldwide. SWDs are not unique for WAG/Rij and GAERS, SWDs were also described in Long-Evans rats, Fisher-344, BN, G and in a variable amount in outbred Wistar rats [15-20]. However, GAERS and WAG/Rij rats, and to some extent Long-Evans rats are well characterized and validated as models for absence epilepsy.

For about 20 years after the description of the genetic models, most of the concepts as proposed by the cortico-reticular theory [3,21-23] were not challenged. This theory suggests that SWDs originate from sleep spindle oscillations. SWDs and sleep spindles have a similar preference to occur during drowsiness and light slow wave sleep [24,25] and both are thought to originate from the RTN [26]. This theory was confirmed by relevant findings that an intact thalamus is necessary for the occurrence of SWDs since lesions of the lateral part including the RTN abolish SWDs [8,27]. RTN cells have an enhanced intrinsic propensity to burst firing as the amplitude of the low threshold Ca2+ current is increased [28]. A crucial role is assigned to the intra-thalamic network, comprising the RTN, and thalamic relay cells which are closely interconnected and act as a rhythm generator. Clinical reports described that SWDs appeared suddenly from a normal appearing background EEG. Strong bilateral synchronization of SWDs implies that an intact corpus callosum is imperative and transection of the corpus callosum showed that each hemisphere contains a machinery for the elicitation of SWDs [29]. Reduced GABAergic inhibition in the cortex was described in WAG/Rij rats [30] and enhanced cortical excitation was described in GAERS [31] All these findings are in line with the concept that absence epilepsy is a cortico-reticular type of epilepsy [3].
THE CONCEPT OF A FOCAL CORTICAL ZONE THAT TRIGGERS SWDs

While studying the spatiotemporal properties of SWDs aiming to elucidate network mechanisms that are involved in the immediate widespread generalization of SWDs, Meeren et al. [32] implanted an electrode grid on the somatosensory part of the neocortex in WAG/Rij rats, recorded local field potentials in cortex and thalamus and then quantified cortico-cortical and cortico-thalamic network relationships. The authors used non-linear association analyses in order to establish the strength of the associations (h2) and the delays between the signals [33]. Together, they determine the degree of functional coupling between underlying neuronal populations. The authors found that during a SWD the strength of association decreased with increasing electrode distance. Moreover, it appeared that during this state in each of their 8 rats there was always one to three electrodes that were leading the others, and that the other electrodes lagged. This leading zone was always in the ventro-lateral parts of the somatosensory cortex; functional topographical data revealed that it was the peri-oral zone of the somatosensory cortex (nose, upper lip, vibrissae), that contains a focal and leading cortical zone. The outcomes of the nonlinear association analyses of thalamic and cortical activity showed that the cortex was leading the thalamus during the first 500 msec of the seizure and after that cortex and thalamus lead each other intermittently. The summarized results indicate the presence of a consistent cortical focus and a fast intracortical spread of SWDs. The cortical focus consistently leads the thalamus at the beginning of SWDs (i.e., the first 500 msec after seizure onset).

Independent, indirect evidence for a hyperexcitable focal zone in the cortex of WAG/Rij rats was subsequently provided by the group of Blumenfeld at Yale [34]. These researchers compared the expression of different subtypes of sodium channel genes in six cortical samples of WAG/Rij and age matched Wistar control rats. More specifically, they studied sodium channel genes (Nav1.1 and Nav1.6) that have been associated with a persistent current (INaP). INaP is thought to underlie neuronal burst firing [35]. They hypothesized that an increased expression of Nav1.1 and Nav1.6 may contribute to the intense epileptic activity seen in anterior cortical regions during SWDs in WAG/Rij rats.

Quantitative polymerase chain reaction and immunocytochemical studies revealed significant focal increases in Na+ channel expression in WAG/Rij rats compared with controls and only in the region of onset (the peri-oral region of the somatosensory cortex, not at the other five cortical regions). This increased expression was not present in younger, presymptomatic animals. A correlation between age and Na+ channel expression was found.

Blocking these upregulated sodium channels with intracortical injections of the sodium channel blocker phenytoin abolished all SWDs, while peripheral injections enhanced SWDs [36,37]. This confirmed that Na+ channels in the targeted region are critically involved in the
control of SWDs. Blocking neuronal activity in the peri-oral area of somatosensory cortex with lidocaine is known to temporally decrease the number of SWDs [38] Microinjections of AMPA-antagonists in the peri-oral region, but not at another location in the somatosensory cortex, reduced number and mean duration of SWDs [39] Similar results were obtained with the positive allosteric modulators of the GABA(A) receptor [40]. Another line of evidence was obtained with local injections of ethosuximide (ETX) in various brain regions in GAERS. ETX is a T-type Ca2+ channel blocker and the preferred drug against absences [41,42]. It was found that ETX is far more effective when it is injected in the perioral region of the somatosensory cortex, than in the area of the presumed thalamic oscillator, the RTN or in the ventral basal complex of the thalamus [43-45]. Injections of ETX in the ventro-basal thalamic complex and in the motor cortex showed little and no effect respectively on the incidence of SWDs. This implies that in order to effectively suppress SWDs with ETX, it is necessary to target the focal area in the somatosensory cortex.

Neurophysiological evidence
Deficit of cortical inhibition is likely to play a role in pathogenesis of absence seizures. Early studies in WAG/Rij rats demonstrated a deficiency in paired pulse suppression in slices of the frontal cortex in WAG/Rij rats compared to Wistar controls [30]. Most likely, in neocortical neurons paired pulse suppression is mediated by presynaptic GABAB receptors [46]. Avoli’s team reported also with optical imaging that the cortex of 6 months old WAG/Rij rats was hyperexcitable to stimulation with the convulsant 4-aminopyridine in comparison with both younger, presymptomatic rats and with age matched non-epileptic control rats [47]. In WAG/Rij rats, the somatosensory cortex revealed alterations in GABA(A) receptor subunit expression and localization, more specifically, WAG/Rij rats exhibited lower mRNA levels for most GABAB(1) subunits when compared with tissue from non-epileptic control (NEC) rats. Additionally, GABAB(1) subunits failed to localize in the distal dendrites of WAG/Rij pyramidal cells, extending in the cortical superficial layers [48]. GABAB receptors regulate neuronal excitability by causing a postsynaptic K+-dependent hyperpolarization and by modulating transmitter release at presynaptic terminals [49]. Moreover, the Avoli group reported that higher doses of the GABAB agonist baclophen were required to depress pharmacologically isolated, stimulus-induced inhibitory postsynaptic potentials (IPSPS) generated by WAG/Rij neocortical neurons as compared to those recorded from cells from non-epileptic control (NEC) cells [50]. A similar decreased sensitivity became evident when baclophen was tested on the synchronous network-driven IPSPS generated by neocortical slices treated with 4AP + glutamatergic receptor antagonists [50].
An increase of cortical excitation could also predetermine absence seizures in WAG/Rij rats. Intracellular neocortical recordings in WAG/Rij rats with sharp electrodes revealed that the late excitatory postsynaptic potentials (EPSP), which appeared in deep layers and led to action potential discharge, could be abolished with N-methyl-D-aspartate (NMDA) receptor antagonist [51]. This documented NMDA mediated increased synaptic excitability in epileptic WAG/Rij rat neurons located in neocortical deep layers. The authors propose that this mechanism may be responsible for initiating and maintaining generalized SWDs in vivo.

Intracellular recordings with sharp electrodes in vivo in GAERS in neurons in the deep (5/6) layers of the somatosensory cortex showed that cells exhibited a specific elevated and regular firing rate during and also between SWDs, the latter were recorded by local field potentials. This neuronal firing pattern correlated with a more depolarized membrane potential than in non-epileptic cortical regions in GAERS and in the somatosensory cortex in NEC rats. This paroxysmal firing in deep layer neurons of the epileptic focus may induce discharges in superficial cortical layers and in distant cortical neurons. These leading neurons showed short periods of suprathreshold oscillations in background EEG and during preictal periods; they seem to provide a cellular scenario for the “cortical focus theory” of the origin of SWDs [52]. Interestingly, ETX is able to modify these aberrant cellular properties and return them to normal level [53].

Preliminary electrical stimulation studies of the deep layers of the somatosensory cortex in WAG/Rij rats yielded indeed larger locally measured evoked responses, and larger than in the motor cortex, while no topographical differences were found in electrically evoked responses in ACI rats (Lüttjohann et al., subm). This demonstrates that there is indeed a local hyperexcitable area in the somatosensory cortex. Earlier Tolmacheva et al.54 could not establish an increase in cortical excitability in the somatomotor cortex in WAG/Rij rats, neither in presymptomatic nor in symptomatic, only in the threshold for the induction of limbic seizures.

Changes in membrane properties of neurons in the neocortical focal epileptic zone

Immunocytochemical studies were, among others, aimed at investigating the role of glutamatergic neurotransmission in the initiation of SWDs. NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the perioral region of the somatosensory cortex in 3 months old presymptomatic and 6 months old symptomatic WAG/Rij rats were compared with age-matched nonepileptic ACI control rats [55]. The presence of NMDA and AMPA receptors was assessed by quantifying cells immunostained with NMDA-NR1 subunit and the AMPA-GluR4 subunit antibodies, respectively. WAG/Rij rats of both ages showed less NMDA-NR1 (-14.7%) and AMPA-GluR4 (-8.7%) subunit
staining than ACI rats. These differences were already present at 3 months of age, suggesting that they might be the cause of the development of SWDs or reflect a strain difference, not necessarily related to the epileptic phenotype. Experimental studies aimed at increasing or preventing the occurrence of SWDs are necessary in order to establish whether changes in these receptors contribute to pathogenesis of the focal zone. Another question is how lower NMDA and GluR4-rich AMPA receptors can be consistent with an increased excitability. The AMPA-GluR4 subunit in the somatosensory cortex is largely restricted to non-pyramidal GABA-ergic cells and a lower number of AMPA receptors would lead to decrease in the strength of inhibition [56-58]. In such a way the low expression of AMPA receptors reduces cortical inhibition, allowing the possibility to initiate SWDs in this region [55]. Whether a similar explanation can be given for the decreased number of NMDA receptors remains to be established.

MGlu receptors are subdivided into three groups on the basis of structural homology, pharmacological profile and transduction pathways: group-I includes mGlu1 and -5 receptors, which are coupled to Gq proteins; group-II (mGlu2 and -3) and group-III (mGlu4, -6, -7 and -8) receptors are coupled to Gi proteins in heterologous expression systems, reviewed by De Blasi et al.59 MGlu1 and mGlu5 receptors positively modulate excitatory synaptic transmission by either amplifying NMDA receptor currents (both subtypes) or by inhibiting GABA release (mGlu1 receptors). MGlu2/3 receptors are preferentially localized on presynaptic nerve terminals, where they negatively modulate both glutamate and GABA release [60]. The putative role of some of the metabotropic glutamate (mGlu2/3, mGlu4, and mGlu1) receptors in the somatosensory cortex was investigated with a comparison between presymptomatic (2-3 months) and symptomatic (6-8 months) WAG/Rij’s and ACI’s. A recent study showed that there were no differences between presymptomatic and symptomatic WAG/Rij and aged matched ACI rats in the mRNA levels of cortical mGlu1 receptors, as determined by in situ hybridization. In contrast, clear differences were found in the thalamus [61]. A different pattern emerged in the cortex for the mGlu2/3 receptors: six-month-old WAG/Rij rats showed an increased expression of mGlu2/3 receptors in the ventrolateral regions of the somatosensory cortex, as assessed by immunohistochemistry and Western blotting in comparison with presymptomatic WAG/Rij and age matched controls [60]. The expression of a representative of another subclass of mGlu receptors, mGlu4 was not differently expressed at the somatosensory cortex [62] The mGlu2/3 receptor signaling was reduced in slices prepared from the somatosensory cortex of both 2 and 6-month-old WAG/Rij rats in comparison with age matched controls, as assessed by the ability of the agonist, LY379268, to inhibit forskolin-stimulated cAMP formation. This was the only abnormality that was found in the somatosensory cortex of pre-symptomatic animals.
Whether an increased adenylyl cyclase activity predisposes WAG/Rij rats to develop SWDs or represents a defensive mechanism during the pre-symptomatic age remains to be determined. The increased receptor expression does not represent a pathological “trait,” but develops as an epiphenomenon of the absences. It seems that mGlu2/3 receptors are in a complex way involved in the generation of SWDs and that an upregulation of these receptors in the somatosensory cortex might be involved in the pathogenesis of absence epilepsy [60].

Hyperpolarization-activated cyclic nucleotide cationic or Ih pacemaker channels, which maintain spontaneous periodic activation, were discovered in the heart and brain and were also called pacemaker channels [63]. Four different isomers of the Ih channels are known (HCN1-HCN4), of which the HCN1 and HCN2 are often coexpressed in the cerebral cortex and hippocampus. These four subunits are thought to assemble to form homomeric or heteromeric functional channels, of which the kinetics, voltage dependency, and cAMP sensitivity are primarily determined by the particular subunit composition [63,64]. It is well known that h and T-type Ca2+ current together are controlling burst firing of thalamo-cortical cells: hyperpolarization opens the Ih channel and cationic current depolarizes the membrane. Hyperpolarization also deinactivates the low threshold T-type Ca2+ channel. The entrance of Ca2+ into the cell induces Ca2+ dependent cAMP synthesis, and a dramatic increase in cellular activity through binding of HCN subunits. The more HCN channels, or better, the more HCN1 subunits in the channel, the more binding and less pacemaker activity [65].

Recently it was established that HCN channels were reduced in the cortex in WAG/Rij rats [66] and that specifically the HCN1 subunit loss occurred early in development and preceded the developmental onset of SWDs [67]. The results of Western blot and PCR analyses corresponded to a decrease in h-current. The outcomes from their [67] elegant and relevant experiments are presented in Figure 1. These authors concluded that reductions in dendritic Ih enhance the activation of dendritic voltage-activated Ca2+ channels, increasing high-frequency burst firing in the deep layer pyramidal neurons, and that changes in the HCN1 subunit can be held responsible for the increase in SWDs.
Figure 1. The age dependent (1, 3 and 6 months of age) expression of Spike-Wave Discharges in the ECoG and changes in spectral parameters in WAG/Rij rats (A, B, C) is followed in time by a decrease in the expression of HCN1 channels (D, Western blot examples). The quantification of HCN1 protein levels was performed by plotting the optical density of HCN1/GAPDH versus age (E). HCN1 expression depends dynamically on age, being larger at 15 days and then reducing from 1 month onwards compared with Wistar HCN1 (for 1, 3 and 6 months. Data shown as mean ± s.e.m. (After ref 67, reprinted with permission).

Others established that neonatal handling and maternal deprivation during the weaning period in WAG/Rij rats affected the SWD-characteristics such as a reduction in seizure number, a decreased interspike interval, and changes in the frequency spectrum of SWDs in
adult rats. Whole cell patch-clamp recordings and from cells of the subgranular layers, in situ hybridization and Western blot analyses showed specifically an increase in the HCN1 level in the somatosensory cortex of these early manipulated rats as compared to untreated WAG/Rij control rats. The increase was selective for the HCN1 subunit and did not affect the expression of the other HCN subunit proteins [68]. Treatment aimed to prevent the age dependent increase, was also able to reduce the age-dependant decrease in the expression of HCN1 channels [69]. Many authors have proposed that HCN1 contributes to enhanced excitability in epilepsy, including absence epilepsy [66-72]. Therefore, the outcomes of the Schridde et al. experiments [68] demonstrate that early environmental changes have long-lasting consequences for SWDs, others found that the prevention of SWDs prevented the decrease in HCN169 and that increased HCN activity is responsible for a decrease in local cortical excitability and subsequent decrease in SWDs. It is also thought that the reduced responsiveness to cAMP, as has been found in the ventral basal complex of the thalamus in presymptomatic WAG/Rij rats and presymptomatic and symptomatic GAERS [64,73] prevents the shift from burst to tonic firing modes. Therefore, the alteration in Ih properties may be the cause of the generation of seizures rather than being induced by seizures, in case similar changes of h current to cAMP stimulation can be found in the focal zone.

Interpretation

It is not always easy to interpret the outcomes of comparative studies between epileptic and non-epileptic rats. Considering that the expression of the HCN and Na2+ channels could be experimentally manipulated by treatments preventing the occurrence of SWDs, it is certain that they are directly linked to the appearance of SWDs [69]. Moreover, the changes in HCN1 subunit loss occurred early in development and preceded the age-related onset of SWDs. Therefore, the causative role of cortical HCN channels in epileptogenesis is well established. Genetic factors are undoubtedly responsible for the age dependent functional changes in at least this type of ion channel dysfunction in the cortex, similar as has been proposed for the recently discovered mutations in GAERS on the gene responsible for the T-type Ca2+ channel [74]. Whether the changes in Na+ channels can be also interpreted as a causative factor for the occurrence of SWDs is still ambiguous: seizure prevention prevented their upregulation, this suggests that SWDs are the causative factor for the upregulation of Na+ channels. On the other hand, a longitudinal study in which pre-symptomatic and symptomatic rats are compared with age matched controls, as was done by Kole et al. [67] for the HNC1 channels, is still missing. Whether the changes in GABAB, iono- and metabotropic glutamate receptors are also genetically programmed or whether they are the
consequence of the hundreds SWDs per day, remains to be established. Indeed, older and recent reports have demonstrated that properties of brain tissue even outside the cortico-thalamo-cortical circuit have been changed in symptomatic absence epileptic rats [75-78]. If differences in cortical characteristic between the epileptic symptomatic and age matched non-epileptic (but not between pre-symptomatic genetic epileptic rats and age matched control rats) are found, then these changes seem strongly correlated with the age dependent increase in SWDs. This has been established in a morphometric study [79] (see section Morphological studies) and in the mGlu2/3 receptor studies [60]. In case both presymptomatic and symptomatic epileptic rats differ from the two age matched (young and older) non-epileptic controls, as has been found in the NMDA-NR1 and AMPA-Glu4 immunoreactivity study [58] then the interpretation depends upon the genetic agreement between the epileptic and non-epileptic strain. In case they are completely similar except the genes for this type of epilepsy, which are only at the beginning of being discovered [74], then the interpretation is that the effect can be considered as a causing factor. In case the epileptic and non-epileptic have many polymorphisms, then the interpretation must be more cautious since other-than-the-epileptic-gene(s) might be involved. Finally, in case only symptomatic and age matched controls are compared, then it remains to be established whether the difference is due to the epilepsy or to other genes, and whether the differences are the cause or caused by the occurrence of SWDs.

**fMRI studies**

fMRI studies (T2*-weighted echo planar imaging at 4.7 Tesla) in WAG/Rij rats were aimed to establish the contribution of sensory (S1), parietal (PtA), and temporal (Te) cortices, and several thalamic nuclei. It was revealed that the BOLD signal was increased in several cortical and thalamic zones including the RTN, mediodorsal nucleus (MD), ventral posteromedial/posterolateral nuclei (VPM/VPL), and posterior thalamic nuclei (Po) during spontaneous absence seizures in awake rats compared with baseline images [80]. Quantitative data are presented in Figure 2. Measurements of BOLD fMRI signals in WAG/Rij rats under fentanylhaloperidol anesthesia during SWDs showed a focal increase in the same anterior brain regions in which electrophysiological studies show the most intense spike-wave discharges, while the occipital cortex was spared [81,82]. This is in agreement with outcomes of cortical mapping studies in WAG/Rij and other strains [18,32,83]. The results of both fMRI studies, also in WAG/Rij rats, are in line with each other and emphasize a preferential role for the somatosensory cortex above other cortical regions.
Morphological studies

Karpova et al. [79] examined the cytoarchitecture of the Golgi stained anterior part of neocortex in WAG/Rij and control rats, paying attention to the frontal (motor) region and to the somatosensory cortex, including its peri-oral region. Typical for both the peri-oral and motor area of WAG/Rij rats was a disorder in distribution of pyramidal cells in the superficial cortical layers (I-III) (only the neurons in these layers were investigated). Apical dendrites of superficial pyramidal cells were often split in two branches, declined and went in non-perpendicular direction. Quantitative morphometric measurements of dendrites such as total length of dendrites, mean length of dendritic segment and size of dendritic arbor were increased in the peri-oral region in comparison to the motor area and in WAG/Rij rats only, indicating an abnormal pattern of dendritic arborization in the epileptic zone in WAG/Rij rats. Disturbances in dendritic trees, a receptive part of pyramidal neurons, may cause an impairment of communications between individual neurons. The superficial pyramidal cells send long-range projections to the remote cortical regions and may synchronize local cortical oscillations. Therefore, the peri-oral area of the somatosensory cortex may express corrupted associations with other cortical areas and this may facilitate synchronization and propagation of SWDs.
SUDDENNESS OF SPIKE-WAVE SEIZURES

Typical absence seizures begin abruptly and have no clinical signs by which an epileptic attack could be predicted, such as, for example, a specific aura preceding complex partial seizures. Although some changes in breathing, cardiac activity and sedation might precipitate absence seizures [84,85], these clinical manifestations are not specific and may appear in non-epileptic states as well, and, therefore, they cannot be used as predictors of an absence seizure. The classical description of the SWDs in the multichannel scalp electroencephalogram (EEG) is that the SWDs appear abruptly from a normal background activity. However, a gradual increase in power in the low frequencies at frontal locations toward the beginning of the SWDs has been found in human patients quite some time ago [85,87]. SWD-like events occurred in the EEG as “poorly developed epileptiform discharges” [88] buried in the background activity prior to the visually recognized epileptic activity. More recently, EEG precursors of spike-wave seizures have been thoroughly investigated in the genetic rat models of absence epilepsy, WAG/Rij and GAERS (reviewed in Section EEG precursor activity of spike-wave seizures). In patients with a mixed form of absence epilepsy (close to petit mal status), Niedermeyer et al. [89,90] first found an increased rhythmical activity and stronger synchronization between bilaterally symmetric (homotopic) cortical areas during a pre-ictal period. In patients with absence epilepsy, Garcia Dominguez et al. [91] and Aarabi et al. [92] described significant changes in intracortical synchrony several seconds before the onset of SWDs. According to Aarabi et al. [92] in 48% of pre-ictal periods a significant drop in synchronization versus background activity was found, in 46% a significant increase in the synchronization and in 6% – no changes in synchronization. It was stated that “the spatiotemporal cortical synchronization is different from patient to patient but reproducible in each patient” (p.216). This implies high inter-individual heterogeneity of EEG synchronization patterns precedes the seizure onset in human patients.

In the WAG/Rij model, individual variations of spatiotemporal synchronization during pre-ictal periods were relatively small [32]. In all subjects, cortico-cortical association strength began to increase a few seconds before the onset of SWDs. The small between-subjects differences in WAG/Rij rats were likely due to the homogeneity of the subjects (they all originate from an inbred strain); such a homogenous subset cannot be assumed for the human population. However, a common prototype of SWD-precursor (preSWD) for all human subjects could not be identified.

The nature of pre-epileptic processes underlying highly stereotypic SWDs is intricate and poorly understood. With EEG power spectrum and coherence analysis substantial differences in amplitude-frequency parameters of pre-SWDs have been found in WAG/Rij rats (see, *Intracortical and thalamo-cortical mechanisms of initiation of spike-wave*
discharges). It is nowadays established that the transition from pre-ictal phase to a fully blown seizure is governed by activity in the cortico-thalamo-cortical neuronal network. The role of the thalamus or subcortical network activity and their interaction with the cortex can only be studied in the genetic models with a sufficient temporal resolution. Specific spatiotemporal synchronization over the thalamocortical system has been examined by means of EEG coherence (see, Intracortical and thalamo-cortical mechanisms of initiation of spike-wave discharges). Another promising tool for examining strength and directionality of cortico-thalamic relationships is Granger causality. Granger causality concept was successfully used to describe the dynamics of thalamo-cortical network associations in the transition from the “pre-ictal phase” → “SWD” → “post-ictal phase” (see, Thalamo-cortical interactions before, during and after spike-wave EEG seizures in terms of Granger causality).

EEG precursor activity of spike-wave seizures
In GAERS, indications were found that pre-seizure EEG epochs exhibit a higher degree of determinism than seizure-free EEG epochs, but lower than those during SWDs [93]. Pinault and coworkers [94,95] recorded local field potentials (LFP), intracellular and extracellular activity in GAERS. These authors noticed that medium-voltage 5-9 Hz oscillations (sometimes in a broader band, 4-12 Hz) were predominant in both the LFP and intraburst frequencies in intracellular recordings prior to SWDs in neurolept anesthetized and free moving animals. Moreover, the medium-voltage 5–9 Hz firing rhythm of cortical cells constantly preceded the onset of SWDs [94]. Cellular mechanisms of medium-voltage 5-9 Hz oscillations were further investigated in anesthetized and nonanesthetized GAERS [95,96]. It was found that absence-related 5-9 Hz oscillations were produced by the somatosensory part of the corticothalamo-cortical system. Second, neurons in layer VI of the somatosensory cortex started firing at 5-9 Hz a few milliseconds earlier than neurons in the corresponding specific thalamic nuclei and RTN. Third, cortical neurons were able to modulate membrane potentials of thalamic neurons throughout dense cortico-thalamic synaptic interactions. All this confirms that layer VI cortical neurons largely affect neuronal activity in functionally related thalamic nuclei. After receiving an initial signal from the periphery and somatosensory cortex [97], the cortico-thalamo-cortical network may enter a pro-epileptic state and produces a 5-9 Hz seizure-precursor rhythm that consequently was followed by SWDs. It is hypothesized that, in epileptic animals, 5-9 Hz pro-epileptic oscillations give rise to SWDs due to modifications of cortico-thalamo-cortical network associations. The Pinault et al.’s 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 [52].
The presence of 5-9 Hz rhythmic precursors of absence seizures has not been confirmed in animal models, other than GAERS, or in humans. Despite the close phenotypic relationship between GAERS and WAG/Rij rat strains [11,13,14,98] precursor activity of SWDs in WAG/Rij rat does not constitute a well-developed 5-9 Hz rhythm [99]. Instead, in WAG/Rij rats, as recorded by local field potentials in freely moving subjects, there was a remarkable diversity in the waveform and frequency characteristics of 1 sec EEG episodes prior to the onset of SWDs (precursor epochs of SWDs, preSWD epochs). It might well be that the anesthesia, as used by Pinault et al. reduces the spontaneous fluctuations in the EEG. We divided the PreSWD epochs in four types based on their time-frequency features EEG and power spectrum (as measured in the frontal EEG), namely, preSWDΔ, preSWDθ, preSWDα and preSWDn. Illustrative examples of different types of SWDs preceding activity are illustrated in Figure 3.

![Figure 3](image-url)

**Figure 3.** Four types of SWD-precursor activity (preSWD epochs) as recorded in the ECoG of a one year old WAG/Rij rat.

These preSWD types were significantly different in EEG power of the characteristic delta-theta-alpha bands. More specifically, preSWDΔ epochs were characterized by the highest
power in delta band and represented ~22% from the total amount of preSWDs, preSWDθ expressed the highest power in theta band (38%), preSWDα - in alpha band (35%). The remaining 5% of precursor epochs were characterized by desynchronized EEG (preSWDn) and showed the least power in all investigated frequency bands. Large heterogeneity or diversity of preictal EEG patterns in WAG/Rij rats are similar to what has been reported in epileptic humans [92].

EEG power spectrum analysis demonstrated that 73% of all SWD precursors in WAG/Rij rats (preSWDθ+preSWDα) exhibited a pronounced theta-component. Besides theta, almost all preSWD (95%) comprised a substantial delta component, suggesting that coexistence of delta and theta activities in the cortex is favorable for the occurrence of SWDs. It is also remarkable that four types of preSWD, which were clearly distinguished in the frontal EEG, did not differ in the thalamus (both RTN and VPN). In all types of preSWD, thalamic counterpart expressed the same power in delta and theta bands [99].

There was also a correspondence between the type of SWD precursor activity and subsequent SWDs in respect to their time-frequency characteristics. For example, for the duration of SWDs it was found that preSWDα and preSWDθ were followed by longer SWDs, and preSWDn and preSWDΔ by shorter absences.

The most significant changes in transition “preSWD”→“SWD” were found in the frontal cortex, whereas changes in the thalamus were less pronounced. The RTN and the VPM were characterized by similar dynamic changes of EEG power. In the frontal cortex, almost all (except SWDn) subtypes of pre-SWD --> SWD transitions demonstrated similar dynamics of power in delta-theta-alpha frequencies: there was an elevation of alpha power accompanied by a decrease of delta(theta) power. A desynchronized precursor preSWDn was followed by SWDs with significantly higher delta in the thalamus, but not in the cortex. Precursor activity with a higher alpha in the frontal EEG (preSWDα) was followed by SWDs with high alpha in the thalamus, but not in the cortex. In the thalamus, an increase of alpha was only significant in preSWDn→SWDn and in preSWDα→SWDα. PreSWDΔ→SWDΔ and preSWDθ→SWDθ displayed a reduction of theta, in addition to that, SWDΔ showed a decrease in delta as compared to preSWDΔ.

In general, the transition from preSWD to a fully blown SWD is characterized by changes in the EEG’s power spectrum and its energy in the various frequency bands change quickly. The predominant presence of delta and theta characterizing almost all preSWD epochs was replaced by a predominant activity in the alpha band during SWDs; this general picture was found in cortex and thalamus. Large differences were also found between various preSWD epochs in EEG power spectra as measured in the frontal and occipital cortex and in the thalamus, however, the differences in cortical power spectra quickly disappear within the first
second of a SWD, and the effects of the preceding epoch quickly vanish and can no longer be detected. In the thalamus, there is a correspondence between frequency profile of SWDs and their precursor epochs: SWDs, whose precursor epochs in the frontal EEG are desynchronized (preSWDn), have an enlarged delta, and seizure-precursors with exaggerated alpha activity in the frontal EEG (preSWDa) are followed by seizures with high alpha activity in the thalamus (while the enlarged alpha component in the frontal cortex has disappeared).

Elevation of delta activity, characterizing the immediate preictal period at the onset of SWDs in WAG/Rij rats, is almost overlooked in genetic animal models. Earlier, Bosnyakova et al. [100] applied wavelet transform for the analysis of EEG in WAG/Rij rats and incidentally noticed high amplitude slow-wave activity (in delta range) before the beginning of SWDs. An increase in cortical delta and high theta in the 2s epoch before the onset of SWDs was noticed in GAERS [101]. Recently, it has been found [98] that power spectrum of 2 sec EEG epochs before the onset of SWDs in GAERS was similar to that in WAG/Rij rats. In both strains, power spectrum of preSWD epochs was compared with baseline EEG spectra as measured in the waking state. In contrast to the baseline EEG, preSWD epochs showed a higher power in the three frequency bands: delta (1-4 Hz), theta (4.5-8 Hz) and alpha (8.5-14 Hz). An increase of EEG low-frequency rhythmic components before SWDs is not surprising, considering that SWDs are more likely to appear when EEG synchronization is somewhat increased while the level of vigilance is decreased. The only significant difference between WAG/Rij rats and GAERS in respect to seizure-precursor activity is that WAG/Rij rats expressed more alpha during preSWD epochs in comparison to GAERS, but power of theta- or delta components did not differ in these two strains. This difference could be explained by differences in the way in which the EEG was measured. We used (quasi) monopolar recordings from the frontal cortex against reference and earth both on the cerebellum and recorded local cortical activity, while Akman et al. [98] used bipolar differential recordings between two active electrodes. Perhaps, theta- or delta components of SWD-precursors are rather local and could better be detected with monopolar recordings of local field potentials [99].

In order to shed more light on EEG dynamics of pre-ictal activity preceding spontaneous absence seizures, we used continuous wavelet analysis of cortical (frontal cortex) and thalamic (VPM) recordings in WAG/Rij rats [102]. This analysis was performed in 3 sec EEG epochs just prior to the onset of SWDs and in 3 sec epochs of background nonepileptic EEG (incl. wakefulness and sleep). Our study was specifically focused on the low frequency bands (from delta to high theta).
The first major finding was that 80-90% of the EEG epochs preceding SWDs as recorded in the frontal cortex were characterized by the co-existence of delta and theta components (a representative example of a SWD that is preceded by 4.2 Hz delta and theta (7.5 Hz) activity is presented in Figure 4).

Figure 4. Example of continuous wavelet transform of SWD (frequency at Y-axis, power in white-grey and black traces), time on the X-axis. The EEG (upper trace) was recorded at the frontal cortex in a one-year-old WAG/Rij rat. Note the elevation of low (3-5 Hz) frequencies during the immediate pre-ictal phase. Left bottom plot (insert) displays instantaneous (momentary) wavelet power spectrum of the pre-ictal episode, in which two peaks could be distinguished in delta and theta frequency range corresponding to delta- and theta precursors of seizure activity. The seizure is characterized by high wavelet power in several frequency ranges, including the main rhythmic component in ~11 to 6 Hz. The frequency decreases over time. The first harmonic can be noticed in ~22 to 11 Hz. Note that frequencies in the wavelet spectrum are given in log-scale. (authors are indebted to Dr. A. Hramov, Saratov State University).

Simultaneous presence of delta and theta components in control EEG epochs, i.e., 10 seconds of interictal EEG, was rare (< 10% of examined epochs).

The second finding was that delta and theta precursors of seizure activity appeared synchronously in the cortex and thalamus. Delta precursor activity in the frontal cortex was found in 90% of all SWDs (82% in the thalamus). Its mean frequency in the cortex was 4.1 Hz, (4.3 Hz in the thalamus) and duration was 0.48 sec in the cortex (0.5 sec in the thalamus). Theta precursor preceded ~92% of SWDs in the frontal cortex (83% in the
thalamus). Its mean frequency in the cortex was 8.6 Hz (8.5 Hz in the thalamus), and its duration 0.46 sec (0.5 sec in the thalamus). There were no differences in the percentages of SWDs that were preceded by theta and delta in cortex and thalamus. The latency of delta precursor to the first spike of the SWDs was longer in the frontal cortex than in the thalamus. This demonstrates that the delta precursor starts earlier in the cortex and this implies that cortical mechanisms are primarily involved in triggering SWDs and that the thalamus seems only secondarily involved.

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. The presence of delta activity during pre-ictal phase is not surprising, SWDs preferably occur when the patients or rats are quiet, during low vigilance state (drowsiness, light slow-wave sleep). These periods are accompanied by a slowing of the EEG frequencies. There are also some neuronal mechanisms that could account for a pro-epileptic nature of brief episodes of delta. It is known that burst firing of thalamo-cortical cells (TC in the thalamus) and cortico-thalamic neurons (CT in the cortex) is attributed to SWDs. The burst firing mode is not only the constituent element of SWDs, also of sleep spindles and delta activity as start to appear during drowsiness. The frequency of the burst firing is determined by the amount of hyperpolarization of TC and CT cells. The intrinsic oscillatory properties of TC neurons are known to underlie burst firing. Each oscillatory cycle includes (1) a depolarization phase induced by a T-type Ca2+ current that triggers a burst of fast Na+/K+ action potentials and (2) a subsequent hyperpolarization phase that activates the hyperpolarization-activated cation Ih channel [96]. Perhaps, pre-ictal delta activity reflects hyperpolarization phase of a sufficient number of CT cells (a critical mass) in the peri-oral region of the somatosensory cortex. CT cells excitatory projections to the posterior thalamic nucleus, the RTN and the ventral basal complex. Next to the activation of CT cells, and slightly later in time, TC cells in the ventral basal complex are hyperpolarized through the activation of GABA-ergic cells in the RTN. This might explain why thalamic precursors are slightly delayed in comparison to cortical ones.

Intracortical and thalamo-cortical mechanisms of initiation of spike-wave discharges

In order to evaluate changes in neuronal synchronization between thalamic and cortical regions at the onset of SWDs, we examined and compared associations (coherence) in two sequential intervals: 1 sec before and 1 sec after the onset of SWDs, once more in freely moving rats (Figure 5-A) [99]. We distinguished five interacting resonant circuits, and showed that each part of circuitry involved operates in a specific oscillatory mode (Figure 5-B) since each circuit was characterized by a peculiar oscillatory pattern.
Figure 5. Outcomes of EEG coherence study characterizing thalamo-cortical network synchronization in transition “preSWD→SWD.” (A) An example of EEG recordings used for coherence calculations. (B) Four neuronal networks (a–d) are involved in the initiation and maintenance of SWD in WAG/Rij rats. This schema is based on EEG coherence data, and it is designed in terms of local/non-local field theory. “Local network” in the neocortex (a) are created by the neighboring areas which are closely interconnected (functional and anatomical connections). “Non-local network” (b) include distant cortical areas, which are functionally heterogeneous and characterized by weak anatomical connections. Development of SWD correlates with high bilateral synchrony in trans-hemispheric cortical networks (c). Thalamo-thalamic networks (d) maintain the overall seizure rhythm in 9.5 Hz. Thalamo-cortical networks (e) are involved in sustaining and augmenting epileptic rhythm.

Local cortico-cortical networks, comprising the cortical surrounding of the epileptic focus, form a “local neuronal circuit” (Figure 5-Ba), were responsible for the initiation of SWDs. These networks showed an increased coherence in frequencies of 8-35.5 Hz and a reduction...
of delta-coherency (1-5 Hz); and they also expressed the highest frequency of the peak of the first harmonic (21 Hz, in the other pairs it was 18.5-20 Hz).

Non-local cortical networks characterized distant connections between fronto-parietal and occipital cortical areas, which were likely to be involved in unilateral spreading of seizure activity (Figure 5-Bb). The SWD-related increase in coherence in these networks was moderate (maximum at 19.3 Hz).

Trans-hemispheric networks were formed by the symmetrical areas in the frontal, parietal and occipital areas. They could be responsible for bilateral propagation and synchronization of seizure activity. In these networks, coherence showed the largest increase as compared to the other pairs and it is characterized by an additional peak in ~16 Hz. This additional 16 Hz peak is likely to reflect seizure propagation through bilateral callosal fibers (transhemispheric synchronization), suggesting a crucial involvement of the corpus callosum in the pathophysiology of absence seizures (see also Vergnes et al. [29]).

Intra-thalamic networks between RTN and VPM seemed to just maintain ~10 Hz seizure rhythm. Initiation of SWDs in these networks was accompanied by an increase of coherence in a narrow frequency window, 8-11.5 Hz, while the gamma-coherence decreased. Probably, a desynchronization (i.e., decrease of coherence) in the gamma band may encourage thalamo-cortical network synchronization in alpha frequencies.

Cortico-thalamo-cortical networks. It was found that the onset of SWDs is associated with an increased coherence in frequencies between 5-60 Hz with two maxima around 10 and 20 Hz, corresponding to the mean frequency of SWDs (8-11.5 Hz) and the harmonic frequency 16-21.5 Hz.

The increase in coherence reflecting the strengthening of network associations showed some frequency constraints: the peak frequencies of coherence in different parts of the oscillatory loops were significantly different, suggesting that each part of the circuit may have its own strategy to sustain seizure activity. Strengthening of corticocortical coherence in the beta range may be associated with genesis of the “spike” component in SWDs. As known, spike component has its maximal expression in the frontal cortex and it is poorly developed in the thalamus and in the occipital cortex [32]. Fronto-thalamic coherence in the beta range was not high, compared with that in cortico-cortical pairs. The increase of beta coherency was not found in occipitothalamic and thalamo-thalamic pairs.

Finally, the frequency profiles of thalamo-cortical synchronization (EEG coherence) were compared for the four different classes of preSWD epochs, i.e., preSWDΔ, preSWDΘ, preSWDα and preSWDn (as described in EEG precursor activity of spike-wave seizures, Figure 3). Yet no features of EEG coherence can be considered as unique for any class of preSWD epoch. In general, each pre-SWD type could be distinguished from others by the
spectral characteristics of the EEG power and by EEG coherence patterns in cortex and thalamus.

It can be concluded that the functional connectivities between different parts of the neuronal network in which SWDs are initiated, amplified, and sustained are frequency-specific changed when the SWDs are affected by the large scale bilateral synchronized and highly rhythmic EEG oscillations. This suggests that each part of the neuronal circuitry operates in its own preferred oscillatory mode during SWDs.

**Thalamo-cortical interactions before, during and after spike-wave EEG seizures in terms of Granger causality**

Directionality of thalamo-cortical interactions during SWDs in WAG/Rij rats has already been explored by means of nonlinear association EEG analysis [32]. These authors showed that directionally of thalamo-cortical coupling varied throughout the seizure and it was the most constant during the first half a second, when the cortical epileptic focus consistently led the thalamus. We applied Granger causality concept in order to investigate directionality of thalamo-(frontal)cortical network associations “pre-ictal period” → “SWD” → “post-ictal period” [105]. In Granger causality, the knowledge about the immediate past of one signal is used to predict the future of another signal. Granger causality is a time domain measure of functional interactions, assuming directionality and information transfer. We first found that the linear estimation of Granger causality provided a good approximation to baseline non-seizure EEG. This encourages us to use the Granger causality concept as a strategy to tackle a challenging problem of predictability of absence seizures in EEG. This yielded new, yet comprehensive information about functional thalamo-cortical interactions during absence epilepsy. The outcomes of our recent study are presented in Figure 6.

1. Linear Granger causalities during pre-ictal phase remained constant and were very low until SWDs were visually recognized in EEG, yet the influence from thalamus to cortex was stronger than vice versa. In other words, information transfer in the direction from “thalamus to frontal cortex” was more intense than in the backward direction. This is the first indication of anisotropy in thalamo-cortical interactions.

2. The onset of SWDs was characterized by a rapid increase of coupling strength in both directions and an amplification of pre-SWD existing tendencies.

3. The increase in coupling strength from “frontal cortex to thalamus” at the onset of SWDs was rapidly restored to the initial level before the cessation of the seizure. The fact that the influence “frontal cortex → thalamus” drops before the end of a seizure could imply that ongoing spike-wave activity is suppressed because of the reduced cortical effect on the thalamus.
(4) The sustained increase in “thalamus to frontal cortex” coupling during SWDs persisted some time after the end of the SWDs.

Figure 6. Granger causality in WAG/Rij rat was accessed between the frontal cortex and the specific thalamus (ventroposteromedial thalamic nucleus) at the onset and the end of SWD. Coefficients of Granger causality, $S_{y|x}$ and $S_{x|y}$, were computed in two successive 10-s epochs (5 + 5 s) including pre-SWD/SWD and SWD/post-SWD. At the onset of SWD, the increase in bidirectional causal coupling between frontal cortex and thalamus was abrupt and significant. Thalamus-to-cortex coupling did not return to the pre-seizure level immediately after the cessation of the epileptic electroencephalographic activity, as did the cortex-to-thalamus coupling. Reprinted with permission [105].

Clinically, both start and end of SWDs are regarded as abrupt and unpredictable, but we observed that changes in Granger causalities at the onset of SWDs were more sharp and fast as compared with that at the end of SWDs (post-SWD periods were characterized by smooth and prolonged changes in Granger causalities). It is hypothesized that the rapid decrease in cortical to thalamus coupling could promote seizure cessation, the propagation
and maintenance of seizure activity could be facilitated under the strong and sustained influence of the thalamus to the cortex.

In terms of Granger causality, the coupling strength between frontal cortex and thalamus remained unchanged until the moment when SWDs could be visually recognized in EEG [105]. This contradicts the outcomes of non-linear association analysis that also takes into account directionality of functional coupling [32]. In this study, a gradual increase in association strength was found between electrodes located at the “focal” epileptic zone in the area of peri-oral projections in the somatosensory cortex already before the onset of SWDs [32].

In general, Granger causality appears to be an effective way to gain more insight into the dynamics of the thalamo-cortical neuronal network mechanism underlying dynamic properties of SWDs. We have to admit that even though the linear estimation of Granger causality appeared suitable for studying cortico-thalamo-cortical directions of interactions, which are involved in maintenance and stopping of SWDs, it was not sufficient for predicting episodes with absence epilepsy. Application of non-linear Granger estimations may help to solve this problem. On the other hand, absence seizures might be unpredictable by nature (e.g., they might have no signs that would allow any kind of prediction). At least, computational model of thalamo-cortical neuronal network [106] explains the unpredictability of SWDs simply by the fact that fluctuations of control parameters (synaptic and cellular properties of thalamic and cortical cells) are unpredictable by definition.

A MULTI-ELECTRODE TECHNIQUE FOR LOCAL FIELD POTENTIALS

The discovery that a focal zone can be found in the deep cortical layers of the somatosensory cortex in both WAG/Rij and GAERS rats, has shifted our attention towards more refined analyses of the local field potentials in the deep layers of the somatosensory area and the changes that occur between especially this local cortical focus and thalamic nuclei, both within the primary somatosensory loop and outside the somatosensory loop. In order to enable such a reinvestigation of network interactions at the start and end of SWDs, a first step was the development of a multichannel electrode system that allowed us to record in different cortical layers and in multiple specific thalamic nuclei. This system was physically implemented in the form of a prefabricated Teflon block, in which isolated electrode wires were inserted through thin holes, that were based on the projections from the desired positions in the brains on the Teflon block, glued and cut at the appropriate length. This block and a head stage are placed stereotactically on the animals head and the electrodes are aimed through the drilled holes in the skull (Figure 7).
The role of the cortico-thalamo-cortical system in absence epilepsy – Chapter 2

Figure 7. New electrode system for multi-channel recording. It consists of a custom made Teflon block with holes in which electrodes are glued. The electrodes are cut at the desired length.

Figure 8. Histological verification of electrode positions: electrodes aimed at layer 4, 5 and 6 of the somatosensory cortex (A); rostral RTN and ATN (B); posterior thalamus and VPM (C); VPM (D); schematic illustration of the multi-electrode system (E).
In first recordings with this new system, electrodes were aimed at layer 5 and 6 of the somatosensory cortex, to obtain LFP recordings of the focal epileptic zone; the fourth layer of the somatosensory cortex as major input layer of the somatosensory loop; the VPM, with its reciprocal connections to the somatosensory cortex; both caudal and rostral RTN, receiving and sending collateral projections to TC and Cortico-Thalamic neurons; anterior nucleus, with its connections to the rostral RTN; and the posterior thalamic nucleus, which directly receives input from layer 5 of the somatosensory cortex without collaterals to the RTN (Figure 8) [107-109]. Recordings were bipolar, differential recordings with ground and reference electrodes on top of the cerebellum and a sample rate of 2048 Hz to ensure a high temporal resolution, needed for an investigation of possibly rapid temporal changes at the onset and offset of SWDs. In the resulting recordings, that now allowed the direct investigation and comparison between LFP activity in the local cortical zone and relevant thalamic nuclei, some phenomena that support both the idea that SWDs do not occur suddenly and the idea that SWDs have a local cortical origin in the deep layers of the somatosensory cortex, can be observed even in the raw EEG traces.

The first phenomenon is displayed in Figure 9a. It can be seen that prior to full-blown SWDs with rhythmic spike and wave activity in both cortex and thalamus there are already strong rhythmic oscillations in the dominant SWD frequency of 8 to 10 Hz. These oscillations are restricted to the deep cortical layers of the somatosensory cortex and start about one second prior to the emergence of the full-blown, generalized SWDs so that at least in recordings of the deep layers of the somatosensory cortex SWDs obviously do not emerge suddenly from a normal background EEG. The restriction of these oscillations to the deep cortical layers is also in good agreement with the concept that SWDs have a local cortical origin instead of a thalamic one.

It seems as if gradually more and more cortical cells become recruited until enough CT cells fire in synchrony, strong enough to entrain the thalamus into SWDs. This process might be reflected in a gradual increase in coupling strength of cortex and thalamus at the start of SWDs. Sitnikova et al. [105] found increases in Granger’s causality coefficient between frontal cortex and ventral basal thalamus, even more interesting would be to analyse this between the cortical focus and the thalamic VPM.

The concept of a local cortical focus in the deep layers of the somatosensory cortex is further supported by additional observations that can be done in the raw EEG traces: When zooming to the first “generalized” spike that can be seen in all cortical layers and all thalamic nuclei, it can be noticed that the spike in the deep somatosensory cortex precedes all thalamic spikes (Figure 9b), which is obviously in agreement with the guiding cortical influence during the first 500ms of a SWD as found by Meeren et al. [32]. Interestingly, this
temporal relationship with the cortical spikes preceding the thalamic one can not only be seen within the first 500 milliseconds but often throughout the whole SWD (Figure 9c). This might indicate that the cortex remains the driving force throughout the whole SWD.

**Figure 9.** Multi-channel EEG recordings of the start of SWD of an adult WAG/Rij rat. A: Strong, rhythmic oscillation in the deep layers of the somatosensory cortex precedes the generalized SWD with rhythmic spike and wave activity in cortex and thalamus. B: Temporal sequence of the first generalized spike. Note that the spike in layer 6 of the somatosensory cortex can be seen a few milliseconds earlier than all other cortical and thalamic ones. C: Temporal sequence of a spike in the middle of the SWD. Note that again the spike in layer 6 of the somatosensory cortex can be seen a few milliseconds earlier than all other cortical and thalamic ones.
Another observation in favor of the cortical focus theory is displayed in Figure 10: Here spike and wave-like oscillations, with most pronounced spiking in cortical layer 6, that remain localized within the layers of the somatosensory cortex and do not involve the thalamus. Incidentally, they even remain localized within a single layer. These localized spike wave-like oscillations have a smaller spike amplitude compared to spikes of a full-blown SWD and even the oscillations preceding the full blown SWD, which might indicate that less CT cells might fire in synchrony, therefore the influence of the cortex on the thalamus is not yet strong enough to also entrain the thalamus into the spike and wave oscillation. Alternatively, the thalamus might not be in the optimal oscillatory state in terms of hyperpolarization of its cells, so that it does not start to reverberate to the cortical oscillatory input. It can be speculated whether these localized oscillations and probably also the oscillations that are seen in the somatosensory cortex preceding the SWDs are similar to the 4-12 Hz middle-voltage oscillations that precede SWDs in GAERS.

Figure 10. Localized spike and wave like discharges in the deep layers of the somatosensory cortex (A) or a single cortical layer (B) from a WAG/Rij rat.
A last observation made in the raw EEG traces concerns the end of SWDs. As can be seen in Figure 11, spike and wave oscillations do stop earlier in the thalamic recordings while the cortical layers continue to oscillate for a few seconds. Given the observation that the cortex seems to be the driving force throughout the seizure, this might mean that a seizure stops because of a decrease in directional coupling from cortex to thalamus. Alternatively, the thalamus either actively engages in another oscillatory pattern or is entrained to another rhythm by a third structure involved in seizure cessation. Signal analytical techniques such as non-linear association analysis, Granger causality or time-frequency analyses, investigating the dynamics of corticothalamic network interactions at the end and start of a seizure need to further elucidate this question as well as the generality for the above described observations done for the start of SWDs.

Figure 11. Multi-channel EEG recordings of the end of a SWD from a WAG/Rij rat. Note that the spike and wave oscillations stop earlier in the thalamus than in the cortex. This might be due to a decrease in cortico-thalamic coupling.
CONCLUSIONS

It is well established that SWDs in WAG/Rij rats and in GAERS are initiated in the peri-oral region of the somatosensory cortex, more precisely, by neurons located in the deep cortical layers. Now the origin has been discovered, the early appearance of SWD-activity can be easily visualized by local field potentials from the deep layers. Also measurements of cortical excitability after local electrical stimulation demonstrate that the focal epileptic area is hyperexcitable.

*In vitro* or combined *in vivo* and *in vitro* studies provide clues to the mechanisms underlying increased cortical excitability and reduced presynaptic inhibition. Expression of two ion channels, the hyperpolarization-activated cyclic nucleotide gated (HCN) and Na+ channels, is directly linked to the age-dependent appearance of SWDs. Proepileptic changes in HCN1 subunit occurred prior to the age-related onset of SWDs. Moreover, an experimental treatment that increases the activity of HCN channels is known to prevent the occurrence of SWDs. It is hypothesized that the loss of cortical HCN channels and a diminished sensitivity of h current to its main modulator cAMP are crucial factors for epileptogenesis in these genetic models. Genetic factors are undoubtedly mainly responsible for the age dependent functional changes. The process of epileptogenesis responsible for the cortical focus is also accompanied by changes in the expression of Na+ channels, GABAB, iono- and metabotropic glutamate receptors and morphometric properties of pyramidal cells. It still remains to be established whether these changes are genetically programmed or they are the consequence of the hundreds SWDs per day.

Several types of EEG signal analysis demonstrate that SWDs in the genetic animal models do not arise suddenly. This has recently been indicated in GAERS by simultaneous electroencephalography-near-infrared spectroscopy (EEG-NIRS) study of the whole cortex [110]. Traditional spectral analysis of preictal EEG epochs in WAG/Rij rats displayed high variability of the immediate seizure-precursor activity. More detailed time-frequency analysis showed that SWDs are preceded by the simultaneous presence of delta and theta activity in cortex and thalamus. EEG coherence study revealed specific frequency associations between the epileptic cortical focus and the closest areas; this “local neuronal circuit” is primarily involved in the initiation of SWDs. The other cortico-cortical, cortico-thalamic and intrathalamic neuronal networks showed frequency-specific changes in coherence, suggesting that each part of thalamo-cortical circuitry operates in an own oscillatory mode during transition from preSWDs to SWDs. Granger causality and non-linear association analyses indicated that SWDs are accompanied by changes in directional coupling between cortex and thalamus. SWDs may terminate when the influence of cortex to thalamus fades away.
REFERENCES


Chapter 3

Electrical Stimulation of the Epileptic Focus in Absence Epileptic WAG/Rij Rats: Assessment of Local and Network Excitability

Published as
Abstract

Objective The study aims to investigate whether there is a higher excitability in the deep cortical layers of the peri-oral region of the somatosensory cortex as compared to other cortical regions in absence epileptic WAG/Rij rats and whether this is unique for this type of epileptic rats, as would be predicted by the cortical focus theory of absence epilepsy.

Methods Excitability of cortical structures was assessed in a double pulse paradigm (inter-pulse interval 400ms, 400µs pulse duration, varying stimulation intensities (20–100 µA)). Electrical stimulation was applied to the subgranular layers of the somatosensory and motor cortex of freely moving WAG/Rij and control Wistar rats. Electrical evoked potentials (EEPs) and afterdischarges (ADs) were recorded during wakefulness, drowsiness and non-REM sleep.

Results WAG/Rij rats, stimulated in the somatosensory cortex, showed higher amplitudes for the N1 and N3 components of the EEPs as compared to WAG/Rij rats stimulated in the motor cortex. This effect was present in all states of alertness and at all tested intensities. In addition, this effect was not (N1) or to much less extent (N3) present in nonepileptic control rats. Stimulation-induced 8 Hz ADs were predominantly found in WAG/Rij rats. ADs were longer after stimulation in the somatosensory than in the motor cortex and preferentially occurred during drowsiness.

Conclusion There is a heightened excitability in the deep layer neurons of the perioral region of somatosensory cortex, which is unique for WAG/Rij rats. Moreover, the presence of 8 Hz ADs might point toward additional changes in the cortico-thalamo-cortical network. Drowsiness is an excellent state for 8 Hz ADs, mimicking spike and wave discharges (SWDs). The results are in good agreement with the cortical focus theory of absence epilepsy.

Keywords somatosensory cortex, electrical stimulation; electrical evoked potentials; WAG/Rij rats, afterdischarges; sleep-wake and drowsiness

Abbreviations ADs: afterdischarges; EEPs: electrical evoked potentials; RTN: reticular thalamic nucleus; SWDs: spike wave discharges; WAG/Rij rats: wistar albino glaxo from Rijswijk; GAERS: genetic absence epileptic rats from Strasbourg; non-REM sleep: non-rapid eye movement sleep; PIR system: passive infrared system.

Introduction

In the past 60 years different theories on the origin of spike and wave discharges (SWDs), the electroencephalographic hallmark of absence epilepsy, have been proposed. These include the centrencephalic theory, proposing a subcortical generator in the intralaminar thalamic nuclei as a continuation of the brainstem reticular activation system (Penfield and Jasper, 1954); the thalamic clock theory suggesting a seizure generator in the reticular thalamic nucleus (RTN), which imposes its rhythm to the thalamo-cortical relay cells (Buzsáki, 1991); the cortical theory which emphasize the role of the cortex in the generation of SWDs as a result of cortical abnormalities (Lüders et al., 1984; Niedermeyer, 1972) or as a secondarily generalized discharge arising near a lesion in the frontal cortex (Bancaud, 1969). Currently the most widely accepted view on the origin of SWDs is that sleep spindles, which are the result of increased activity of GABAergic RTN cells that hyperpolarize thalamocortical cells and set them into a burst firing mode (Steriade, 2003), are modified by a
globally, hyperexcitable cortex into pathological SWDs (cortico-reticular theory) (Gloor, 1968; Kostopoulos, 2000; Meeren et al., 2005).

Recent data in two genetic animal models of absence epilepsy (WAG/Rij (Wistar Albino Glaxo from Rijswijk) and GAERS (Genetic Absence Epileptic Rats from Strasbourg)), however, question these ideas: signal analytical techniques, applied on ECoG signals containing SWDs obtained from a cortical grid in combination with thalamic recordings, demonstrated that the start of SWDs can be observed in the perioral region of the somatosensory cortex and that only subsequently the rest of the cortex and thalamus get involved (Meeren et al., 2002; van Luijtelaar et al., 2011). In addition, Polack et al. (2007, 2009) described in GAERS that cells in cortical layer V and VI of the somatosensory cortex are depolarized interictally and show an increased firing rate already before SWDs can be seen in local field potentials. These results fit excellently in the cortical focus theory on absence epilepsy stating that the perioral region of the somatosensory cortex contains a “hot spot” where SWDs are generated (Meeren et al., 2002; van Luijtelaar and Sitnikova, 2006). This theory implies that the excitability in the cortical focus should be higher as compared to other cortical regions. On top, this should be unique for absence epileptic rats. This was tested in the current experiment in vivo. Freely moving rats received electrical double pulse stimulation in the either the focal zone or the motor cortex, outside but adjacent to the proposed epileptic focus. Resulting electrical evoked potentials (EEPs) were recorded locally at the somatosensory and motor cortex. Local excitability in the deep cortical layers was determined by measuring the amplitude of the early components of these EEPs. The slower components of the evoked potentials might reflect cortical network or even subcortical network activity.

Since the amplitude of auditory and visual evoked potentials is known to change with vigilance states (Coenen, 1995; Meeren et al., 1998) and since it is not known whether this is also the case for EEPs, measurements were done in three different states of vigilance. This allowed us to investigate whether excitability may change during the natural vigilance states and to control for this putative confounding factor on EEP amplitude.

Electrical stimulation of the sensorimotor cortex may be followed by different types of afterdischarges (ADs); their type and nature depends on stimulation intensity (Mares and Kubova, 2006). The amount and duration of electrically-induced ADs is used as an index for cortical and subcortical network excitability and recruitment.

In accordance with the cortical focus theory it is expected that the amplitude of the early components of EEPs, indicating excitability, is higher in WAG/Rij rats stimulated in the somatosensory cortex as compared to WAG/ Rij rats stimulated in the motor cortex. In
addition, the probability of occurrence and duration of ADs will be longer when stimulated in the somatosensory cortex versus motor cortex and will depend on the vigilance state. All measurements were done in WAG/Rij rats and non-epileptic control Wistar rats in order to test whether these effects are unique for rats with absence seizures.

EXPERIMENTAL PROCEDURES

Animals

Subjects, male WAG/Rij rats and Wistar rats, age 12–14 months, mean body weight of 350 g (range 324–380 g) were born and raised in the laboratory of Biological Psychology, Donders Centre for Cognition at Radboud University Nijmegen, the Netherlands. Housing conditions were in accordance with standard laboratory conditions (two animals per cage including cage enrichment (Enviro-dri)). Rats were kept at 12:12 light/dark cycle (light off phase between 8.00 and 20.00 h) and had free access to food and water. After surgery rats were housed individually. The experiment was approved by the Ethical Committee on Animal Experimentation of Radboud University Nijmegen (RU-DEC). Efforts were done to minimize the number of used animals and to make the discomfort for the animals as minimal as possible.

Stereotactic surgery

Stereotactical surgery was performed under isoflurane (Pharmachemie BV, Haarlem, the Netherlands) anesthesia: WAG/Rij and Wistar rats were divided in two groups. Group 1 (eight WAG/Rij and eight Wistar rats): two stimulation electrode wires (anode and cathode) were implanted in the deep layers of the somatosensory cortex, right hemisphere (A/P: -0 mm, M/L: -5 mm, H: -4.5mm). The tip distance of anode and cathode was about 1 mm in order to achieve local stimulation of the cortical focus. Two active EEG recording electrodes were additionally implanted in these rats: one of these recording electrodes was placed near the stimulation site in the deep layers of the somatosensory cortex (tip distance between recording and the nearest stimulation electrode was 1 mm, all three wires of the tripolar electrode set (Plastic One, Roanoke, VA, USA) were oriented in A/P direction). The second recording electrode was epidurally implanted on the remote motor cortex of the right hemisphere (A/P: +2 mm, M/L: -2 mm). A reference and a ground electrode were implanted on top of the cerebellum.

Group 2 (nine WAG/Rij and eight Wistars): two stimulation electrode wires (anode and cathode) and one EEG recording electrode were implanted in the deep layers of the motor cortex, right hemisphere (A/P: +2 mm, M/L: -2 mm, H: -3 mm). Tip distance between the recording and the nearest stimulation electrodes was again 1 mm; the orientation (A/P) of the
three depth electrodes was the same as in animals from group 1. This electrode configuration allowed stimulation and recording of the control area adjacent to the cortical focus. A second EEG recording electrode was epidurally implanted on the remote somatosensory cortex (A/P: -0 mm, M/L: -5 mm). Again a reference and a ground electrode were implanted on top of the cerebellum. All coordinates were determined according to the stereotactic atlas of Paxinos and Watson (1998). Electrode assemblies were fixed to the skull with the aid of dental cement. The rats were preoperative injected with Atropine (Pharmachemie BV, Haarlem, the Netherlands) and Rimadyl (Pfizer Animal Health B.V., Capelle a/d Ijssel, the Netherlands), and again with Rimadyl® postoperatively (24 and 48 hours after surgery). Rats were allowed to recover for 2 weeks.

**EEG recording and stimulation**

Rats were placed individually in a 20_35_25 cm³ Plexiglas registration box and connected to the recording leads for EEG recording and electrical brain stimulation. The leads were attached to a swivel-contact, which allowed registration and stimulation in the freely moving animals. The EEG signals were amplified with a physiological amplifier (TD 90087, Radboud University Nijmegen, Electronic Research Group), filtered by a band pass filter with cut-off points at 1(HP) and 100(LP) and a 50 Hz Notch filter, and digitalized with a constant sample rate of 256 Hz by WINDAQrecording-system (DATAQ-Instruments,Inc., Akron, OH, USA). The movements of the rat were registered by a Passive Infrared Registration system (PIR, RK2000DPC LuNAR PR Ceiling Mount, Rokonet RISCO Group S.A., Drogenbos, Belgium). Electrical stimulation was provided with the aid of a programmable Stimulus Generator (TD 10075 Electronic Research Group, Radboud University Nijmegen), and an Isolated Constant Current unit.

All rats received electrical double pulse stimulation with a pulse-duration of 0.4 ms, and an interpulse interval of 400 ms. Stimulation in WAG/Rij rats was given during wakefulness, drowsiness and non-REM sleep by a trained EEG analyst. Double pulses of five different intensities (20, 40, 60, 80,100 µA) were given per state of alertness resulting in a total of 15 stimulation conditions. The order of the stimulation conditions was randomized. WAG/Rij rats received a mean of 30 (range 10–76) subsequent double-pulse pairs during each stimulation condition with a variable inter pair interval of at least ten seconds.

All responses to stimuli of the same stimulation condition (stimuli of the same intensity and delivered during the same state of alertness) were offline averaged resulting in 15 EEPs per rat. Time-points of stimulation were retained by time point markers which were conveyed by the stimulation unit to the WINDAQrecording-system and displayed in the form of block pulses in an auxiliary channel in the data acquisition system. However, given that the signal
of the time point markers was, in contrast to the EEG, unfiltered, a time delay of about 4 ms was introduced in the EEG signal (this was empirically verified). This implies that the recorded latencies of the resulting EEPs are delayed by 4 ms. Furthermore, for EEPs typical stimulation artifact is not visible in the recordings due to the filtering- and sampling properties.

Wistar rats, which were used to determine whether a possible difference in the excitability between somatosensory and motor cortex is a unique property of absence epileptic rats or a general phenomenon for sensory and motor cortices, received only double pulse stimulation of 60 μA during wakefulness.

The state of alertness was determined during recording, by observing the animal’s behavior and EEG. The criteria for wakefulness were a moving animal visualized in the recordings by an active PIR and a high frequency, low amplitude EEG. For non-REM sleep the animal was immobile with a flat PIR and a low frequency, high amplitude EEG. Drowsiness was scored if the animal was motionless with a flat PIR with only minor movements and an intermediate EEG with respect to amplitude and presence of slow activity for at least 10 s before stimulation.

After termination of the experiment, all rats were perfused for histological verification of the electrode locations. Brains were fixed in a 30% sucrose solution, 0.1 ml TBS, cut in 40 μm coronal slices with a microtome, and stained with Cresyl Violet.

Data analysis

BrainVision analyzer (Brain Products GmbH, Gilching, Germany) was used for the offline averaging of EEPs. Single trial responses were averaged per intensity and vigilance state resulting in 15 EEPs per rat. A mean of 30 responses to stimulation (range 10–76) were averaged to obtain one EEP. The main components of the EEPs were identified and categorized by their latencies. The amplitudes of these components were statistically analyzed.

A repeated measures ANOVA with amplitude as dependent variable, stimulation site (somatosensory and motor cortex) as between subjects factor and state of vigilance (wakefulness, drowsiness and sleep), intensity (20–100 μA) and stimulus (stimulus 1, stimulus 2) as within subjects factors was used for the EEP data of WAG/Rij rats.

EEP data of the Wistar rats, which were, due to their control group status only exposed to one intensity during one state of alertness, were analyzed by means of a repeated measures ANOVA with amplitude as dependent variable, stimulation-site (somatosensory and motor cortex) as between and stimulus (1, 2) as within subjects factor.
ADs were subject to analysis if they fulfilled the following criteria: oscillations which occurred immediately after stimulation (within 5 ms after the first pulse), have a spike amplitude of at least twice the background EEG and a duration of at least 1 s.

Frequency spectra of ADs as recorded next to the stimulation site in either the somatosensory cortex or motor cortex were generated for WAG/Rij and Wistar rats. AD spectra of WAG/Rij rats were analyzed by means of a repeated measures ANOVA with amplitude (power in a given frequency band) as dependent variable, stimulation site (somatosensory and motor cortex) as between subjects factor and frequency band (3–5, 7–9, 15–17, 23–25 Hz) as within subjects factor. Secondly, the duration of WAG/Rij ADs were analyzed by a 3-way ANOVA with stimulation site, intensity as well as state of alertness as between subjects factors.

Furthermore, the probability of AD occurrence was calculated for ADs induced with a stimulation intensity of 60 µA for both WAG/Rij rats and Wistar rats. Probability of ADs in WAG/Rij rats were analyzed with repeated measures ANOVA with stimulation site as between subjects factor and state of vigilance as within subjects factor.

Lastly, the correlation between N1 amplitude and AD probability was calculated for all rats and for WAG/Rij and Wistar rats separately, in order to investigate the relationship between the indicators of local excitability (N1 amplitude) and network activity (AD probability).

EEG parts with movement artifacts were not included in the analysis. In addition only animals with verified electrode positions in the deep layers (IV–VI) of the somatosensory and motor cortex were included in the statistical analyses.

RESULTS

Electrical evoked potentials in WAG/Rij rats

Stimulation-induced evoked potentials in WAG/Rij rats were mainly visible in the EEG-channel recorded in the deep layers (IV–VI) of either somatosensory or motor cortex, next to the stimulation site. These EEPs consisted of various components: N1 (latency 15 ms), P1 (latency 23 ms), N2 (latency 27 ms), P2 (latency 31 ms) and N3 (40ms). An exemplary evoked potential as measured in the somatosensory and motor cortex is shown in Fig. 1 (lower panel).

The ANOVA of the N1, the most direct measurement of local excitability, revealed a significant main effect for stimulation site ($F(1,15)=5.284; P<0.05$): its amplitude was higher in rats stimulated in the deep somatosensory cortex than in rats stimulated in the deep motor cortex (see Fig. 1 upper panel).

In addition, the N1 also showed a main effect of state ($F(1,16)=5.294; P<0.05$) and a main effect of intensity (N1: $F(1,24)=7.303; P<0.01$). Post hoc comparisons demonstrated lower
amplitudes during wakefulness as compared to drowsiness and sleep (P's<0.05) and increasing amplitudes with increasing stimulation intensities (see Fig. 2). The main effect of stimulus was not significant (F(1,15) =0.469; P>0.05): the amplitude of N1 was equally high for the first and second pulse (see Fig. 1 lower panel).

![Graphs showing electrical evoked potentials (EEPs) in WAG/Rij and Wistar rats.](image)

**Fig. 1.** Electrical evoked potentials (EEPs) in WAG/Rij and Wistar rats. Lower panel: Exemplary EEPs induced by stimulation in either the deep layers of the somatosensory cortex (left panel) or the deep layers of the motor cortex (right panel) of a WAG/Rij rat. Induction stimuli had an intensity of 40 µA and were applied during non-REM sleep. ECoG recordings were made from electrodes near (1 mm) the stimulation site. Upper panel: Amplitude (Mean and s.e.m.) of components N1 (left) and N3 (right) for evoked potentials induced in the somatosensory cortex or motor cortex (averaged across states of alertness) of either absence epileptic WAG/Rij rats or non-epileptic Wistar rats. Note that Wistar rats were only stimulated with an intensity of 60 µA while WAG/Rij data were averaged across the whole intensity (20–100 µA) range.

Neither the stimulation-siteXstate nor the stimulationsiteXintensity interaction turned out to be significant (see Fig. 2) demonstrating that the difference in N1 amplitude between
somatosensory and motor cortex stimulation was equally strong at all states of alertness and all tested intensities.

The slower component N3 also displayed a significant main effect for stimulation site ($F(1,15) = 7.538; P<0.05$). Again, the amplitudes were higher in the somatosensory than in the motor cortex (see Fig. 1 upper panel). Also, the main effect of state ($F(1,16) = 5.703; P<0.05$) and intensity ($F(1,23) = 12.197; P<0.01$) were significant. Post hoc comparisons demonstrated lower amplitudes during wakefulness as compared to drowsiness and sleep ($P<0.05$) and increasing amplitudes with increasing stimulation intensities (see Fig. 2).

![Graphs showing amplitude of components N1 and N3 for somatosensory and motor cortex stimulation during different states of alertness and stimulation intensities.](image)

**Fig. 2.** Amplitude (Mean and s.e.m.) of components N1 and N3 for somatosensory as compared to motor cortex induced EEPs of WAG/Rij rats during different states of alertness (left) and for different stimulation intensities (right).
The main effect of stimulus was not significant ($F(1,15)=0.122; P>0.05$): the amplitudes of N3 were equally high for the first and second pulse (see Fig. 1 lower panel). The N3 showed a significant stimulationsiteXstate interaction ($F=7.271; P<0.05$). Post hoc tests demonstrated that stimulation of the somatosensory cortex always (during all three states of alertness) resulted in higher amplitudes compared to stimulation of the motor cortex (all $P_s<0.05$), but that the difference between brain structures was stronger during drowsiness and sleep as compared to active wakefulness (see Fig. 2).

The N3 displayed a significant stimulation-siteXintensity interaction ($F=8.808; P<0.01$) as well. Post hoc tests revealed that stimulation of the somatosensory cortex resulted into higher amplitudes compared to stimulation of the motor cortex in all tested intensities ($P_s=0.05$) except for the intensity 20 $\mu$A. Furthermore, the difference between brain structures became larger with increasing stimulation intensities (see Fig. 2).

The remaining components P1, P2 and N2 neither showed a main effect for stimulation-site nor a main effect for state, nor a main effect for stimulus ($P_s>0.05$). N2 showed a significant main effect for intensity ($F(1,18)=4.826; P<0.05$); its amplitude increased with increasing stimulation intensities.

**Electrical evoked potentials in Wistar rats**

EEPs in Wistar rats displayed similar waveforms with similar latencies as were found in WAG/Rij rats (see caption Electrical evoked potentials in WAG/Rij rats).

In contrast to WAG/Rij rats, the ANOVA of the N1 did not reveal a significant main effect of stimulation-site ($F(1,14)=0.511; P=0.487$). Amplitudes of the N1 did not differ for Wistar rats stimulated in the somatosensory as compared to Wistar rats stimulated in the motor cortex (Fig. 1 upper panel). The main effect of stimulus and the stimulation-siteXstimulus interaction for component N1 turned out to be not significant, too.

A significant main effect of stimulation-site was found ($F(1,14)=15.615; P<0.01$) for N3, as was the case in WAG/Rij rats. N3 showed a significantly higher amplitude for the somatosensory as compared to the motor cortex, but this effect was less pronounced than in the WAG/Rij rats: a difference of 70% between somatosensory and motor cortex was found for Wistar rats as compared to a difference of 90% for WAG/Rij rats (comparison controlled/corrected for intensity of stimulation) (Fig. 1 upper panel).

No other main or interaction effect was found for N3 or any other EEP component in Wistar rats.
ADs in WAG/Rij rats
ADs were noticed in the EEG of WAG/Rij rats after the presentation of the double pulse stimuli. In contrast to the EEPs, ADs were always visible in both the recording channel close to the stimulation site and the more remote recording site (i.e. the motor cortex for rats stimulated in the somatosensory cortex and the somatosensory cortex for rats stimulated in the motor cortex). Between 33% and 87% of WAG/Rij rats displayed a mean of 14 (range 1–68) ADs, depending on the stimulation site and vigilance state. More details can be found in Table 1.

Table 1. Number of rats showing stimulation-induced afterdischarges during three different states of alertness

<table>
<thead>
<tr>
<th>Group</th>
<th>Wakefulness</th>
<th>Drowsiness</th>
<th>Non-REM sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAG/Rij stimulated in somatosensory cortex</td>
<td>six out of eight =&gt; 75%</td>
<td>seven out of eight =&gt; 87%</td>
<td>six out of eight =&gt; 76%</td>
</tr>
<tr>
<td>WAG/Rij stimulated in motor cortex</td>
<td>five out of nine =&gt; 55%</td>
<td>seven out of nine =&gt; 77%</td>
<td>three out of nine =&gt; 33%</td>
</tr>
<tr>
<td>Wistars stimulated in somatosensory cortex</td>
<td>three out of eight =&gt; 37.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wistars stimulated in motor cortex</td>
<td>one out of eight =&gt; 12.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The morphological appearance of ADs in WAG/Rij rats was spike–wave-like, rather similar to spontaneous SWDs. Frequency spectra generated via Fast Fourier Transformation showed for both ADs, induced by stimulation in the somatosensory and motor cortex, a dominant frequency of 7–9 Hz as is the case for spontaneous SWDs. In addition to the major peak at 8 Hz, smaller peaks were sometimes present at the frequency of the first (15–17 Hz) and second harmonic (23–25 Hz).

The ANOVA on the amplitude of AD spectra revealed a significant main effect of stimulation site ($F(1,11)=8.826; P<0.05$): spectra of ADs induced by stimulation of the somatosensory cortex had higher amplitudes as compared to spectra of ADs induced by stimulation of the motor cortex (Fig. 3 left panel). In addition, a main effect of frequency band ($F(2,24)=37.01; P<0.01$) was found: the 7–9 Hz band showed higher amplitudes compared to all other frequency bands. Lastly, an interaction between stimulation site and frequency band ($F(1,24)=14.66; P<0.01$) was found. Post hoc tests showed higher amplitude values for the 7–9 Hz band for ADs induced by stimulation in the somatosensory cortex as compared to ADs induced by stimulation in the motor cortex ($P<0.01$), but no significant area differences for the other frequency bands (Fig. 3).
In addition, differences in the duration of the ADs were noticed. The ANOVA revealed a significant main effect of stimulation site ($F(1,560)=17.47; P<.001$): ADs lasted longer when the induction stimulus was given in the somatosensory compared to stimulation in the motor cortex.

Duration of the ADs also depended on the intensity of stimulation ($F(2,560)=50.43, P<.001$), intense stimuli produced longer ADs, and were also dependent on the state of the animals ($F(2,560)=1239.6, P<.0001$). Post hoc tests showed that the duration was longer during the drowsy state compared to wakefulness and sleep.
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The stimulation-siteXstate interaction (Fig. 4, left panel) was not significant indicating that the difference in duration between somatosensory and motor cortex induced ADs were present irrespective of the state of alertness. Differences were also seen for the probability of AD induction: the chance to induce an AD was dependent on the vigilance state ($F(1,18)=10.66; P<0.01$). The probability of an AD was higher during drowsiness compared to wakefulness and sleep (Fig. 4, right panel). No significant effect was seen for stimulation site and stimulation-siteXvigilance state.

![Fig. 4. Duration (mean and s.e.m.) (left) and mean probability (right) of Afterdischarges (ADs) induced by stimulation in the somatosensory and the motor cortex during three behavioral states (wake, drowsiness, non-REM sleep). Depending on the vigilance state, data are based on ADs of 33% to 87% of WAG/Rij rats that display a mean of 14 (range 1 to 68) ADs.](image)

**ADs in Wistar rats**

Only sporadic and short lasting ADs were seen in four of the 16 Wistar rats (Table 1). A representative AD, and a frequency spectrum of such an AD as seen in a Wistar rat, is displayed in Fig. 3, right panel. It illustrates that ADs of Wistar rats are more irregular compared to those of WAG/Rij rats. Likewise, the spectrum of a Wistar AD has no highamplitude peak at 8 Hz as is the case for WAG/Rij rats but present double peaks at 7 and 9 Hz, with a medium to low amplitude. No further statistical analyses were applied to them considering the small number of Wistar rats displaying ADs.

**Relation between local excitability and network excitability**

A correlation analysis between N1 amplitude and AD probability for a unified group of WAG/Rij and Wistar rats revealed a non significant result ($P=0.577, r=0.098$). This also held
DISCUSSION
The study aimed to investigate whether there is higher cortical excitability in the deep somatosensory in comparison to the deep motor cortex in WAG/Rij rats, and not in nonepileptic control rats, as would be in line with the cortical focus theory of absence epilepsy (Meeren et al., 2002; van Luijtelaar and Sitnikova, 2006). Electrical stimulation was used to assess local excitability, the amplitude of the early component(s) of the EEP was used as such. The slower components of the EEPs might reflect cortical network or subcortical network activity, while properties of ADs are assumed to predominantly reflect rhythmic cortical- subcortical network oscillations (see “Similarities between ADs and SWDs” for argumentation).

Excitability as assessed by electrical evoked potentials
The major outcome of the present study was the larger amplitude of the N1 in the electrical stimulation elicited evoked response in the deep layers of the somatosensory vs. the same response in the motor cortex of WAG/Rij rats.

Since the latencies of the N1 but also the other components of the EEPs, as elicited and measured in both deep layers, were rather similar they can be directly compared and interpreted. Given this, the outcome clearly demonstrates a higher excitability of the epileptic zone in the somatosensory cortex. The non-significant correlation between N1 amplitude and AD probability further demonstrates and ensures that the higher excitability in WAG/Rij somatosensory cortex is a local property. Since no difference in N1 amplitude was found in nonepileptic control rats stimulated with 60 µA, it is concluded that that this heightened excitability represents a characteristic of an epileptic focus rather than a normal difference in excitability between somatosensory and motor cortices of healthy individuals. Whether Wistar rats might show a difference in N1 EEP-amplitude between somatosensory and motor cortex when stimulated with a higher intensity of, for example, 100 µA is not clear from this experiment. This is however unlikely since differences in N1 amplitude between somatosensory and motor cortex were equally strong for all tested intensities in WAG/Rij rats. In addition such a result would not change our conclusion since a difference in N1 amplitude between somatosensory and motor cortex was found also for WAG/Rij rats stimulated at 60 µA but not for Wistar rats.

Measurements of EP amplitude were done during three states of alertness in order to investigate whether the epileptic excitability may change during the natural vigilance states and to control for by vigilance induced possible confounding factor on EEP amplitude (van...
Luijtelaar et al., 1998; Meeren et al., 1998; Loewy et al., 2000; Coenen, 1995). The degree of local hyperexcitability, as expressed in the amplitude of N1, was independent of state, as can be concluded from the fact that the difference between somatosensory and motor cortex was equally strong during all states of vigilance. Therefore it seems that the high amplitude of the N1 reflects increased cortical excitability given the state independency of the degree of hyperexcitability of the somatosensory cortex.

The excitability of the sensory-motor cortex was earlier studied in WAG/Rij rats (Tolmacheva et al., 2004; Mares and Tolmacheva, 2007). These authors compared presymptomatic and symptomatic WAG/Rij rats with age matched nonepileptic control rats but from their studies no compelling evidence was obtained for an increased cortical excitability considering that both ACI (nonepileptic controls) and WAG/Rij rats showed reduced thresholds for various types of electrical stimulation induced ADs and seizures in comparison to Wistar controls. Various differences including the electrode location and stimulation parameters explain the difference between the studies. Other results in vitro and in vivo are in good agreement with our current findings. Those studies hint to the possible underlying causes of the increased hyperexcitability found for the somatosensory cortex of WAG/Rij rats including a reduction in hyperpolarization-activated cation current (Ih) (Strauss et al., 2004; Kole et al., 2007), an upregulation of sodium channels (Klein et al., 2004; Blumenfeld et al., 2008), increased NMDA receptor-mediated activity (Luhmann et al., 1995; D’Antuono et al., 2006; Pumain et al., 1992), a decrease in the efficacy of GABAA or GABABergic inhibition (Merlo et al., 2007; Inaba et al., 2009; van Luijtelaar and Sitnikova, 2006) and an increased expression of mGlu2/3 receptors (Ngomba et al., 2005). Therefore, it seems now well established from both in vivo and in vitro studies that there is indeed an increased excitability or decreased inhibition in the somatosensory cortex in genetic epileptic rats; however, not or less in other parts of the cortex.

A difference between somatosensory and motor cortex of WAG/Rij rats was also found for the amplitude of the slower component N3. While the N1 represents local properties (early components are primarily determined by stimulus characteristics (Coenen, 1995)), the slower N3 can be seen to reflect network properties, since later EP components can also be influenced by higher order, top down processes via cortico-thalamo-cortical network interactions (Coenen, 1995). The difference in N3 amplitude between somatosensory and motor cortex induced EEPs was present for both rat strains but this difference between somatosensory and motor cortex was much more pronounced in WAG/Rij rats than in the Wistar rats. This might either be the result of the increased excitability in WAG/Rij somatosensory cortex or might point toward additional differences in cortical or cortico-thalamo-cortical network properties and activity involved in shaping the morphology of EEPs.
between the two strains.

**Similarities between ADs and SWDs**

ADs in the WAG/Rij rat, induced by both, somatosensory and motor cortex stimulation, showed remarkable similarities to spontaneous SWDs: ADs had a spike wave like morphology with equal amplitudes throughout the whole oscillation. Furthermore, their frequency spectrum was rather similar with a major peak around 8 Hz, representing the dominant frequency of ADs and SWDs, and smaller peaks at the first and second harmonic (see Sitnikova et al., 2009 for the frequency spectrum of spontaneous SWDs). Moreover, in contrast to the evoked potentials, the ADs were clearly present in the remote recording channel. It seems that these 8 Hz ADs quickly generalize as is the case for SWDs.

Another striking similarity between both epileptic oscillations is their strong preference to occur during the drowsy state. AD duration and probability of occurrence during drowsiness was more than three times higher compared to the wake and non-REM sleep state. This was independent of the location where ADs were elicited, considering that there was no interaction between state and location. A similar strong preference for the drowsiness state is also well known for the occurrence of the spontaneous SWD (Drinkenburg et al., 1991). Neither the existence of this type of 8 Hz ADs, nor the large effects of vigilance on this type of ADs were described earlier.

Given these strong similarities between spontaneous SWD and stimulation-induced ADs it can be assumed that the ADs, recorded in this experiment, represent corticothalamo-cortical network activity. This might also be supported by the fact that SWD-like oscillations can be found after low frequent thalamic stimulation (Mirski et al., 1997) or by single pulse stimulation of the thalamus, which induces rhythmic ADs in adult rats (Mares et al., 1982). In addition, unpublished data from our group on the effects of stimulation of the somatosensory cortex combined with multiple cortical and thalamic local field potentials revealed that this type of ADs were simultaneously present in cortex and thalamus.

**Network activity as assessed by afterdischarges**

The ADs are supposed to reflect oscillations in a corticothalamo-cortical network (see paragraph Similarities between ADs and SWDs; Mirski et al., 1997; Mares et al., 1982). Some of the effects on ADs were specific for the region of stimulation, other effects were region independent. Therefore both local properties of the brain-structure and global properties of the cortico-thalamic network (loop) can be inferred from them.

Location independent effects: All ADs have a strong preference to occur during the drowsy state and spectra of all AD showed a dominant frequency of 8 Hz. Since this is also the case
for SWDs (see upper paragraph), it is concluded that 8 Hz is the optimal frequency for both types of epileptic oscillations in the cortico-thalamo-cortical system given the drowsy state. This preferred frequency is not likely to be driven by the stimulation frequency, considering that only two stimuli were presented with an interstimulus interval of 400 ms (2.5 Hz). Whether stimulation with a stimulus train of 8 Hz might further increase the probability of ADs during drowsiness needs to be investigated. The changes in firing pattern of the pyramidal neurons in the cortico-thalamo-cortical network from a tonic to a bursting firing mode, which are due to changes in the membrane potential of these cells (more hyperpolarization), might underlie the preferred occurrence of ADs during drowsiness.

The probability to induce ADs was also independent of the stimulation site. This was unexpected since stimulation within the hyperexcitable epileptic zone was expected to result in more ADs. Perhaps stimulation of the motor cortex quickly involves the epileptic zone since high coherence values between motor and somatosensory cortex during SWD suggest strong intrahemispheric coupling (Sitnikova and van Luijtelaar, 2006). Furthermore, a role in cortical subcortical interaction cannot be excluded. The lack of correlation in WAG/Rij rats between the amplitude of the N1, indicating local excitability, and AD probability, indicating oscillatory activity in the cortico-thalamo-cortical network, suggests that additional changes exist at a thalamic level that favor the occurrence of ADs in WAG/Rij rats.

Also the sparse presence of ADs in Wistar rats with the current chosen low intensities testifies for additional changes in the cortico-thalamo-cortical network of WAG/Rij rats. Whether these network changes responsible for the absence or presence of ADs are epilepsy related or related to another non-epileptic strain difference is yet unclear. In an earlier study, Tolmacheva et al. (2004) found large differences in the duration of slow 3 Hz ADs between WAG/Rij and non-epileptic control rats but no difference between symptomatic and presymptomatic WAG/Rij rats. During this period the epileptic phenotype, as expressed by the age dependent increase of SWDs, increased, while the duration of the 3 Hz ADs remained constant (Tolmacheva et al., 2004). This suggests that the duration of 3 Hz ADs are dependent on the strain but not related to the epileptic phenotype. It might well be that 3 (Mares and Kubova, 2006; Tolmacheva et al., 2004) and 8 Hz ADs, as found in the present study, are difficult to compare considering quite some methodological differences between the studies done in the Mares laboratory and in ours. It remains thus possible that the large probability of these newly discovered 8 Hz ADs in WAG/Rij rats can be explained by epilepsy related changes in the cortico-thalamocortical network, including the RTN. Changes in properties of GABA and glutamate and their modulators in the RTN have been described in the WAG/Rij rat (van de Bovenkamp-Janssen et al., 2006; Ngomba et al., 2005; Liu et al., 2007; van Rijn et al., 2010), while an upregulation in T-type Ca2+ channels has been
described in GAERS (Tsakiridou et al., 1995). The lateral thalamus, including the RTN, has an indispensable contribution in propagation and maintenance of SWD (Liu et al., 1992, 2007; Sohal et al., 2003; Meeren et al., 2009), and most likely also of 8 Hz ADs.

Location dependent effects: the 8 Hz ADs elicited at the somatosensory cortex lasted longer than those induced in the motor cortex. This difference might be seen as support for the differences in excitability between the two regions. Alternatively, the difference in AD duration might reflect properties of different sub-loops of the corticothalamo-cortical system.

Also spectral differences between somatosensory and motor cortex elicited ADs were found as indicated by a significant main effect of stimulation site for the amplitudes of AD spectra. The remarkably larger peak in the 7–9 Hz band of ADs elicited in the somatosensory cortex points toward the involvement of a large number of cortical neurons firing synchronously in a stable rhythm. The stability of the 8 Hz rhythm also helps to explain the longer duration of somatosensory cortex induced ADs as compared to motor cortex induced ADs. Under the assumption that cortex and thalamus fire synchronously during SWDs (or show increased coupling, Sitnikova et al., 2008), a seizure initiator (cortex) imposing a strong and stable rhythm to the rest of the system (thalamus) is less likely to be disrupted by phase desynchronization, and an abortion of the epileptic oscillation. Alternatively, the large 8 Hz spectral peak from the somatosensory ADs might reflect a more stable 8 Hz rhythm within the whole somatosensory loop as compared to the motor-loop of the cortico-thalamic system, the latter serving a less favorable condition for long ADs.

In sum, there is a focally heightened excitability in the deep layers of the perioral region of somatosensory cortex in WAG/Rij rats, the region from which the SWDs are emerging. The increased local excitability is expressed in the larger amplitude of the N1. These results are in agreement with the cortical focus theory for absence epilepsy.

The differential properties of the ADs suggest differences in cortico-thalamo-cortical circuits between epileptic and non-epileptic rats. The longer duration and higher probability for the 8 Hz ADs in the drowsy state and the well known high prevalence of SWDs during drowsiness demonstrates that drowsiness is rather favorable for the occurrence of epileptic cortico-thalamo-cortical oscillations and suggests that 8 Hz ADs and SWDs share common network properties. Their constituent cells might have the same neurophysiological properties, such as burst firing and membrane potential for their occurrence.

REFERENCES


Chapter 4

An algorithm for real-time detection of spike-wave discharges in rodents

Published as
Abstract

The automatic real-time detection of spike-wave discharges (SWDs), the electroencephalographic hallmark of absence seizures, would provide a complementary tool for rapid interference with electrical deep brain stimulation in both patients and animal models. This paper describes a real-time detection algorithm for SWDs based on continuous wavelet analyses in rodents. It has been implemented in a commercially available data acquisition system and its performance experimentally verified. ECoG recordings lasting 5–8 h from rats (n = 8) of the WAG/Rij strain were analyzed using the real-time SWD detection system. The results indicate that the algorithm is able to detect SWDs within 1 s with 100% sensitivity and with a precision of 96.6% for the number of SWDs. Similar results are achieved for 24-h ECoG recordings of two rats. The dependence of accuracy and speed of detection on program settings and attributes of ECoG are discussed. It is concluded that the wavelet based real-time detecting algorithm is well suited for automatic, real-time detection of SWDs in rodents.

Keywords Absence seizures; Detection; ECoG; Real time; WAG/Rij rats; Continuous wavelet

Introduction

Spike-wave-discharges (SWDs) are seen in the electroencephalogram of different forms of absence epilepsy (for review see Niedermeyer, 1993) and in some inbred rodent strains, such as rats of the WAG/Rij strain, in Fischer 344 rats, in selection lines such as GAERS (now fully inbred), and also, to a lesser extent, in some outbred lines such as the Wistar and Long-Evans lines, in mice with spontaneous mutations, or in transgenic or knockout mice (Depaulis and van Luijtelaar, 2006; Willoughby and Mackenzie, 1992; Shaw, 2004; Burgess, 2006; Noebels, 2006). A real-time spike-wave discharge (SWD) detection method could be used to trigger the presentation of external stimuli to study information processes during periods of reduced consciousness (Drinkenburg et al., 2003), or to trigger automatic electrical brain stimulation contingent upon the appearance of SWDs (a closed-loop brain computer interface). Recent reviews of different approaches for the off-line analyses of SWDs and of SWD prediction techniques suggested generic approaches to algorithm testing and verification (Mormann et al., 2007) but did not cover the subject real-time analyses (Mohseni et al., 2006). Here we present both a description and an experimental verification of a continuous wavelet based real-time detection algorithm for SWDs in the ECoG of genetic rodent models.

This algorithm is based on the continuous wavelet transform (CWT) and is used here for real-time analysis of ECoG recordings. 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 normal EEG contains non-stationary phasic events such as sleep spindles and K-complexes, as well as interictal and ictal activity in epileptic
patients. Wavelet analyses is, therefore, theoretically suited for characterization and identification of short lasting (phasic) nonstationary events, as has been demonstrated offline for sleep spindles and SWDs in ECoG data (Korovinskii and Hramov, 2003; Sitnikova et al., 2009). CWT-based analysis also exhibits high robustness to noise due to the fact that it is possible to focus on specific relevant frequencies. It is also possible to reduce the contribution of less relevant properties of the signal. This is helpful in case of signals with low signal-to-noise ratios, such as EEGs. Finally, CWT can be implemented in a computationally efficient manner. This is especially crucial for real-time analysis of several EEG’s at the same time.

Wavelets extracted from offline data have previously been used in a number of studies describing the time-frequency characteristics of SWDs and sleep spindles in humans and rats (Adeli et al., 2003; Gabova et al., 2004; Bosnaykova et al., 2006, 2007; Ubeyli et al., 2009; Sitnikova et al., 2009). These studies show that SWDs are not constant phasic events. Rather, SWDs reveal clear dynamics in the frequency domain for both species. Its frequency is highest at the beginning of SWDs and quickly drops to the commonly reported frequencies (3–4 Hz in humans and 7–8 Hz in rats), with periodical fluctuations during the second part of the discharge. The frequency remains stable during the middle portion of the discharge and drops towards its end. Clear regional differences in amplitude have also been reported. For instance, larger amplitudes for both spike and wave have been shown for frontal regions relative to the occipital cortex.

A number of techniques exploring the dynamics of ECoG spectral components for SWD detection have been proposed, applied and compared (Ubeyli et al., 2009; Sitnikova et al., 2009); for the results of the comparisons see van Hese et al. (2003, 2009). Although some were clearly superior to others, most methods showed sufficiently high sensitivity and precision. However, all of them lack the ability to process real-time data. While some reports have described real-time SWD detection (Raghunathan et al., 2009; Talathi et al., 2008), the proposed techniques have all shown relatively low performance.

Here we propose and evaluate a CWT method for real-time detection of SWDs in ECoG using rats of the WAG/Rij strain (van Luijtelaar and Coenen, 1986; van Luijtelaar and Sitnikova, 2006), and compare its performance against visual scoring.

2. Materials and methods

2.1. Animals

Eight male WAG/Rij rats between 8 and 12 months old were used as experimental subjects. They were bred in the laboratory of the department of Biological Psychology in the Donders Centre for Cognition at Radboud University Nijmegen, The Netherlands. Housing conditions
were in accordance with standard laboratory conditions including cage enrichment (Enviro Dry home cage®, Plexx, Elst, The Netherlands) with free access to food and water, individual housing after surgery and a 12:12 light/dark cycle (white light on at 7.00 a.m.). The experimental protocols were authorized by the Ethical Committee on Animal Experimentation of the Radboud University Nijmegen (RU-DEC).

2.2. Surgery
A tripolar ECoG recording electrode (Plastic One, Roanoke, VA, USA; MS 333/2A) was implanted stereotactically under isoflurane anesthesia. One active electrode wire was positioned epidurally on the frontal cortex of the right hemisphere (A/P: +2; M/L: −3.5) and a second epidural electrode was positioned on the occipital cortex (A/P: −6; M/L: −4). The third electrode wire (ground electrode) was placed on top of the cerebellum (all coordinates were determined according to the stereotactic atlas of Paxinos and Watson (1998)). The electrode assembly, see Fig. 1, was fixed to the skull with the aid of dental acrylic cement (Dental Union, Groningen, The Netherlands). Following surgery, rats were allowed to recover for 2 weeks.

![Electrode positions for ECoG recording on the skull and referring to positions in the rat brain atlas (Paxinos and Watson, 1998). Differential recordings were made between the frontal-cerebellar and parietal-cerebellar derivations.](image)

**Fig. 1.** Elect rode positions for ECoG recording on the skull and referring to positions in the rat brain atlas (Paxinos and Watson, 1998). Differential recordings were made between the frontal-cerebellar and parietal-cerebellar derivations.

2.3. ECoG recording
Differential recordings were made. The ECoG of all eight rats was recorded continuously for 5 h during the light phase (5−15 h). In addition, two of the rats were subject to a continuous 24-h recording. Rats were connected to recording leads for ECoG recording attached to a
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swivel-contact, which allows registration of the ECoG in freely moving animals. Signals were digitally recorded by the means of WINDAQ-recording-system (DATAQ-Instruments, Akron, OH, USA) at a constant sample rate of 500 Hz. Before digitizing, signals were amplified and filtered by a band pass filter with cut-off points at 1 (HP) and 100 Hz (LP), along with a 50Hz Notch filter. The digitized signal was sent to the real-time SWD detection system (“online SWD detection system”—OSDS), which performed the wavelet convolution of the signal. Whenever a SWD was detected in the ECoG, a square-wave marker was sent to an additional channel of the WINDAQ system for the duration of the SWD. The decision of the OSDS-program was based on the instantaneous power of the EEG as extracted from the wavelet analysis. Whenever this instantaneous power reached a predefined threshold, a SWD was presumed to have occurred. The decision threshold was set for each rat individually. Individual decision thresholds for most adequate SWD detection were determined based on the offline analysis of ECoG traces of a given rat, which were recorded before (retrospective evaluation). This value varied between 0.6 and 1.0 for the different animals. Next, eight rats were prospectively evaluated: the optimal values, as determined in the retrospective part of the study, were now used in the 5 h study. Finally a 24-h prospective study was carried out for two animals. One animal was recorded at the same time during the real-time SWD detection part of the experiment.

2.4. OSDS algorithm

The automated real-time SWD detection algorithm is based on the CWT. A wavelet transform is a special form of time-frequency representation of an analogue signal which is obtained by convolving a signal (e.g. ECoG signal) $x(t)$ with a given wavelet basis function $\psi_s(t)$ (Daubechies, 1992; Koronovskii and Hramov, 2003)

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

where * denotes a complex conjugation. The wavelet basis function can be produced from the mother wavelet function:

$$\psi_{s,\tau}(t) = \frac{1}{\sqrt{s}}\psi_0 \left( \frac{t - \tau}{s} \right)$$

Here $s$ is a timescale (as specified as a given compression or dilation of a mother function), $\tau$ is the time shift of the wavelet transform and $\psi_0$ is a prototype ‘mother’ wavelet function. One possible mother function is the complex Morlet wavelet.

Recently it was found that this mother wavelet is much better suited for being incorporated in an algorithm that recognizes SWDs than other mother wavelet functions (Sitnikova et al.,
Therefore it was chosen as the ‘mother’ wavelet in the present SWD detection algorithm. The Morlet wavelet function is presented below:

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

(3)

Here, parameter $\omega_0$ determines the shape and width of the wavelet function. Therefore one may state that $\omega_0$ determines the functional relation between time scales $s$ of the wavelet transform and frequencies $f$ in the original signal (Koronovskii and Hramov, 2003): if $\omega_0 = 2\pi$, then $s$ corresponds to $s = 1/f$. This comparison is useful in the initial phase of the investigation of the signal as it allows making assumptions of which timescale range are most adequate. According to previous research (Sitnikova et al., 2009), $\omega_0 = 2\pi$ is the best choice for the recognition of SWD, as it provides the optimal timefrequency resolution of an ECoG signal; it facilitates the precise localization of oscillatory events in the time and frequency domains for complex signals containing multiple frequencies that vary over time, as is the case for ECoG signals.

High frequencies (30–80 Hz) appear in the ECoG during SWDs (Sitnikova et al., 2009). The wavelet power for this frequency range at each moment in time will show 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 OSDS program for SWD detection calculates the corresponding wavelet power for a given frequency range each time that a new sample is acquired, so every 2ms. More precisely, the OSDS performs the wavelet transform on a total of 15 scales proportional to 15 frequencies. The sum of the calculated wavelet power values for each frequency is the absolute wavelet power over the frequency domain (“power over domain”—POD). The 15 scales are equally distributed between 30 and 80 Hz. Processing of additional (more than 15 scales) time scales does not significantly affect the sensitivity and precision of the algorithm, but will result in an increase of calculation time.

A flowchart of the algorithm is presented in Fig. 2. Each calculated POD value is subsequently compared to the threshold value, which should be determined for each animal individually. A value that is 2.5–3.5 times greater than the mean power of normal (=nonepileptic, background) activity can be chosen as an initial threshold.
As soon as the POD exceeds this threshold, it is assumed that a SWD takes place; this is marked in an additional channel of the WINDAQ acquisition system. This channel consists of a digital–analog converter (DAC) output with two possible levels: high (output voltage is equal to +2.5 V) and low (output voltage is equal to −2.5 V). The output is set to “low” when the detection program starts. In case the POD exceeds the threshold value and the output is low, the latter is set to high. In case the D-A auxiliary channel is high and POD is below the threshold, the output is switched to low. This guarantees that the output of this auxiliary channel is always high during SWD and low otherwise.

Due to the appearance of short lasting periods with high frequencies in the ECoG during spiky phenomena, sharp sleep spindles, isolated spikes or polyspikes, the POD may indicate

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**Fig. 2.** Flow chart of online seizure detection algorithm.
short-term bursts that are not SWDs. The magnitude of these bursts might be big enough to reach the preset threshold value and a false detection takes place. Moreover, these short-term POD bursts seem to be relatively common. In order to prevent these false detections, the wavelet transform is appended with a smoothing procedure.

The OSDS program smoothes POD over a window of a certain width. The smoothing window width or size directly affects speed and precision of SWD detection. The shorter the window, the less time it takes for the POD average to reach the threshold, but the probability of false detection increases. On the other hand, wider smoothing windows yields a better precision, but it takes longer to detect a SWD. The size of the smoothing window was systematically varied and it was found that, given a 500 S/s sampling rate, a window size of 400–600 points works best (acceptable speed and highest sensitivity and precision), providing a reasonable compromise between speed of SWD detection and number of errors.

Fig. 3 shows the dependence of false detections, true detections and missed SWDs on window size and threshold value as established during a 5 h ECoG recording of a representative subject. It can be inferred from the maps in Fig. 3 that there are some areas (combinations of threshold values and smoothing window sizes) for which all SWDs have been detected while the number of false detections and missed events was close or equal to zero.

**Fig. 3.** (a) (Normalized) numbers of true detections of SWDs, (b) missed SWDs, (c) false detections of SWDs as functions of window size and threshold.
It is also easy to see that these areas correspond to threshold and window size values in the range indicated above. It should be noted that the maps provide all knowledge needed for the appropriate choice of the two parameters. However it is not necessary to compute the whole surface to find the best parameters as the area of feasible window sizes does not vary much from animal to animal. It is more practical to establish the number of false detections and missed events for the chosen threshold value and to use the median value of the range as a suitable window size.

The proposed algorithm for SWD detection was implemented in such away that it was possible to run it real-time; this was feasible due to the properties of the wavelet transform. As mentioned above, the wavelet transform allows one to observe the amount of energy belonging to a certain frequency band at a certain time. The transform is defined by (1). At first glance this function seems impractical for a fast real time implementation, as it requires the integration over an infinite time domain. However, a wavelet function is limited in time by the period in which most of energy is amassed. In other words, the value of a wavelet function outside this interval is close to zero, while inside it is sufficiently greater than that. Therefore, integration over the duration of the wavelet function can be substituted by integration over an infinite time domain without loss of precision (Koronovskii and Hramov, 2003).

In the case of wavelet analyses of the EEG, one deals with signal samples instead of a continuous function. Integration in (1) should be replaced with summation over a set of samples multiplied by a sampling increment in order to perform a wavelet transform of a time series:

\[ W(s, t_0) = \sum_{i=x_{\text{start}}}^{x_{\text{end}}} x_i h \psi_s t_0 \]  

Reasonable limits \([x_{\text{start}}, x_{\text{end}}]\) are \(-4 \text{ s/h and } +4 \text{ s/h, respectively, where } s \text{ is the timescale, } h \text{ is the sampling increment, } -\text{ and } +\text{ signs denote the fact that, in order to perform the transform, one needs to take into account a number of samples taken prior to the one under consideration (corresponding to the moment } t_0 \text{) along with a number of samples thereafter. The need for } +\text{ samples is a drawback of the proposed technique: the point currently under consideration is always a point collected several time steps ago. However in case of SWD detection, this delay in processing is sufficiently smaller than the characteristic length of events of interest. Considering the fact that frequencies of interest fall into 10–100 Hz range and a sampling rate of 500 s/s, one can estimate the number of points} \]
needed to be collected after the current one as 200, which takes 400 ms. As the characteristic length of the event to be detected is more than 1 s, this computational delay should not be considered a serious issue.

The algorithm speed is determined by the programming techniques used for data processing and threshold detection. A significant speed gain can be obtained by replacing function calls for computation of wavelet function values by array referencing. This was done by shifting the signal along the fixed wavelet function rather than shifting the wavelet function along the signal. Using an array to store wavelet function values and a FIFO (first in/first out) buffer to store a part of the ECoG signal yields a dramatic increase of processing speed.

The program can be applied to prerecorded data, but the described technique can also be used for real-time fast detection of SWDs.

2.5. Method evaluation
An optimal threshold value was chosen for each animal on the basis of preliminary recorded data. Then a 5 h recording was made with OSDS analyzing ECoG in real-time using the optimal threshold. Each ECoG file was visually inspected by a trained electrophysiologist.

Criteria for SWDs visual recognition (the amplitude of the spike should be twice the amplitude of the background ECoG, the presence of sharp spikes and slow waves, the minimal duration of a train of SWDs should be 1 s, the frequency of the SWDs should be between 11 and 7 Hz) are well known (van Luijtelaar and Coenen, 1986; Midzianovskaya et al., 2001; van Hese et al., 2003). The outcome of this inspection in terms of the number of recognized SWDs was compared with the outcome provided by the OSDS system in order to determine the amount of correctly detected SWDs, falsely detected events and undetected SWDs. In order to investigate performance of the algorithm, its precision and sensitivity were calculated. False positive, true positive and false negative detection numbers were determined as the number of events wrongly identified as SWDs, events correctly identified as SWDs and missed SWDs (genuine SWDs not detected by the program).

The detection of a SWD by the OSDS was indicated by an upward deflection in the auxiliary channel. The only purpose of the algorithm was to determine the moment of onset of SWDs. Therefore, the duration of the various events (false and true positive and missed events) was not analysed. In other words, we have evaluated the number of leading pulse edges without taking the length of the detected events into consideration.

3. Results
The number of visual detected SWDs per hour was the same as previously reported, 5–20 h⁻¹, typical for ECoG recordings in the light phase of the 24 h light-dark cycle (e.g. van
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Luijtelaar and Coenen, 1988; Ngomba et al., 2005). The OSDS was able to detect SWDs with a mean sensitivity as high as 100% and a mean precision of 96.9% (range 94.3–100%) in our data set of eight different ECoG recordings (n = 8) of 5 h (see Table 1 for details).

Table 1 SWD detection rates of 8WAG/Rij rats recorded for 5 h.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Threshold</th>
<th>Window size, data points</th>
<th>Number of visual detections</th>
<th>Number of automated detections</th>
<th>Sensitivity, %</th>
<th>Precision, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6</td>
<td>600</td>
<td>101</td>
<td>101</td>
<td>100</td>
<td>97.1</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>500</td>
<td>29</td>
<td>29</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>500</td>
<td>43</td>
<td>43</td>
<td>100</td>
<td>95.6</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>600</td>
<td>66</td>
<td>66</td>
<td>100</td>
<td>98.5</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>500</td>
<td>44</td>
<td>44</td>
<td>100</td>
<td>95.7</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>500</td>
<td>66</td>
<td>66</td>
<td>100</td>
<td>94.3</td>
</tr>
<tr>
<td>7</td>
<td>0.85</td>
<td>500</td>
<td>115</td>
<td>115</td>
<td>100</td>
<td>97.5</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
<td>600</td>
<td>56</td>
<td>56</td>
<td>100</td>
<td>96.6</td>
</tr>
</tbody>
</table>

Mean +/- SD 65 +/- 29 65 +/- 2.1 +/- 29 2.1 +/- 1.3 0 100 +/- 0 96.9 +/- 1.8

Sensitivity = TP/(TP + FN)×100%; precision = TP/(TP + FP)×100%, where TP is the number of true positive detections, FP is the number of false positive detections, i.e. the number of events which are recognized as SWD by OSDS but considered as a different type of event by the expert, FN is the number of SWDs not recognized by OSDS (missed SWDs).

Fig. 4 illustrates examples of correctly detected and rejected events. Events marked as “A” are correctly identified SWDs. A significant increase of POD, above the values obtained during background activity, could be easily seen during these events. The event marked as “B” is a high amplitude noise-like oscillation that takes place in low frequency range; here, the POD does not change significantly. “C” corresponds to a false detection of an underdeveloped SWD: despite the fact that the POD increase was not as large as during a genuine SWD, the POD rose above the threshold, leading to a false alarm. D-marked pieces of the ECoG illustrate common recording artifacts. Phasic ECoG events or by the OSDS falsely detected SWDs were, upon visual inspection, identified as “intermediate state” or as spiky phenomena (Gottesmann, 1996).
Fig. 4. Illustrative examples of the ECoG (first trace) the through wavelet analyses of the power in the 30–80 Hz band (POD, second trace), and the result of the detection algorithm (OSDS, third trace), four types of phasic events and their detection and rejection by the OSDS system. (A) SWD, 10 correct detections, (B) SWD-like event, however too short (only two large amplitude spikes and waves) to be scored as SWD, correctly rejected, later sleep spindle, (C) intermediate state, falsely detected as SWD, (D) movement artefacts, correctly not recognized as SWD.

The average time needed for SWD detection was 1.0±0.55 (SD) seconds following the SWD onset (given a smoothing window size of 500 data points to obtain the average the POD, and a sample rate of 500 S/s).

In a second experiment the ECoG’s of two rats were analyzed with the OSDS for 24 h. The results are presented in Fig. 5; it illustrates thenumberof true and false detections, as well as thenumber of missed events. The data show that the rats had 300–400 SWDs during the 24h period, with a mean duration 6.6 s. The outcomes are in line with what was found earlier in WAG/Rij rats of the same age (van Luijtelaar and Coenen, 1986; van Luijtelaar and Sitnikova, 2006).
4. Discussion
The aim of the study was to describe and verify a continuous wavelet based real-time SWD detecting algorithm. It was found that this algorithm is able to detect SWDs with a high sensitivity and precision as far as the number of SWDs is concerned. Short lasting movement artifacts, as illustrated in Fig. 4, characterized by large amplitude sharp non-physiological deflections, were mostly rejected due to the smoothing window. This window was introduced in order to meet the condition that an SWD should last minimally 1 s. Longer lasting trains of artifactsmaybe falsely identified as SWDs, but were not found in the present data set. Some of the false detections were in fact sleep spindles belonging to the intermediate state. The intermediate state is characterized by 8Hz sharp large amplitude spindles occurring at the transition from non-REM sleep toREMsleep in rats. Its presence is somewhat more expressed (larger amplitude, sharper oscillations) in the ECoG of WAG/Rij rats (Gandolfo et al., 1990). Other false detected SWDs could be so called spiky phenomena: 8Hz oscillations mimicking SWD but not fulfilling the strict criteria of SWDs. They are shorter, have smoother peaks and less pronounced waveforms when compared to genuine SWDs. They have, also in contrast to SWDs, a waxing and waning pattern more reminiscent to that of the classic sleep spindle (Drinkenburg et al., 1993). It can be concluded that this algorithm, with its presently explored parameters and given its high sensitivity and specificity, can be used for further studies of spontaneous SWDs.

![Fig. 5. SWD (spike-wave discharge) detection rates for 24 h recording: red = true detections, green = false detections, blue = falsely undetected SWDs.](image)
It was also experimentally verified that a SWD can be detected approximately 1 s after the visual determined onset of the event (the first sharp spike, at least twice the background amplitude followed by series of waves and spikes with an interspike frequency between 7 and 11 Hz (common criteria). Therefore it seems that the algorithm is fast enough for rapid intervention during SWDs with either external stimuli or electrical brain stimulation.

The speed of detection as well as sensitivity and precision are dependent on certain characteristics such as the individual properties of the sharpness of the spikes in the trains of SWDs, the amplitude of the background EEG and parameter settings (i.e. window size) and the threshold value. Using a smaller smoothing window size and a lower threshold value one can reduce the detection time. However it should be noted that attempts to decrease detection time may lead to significant increase of false detections. Different speed-accuracy settings can be chosen, depending on whether speed (=fast detection) or accuracy is preferred within a given experimental setting. Attempts to attain the highest sensitivity and precision rates as possible clearly require the prior analysis of off-line data for a careful selection of optimal threshold values.

Up to this point, the experiment was performed with only one animal under study at a time; however, the algorithm is efficient enough to handle several signals simultaneously without a significant loss of performance considering that is takes only ca. 10% of time needed to collect single readout for instantaneous POD calculation. In the present experiment, the properties of the hardware used for data acquisition represented the biggest obstacle for increasing the number of channels under observation.

A previous SWD detection system was based on the absolute difference of two consecutive digitized samples, the steepness of the EEG (Westerhuis et al., 1996). On the basis of this steepness, the maximum steepness over a time period of 0.25 s was derived. If the average value over a time period of 0.25 s exceeded a certain threshold value for the duration of 1 s, a SWD was detected. This system has been evaluated by comparing its off-line detection of aberrant EEG phenomena to the consensus of two experts. The consensus detected in total 405 SWDs. The automatic system detected 392 phenomena correctly, 97%. Thirty-six incorrect detections were made (false positives), mainly consisting of longer than 1 s lasting movement artifacts.

It can be concluded that the present system is more sensitive and precise than the previous one. Its implementation as real-time system has benefits for closed loop deep brain stimulation systems. Finally, it is computationally more efficient than our previous system.
References


Abstract

Purpose: The site specific effects of two different types of electrical stimulation of the thalamus on electroencephalic epileptic activity as generated in the cortico-thalamo-cortical system were investigated in genetic epileptic WAG/Rij rats, a well characterized and validated absence model.

Methods: First, 12 male rats received low frequency (double-pulse pairs of 2.5 Hz, 150 μA intensity and 30 s inter-pair-interval) open-loop stimulation to either the Ventral-Postero-Medial (VPM) or the Anterior Thalamic Nucleus (ATN) for 8 h. Second, rats received high frequency (130 Hz, pulse train of 1 s) closed-loop stimulation applied to either VPM or ATN whenever an aspike-wave discharge (SWD) was automatically detected.

Results: Low frequency stimulation induced 8 Hz SWD-like afterdischarges (AD). AD were frequently seen in VPM but rarely in ATN stimulated rats. AD, recorded in cortex and thalamus, showed a strong temporal coherence (visually assessed) and opposite spike polarities. Properties of AD and spontaneous SWD were equally affected by the stimulation. Closed-loop high frequency stimulation disrupted spontaneous SWD with no difference between ATN and VPM stimulated rats. 89% of SWD could be disrupted leading to a decrease in average SWD duration from 9 to 1.5 s.

Conclusion: Low frequency stimulation induced AD, which strongly mimic SWD. Moreover, the effects were site-specific. High frequency thalamic stimulation disrupts ongoing SWD probable by interfering with the slow firing pattern of cortico-thalamo-cortical neurons seen during SWD cycle. The absence of stimulation site specificity for high frequency stimulation might be due to the fact that stimulation only started on average 1 s after SWD onset when SWD are already fully expressed in the bidirectional cortico-thalamo-cortical resonance system.

Keywords Absence epilepsy; Spike-wave discharges; Afterdischarges; Electrical stimulation; Anterior thalamus; Ventral-Postero-Medial thalamus; Cortico-thalamo-cortical system; WAG/Rij rats

Introduction

The generation of generalized spike and wave discharges (SWD), the electrophysiological hallmark of absence epilepsy, is known to rely on an intact cortico-thalamo-cortical network (Crunelli and Lerescue, 2002; Danober et al., 1998; Onat et al., 2012; van Luijtelaar and Sitnikova, 2006; van Luijtelaar et al., 2011b). Pathophysiological mechanisms of SWD generation are commonly studied into two well described and validated genetic animal models, WAG/Rij and GAERS, which show spontaneous periods of reduced responsiveness, accompanied by SWD (Coenen and Van Luijtelaar, 2003; Depaulis and van Luijtelaar, 2006). In these models an epileptic-focus, located in the deep layers of the somatosensory-cortex, was identified as the site of SWD origin (Meeren et al., 2005, 2002; Polack et al., 2007, 2009). Recently it was shown that low-frequency electrical stimulation of this epileptic-focus in WAG/Rij and GAERS rats could induce afterdischarges (AD), which strongly resembled SWD (Lüttjohann et al., 2011; Zheng et al., 2012). Like SWD, local-field-potential recordings of AD, measured in the deep somatosensory and on the motor-cortex, showed a rhythmic spike and wave pattern with a frequency of 8 Hz. Furthermore, AD were most
frequently induced during drowsiness and only seldom during deep sleep and active-wakefulness, indicating the same vigilance preference known for SWD occurrence (Drinkenburg et al., 1991; Lüttjohann et al., 2011; Smyk et al., 2011). Given this strong similarity between SWD and AD, the authors assumed that these AD, like SWD reflect a cortico-thalamo-cortical phenomenon, and that based on characteristics of AD, network properties relevant for the generation of SWD could be inferred. However, the recordings in our previous study were restricted to the cortex and it needs to be established whether AD are indeed mimicking SWD as far as the involvement of the thalamus is concerned. Therefore, it is investigated whether AD, similar to SWD, can be measured in both cortex and thalamus and whether thalamic stimulation is able to affect characteristics of SWD and AD similarly. Although the role of the cortex and thalamus in SWD initiation and spreading is widely recognized, the exact interactions between a cortical focus and different thalamic nuclei and their contributions in these processes still need to be elucidated (van Luijtelaar et al., 2011b). The VPM (ventral-postero-medial) thalamic nucleus is thought to be part of the key-network of SWD generation. It sends sensory information to and receives information from the somatosensory cortex via direct thalamo-cortical and cortico-thalamic connections. These cells project collaterals to the caudal reticular-thalamic-nucleus (RTN), which in turn sends GABAergic projections to the VPM (Deschenes et al., 1998; Lu and Lin, 1993; Pinault and Deschenes, 1998). The anterior-thalamic-nucleus (ATN), by contrast, does not have any direct connections to the epileptic-focus. It is part of the limbic-loop and predominantly projects to the cingulate and the retrohippocampal cortex (Shibata, 1993a,b; Van Groen and Wyss, 1995). Furthermore, the ATN sends and receives projections to the rostral RTN (Gonzalo-Ruiz and Lieberman, 1995; Pinault and Deschenes, 1998). The sensitivity of stimulation to induce AD can be compared between VPM and ATN. If AD are indeed a type of SWD (Lüttjohann et al., 2011; Zheng et al., 2012), they should be recorded in the VPM and more readily induced via stimulation of the VPM than via ATN. While low-frequency thalamic stimulation is expected to induce epileptic activity in the form of AD, high-frequency thalamic stimulation (130 Hz) has antiepileptic effects and should abort ongoing SWD (Vercueil et al., 1998). The location specificity/sensitivity (VPM, ATN) of this type of stimulation will be investigated in the second part of the study. It is hypothesized that it is easier to disrupt SWD by VPM as compared to ATN stimulation. Whereas in the first part of the study low-frequency stimulation will be applied in an open-loop fashion, i.e. is continuously applied, high-frequency stimulation will be applied in a closed-loop fashion, i.e. only in reaction to SWD.
Method

Subjects

12 male WAG/Rij rats, age 6 months, were used as experimental subjects. They were born and raised at the department of Biological Psychology, Donders Centre for Cognition, Radboud University Nijmegen, The Netherlands. Prior to surgery rats were housed in pairs (High Makrolon cages with Enviro-Dri bedding-material and cage-enrichment) with free access to food and water and were kept at a 12—12 h light—dark cycle (light off at 8.30 A.M.). After surgery rats were housed individually. The experiment was approved by the Ethical Committee on Animal Experimentation of Radboud University Nijmegen. Efforts were made to minimize the amount of discomfort and restrict the number of animals.

Surgery

Stereotactic surgery was performed under isoflurane anesthesia: WAG/Rij rats were divided in two groups, which differed with respect to the location of the stimulation electrodes (Group 1: local stimulation in the VPM; Group 2: local stimulation in the ATN). In both groups two tripolar electrode sets (Plastic-OneMS333/2a) were implanted. Each tripolar electrode set consisted of three stainless steel wires, isolated with polyimide, with a diameter of 0.2 mm. Only the tip of each electrode wire was un-isolated. In group 1 (n = 7) the three electrode wires of the first tripolar assembly were implanted in the VPM, right hemisphere (A/P: −3.3 mm, M/L: −2.5 mm, H: −7.2 mm). Wires were oriented in an anterior-posterior direction and had the following configuration: stimulation anode, stimulation cathode, active recording electrode. Tip distance between electrode tips was 0.3 mm to ensure localized stimulation and recording within the VPM. The first wire of the second electrode set was implanted epidurally on the motor cortex (A/P: +2 mm, M/L: −2 mm), right hemisphere and was used as active recording electrode. The second and third wire of this set were implanted on top of the cerebellum and functioned as ground and reference electrode respectively. In group 2 (n = 5) two electrode-wires of the first tripolar-assembly were implanted in the ATN right hemisphere (A/P:−1.4 mm, M/L: −1.5 mm, H: −6.4 mm) and functioned as stimulation anode and cathode. Again, wires were oriented in an anterior-posterior direction and had a tip distance of 0.3 mm to ensure localized stimulation. The third wire of this electrode set was epidurally implanted on the motor cortex (A/P: +2 mm, M/L: −2 mm), right hemisphere, and functioned as the active recording electrode. Of the second electrode set one wire was implanted in the VPM, right hemisphere (A/P: −3.3 mm, M/L: −2.5 mm, H: −7.2 mm) and functioned as the active recording electrode whereas the other two wires were implanted on top of the cerebellum as the ground and reference electrodes respectively. All coordinates were determined according to the stereo-tactic atlas of Paxinos and Watson (Paxinos and
Watson, 1998). Electrode-assemblies were fixed to the skull with dental cement. Rats were preoperatively injected with Atropine and Rimadyl, and again with Rimadyl postoperatively (24 and 48 h after surgery). Rats were allowed to recover for 2 weeks.

**Data-acquisition, electrical stimulation and experimental procedure**

Rats were placed individually in a 20 inch × 35 inch × 25 inch Plexiglas registration box and connected to the recording leads for LFP recording and electrical brain-stimulation. Leads were attached to a swivel-contact, which allowed registration and stimulation in the freely moving animals. The LFP signals were amplified with a physiological amplifier (TD90087, Radboud-University-Nijmegen, Electronic-Research-Group), filtered by a band-pass filter with cut-off points at 1(HP) and 100(LP) and a 50 Hz Notch filter, and digitalized with a constant sample-rate of 500 Hz by WINDAQ-recording-system (DATAQ-Instruments, Inc., Akron, OH, USA). Movements of the rat were registered by a Passive-Infrared-Registration system (PIR, RK2000DPCLuNAR PR-Ceiling-Mount, Rokonet RISCO-Group S.A., Drogenbos, BE).

Experimental procedure part 1 (open-loop, low-frequency, thalamic stimulation): on experimental day 1 (two weeks after surgery) a baseline recording was performed for 8 h during the dark phase using the above described recording settings (Baseline-LFS). The next day LFP recordings were recorded for 8 h and open-loop electrical stimulation was applied using the following stimulation settings (LFS-stim): double-pulse stimulation with a pulse-duration of 0.4 ms, an interpulse-interval of 400 ms (both previously used in our study on cortical stimulation (Lüttjohann et al., 2011)) and an intensity of 150 µA. Double-pulses were administered for a total of 8 h during the dark phase with a 30 s interval between each double-pulse pair; a total number of 960 double pulse pairs were administered to the rats.

Experimental procedure part 2 (closed-loop, high-frequency, thalamic stimulation): two weeks after experiment one, rats underwent baseline recording during the first 3 h of the dark phase (Baseline-HFS). Next, rats were exposed to an intensity-threshold-test (ITT). In this test the LFP signal of the motor cortex-channel was analyzed by a previously developed and validated online SWD detection algorithm, which was shown to detect SWD with a sensitivity of 100% and a specificity of 96% (Ovchinnikov et al., 2010). It was implemented into a closed-loop DBS system: as soon as a SWD was detected (within 1 s) high-frequency stimulation (130 Hz, bi-phasic pulses, width 0.4 ms, 1 s pulse-train) was immediately administered to the rat. Stimulation started with a low intensity of 20 µA, and was increased in steps of 10 µA until 3 consecutive SWD were interrupted. The behavior of the rats during this intensity-test was closely observed. Next the rat was stimulated with the determined intensity during 1 h. If the rat had a sufficient amount of SWD (10 or more) and at least 90%
were disrupted, that specific intensity was considered the SWD-interruption-threshold. When less than 90% were disrupted, the intensity was increased with 10 µA and the rat was stimulated for another hour. The next day rats were subject to the final stimulation-session (HFS-stim). In this stimulation session LFP signals were recorded during the first three hours of the dark phase (time-locked to the Baseline-HFS session to exclude circadian effects on the number of SWD) and closed-loop high-frequency stimulation (130 Hz, bi-phasic, pulse-width 0.4 ms, 1 s pulse-train, and previously deter-mined interruption-threshold as intensity) was administered to the rat whenever a SWD was detected with the aid of the online-SWD detection algorithm, functionally connected to the constant-current stimulator (Fig. 1).

![Image](image.png)

**Figure 1** Scheme of experimental procedures along time axis. Note that rats in this experiment were exposed to a reverse12/12 h light-dark-cycle with light off between 8.30 h and 20.30 h. Abbreviations: LFS: low frequency stimulation, open loop; HFS: high frequency stimulation, closed loop. ITT: intensity threshold test or determination of stimulus intensity needed for the disruption of SWD to be used in the HFS stimulation session (See text for more details).

**Histology**

At the end of both experimental subparts electrode positions were histologically verified. To this end a direct-current (9 V, 25 µA, 10 s duration) was passed through each electrode in the deeply anaesthetized rat. Rats were perfused with a potassiumferrocyanide-formaldehyde-phosphate solution. Brains were fixed in a 30% sucrose solution, 0.1 ml PBS, cut in 40 µm coronal slices with the aid of a microtome, and stained with Cresyl violet. Only animals with a confirmed electrode tip of the stimulation electrodes in the target regions were included in the statistical analyses (Fig. 2).

**Data analysis**

The number and duration of SWD and AD during baseline and open-loop LFS-stimulation were quantified in the first experiment. Coherence, synchronicity between recording channels and general morphology of SWD and AD were assessed based on visual inspection. Behavioral activity was quantified using the PIR values. SWD data were analyzed with a repeated-measures-ANOVA with either the number of SWD or SWD duration as dependent variable, group (ATN, VPM)as between-subjects-factor, session (baseline, stimulation-session) as within-subjects-factor 1 and time (hour 1—hour8) as within-subjects-factor 2. The same was done with the PIR data. The number of AD during the stimulation-session was
analyzed via a repeated-measures ANOVA with the number of AD as dependent variable, group (VPM, ATN) as between-subject-factor and time (hour 1 - hour 8) as within-subjects-factor. Since not all rats showed AD during all hours of stimulation, AD-duration was analyzed via a 2-way ANOVA with group (VPM, ATN) as between-subjects-factor 1 and time (hour1 - hour8) as between-subjects-factor 2. For the second experiment, intensity to disrupt SWD (intensity-threshold) was compared via an unpaired t-test with group (VPM, ATN) as grouping-factor. The percentage of disrupted SWD was analyzed via a repeated-measures analysis with session (intensity-threshold-test, HFS-stimulation-session) as within-subject-factor and group (VPM, ATN) as between-subjects-factor. Number, mean duration and total duration of SWD as recorded during the HFS-baseline-session and HFS-stimulation session were analyzed via a repeated-measures ANOVA with group (VPM,ATN) as between-subjects-factor and session (baseline, HFS-stim) as within-subjects-factor. All statistical analyses were performed using SPSS19-software (SPSSinc., Chicago, USA).

Figure 2 Histological verification of electrode position (pictures adapted from Paxinos and Watson (1998)). Blue dots indicate the position of the electrode tips within the various thalamic nuclei, only rats with a verified electrode tip in the targeted region were included in the statistical analyses.
Results
Low-frequent, open-loop, thalamic stimulation induces afterdischarges (AD)
Low-frequency (2.5 Hz) thalamic stimulation induced AD: they were characterized by a rhythmic spike and wave pattern of 8 Hz which was immediately (without obvious latency) expressed following the stimulation induced evoked potentials. AD were always recorded in both the VPM and the motor-cortex recording-channel. Upon visual inspection recordings in both channels showed a strong temporal coherence. As is known for SWD (Meeren et al., 2002; Sitnikova and van Luijtelaar, 2007), spike-polarity in cortex and thalamus were opposite (Fig. 3A). Rats were always motionless during AD except for rhythmic twitching movements of the whiskers.

![Figure 3](image)

**Figure 3** Afterdischarges (AD) induced by low frequency thalamic stimulation. (A) Exemplary recording of an AD induced via stimulation of the VPM. (B) Average number of stimulation induced AD via stimulation of either VPM or ATN. (C) Average duration of ATN-stimulation or VPM-stimulation induced AD across stimulation-time.

The ANOVA on AD number revealed a significant main effect of group \( F(1,10) = 10.45; p < 0.01 \). VPM stimulated rats showed a higher \( (37.2 \pm 6.8) \) number of AD compared to ATN.
30% of the 960 stimulations in VPM induced AD, but only in 2.3% of cases AD were induced in ATN stimulated rats. A marginal groupXtime interaction-effect \( F(7,66) = 1.819, p = 0.098 \) was seen for AD duration: AD duration for VPM stimulated rats remained on 7.3 ± 0.5 s throughout the 8 h of stimulation, AD duration of ATN stimulated rats increased over time from 4 ± 1.8 s during the first stimulation hour to 14 ± 2.6 s during the last stimulation hour (Fig. 3c). Post hoc test showed that during the first hour of stimulation ATN stimulated rats had significantly shorter AD than VPM stimulated rats, whereas during the last hour of stimulation ATN induced AD were significantly longer than VPM induced AD (\( p < 0.05 \)). The main effects of time and group by contrast remained far from being significant.

**Effect of low-frequent, open-loop, thalamic stimulation on SWD and behavior**

The ANOVA on SWD number revealed a significant session effect (\( F(1,6) = 7.022, p < 0.05 \) (there were more SWD during the stimulation day) and a timeXgroupXsession interaction effect (\( F(1,70) = 2.417, p < 0.05 \)). Post hoc tests revealed that VPM stimulated rats showed a higher number of SWD during the first two hours of stimulation (\( p < 0.05 \)) but that SWD number decreased over time and returned to baseline level thereafter (Fig. 4b). For ATN stimulated rats, SWD number during stimulation did not reach significantly higher levels compared to baseline (\( p > 0.05 \)). The main-effects of group and time were also non-significant. Like for the AD, ANOVA on the mean duration of SWD during the stimulation-session revealed a significant groupXtime interaction effect (\( F(7,4) = 8.394, p < 0.05 \)): whereas SWD duration for the VPM group remained on an average of 8.6 ± 0.61 s throughout the 8 h of stimulation, SWD duration of the ATN group increased over time from 6.7 ± 0.98 s during the first hour of stimulation to an average of 9.4 ± 1.02 s during the last hour of stimulation (Fig. 4a). Neither the main-effect of time, nor the main-effect of group, nor the main effect of session (baseline, stimulation) reached significance.

The ANOVA on behavioral-activity revealed a significant timeXgroupXsession interaction-effect (\( F(6,61) = 3.104, p < 0.05 \)), similar to what was found for SWD number. The post hoc test also showed similar effects to SWD number, no significant change in behavioral activity between baseline and stimulation session was found for ATN stimulated rats. For VPM stimulated rats a significant decrease in behavioral activity was seen during the first hour of stimulation but returned to its baseline-level in the following hours (Fig. 4c). It can be noted, that this is almost mirror-reversed to the effect of SWD number seen for VPM stimulated rats.
Figure 4 Effects of low frequent, open loop thalamic stimulation on SWD. (A) Mean duration of SWD during 8 h of ATN or VPM stimulation. (B) Mean number of SWD seen during baseline and low frequency stimulation for VPM stimulated rats. (C) Average behavioral activity seen during baseline and low frequency stimulation for VPM stimulated rats.

Closed-loop, thalamic high-frequency stimulation to disrupt SWD
The online SWD detection algorithm detected SWD within about 1 s and high-frequency stimulation was applied gently upon SWD detection. Both VPM (71.6 µA ± 26.2) and ATN (113.6 µA ± 54.4) high-frequency stimulation disrupted SWD (Fig. 5a). The comparison between the intensity-thresholds needed to disrupt at least 90% of SWD as determined in the intensity-threshold-test did not show a significant difference.

ANOVA on SWD duration showed that high-frequency, closed-loop stimulation was able to significantly shorten SWD from 9.04 ± 0.87 s recorded during baseline to 1.52 ± 0.09 s recorded during the stimulation-session (main-effect session: \( F(1,11) = 80.919, p < 0.05 \)) (Fig. 5b). This was the case for both VPM and ATN stimulated rats as was demonstrated by a non significant main-effect of group. The abortion of SWD was in 40% of the cases.
accompanied by an orientation-reaction like head-movement, no movement was induced at the onset of stimulation.

**Figure 5** Disruption of SWD by closed loop, high frequency thalamic stimulation. (A) Exemplary recording of a SWD, disrupted by ATN stimulation. (B) Average SWD duration during baseline and high-frequency stimulation session.

The percentage of disrupted SWD during stimulation did not differ between VPM and ATN stimulated rats. However, for both groups the percentage disrupted SWD during the stimulation-session was significantly decreased as compared to the intensity-threshold-test (intensity-threshold-test = 97.03% ± 0.96; stimulation-session = 89.8% ± 5.60, as was revealed by a main session effect (intensity-threshold-test, stimulation-session $F(1,8) = 7.016, p < 0.05$). A slight increase between HFS-baseline session and HFS-stimulation session was noticed for SWD number but both main and interaction effect remained non-significant (session: $p = 0.25$; sessionXgroup: $p = 0.4$). The ANOVA on total SWD duration revealed a significant main effect of session $F(1,9) = 23.96, p = 0.001$): the time spent in SWD activity during baseline was $497 ± 52.6$ s, this was reduced to $129 ± 33.5$ s during HFS. The main effect of group as well as all interaction effects remained non-significant. In addition, no significant main or interaction-effects were found for overall behavioral activity.

**Discussion**

The current study investigated effects of two different types of electrical-stimulation (open-loop, low-frequency stimulation and closed-loop, high-frequency stimulation) of the thalamus on epileptic activity within the cortico-thalamo-cortical network of absence epileptic WAG/Rij rats. Specificity of stimulation effects was assessed by comparing stimulation to either VPM or ATN.
Induction of epileptic activity via low-frequency, open-loop, thalamic stimulation

Low-frequency, open-loop thalamic stimulation was found to induce epileptic activity in the form of rhythmic after-discharges (AD). AD were characterized by a spike and wave pattern of 8 Hz and did not induce behavioral alterations but was seen to occur in a motionless rat that only produces rhythmic whisking movements. Self-sustained afterdischarges with spike and wave morphology were reported earlier after stimulation of the specific-somatosensory-thalamic-nucleus or the sensory-motor cortex of Wistar and WAG/Rij rats (Pohl et al., 1986; Tolmacheva et al., 2004). However these AD were of lower frequency and accompanied by behavioral alterations like head- and forelimb-clonus, so that the 8 Hz AD can be regarded to be of a different typology. The same type of rhythmic 8 Hz spike and wave AD, occurring without behavioral alterations, were recently reported after low-frequency stimulation of the deep somatosensory-cortex (the epileptic-focus) of absence epileptic WAG/Rij and GAERS rats (Lüttjohann et al., 2011; Zheng et al., 2012). Since AD showed a strong similarity to SWD, the electroencephalographic hallmark of absence epilepsy (i.e. same morphology, same frequency, same preference to occur during drowsiness, easily induced in WAG/Rij rats but not in Wistar rats), it was proposed that these AD are a sort of stimulation induced SWD and thus a cortico-thalamo-cortical phenomenon, although recordings were not performed in the thalamus.

Results of our current study strongly support this proposal since the same type of AD induced by thalamic stimulation in WAG/Rij rats could be recorded at the same time in thalamus (VPM) and (motor)-cortex. Like for SWD (Sitnikova and van Luijtelaar, 2007), recordings of the regular 8 Hz spike and wave AD pattern showed a strong temporal synchrony between recording sites (visually assessed) and spikes of cortex and thalamus were of opposite polarity. Furthermore, it can be noted that both oscillations go along with the same behavioral response (SWD, like AD, occur in a passive rat that performs rhythmic whisking movements (Semba and Komisaruk, 1984; van Luijtelaar and Coenen, 1986; van Luijtelaar and Sitnikova, 2006). Based on these electrophysiological and behavioral characteristics of AD, it can safely be concluded that AD strongly resemble/mimic SWD (Depaulis and van Luijtelaar, 2006).

It was further hypothesized that if AD are stimulation induced SWD, they should be more readily induced by stimulation in the VPM than by stimulation of the ATN, since the VPM is part of the brain-circuitry in which SWD are initiated. This hypothesis was also confirmed: low-frequency stimulation of the VPM could frequently induce AD oscillations. ATN stimulation by contrast only rarely induced AD, with VPM induced AD outreaching ATN induced AD by a factor of 14.
The preference for AD induction within the circuitry in which SWD are initiated is thought to be related to the anatomical connectivity profile of the two nuclei. The VPM has direct connections to the somatosensory-cortex and electrical stimulation of the VPM might lead to a postsynaptic activation in the epileptic-focus. This might trigger an epileptic oscillation. Likewise stimulation of the motor-cortex, also possessing direct connections to the epileptic-focus (FitzGerald and Folan-Curran, 2002) was shown to easily induce AD (Lüttjohann et al., 2011). The importance of a trigger from the VPM to elicit SWD was recently suggested (Abbasova et al. (2010). Peripheral inactivation of the trigeminal-nerve which projects to the VPM blocked SWD occurrence. The ATN, by contrast, does not possess direct connections to the epileptic-focus. Therefore it is less likely that its stimulation can function as an effective trigger to activate the epileptic-focus.

Low-frequency thalamic stimulation was also found to influence properties of SWD and also this was stimulation-site specific: whereas ATN stimulation prolonged the duration of SWD over time, VPM stimulation did not. Interestingly, the same site-specific effects of long-lasting stimulation were found for AD duration: ATN stimulation prolonged the duration of AD over time, VPM stimulation did not. It can therefore be noted that AD and SWD react in the same way towards these experimental manipulations. Since the ATN does not possess direct connections to the cortical-focus, it is possible that the effects of ATN stimulation on SWD and AD duration are achieved via its connections to the rostral RTN; ATN stimulation may result in a postsynaptic activation of the rostral RTN. Effects of RTN activity on SWD duration was reported earlier by Sohal et al. (2003) in slice preparations. Furthermore, the paradoxical effect, that rostral RTN inhibition or lesions (and not the caudal part), although not being part of the somatosensory-loop, led to a decrease of SWD duration was reported in the same (Berdiev et al., 2007) but also in an almost similar genetic absence model (GAERS) (Aker et al., 2006).

Another agreement between SWD and AD was that low-frequency VPM stimulation increased the number of SWD compared to baseline, similar to the high number of AD seen with VPM stimulation. ATN stimulation by contrast only rarely induced AD and was not able to significantly increase the number of spontaneous SWD. Again, a trigger from the VPM might activate the cortical epileptic-focus leading to the increase in SWD and AD, whereas ATN stimulation is much less likely to provide an effective trigger, given the absence of direct neuronal connections between ATN and somatosensory-cortex. The only dissociation between SWD and AD noted in this study was that there was a decrease over time in the number of SWD during the 8 h VPM stimulation, while AD induction remained high-level throughout the 8 h of stimulation. The decrease of SWD number over time might be related to the behavioral activity of rats which increased over time for VPM stimulated rats. Also for
AD number such a relation to behavior and the level of vigilance has been reported; the slightly higher intensity of the induction stimulus (100 µA used by Lüttjohann et al. (2011) vs. 150 µA used in the current study) might explain the difference with 150 µA, being a more potent trigger, able to induce AD even in a more active rat.

It can be concluded that 8 Hz AD, induced by either stimulation of the cortex and VPM strongly mimic SWD. These AD can be recorded in cortex and thalamus, and are more easily induced within the loop in which SWD are elicited.

**Disruption of ongoing epileptic activity via high-frequency, closed-loop thalamic stimulation**

Given the outcome that low-frequency thalamic stimulation induces epileptic activity (SWD and AD), it was next investigated whether thalamic, closed-loop or responsive, high-frequency (130 Hz) stimulation is able to disrupt SWD, and whether this effect is also dependent on the site of stimulation. It was hypothesized that it might be easier to disrupt SWD by VPM stimulation as compared to ATN stimulation. Surprisingly no indication for the specificity of stimulation-site was found for closed-loop, high-frequency stimulation. Stimulation of both nuclei disrupted SWD and neither a significant difference in the required intensity for disruption nor a significant difference in the percentage of disrupted SWD was found. Both groups showed a small reduction between intensity-test and stimulation-session, which might indicate some diurnal variation (the intensity-threshold-test was performed in the afternoon, stimulation-session took place the next morning) of intensity-thresholds. Next to our closed-loop high frequency results, bilateral high-frequency stimulation applied to structures that send afferent-projections to the cortico-thalamo-cortical system (subthalamic-nucleus (STN), superior-coliculus (SC)) in GAERS rats was also reported to disrupt SWD (Nail-Boucherie et al., 2002; Vercueil et al., 1998) with intensities similar to those of the current study (VPM (71.6 µA), ATN (113.6 µA), STN (100 µA), SC (76 µA)). In our study, however, stimulation was only applied unilaterally and it is likely that bilateral stimulation might further lower the required intensity as supported by the fact that unilateral STN stimulation with the intensity found to be efficient for bilateral stimulation did not disrupt SWD (Vercueil et al., 1998).

In the light of these results an important question, worthy to be discussed, regards the potential mechanisms of action of DBS. In all these studies it is unlikely that the disruption effect of SWD can be attributed to an induction of behavioral-activity. Vercueil et al. (1998) demonstrated that the intensity to induce behavioral reactions was significantly different to the intensity required to disrupt SWD. In our study stimulation itself did not induce behavioral reactions and only in 40% cases the end of SWD (seen on average 0.5 s after stimulation
onset) induced an orientation reaction like head movement of the rat. Rather, the neuronal firing pattern might be directly changed by stimulation. In line with this suggestion Lee et al. (2004) demonstrated that high frequency stimulation of the subthalamic nucleus (STN) resulted in an increased firing rate in both the STN and the STN target structure, the substantia nigra, which was shown to be mediated by synaptic transmission. In addition Berenyi et al. (2012) reported a phase dependent increase of neuronal firing with 1 Hz sinusoidal stimulation. Whereas VPM stimulation has the potential to directly affect neuronal firing behavior of the epileptic-focus, ATN stimulation, like might have been the case for low-frequency (see paragraph 4.1) stimulation, might act via its projections to the rostral RTN, which is often mentioned as a structure controlling SWD duration (Aker et al., 2006; Sohal et al., 2003). Furthermore, whereas low-frequency, open-loop ATN stimulation might activate rostral RTN neurons in a slow and rhythmic way leading to an increase of the duration of SWD and AD, the short temporal distance between pulses of high-frequency stimulation might activate rRTN neurons in such a way that it interferes with the slow (8 Hz), rhythmic, firing patterns required for the maintenance of SWD (desynchronization of the LFP). Alternatively, high frequency DBS might act via a depolarization blockage or a depletion of the neurotransmitter pool (Schiller and Bankirer, 2007). Certainly, additional neurophysiological studies on the neuronal mechanisms of DBS are required.

Interestingly, recently low-frequency, closed-loop, trans-cranial stimulation, was shown to also successfully disrupt SWD (Berenyi et al., 2012). This, however, might crucially depend on the phase/timing of stimulation: the authors argued that stimulation activates silent neurons during the wave component. These neurons should normally be active to create the spike but cannot do so because of being refractory after being activated during the wave. It can be noted that this argumentation is in line with our interference proposal of high frequency stimulation noted above. The lack of stimulation-site specificity for high-frequency stimulation as found in the present study might be due to the relatively late onset of stimulation. Since high-frequency stimulation on average started 1 s following the onset of SWD, it might be possible that a stimulation-site specificity is only apparent at the very onset of SWD. Likewise, Meeren et al. (2002) reported that the initial driving force of the epileptic-cortical-focus onto thalamic nuclei was restricted to the first 500 ms following the onset of SWD but that there-after cortex and thalamus form a bidirectional resonance circuitry. Early disruption (within the first 500 ms) or even prevention of SWD by stimulation triggered by detection of SWD precursor activity (van Luijtelaira et al., 2011a) might, by contrast, require desynchronizing stimulation of either the epileptic-focus itself, or the primary thalamic reverberator, needed for SWD generalization.
Electrical stimulation to deep brain structures is proposed as a new therapeutic treatment approach for epileptic seizures. Interestingly, a large clinical trial was recently started targeting the ATN (Lega et al., 2010). The application of electrical pulses to relevant brain-structures is aimed to modulate or even prevent the abnormal brain-activity. The search for optimal therapeutic stimulation protocols and inclusion criteria is still in its infancy (Montgomery and Gale, 2008; Pollo and Villemure, 2007; Theodore and Fisher, 2004). In the present study it was established that low-frequency electrical stimulation of the ATN may also trigger AD and prolong AD and SWD duration. This suggests that at least open-loop, low-frequency stimulation must be avoided in patients with SWD in their EEG. Closed-loop, high-frequency, stimulation by contrast, seems a fruitful approach for seizure disruption. However, longer lasting (e.g. 24 h or more) stimulation sessions are relevant to establish the stability of stimulation effects and to exclude the risk of SWD rebound, which has been noted in the current experiment only with a non-significant increase of SWD, but has been reported to happen to a significant degree after seizure triggered high frequency stimulation of other brain structures in GAERS (Feddersen et al., 2007).

References


Chapter 6

The dynamics of cortico-thalamo-cortical interactions at the transition from pre-ictal to ictal LFPs in absence epilepsy

Published as
Abstract

Purpose: Generalized spike and wave discharges (SWD) are generated within the cortico-thalamo-cortical system. However, the exact interactions between cortex and different thalamic nuclei needed for the generation and maintenance of SWD are still to be elucidated. This study aims to shed more light on these interactions via multisite cortical and thalamic local-field-potential recordings.

Methods: WAG/Rij rats were equipped with multiple electrodes targeting layers 4 to 6 of the somatosensory cortex, rostral and caudal RTN, VPM, anterior (ATN)- and posterior (Po) thalamic nucleus. The maximal association-strength between signals was calculated for pre-ictal→ictal transition periods and in control periods using non-linear-association-analysis. Dynamics of changes in coupling-direction and time-delays between channels were analyzed.

Results: Earliest and strongest increases in coupling-strength were seen between cortical layers 5/6 and Po. Other thalamic nuclei became later involved in SWD activity. During the first 500 ms of SWDs the cortex guided most thalamic nuclei while cortex and Po kept a bidirectional crosstalk. Most thalamic nuclei started to guide the Po until the end of the SWD. While the rostral RTN showed increased coupling with Po, the caudal RTN decoupled. Instead, it directed its activity to the rostral RTN.

Conclusions: Next to the focal cortical instigator zone of SWDs, the Po seems crucial for their occurrence. This nucleus shows early increases in coupling and is the only nucleus which keeps a bidirectional crosstalk to the cortex within the first 500 ms of SWDs. Other thalamic nuclei seem to have only a function in SWD maintenance. Rostral and caudal-RTN have opposite roles in SWD occurrence.

Keywords Somatosensory cortex; Cortico-thalamo-cortical system; Network interactions; Non-linear association analyses; Posterior thalamic nucleus; Spike–wave discharges; WAG/Rij rats

Abbreviations SWD: spike-wave-discharges; ctx4: layer 4 of the somatosensory cortex; ctx5: layer 5 of the somatosensory cortex; ctx6: layer 6 of the somatosensory cortex; ATN: anterior thalamic nucleus; Po: posterior thalamic nucleus; VPM: Ventral–Postero-Medial nucleus of the thalamus; cRTN: caudal reticular thalamic nucleus; rRTN: rostral reticular thalamic nucleus; FCTS: first cortico-thalamic spike

Introduction

Spike and wave discharges (SWDs) are the electrophysiological hallmark of absence epilepsy. Today it seems well established that SWDs in rodent models are generated by a local hyperexcitable instigator zone located in the deep layers (5/6) of the perioral-region of the somatosensory-cortex of absence epileptic WAG/Rij and GAERS rats (Manning et al., 2004; Meeren et al., 2002; Pinault, 2003; Polack et al., 2007, 2009; Sitnikova and van Luijtelaar, 2004), two well validated models of absence epilepsy (Depaulis and van Luijtelaar, 2006; van Luijtelaar and Sitnikova, 2006; van Luijtelaar et al., 2011b).

Lesion studies, however, demonstrate that an excitable region is not sufficient for the occurrence of SWDs and indicate that some thalamic nuclei (lateral and lateral posterior nucleus group) seem to be important for SWD occurrence (Pellegrini and Gloor, 1979; Vergnes and Marescaux, 1992) and might have different functional roles (rostral- vs. caudal
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reticular-thalamic-nucleus (RTN)) in the occurrence of SWD (Aker et al., 2006; Berdiev et al., 2007; Meeren et al., 2009).

It is generally accepted that SWDs occur in an intact corticothalamo- cortical network, although the exact interactions between the cortex and different thalamic nuclei necessary for the generation and maintenance of SWDs are far from completely understood (Seidenbecher and Pape, 2001). Only a few studies have investigated interactions between cortex and thalamus. Non-linear-association analysis applied on local field potentials (LFP’s) recordings from an epidural cortical grid and Ventral–Postero-Medial (VPM), Ventral–Postero-Lateral (VPL) and Lateral–Dorsal (LD) thalamic nucleus showed that (1) the start of SWDs was associated with an increase in association-strength between cortical focus and other cortical and thalamic sites and that (2) the cortex led the thalamus during the first 500 ms of a SWD but later on coupling direction alternated (Meeren et al., 2002). Likewise Polack et al. (2009) showed that SWD associated unit firing in the somatosensory cortex preceded cell firing in motor cortex and thalamus. Sitnikova et al. (2008) reported sudden and rapid increases in coupling between frontal cortex and VPM at the onset of SWDs. Finally, frequency specific changes in coherence were found for local-cortical, non-local-cortical, thalamo-thalamic and cortico-thalamic networks suggesting that each circuit oscillates in its preferred frequency during SWDs (Sitnikova and van Luijltelaar, 2006). In none of these network studies, however, recording electrodes have targeted the focal epileptic zone in the deep layers of the somatosensory cortex together with multiple thalamic sites.

The focal cortical epileptic zone is part of a larger corticothalamo- cortical network: layer 6 of the somatosensory cortex sends projections to the VPM, which give collaterals to the caudal part of the RTN (Bourassa et al., 1995; Deschenes et al., 1998). The caudal part of the RTN has GABAergic projections to Thalamo-Cortical (TC) cells which relay from the VPM to cortical layer 4 of the somatosensory cortex (Ghazanfar et al., 2001; Oda et al., 2004; Pinault and Deschenes, 1998), thereby closing the thalamic somatosensory loop.

SWD activity in layer 5 of the somatosensory cortex can spread via its reciprocal connection towards the posterior thalamic nucleus (Po) (Deschenes et al., 1994; Nothias et al., 1988; Veinante et al., 2000). This is a higher order nucleus that is thought to be involved in corticocortical communication (Sherman and Guillery, 2005). The rostral part of the RTN projects towards the anterior thalamic nucleus (ATN) (Aker et al., 2006; Gonzalo-Ruiz and Lieberman, 1995), a possible target of intra-thalamic spread enabling information to exit the somatosensory cortico-thalamic loop.

In the current experiment local-field-potential recordings including SWDs were obtained from all these structures and analyzed on their functional connectivity in order to reveal the
dynamics of detailed network interactions within this cortico-thalamo-cortical circuitry relevant for the generation and maintenance of SWD.

Method

Subjects
16 male WAG/Rij rats, 6 to 9 months of age were used as experimental subjects. They were born and raised at the Department of Biological Psychology, Donders Centre for Cognition, Radboud University Nijmegen, The Netherlands. Prior to surgery the rats were housed in pairs (High Makrolon® cages with Enviro Dri® bedding material and cage enrichment) with free access to food and water and were kept at a 12–12 h light–dark cycle (light off at 8:30 AM). After surgery the rats were housed individually. The experiment was approved by the Ethical Committee on Animal Experimentation of Radboud University Nijmegen (RU-DEC). Efforts were done to keep the discomfort of the animals as minimal as possible.

Construction of an electrode system suited for multi-site LFP recordings
A self-constructed electrode system was manufactured for multisite local field potential recordings at specified brain locations (van Luijtelaar et al., 2011b). It consisted of a 4 mm×5 mm, 3 mm thick Teflon block, which contained small holes located at the relative A/P and M/L coordinates of the electrode target structures as determined by the rat-brain atlas of Paxinos and Watson (1998). This block was fixed in a micro manipulator, which allowed us to insert electrode wires (stainless steel electrodes insulated with poliamide, diameter: 127 μm, only the cutting edge of the electrode was not isolated) through the holes and set the wire to its accurate depth coordinate. Wires were fixed to the Teflon-block by glue. The electrodes were fixed at the top-site to a connector pin which was entered into an electrode pedestal suitable for the connection to a multi-lead electrode cable.

Surgery
Implantation of the LFP recording electrodes was done in a stereotactic frame under isoflurane anesthesia. At the start of surgery, rats received a subcutaneous injection of the analgesic Rimadyl and an intramuscular injection of atropine to prevent excessive salivary production. Body temperature was controlled and conserved via a heating pad. The local anesthetic Lidocaine was used on the incision points. Holes were drilled into the skull on top of the right hemisphere for the insertion of electrode wires at the following positions: somatosensory cortex: A/P=0, M/L=−4.6 depth=−2.8 (layer 4), −3.1 (layer 5), −3.6 (layer 6); anterior thalamus: A/P=−1.4 M/L=−1, depth=−6.2; rostral RTN: A/P=−1.4, M/L=−1.9, depth=−6.6; posterior thalamic nucleus: A/P=−3.6, M/L=−2, depth=−5.4; VPM: A/P=−4.16,
M/L=−2.8, depth=−6 and caudal RTN: A/P=−3.1, M/L=−3.5, depth=−6.6. All coordinates were determined relative to Bregma according to the rat-brain atlas of Paxinos and Watson (1998). Electrode wires, fixed in the Teflon block at the appropriate position and length, were simultaneously entered into the brain. Ground and reference electrodes were positioned epidurally on top of the cerebellum. The electrode assembly was fixed to the skull via dental cement. Postoperative analgesic Rimadyl (24 and 48 h after surgery) was administered and rats were allowed to recover for 2 weeks.

**Recording of local field potentials**

Two weeks after surgery the rats were placed individually in a 20×35×25 cm Plexiglas registration box and connected to the recording leads for multi-channel LFP recordings. These were attached to a swivel-contact, which allowed recording in freely moving animals. The LFP signals were amplified with a physiological amplifier (TD 90087, Radboud University Nijmegen, Electronic Research Group), filtered by a band pass filter with cut-off points at 1(HP) and 100(LP) and a 50 Hz Notch filter, and digitalized with a constant sample rate of 2048 Hz by WINDAQ-recording-system (DATAQ-Instruments). In addition to the LFP signals the movements of the rat were registered by means of a Passive Infrared Registration system (PIR, RK2000DPC LuNAR PR Ceiling Mount, Rokonet). Each rat was recorded for a period of 4 h during the dark phase.

**Signal analysis**

Connectivity between brain structures was studied with the aid of the non-linear association analysis (Pijn, 1990; Pijn et al., 1989). This signal analytical technique has frequently been shown to be a reliable measure for functional coupling of brain signals including SWDs (Gupta et al., 2011; Meeren et al., 2002; Wendling et al., 2001; Westmijse et al., 2009). Its main advantage above several other connectivity measures is that it does not presume a linear relationship between signals and is able to reveal information about the direction of coupling. It is a time-domain analysis, which can reveal three different parameters of interest:

1. The strength of functional coupling or maximal association between two brain signals h²:

   The strength of functional coupling is seen as the degree of dependence between two signals, which is expressed in the nonlinear correlation coefficient h², describing the degree to which amplitude values of signal Y can be predicted based on the amplitude values of signal X according to a non-linear regression curve. The variance of Y according to the regression curve is called the explained variance, i.e., it is explained or predicted on the basis of X. By subtracting the explained variance from the total variance the unexplained variance is obtained. The correlation coefficient expresses the reduction of variance of Y that
can be obtained by predicting the values according to the regression curve: $h^2 = (\text{total variance} - \text{unexplained variance}) / \text{total variance}$. This correlation coefficient can vary between 0 (Y is totally independent of X) and 1 (Y is completely determined by X). In case of a linear relationship between both signals $h^2$ is equal to the well known linear correlation coefficient $r^2$. In practice, the non-linear regression curve is obtained by describing the scatterplot of Y versus X by segments of linear regression curves. The variable X is subdivided into bins; for each bin the X value of the midpoint ($p_i$) and the average value of $y(q_i)$ are calculated, and the resulting points ($p_i,q_i$) are connected by segments of straight lines (= linear regression curves). The non-linear correlation coefficient $h^2$ can now be computed as the fraction of total variance that can be explained by the segments of linear regression lines via:

$$h^2 = \frac{\sum_{i=1}^{N}(y_i - <y>)^2 - \sum_{i=1}^{N}(y_i - f(x_i))^2}{\sum_{i=1}^{N}(y_i - <y>)^2}$$

with N being the number of samples and $<y>$ being the average of all $y_i$ (Meeren et al., 2002; Pijn, 1990).

(2) 2nd parameter of interest = time-delays of a signal between brain structures or signal transduction time and

(3) 3rd parameter of interest = the direction of functional coupling:
Similarly, as in the case of the cross-correlation, one can estimate $h^2$ as a function of time shift ($\tau$) between signal X and Y or vice versa. That shift for which the maximum value for $h^2$ is reached is used as an estimate of the time lag or time delay between the two signals, it is the second parameter of interest of this study. In addition, it also reveals the third parameter of interest: the direction of coupling by telling us whether signal Y lags behind signal X (so signal X can predict the future of signal Y, i.e. Y is dependent on signal X) or signal X lags behind signal Y (so signal Y can predict the future of signal X, i.e. X is dependent on signal Y) (Meeren et al., 2002; Pijn et al., 1989). Based on this third parameter the analysis aims to extract the existence of a causal relationship between brain structures (a change of the signal in brain structure A causes a change of the signal in structure B a few milliseconds later). However, one has to be aware that the interpretation of causality needs to be taken with some caution since a differentially delayed influence by a third non-recorded area cannot completely be excluded. This risk is aimed to be minimized in this study by targeting/recording from multiple anatomically connected brain areas.
For more details of the theoretical and practical aspects of this nonlinear association method see (Lopes da Silva et al., 1989; Pijn et al., 1989, 1990). The code of the non-linear association analyses is available in FieldTrip, a Matlab oriented toolbox (Oostenveld et al., 2011). Fieldtrip is released as open source under the GNU general public license.
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In the current experiment $h^2(\tau)$ was calculated for each channel combination. The data analysis window for the correlation of amplitude values had a size of 500 ms and was subdivided into 10 bins, the maximum time shift was 40 ms, and the minimum time shift was 0.5 ms. Moreover, instead of a single window describing the maximal association between channels for this time period of 500 ms, $h^2(\tau)$ was calculated dynamically for moving time windows (shifting in steps of 125 ms) along a complete time period of 6 s describing the transition period from pre-ictal to ictal LFPs. The first epileptic spike (sharp spike of at least twice the background LFPs) which is visible in all cortical and thalamic recordings and was followed by rhythmic SWD activity was chosen as reference point for the selection of this transition period. This reference-point, further referred to as first cortico-thalamic spike (FCTS), marked the midpoint of the pre-ictal–ictal transition period (Fig. 1). This resulted in a total of 45 values of coupling along the transition period. For calculation of channel-coupling during non-epileptic control periods, which was compared to coupling during the transition periods, LFP segments of 500 ms+40 ms duration were selected from passive wakefulness distant (by at least 5 min) to a SWD. These were characterized by a low amplitude desynchronized high frequency LFPs recorded in a motionless rat. To avoid the possibility of selecting REM-sleep LFPs, LFP segments containing clear signs of theta activity in a motionless rat were avoided. In addition, control periods were preceded or followed by periods of active wakefulness. For each rat a total of 10 pre-ictal–ictal transition periods and 10 control periods were analyzed and the outcomes were averaged per rat in order to obtain representative values of coupling strength, coupling direction and time delays. Only data from brain structures with a histologically verified proper electrode position were included in the statistical analysis (see Supplement I). To this end a direct current (9 V, 25 µA, 10 s duration) was passed through each electrode in the deeply anesthetized rat at the end of the experiment. Rats were perfused with a potassium-ferrocyanide–formaldehyde–phosphate solution, coloring these lesions at the end of each electrode tip. Brains were fixed in a 30% sucrose solution, 0.1 ml PBS, cut in 40 µm coronal slices with the aid of a microtome, and stained with Cresyl violet. Only electrodes for which the midpoint of the small lesion was located within the target structure were considered as properly implanted and included in statistical analysis.
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Fig. 1. Upper panel: multi-site local field potentials recording showing the transition from pre-ictal LFP recordings; black vertical bar indicates the time-point of the first cortico-thalamic spike (FCTS). Lower panel: temporal changes in cortico-thalamo-cortical connectivity were determined via a moving time/analysis window of 500 ms, which was shifted in steps of 125 ms along a transition spanning from 3 s prior until 3 s following the first cortico-thalamic spike of a SWD. Lower panel shows a schematic illustration of these performed time-shifts for signal analysis. Abbreviations: ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: Posterior thalamic nucleus, VPM: Ventral–Postero-Medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
For each channel combination coupling data were statistically analyzed using a repeated measures analysis of variance with $h^2(\tau)$ as dependent variable and time point (control period and 45 along the transition period) as within subjects factor. The percentage of segments in which either signal A is leading signal B, signal B is leading signal A and signal A and B being synchronous in time (maximal association is reached at $\tau=0$) was calculated for each time point for analysis of coupling direction and was subject to a repeated measures analysis as well.

To avoid the problem of type I errors, the results of the multiple comparisons in the post-hoc analyses were only regarded as significant if they fulfilled the following criteria: (1) a significant main effect was seen one-way repeated measures ANOVA (2) comparisons were restricted to the control period and (3) only clusters of minimal 3 subsequent points were considered as significant (Field, 2009; Maris and Oostenveld, 2007).

We referred to the midpoint of a time-window as the representative time-value of changes in a given time-window for graphical and textual representation/documentation.

Supplement I: Results of histological verification of electrode position. X indicates correctly located electrode.

<table>
<thead>
<tr>
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<th>Ctx6</th>
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**Results**

**Coupling strength, coupling direction and time delays in the non-epileptic passive wakefulness state**

Coupling strength $h^2(\tau)$ and time delays as well as the pattern of coupling direction between signals during control periods (nonepileptic LFP signals recorded during passive wakefulness distant to an SWD) strongly depended on the channel-combination ($F(34,318)=40.435$, 40.435,
In general thalamo-thalamic and cortico-cortical time delays with a mean of 1.7 ms were significantly shorter than cortico-thalamic delays with a mean signal transduction time of 10.3 ms ($F(1,346)=168.106$, $p<0.01$). In addition, cortico-thalamic signal transduction time was significantly shorter than thalamo-cortical (8.9 ms and 11.4 ms respectively) ($F(1,173)=9.68$, $p<0.01$).

Most cortico-thalamic channel pairs did not show a dominant leading structure (coupling direction) during control periods (Table 2). For these pairs the percentage of LFP-segments in which either signal or structure A is leading signal or structure B is not different to the percentage of segments in which signal or structure B is leading signal or structure A ($p>0.05$). Exceptions to this were channel pairs ctx4→ATN, ctx5→ATN, ctx5→rRTN and ctx6→rRTN: for these pairs the percentage of LFP-segments in which the cortex leads the thalamus was significantly higher than the percentage of LFP-segments in which the thalamus leads the cortex ($p<0.05$). For all cortico-thalamic pairs it was only rarely seen (only in a mean of 9% of cases) that cortex and thalamus were running equal in time (i.e. 

Table I: Coupling strength $h^2(\tau)$ and time delays in milliseconds during non epileptic control periods as found for the different channel combinations

<table>
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<th></th>
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In general thalamo-thalamic and cortico-cortical time delays with a mean of 1.7 ms were significantly shorter than cortico-thalamic delays with a mean signal transduction time of 10.3 ms ($F(1,346)=168.106$, $p<0.01$). In addition, cortico-thalamic signal transduction time was significantly shorter than thalamo-cortical (8.9 ms and 11.4 ms respectively) ($F(1,173)=9.68$, $p<0.01$).
maximal association is found at time-point zero).

Half of the intra-thalamic and intra-cortical channel pairs also did not show a dominant leading structure (Table 2). In addition and in contrast to the thalamo-cortical pairs, these pairs also regularly showed LFP periods in which both signals were running equal in time (i.e. maximal association is found at time-point zero): the percentage of LFP-parts in which either structure A is leading B, structure B is leading A and structure A and B being equal in time, did not significantly differ (p's>0.05) (Table 2).

The other half of intra-thalamic and intra-cortical connections for which a dominant leading structure was identified included the following channel pairs with the following pattern of guidance: cRTN guiding \(\rightarrow\) VPM, ATN\(\rightarrow\)VPM, ATN\(\rightarrow\)Po, rRTN\(\rightarrow\)Po, cRTN\(\rightarrow\)Po and ctx4\(\rightarrow\)ctx6 (p's<0.01) (Table 2).

Table II: Coupling direction during non-epileptic control EEG as expressed in mean percentage of structure A leading structure B, structure B leading structure A and signals of both structures being equal in time; * indicates sign. higher values

<table>
<thead>
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<th>Signal B</th>
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<th>B&gt;A</th>
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Spike and wave discharges, electrophysiological observations

All rats showed SWD’s, mean 10 per hour, mean duration SWDs 7 s. Rhythmic spike and wave activity in the typical frequency of 8–10 Hz was present in all recorded channels.
Spikes had a smaller amplitude in thalamus than in cortex (500 vs 700 µV) and had a less sharp waveform, as described earlier (Sitnikova and van Luijtelaar, 2007) (Fig. 1). In 20% of the rats SWD like activity could be seen by visible inspection earlier in the cortex than in the thalamus. In these rats the local start of SWD activity preceded the thalamic involvement by up to 1 s (see van Luijtelaar et al., 2011b for figure).

**Coupling strength during the pre-ictal→ictal transition period**

The pre-ictal→ictal transition period includes the analyses of the local field potential recordings lasting from 3 s prior to the first cortico-thalamic spike (FCTS) of the SWD until 3 s following it. Coupling strength during this period was calculated for shifting time windows (Fig. 1) and compared to the coupling strength found during control segments in order to sketch the temporal evolution of changes in coupling that are associated with SWD generation. The following pattern of changes could be observed (see Fig. 2 for a graphical representation of all changes in $h^2(\tau)$ as well as Table 3):

1. As early as 1.25 s prior to FCTS a significant increase in coupling was found between cortical layer 6 and Po ($F(2,31) = 14.915, p<0.01$).
2. Immediately thereafter (1.125 s prior to FCTS) a decrease in coupling was seen between caudal RTN and Po ($F(3,41) = 5.487, p<0.01$). This decrease reached its maximum at about 750 ms prior to FCTS and returned to its baseline coupling level at around 250 ms to FCTS.
3. Next, coupling between cortical layer 5 and Po ($F(3,48) = 9.399, pb0.01$) as well as cortical layer 4 and Po ($F(3,40) = 12.268, p<0.01$) were found to start increasing at 500 ms and 375 ms before FCTS respectively.
4. The majority of channel pairs, including ctx6–VPM, rRTN–cRTN, rRTN–ATN, cRTN–ATN and rRTN–Po, showed an increase in coupling within the time windows from 250 ms prior FCTS until 125 ms following FCTS ($p$ of rRTN–cRTN<0.05; all other $p$'s<0.01).
5. Later changes in coupling strength were seen for channel pair ATN–Po ($F(3,31) = 9.122, p<0.01$) and ctx4–VPM ($F(3,33) = 3.783, pb0.02$). These showed an increase in coupling at 250 ms and 375 ms after FCTS, respectively.

In general, strongest (>16%) increases in coupling strength were found between all three cortical layers and the posterior thalamic nucleus (ctx6–Po=22.5%; ctx5–Po=16.6%; ctx4–Po=16.6%) as well as Po and rostral RTN (23.2%); moderate (10–16%) increases were found for channel pairs ctx6–VPM (15.3%), ctx4–VPM (11.0%) and Po–ATN (13.2%); and small (b10%) but significant increases were found for rRTN–cRTN (4.6%), ATN–rRTN (7.1%) and ATN–cRTN (8.8%). In addition, most channel pairs reached a plateau in coupling strength at around 375 ms after the FCTS and remained on this level until the end of analysis window (= 3 s after FCTS).
The role of the cortico-thalamo-cortical system in absence epilepsy – Chapter 6

Fig. 2. Coupling strength $h^2(\tau)$ during the pre-ictal→ictal transition period displayed for all channel combinations that show significant SWD related changes. Filled circles indicate significantly different values of $h^2(\tau)$ as compared to non-epileptic control periods (first data value indicated by a ‘c’ on the x-axis). Per channel-pair average SE values (averaged over timepoints) are given in the legend on the right-hand site. Note: Earliest SWD related increases in coupling strength, found for channel combination ctx6–Po nucleus do already start to occur 1.25 s prior to the first cortico-thalamic spike of SWD, whereas other channel combinations show later changes in coupling strength (most upper graph displaying channel-pairs showing earliest changes in coupling strength to lowest graph displaying channel-pairs showing latest changes in coupling strength). Channel-combination cRTN–Po is the only pair for which a SWD related decrease in $h^2(\tau)$=decoupling can be found. Since only data from brain structures with an histologically verified proper electrode position were included to analysis, channel pairs vary with respect to the number of included animals and SWD: ctx6–Po: 14 rats and 140 SWD; ctx5–Po: 14 rats and 130 SWD; ctx5–Po: 14 rats and 140 SWD; ctx4–Po: 14 rats and 140 SWD; ctx6–VM: 12 rats, 120 SWD; rRTN–ATN: 7 rats, 70 SWD; rRTN–Po: 9 rats, 90 SWD; rRTN–cRTN: 8 rats, 80 SWD; cRTN–ATN: 9 rats, 90 SWD. Abbreviations: c: non-epileptic control period, ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: Ventral–Postero-Medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
Coupling direction during the pre-ictal→ictal transition period

Almost all cortico-thalamic channel pairs for which SWD related increases in coupling strength have been found (ctx6–Po, ctx4–Po, ctx6–VPM and ctx4–VPM) also showed SWD related changes in coupling direction (p's<0.05). For all these pairs the cortex started to guide the thalamus in the majority of SWDs. This cortical guidance started to occur around FCTS and lasted only 250 ms to 750 ms, depending on the channel pair (Fig. 3A). The only exception was channel pair ctx5–Po, where no change in coupling direction was seen (p>0.05) despite increases in $h^2(\tau)$. In this case cortex and posterior thalamic nucleus kept a bidirectional crosstalk during the complete pre-ictal→ictal transition period.

Of the five intra-thalamic channel pairs that showed SWD related increases in $h^2(\tau)$, three pairs also showed SWD related changes in coupling direction: the caudal RTN started to guide the rostral RTN (p<0.01) in the majority of SWDs, and both rostral RTN and ATN significantly increased their guidance to the Po (rRTN→Po, p<0.05; ATN→Po, p<0.01). This change in guiding pattern occurred around FCTS for the latter two pairs (Fig. 3B).

For channel pair rRTN–cRTN on the other hand, the caudal RTN already started to guide the rostral RTN at 875 ms prior to FCTS (Fig. 3C, Table 3). It can be noted that this early change in coupling direction occurred shortly after the first increase in coupling strength found between ctx6 and Po (change in $h^2(\tau)$ at 1.25 s prior to FCTS) and the early decrease in coupling strength between caudal RTN and Po (change in $h^2(\tau)$ at 1,125 s prior to FCTS).

In contrast to the cortico-thalamic pairs, the change in coupling direction persisted until the end of the analysis window (3 s after FCTS) for all three intra-thalamic pairs. In addition to the channels for which SWD related increases in $h^2(\tau)$ were found, many channel pairs for which no changes in $h^2(\tau)$ were seen, still did show changes in coupling direction (Table 3).

Among the intrathalamic pairs, again the Po got led by another thalamic nucleus (VPM), which started to occur at FCTS and continued until the end of the analysis window (p<0.05). It can thus be noted that the posterior nucleus became guided by all other thalamic nuclei (except cRTN which also showed a decrease in coupling strength with the Po (see prior paragraph)), starting from FCTS until 3 s following it. Furthermore, the cRTN started to guide (→) the VPM and VPM→the ATN. Among the cortico-thalamic pairs ctx6 and ctx5 increased their guidance of the ATN and ctx6 started to guide the caudal RTN at the point of FCTS (p's<0.05). For the latter cortico-thalamic pair this change in coupling direction persisted until 3 s after FCTS. Early changes in coupling direction for the intra-cortical pairs were already seen 1.875 s prior to FCTS; cortical layer 4 (strongly) increased its guidance upon ctx5 and ctx6. In both cases, however, coupling direction abruptly reversed at the point of FCTS with
ctx5 and ctx6 guiding ctx4 in the majority of SWDs until the end of the analysis window (3 s after FCTS) (p's<0.05) (Fig. 3D).

**Fig. 3.** Changes in coupling direction seen during the pre-ictal→ictal transition period expressed as the percentage of all analyzed SWD for which either structure A guides structure B or vice versa. Filled circles indicate significantly differences as compared to non-epileptic control periods (first data value indicated by a 'c' on the x-axis). A: temporal increase in cortical guidance at SWD generalization (a representative example of cortico-thalamic channel pairs). Channel pair ctx6–VPM is representative displayed for ctx4–VPM, ctx5–ATN, ctx6–ATN, ctx6–Po and ctx4–Po; note that channel pair ctx5–Po is an exception to this keeping a bidirectional crosstalk with the cortex (see text for details) (data include 12 rats and 120 SWD); B: Persistent change in guidance pattern starting at SWD generalization (a typical picture seen for the intra-thalamic channel pairs) (channel pair ATN–Po is representative displayed for VPM–Po, rRTN–Po and ATN–VPM) (data include 10 rats and 100 SWD); C: caudal and rostral RTN showing an earlier change in guiding pattern occurring prior to SWD generalization (data include 8 rats 80 SWD); D: complex intracortical changes in guiding pattern: prior to FCTS cortical layer 4 guides the deeper layers but at SWD generalization the reverse is the case (data include 16 rats, 160 SWD). Abbreviations: c: non-epileptic control period, ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, cRTN: caudal reticular thalamic nucleus, Po: posterior thalamic nucleus, VPM: Ventral–Postero-Medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
Time-delays during the pre-ictal—ical transition period
Changes in time delays (see Table 3 for summary) were seen between all except two (ctx4–Po, ctx5–VPM p's>0.05) cortico-thalamic channel pairs (all p's<0.01) independent of whether they also showed SWD related increases in coupling strength h²(τ) and or changes in coupling direction. In general a gradual increase of time-delays could be seen, which started to appear prior to FCTS and earlier than the changes found in coupling strength and/or coupling direction (timedelay increases were seen at a mean of 1.077 s prior to FCTS with a range between 2 s prior to FCTS to 500 ms prior to FCTS) (Fig. 4A). These increased time-delays were present until the end of the analysis window. The only exception was seen for channel pair ctx5–Po that showed a temporary decrease of its time delay at around FCTS (Fig. 4B). Intrathalamic pairs: 50% (Table 3) showed also significant increases in time-delays (p's<0.01). They started to occur only around FCTS and remained present until the end of the analysis window (Fig. 4C). Timeshifts of the intra-cortical pairs ctx6–ctx4 and ctx5–ctx4 gradually started to increase at 1 s prior to FCTS and 375 ms prior to FCTS, respectively and also continued to remain present until the end of the analysis window.

Table III: Summery of significant changes in preictal -> ictal transition period. Indicated is time point of change: early, late, at first cortico-thalamic spike (FCTS), prior to FCTS, earlier than h²(τ) changes; direction of change: ↑increase, ↓decrease; direction of coupling between brain structures: structure A -> (guiding) structure B

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Fig. 4. Changes in time-delays found during the pre-ictal–ictal transition period. Filled circles indicate significantly different values as compared to non-epileptic control periods (first data value indicated by a ‘c’ on the x-axis): A: typical thalamo-cortical channel pair showing increases in time-shifts before changes in coupling strength or coupling direction (Channel pair ctx6–VPM is representative displayed for ctx4–VPM, ctx4–cRTN, ctx6–cRTN, ctx4–rRTN, ctx4–ATN, ctx5–ATN, ctx5–cRTN, ctx5–rRTN, ctx6–ATN, ctx6–Po, ctx6–rRTN) (data include 12 rats and 120 SWD); B: temporal decrease of time-shifts between cortical layer 5 and posterior nucleus (data include 14 rats and 140 SWD); C: typical intrathalamic increase of time-shifts starting at around SWD generalization (Channel pair cRTN–rRTN is representative displayed for ATN–rRTN, VPM–cRTN, Po–ATN and Po–VPM) (data include 12 rats and 120 SWD) (data include 8 rats and 80 SWD). Abbreviations: c: non-epileptic control period, ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: Ventral–Postero-Medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
Discussion

This study investigated the dynamics of cortico-thalamo-cortical interactions at the transition from pre-ictal to ictal LFP recordings in a genetic model of absence epilepsy. For this aim changes in the degree of coupling between brain structures $h^2(\tau)$, the direction of coupling and the signal delay time between brain structures (time-shifts) during a six-second lasting pre-ictal→ictal transition period were sketched in contrast to non-epileptic control periods. The midpoint of our transition period was marked by the first epileptic spike (sharp spike of at least twice the background LFP) which was visible in all cortical and thalamic recordings and was followed by rhythmic SWD activity (first cortico-thalamic spike or FCTS). We regard this timepoint as moment of “generalization” or start of “full blown” cortico-thalamic SWD, although the existence of earlier, more subtle, synchronous activity in cortex and thalamus which is not manifested in the waveform of the local field potential cannot completely be excluded since no multiple or single cell unit recordings are done in parallel.

Nevertheless, by measuring an extended part of the corticothalamic system including the deep layers of the perioral-region of the somatosensory-cortex (the local instigator zone of SWDs) and several important (based on anatomical, lesion, neurophysiological and modeling studies) thalamic nuclei with a high temporal resolution, this study was able to reveal detailed and exact network interactions relevant for the generation and maintenance of SWDs and to disentangle the relative contribution of different brain-structures.

Generation and generalization of SWD, the role of the cortex

Analysis of the extended network revealed that SWD related increases in coupling-strength between cortex and thalamus can already be observed up to 1.25 s prior to the first cortico-thalamic spike of SWDs. At this point the spike–wave activity is not yet seen in the thalamus, whereas localized rhythmic activity can sometimes already be noted in layers 4 to 6 of the somatosensory-cortex. At the same time point as SWD activity generalizes to the thalamus and a full blown cortico-thalamic SWD arises (at FCTS), these increases in coupling-strength can be seen to become accompanied by changes in coupling-direction at FCTS. At this time-point of generalization the cortex guides the thalamus for all except one (see discussion in the next paragraph) thalamic nuclei that show SWD related increases in coupling-strength. This cortical guidance was restricted to the first 500–750 ms after FCTS whereas thereafter cortex and thalamus returned to a bidirectional crosstalk. The cortical guidance at the onset of SWDs is in good agreement with our earlier findings (Meeren et al., 2002). Whether the moment of SWD generalization to the thalamus (FCTS) also corresponds to the moment of bilateral cortical generalization still needs to be investigated since in this study only unilateral recordings within different layers of the somatosensory cortex were obtained.
Earlier SWD-related changes in coupling-direction were only seen in the local intra-cortical circuit. Layer 4 massively guides layers 5 and 6 as early as 1.875 s prior to FCTS. This and the already high intracortical coupling-strength between the cortical layers might explain why sometimes localized cortical SWD activity can be seen prior to FCTS. Alternatively it might indicate that the deep layers receive an increased amount of input from layer 4 prior to SWD occurrence. Most likely, peripheral input might be necessary for the occurrence of SWD since it has been demonstrated that temporary deactivation of the trigeminal-nerve went along with a decrease in SWD (Abbasova et al., 2010). At FCTS, however, coupling-direction again abruptly changes to guidance of layers 5 and 6. These intracortical dynamics preceding SWDs might represent the dominance of especially these deep layers in SWD generation.

In summary, we demonstrate the early involvement of the deep layers in SWD generation and show that they are the initial force for SWD generalization to the thalamus. These results are in agreement with the cortical focus theory of absence epilepsy (Lüttjohann et al., 2011; Meeren et al., 2002; Pinault, 2003; Polack et al., 2007; van Luijtelaar and Sitnikova, 2006; van Luijtelaar et al., 2011b).

The special role of the posterior thalamic nucleus

Since the time-course of SWD related changes differed for the various thalamic nuclei it might be inferred that the thalamic nuclei also differ in their contribution to SWD occurrence. The VPM has been considered as the primary thalamic target for SWD activity in previous network-studies (Meeren et al., 2002; Sitnikova and van Luijtelaar, 2006; Sitnikova et al., 2008). The extended network analysis in the current study highlights the importance of the Po nucleus, instead. This nucleus not only shows the earliest increase in coupling-strength with the cortex but also one of the strongest increases. For the VPM on the other hand increases in coupling strength only took place at around FCTS, together with the majority of cortico-thalamic and thalamo-thalamic pairs, and were only of moderate degree.

The special role of the Po nucleus is also highlighted by the fact that this nucleus is the only one that shows SWD related increases in coupling-strength with cortical layer 5 and stays in bidirectional crosstalk with this layer within the first 500 ms after FCTS. It seems that cortex and Po guide each other. This bidirectional crosstalk might be crucial for the generation of a full blown cortico-thalamocortical oscillation (SWD) in the genetic absence models, since lesion studies indicate that an intact cortico-thalamic network, which can provide/function as a resonance circuitry, is needed for the occurrence of SWD (Avoli and Gloor, 1981; Meeren et al., 2009; Sitnikova and van Luijtelaar, 2004). In this study, during the first 500 ms of the SWD, such a closed-loop resonatory circuit is only seen/present between layer 5 of the
somatosensory cortex and the Po nucleus and this demonstrates the unique position of the Po in the pre-ictal period and in the first 500 ms of a SWD in spreading oscillations to the cortex. Interestingly, all thalamic nuclei start to guide the Po from FCTS onwards and remain doing this throughout the whole SWD. This suggests an important role for the Po in “channeling” the thalamic output to the cortex and also in synchronizing thalamic and cortical oscillations considering the widespread cortical projections of the Po.

Other closed-loop circuits between cortex and thalamus (re)appear following the first 500 ms, demonstrating an increase in bidirectional crosstalk between cortex and thalamus in the phase of SWD maintenance. The extension of the crosstalk between cortex and thalamus indicates that other thalamic nuclei now take part in guiding the cortex. It can also be observed that the Po remains guided during the whole SWD by all other thalamic nuclei while keeping its bidirectional crosstalk to the cortex. This suggests that the Po keeps on “channeling and synchronization” the thalamic output to the cortex throughout the duration of the SWD, and thereby supporting the maintenance of SWDs. Selective lesioning of the various afferents of the Po might provide an answer to their role in both spreading and maintenance of SWDs.

In all prior theoretical and computational models on the origin and spreading of SWDs the Po nucleus has only received little to no attention. This seems rather surprising considering its large and widespread cortico-thalamo-cortical projections (Sherman, 2005a). Earlier EEG mapping and noninvasive imaging studies only demonstrated its involvement in SWD occurrence (Nersesyan et al., 2004; Tenney et al., 2004; Vergnes et al., 1990), whereas some researchers (Kostopoulos, 2001; Polack et al., 2007) already speculated that SWD activity generated in layer 5 and 6 of the somatosensory-cortex might primarily spread to the thalamus via layer 5 axonal projections which are known to terminate in Po and speculated about the Po as a optimal candidate for the spreading and generalization of the SWD rhythm given its diffuse projections to superficial cortical areas. The outcomes of this study support the hypothesis of Polack et al. (2007; 2009) and Kostopoulos (2001) and emphasize an early and necessary role of the Po in the occurrence of “fully generalized” cortico-thalamic SWDs.

The role of the VPM, by contrast, seems only to be related to the maintenance of SWDs, given its time point in the occurrence of SWD and the modest degree in coupling increase. Questions about the main role of the VPM were also raised by the outcomes of a study which found that local, bilateral infusion of Ethosuximide in the VPM has only weak and delayed effects on SWD occurrence (Richards et al., 2003). Likewise Polack et al. (2009), these authors recorded intracellularly in VPM and Po neurons, reported that VPM neurons only showed single action potential firing during SWDs, while Po neurons, displaying a larger hyperpolarization could trigger burst of action potentials. Whether application of
Ethosuximide to the Po has stronger effects than in the VPM on SWD occurrence needs to be investigated. In the human condition, yet, only one single fMRI study has compared the time course of SWD involvement of different thalamic nuclei (Tyvaert et al., 2009). These authors demonstrated a relatively late SWD involvement of the anterior thalamic nucleus as compared to centromedian and parafascicular nucleus using fMRI-haemodynamicresponse-function and concluded that the ATN seems only to have a maintenance function for SWDs. In our study the ATN also only seems to have a minor role. This nucleus did not show increases in coupling-strength with the cortex. At FCTS it only showed changes in coupling direction (became guided by the cortex) and an increase in coupling with other thalamic nuclei. It thus might be concluded that the ATN is only passively/secondarily involved in SWD activity.

This sparse involvement can be seen to be in line with prior lesion studies, in which lesions to the ATN did not affect SWD occurrence in GAERS (Vergnes and Marescaux, 1992).

**Opposite role of caudal- and rostral-RTN**

Whereas the rostral-RTN showed strongest increases in coupling strength with Po at FCTS, the caudal-RTN was the only nucleus, for which a decrease in coupling with Po was found. This uncoupling of the caudal-RTN was seen as one of the earliest changes in $h^2(\tau)$ at 1,125 s prior to FCTS. Interestingly, immediately thereafter (875 ms prior to FCTS) the caudal-RTN started to guide the rostral-RTN, which was accompanied by an increase in coupling-strength at FCTS. It seems that the rostral- and caudal-RTN have opposite roles for SWD occurrence. This seems in agreement to outcomes of pharmacological studies in GAERS and lesion studies in WAG/Rij rats (Aker et al., 2006; Meeren et al., 2009). The latter authors found an increase in SWD activity after lesions to the ventral–lateral part of the thalamus, including the caudal-RTN (Meeren et al., 2009). Temporal deactivation of the caudal-RTN via bicuculline increased SWDs (Aker et al., 2006), but led to a decrease after temporal inhibition of the rostral-RTN or lesions to the lateral-thalamus including the rostral-RTN (Berdiev et al., 2007; Meeren et al., 2009). Results of the current study describing the network behavior of both RTN subparts at the transition from pre-ictal to ictal LFPs may help to explain these results. Our data hint towards the following scenario: in the unlesioned state the rostral-RTN might be engaged in hyperpolarizing thalamo-cortical cells (as seen in the increase in $h^2(\tau)$ between rRTN–Po and rRTN–ATN). This makes them more prone to fire in a burst like fashion and to rhythmically respond to the cortical volleys typical for cortical elicited SWDs. The inhibitory action of the caudal-RTN on the other hand is redirected to the rostral-RTN, which may dampen the synchronizing effect of the rRTN onto the thalamus. In case of caudal-RTN damage this dampening effect is missing, resulting in an increase in SWD...
activity. Oppositely, rostral-RTN lesion results in a decrease of thalamic synchronization. As a consequence thalamo-cortical cells are less hyperpolarized and less prone to burst firing in reaction to cortical volleys, resulting in less or shorter SWDs.

**Time delays and vigilance**

Lastly, next to changes in coupling strength and coupling direction also changes (mainly increases) in time delays were found to occur during the pre-ictal—ictal transition period. Since large scale structural changes are not likely to occur within a few seconds, changes in time shifts need to be interpreted as indicating a phase shift between signals of different brain structures. Given the fact that most of the changes already took place before the changes in coupling strength and were also found in channel pairs, for which no significant change in \( h^2(\tau) \) was found, these phase shifts might be due to changes in neuronal firing patterns accompanying changes in the level of vigilance. It is well known that an intermediate level of vigilance is a beneficial condition for the occurrence of SWDs (Drinkenburg et al., 1991; van Luijtelaaar and Bikbaev, 2007). We established also that SWDs were indeed preceded by both cortical and thalamic delta precursors closely co-occurring in time (van Luijtelaaar et al., 2011a).

Changes in vigilance throughout the pre-ictal period could theoretically also explain part of the early increases in coupling strength since about 40% of SWD tend to occur during light sleep (Drinkenburg et al., 1991). However, considering that these early changes were seen only for some channel-pairs and not in all, and since vigilance related changes might be present in all channel pairs, it is rather likely that the early changes in coupling strength can be considered as pre-ictal activity.

Given the properties of the non-linear association analysis being an amplitude based method in the time domain, it remains to be answered, whether the found changes in time-shifts or in the phase relationships between signals also indicate a change in synchronization. Put into other words: Are the cortex and thalamus getting more ‘in phase’ with each other? Others investigating spectral properties of periods before and during SWD (Sitnikova et al., 2009) or of the neuronal firing pattern (Seidenbecher and Pape, 2001) already detected an increase in the gamma frequency range of two thalamic nuclei preceding the start of SWD. Changes in frequency spectra of the LFPs might also be a potential underlying explanation for changes in \( h^2 \). Since the non-linear association analysis does not selectively focus on the predominant SWD frequency of 8 to 10 Hz, it is also relevant to see whether \( h^2 \) changes are attributable to spectral changes inside or outside of the SWD relevant frequency range. The usage of different signal analytical tools of neurophysiological data obtained in a large network such that interactions between brain structures can be investigated might reveal important and
interesting additional information on the generation, generalization and maintenance of SWDs. In any study correlating monopolarly derived signals one has to be aware of the risk of volume conduction. In our study, however, the volume conducted component appears to be relatively small as indicated by the variability in the pattern of coupling direction. Both during control periods as well as during SWD (except for the first 500 ms) coupling direction constantly changed between cortex and thalamus, whereas for volume conduction a more stable pattern would be expected. Moreover, we compared mainly changes within channel pairs (although not exclusively) and changes within a pair are not influenced by volume conduction.

In summary, this study demonstrated the following:
- SWD related changes in local field potentials can be found more than a second prior to SWD generalization.
- At the same time point as SWD activity generalizes to the thalamus and a full blown cortico-thalamic SWD arises (at FCTS), the increases in coupling-strength can be seen to become accompanied by changes in coupling-direction with the cortex driving the thalamus.
- The Po nucleus seems the most important of the thalamic nuclei recorded in the occurrence of SWD while other nuclei only seem to have a maintenance function.
- Time point of involvement in the SWD differs between thalamic nuclei.
- Rostral- and caudal-RTN have opposite roles in SWD occurrence

References


Chapter 7

Peri-ictal network dynamics of spike-wave discharges:
Phase and spectral characteristics

Published as
Abstract

Purpose: The brain is a highly interconnected neuronal assembly in which network analyses can greatly enlarge our knowledge on seizure generation. The cortico-thalamo-cortical network is the brain-network of interest in absence epilepsy. Here, network synchronization is assessed in a genetic absence model during 5 s long pre-ictal->ictal transition periods.

Method: 16 male WAG/Rij rats were equipped with multiple electrodes targeting layer 4 to 6 of the somatosensory-cortex, rostral and caudal RTN, VPM, anterior-(ATN) and posterior (Po) thalamic nucleus. Local field potentials measured during pre-ictal->ictal transition and during control periods were subjected to time-frequency and pairwise phase consistency analysis.

Results: Pre-ictally, all channels showed spike-wave discharge (SWD) precursor activity (increases in spectral power), which were earliest and most pronounced in the somatosensory cortex. The caudal RTN decoupled from VPM, Po and cortical layer 4. Strong increases in synchrony were found between cortex and thalamus during SWD. Although increases between cortex and VPM were seen in SWD frequencies and its harmonics, border spectral increases (6–48 Hz) were seen between cortex and Po. All thalamic nuclei showed increased phase synchronization with Po but not with VPM.

Conclusion: Absence seizures are not sudden and unpredictable phenomena: the somatosensory cortex shows highest and earliest precursor activity. The pre-ictal decoupling of the caudal RTN might be a prerequisite of SWD generation. Po nucleus might be the primary thalamic counterpart to the somatosensory-cortex in the generation of the cortico-thalamic-cortical oscillations referred to as SWD.

Keywords Cortico-thalamo-cortical system; Network interactions; Pairwise-phase-consistency; Time-frequency-analysis; Epileptic precursor activity; Reticular-thalamic-nucleus; Posterior thalamus; Somatosensory cortex; Spike-wave discharges; WAG/Rij rats

Abbreviations SWD: spike-wave-discharges; ctx4: layer 4 of the somatosensory cortex; ctx5: layer 5 of the somatosensory cortex; ctx6: layer 6 of the somatosensory cortex; ATN: anterior thalamic nucleus; Po: posterior thalamic nucleus; VPM: ventral-postero-medial nucleus of the thalamus; cRTN: caudal reticular thalamic nucleus; rRTN: rostral reticular thalamic nucleus; FCTS: first cortico-thalamic spike; PPC: pairwise-phase-consistency; TFA: time-frequency analysis.

Introduction

Childhood absence epilepsy is a neurological disorder found in children between the ages of 5 to 12. Its main clinical symptom is the frequent occurrence of periods of reduced consciousness and unresponsiveness which are accompanied by concomitant, highly synchronous, bilateral spike and wave discharges (SWD), recorded in the electroencephalogram (Depaulis and van Luijtelaar, 2006). For a long time, absence seizures have been categorized as a primary generalized form of seizures (ILAE, 1989) occurring unpredictably and simultaneously in the whole brain. However, with advances in techniques it becomes more and more apparent that all kinds of seizures, including absence seizures, can better be regarded as occurring in (selective) rapidly interacting brain networks, currently termed ‘system epilepsy’ (Berg et al., 2010; Capovilla et al., 2009).
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The study of such seizure networks can greatly enlarge our knowledge on seizure generation. It can help to elucidate the mechanisms of communication between and within brain networks involved in seizure occurrence (how different brain regions communicate (interact) during seizure generation) and might show whether there are crucial brain regions (hubs) and loops that are more important than others.

One frequently suggested way of neuronal communication is through coherent brain oscillations (coherence) (Buzsaki, 2006). Coherence estimates the phase-stability between two signals across trials and might therefore be seen as a measure of neural synchrony (Vinck et al., 2010).

For absence epilepsy, strong cortico-thalamic coherence and neuronal firing phase locked to the rhythmic spike and wave cycle was described during SWD (Avoli et al., 1983; Pinault and O'Brien, 2005; Seidenbecher et al., 1998; Vergnes et al., 1989), but few studies investigated coherence during the periictal transition phase. In GAERS (genetic absence epileptic rats from Strasbourg) SWD emerge from 5 to 9 Hz sensory-motor like oscillations. These type of oscillations emerge from a desynchronized background EEG and show a medium to strong cortico-thalamic coherence, indicating a gradual process of neuronal synchronization accompanying SWD generation (Pinault and O'Brien, 2005; Pinault et al., 2001, 2006). On the other hand, these oscillations are not sufficient for the generation of SWD since non-epileptic control rats also possess these oscillations.

Sitnikova and van Luijtelaar (2006) compared unilateral, cortical, local, and long range intrahemispheric coherence with corticaltranshemispheric, unilateral intrathalamic, and unilateral corticothalamic coherence in genetic absence-epileptic WAG/Rij rats (Depaulis and van Luijtelaar, 2006; van Luijtelaar and Sitnikova, 2006) and reported that each network shows different frequency specific changes with SWD occurrence. The coherence analysis in this study, however, was restricted to a comparison 1 s prior as compared to 1 s following SWD onset whereas subsequent studies showed that this pre-ictal interval already contains SWD related changes in the form of spectral delta-theta precursor activity in frontal cortex and VPM (Sitnikova and van Luijtelaar, 2009; van Luijtelaar et al., 2011a). Moreover, cortico-thalamic coherence, thought to be most relevant for SWD occurrence (Depaulis and van Luijtelaar, 2006), was only investigated between frontal cortex and thalamus rather than between the more important SWD-instigator zone in the somatosensory cortex and its recipients, the VPM, RTN and Po (Meeren et al., 2002).

In the current study the dynamics of coherent neuronal network activity and spectral power changes in a longer transition from the pre-ictal to the ictal state are investigated in an extended part of the cortico-thalamo-cortical network. This enables us to sketch a potentially
gradual developmental process of SWD, to investigate the relative contribution of different channel-pairs in SWD generation, and probably to detect (predictive) SWD precursor activity. For the spectral power, special attention is paid to the deep somatosensory cortex, where spectral precursor activity has not been studied before in this genetic model.

**Methods**

**Subjects**

16 male WAG/Rij rats, 6 to 9 months of age were used as experimental subjects. They were born and raised at the Department of Biological Psychology, Donders Centre for Cognition, Radboud University Nijmegen, The Netherlands. Prior to surgery rats were housed in pairs (High Makrolon cages with Enviro Dri® bedding material and cage enrichment) with free access to food and water and were kept at a 12–12 h light–dark cycle (lights off at 8.30 AM). After surgery rats were housed individually. The experiment was approved by the Ethical Committee on Animal Experimentation of Radboud University Nijmegen (RU-DEC). Efforts were made to keep the discomfort for the animals as minimal as possible.

**Surgery**

Implantation of the LFP recording electrodes was done in a stereotactic frame under isoflurane anesthesia. At the start of surgery, rats received a subcutaneous injection of the analgesic Rimadyl and an intramuscular injection of atropine to prevent excessive salivary production. Body temperature was controlled and conserved via a heating pad. The local anesthetic Lidocaine was used on the incision points. Holes were drilled into the skull on top of the right hemisphere for the insertion of recording electrodes at the following positions: Somatosensory cortex: A/P=0, M/L=−4.6 depth=−2.8 (layer 4), −3.1 (layer 5), −3.6 (layer 6); anterior thalamus: A/P=−1.4 M/L=−1, depth=−6.2; rostral RTN: A/P=−1.4, M/L=−1.9, depth=−6.6; posterior thalamic nucleus: A/P=−3.6, M/L=−2, depth=−5.4; VPM: A/P=−4.16, M/L=−2.8, depth=−6 and caudal RTN: A/P=−3.1, M/L=−3.5, depth=−6.6 (Fig. 1). All coordinates were determined relative to Bregma according to the rat-brain atlas of Paxinos and Watson (Paxinos and Watson, 1998). Electrode wires, assembled in a self-constructed electrode system (Lüttjohann and van Luijtelaar, 2012; van Luijtelaar et al., 2011b) were simultaneously inserted into the brain. Ground and reference electrodes were positioned epidurally on top of the cerebellum. The electrode assembly was fixed to the skull via dental cement. Postoperative analgesic Rimadyl (24 and 48 h after surgery) was administered and rats were allowed to recover for two weeks.
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Recording of local field potentials
Two weeks after surgery rats were placed individually in a 20×35×25 cm Plexiglas registration box and connected to the recording leads for multi-channel LFP recordings. These were attached to a swivel-contact, which allowed recording in freely moving animals. The LFP signals were amplified with a physiological amplifier (TD 90087, Radboud University Nijmegen, Electronic Research Group), filtered by a band pass filter with cut-off points at 1 (HP) and 100 (LP) and a 50 Hz Notch filter, and digitized with a constant sample rate of 2048 Hz by WINDAQ-recording-system (DATAQ-Instruments). Also the movements of the rat were registered by means of a Passive Infrared Registration system (PIR, RK2000DPC LuNAR PR Ceiling Mount, Rokonet). Each rat was recorded for a period of 4 h during the dark phase of the 12–12 LD cycle.

Signal analysis
A randomly chosen subset of 10 pre-ictal->ictal transition periods of 5 s duration were selected for each rat for signal analysis. As in an earlier study (Lüttjohann and van Luijtelaar (2012)), these 5-second epochs were centered around the occurrence of the first epileptic cortico-thalamic spike (FCTS). This is 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 (Fig. 2). 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-thalamic SWD (Lüttjohann and van Luijtelaar, 2012).

Selected epochs of non-epileptic activity were used as control data. For each rat 10 epoch of 500 ms duration were randomly selected during passive wakefulness distant (by at least 5 min) to SWD. Passive wakefulness is characterized by low amplitude, desynchronized, high frequency LFP signals recorded in a motionless rat. A major proportion of SWD tends to emerge during this state of vigilance (Drinkenburg et al., 1991).

Spectral properties (amplitude and phase) of LFP signals during transition and control periods were assessed via a time frequency analysis (TFA) using Hanning tapering. Since, for SWD, changes in connectivity are known to occur within timeframes as short as 500 ms (directional coupling from cortex to thalamus was found only during the first 500 ms of SWDs (Meeren et al., 2002)), assessment of spectral power in this study was also performed in 500 ms timeframes shifting along the pre-ictal->ictal transition period in steps of 50 ms. Given the size of the analysis window, frequency resolution was restricted to an accuracy of 2 Hz. TFA was performed for the frequency-range 2 to 60 Hz.

Coherent neuronal network activity (network communication) was quantified with pairwise-phase consistency analysis (PPC) (Vinck et al., 2010). The PPC metric quantifies the
consistency of the phase difference between two signals, across observations (SWD). It is a normalized measure, and it is bounded between 0 (signifying the lack of synchronization) and 1 (signifying perfect synchronization). In these respects the PPC metric resembles the well-known coherence coefficient. However, the coherence coefficient is a metric that is biased, where the size of the bias depends on the number of observations. This is not the case with the PPC metric, which makes it particularly suitable in a setting where the number of observations (SWD) is relatively low (and consequently the size of the estimation bias in the coherence is high). This difference in bias is due to a difference in the calculation of the estimates: Both coherence and PPC are calculated from the single trial cross-spectral densities, which quantify the phase difference of oscillations between a pair of signals at a given frequency. The cross-spectral density can be visualized in a 2-dimensional Cartesian plane as a vector from the origin, where the angle relative to the positive X-axis represents the phase difference. The coherence is computed from the length of the average cross-spectral density vector. This average is obtained by putting all single-trial cross-spectral density vectors head to tail, and dividing the resulting vector by the number of observations. In the presence of true phase synchronization (i.e. a consistent phase difference across observations), the single trial cross-spectral density vectors have a tendency to point in the same direction, yielding a large average cross-spectral density vector, and hence a large coherence coefficient. In the absence of true phase synchronization (i.e. the cross-spectral density vectors are pointing into random directions), the average cross spectral density vector will be much smaller, but will always have a non-zero value. Therefore, when trying to estimate the true coherence (which is 0 in the absence of phase synchronization) from a finite number of observations, the estimate will always be too high (hence biased). Moreover, the size of the bias will be inversely related to the number of observations, because one gets ‘closer to zero’ with the average of the cross-spectral densities when there are more observations.

The PPC is computed from the average angular distance between all pairs of cross-spectral density vectors. This means that for each pair of observations, the difference between the estimated phase difference is computed (yielding the angular distance), and the cosine of the angular distances is averaged across all pairs of observations. In the presence of true phase synchronization, the single-trial cross-spectral density vectors point into the same direction, and the pairwise angular distances are small (i.e. close to 0, and hence with a cosine close to 1), yielding a large PPC value. In the absence of true phase synchronization, the single-trial cross-spectral density vectors point into random directions, yielding a distribution of pairwise angular distances between 0 and 180°, with cosines between 1 and −1, yielding a small PPC value, because the negative cosines cancel the positive cosines in the average. It
was shown that this metric is not (or much less) biased by the number of observations (Vinck et al., 2010). For more detailed information on the PPC and coherence analysis and their bias properties the reader is referred to Vinck et al. (2010) and Maris et al. (2007).

PPC data as well as spectral power during pre-ictal and ictal periods were compared to control periods with the aid of a non-parametric cluster based permutation test (Maris and Oostenveld, 2007). This test has been shown to be a reliable statistical method for the analysis of neurophysiological data requiring comparison along multiple time and frequency bins, with efficient control of type II errors (Maris et al., 2007). Basically the following steps are performed:

1) First the datasets of condition 1 (transition period) and condition 2 (control period) are compared by Students' t-test. All datapoints (time-frequency points) reaching significant difference are grouped to a cluster based on temporal adjacency. For each cluster the sum of the T-values (test-statistic of the previous t-test) is calculated.

2) Next, the original time-frequency data-sets are randomly distributed to either condition 1 or 2. By this the difference between control and transition period is destroyed.

3) For each random distribution (in our case 500) step 1 is performed.

4) Lastly the summed t-values of a cluster (calculated in step 1) for the real data-set and the random distributions are displayed in a histogram.

In case the summed t-value computed from the real dataset is positioned below the 2.5-th or above the 97.5-th quantile of the histogram (pb0.05 two-sided), the real dataset deviates (with a certainty of 95%) from randomized datasets, which do not have a difference between control and transition period. The real cluster can thus be considered as representing a significant difference between control and transition period.

In addition to the permutation test, the percentage of pre-ictal periods showing power increases more than 2-times the background power within the delta and theta frequency range were registered for time frequency data. This operating scheme has been used earlier to quantify spectral SWD precursor activity (van Luijtenaar et al. (2011a)).

All statistical and signal analyses were performed with FieldTrip, an open-source Matlab-based toolbox for advanced analysis of e.g. electrophysiological data (Oostenveld et al., 2011).

Only data from brain structures with a histological verified proper electrode position were included in the statistical analysis (Fig. 1). To facilitate the finding of the location of the tip of the recording electrodes, a direct current (9 V, 25 μA, 10 s duration) was passed through each electrode in the deeply anesthetized rat at the end of the experiment. Next, rats were perfused with a potassiumferrocyanide–formaldehyde–phosphate solution, coloring these lesions at the end of each electrode tip. Brains were fixed in a 30% sucrose solution,
0.1 ml PBS, cut in 40 µm coronal slices with the aid of a microtome, and stained with Cresyl violet. Only electrodes for which the midpoint of the small lesion was located within the target structure were considered properly implanted and included in statistical analysis (Fig. 1).

**Results**

**Electrophysiological and spectral characteristics: the presence of precursor activities**

All rats showed SWD: mean 10 per hour, mean duration SWD 7 s. Rhythmic spike and wave activity in the typical frequency of 8–10 Hz was present in all recorded channels (Fig. 2a). Time-frequency spectra of all recorded channels showed highest power values in the SWD characteristic frequency band of 8–10 Hz during the ictal period (Fig. 2b). Frequency modulation from 10 to 12 Hz at the beginning of the SWD, quickly followed (within 1 s) by oscillations in the 7–8 Hz band was noticed (Bosnyakova et al., 2006). Somewhat less intense maxima in power were seen at the first (around 20 Hz) and sometimes second harmonic (around 30 Hz).

Non-parametric cluster based permutation test revealed that all channels showed a significant increase in power in the delta (2–4 Hz) and theta (6–12 Hz) frequency range as compared to the control period (all p's<0.005) during the pre-ictal interval (2.5 s prior to FCTS until FCTS). Channels, however, differed regarding their pattern of maximal pre-ictal power values (increases in power larger than 2-times the background power): Earliest and most pronounced pre-ictal power was noticed for the deep layers of the somatosensory cortex (all 16 rat, 160 SWD), with ctx5 showing delta and theta precursor activity simultaneously in 75% of pre-ictal periods. These started on average 2 s prior to FCTS (Fig. 2b). In thalamic recordings, on the other hand, maximal power values started at 0.75 s (range 0.25 to 1 s) prior to FCTS. Whereas for the cRTN (13 rats, 130 SWD) combined delta-theta precursor was most common (44.4% of SWD), VPM (12 rats, 120SWD) and Po (14 rats, 140 SWD) precursor activity was most often (54.7 and 53.2% of SWD) seen to be restricted to the delta band (see Fig. 2c for more details).
Fig. 2. Electrophysiological and spectral power characteristics of SWD: A: Exemplary multi-site local field potentials recording showing the transition from pre-ictal to ictal LFP recordings; black vertical bar indicates the time-point of the first cortico-thalamic spike (FCTS). B: Average time-frequency power spectra of the pre-ictal->ictal transition period seen for layer 5 somatosensory cortex as compared to ventral-postero-medial thalamic nucleus. Note that SWD precursor activity (prominent pre-ictal power values greater than 2 times the background power) is more pronounced in the somatosensory cortex than in the ventral-postero-medial thalamic nucleus. C: Pattern of SWD precursor activity found for different channel-pairs. Since only data from brain structures with a histologically verified proper electrode position were included in the analysis, channels vary with respect to the number of included animals and SWD: ctx4: 16 rats, 160 SWD; ctx5: 16 rats, 160 SWD; ctx6: 16 rats, 160 SWD; ATN: 12 rats, 120 SWD; Po: 14 rats, 140 SWD; VPM: 12 rats, 120 SWD; cRTN: 13 rats, 130 SWD; rRTN: 9 rats, and 90 SWD. Also note the different scaling of the power-scale. Abbreviations: ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: ventral-postero-medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
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**PPC during the pre-ictal period**

Four of the twenty-eight channel-pairs showed significant changes in PPC (Fig. 3, Table 1) during the pre-ictal period.

![Graphs showing significant changes in pairwise-phase-consistency (PPC) values during the pre-ictal period.](image)

**Fig. 3.** Significant changes in pairwise-phase-consistency (PPC) values during the pre-ictal period displayed as absolute difference between control, non-epileptic PPC value and pre-ictal PPC value (pre-ictal PPC minus control PPC). Non-significant differences are displayed as a zero (white) difference. Since only data from brain structures with a histologically verified proper electrode position were included in analysis, channel-pairs vary with respect to the number of included animals and SWD: ctx4-cRTN: 13 rats, 130 SWD; Po-cRTN: 13 rats, 130 SWD; VPM-cRTN: 9 rats, 90 SWD; rRTN-ctx5: 9 rats, and 90 SWD. Abbreviations: ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, Po: posterior thalamic nucleus, VPM: ventral-postero-medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
Interestingly, in all cases a reduction compared to non-epileptic control periods was found and in three out of these four pairs the caudal reticular thalamic nucleus was involved:

- At 1.2 s prior to FCTS, channel-pair cRTN-ctx4 (13 rats, 130 SWD) showed a significant reduction of 0.2 (p<0.025). PPC reduced from 0.35 (control) to 0.15 for frequencies around 20 Hz.
- Shortly thereafter (about 1 s prior to FCTS) a reduction from 0.5 (control) to 0.25 was found for channel-pair cRTN-Po (13 rats, 130 SWD) at the 20–30 Hz (p<0.025) and of 0.3 around 2 Hz (p<0.025).
- The third channel-pair showing a significant pre-ictal reduction was cRTN-VPM (9 rats, 90 SWD). For this pair, decreases were found in high frequencies but also in the SWD characteristic 10 Hz band. At 10–12 Hz a reduction of 0.22 (from 0.6 control to 0.38 pre-ictal) was seen 0.8 s prior to FCTS (p<0.025). At 14–20 Hz this channel-pair showed a reduction of 0.35 (p<0.025).
- Lastly, channel-pair rRTN-ctx5 (9 rats, 90 SWD) showed a significant decrease in pre-ictal PPC at 1.2 s prior to FCTS. PPC values dropped by 0.15 from 0.52 (control) to 0.37 (p<0.01). This decrease was seen around 4 Hz.

**PPC during the ictal period**

Most channel-pairs showed increases in PPC at the transition to the ictal period. This transition took place in a strong and rather abrupt fashion and was found to be channel-pair and frequency specific (Table 1):

**Cortico-thalamic channel-pairs:**

Earliest increases in PPC were found between all three layers of the somatosensory cortex and the posterior thalamic nucleus (all channel pairs 14 rats, 140 SWD). They started to occur 0.2 s prior to FCTS and lasted until the end of the analysis window (2.5 s following FCTS) (all p's<0.01). These early increases in PPC between cortex and Po were quickly followed by increases in PPC in channel-pairs ctx4-VPM, ctx5-VPM, ctx6-VPM, ctx4-ATN, ctx5-ATN and ctx6-ATN (all 12 rats, 120 SWD). For these channel-pairs, significantly higher PPC values as compared to control periods were reached between 0.15 s to 0.05 s prior to FCTS, depending on frequency (all p's<0.01), and also lasted until the end of the analysis window (2.5 s following FCTS). PPC increases of cortex-ATN and cortex-VPM channel-pairs were restricted to 10–12 Hz and its two harmonics.

Cortex-Po channel-pairs, on the other hand, demonstrated PPC increases in a much more extended frequency range spanning from 8 to 48 Hz (Fig. 4). The average absolute increase in PPC for these channel-pairs ranged from 0.3 to 0.65.
Fig. 4. Significant cortico-thalamic changes in pairwise-phase-consistency (PPC) values during the ictal period displayed as absolute difference between control, non-epileptic PPC value and ictal PPC value (ictal PPC minus control PPC). Non-significant differences are displayed as a zero (white) difference. Note the difference in the frequency-range displaying significant changes between ctx4-Po (representatively plotted for ctx5-Po and ctx6-Po) and ctx5-VPM (representatively plotted for ctx4-VPM, ctx6-VPM, ctx4-ATN, ctx5-ATN and ctx6-ATN). Since only data from brain structures with a histologically verified proper electrode position were included in the analysis, channel-pairs vary with respect to the number of included animals and SWD: ctx4-Po: 14 rats, 140 SWD; ctx5-VPM: 12 rats, and 120 SWD. Abbreviations: ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: ventral-postero-medial thalamic nucleus.

The two cortico-thalamic channel-pairs cRTN-ctx4 (13 rats 120 SWD) and rRTN-ctx5 (9 rats, 90 SWD), for which a decrease in PPC was seen in the pre-ictal interval, did not show significantly decreased PPCs in the ictal-period anymore. For both channel-pairs the decreased PPC values returned to control values, at 0.1 s and 0.45 s prior to FCTS respectively. Caudal RTN-ctx4 remained at its baseline level during the complete ictal period and did not show any increases in PPC during the ictal interval; rRTN-ctx5, by contrast, showed a significant increase in PPC around 20 Hz starting 0.1 s prior to FCTS (p<0.001). The same was found for channel-pair rRTN-ctx4 and rRTN-ctx6 (9 rats, 90 SWD and p's<0.02 for both). Similar to channel-pair cRTN-ctx4, channel-pairs cRTN-ctx5 and cRTN-ctx6 (all 13 rats, 130 SWD) also showed baseline PPC levels at the beginning of the ictal period. Only towards the end of the ictal period did both channel-pairs show an increase in PPC values in the SWD characteristic 10–12 Hz band, starting 1.5 and 1 s after FCTS respectively (both p's<0.025).
Table 1 Overview of significant changes in PPC per channel pair: indicated is either a significant increase (↑) or decrease (↓) in the pre-ictal and ictal period as compared to control period, and the frequencies for which this change is found. For details on the exact timing (onset and stop of changes see main text). Abbreviations: ctx4: layer 4 of the somatosensory cortex, ctx5: layer 5 of the somatosensory cortex, ctx6: layer 6 of the somatosensory cortex, ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: ventral-postero-medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.

<table>
<thead>
<tr>
<th>channel-pair</th>
<th>no. rat/SWD</th>
<th>pre-ictal</th>
<th>ictal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctx4-Po</td>
<td>14, 140</td>
<td>↑ 8-48Hz</td>
<td></td>
</tr>
<tr>
<td>ctx5-Po</td>
<td>14, 140</td>
<td>↑ 8-48Hz</td>
<td></td>
</tr>
<tr>
<td>ctx6-Po</td>
<td>14, 140</td>
<td>↑ 8-48Hz</td>
<td></td>
</tr>
<tr>
<td>ctx4-VPM</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>ctx5-VPM</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>ctx6-VPM</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>ctx4-ATN</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>ctx5-ATN</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>ctx6-ATN</td>
<td>12, 120</td>
<td>↑ 8-12Hz &amp; harmonics</td>
<td></td>
</tr>
<tr>
<td>cRTN-ctx4</td>
<td>13, 130</td>
<td>↓ 20Hz</td>
<td>back to baseline level</td>
</tr>
<tr>
<td>cRTN-ctx6</td>
<td>13, 130</td>
<td>late (at 1.5sec) ↑ 10-12Hz</td>
<td></td>
</tr>
<tr>
<td>rRTN-ctx4</td>
<td>9, 90</td>
<td>↑ around 20Hz</td>
<td></td>
</tr>
<tr>
<td>rRTN-ctx5</td>
<td>9, 90</td>
<td>↑ around 20Hz; at 4Hz back to baseline level</td>
<td></td>
</tr>
<tr>
<td>cRTN-rRTN</td>
<td>8, 80</td>
<td>late (2sec) ↑ 8-10Hz</td>
<td></td>
</tr>
<tr>
<td>cRTN-ATN</td>
<td>9, 90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cRTN-VPM</td>
<td>9, 90</td>
<td>↓ 10-12Hz &amp; ↓ 14-20Hz</td>
<td></td>
</tr>
<tr>
<td>cRTN-Po</td>
<td>13, 130</td>
<td>↓ 20-30Hz &amp; 2Hz</td>
<td></td>
</tr>
<tr>
<td>ATN-Po</td>
<td>10, 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rRTN-Po</td>
<td>9, 90</td>
<td>↑ 8-10Hz; 2, 20-30Hz back to control; new ↓ at 30-46Hz</td>
<td></td>
</tr>
<tr>
<td>VPM-Po</td>
<td>10, 100</td>
<td>↑ 8-10Hz</td>
<td></td>
</tr>
<tr>
<td>VPM-ATN</td>
<td>9, 90</td>
<td>↑ 10-12Hz &amp; harmonic</td>
<td></td>
</tr>
<tr>
<td>VPM-rRTN</td>
<td>7, 70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATN-rRTN</td>
<td>7, 70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctx4-ctx5</td>
<td>16, 160</td>
<td>↑ 8-48Hz</td>
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<tr>
<td>ctx4-ctx6</td>
<td>16, 160</td>
<td>↑ 8-48Hz</td>
<td></td>
</tr>
<tr>
<td>ctx5-ctx6</td>
<td>16, 160</td>
<td>↑ 8-48Hz</td>
<td></td>
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</tbody>
</table>

Intra-thalamic channel-pairs:
The two intra-thalamic channel-pairs that already showed a decrease in PPC in the pre-ictal period again showed changes in PPC when entering the ictal period: Channel-pair cRTN-VPM (9 rats, 90 SWD) returned from its decreased pre-ictal PPC value to baseline levels for the 10–16 Hz band, while keeping its decreased pre-ictal PPC values at 20 Hz. In addition, a decrease from 0.6 (control) to 0.25 (ictal) was found for 30 to 40 Hz (all p's<0.025).

Channel-pair cRTN-Po (13 rats, 130 SWD) also returned its decreased pre-ictal PPC values for 20–30 Hz towards control, and switched towards decreased PPC values (baseline 0.4 to ictal=0.2) for the higher (30–46 Hz) frequencies (p<0.01). However, this channel-pair also suddenly showed increases in PPC (0.5 control to 0.8 ictal) in the 8–10 Hz band starting at 0.035 s prior to FCTS (p<0.01). Besides the cRTN, ATN and rRTN also showed an increase in PPC with the Po (ATN-Po: 10 rats, 100 SWD; rRTN-Po: 9 rats, 90 SWD) (8–12 Hz band, all p'sb0.01) (Fig. 5).
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Fig. 5. Significant thalamo-thalamic changes in pairwise-phase-consistency (PPC) values during the ictal period displayed as absolute difference between control, non-epileptic PPC value and ictal PPC value (ictal PPC minus control PPC). Non-significant differences are displayed as a zero (white) difference. Note that the Po shows increased PPC-values with all thalamic nuclei except VPM, which does not couple with any other thalamic nuclei. Since only data from brain structures with a histological verified proper electrode position were included to analysis, channel-pairs vary with respect to the number of included animals and SWD: Po-VPM: 10 rats, 100 SWD; Po-cRTN: 13 rats, 130 SWD; Po-rRTN: 9 rats, 90 SWD; Po-ATN: 10 rats, 100 SWD; VPM-cRTN: 9 rats, 90 SWD; VPM-rRTN: 7 rats, 70 SWD; VPM-ATN: 9 rats, and 90 SWD. Abbreviations: ATN: anterior thalamic nucleus, Po: posterior thalamic nucleus, VPM: ventral-postero-medial thalamic nucleus, cRTN: caudal reticular thalamic nucleus, rRTN: rostral reticular thalamic nucleus.
The only thalamic nucleus that did not show an increase in PPC with Po was the VPM (VPM-Po: 10 rats, 100 SWD). During the ictal period the VPM only showed increased PPC values with the three layers of the somatosensory cortex (all 12 rats, 120 SWD) (p's<0.025), but not with any other thalamic nucleus (all p's>0.05) (Fig. 5).

Lastly, ATN-rRTN (7 rats, 70 SWD) and rRTN-cRTN (8 rats, 80 SWD) showed increased PPC values (8–10 Hz) that started to occur at 0.5 s and 2 s after FCTS respectively (p's<0.025). No changes in PPC were found for channel pair cRTN-ATN (9 rats, 90 SWD) (p>0.05).

**Cortico-cortical channel-pairs:**

All intra-cortical channel-pairs (all 16 rats, 160 SWD) showed abrupt increases in PPC, from control values of around 0.7 to 0.9 ictally, within the frequency range of 8 to 48 Hz (all p's<0.01). Significant changes were seen somewhat earlier for the higher frequencies (0.1 s prior to FCTS, 20–48 Hz) as compared to lower frequencies (8–20 Hz). Increased PPC values were maintained until the end of the analysis window (2.5 s following FCTS).

**Discussion**

The study of network interaction has the potential to unravel crucial changes in the communication between brain structures, which underlie/go along with the generation of generalized absence seizures. In the current study, dynamics of spectral power and phase characteristics were described for pre-ictal->ictal transition periods within an extended part of the cortico-thalamo-cortical system of absence epileptic WAG/Rij rats, including the deep layers of the perioral-region of the somatosensory-cortex (the local instigator zone of SWDs) and several important (based on anatomical, lesion, neurophysiological and modeling studies) thalamic nuclei. PPC analysis (Vincx et al., 2010) was used to study the role of coherent neuronal network activity, a proposed mechanism of neuronal communication (Buzsaki, 2006; Schnitzler and Gross, 2005), in the generation of SWD. Time-frequency analysis was used to study changes in spectral power properties and to assess the presence of SWD precursor activity. For the latter analysis, special attention was paid to the somatosensory cortex (SWD instigator zone), where spectral precursor activity was not studied before in this genetic model. The combination of both analyses enabled us to draw the following conclusions regarding the generation of SWD:

**Network communication during the pre-ictal period: decoupling of the caudal reticular thalamic nucleus**

A remarkable result of the current study was that the pre-ictal period was characterized by decreases in PPC, it was found in 4 out of 28 channel-pairs. Here, the cRTN is especially
noteworthy: it decouples from Po, VPM and ctx4. A pre-ictal decrease in (long-range) synchrony as compared to interictal periods was also reported in patients with partial seizures and absence seizures, which was seen several seconds prior to SWD onset (Amor et al., 2009; Le Van Quyen, 2005; Mormann et al., 2003a,b). These authors gave three different suggestions on how to interpret such a pre-ictal desynchronization:

1) desynchronization might provide an ‘idle’ population of neurons, that can be recruited into the epileptic process more easily; 2) desynchronization might reflect a depression of synaptic inhibition and 3) desynchronization might isolate the epileptic focus from regulatory influences of other brain structures and thereby facilitate local pathological recruitment (Le Van Quyen, 2005).

The decoupling of the caudal RTN found in the current study provides support for the first and second suggestion: The reticular thalamic nucleus is known to strongly regulate the activity of thalamo-cortical and cortico-thalamic relay cells via its GABAergic projections, and is often regarded/described as an attentional gate or spotlight (Coenen, 1995; Crick, 1984; Guillery et al., 1998; Pinault, 2004). It might be that only when the inhibitory regulation of the cRTN onto the thalamo-cortical system is gone (disinhibition), the local cortical instigator zone has the chance to entrain the thalamus into SWD activity. The decoupling from the Po might especially be seen as crucial. This nucleus has been proposed to be strongly involved in the generation of SWD given early and strong increases in coupling (as measured with non-linear association analysis) with the cortical focus (Lüttjohann and van Luijtelaraar, 2012).

Less evidence is provided for the third possibility; which would imply a gradual increase in local cortical PPC indicating a local preparation/recruitment towards enhanced excitability, which is only possible when the cortical zone is isolated from surrounding influences. Such a state has been reported in the human condition several hundred milliseconds prior to SWD onset in whole head MEG studies (Amor et al., 2009; Gupta et al., 2011). A local preparation state, as found in patients, might not be necessary but cannot be excluded. Electrical evoked potential studies in this model demonstrated that the deep layers of the somatosensory cortex show an increased excitability as compared to motor cortex and healthy control animals independent of alertness (Lüttjohann et al., 2011). However, it is not known whether the local excitability changes in the pre-ictal period, as would have been predicted by interpretation possibility 3; single trial evoked potentials preceding the onset of SWD might be an option to investigate putative dynamics of cortical excitability.

Next to the three interpretation possibilities proposed in human studies (see above) we want to propose a fourth possibility on how decoupling of the caudal RTN might facilitate SWD occurrence: Decoupling of the caudal RTN might change the way in which external stimuli reach the hyperexcitable somatosensory cortex. Such external inputs are imperative
for SWD occurrence: Abbasova et al. (2010) accomplish a complete abolishment of SWD by pharmacological blockage of the peripheral trigeminal nerve. Therefore, decoupling of the cRTN might change trigeminal input into an effective trigger for a somatosensory ‘hyperdischarge’. Especially the decoupling of the cRTN from cortical input layer 4 and the VPM, which is the primary relay nucleus of incoming somatosensory stimuli, might be in favor of this latter interpretation since disinhibition allows a better transfer of information through the thalamus.

Independent of the four different possibilities of interpretation, we propose that the decoupling of the cRTN might be a prerequisite for SWD occurrence; it prepares the cortico-thalamo-cortical network towards a pro-epileptic state. Earlier pharmacological studies by Aker et al. (2006), and a lesion study by Meeren et al. (2009), demonstrating an increase in SWD activity after deactivation of the cRTN, which might be seen as an artificially created decoupling of the cRTN from all other thalamic nuclei, strongly support this conclusion. In the same line of reasoning, it would be interesting to investigate whether the cRTN is also involved in the disruption or termination of an ongoing SWD. Based on the above proposed role of the cRTN, as having a dragging effect towards epileptic oscillations, one might expect an increase in PPC between cRTN and cortex towards the end of the SWD. The late ictal increase between cRTN and ctx5/6 found in the current study as well as earlier work in slices demonstrating that the RTN is involved in the regulation of SWD duration (Sohal et al., 2003) are in line with this idea. In addition, in in vivo experiments changes in the intercellular communication within the RTN via gap junctional coupling were shown to influence SWD duration (Proulx et al., 2006). Detailed network investigations focusing on the end of SWD might further enlarge our knowledge on this topic.

It is theoretically possible that parts of the pre-ictal decreases in PPC can be attributed to changes in vigilance, given the preference of SWD to occur in an intermediate level of alertness (Drinkenburg et al., 1991; Smyk et al., 2011). It is, however, more likely that the decoupling of the cRTN is a phenomenon that is specific to SWD generation. In favor of this argument is the channel-pair specificity of PPC decreases (vigilance related changes might be less specific). Also, the subtle increases in power (see the Electrophysiological and spectral characteristics: the presence of precursor activities section) and their temporal proximity to the FCTS are in favor of our view that they are SWD related and not vigilance related. In addition, the similar pre-ictal desynchronization in humans could even be used for automated seizure prediction with a specificity up to 100% (Mormann et al., 2003a,b).

There was no clear increase in PPC between cortex and thalamus in the 2–3 s pre-ictally; strong increases in phase synchronization in the SWD's frequency band and its
harmonics occurred 100 ms before FCTS. Results from the GAERS model of absence epilepsy, showing medium to strong cortico-thalamic coherence during their SWD preceding 5–9 Hz oscillations (Pinault and O'Brien, 2005), suggest that the cortico-thalamo-cortical system also prepares toward a pro-epileptic state by facilitating and alleviating network communication between epileptic focus and given thalamic counterparts. Non-linear association analyses (amplitude coupling) of cortico-cortical network in WAG/Rij rats also revealed gradual changes preceding the onset of FCTS by about 1 s (Meeren et al., 2002). Likewise, a pre-ictal increase in amplitude coupling between cortical focus and the posterior thalamic nucleus, together with changes in time delays (Lüttjohann and van Luijtelaar, 2012), hinted towards a gradual increase in cortico-thalamic network communication prior to SWD onset. Apparently these increases in cortico-thalamic and thalamo-cortical coupling in WAG/Rij rats are not established via linear, stable, phase relationship between signals — but might be of non-linear nature (which remain undetected by a linear method like PPC). Another option is that they might better be explained by early changes in spectral power (see Fig. 2 and the SWD are not sudden and unpredictable phenomena section), rather than by changes in phase relationships as measured with PPC. Moreover it is possible that these pre-ictal changes finally result in the rhythmic cortical and thalamic SWD activity and that only then stable phase-locked rhythmic activity reflected by high PPC values, can be seen.

In all, we propose that the decoupling of the caudal RTN might play a crucial part in the generation of SWD. It prepares the cortico-thalamo-cortical network for a pro-epileptic state which starts with decoupling of the cRTN. Pre-ictal decoupling is in good agreement with the descriptions of a pre-ictal long-range desynchronization in humans with absence seizures (Amor et al., 2009).

**Frequency and channel specific ictal synchronization**

Changes in ictal PPC were found to be channel-pair and frequency specific. This might indicate differences with respect to the relative contribution of channel-pairs in SWD generation and generalization. In general two noticeable observations could be made:

a) For channel-pairs ctx6-Po, ctx5-Po, and ctx4-Po, synchronization was seen in a huge frequency range (8–48 Hz), whereas other cortico-thalamic channel-pairs only showed increased PPC values in the SWD specific 8–10 Hz band and its harmonics. This broad spectral synchronization can be linked to the sharpness of the spikes in both cortex and posterior nucleus. Sharp spikes in the local field potential of SWD are built up by a collection of high frequencies with a similar and constant phase difference. Physiologically, this implies that a huge number of neurons in posterior thalamus and cortex fire in a synchronous burst
like fashion. Such a firing pattern is generally believed to be a mechanism of neuronal synchrony and to favor epileptic oscillations (Blumenfeld, 2003). Alternatively, the sharp spikes of the Po nucleus might be the result of a more symmetrical orientation of Po cells, as compared to other thalamic nuclei, which results in strong open field dipoles, easily picked up by the recording electrode. The outcome of studies by Polack et al. (2009), showing Po neurons to be more likely to fire in bursts, phase locked to the spike components of SWD, as compared to VPM cells, and by Cavdar et al. (2012) reporting an increase of so called driver terminals in the posterior nucleus of absence epileptic GAERS rats as compared to healthy Wistar control rats, might be seen to be in line with an important role for the Po in the occurrence of the widespread cortico-thalamo-cortical oscillations named SWD.

A differential role of the different thalamic nuclei with respect to the occurrence of SWD was also proposed based on the outcomes of fMRI studies in patients (Tyvaert et al., 2009).

b) Next to this broad synchronization with the deep somatosensory cortex, the Po increased its ictal phase consistency to all other thalamic nuclei. One exception to this was the VPM, which did not show increased PPC values with any other thalamic nuclei and only shows ictal increases in PPC with the cortex. It seems that the VPM stays in a separate loop with the cortical focus whereas the Po takes the role of the major thalamic (resonating) counterpart that takes the responsibility/function of “channeling and synchronization” the thalamic output to the cortex and therefore greatly supports the maintenance of SWDs. This was also proposed in an earlier study by Lüttjohann and van Luijtelaar (2012), who found that the Po was guided by other thalamic nuclei but kept a bidirectional crosstalk with the cortex. This also implies a relatively minor role of the VPM in SWD occurrence/maintenance as compared to the higher order Po nucleus. It provides/relays the initial, necessary input to the cortical focus, but does not seem to play the primary role in cortico-thalamic resonance feedback. Such a relatively minor role of VPM in SWD occurrence was also indicated by a study of Richards and colleagues (Richards et al., 2003), showing that local injections of Ethosuximide in the VPM/VPL were not very effective in suppressing SWDs; and by studies of Lerescue et al. (2012) and Polack et al. (2009) showing that the VPM only rarely fires in a burst-like fashion during SWD. Indeed it is known that the cortico-thalamic neurons from somatosensory cortex layer 6 to VPM are so called ‘modulators’, which are defined by their action via slow postsynaptic metabotropic receptors and showing weak/broad input–output firing correlograms, indicating weak responsiveness to cortical input. The Po, by contrast, primarily receives so called driver inputs from somatosensory layer 5, which are defined by their action via fast acting ionotropic receptors and showing a sharp peak in input–output firing correlograms indicating strong responsiveness to cortical input (Sherman and Guillery, ...
1998, 2002). Whether local application of Ethosuximide to the Po might be more effective to suppress SWD needs to be investigated.

In summary it can be concluded that the Po might be important for the occurrence and generalization of SWD. We propose that this nucleus might be the primary thalamic counterpart to the somatosensory cortex in the generation of cortico-thalamic oscillations of SWD, which is engaged in “channeling and synchronization” of the thalami output to the cortex and thereby supporting the maintenance of SWD.

**SWD are not sudden and unpredictable phenomena**

It is now firmly established that SWD in rodent models are not sudden and unpredictable events. Changes in network communication (see the Network communication during the pre-ictal period: decoupling of the caudal reticular thalamic nucleus section) and spectral (power) SWD precursor activity were found in the current study. Earlier studies reported early, SWD preceding, enhanced firing of neuron in the deep layers of the somatosensory cortex of GAERS rats (Polack et al., 2007), cortical long range desynchronization in humans (Amor et al., 2009) and spectral power changes in humans and animals (Inouye et al., 1992, 1994; Sitnikova and van Luijtelaar, 2009). Rather detailed precursor activity was found in LFP recordings of frontal cortex and VPM of WAG/Rij rats, which were uniquely related to the occurrence of SWD (van Luijtelaar et al., 2011a).

Time frequency analysis in the current study was performed to validate the existence of such precursor activity (in spectral power) in an extended part of the cortico-thalamo-cortical system with special attention to the deep layers of the somatosensory cortex (the instigator zone of SWD). Looking at this extended network, precursor activity was found to be channel specific. Strong precursor activity was found in the deep layers of the somatosensory cortex. As would be predicted by the cortical focus theory of absence epilepsy, this precursor activity started earlier in the deep somatosensory cortex than in the thalamic recordings. Precursor activity in the cortex and caudal RTN was mostly a combination of delta–theta activity. VPM and Po, on the other hand, predominantly showed precursor activity restricted to the delta band. Whether these channel specific changes in spectral power also affect network communication remains to be investigated by e.g. cross correlation of spectral power.

All in all, the early pre-ictal changes in spectral power (current study, Inouye et al., 1994; Inouye et al., 1992; Sitnikova and van Luijtelaar, 2009; van Luijtelaar et al., 2011b), changes in network communication (current study; Lüttjohann and van Luijtelaar, 2012; Amor et al., 2009; Westmijse et al. (2009); Gupta et al., 2011) and neuronal firing (Polack et al., 2007) raise the possibility of SWD prediction and an early intervention prior to generalization from cortex to thalamus.
A proposed scenario for SWD generation and generalization

Based on results in the current study and earlier studies in genetic rodent models of absence epilepsy, we propose the following scenario for the generation and generalization of SWD as occurring in WAG/Rij rats:

- The caudal RTN decouples from three structures (VPM, ctx4, Po) that are all part of the somatosensory cortico-thalamo-cortical network. The decoupling prepares the network for a proepileptic state (current study). The decoupling of the cRTN has two consequences which might be prerequisites for SWD generation:

  Prerequisite 1 -> Decoupling between cRTN-Po, creates ‘idle’ Po neurons. In this way the cortical focus can increase its communication with the Po and can quickly entrain it in SWD activity. This probably takes place in a nonlinear fashion (Lüttjohann and van Luijtelaar, 2012).

  Prerequisite 2 -> Decoupling of cRTN from VPM and input layer ctx4 changes the way external stimuli reach the hyperexcitable somatosensory cortex. Input to the deep cortical layers of the somatosensory cortex can then function as an initializing trigger to elicit oscillations in the 7–12 Hz range, triggering local SWD. This local activity is picked up by the posterior thalamic nucleus and involves the thalamus (see prerequisite 1).

The Po functions as a thalamic reverberator, it keeps a bidirectional crosstalk to the cortex (Lüttjohann and van Luijtelaar, 2012) and may spread SWD activity to remote cortical areas via its widespread connections to the cortex (Kostopoulos, 2001; Polack et al., 2007; Sherman and Guillery, 2002). Furthermore it might be involved in “channeling and synchronization” the thalamic output to the cortex and therefore greatly supporting the maintenance of SWD (current study+Lüttjohann and van Luijtelaar, 2012).

On the validity of extrapolation of findings in animal models to the human condition

It is crucial to ask how far results on SWD generation from animal models can be extrapolated to the human condition since SWD of rats and humans can easily be seen to differ from each other in terms of their fundamental frequency (2.5–4 Hz in humans vs. 7–11 Hz in rats). Modeling experiments, however, show that this difference can either simply be explained by differences in brain size (with longer axons and dendrites of C–T and T–C cells in humans as compared to rats and thus longer signal-conduction times) (Roberts and Robinson, 2008), or by a difference in GABA-ergic conductance profiles within the cortico-thalamo-cortical system (in species with a GABAB conduction dominance showing 2.5–4 Hz SWD and species with a GABAA conduction dominance showing 6–10 Hz SWD) (Destexhe, 1999).
In general the genetic rat models like the WAG/Rij rat, used in this study, and GAERS rats, are the best characterized models for absence epilepsy in humans and are shown to possess a high face and predictive validity as reviewed in detail in (Marescaux et al., 1992; Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006; van Luijtelaar et al., 2011b).

In terms of brain networks, it can be noted that for children with childhood absence epilepsy also, more and more evidence accumulates that SWD are not a 'primary generalized' type of seizure but that there are local, cortical, susceptible regions that functions as the initiator of SWD and then rapidly entrains selective corticothalamo-cortical networks to form a resonator circuitry for SWD generalization and maintenance (Gupta et al., 2011; Tyvaert et al., 2009; Westmijse et al., 2009). It needs to be pointed out, however, that the location of this susceptible zone in children was found to be located in the parietal and frontal cortex and not in the somatosensory cortex as is the case for the genetic rat models. Consequently, the exact secondarily involved thalamic nuclei might also be different.

The basic principles, however, as outlined in detail for the WAG/Rij rat in the A proposed scenario for SWD generation and generalization section, are likely to be the same or at least similar. A pre-SWD change in the inhibitory and excitatory balance in the connected brain network (decoupling of the RTN), enables a situation of increased communication between a susceptible zone and its anatomically connected thalamic counterparts (prerequisite 1). It enables that also a trigger-input can reach this susceptible zone (prerequisite 2) starting a localized epileptic discharge that is picked up and generalized by the thalamic counterpart. Whether in the human condition this thalamic counterpart is also a higher order thalamic nucleus (as the Pn found for the WAG/Rij rat) needs to be investigated. Interestingly, a recent imaging study (Tyvaert et al., 2009) inspecting activation patterns of different thalamic nuclei in children with childhood absence epilepsy reported early increases of the fMRI bold signal for the centromedian–parafascicular nuclei (two higher order nuclei) as compared to a later activation of the anterior nucleus. Likewise, the RTN is reported to monitor and affect all reciprocal communications between thalamus and cortex and plays a crucial role in all theoretical models (an exception is the bicuculline model in cats, Steriade) on how absences are generated (Guillery et al., 1998; Huguenard and McCormick, 2007; Meeren et al., 2005). However, our data suggest a more complex and even an opposite role for the caudal part of the RTN as was previously assumed. Certainly, however, future translational studies, preferably with improved non-invasive imaging and source localization methods, are desirable to establish network mechanisms in patients.

In summary: via combined investigation of dynamics of spectral power in individual channels and network communication (synchrony/ stability of phase relationships) between
channels in the corticothalamo-cortical system of absence epileptic WAG/Rij rats as seen during SWD generation, the following conclusions can be drawn:

- SWD do not occur sudden but are preceded by changes in network communication and spectral (power) precursor activity of pre-ictal LFPs.
- Spectral precursors are clearly present in the instigator zone of SWD, which is in good agreement with the cortical focus theory of absence epilepsy.
- A pre-ictal decoupling of the cRTN with other thalamic nuclei and cortex might be a prerequisite for SWD generation, which prepares the cortico-thalamic-cortical network in a pro-epileptic state.
- The posterior thalamic nucleus shows strongest (all frequencies) and widest (to all other channels) increases in network synchrony and might therefore be regarded as a key player for SWD generation and maintenance.

References


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Chapter 8

Cortico-thalamo-cortical changes of Granger Causality at on- and offset of spontaneous Spike-Wave Discharges

Lüttjohann A., Schoffelen JM., van Luijtelaar G. (submitted)
Abstract

Purpose Coupling-dynamics at the onset and termination of spontaneous spike and wave discharges (SWD) were studied in an extended part of the cortico-thalamo-cortical system of freely moving, genetic absence epileptic WAG/Rij rats.

Methods Local-field potential recordings of 16 male WAG/Rij rats, equipped with multiple electrodes targeting layer 4 to 6 of the somatosensory-cortex, rostral and caudal RTN, VPM, anterior-(ATN) and posterior (Po) thalamic nucleus, were obtained. Six seconds lasting pre-SWD->SWD, SWD->post SWD and control periods were analyzed with time-frequency methods and between-region interactions were quantified with frequency-resolved Granger Causality (GC) analysis.

Results Most channel-pairs showed increases in GC lasting from onset to offset of the SWD. While for most thalamo-thalamic pairs a dominant coupling direction (stronger increases in GC for direction A->B as compared to direction B->A) was found during the complete SWD, most cortico-thalamic pairs only showed a dominant directional drive (always from cortex to thalamus) during the first 500ms of SWD. Channel-pair ctx4-rRTN showed a longer lasting dominant cortical drive, which stopped 1.5 sec prior to SWD offset. This early decrease in directional coupling was followed by an increase in directional coupling from caudal RTN to rostral RTN 1 sec prior to SWD offset. For channel pairs ctx5-Po and ctx6-Po the heightened cortex->thalamus coupling remained until 1.5 sec following SWD offset, while the thalamus->cortex coupling for these pairs stopped at SWD offset.

Conclusion The high directional coupling from somatosensory cortex to the thalamus at SWD onset is in good agreement with the idea of a cortical epileptic focus that initiates and entrains other brain structures into seizure activity. The decrease of cortex to rRTN coupling as well as the increased coupling from caudal RTN to rostral RTN preceding SWD abortion demonstrate that SWD termination is a gradual process that involves both cortico-thalamic as well as intrathalamic processes. The rostral RTN seems to be an important resonator for SWD and relevant for maintenance, while the caudal RTN might inhibit this oscillation. The somatosensory cortex seems to attempt to reinitiate SWD following its offset via its strong coupling to the posterior thalamus.

Keywords Cortico-thalamo-cortical system; network interactions; Granger causality; reticular-thalamic-nucleus; posterior thalamus; somatosensory cortex; spike-wave discharges; WAG/Rij rats; genetic models; seizure termination

Abbreviations SWD: spike-wave-discharges; ctx4: layer 4 of the somatosensory cortex; ctx5: layer 5 of the somatosensory cortex; ctx6: layer 6 of the somatosensory cortex; ATN: anterior thalamic nucleus; Po: posterior thalamic nucleus; VPM: ventral-poster-medial nucleus of the thalamus; cRTN: caudal reticular thalamic nucleus; rRTN: rostral reticular thalamic nucleus; FCTS: first cortico-thalamic spike; LCTS: last cortico-thalamic spike; GC: Granger Causality; TFA: time-frequency analysis.

Introduction

The major electrophysiological characteristic of absence epilepsy are the rhythmic, generalized, bilateral synchronous spike and wave discharges (SWD). The occurrence of SWD is dependent on an intact cortico-thalamo-cortical system. Despite decades of debate, on whether the cortex or the thalamus is the initiator of SWD, it is today more and more assumed, that a focal zone triggered cortical input to the various parts of the thalamus, including the posterior nucleus and the sensory regions, elicits both excitation and inhibition.
during the SWD, the latter via feed-forward synaptic inhibition of thalamo-cortical cells via the reticular thalamic nucleus (RTN) (Steriade, 1998, Meeren et al., 2002, Pinault and O’Brien, 2005, Huguenard and McCormick, 2007). The cortical onset zone (a hyperexcitable epileptic focus) in the genetic absence models such as WAG/Rij and GAERS (Marescaux et al., 1992, Coenen and Van Luijtelaar, 2003, Depaulis, 2006) is in the deep layers of the facial somatosensory cortex (Meeren et al., 2002, Polack et al., 2007, Polack et al., 2009). Next to several independent indications of an selectively altered excitability (van Luijtelaar et al., 2011)) this region shows an increase in rhythmic burst firing shortly before SWD onset (Polack et al., 2007) and LFP recordings of both SWD and their precursor rhythm (in GAERS) are found to start earlier in this focal area as compared to other adjacent and distant cortical and thalamic sites (Meeren et al., 2002, Pinault and O’Brien, 2005, Polack et al., 2009, Zheng et al., 2012). Most importantly, using non-linear association analysis, an analysis with which not only the strength of coupling but also the direction of coupling can be inferred (Lopes da Silva et al., 1989, Pijn, 1990), it was shown, that the focal cortical zone drives all other recorded cortical and thalamic sites during the first 500 ms of a SWD, whereas thereafter coupling direction alternates between cortex and VPM and VPL (Meeren et al., 2002). Recently is was established that this is not the case for the posterior thalamic nucleus (Po): the association strength between the deep layers of the somatosensory cortex and Po increased already 1.25 seconds prior to SWD onset and cortex and Po kept a bidirectional crosstalk during the complete SWD. It was argued that the Po is the only thalamic nucleus (recorded in this study), which is able to respond/give feedback to the epileptic focus within the first 500 ms of an SWD, which might be crucial for the generation of cortico-thalamo-cortical SWD (Lüttjohann and van Luijtelaar, 2012).

In contrast to SWD generation, relatively little attention has been paid to the investigation of network interactions associated with the spontaneous termination of SWD: Sitnikova et al (2008) studied the interaction between the frontal cortex and VPM with a non-frequency resolved linear estimation of Granger Causality (GC) during transition periods surrounding the onset and offset of SWD. They reported a gradual decrease in cortico-thalamic coupling, which started prior to the end of SWD and a strong thalamo-cortical coupling which remained present throughout the SWD. It was proposed that the reduced influence of the cortex on the thalamus might be related to the cessation of SWD, whereas the thalamus is not much involved in SWD termination.

Sohal et al (2003) as well as Proulx et al. (2006), by contrast, proposed the thalamus (RTN) to be involved in the control of SWD duration. They showed that selective pharmacological manipulations, which effect the intra RTN communication, significantly shortened SWD duration.
Furthermore, also arousal regulatory structures that send afferents to the cortico-thalamo-cortical system, such as the substantia nigra, the locus coeruleus or the nucleus basalis of Meynert might be involved in SWD termination (Danöber et al., 1998, Berdiev and van Luijtelaar, 2009).

In the current study local field potentials of pre-SWD -> SWD and SWD -> post-SWD transition periods are obtained from an extended part of the cortico-thalamo-cortical system of absence epileptic WAG/Rij rats (including the deep layers of the somatosensory cortex and multiple relevant thalamic nuclei) and signals are analyzed by a frequency resolved Granger Causality analysis. Like the non-linear association analysis (Lopes da Silva et al., 1989, Pijn, 1990) Granger Causality is a directed connectivity analysis, which tries to infer causal relationships between signals. It is aimed to validate previous network results (reported above) on SWD generation using another ‘directed’ connectivity analysis and to describe changes in network interactions associated with the termination of SWD. The latter analysis might reveal whether there is a dominant structure (cortex or a thalamic nucleus) that drives the termination of a SWD.

2. Methods
2.1 Subjects

16 Male WAG/Rij rats, 6 to 9 month of age were used as experimental subjects. They were born and raised at the department of Biological Psychology, Donders Centre for Cognition, Radboud University Nijmegen, The Netherlands. Prior to surgery rats were housed in pairs (High Makrolon® cages with Enviro Dri® bedding material and cage enrichment) with free access to food and water and were kept at a 12-12h light-dark cycle (light off at 8.30AM). After surgery rats were housed individually. The experiment was approved by the Ethical Committee on Animal Experimentation of Radboud University Nijmegen (RU-DEC). Efforts were made to keep the discomfort for the animals as minimal as possible.

2.2 Surgery

Implantation of the LFP recording electrodes was done in a stereotactic frame under isoflurane anesthesia. At the start of surgery, rats received a subcutaneous injection of the analgesic Rimadyl® and an intramuscular injection of atropine to prevent excessive salivary production. Body temperature was controlled and conserved via a heating pad. The local anesthetic Lidocaine was used on the incision points. Holes were drilled into the skull on top of the right hemisphere for the insertion of recording electrodes at the following positions: Somatosensory cortex: A/P=0, M/L=-4.6 depth= -2.8 (layer 4), -3.1 (layer 5), -3.6 (layer 6);
anterior thalamus: A/P=-1.4, M/L=-1, depth=-6.2; rostral RTN: A/P=-1.4, M/L=-1.9, depth=-6.6; posterior thalamic nucleus: A/P=-3.6, M/L=-2, depth=-5.4; VPM: A/P=-4.16, M/L=-2.8, depth=-6 and caudal RTN: A/P=-3.1, M/L=-3.5, depth=-6.6 (Figure 1). All coordinates were determined relative to Bregma according to the rat-brain atlas of Paxinos and Watson (1998) Electrode wires, assembled in a self-constructed electrode system (van Luijtelaar et al., 2011, Lüttjohann and van Luijtelaar, 2012, Lüttjohann et al., 2013) were simultaneously inserted into the brain. Ground and reference electrodes were positioned epidurally on top of the cerebellum. The electrode assembly was fixed to the skull via dental cement. Postoperative analgesic Rimadyl® (24 and 48 hours after surgery) was administered and rats were allowed to recover for two weeks.

2.3 Recording of local field potentials

Two weeks after surgery rats were placed individually in a 20x35x25 inch Plexiglas registration box and connected to the recording leads for multi-channel LFP recordings. These were attached to a swivel-contact, which allowed recording in freely moving animals. The LFP signals were amplified with a physiological amplifier (TD 90087, Radboud University Nijmegen, Electronic Research Group), filtered by a band pass filter with cut-off points at 1 (high pass) and 100 (low pass) and a 50 Hz Notch filter, and digitized with a constant sample rate of 2048 Hz on a WINDAQ recording system (DATAQ-Instruments). The movements of the rat were registered by means of a Passive Infrared Registration system (PIR, RK2000DPC LuNAR PR Ceiling Mount, Rokonet). Each rat was recorded for a period of 4 hours during the dark phase of the light-dark cycle.

2.4 Signal analysis

A randomly chosen subset of 10 pre-ictal -> ictal transition periods and 10 ictal -> post ictal transition periods of 6 seconds duration were selected for each rat for signal analysis.

As in earlier studies (Lüttjohann and van Luijtelaar, 2012, Lüttjohann et al., 2013) the onset of the SWD in the pre-ictal -> ictal transition period was represented by the occurrence of the first epileptic cortico-thalamic spike (FCTS). This is 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 (see for example Figure 1 or (Lüttjohann and van Luijtelaar, 2012, Lüttjohann et al., 2013)). Likewise the last sharp spike of at least twice the background LFPs, visible in all cortical and thalamic recordings, which is preceded by rhythmic SWD activity, was defined as moment of SWD offset (LCTS) (Figure 1).
Selected epochs of non-epileptic activity were used as control data. For each rat ten epochs of 500 ms duration were randomly selected during passive wakefulness distant (by at least five minutes) to SWD. Passive wakefulness is characterized by low amplitude, desynchronized, high frequency LFP signals recorded in a motionless rat. A major proportion of SWD tend to emerge during this state of vigilance (Drinkenburg et al., 1991).

Ten epochs (per rat) of 500 ms duration, taken in the middle of an SWD, functioned as a ‘stable SWD’ control.

Since, for SWD, changes in connectivity are known to occur within timeframes as short as 500 msec (Meeren et al., 2002), signals were analyzed in timewindows of 500 ms, shifting along the transition periods in steps of 125 ms. First, spectral decomposition was performed using a multi-taper approach (Percival and Walden, 1993) in the frequency range of 2 to 60 Hz. Given the size of the analysis window, the frequency resolution was restricted to an accuracy of 2 Hz. Next, frequency resolved Granger Causality was computed using non-parametric spectral factorization (Dhamala et al., 2008a, b), as implemented in FieldTrip (Oostenveld et al., 2011), for all cortico-thalamic and thalamo-thalamic channel pairs. In general, GC investigates whether the prediction of signal A based on its own past can be improved by also including information of the past of signal B. In this way GC is assumed to detect causal influences between two signals. In the current experiment a causal influence or directional coupling was only inferred if GC values for the coupling direction A->B were significantly higher than for coupling direction B->A or the other way around. It is assumed that changes (improvements) in the signal to noise ratio (SNR) occurring with SWD onset are equally strong in cortex and thalamus, so that such unequal increases in GC cannot be attributed to unequal changes in SNR, but indicate a true directional drive. An increase (e.g. non-epileptic control vs. SWD) of GC of equal strength for both coupling directions (A->B and B->A) is regarded as an increase in ‘bidirectional’ coupling without a dominant, guiding structure.

Statistical comparison of GC values between coupling directions (A->B vs B->A) was done with a non-parametric cluster based permutation test (Maris and Oostenveld, 2007). This test has been shown to be a reliable statistical method for the analysis of neurophysiological data requiring comparison along multiple time and frequency bins, with efficient control of type II errors (Maris et al., 2007). The same statistical test was used to compare GC values of

- pre-ictal -> ictal transition periods to non-epileptic control periods,
- ictal -> post-ictal transition periods to non-epileptic control periods and
- ictal -> post-ictal transition periods to ‘stable SWD’ control periods.
All statistical and signal analyses were performed with FieldTrip, an open-source Matlab-based toolbox for advanced analysis of e.g. electrophysiological data (Oostenveld et al., 2011).

Only data from brain structures with a histologically verified proper electrode position were included in the statistical analysis (Table 1). To facilitate the finding of the location of the tip of the recording electrodes, a direct current (9V, 25µA, 10sec duration) was passed through each electrode in the deeply anaesthetized rat at the end of the experiment. Next, rats were perfused with a potassiumferrocyanide - formaldehyde - phosphate solution, coloring these lesions at the end of each electrode tip. Brains were fixed in a 30% sucrose solution, 0.1 ml PBS, cut in 40 µm coronal slices with the aid of a microtome, and stained with Cresyl violet. Only electrodes for which the midpoint of the small lesion was located within the target structure were considered properly implanted and included in statistical analysis.

| Table 1: Results of histological verification of electrode position. X indicates correctly located electrode. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| ctx4 | ctx5 | ctx6 | ATN | Po | VPM | cRTN | rRTN |
| Rat1 | x | x | x | x | x | x | x | x |
| Rat2 | x | x | x | x | x | x | x | x |
| Rat3 | x | x | x | x | x | x | x | x |
| Rat4 | x | x | x | x | x | x | x | x |
| Rat5 | x | x | x | x | x | x | x | x |
| Rat6 | x | x | x | x | x | x | x | x |
| Rat7 | x | x | x | x | x | x | x | x |
| Rat8 | x | x | x | x | x | x | x | x |
| Rat9 | x | x | x | x | x | x | x | x |
| Rat10 | x | x | x | x | x | x | x | x |
| Rat11 | x | x | x | x | x | x | x | x |
| Rat12 | x | x | x | x | x | x | x | x |
| Rat13 | x | x | x | x | x | x | x | x |
| Rat14 | x | x | x | x | x | x | x | x |
| Rat15 | x | x | x | x | x | x | x | x |
| Rat16 | x | x | x | x | x | x | x | x |
3 Results

3.1 Spike and Wave Discharges, electrophysiological observations

All rats showed SWD’s, mean 10 per hour, mean duration SWDs 7 seconds. Rhythmic spike and wave activity in the typical frequency of 8-10 Hz was present in all recorded channels. SWD-like activity could be seen by visible inspection earlier in the cortex than in the thalamus in 20% of the rats. In these rats the local start of SWD activity preceded the thalamic involvement by up to 1 second (see van Luijtelaar et al., 2011b for figure). In addition, in about 20% of rats SWD activity was found to terminate about 1 second earlier in the thalamic recordings as compared to the cortical recordings (see van Luijtelaar et al., 2011b for figure).

3.2 Cortico-thalamic changes in Granger causality (GC) at SWD onset and SWD offset

All cortico-thalamic pairs showed low GC (values between 0.01 and 0.05) during the non-epileptic control as well as during the pre-ictal period. During the complete SWD all channel pairs showed a significant increase in GC as compared to control (all p’s<0.025), which was the case for both coupling directions (see Figure 1 for an example).

![Figure 1](image_url)

**Figure 1** Exemplary local-field potential (LFP) recording of a pre-SWD->SWD and SWD->post-SWD transition period displayed for channel combination somatosensory cortex layer 4 (ctx4) – Posterior thalamic nucleus (Po). The black arrows indicate the moment of SWD onset and SWD offset. On each side of the arrow 3 seconds of either ictal or non-ictal LFP signals are displayed (upper panel). The lower panel shows the average (across 14 rats and 140 SWD) values of Granger Causality for the same channel pair found for the coupling direction ctx4 -> Po.
For most cortico-thalamic pairs (ctx6-VPM, ctx5-VPM, ctx6-Po, ctx4-Po, ctx6-ATN, ctx5-ATN, ctx5-cRTN) (Table 2) a higher GC was seen for the coupling direction cortex->thalamus as compared to coupling direction thalamus->cortex (all p-values<0.025), but this directional coupling was restricted to the onset (first 500 ms following FCTS) of the SWD (Figure 2a). Whereas for channel-pairs ctx6->ATN, ctx5->ATN, ctx5->cRTN this increase was found for the higher (20-40 Hz) frequencies, for the other channel pairs (ctx6->VPM, ctx5->VPM, ctx6->Po, ctx4->Po) this increase was seen for the low (4-12 Hz) frequencies including the typical SWD frequency range of 8-10 Hz. Channel pair ctx4->ATN showed higher GC values for the coupling direction cortex->thalamus in the low frequencies, which was restricted to the onset of SWD. In addition, this channel pair (ctx4-ATN) also showed higher GC-values for the coupling direction cortex->thalamus in the frequency range 20-50 Hz, which was not restricted to SWD onset but was seen persistently during the entire SWD. Channel pairs ctx4->cRTN, and ctx4->VPM showed a persistent (during the entire SWD) higher GC values for the coupling direction cortex->thalamus in the high frequencies (20-50 Hz) (Figure 2b).

Channel pair ctx4-rRTN showed a higher directional GC value for the cortex->thalamus, which was persistently present throughout the entire SWDs in the 20-50 Hz frequency range. In addition, this was the only cortico-thalamic channel pair for which a persistent (not restricted to SWD onset) dominant drive from the cortex was also seen in the SWD frequency band 6-12 Hz. However, it only remained present until 1.5 second prior to SWD offset (Figure 3a). The lack of a higher cortex->thalamus coupling during the last 1.5 sec as compared to thalamus->cortex coupling was due to a decrease of cortex->thalamus coupling rather than an increase in the thalamus->cortex coupling. This was verified by significant difference between GC in SWD transition period as compared to stable SWD-control period which started to occur at the same time-point, whereas for all other channel-pairs this only started to be significant at SWD offset.
Figure 2 Typical pattern of directional coupling (one dominant guiding (->) structure) seen for most cortico-thalamic and thalamo-thalamic channel pairs. A directional coupling is inferred only if Granger Causality of one coupling direction is significantly higher than for the other; these timepoints are displayed in red. Note that for most cortico-thalamic channel pairs a dominant guiding structure (the somatosensory cortex) was only seen at SWD onset (panel A), whereas for most thalamo-thalamic pairs a dominant guiding structure was found continuously throughout the SWD (panel C). For channel pair ctx4-ATN, ctx4-cRTN and ctx4-VPM a continuous directional drive was found for high frequencies (panel B). Abbreviations: ctx4 and ctx6: layer 4 or 6 of somatosensory cortex; ATN: Anterior thalamic nucleus; VPM: Ventral-Postero-Medial thalamic nucleus; rRTN: rostral Reticular Thalamic Nucleus.
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Figure 3 Special patterns of directional coupling (one dominant guiding (->) structure) seen during the SWD->post SWD transitional period. A directional coupling is inferred only if Granger Causality of one coupling direction is significantly higher than for the other; these timepoints are displayed in red. A: Channel pair ctx4->rRTN was the only channel pair for which the cortex constantly drove the thalamus within the SWD relevant 8-12 Hz frequency band. In addition it is the only pair for which an early (1.5 sec prior to SWD offset) decrease in GC values is seen. B: Channel pair cRTN->rRTN is the only channel pair for which an increase in directional coupling is seen shortly prior to SWD offset. C: Channel pair ctx5->Po and ctx6->Po are the only channel pairs where an increase in directional coupling is seen immediately following SWD offset. See text for more details. Abbreviation: ctx4 = layer 4 of somatosensory cortex; ctx5 = layer 5 of somatosensory cortex; rRTN = rostral Reticular Thalamic Nucleus; Po = Posterior Thalamic Nucleus.
Most cortico-thalamic channel pairs showed low (no significant difference as compared to non-epileptic control periods) GC values during the post-ictal period, similar to the pre-ictal and control period. The only two exceptions were channel pair ctx6-Po and ctx5-Po: they showed higher directional cortex->thalamus coupling 0.25 to 1.5sec following the offset of SWD (Figure 3c). This higher cortex->thalamus coupling as compared to thalamo->cortical coupling could be attributed to the fact that the thalamo-cortical coupling decreased to baseline valued following the SWD offset, whereas the cortex->thalamus coupling remained on an increased GC level. It can be noted that the latter channel pair (ctx5-Po) is not only special regarding its GC pattern in the post ictal period. In contrast to most channel pairs (as described above) ctx5-Po does not show a higher cortex->thalamus directional coupling as compared to thalamus->cortex coupling at SWD onset, but GC coupling for both directions was equally strong (bidirectional).

3.3 Thalamo-thalamic changes in Granger causality at SWD onset and SWD offset

Thalamo-thalamic GC values during the non-epileptic control as well as during the pre-ictal period were low (0.01 to 0.05). In contrast, GC values during SWD were increased compared to the non-epileptic control period for both coupling directions (all p’s<0.025). For most channel pairs a dominant coupling direction (stronger increase in GC for one direction compared to the other) could be identified during SWD (Table 2). While most of the cortico-thalamic channel pairs revealed this directed or dominant coupling direction only at SWD onset (first 500 ms), this restriction to SWD onset was seen for only two intra-thalamic pairs: VPM->Po and VPM->ATN.

In contrast most thalamo-thalamic channel pairs showed persistent directional coupling throughout the whole SWD. This was observed for the following pairs (arrow indicates the direction of interaction): cRTN->VPM, cRTN->Po, rRTN->Po, ATN->Po. In all these cases dominant coupling was seen in the frequency band between 4-12 Hz, including the major SWD frequency range (Figure 2c).

An exception was found for channel pair cRTN-rRTN: the SWD related increase in GC was equally strong for both coupling directions at SWD onset and during SWD, while a directional dominance was found to occur 1 sec prior to SWD offset until 0.2 sec following its offset. In this period significantly higher GC values were found for the direction cRTN->rRTN in the 2-12 Hz frequency range (Figure 3b). This dominance was attributed to an increase in GC for cRTN-rRTN towards the end of the SWD as well as a slight decrease of rRTN->cRTN GC. It can be noted that the time-point of change in coupling dominance is immediately following the earlier described decrease in GC seen for the ctx4->rRTN and immediately prior to the increase noted for ctx5-Po and ctx6->Po (see Figure 3 for temporal preceding at SWD offset).
Table 2 Pattern of changes in directional coupling and bidirectional-coupling seen for cortico-thalamic and thalamo-thalamic channel pairs at on- and offset of SWD. A directional coupling (one dominant guiding (->) structure) is inferred only if Granger Causality of one coupling direction is significantly higher than for the other. Bidirectional coupling on the other hand is inferred if the increase in GC compared to preictal control values is equally strong for both coupling directions. A change indicated to be temporarily present represents a change of about 500ms duration. Abbreviations: ↑ = increase, BC = Bidirectional coupling, ctx4 = layer 4 of the somatosensory cortex, ctx5 = layer 5 of the somatosensory cortex, ctx6 = layer 6 of the somatosensory cortex, ATN = anterior thalamic nucleus, Po = Posterior thalamic nucleus, VPM = Ventral-Posterior-Medial thalamic nucleus, cRTN = caudal reticular thalamic nucleus, rRTN = rostral reticular thalamic nucleus, C = Cortex, T = Thalamus.

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<td>ctx5-Po</td>
<td>BC</td>
<td>↑ ctx5-&gt;Po, 2-10 Hz, 0.25 to 1.5 sec following LCTS</td>
</tr>
<tr>
<td>ctx6-Po</td>
<td>↑ ctx6-&gt;Po, onset, 4-12 Hz, temporary</td>
<td>↑ ctx6-&gt;Po, 2-10 Hz, 0.25 to 1.5 sec following LCTS</td>
</tr>
<tr>
<td>ctx4-Po</td>
<td>↑ ctx4-&gt;Po, onset, 8-20 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx6-VM</td>
<td>↑ ctx6-&gt;VPM, onset, 4-10 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx5-VM</td>
<td>↑ ctx5-&gt;VPM, onset, 4-10 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx6-ATN</td>
<td>↑ ctx6-&gt;ATN, onset, 8.12 Hz and 25-35 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx5-ATN</td>
<td>↑ ctx5-&gt;ATN, onset, 25-35 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx5-cRTN</td>
<td>↑ ctx5-&gt;cRTN, onset, 20-40 Hz, temporary</td>
<td>BC</td>
</tr>
<tr>
<td>ctx4-ATN</td>
<td>↑ ctx4-&gt;ATN 8-12 Hz temporary at onset, 20-50 Hz persistent from onset till end of SWD</td>
<td></td>
</tr>
<tr>
<td>ctx4-cRTN</td>
<td>↑ ctx4-&gt;cRTN 20-50 Hz persistent from onset till end of SWD</td>
<td></td>
</tr>
<tr>
<td>ctx4-VPM</td>
<td>↑ ctx4-&gt;VPM, 40-50Hz, persistent till end of SWD</td>
<td></td>
</tr>
<tr>
<td>ctx4-rRTN</td>
<td>↑ ctx4-&gt;rRTN 20-50 Hz persistent from onset till end of SWD and 8-12 Hz from onset SWD till 1.5 sec before end of SWD</td>
<td></td>
</tr>
<tr>
<td>ctx6-cRTN</td>
<td>BC</td>
<td>BC</td>
</tr>
<tr>
<td>ctx5-rRTN</td>
<td>BC</td>
<td>BC</td>
</tr>
<tr>
<td>ctx6-rRTN</td>
<td>BC</td>
<td>BC</td>
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<tr>
<td><strong>T-T</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cRTN-rRTN</td>
<td>BC</td>
<td>↑ cRTN-&gt;rRTN 2-12 Hz, 1sec prior to LCTS to 0.25 following LCTS</td>
</tr>
<tr>
<td>cRTN-Po</td>
<td>↑ cRTN-&gt;Po 4-12 Hz persistent</td>
<td></td>
</tr>
<tr>
<td>rRTN-Po</td>
<td>↑ rRTN-&gt;Po 4-12 Hz persistent</td>
<td></td>
</tr>
<tr>
<td>ATN-Po</td>
<td>↑ ATN-&gt;Po 4-12 Hz persistent</td>
<td></td>
</tr>
<tr>
<td>cRTN-VPM</td>
<td>↑ cRTN-&gt;VPM 4-12 Hz persistent</td>
<td></td>
</tr>
<tr>
<td>VPM-Po</td>
<td>↑ VPM-&gt;Po 25-45 Hz temporarily</td>
<td></td>
</tr>
<tr>
<td>VPM-ATN</td>
<td>↑ VPM-&gt;ATN 30-35 Hz temporarily</td>
<td></td>
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<tr>
<td>rRTN-ATN</td>
<td>BC</td>
<td></td>
</tr>
<tr>
<td>cRTN-ATN</td>
<td>BC</td>
<td></td>
</tr>
<tr>
<td>rRTN-VPM</td>
<td>BC</td>
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</tbody>
</table>

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Discussion

The study aimed to investigate dynamics of cortico-thalamo-cortical network interactions, which are seen during the initiation and termination of SWD, using frequency resolved Granger Causality, quantifying directional coupling between cortex and different thalamic regions. In the current study directional coupling was inferred only if GC values for one coupling direction (e.g. A->B) was significantly higher than for the other coupling direction (B->A). An increase in GC (e.g SWD vs. control) of equal strength for both coupling directions (A->B and B->A) was regarded as an increase in ‘bidirectional’ coupling without a dominant, guiding structure.

To achieve a complete/extended picture of network changes associated with SWD initiation and termination, signals were obtained from multiple thalamic sites, which have been proposed to play an important role in SWD generation, as well as from the deep layers of the somatosensory cortex, the SWD instigator zone.

The onset of SWD (first 500 ms) was characterized by a strong increase in directional coupling (higher increase of GC for coupling direction cortex->thalamus as compared to thalamus->cortex) from the somatosensory cortex to most but not all thalamic nuclei. It is assumed that changes (improvements) in the signal to noise ratio (SNR) occurring with SWD onset are equally strong in cortex and thalamus, so that these unequal increases in GC cannot be attributed to unequal changes in SNR, but indicate a true change in directional drive. The majority of the cortical thalamic coupling results including their directions and dynamics are in strong concordance with earlier results on the dynamics of network interactions for the generation of SWD using a different, ‘directed’ connectivity analysis (non-linear association analysis) (Meeren et al., 2002, Lüttjohann and van Luijftelaar, 2012).

Sitnikova et al (2008) also used Granger Causality to study changes directional coupling between frontal cortex and VPM in pre-SWD->SWD transition periods and these authors did not find a dominant leading role of the frontal cortex at SWD onset. On the other hand, David et al (2008) applying GC on fMRI data of GAERS rats did find a cortical drive at SWD onset between the somatosensory cortex and thalamus as well as between somatosensory cortex and striatum. Together it seems that a dominant cortical drive can only be revealed for the somatosensory cortex but not for other cortical sites. Therefore it seems that the results reveal full support for the cortical focus theory of absence epilepsy, which states that there is an epileptic focus in the deep somatosensory cortex, which functions as initiator/generator of SWD and drives other cortical regions and thalamus into SWD activity.

For some cortico-thalamic channel pairs the directed cortical drive was found in high frequencies (20-40 Hz) above the SWD characteristic 8-12 Hz band. Changes in the beta and gamma band are well known to occur after the administration of GABAergic drugs...
(Coenen and van Luijtenaar, 1989, Halonen et al., 1992), so that also the changes in high frequency coupling might be related to a GABAergic influence. At the same time one needs to realize that a spike component of LFP signal is constituted out of multiple, including high frequencies. The exact meaning of such a coupling, which is only present in these high frequencies (as e.g. seen for ctx5-ATN, ctx4/5-cRTN and ctx4-VPM) but not in the main SWD frequency band of 8-12Hz, is not entirely clear.

Most intrathalamic pairs showed a persistent (throughout the complete SWD) drive in SWD frequencies, whereas such a coupling pattern was only found for one cortico-thalamic channel pair. Interestingly, in all except one (cRTN-VPM) of these cases the Po was driven by another thalamic nucleus. This is in line with earlier network analytical studies, which associated the Po as having a role of synchronizing and channeling thalamic output to the cortex (Lütjohann and van Luijtenaar, 2012, Lütjohann et al., 2013). The persistent drive of the cRTN to the VPM is in agreement with classical neurophysiological and computational data, reporting a tonic hyperpolarization of the thalamic relay cells during SWD, which is brought about by the GABAergic influence (Destexhe, 1999, Steriade, 2003, Huguenard and McCormick, 2007).

Comparatively few studies have investigated the spontaneous termination of SWD, which was the other major focus of the current study. Connectivity results on SWD termination are graphically summarized in Figure 4. The most important outcomes of this part of the study are the following: 1) Whereas most cortico-thalamic and thalamo-thalamic channel-pairs showed an abrupt return from high GC values during SWDs to low non-epileptic control GC values at LCTS, channel-pair ctx4-rRTN showed a more gradual decrease of its directional coupling (from layer 4 of the somatosensory cortex to the rostral RTN). It started as early as 1.5 seconds prior to LCTS (Figure 4). 2) This was followed by an increase in directional coupling from the caudal RTN to the rostral RTN; it started at about 1 second prior to LCTS and lasted until 0.25 seconds following it (compare Figure 4a and Figure 4b). 3) A directional coupling from cortical layer 5 and 6 to the Po was noticed: it lasted from 0.25 until 1.5 sec following LCTS (Figure 4b). This was due to the continuation of the increased SWD GC in cortex-thalamus direction whereas GC in the opposite direction already returned to non-epileptic control levels at LCTS.
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Figure 4 Summary of connectivity during the SWD -> post SWD transition period. The left two panels represent coupling profiles prior to LCTS, whereas the right panel illustrates coupling following LCTS. Connectivity is indicated as either unidirectional (->) or bi-directional (<>). Solid lines represent coupling between structures which possess a direct anatomical connection, dashed lines indicate coupling between structures that are anatomically only indirectly connected. Black lines indicate a coupling strength at the level of the ‘stable SWD control’ period and blue lines indicate coupling which is significantly lower than during the ‘stable SWD control’ period but significantly higher than during the ‘non-epileptic control’ period. In the middle panel stars indicate changes relative to the previous (left) coupling profile. Exact timepoints of changes are given below. Abbreviation: ctx4 = layer 4 of somatosensory cortex; ctx5 = layer 5 of somatosensory cortex; ctx6 = layer 6 of somatosensory cortex; rRTN = rostral Reticular Thalamic Nucleus; cRTN = caudal Reticular Thalamic Nucleus; Po = Posterior Thalamic Nucleus; VPM = Ventral Postero Medial Thalamic Nucleus; ATN = Anterior Thalamic Nucleus; LCTS = Last Cortico-Thalamo-Cortical Spike of SWD.

Interpretation of result 1 and 2:
Since both findings occur in close temporal proximity to the offset of SWD they may be related to SWD termination. At the same time, since these findings occur relatively long (1.5 and 1 seconds) prior to the LCTS, it may be justifiable to conclude that the termination of SWD is a gradual process, which is already initiated 1.5 seconds prior to SWD offset. In addition, these results show that there is not one ‘single’ structure that ‘takes the initiative’ in SWD termination, but suggest that SWD termination relies on a combination of both cortico-thalamic as well as intrathalamic processes.

Changes in the intra-RTN communication were also related to the control of SWD duration based on the outcomes of two pharmacological experiments (Sohal et al., 2003, Proulx et al., 2006). Interestingly, whereas Sohal et al (2003) achieved a shortening of SWD duration by enhancing intra-RTN communication via the local administration of the benzodiazepine clonazepam, Proulx et al. (2006) achieved a reduction of SWD duration via the decrease of intra-RTN communication by blocking gap-junctions. Others reported that the RTN of absence epileptic WAG/Rij rats is characterized by changes in GABA<sub>A</sub> receptor

* 1.5 sec prior to LCTS  ** 1 sec prior to LCTS  *** till 1.5 sec following LCTS
expression (a specific loss of alpha3 subunit immunoreactivity at inhibitory synapses) as compared to non-epileptic control rats (Liu et al., 2007). The outcomes of the present study now demonstrate for the first time the involvement of intra RTN communication in the control of SWD duration (SWD termination) in the natural context of spontaneously terminating (without pharmacological manipulations) SWD, in an in vivo genetic rat model under physiological circumstances. In addition, our study clarifies the nature of intra RTN changes towards the spontaneous abortion of SWDs showing that an increase in coupling (needs to) occur(s) from caudal to rostral RTN, whereas a decrease in coupling (needs to) occur(s) in the direction from rostral to caudal RTN.

An early, gradual decrease (shortly after SWD onset) in GC was also reported between frontal cortex and VPM in the Sitnikova et al (2008) study. Such a gradual decrease in cortex->thalamus coupling might be relatable to the gradual slowing of SWD frequencies seen in wavelet and other time-frequency analyses of SWD in patients and rats (Bosnyakova et al., 2006, Bosnyakova et al., 2007), which seem ultimately to lead to the termination of SWD. In longer lasting SWD, a repetition of this frequency modulation can be observed, which would also anticipate a waxing and waning pattern of the cortex->thalamus GC coupling strength. Such a pattern, however might be obscured by the averaging across seizures, as was performed in this study.

It remains to be established, whether the early decrease between frontal cortex and VPM reported by Sitnikova et al. and the decrease between ctx4 -> rRTN found in the current study are of equal relevance for the termination of SWD. As noted above ctx4 -> rRTN was the only cortico-thalamic pair in this study, for which a persistent (from FCTS until 1.5 sec prior to LCTS) dominant cortical drive was found. Such a consistent drive might be relevant for the maintenance of SWD, while its termination at 1.5 sec prior to LCTS initiates SWD termination.

It needs to be mentioned, however, that no direct anatomic connections exist between the somatosensory cortex and rostral RTN. Therefore, it cannot be excluded that also a third structure, like e.g. the intralaminar nuclei, that have been proposed to play a role in SWD maintenance (Seidenbecher and Pape, 2001, Gorji et al., 2011) and which are known to receive and send input from the somatosensory cortex as well as to project to the rostral RTN (Kaufman and Rosenquist, 1985, Cornwall et al., 1990), is involved in this maintenance and termination process. In this way the intralaminar nuclei might either function as an additional, non-recorded relay station (ctx4 driving ->) intralaminar nuclei -> rostral RTN), or as a common input to both structures that drives both with a different time delay (intralaminar nuclei ->ctx4 with signal shorter signal transduction time than intralaminar nuclei ->rostral
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RTN). The same might hold for other (non-directly) connected structures shown as dashed lines in figure 4.

In our study it can be noted that in both seizure termination processes (decrease in directional coupling ctx4->rostral RTN and increase in directional coupling caudal RTN->rostral RTN) the rostral RTN is involved: a reduction of cortical excitatory drive might result in a reduced oscillatory activity of the RTN. The increase of GABAergic coupling from caudal RTN onto the rostral RTN might have the same effect. Two more conclusions might be justified: a) The rostral RTN might function as an important resonator, which is of crucial relevance for the maintenance of SWD; and b) rostral and caudal RTN seem to have opposite effects on SWD, whereas increased oscillatory activity of the rostral RTN facilitates long SWDs, the caudal RTN rather functions as a break and increased activity of the caudal RTN hampers long lasting SWD.

These views are supported by earlier studies: Electro-microscopic inspection of the RTN by Liu and Jones (1999) revealed differences in receptive fields between RTN subparts (rostral and the more caudal ventral lateral RTN) and pharmacological and lesion studies revealed that a lesioning/inhibition of the caudal RTN led to an increase in SWD activity (Aker et al., 2006, Meeren et al., 2009), whereas a lesioning/inhibiting of the oscillator rostral RTN resulted in an increase of SWD activity (Aker et al., 2006, Berdiev and van Luijtelaar, 2009, Meeren et al., 2009).

Interpretation of result 3:
Since this change is seen immediately after SWD termination it seems not to be related to the termination process itself any more. Rather, the increased deep somatosensory cortex->Po coupling, which remains present until 1.5 sec following LCTS, seems to indicate that epileptic focus, seated in the deep layers of the somatosensory cortex, tries to re-initiate the SWD. Such a re-initiation attempt of the somatosensory cortex might be in accordance with signal analytical results of Maris et al (2006) and Bouwman et al., (2007) showing that the chance of getting a new SWD is highest immediately after the previous one. In addition, it seems also be in line with the above mentioned repetition of SWD frequency modulation that has been found for WAG/Rij rats and in patients during long lasting SWDs (Bosnyakova et al., 2006, Bosnyakova et al., 2007).

Interestingly, this enhanced cortico->thalamic coupling is maintained (for another 1.5 sec following the SWD) with the Po. This nucleus has been proposed to play an important role in SWD initiation (Lüttjohann and van Luijtelaar, 2012). In a network-analytical study investigating the onset of SWD the Po showed highest increases in coupling with layer 5 of the somatosensory cortex, as established with the aid of a non-linear association analysis.
and was the only nucleus recorded in this study that showed a bidirectional-crosstalk to the cortex even within the first 500ms. In this way the Po was the only thalamic nucleus which could respond/give feedback to the epileptic focus in this onset time, which was regarded crucial for SWD generation (Lüttjohann and van Luijtelaar, 2012).

Channel-pair ctx5-Po showed a strong bidirectional (without a dominant guiding force from the cortex) coupling also in the current study, but also for three other channel-pairs such a bidirectional coupling was seen. It needs to be mentioned however that in the case of Granger Causality an equal increase in GC for both coupling directions needs to be interpreted with some caution, since an increase in GC between control and SWD might also be related to the improvement of the signal to noise ratio seen during SWD.

Following the end of SWD only the deep somatosensory cortex kept is increased coupling towards the Po, whereas the Po->deep somatosensory cortex coupling stopped at LCTS. In case a strong bidirectional coupling between deep somatosensory cortex and Po is indeed relevant for SWD initiation, the current results should be interpreted in the following way: The deep somatosensory cortex attempts to reinitiate an SWD (as represented by the increased GC value, which is still present following LCTS) but this attempt/effort fails since the posterior thalamic nucleus does not respond/give feedback to the cortex anymore (increased GC values Po->ctx stop at LCTS). Likewise, in the case of longer lasting SWD, showing repetitions of the SWD typical frequency modulation, for which a waxing and waning pattern of cortex->thalamus GC coupling strength might be expected (see above) a re-initiation (or continuation) succeeds, since the Po might keep responding to the somatosensory cortex.

More direct measures of neuronal activity like multiple or single cell recordings in Po and deep cortical layers might help to verify this suggestion.

In summary,

- Frequency derived GC cortico-thalamic and intrathalamic network analyses showed that the onset of SWD (first 500 ms) is characterized by a strong directional drive of the deep somatosensory cortex to most but not all thalamic nuclei.
- The Po is constantly driven by other thalamic nuclei during the entire SWD.
- The termination of SWD is a gradual process, which is already initiated up to 1.5 seconds prior to SWD offset.
- SWD termination involves both cortico-thalamic as well as intra-thalamic processes.
- The intra-RTN communication is involved in the regulation of SWD duration.
- The rostral RTN might have a resonatory function, which is crucial for SWD maintenance, whereas the caudal RTN might function as a break of this oscillatory activity (see also (Meeren et al., 2009));
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- The deep somatosensory cortex tries to re-initiate an SWD but does not succeed since the Po does not responds back to the cortex

References


Chapter 9

GENERAL DISCUSSION
General Discussion

There is wide agreement that spike and wave discharges (SWD), the electroencephalographic hallmark of absence epilepsy, are generated within the cortico-thalamo-cortical system and that the integrity of the system is a prerequisite for the occurrence of ‘full blown’, bilateral, symmetrical and seemingly primary generalized SWD (Jasper and Fortuyn, 1947, Gloor et al., 1990, Danober et al., 1998, Seidenbecher et al., 1998, McCormick and Contreras, 2001, Coenen and Van Luijtenaar, 2003, Meeren et al., 2005, Pinault and O'Brien, 2005, van Luijtenaar and Sitnikova, 2006, Huguenard and McCormick, 2007).

Network interactions within the system, on the other hand, as well as the relative contribution of network structures to SWD generation and sustaiment, are still enigmatic and highly debated. Among several theories on SWD origin (Meeren et al., 2005) the most popular two (the cortico reticular theory (Gloor, 1968) and cortical focus theory (Meeren et al., 2002, Polack et al., 2007)), can be said to 'network theories'. That is, both attribute a functional role for SWD occurrence to the cortex and thalamus, which interact with each other. However, the functional weighing that these theories give to cortex and thalamus respectively differs extensively (Chapter 1). Whereas for the cortico-recticular theory some inconsistent experimental results have been accumulated in the past years (Chapter 1, (Pinault and O'Brien, 2005, van Luijtenaar and Sitnikova, 2006, Leresche et al., 2012), the cortical focus theory is a more recent theory on SWD origin, which is currently in the focus of experimental investigations (Chapter 2,(van Luijtenaar et al., 2011b)). Nevertheless, this theory is also only a beginning of a description (a new idea) on how the cortico-thalamo-cortical system is involved in the generation of SWD. This idea remains to be validated and several questions are still left to be answered.

This thesis aimed to investigate the role of the cortico-thalamo-cortical system in the generation, maintenance and termination of spike and wave discharges in greater detail. In particular it was aimed to:

1) Perform an experimental test of the cortical focus theory, which states that there is a local hyperexcitable epileptic focus in the deep layers of the perioral somatosensory cortex. This epileptic zone is thought to initiate a cascade of events that ultimately leads to the occurrence of the bilateral and generalized SWD, if the thalamo-cortical circuitry is in an appropriate state. It drives other cortical and thalamic sites during the first 500 ms of a SWD, while thereafter the thalamus is thought to function as a resonator circuit for SWD sustaiment (Meeren et al., 2002, Meeren et al., 2005, van Luijtenaar and Sitnikova, 2006, Polack et al., 2007, van Luijtenaar et al., 2011b)
2) To give a better description of the above mentioned ‘cascade of events’, which ultimately lead to the occurrence of SWD, by investigating the dynamics of cortico-thalamo-cortical interactions in pre-SWD->SWD transition periods, which might be necessary for the generation, generalization, and maintenance of SWD; as well as to investigate cortico-thalamo-cortical network dynamics during SWD->post SWD transition periods, in order to learn about relevant network mechanisms of SWD termination.

3) To investigate whether there are differences in functional contribution between cortex and thalamus as well as and in particular between different thalamic nuclei for the generation, maintenance and termination of SWD and to disentangle these functions.

Given the wide agreement that an intact cortico-thalamo-cortical system is necessary for the occurrence of SWD, mentioned above (Jasper and Fortuyn, 1947, Gloor et al., 1990, Danober et al., 1998, Seidenbecher et al., 1998, McCormick and Contreras, 2001, Coenen and Van Luijtelalaar, 2003, Meeren et al., 2005, Pinault and O'Brien, 2005, van Luijtelalaar and Sitnikova, 2006, Huguenard and McCormick, 2007), it comes as no surprise that earlier studies also started to investigate network interactions at SWD onset and tested the proposals of a network theory like the cortical focus theory.

Chapter 2 of the thesis gave a review on these earlier attempts. Next to presenting the state of the knowledge at the beginning of this thesis, this review also illustrates that there are several shortcomings in prior network studies, including that some studies did not investigate dynamical changes of cortico-thalamo-cortical interactions, while others did not investigate interactions between the deep layers of the cortical focus and several thalamic nuclei.

To overcome the mentioned shortcomings and to bring our knowledge of the above mentioned topics to a more advanced level, the current thesis performed a refined network analysis using the experimental technique of deep brain stimulation as well as several signal analytical methods. Refined network analysis thereby implied that:

- LFP signals for the signal analytical studies were gathered in an extended part of the cortico-thalamo-cortical system, including the deep layers of the somatosensory cortex and multiple relevant thalamic nuclei.
- Signals were analyzed in a dynamical fashion with a good temporal resolution along transition periods.
- The anatomical connectivity profile of the cortico-thalamo-cortical system, described in section 1.2, was kept in mind for the selection of appropriate recording and stimulation sites, as well as for the interpretation of results.
In the following, the above mentioned topics (main aims of this thesis) are discussed in light of the major results revealed from the refined network analysis performed in this thesis, and incorporated with the earlier literature.

9.1 Evaluation of the cortical focus theory part 1: the deep perioral somatosensory cortex as ‘initiator’ of SWD

In contrast to the cortico-reticular theory, which attributed the role of the rhythm generator to the intrathalamic RTN-thalamo-cortical relay cells network (this theory assumes that the sleep-spindle-rhythm is the basis of SWD) and held a generally hyperexcitable cortex responsible for the transformation of the physiologically normal sleep-spindle oscillations into pathological SWD (Chapter 1, (Kostopoulos et al., 1981, Gloor et al., 1990)), the cortical focus theory attributes the role of both the rhythm generator and the role of the initiator of SWD to a local cortical area: it states that the deep perioral somatosensory cortex contains a hyperexcitable focal area, the origin of SWD.

In the following part of the Discussion, experimental, observational, and signal analytical results acquired throughout the entire thesis will be evaluated on their consistency with this proposal.

9.1.1 Experimental results: Comparison of local, cortical, excitability

The most direct experimental test of the existence of a hyperexcitable spot that can function as initiator of SWD was performed in Chapter 3 of this thesis. It was hypothesized that, if there is indeed a local, hyperexcitable area, the amplitude of an electrical evoked potential (EEP), measuring excitability, evoked and recorded in the deep somatosensory cortex of the absence epileptic WAG/Rij rat, should be higher than the amplitude of an EEP, evoked and recorded in an adjacent cortical area like the deep motor cortex. This hypothesis was found to be true for the early N1 component of the EEP as well as for the later N3 component. Since early components of an evoked potential are known to be mainly dependent on stimulus properties while later components can also be influenced by network and top-down processes (Coenen, 1995), the increased amplitude of the N1 is, especially, a good indicator for the selective increase in local excitability of the deep somatosensory cortex. The increase of the late N3 component, on the other hand, might indicate an additional heightened excitability of the network.

An alternative explanation - that the difference in N1 EEP amplitude between deep somatosensory cortex and deep motor cortex might simply be the result of a normal difference in excitability between sensory and motor cortices rather than indicating an epileptic abnormality - could be ruled out by performing the same EEP comparison in healthy Wistar control rats. Since in these healthy rats no difference in N1 EEP amplitude could be
found, it can thus be safely concluded that the increased excitability of the deep somatosensory cortex is indeed specific for an absence epileptic animal. Since the motor cortex is a directly adjacent cortical area to the somatosensory cortex, the proposal that such an increased excitability is a local phenomenon is reasonable. The exact extent of this local zone remains to be investigated, however, since no EEPs were evoked in other adjacent cortical areas, like e.g. the secondary somatosensory cortex. Zheng et al., (2012), for example, propose a somewhat broader initiation area including the deep cortical layers of primary and secondary somatosensory cortex as well as the insular cortex in GAERS rats, since these areas showed increases in coherence shortly before SWD onset.

The increase in N1 EEP amplitude was found to be independent of the level of alertness (wakefulness, drowsiness and deep slow wave sleep), showing that the enhanced excitability is a ‘permanent’ characteristic of the deep somatosensory cortex of adult (older than 6 months) WAG/Rij rats. As a consequence, the underlying cause of this higher excitability needs to be attributed to a ‘permanent’ factor.

The nature of this increased excitability is most likely attributable to a sum of local changes promoting excitation in itself as well as factors that indirectly lead to an increased excitation by inducing a local lack of inhibition. As reviewed in Chapter 2, several such changes have been reported to be present in the somatosensory cortex of absence epileptic rats, including an upregulation of particular subtypes of sodium channels (Klein et al., 2004), increased NMDA receptor-mediated activity (Pumain et al., 1992, Luhmann et al., 1995, D’Antuono et al., 2006), a decrease in the efficacy of GABA_A or GABA_B-ergic inhibition (Merlo et al., 2007, Inaba et al., 2009) and an increased expression of mGlu2/3 receptors (Ngomba et al., 2005). Probably one of the most elegant documented changes in the somatosensory cortex of WAG/Rij rats is a reduction in hyperpolarization-activated cation current (I_h) (Strauss et al., 2004, Schridde et al., 2006, Kole et al., 2007). Like SWD, which show an age dependent expression (increase), the reduction (as compared to aged matched Wistar control rats) of HCN1 channels was also found to be age related but the time-course of the receptor decrease was found to shortly precede the time-course of SWD ontogeny (Kole et al., 2007). Thus, the decrease in HCN1 cannot be attributed to the exposure of the brain to hundreds of SWD per day, but might rather be the result of a genetic predisposition towards a diminishment of HCN1 channels. This in turn makes the rats more prone to develop SWD, which becomes manifested during ontogeny. Next to this, Blumenfeld and colleagues demonstrated that an early onset long-term treatment with the anti-absence drug ethosuximide prevents (or delays) both the ontogeny of SWD as well as the HCN1 channel loss within the somatosensory cortex of WAG/Rij rats (Blumenfeld et al., 2008), so that, in combination, even a causal role between HCN1 channel loss, resulting in enhanced
excitability of the somatosensory cortex as well as SWD ontogeny, may be justified. Such an ontogenetic evolution and possibility of prevention by e.g. an early long-term treatment, however, remains to be tested for the enhanced N1 EEP amplitude that demonstrates local hyperexcitability of the deep somatosensory cortex of WAG/Rij rats, as described in Chapter 3.

9.1.2 Observational and signal analytical results: Comparison of somatosensory-cortical and thalamic signal properties

Next to the direct experimental test of the cortical-focus theory in Chapter 3, in which the excitability of different (adjacent) cortical areas was assessed and compared, a comparison between the assumed cortical focus (in the deep layers of the somatosensory cortex) and several thalamic nuclei was performed in the signal analytical studies in Chapter 2, 6, 7 and 8. To this end a new, flexible, electrode system for the simultaneous recording of local field potentials from multiple, specific brain structures was developed and its stability for data acquisition suited to the investigation of multi-site network analysis was verified (Chapter 2).

Based on the anatomical connectivity profile of the somatosensory cortex, and to cover (record from) an extended part of the potentially relevant SWD network, signals were obtained from 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, Sherman and Guillery, 2005), rostral and caudal RTN, as potential synchronizer of the system (Huguenard and McCormick, 2007), and the anterior thalamic nucleus, as a thalamic control site outside the somatosensory loop.

Visual inspection (i.e. without the application of a signal analytical method) and quantification of these recordings revealed that in about 20% of rats SWD activity can be seen to start up to 1 second earlier in the deep somatosensory cortex as compared to all other thalamic nuclei (Chapter 2, 6, 7 and 8). In addition, in about 20% of the rats local SWD-like activity could be found (Chapter 2), this was only present in the deep layers (IV-VI) of the somatosensory cortex, while the thalamic recordings were unaltered. The absence of these observations in other rats might be explained by an inter-individual variability in the location of the focus between rats as found in the study of Meeren et al (2002); in the studies of the current thesis identical electrode coordinates were used for all rats.
Recordings of localized thalamic SWD activity or SWD activity that can be seen, on visual inspection, to start earlier in the thalamus than in the cortex, were never encountered. Therefore it can be concluded that both observations provide good support for the statement that the deep somatosensory cortex functions as SWD rhythm generator, which is, next to the increased excitability (Chapter 3), a second characteristic of an epileptic focus.

So called ‘embryonic SWD’, reminiscent to the localized SWD in the current thesis, were also reported by Seidenbecher et al. (1998) in anaesthetized GAERS rats. These ‘embryonic SWD’ were recorded concomitant to SWD correlated firing in layer IV and V of the somatosensory cortex, which precede the main, generalized, cortico-thalamo-cortical SWD, (Seidenbecher et al., 1998). In GAERS rats SWD are reported to arise out of 5-9 Hz medium voltage oscillations, reminiscent to the sensory-motor-rhythm (Pinault et al., 2001, Pinault et al., 2006), another cortically generated rhythm (Nicolelis et al., 1995). In a recent study by Zheng et al. (2012) these oscillations were related to local increases in interactions between primary and secondary somatosensory cortex as well as the adjacent insular cortex. Whether this is also the primary network for these localized embryonic ‘SWD’ in WAG/Rij rats remains to be investigated.

The observation that SWD can be present in the cortex before the thalamus is involved highlights the need for a better definition on what can be regarded as the timepoint of SWD onset. In accordance with the general concept of a SWD as being a generalized, pathophysiological, cortico-thalamo-cortical oscillation, rather than a localized cortical oscillation, the first cortico-thalamic spike (FCTS), defined as a first sharp spike of at least twice the amplitude of background LFPs, visible in all cortical and thalamic recordings and followed by rhythmic SWD activity, was defined as the moment of “generalization” of epileptic activity. It is considered the start of a “full blown” cortico-thalamo-cortical SWD and was taken as a zero reference point in all signal analytical studies.

Our signal analytical studies also revealed supporting evidence for the cortical focus theory: Time frequency analysis during pre-SWD->SWD transition periods, centered around FCTS, revealed that for 75% of SWD strong increases in combined delta/theta power (more than 2 times the background power) were seen to occur in the deep somatosensory cortex (especially layer V) as early as 2 sec prior to FCTS, while such increases in the thalamic recordings were only seen at a later time-point.

Such increases in power could not be attributed to changes in local connectivity between the three deep layers of the somatosensory cortex: the local connectivity between the layers, as expressed by the non-linear association coefficient $h^2$, did not change and remained rather high (Chapter 6). Whether the pre-FCTS power increase can be attributed to changes in
connectivity between adjacent somatosensory areas (S1,S2) as in the case of Zheng et al (2012) remains to be investigated.

Lastly, both directed connectivity analysis (Nonlinear association analysis and Granger Causality), as performed in Chapter 6 and 8, showed that at the moment of generalization from cortex to thalamus (FCTS) the deep layers of the somatosensory cortex drive most thalamic nuclei (see Paragraph 9.2 and 9.3 for a discussion on the exceptions), within the first 500ms following FCTS. These results replicate and extend the original findings by Meeren et al. (2002) and validate (next to the characteristic of local hyperexcitability and rhythm generating abilities presented above) a third characteristic of an epileptic focus of actually secondary generalized seizures: the ability to lead and to drive other structures into SWD activity.

Others using Granger Causality analysis of LFP data did not find a leading role of the frontal cortex (Sitnikova et al., 2008), while Granger Causality on fMRI data revealed a leading role for the somatosensory cortex (David et al., 2008), supporting the selectivity for this driving capacity to the somatosensory cortex.

The observation that at FCTS in the intracortical circuit (between the layers of the somatosensory cortex) a change in coupling direction from ctx4 driving ctx5 and ctx6 towards ctx5->ctx4 and ctx4->ctx6 also takes place (Chapter 6), is thereby in good agreement with the refinement of the focus location towards the deeper cortical layers (V-VI) as was established in the GAERS model (Pollack et at (2007). It represents the dominance of especially these deep layers in SWD generation.

In summary: In the current thesis a mixture of cortico-cortical comparisons (Chapter 3) as well as cortex-thalamus comparisons (Chapter 2, 6-8) demonstrated that the deep somatosensory cortex possess three characteristics (locally enhanced excitability, local rhythm generating abilities and ‘driving’ forces onto other structures), which can be seen as a prerequisite for an ‘initiator’ of SWD. This justifies the conclusion that this mixture of results as presented in this thesis is in full support for the cortical focus theory stating that the deep somatosensory cortex contains a local epileptic focus, which is the ‘initiator’ of SWD.

### 9.1.3 Afterdischarges

Chapter 3, where the hypothesis of an increased excitability of the deep somatosensory cortex of WAG/Rij rats via paired pulse electrical stimulation (see Paragraph 9.1.1) was tested, also provided functional evidence that the somatosensory cortex is able to produce epileptic discharges. It was found that in about 20% of cases, stimulation of the deep somatosensory cortex of WAG/Rij rats, but not (or to a much lesser extent) of healthy Wistar control rats, was able to produce rhythmic, self sustained afterdischarges (AD) lasting
several seconds, which strongly resemble spontaneous SWD. The same phenomenon was recently confirmed in the GAERS model (Zheng et al., 2012).

AD are a well known result of electrical stimulation, they are often used to measure brain excitability and to assess the effectiveness of antiepileptic drugs (Mares and Kubova, 2006). Low frequency stimulation of e.g. the sensory-motor cortex or somatosensory thalamus with intensities around 1mA are known to induce 2-3 Hz, self-sustained spike and wave AD accompanied by clonic convulsions (Pohl et al., 1986, Tolmacheva et al., 2004) and stimulation of limbic structures can induce local, non-convulsive AD of 1Hz which develop into more complex, generalized, notch and polyspike AD followed by a post ictal depression and accompanied by convulsions of increasing intensity after repeated exposure to stimulation (kindling) (Racine, 1972), the kind of AD recorded in Chapter 3, has, to the best of our knowledge, not been described before. AD recorded in this thesis have a spike and wave morphology with a main frequency of 8 Hz (like SWD (Bosnyakova et al., 2006)), can be induced with intensities as low as 20-100µA, preferentially occur during drowsiness (similar to SWD in genetic rat models (Drinkenburg et al., 1991) and occur in a passive motionless rat, which only performs rhythmic whisking movements (Semba and Komisaruk, 1984).

All these characteristics make this kind of AD strongly reminiscent of spontaneous SWD. It was therefore hypothesized that the 8Hz AD recorded in Chapter 3 are stimulation induced SWD and thus a cortico-thalamo-cortical phenomenon. While in Chapter 3 it was only shown that the AD could be recorded in the somatosensory cortex as well as the frontal (motor) cortex, the involvement of the thalamus was confirmed in Chapter 5, where the same type of AD, this time induced via (the same type of) stimulation of the thalamus (VPM/ATN), could be recorded in both cortex and thalamus (VPM). Following this, AD, like SWD (Sitnikova and van Luijtelaar, 2007), showed an opposite spike polarity in cortex and thalamus and showed a strong temporal coherence between recording sites (visually assessed). Moreover, AD reacted in the same way as SWD in experimental manipulations: i.e. longer lasting stimulation of the ATN increased the duration of both SWD and AD over time. Stimulation of the VPM was much more efficient to induce AD as compared to stimulation of the ATN and only stimulation of the VPM but not the ATN led to a significant increase of the number of spontaneous SWD. Whether AD also react like SWD to other manipulations, e.g. the systemic administration of the anti-absence drug Ethosuximide, remains to be investigated. However, based on the multitude of analogies between SWD and AD, as listed above, it is justifiable to conclude that AD are stimulation induced SWD.

It is therefore assumed that network properties relevant for the generation of SWD can be inferred based on characteristics of AD.
Chapter 3 of this thesis thus provided functional evidence that the somatosensory cortex of WAG/Rij but not Wistar rats is able to produce SWD (type of oscillations).

It needs to be mentioned, however, that motor cortex stimulation was also able to induce AD. These AD however, were shorter and had a less stable 8Hz rhythm than AD elicited in the somatosensory cortex. This might either be the result of the excitability difference of the cortical areas or differences in sub-loop (somatosensory loop vs. motor loop) properties. A recent modeling study demonstrated that heterogeneity of cortical tissue can explain differences in AD properties, as described in this thesis (Goodfellow et al., 2012). The loop specificity for SWD like AD was confirmed in Chapter 5 since stimulation of the VPM, which is like the epileptic focus part of the somatosensory loop, could easily induce AD, while stimulation of the ATN, a nucleus that belongs to the limbic system, only rarely (in 2% of cases) induced AD. The anatomical connectivity profile of the stimulated areas (motor cortex and VPM, but not ATN, possess direct connections to the epileptic focus) also allows the possibility that in the case of motor cortex and VPM stimulation AD induction might be the result of a postsynaptic activation of the epileptic focus in the somatosensory cortex. Such an activation of both the locally stimulated structure as well as the postsynaptic reception structure was demonstrated for electrical stimulation of the substantia nigra (Lee et al., 2004).

In any case, the AD results as well as the observational results as described in Chapter 2, showing localized SWD in the somatosensory cortex of WAG/Rij rat, raise the questions of why and how SWD activity sometimes generalizes to the thalamus to start a ‘full blown’ SWD or why cortico-thalamo-cortical AD are elicited, while at other times the SWD activity stays local or AD are not induced. Answers to the questions on the spontaneous SWD were obtained in the analyses of the network, see Chapters 6 to 8 (Paragraph 9.2 and 9.3).

9.2 The cascade of events: Dynamics of cortico-thalamo-cortical network interactions

Dynamics of network interactions which ultimately lead to the generation or termination of ‘full blown’ SWD were investigated in the signal analytical studies (Chapter 6 to 8), with the aid of different, partly complimentary, advanced connectivity analyses.

Local field potential recordings were obtained in an extended part of the cortico-thalamo-cortical system including layer IV, V and VI of the somatosensory cortex, VPM, the posterior thalamic nucleus, and the caudal RTN, (all parts of the SWD/AD susceptible somatosensory loop and including the proposed epileptic focus), as well as the rostral RTN and the ATN.

Network interactions between these structures were assessed along pre-SWD->SWD and SWD->post SWD transition periods, which were centered around FCTS or equivalently around the last cortico thalamic spike (LCTS), respectively, and were, given the vigilance
preference of SWD (and AD) occurrence, compared to network connectivity during non-epileptic passive wakefulness control periods.

In addition, SWD->post SWD transition periods were also compared to so called 'stable SWD' periods, acquired from the middle of SWD.

These comparisons revealed a total number of 5 different profiles of network connectivity, which are displayed in Figures 9.1a and 9.1b, 9.2 and 9.3a and 9.3b. In these figures, significant changes in network connectivity are either indicated as solid lines, for connectivity changes between anatomically connected structures or as dashed lines. The direction of coupling is indicated by arrowheads, which can either be unidirectional (->) or bidirectional (<->). For results of the pairwise phase consistency analysis (PPC) as performed in Chapter 7, from which no directionality can be inferred, a line without arrowhead is used, if the direction of coupling cannot be inferred from one of the other 'directed' analyses. The strength of connectivity changes is color coded with orange indicating significant increases compared to non-epileptic controls that did not reach a plateau, red indicating increases in coupling reaching a plateau level and blue indicating decreases in coupling.

Given the increased signal to noise ratio during the ictal period, which heightens the chance of 'spurious' results, only those changes that were consistently found (i.e. by at least two of the three analyses methods) are included in the ‘first 500 ms’ and the ‘during SWD’ profiles (Figure 9.1b and 9.2) (Table 1,2). Inclusion decisions for these coupling profiles are outlined in Table I and Table II for all channel-pairs. The ‘pre LCTS’ profile, also prone to spurious correlations due to an increased signal to noise ratio, can only be based on a single analysis (Chapter 8) (Figure 9.3b). In order not to over-interpret changes detected in this profile, only those connections were considered which fulfilled the above described criterion (i.e. revealed consistent results in at least two of the three analyses methods) in the previous ‘during SWD’ profile.

The five connectivity profiles are grouped into 3 consecutive figures, characterizing ‘network’ processes of SWD generation, maintenance and termination, respectively. It is proposed that these changes might perhaps be prerequisites for either the generation or termination of SWD (and probably also AD), which determine whether an SWD remains a localized discharge (Chapter 2) or develops into a full blown cortico-thalamo-cortical oscillation.

In the tightly connected next paragraph (Paragraph 9.3), which unravels the different functional roles of the network structures, an interpretation is given on why these changes are important for the initiation or termination of SWD respectively.
Table I: Inclusion criteria for network changes during the ‘first 500ms’ interval. Type of decision is specified in number: 1= included, 2= not included (only one analysis, since BC increase seen in GC analysis can be due to changes in signal to noise ratio), 3= not included (change just in one analysis detected), 4= not included (inconsistent results). Abbreviations: $h^2$ = non-linear association analysis; ppc = pairwise phase consistency analysis; GC = Granger Causality analysis; BC=bidirectional coupling increase, D=Directional coupling increase; = cc = change in coupling direction without increases in coupling; ctx4=layer 4 of somatosensory cortex; ctx5=layer 5 of somatosensory cortex; ctx6=layer 6 of somatosensory cortex; ATN=anterior thalamic nucleus, rRTN=rostral reticular thalamic nucleus; cRTN=caudal reticular thalamic nucleus; Po=posterior thalamic nucleus; VPM=ventral-postero-medial thalamic nucleus

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Table II: Inclusion criteria for network changes during the ‘during SWD’ interval. Type of decision is specified in number: 1= included, 1b=included (also the bidirectional coupling of the Granger Causality can be seen as real increase, since a directional drive was seen earlier, therefore there is more than one analysis in favor for the increased coupling); 2= not included (only one analysis, since BC increase seen in GC analysis can be due to changes in signal to noise ratio), 3= not included (change just in one analysis detected), 4= not included (inconsistent results). Abbreviations: $h^2$ = non-linear association analysis; ppc = pairwise phase consistency analysis; GC = Granger Causality analysis; BC=bidirectional coupling increase, D=Directional coupling increase; $= cc$ = change in coupling direction without increases in coupling; ctx4=layer 4 of somatosensory cortex; ctx5=layer 5 of somatosensory cortex; ctx6=layer 6 of somatosensory cortex; ATN=anterior thalamic nucleus, rRTN=rostral reticular thalamic nucleus; cRTN=caudal reticular thalamic nucleus; Po=posterior thalamic nucleus; VPM=ventral-postero-medial thalamic nucleus

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9.2.1 A scenario of SWD generation

The first connectivity profile containing significant changes in cortico-thalamo-cortical connectivity associated with SWD generation displays changes prior to the onset of the ‘full blown’ SWD, and is therefore called ‘pre-FCTS’ (Figure 9.1a). Although at this state SWD activity is not yet present in the thalamus, significant increases in the communication between cortex and thalamus can already be seen. Precisely, as early as 1.25 seconds prior to FCTS the deep layers of the somatosensory cortex and the strong, reciprocally connected posterior thalamic nucleus, start to gradually increase their communication in a bidirectional fashion, until reaching a maximal value at around 375ms following FCTS. Since these increases are only detected by the non-linear association analysis (Chapter 6) but not by the PPC and GC analysis, it is likely that these increases in communication take place in a non-linear fashion, which cannot be detected by linear connectivity methods like PPC or GC. Indeed epileptic seizures are often regarded to be of non-linear nature and signal analyses derived from the theory/mathematics of non-linear dynamical systems are proposed to be of particular value for the understanding of seizure generation mechanism as well as to open the possibility of seizure prediction (Le Van Quyen et al., 1999, Lehnertz et al., 1999, Litt and Echauz, 2002, Lopes da Silva et al., 2003a, Lopes da Silva et al., 2003b, Stefan and Lopes da Silva, 2013). In line with this, several nonlinear changes have been reported for different kinds of epileptic seizures (Martinerie et al., 1998, Bettus et al., 2011). Also in the original study by Meeren et al (2002), that detected the existence of an epileptic focus in
absence epileptic WAG/Rij rats, cortico-thalamic coupling was found to be of nonlinear nature while cortico-cortical coupling increases seen during SWD were of linear nature. Linear coupling changes were also detected (Chapter 7) in the pre-ictal period. At 1.2 sec prior to FCTS a phasic decoupling between the caudal RTN and layer IV of the somatosensory cortex and rostral RTN and layer V of the somatosensory cortex (Chapter 7), was observed, shortly followed by a phasic decoupling between cRTN and Po (Chapter 7 and 6 (the directional non-linear association analysis demonstrated that the cRTN reduces its communication to the VPM)) at about 1 sec prior to FCTS, and a decoupling between cRTN and VPM at 0.8 seconds prior to FCTS. It can be noted that in all cases decreases in coupling are revealed. The coupling between caudal RTN and VPM can be especially regarded as another argument against the cortico-reticular theory, which assumes sleep spindles to be the natural basic rhythm of SWD. Sleep spindle generation, however, requires an increased influence of the RTN onto thalamo-cortical relay cells (Steriade, 2003), which is at odds with a decoupling between cRTN and VPM found in the current study.

In any case based on the above reported dynamics of pre-ical changes it can be concluded that, in contrast to a long lasting view that SWD are sudden and unpredictable events (1981), 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 in network activity might also open the possibility for SWD prediction. Pre-ictal decreases in cortical, long range synchronization were also reported in the MEG of patients with juvenile absence seizures (Amor et al., 2009), and similar decreases in long range desynchronization were successfully used for seizure prediction in patients with partial seizures with a specificity of 100% (Mormann et al., 2003). Figure 9.1b: The decreased coupling values of the pre-ical epoch return to normal baseline values at around FCTS, while the increased coupling between cortex and Po remains. At FCTS, however, additional increases in coupling between the deep layers of the somatosensory cortex and several thalamic nuclei (VPM, ATN, rRTN) can be seen. In line with the cortical focus theory, it can be noted that in all these cases coupling increases are of unidirectional nature with the cortex driving the thalamus. Granger Causality revealed that for the anatomically connected ctx6-VPM this drive could be attributed to a drive in the frequency range between 4-10Hz, while for the connection ctx6-ATN the drive was seen in higher frequencies (40-50Hz).

An exception for the unidirectional cortical drive was channel pair ctx5-Po, which kept a bidirectional crosstalk reaching maximal increases (stronger than the other cortico-thalamic pairs (Chapter 6) at about 375ms following FCTS. This observation can be seen as an extension of the cortical focus theory by showing that the thalamus is not homogeneous in its interactions with the cortical focus (Paragraph 9.3).
PPC analysis for this pair revealed increases in phase-coupling in the broad frequency range 8-48Hz, while for the other cortico-thalamic pairs, noted above, PPC increases were seen in the 8-12 Hz band and its harmonics.

In addition, unidirectional increases in thalamo-thalamic coupling also took place at FCTS. Except for the VPM (for which inconsistent results were revealed by the different analyses) all thalamic nuclei (ATN, cRTN and rRTN) consistently started to drive the Po. This drive, as well as increases in PPC, were found in the 4-12 Hz band.

**9.2.2 SWD sustainment**

About 500 ms following FCTS the cortico-thalamo-cortical system proceeds into the third, more stable (i.e. longer lasting) coupling profile (Figure 9.2), which characterizes SWD sustainment.

During this phase all intra-thalamic coupling connections described in the ‘first 500 ms’ coupling period remain the same. An additional increase of coupling was observed between cRTN–rRTN, ATN-rRTN and ctx5-cRTN. In line with the predictions of the cortical focus theory, that the cortical focus only drives the thalamus during the first 500ms, most cortico-thalamic connections turn into a bidirectional crosstalk, with cortex and thalamus taking turns in driving each other (Chapter 6, 8). However, there are two exceptions: channel pair ctx4-rRTN and ctx4-ATN; here the cortical drive is maintained. As was the case for the bidirectional crosstalk between ctx5-Po seen during the first 500ms, this observation extends the cortical focus theory by showing that the thalamus is not homogeneous in its interactions with the cortical focus (Paragraph 9.3). Whereas for channel pair ctx4->ATN this drive was found in higher frequencies 20-50Hz, the driving force between ctx4->rRTN was also found in the SWD characteristic 8-12 Hz band and in the 20-50Hz band.
9.2.3 A scenario of SWD termination

Visual inspection around the offset of SWD already revealed differences in the timepoint of SWD termination between cortex and thalamus in about 20% of rats, as was the case for the onset of SWD; the thalamic SWD activity was found to stop about 1 second earlier than cortical SWD activity (Chapter 2, 8). Again, this raised the need to determine a definition of a SWD offset point for signal analysis. In accordance with the prior definition of the SWD onset point, the last cortico-thalamic spike (LCTS), i.e. the last spike of at least twice the background LFP, visible in all cortical and thalamic recording sites and preceded by rhythmic SWD activity, was defined as the end of a ‘full blown’ cortico-thalamo-cortical SWD.

Dynamics in network-coupling relevant for the termination of SWD, which have received little attention in past research, were studied along SWD-post SWD transition periods, centered around FCTS. Within the pre-LCTS period (Figure 9.3a) two changes were noted: First, the directional drive from ctx4 onto rRTN found for the SWD relevant 8-12Hz band in the SWD sustainment coupling profile (Figure 9.2), diminished at about 1.5 seconds prior to LCTS. Second, an increase in the directional drive between cRTN driving rRTN was observed 1 second prior to LCTS lasting until 0.25 seconds following LCTS. This was followed (Figure 9.3b) by a return to baseline coupling values seen for all except two channel pairs (ctx5-Po and ctx6-Po) at FCTS. Only ctx5-Po and ctx6-Po remained on an increased coupling strength for another 1.5 seconds following FCTS. In contrast to the SWD sustainment and the (ctx5-Po) SWD initiation period, the increased coupling between cortex and Po was not bidirectional, with cortex and Po
performing a bidirectional crosstalk, but of unilateral nature with the cortex driving the Po (Figure 9.3b) (see Paragraph 9.3.1a for interpretation).

Given the early changes found in the pre-LCTS period (decrease in directional drive ctx4-rRTN 1.5 secs prior to LCTS and increased directional drive cRTN-rRTN), which seem to initiate the massive coupling loss at FCTS, it can be concluded that comparable to SWD generation (Paragraph 9.2.1) SWD termination is also a gradual rather than an abrupt process. Sitnikova et al (2008) also reported an pre-LCTS reduction in the strength of frontal cortex – VPM coupling, suggesting that other cortical regions also reduce their communication to the thalamus prior to SWD offset.

Furthermore, it can be concluded that both cortico-thalamic as well as intra-thalamic processes participate in this initiation of SWD termination. It can be noted, however, that in the current thesis, both the cortico-thalamic and the intra-thalamic coupling changes associated with SWD termination include the rostral RTN (Paragraph 9.3.1c).

In contrast to the network analysis on SWD termination performed in the current thesis, network analysis on sleep spindle termination (Timofeev et al., 2001), demonstrated a major role of, predominantly, the cortex - i.e the cortical cells were found to start firing out of phase and a computer model lacking the cortico-thalamic input was found to allow continuous spindle oscillations within the thalamus RTN network. This again shows that sleep spindles and SWD are fundamentally different types of oscillations. This adds a counter-argument to the cortico-reticular theory.

9.3 The thalamus is not homogeneous: Unraveling different functional contributions and interpretation of the SWD generation and termination scenario

From the above presented dynamics of network interaction seen with SWD generation, sustainment and termination, as well as from the electrical stimulation study performed in Chapter 5, it can be seen that not only the cortex as compared to the thalamus, but also the different thalamic nuclei behave differently within particular SWD transition periods and towards experimental manipulations. Electrical stimulation to some thalamic structures (VPM) can easily induce SWD-like AD, this is only rarely the case with stimulation of other structures (ATN). Some thalamic structures such as Po and cRTN are involved in the SWD generation process earlier than others, while the cRTN and rRTN are involved early in SWD termination processes. It can thus be safely concluded that the thalamus is not a homogeneous structure, which in turn indicates that the different thalamic nuclei fulfill different tasks in the overall processes of SWD generation, sustaiment and termination. Whilst the function of the SWD ‘initiator’ has already been attributed, discussed and validated to and for the deep somatosensory cortex (Paragraph 9.1), the functional roles of the different thalamic nuclei remain to be determined.
In the following paragraph, different tasks or functional roles are proposed for the nuclei, based on the experimental and signal analytical results gathered throughout this thesis. In addition, an interpretation of the above described SWD generation and termination scenario is offered by discussing the relevance of the attributed functions for the generation, sustainment and termination of SWD.

9.3.1 Attribution of functions

a) The ‘Reverberator’

One of the surprising results of the current thesis was the large role of the posterior thalamic nucleus (Po). Although this nucleus possesses strong reciprocal connections to the epileptic focus in layer V of the somatosensory cortex (Deschênes et al., 1994) and although earlier invasive and non-invasive studies already indicated its participation in SWD (Vergnes et al., 1987, Tenney et al., 2004) this nucleus has received little to no attention in previous studies on SWD generation. To the best of our knowledge this nucleus was not included in any computational model of SWD generation and received only little attention in neurophysiological studies on SWD generation. Most studies predominantly focused on the role of the VPM and RTN and only a few researchers proposed, in theoretical scenarios, that the Po with its widespread connections to other cortical areas might help to spread and generalize SWD activity (Kostopoulos, 2001, Polack et al., 2007, Polack et al., 2009).

In the current thesis the Po jumped into the focus of attention, as it was the nucleus that showed the earliest pre-ictal, as well as one of the strongest ictal, increases in coupling to the epileptic focus (Chapter 6). Both characteristics (earliest and strongest increases with the cortical focus) make it an interesting candidate for the initiation of ‘full blown’ SWD. Until now it could not be excluded that part of these early increases in $h^2$ might be attributable to changes in the level of vigilance. However, the Po is also characterized by a third behavioral characteristic, which makes it highly likely to be of crucial relevance for SWD generation: The Po is the only nucleus, recorded in this study, which kept a bidirectional crosstalk to the epileptic focus during the first 500ms of SWD. In other words the Po was the only thalamic nucleus which was able to ‘respond’ or give feedback to the cortical epileptic focus within the early SWD generation period. The importance of such a ‘responder’ is nicely expressed in a review by Huguenard and McCormick (2007). After describing the ideas of the cortical focus theory these authors state:

“This model would require that the neocortical network be receptive to the feedback excitatory TC activity, that is, that it not produce destructive interference that would destabilize the overall development of a global oscillation. This hypothesis remains to be tested.” (Huguenard and McCormick, 2007, Trends in Neurosciences, 30(7), p. 354)
The strong bidirectional coupling between cortex and Po, as revealed in this thesis, exactly fulfills this prerequisite. In other words, a seizure generator (either in cortex or in thalamus) alone is not enough for the occurrence of bilateral, symmetrical and synchronized SWDs. Rather an intact, closed cortico-thalamo-cortical loop, is required (see e.g. (Avoli and Gloor, 1981, Sitnikova and van Luijtelaar, 2004). During the first 500 ms such a closed loop of signal reverberation is only provided via the Po, either in the form of small, direct loop (Figure 9.4, green) established in the direct bidirectional ctx5<->Po connection, or, in a longer loop (Figure 9.4, blue) where the cortex sends activity to other thalamic nuclei, they send this activity to the Po and the Po in turn returns the activity back to the cortex.

Interestingly, following the end of SWD, as described in Paragraph 9.2.3, the cortex keeps an increased connectivity to the Po for another 1.5 seconds but at this state the Po does not respond to the cortex anymore (Figure 9.3). Since it is known that the chance to get a new SWD is highest following the end of SWD (Maris et al., 2006), this behavior might be interpreted as a SWD re-initiation attempt of the cortical epileptic focus. The prolonged local SWD activity in the deep somatosensory cortex, seen in about 20% of rats (Chapter 2, 8) might also favor this suggestion. The re-initiation attempt, however, fails, which might be attributable to the fact that the Po does not respond to the cortex anymore. During the timecourse of short-lasting SWD a frequency modulation from high (about 11 Hz) to lower (about 6 Hz) frequencies can be seen (Bosnyakova et al., 2006). For longer lasting SWD such a frequency modulation is repeatedly present (a sort of SWD repacking). At the end of each individual repetition element, the SWD seems to be at its highest chance for termination but continues. It is hypothesized that network analyses clustered around such repetition endpoints would show the same network changes as seen for the final termination, except for the fact that the Po keeps responding to the thalamus, so that the re-initiation or

![Figure 9.4: Schematic interpretation of the importance of a bidirectional crosstalk between cortical, epileptic focus and posterior thalamic nucleus (Po) for the generation of ‘full blown’ SWD (see text for details).]
continuation is successful. Positive validation of this hypothesis in future network studies would confirm the important role of the Po for SWD generation and ‘re-initiation’.

From an anatomical point of view it is not surprising that the Po is the primary thalamic counterpart of the cortical focus. It is the higher order thalamic nucleus of the somatosensory system, which receives its main driving input from the somatosensory cortex via the so-called ‘driver’ cortico-thalamic cells which act via fast ionotropic receptors. Interestingly, a recent study by Cavdar (2012) revealed that the Po nucleus of absence epileptic GAERS rats contains a significantly higher number of driver terminals, as compared to healthy non-epileptic control rats. This difference was not found in other thalamic nuclei. Furthermore, higher order nuclei like the Po, as compared to first order nuclei such as the VPM, have been demonstrated to be more prone to burst firing (Ramcharan et al., 2005), a property which is generally believed to be favorable for SWD generation (Avoli et al., 2001).

Since the Po stays in the bidirectional crosstalk to the cortex, and continues to receive input from other thalamic nuclei during SWD sustainment (Chapter 6,7,8), it might be possible that the Po remains the major responder to the cortex. The Po seems to gather the intra-thalamic activity to generate a common synchronous feedback to the cortex, which might support SWD sustainment. This, however, remains to be validated, since at this stage additional ‘closed loop circuits’ are active (e.g. ctx<->VPM). Lastly, given its widespread cortical connections (Kostopoulos, 2001), the Po might also be involved in SWD generalization (Kostopoulos, 2001). Since no additional frontal cortical electrodes were implanted in the studies of Chapter 6-8 to investigate SWD generalization, the importance of the Po and other thalamic nuclei in SWD generalization also remains a question to answered in future studies (Paragraph 9.5).

b) The ‘(trigger) input relay’

The VPM, in contrast to the Po, has always been regarded as belonging to the key-network of SWD generation (Depaulis and vanLuijtelaar, 2006). This is probably a remainder of the cortico-reticular-theory, which assumes sleep spindles to be the underlying rhythm of SWD. Sleep spindles are generated in the intra-thalamic circuit, in which the GABAergic influence of the RTN hyperpolarizes thalamic relay cells and brings them into a burst firing mode. The VPM, as one of the major sensory relay cells was therefore, according to this theory, crucially involved in rhythm generation.

Results of the current thesis, however, indicate that the role of the VPM for SWD generation, as compared to the Po, might be of smaller or at least of a different nature: In contrast to the Po, the VPM only showed late (earliest at FCTS) increases in coupling with the cortical focal area and these were only of small to moderate degree (Chapter 6).
In addition, during the SWD sustainment phase the VPM only showed consistent (verified by multiple connectivity methods) increases in coupling with the three layers of the somatosensory cortex, but, in contrast to the Po, not with other thalamic nuclei (Table 9.1). Given the fact that the VPM is a first order thalamic nucleus, or in the categorization schema of Jones a ‘core’ cell that projects only to a localized region of the cortex (Jones, 2009), it is unlikely that the VPM is crucially involved in the sustainment or generalization of the SWD, which might, given the generalized nature of SWD, require a broader contact to the cortex. The Po on the other hand is a higher order nucleus that sends ‘matrix’ cells to the cortex, i.e. it projects to multiple cortical areas.

In line with a more modest role of the VPM in SWD generation and sustainment, local application of the anti-absence drug ethosuximide to the VPM of GAERS rats was shown to be insufficient to prevent SWD (Richards et al., 2003). Furthermore multiple unit recordings in the VPM in GAERS rats demonstrated that the VPM only seldom fires in a seizure-favorable burst-like fashion during SWD (Pinault and O’Brien, 2005, Leresche et al., 2012). Interestingly, Polack et al (2009) demonstrated that the ratio of burst firing during SWD is higher in the Po as compared to the VPM. Likewise, direct comparison between Po and VPM (Chapter 7) revealed that strong increases in phase coupling between cortex and Po were seen in a much broader spectral domain as compared to the VPM (Figure 7.4). It was argued that the sharper form of the SWD spikes, recorded from the Po, might explain this difference.

From a signal analytical point of view it is known that such a sharp signal is composed out of multiple frequencies (Rosen and Howell, 1991), which all run in phase during the SWD. From a physiological point of view it can be added that the creation of such spikes requires multiple cells to fire synchronously in a burst like fashion (Coenen, 1995).

On the other hand the VPM can also not be said to be completely unimportant for SWD generation: A recent study by Abbasova and colleagues (2010) demonstrated the importance of external (somato)-sensory input for the occurrence of SWD. These authors accomplished an almost complete abolishment of SWD via an inhibition of the peripheral trigeminal nerve. Given this importance of external input for the generation of SWD, the VPM gets a crucial role in SWD generation from an anatomical point of view. The VPM is the first order nucleus, which relays such external stimuli, transported to the VPM by the trigeminal nerve, to layer IV of the somatosensory cortex. From layer IV intra-cortical connections (Thomson and Bannister, 2003) can transport the trigger input to the deep-layers (V and VI), which contain the epileptic focus. In line with this, $h^2$ analysis showed a directional drive from layer IV to layer V and VI prior to FCTS. The function of a ‘trigger input relay’ is therefore proposed for the VPM. Supporting this function, Chapter 5 of this thesis showed that low frequency electrical stimulation was able to
induce SWD-like AD, whereas stimulation of the ATN, a nucleus, that does not relay information to the somatosensory cortex, did so much less efficiently. The somatosensory cortex is known to contain so-called 'intrinsically bursting' cells (Connors and Gutnick, 1990), which react to incoming stimuli with a stereotyped burst firing pattern. In the secondary somatosensory cortex of GAERS rats (the slightly different location of the epileptic focus in this strain) these cells were recently found to be more numerously present as compared to the adjacent primary somatosensory cortex and insular cortex (Zheng et al., 2012). Such cells reacting stereotypically and vigorously to an incoming trigger sent by the VPM might therefore form the basis of a first local epileptic hyperdischarge of the somatosensory epileptic focus.

c) The ‘resonator’

Another network behavior was detected for the rostral RTN. This nucleus was found to be continuously guided by the somatosensory cortex (layer IV) in the high frequencies (20-50Hz) and until 1.5 secs prior to LCTS in the SWD typical 8-12Hz band (Chapter 8). While an 8-12Hz firing rate is known to be the intrinsic firing rhythm of T-C and C-T cells (Golshani and Jones, 1999) the higher frequency drive might be a result of active GABAergic neurons, which are known to be able to fire at a higher frequency. In line with this, changes (increases) in the spectral EEG content by GABAergic drugs are predominantly seen in this (20-50Hz) frequency range (Coenen and van Luijtelaar, 1989).

Since for this channel pair (ctx4->rRTN) a bidirectional crosstalk was never encountered, the rRTN can better be regarded as a resonator, which (passively) picks up the SWD activity send by the somatosensory cortex. Still, the activation of the rostral RTN is apparently relevant for SWD sustainment and a reduction of this activity, brought about by a termination of the cortical drive and an increase in inhibition by the caudal RTN, (see below) is involved in the termination of SWD. By contrast, post synaptic activation of the rRTN, brought about by ATN stimulation, was shown to be able to prolong the duration of both stimulation induced AD as well as spontaneous SWD (Chapter 5).

The relevance of the RTN for SWD sustainment was already indicated in the late eighties and early nineties by lesion and pharmacological inhibition studies (Buzsaki et al., 1988, Avanzini et al., 1992, Avanzini et al., 1993) and several changes have been reported in the RTN of absence epileptic rats, like a specific GABA α3 subunit loss in the RTN of absence epileptic WAG/Rij rats (Liu et al., 2007) and an increase in the T-type calcium conductance in the RTN of GAERS (Tsakiridou et al., 1995)

Whereas early studies did not explicitly differentiate between the rostral and caudal part of the RTN, studies by Meeren et al. (2002; 2009) and Aker et al. (2009) first highlighted the
special role of the rostral RTN: These studies demonstrated that an inhibition or lesion including the rostral RTN significantly reduced SWD activity in WAG/Rij and GAERS rats, whereas this was not the case if the rostral part was still functionally intact (Meeren, 2002, Aker et al., 2006, Berdiev and van Luijtelaar, 2009, Meeren et al., 2009).

The current thesis confirms this involvement of the rostral RTN in the control of SWD duration for the first time in a natural context (i.e. without experimental manipulations) of spontaneously occurring and terminating SWDs. The above mentioned changes (GABA $\alpha_3$ subunit loss (Liu et al., 2007) increase in IT conductance (Tsakiridou et al., 1995) as well as differences in receptive fields between RTN subparts (rostral and the more caudal ventral lateral RTN) (Liu and Jones, 1999) might explain the susceptibility of the rostral RTN to function as a resonator for SWD sustainment.

d) The ‘gate-keeper and break’

Next to a ‘reverberator’, a ‘resonator’ and a ‘trigger input relay’, the behavior of a third thalamic nucleus, recorded in this thesis, the caudal RTN, can best be interpreted as representing a ‘gate-keeper’ or ‘break’ for SWD activity. The caudal RTN was found to decouple (show a phasic desynchronization with) from the VPM (trigger input relay), cortical layer IV (the major sensory input layer of the somatosensory cortex) and the reverberator Po during the pre-FCTS interval (Chapter 7). The relevance of such a decoupling for SWD generation was nicely described in a study by Amor et al. (2009) and a related review by Le Van Quyen (2005). As mentioned in Paragraph 9.2.1 a pre-ictal decrease in cortical long range synchronization was also found in patients with SWD (juvenile absence epilepsy) (Amor et al., 2009). The authors assume that such a desynchronization might prepare a brain network towards a pro-epileptic state, since such a desynchronization might reflect a depression of synaptic inhibition and might provide an ‘idle’ population of neurons that can easily be recruited by an epileptic focus. In line with this interpretation, the decoupling of the inhibitory caudal RTN might also bring the cortico-thalamo-cortical network into a pro-epileptic state, and might therefore be a prerequisite for SWD generation: Precisely, as a consequence of a reduced inhibitory influence of the caudal RTN onto the Po, a population of ‘idle’ Po neurons might be created, which then allows the epileptic focus to increase its communication to the important ‘reverberator’. Secondly, the short lasting reduction of ‘gate-keeping/gate closing’ activity of the cRTN on the VPM input relay and cortical input layer IV might change the way in which an external stimulus (or electrical pulse provided in Chapter 5), might reach the hyperexcitable, focal, cortical area.

It is known that during low levels of vigilance the increased inhibition of the cRTN onto thalamic relay cells like the VPM reduces/blocks the amount of externally relayed signal to
the cortex as expressed in a reduced transfer ratio (Coenen, 1995). Furthermore it is known that during such a low level of vigilance, cortical cells react in a synchronous burst like fashion towards an incoming stimulus (Coenen, 1995). The short lasting decoupling of the cRTN might now allow such an external stimulus to reach the epileptic focal zone and function as an effective trigger for an epileptic hyperdischarge.

The above claimed assumption, that such a decoupling and its resulting preparation of the system into a pro-epileptic state is a prerequisite for SWD generation, might be supported by the fact that such a decoupling in patients with focal epilepsy could already be used to predict seizures with a sensitivity of up to 100% (Mormann et al., 2003).

While the pre-ictal decoupling of the cRTN might already be regarded as the removal of a break for SWD activity, the function of the break, as attributed here to the cRTN, becomes especially visible during the ‘pre-LCTS’ period. As one of the two changes that seem to initiate SWD termination, the cRTN is found to strongly increase its directional coupling onto the rRTN. This increased inhibition onto the rRTN, might, like the decreased cortical drive to this nucleus as discussed above, result in a decreased activity of the ‘rRTN resonator’, important for SWD sustainment. The caudal RTN breaks the resonating rRTN activity and therefore initiates SWD termination. Pharmacological in vivo and in vitro studies have suggested that intra-RTN communication is involved in the control of the duration of SWD (Sohal et al., 2003, Proulx et al., 2006). Again, this thesis supports this idea about the function of the cRTN and it is, to the best of our knowledge, the first time that this function is proposed based on data as obtained in a natural (unmanipulated) context of spontaneously occurring and terminating SWD.

At this stage it can also be concluded that although they are part of the same structure, caudal RTN and rostral RTN have opposite roles: one supporting SWD activity (resonator rRTN) and the other dragging/breaking SWD activity. The selective lesion or pharmacological inhibition studies are in line with this (Aker et al., 2006, Berdiev and van Luijtelaar, 2009, Meeren et al., 2009).

9.3.2 The key network of SWD

The distribution of different functional roles to the different thalamic nuclei, for SWD generation, sustainment and termination, presented above allows some comments with respect to the key-network of SWD:

As already mentioned (Paragraph 9.3.1b) the commonly regarded key-network in which SWD are predominantly studied and thought to be elicited, propagated, and sustained include the RTN, the VPM and the somatosensory cortex (McCormick and Contreras, 2001, Pinault and O'Brien, 2005, van Luijtelaar and Sitnikova, 2006, Huguenard and McCormick,
2007). Results of the current thesis, however, require the conclusion that this network is more extensive:

The discovery of the remarkable role of the Po, for example, claims that this nucleus clearly should also be regarded as a member of the key-network of SWD. This thesis therefore recommends that the Po should be included in future studies on the mechanism of SWD occurrence.

The description of a dual/opposite role of the RTN (with the rostral RTN as the important resonator for SWD sustainment - having an SWD promoting effect - and the caudal RTN having a dragging effect), as previously proposed by lesion and pharmacological studies and verified in a natural (i.e. unmanipulated) context of SWD generation in the current thesis, as well as the proposal that the main function of the VPM is only to relay an external trigger input (Abbasova et al., 2010) to the epileptic focus, might even allow the proposal that the key-network of SWD, and therefore the focus of experimental investigation, can better be shifted from the classical ‘somatosensory cortex, RTN and VPM’ network towards the SWD promoting ‘somatosensory cortex-Po-rRTN’ network.

The dashed lines in Figure 9.1 to 9.3 illustrate connectivity changes between structures that do not possess direct anatomical connections. Examples are the continuous drive between ctx4->rRTN, or that all thalamic nuclei drive the Po during SWD. It remains possible that other structures, which were not recorded in this thesis, might be the basis for these ‘spurious’ (non-anatomical) connections and might also be part of the ‘primary’ SWD network. These structures might, for example, be represented by the intra-laminar nuclei, which have been proposed to play a role in SWD sustainment by the group of Pape (Seidenbecher and Pape, 2001, Staak and Pape, 2001, Gorji et al., 2011), and which receive input from the somatosensory cortex and project to the rRTN (Kaufman and Rosenquist, 1985, Cornwall et al., 1990). Alternatively, a signal from the somatosensory cortex might first be transmitted via strong cortico-cortical connections (Meeren et al., 2002) to the cingulate and infralimbic cortex and from there via efferent projections to the rostral RTN (Cornwall et al., 1990). Lastly, the non-anatomical driving connections between ATN->Po and rRTN->Po, could go via an intra-RTN transmission (ATN->rRTN->cRTN-Po and rRTN->cRTN->Po) (Crabtree and Isaac, 2002).

Future studies using a combination of high temporal and spatial imaging methods might help to guide the decision of which structures are interesting to target with invasive electrophysiology.
9.4 Clinical relevance of results

Two forms of electrical stimulation were applied to the ATN or the VPM in order to, inter alia, explore differences between network structures (for SWD generation and termination) via the investigation of location specificity of stimulation effects (Chapter 5). Such an application of electrical pulses to specific parts of the brain, currently best known under the name deep brain stimulation (DBS), is also regarded as a promising new treatment approach for several neurological disorders including epilepsy (Theodore and Fisher, 2007, Huang and Luijtelaar, 2013). Like a pacemaker, these pulses are hoped to normalize abnormal brain rhythms in disease. The search for optimal stimulation protocols with maximal therapeutic efficacy and fewest side effects, as well as the exploration of the underlying mechanism of action of DBS, however, are still in their infancy. Knowledge on the network mechanisms of seizure generation (i.e. knowledge of which changes in network communication should be prevented by DBS) as well as knowledge on the mechanism of seizure termination (i.e. knowledge of which network changes should be achieved by DBS), as acquired in the current thesis, can be regarded as helpful to guide the selection of appropriate target structures for DBS and to help to interpret stimulation effects:

In Chapter 5 it was revealed that high frequency closed loop stimulation (i.e. stimulation contingent upon a seizure), applied to the ATN or VPM is able to reliably (in on average 89% of cases) terminate ongoing SWD with stimulation intensities as low as 71.6 to 113.6 μA and, by this, to shorten their mean duration from about 9 seconds to about 1.5 seconds and, consequently, to shorten the total SWD duration (average SWD duration times SWD number) from 497 seconds (in three hours of recording) to only 129 seconds. Such a closed loop paradigm of stimulation was made possible by the development of a wavelet based SWD detection algorithm which was functionally connected to a stimulation unit to form a closed loop BCI system (Chapter 5). The performance of this algorithm to detect SWD online (i.e. automatically on real time data) was previously validated and it was established that this algorithm can detect SWD within about 1 second and with a specificity of 96% and a sensitivity of up to 100% (Chapter 4).

Since the VPM was a key structure of the previously proposed key-network of SWD, it was hypothesized that VPM stimulation would be more efficient than ATN stimulation for the disruption of SWD. This, however, was not found - there were no significant differences in the percentage of successfully disrupted SWD nor in the required stimulation intensity for disruption. From the newly proposed key-network of SWD (Paragraph 9.3.2) the unexpectedly high effectiveness of ATN stimulation might be explained by the fact that this nucleus sends direct connections to the rRTN, which has been proposed to be an important resonator for SWD sustainment (Paragraph 9.3.1c). The possibility of such an activation of
postsynaptically connected structures by DBS was recently shown for STN (= subthalamic nucleus) stimulation, which led to an increased cell firing in both the STN as well as the postsynaptic STN target structure, the substantia nigra (Lee et al., 2004). In the case of the high frequency closed loop stimulation (Chapter 5) the high frequency of pulse delivery to the rRTN might then interfere with the classical SWD 8-12Hz rhythms imposed to this nucleus by the somatosensory cortex (Figure 9.2 and Figure 3 of Chapter 8) and by this terminate the ongoing SWD.

It remains possible, however, that a (stronger) location specificity for this type of stimulation can be revealed, if stimulation is applied at an earlier timepoint in the train of the SWD. In Chapter 5 stimulation was only applied at about 1 second following SWD onset. Paragraph 9.2 of this thesis tells us that at this time-point the SWD is already in a stable SWD sustainment connectivity stage, with multiple interacting cortical-thalamic and intra-thalamic circuits. At an earlier stage (i.e. within the first 500 ms or even prior to FCTS) Figures 9.1a and 9.1b would predict that some stimulation targets might be more promising than others: Here the stimulation of deep somatosensory cortex, as SWD initiator, or the Po, which needs to reverberate for the generation of a full blown SWD (see Paragraph 9.2.1a), would be recommendable.

The quite late time-point of stimulation can be attributed to the chosen SWD detection threshold. This is a threshold value for the averaged instantaneous power within the 60-80Hz band, based on which the online SWD detection algorithm decides whether a SWD is present or not. With the current algorithm the detection time can be reduced to about 500 ms but this comes at the cost of detection specificity (Chapter 4).

The existence of the early pre-FCTS network changes (Chapter 6,7) and the existence of early local increases in delta and theta power in the deep somatosensory cortex (Chapter 7), might suggest the feasibility of a closed loop seizure prevention system. It should allow the delivery of desynchronizing high frequency stimulation in the pre-FCTS stage, and by this, if delivered to one of the early involved structures, it might be able to completely prevent SWD generation. The sensitivity and specificity for such a precursor detection system remain to be established. The study by van Luijtelaaar et al. (2011a), describing that wavelet detected combined delta-theta precursor activity is specific to the pre-ictal period and is rarely found in interictal states makes such a development of a SWD precursor detection algorithm a promising approach.

In contrast to high frequency stimulation, the low frequency closed loop stimulation (Chapter 5) was found to have pro-epileptic effects and the type of effect was revealed to be location specific. Whereas ATN stimulation projecting to the SWD resonator rRTN prolonged SWD and AD duration, VPM and motor cortex stimulation projecting to the epileptic instigator
zone induced epileptic SWD-like AD. At first glance this should lead to the conclusion that such stimulation should be avoided in patients with SWD. A recent study by Berenyi and colleagues (2012), however, demonstrated that low frequency, closed loop stimulation applied to the skull surfacing the somatosensory cortex, is also able to shorten SWD duration. While these results seem to be completely at odds with the results of Chapter 3 and 5, closer inspection reveals that together these studies demonstrate that next to the stimulation location also the timing of stimulation matters much more than in the case of high frequency stimulation. Berenyi and colleagues (2012) argue that for their stimulation to be effective, silent neurons during the wave components need to be activated (i.e. stimulation needs to be applied during the wave) so that these neurons are refractory during their duty phase, where they would need to burst-fire in synchrony for the creation of a new epileptic spike. It can be noted that this argumentation is in agreement with the interference hypothesis for the mechanism of action of high frequency stimulation proposed above. Wrongly timed, however, i.e. in moments where network connectivity is in a SWD favorable state (see Paragraph 9.2 and Figure 9.1 for a detailed description of this state), a low frequency stimulus applied to the epileptic focus, or to structures which send direct projections to the focus, can act as an effective trigger to induce an epileptic hyperdischarge. High frequency stimulation, on the other hand, is less time dependent, given its desynchronizing nature, and can therefore be concluded to be safer in the context of a therapeutic intervention. Stability of stimulation and long term effect remain to be evaluated prior to a clinical application. Lastly it remains to be noted that of course for children with absence epilepsy a more non-invasive stimulation, as proposed by Berenyi (2012) might be desirable. Since, for children with absence epilepsy, more and more evidence for a local cortical epileptic focus accumulates (Westmijse et al., 2009, Gupta et al., 2011, Ossenblok et al., 2013) a noninvasive (local) brain stimulation (Berenyi et al., 2012, Zobeiri and van Luijtel, 2013) might not be unthinkable. More invasive brain stimulation, on the other hand might still be an option for therapy of other epileptic syndromes with absence seizures which are difficult to control by antiepileptic drugs like e.g. juvenile myoclonic or atypical epilepsy.

9.5 Verification and extension of thesis results (suggestions for further research)

A part of this thesis (the network analyses of cortex and different thalamic nuclei) is merely descriptive and the functional distribution proposed in Paragraph 9.3 is also inspired by this observed network behavior of the somatosensory cortex and the thalamic nuclei during transition periods. Therefore it is warranted to propose that the functional distribution proposed in Paragraph 9.3 benefits from verification in manipulatory future experiments. For example, if it is true that the main function of the VPM is to provide an input trigger to the
somatosensory cortex, whereas the Po, as ‘reverberator’, plays the major role for SWD generation via the provision of a closed loop circuit (see Paragraph 9.3.1a), functional inhibition (bilateral) of both the VPM as well as the Po should result in an abolishment of SWD. AD, however, for which it has been argued that network properties relevant for SWD can be inferred (see Paragraph 9.1.3), since they have the same or at least similar properties as SWD, induced by electrical stimulation of the somatosensory cortex, should still be visible in rats with a functionally inhibited VPM (since the input trigger is artificially provided) but not in rats with a functionally inhibited Po.

A second remaining issue to be investigated is SWD generalization. Since in the current thesis local field potential recordings were, next to multiple thalamic recordings, only recorded from one cortical area (three layers of the somatosensory cortex), it remains to be answered whether the moment of SWD generalization from cortex to thalamus (FCTS) is equivalent to the moment of widespread cortical generalization. For this, similar signal analytical studies as performed in the current thesis with multiple thalamic but also multiple cortical recording sites are already valuable. The infliction of additional unit recordings combined with local field potential recording could exclude the risk of detecting spurious correlations that are only attributable to volume conduction. Furthermore, the registration of additional brain structures might reveal other relevant network interactions for SWD occurrence. Meeren (2002) argued for a predominant generalization via cortico-cortical pathways (she found strong cortico-cortical coupling dynamics, which could not be explained by her thalamic recordings in VPM and LD). By contrast Sherman and Guillery (2005) point to a prominent role of higher order nuclei like the Po for the transfer of information from one cortical site to another. Recently this group was able to demonstrate in slices that cortico-cortical signal transfer (cortico-cortical communication), as visualized with voltage sensitive dye imaging, was completely abolished after inhibition of the Po (Theyel et al., 2010). Furthermore, also the intralaminar nuclei, with their widespread cortical projections to multiple cortical areas might be interesting candidates to contribute in SWD generalization. The Central Lateral nucleus, for example, has been shown to fire during the wave component of SWD (Seidenbecher and Pape, 2001, Gorji et al., 2011), a timepoint that has been seen in human MEG studies to go along with widespread generalization of SWD (Westmijse et al., 2009, Gupta et al., 2011, Ossenblok et al., 2013).

Again manipulatory experiments, as described above, with functional inhibition of particular thalamic nuclei, or selective cuts of particular cortico-cortical or thalamo-cortical connections preferentially with surgery guidance based on individualized DTI images, might help to reveal the contribution of these connections.
Network analysis in the current thesis was performed in a well validated genetic animal model of childhood absence epilepsy, the WAG/Rij rat (Coenen and Van Luijtenaar, 2003, Depaulis and vanLuijtenaar, 2006). As with all results from animal models, the question on the generalizability of results to the human condition is a justified and relevant one. It can be noted that in humans with absence epilepsy more and more evidence accumulates for the existence of a local cortical epileptic focus, which seems to be located in frontal parietal regions (Westmijse et al., 2009, Moeller et al., 2010, Gupta et al., 2011, Ossenblok et al., 2013). Given this difference in focal location as compared to the WAG/Rij rat, it is likely, however, that the primary thalamic counterpart might not be the Po but a different one. It is proposed however, that in the case of human absence epilepsy, this might be a higher order thalamic nucleus, since those possess diffuse connections to multiple cortical areas and are therefore rightly suggested to be involved in cortico-cortical communication. Furthermore, it is proposed (Chapter 7) that the basic principles of SWD generation, outlined for the WAG/Rij rat in Paragraph 9.2, 9.3 and Chapter 7, might also hold for the human condition: Like in the rat, SWD generation in the human might require a pre-SWD change in the inhibitory and excitatory balance in the connected brain network ((like the decoupling of the RTN found in the current thesis and the decrease in long range synchronization found by Amor et al. (2009)). This change could then result in a situation which enables an increased communication between an epileptic focus and its anatomically connected thalamic counterparts (prerequisite 1) or, in other words, might create an idle population of neurons that can easily be entrained in the seizure activity (present manuscript and Amor et al. 2009)). In addition, this change in inhibitory and excitatory balance might enable a trigger-input that is able reach this susceptible zone (prerequisite 2) and initializes a localized epileptic discharge that can quickly picked up and generalized by the thalamic counterpart (prerequisite 1).

The exact cortico-thalamo-cortical coupling dynamics in human absence epilepsy remain to be investigated, however, while cortico-cortical coupling dynamics are well described: In accordance with the cortical focus theory, it was found that the onset of SWD is associated with an increase in local clustering in a frontal or parietal region, which is followed by a repetitive pattern of global/generalized coupling during the wave and local coupling during the spike (Westmijse et al., 2009, Ossenblok et al., 2013). This is thought to be mediated via cortico-thalamo-cortical reentrance circuits (Ossenblok et al., 2013). By contrast, the description of cortico-thalamic coupling dynamics is still at a preliminary stage. So far, only a single study compared timepoints of SWD activation between thalamic nuclei using combined EEG-fMRI recordings: Interestingly, here non-specific nuclei (parafascicular and centromedian) were also found to be activated earlier than the specific (first order) ATN
Changes in BOLD signal, however, as described in this and other studies aimed to investigate cortical-subcortical coupling dynamics, are slow processes, while animal studies show (this thesis and Meeren et al. 2002) that network changes can occur in the range of hundreds of milliseconds. A chance for improvement to the investigation of cortico-thalamic coupling dynamics might be seen in the development of improved source-modeling techniques e.g. Dynamic Imaging of Coherent Sources (DICS), proposed to be able to also detect deeper sub-cortical sources (Gross et al., 2001): In a recent study by Moeller et al (2012), e.g. it was demonstrated that DICS was able to detect similar sources to fMRI, including deep thalamic sources. Spatial localization of sources might even further be improved by applying DICS in highly realistic anisotropic, six compartment, Finite Element Models. The use of such head-models, where the individual tissue conductivity, determinable by DTI, is also taken into account, has already been shown to improve localization of epileptic sources in focal epilepsy (Wolters et al., 2006, Rullmann et al., 2009).

With such source modeling techniques, the dynamics of interacting sources could be described in (small) analyses windows, which are shifted in small time-steps along pre-SWD -> SWD transition periods, improving the temporal precision. In addition, using analysis methods such as Partial Directed coherence even the most interesting question of coupling direction (which source drives which) might be approachable.

A prior validation of the quality of results that can be revealed with such new source modeling techniques could be performed in the rat (WAG/Rij and GAERS) where SWD coupling dynamics are already well described (see Paragraph 9.2).

If proven to be able to reveal correct results, such a non-invasive method to detect the epileptic focus might even help to improve invasive network analysis in the rat. As already noted above, within rats there is also some inter-individual variance with respect to the location of the epileptic focus. Prior non-invasive source modeling might enable an individualized implantation of recording electrodes to the epileptic focus, and (determined with DTI) the exact connected thalamic target areas.

Lastly, as already mentioned in Paragraph 9.4, a fourth interesting future trajectory, possibly the one with most clinical translatability and applicability is the development and validation of an online SWD-precursor detection algorithm. If shown to be able to detect SWD precursor with a high sensitivity and specificity, such an algorithm could be implemented in a closed loop BCI system to provide desynchronizing electrical stimulation to prevent SWD. Given the existence of a cortical epileptic focus in humans, such a closed loop precursor electrical stimulation paradigm might even be feasible in a non-invasive manner (Berenyi et al., 2012), however long-term efficacy of stimulation (e.g. the exclusion of SWD
rebound effects (Feddersen et al., 2007)) and safety of stimulation remains to be established prior to application in humans.

9.6 Evaluation of the cortical focus theory part two

The advent of the cortical focus theory in 2002, stating that the deep somatosensory cortex of absence epileptic rats contains a local epileptic focus, which is the origin of SWD, while the thalamus, functions as a resonator for SWD sustainment, has led to a shift in the distribution of functional significance. It changed from an almost equal distribution between cortex and thalamus found in the cortico-reticular theory (i.e attributing the thalamus with the function of the rhythm generator and the cortex with the function of a transformer (see Chapter 1)) to a much stronger weighting for the somatosensory cortex in the cortical focus theory. As a consequence the attention of subsequently performed research shifted towards the somatosensory cortex.

While in the current thesis quite some supporting evidence in favor of the deep somatosensory cortex as initiator of SWD could be obtained (Paragraph 9.1), the present results on the other hand also demonstrate that the thalamus is more than just a ‘passive’ resonator for SWD sustainment, and by this can pull part of the functional significance back to the thalamus: The deep cortical layers might initiate an SWD but early pre-ictal changes in cortico-thalamic as well as intra-thalamic coupling prepare the system towards a proepileptic state and might be responsible for whether the cortical focus gets the chance to entrain the cortex in SWD activity and decide whether a ‘full blown’ cortico-thalamo-cortical SWD can arise.

A recent review by Avoli (2012), giving a short historical overview on the theories of SWD generation noticed that the significance which is given to the cortex or thalamus in SWD generation has proceeded in cycles from thalamus (centrencephalic theory) to cortex (cortical theory), to thalamus and cortex (cortico-reticular theory, and back to cortex (cortical-focus theory), and predicted that the next epoch will again be a thalamic one. While the description of e.g. the Posterior thalamic nucleus, as an important ‘reverberator’, crucially relevant for SWD generation (see Paragraph 9.3.1a) might be seen to be in line with this prediction, I want to disagree or better warn for such a development. Even local changes have an effect on a complete network. Therefore the most appropriate way to learn about SWD generation, generalization, sustainment and termination is by studying network interactions.

In summary: an extended (refined) network analysis, employing a combination of experimental deep-brain stimulation studies and several, advanced, complementary signal
(connectivity) analytical methods applied to local field potential recordings, obtained within an extended part of the cortico-thalamo-cortical system with the aid of a newly developed electrode system, this thesis was able to:

- Verify the statement of the cortical focus theory that the deep somatosensory cortex is the initiator of SWD by showing that:
  - the deep somatosensory cortex is more excitable than the adjacent motor cortex and this difference is typical for absence epileptic WAG/Rij rats.
  - the deep somatosensory cortex has rhythm generating abilities (i.e. can generate local SWD, shows an earlier onset of SWD activity than other thalamic sites and can generate SWD-like self sustained AD).
  - the deep somatosensory cortex has driving capacities (drives most thalamic nuclei during the first 500ms of SWD)

- Extend and correct the cortical focus theory by:
  - Showing that the thalamus is more than a ‘passive’ resonator, some thalamic nuclei prepare the cortico-thalamo-cortical system into a pro-epileptic state
  - Showing that the thalamus is not a homogeneous structure and unraveling different functional roles of thalamic nuclei for the generation, sustainment and termination of SWD
  - Proposing a SWD generation scenario based on investigations of dynamical network (connectivity) changes
  - Proposing a SWD termination scenario based on investigations of dynamical network (connectivity) changes

- Show that SWD termination is not a sudden, unpredictable event, dominated by a single structure, but rather a gradual network process.
- Show that SWD generation is not a sudden, unpredictable event leading to, inter alia a new definition of the timepoint of SWD onset
- to correct the view of what should be regarded as key-network of SWD in the genetic rat models
- Bring up interesting new, scientific and clinically relevant research hypothesis for future studies and
- To extract clinical relevant information for the search of stimulation parameters and stimulation mechanisms for the newly proposed treatment option of DBS.
References


Addendum
English Summary

Bilaterally generalized spike and wave discharges (SWD) are the major electroencephalographic hallmark of absence epilepsy, a non-convulsive type of epilepsy found in young children. While there is a wide agreement that SWD are generated within the cortico-thalamo-cortical system, network interactions between cortex and different thalamic nuclei as well as the relative contributions of these structures for SWD generation, maintenance and termination are still enigmatic and highly debated, resulting in multiple theories on the origin of SWD. In the general introduction (Chapter 1) the clinical characteristics of absence epilepsy are introduced, the most commonly used animal models to study disease mechanisms of absence epilepsy, the basic anatomy of the cortico-thalamo-cortical system and the two most accepted competing ‘network theories’ on the origin of SWD. The most recent theory, the cortical focus theory, is currently in the focus of experimental investigation. It proposes that the deep layers of the perioral somatosensory cortex of genetic absence epileptic rats contain an epileptic area where SWD are generated, whereas the thalamus functions as a resonator for SWD sustainment. However, this theory is also only the beginning of a description (a new idea) on how the cortico-thalamo-cortical system is involved in the generation of SWD. Aim of the current thesis is to investigate the role of the cortico-thalamo-cortical system in the generation, maintenance and termination of SWD to a greater detail by (i) performing experimental test of the cortical focus theory; (ii) Describing dynamics of network interactions between cortex and different thalamic nuclei during pre-SWD->SWD and SWD->post SWD transition periods; and to (iii) investigate whether there are differences in functional contribution between cortex and thalamus as well as and in particular between different thalamic nuclei for the generation maintenance and termination of SWD and to disentangle these functions. Two study approaches, electrical deep brain stimulation and mathematical signal analysis, suited to achieve these aims are further introduced in chapter 1.

Chapter 2 starts the search for answers to the three above mentioned topics with a review of current studies offering experimental support for the cortical focus theory as well as earlier network studies which also tried to better describe ‘network changes’ occurring with SWD generation. In none of these earlier network studies interactions between the assumed cortical focus in the deep somatosensory cortex and thalamic nuclei have been investigated. A new multi-electrode system, developed in this thesis, suited for the simultaneous recording of LFP signals at multiple specific brain structures is introduced. Early hypotheses about the relative contribution of different structures of the cortico-thalamo-cortical system in the generation and disruption of SWD, based on exemplary observations of LFP signals of SWD obtained with this new electrode system are discussed.
The role of the cortico-thalamo-cortical system in absence epilepsy – Addendum

The idea of a local hyper-excitability epileptic focus, as proposed by the cortical focus theory, is investigated with the aid of local stimulation protocol (Chapter 3). It was demonstrated that stimulation of the deep somatosensory cortex resulted in larger, locally recorded, Electrical Evoked Potentials as compared to stimulation of the deep motor cortex of absence epileptic WAG/Rij rats, whereas no such difference between cortical areas was seen in healthy Wistar control rats. This indicated that the deep cortical layers of the somatosensory cortex are indeed highly excitable. Stimulation of the cortex in WAG/Rij rats but not in Wistar rats resulted also in self sustained afterdischarges (AD), which strongly resembled SWD in terms of their morphology, frequency and preferred state of occurrence during the drowsy state. Although thalamic recordings were missing, these AD were interpreted as cortico-thalamo-cortical oscillation and a sign of network excitability in WAG/Rij rats.

In Chapter 4 an online SWD detection algorithm, which might be suited for the implementation into a closed loop DBS system for the rapid and automatic application of responsive/closed loop electrical stimulation, is described and evaluated. This algorithm, which employs wavelet analysis (section 1.3) to estimate the power of LFP signals in the frequency range between 30-80Hz, is able to quickly (within 1 second) and reliably detect SWD with a specificity of about 96% and a sensitivity of up to 100%.

In the first part of Chapter 5 low frequency stimulation as used in Chapter 3 was applied to two thalamic nuclei (VPM and ATN). This was done in order to investigate whether the same type of SWD-like AD, as reported after stimulation of the somatosensory cortex in WAG/Rij rats can also be induced via thalamic stimulation and, like SWD, can be recorded simultaneously in cortex and thalamus. Next it was investigated whether stimulation of the VPM, which has direct connections to the focal epileptic area in the deep somatosensory cortex, is more effective in inducing AD as compared to stimulation of the ATN, which does not possess direct connections to the somatosensory cortex. It was found that thalamic stimulation induces SWD-like AD and that this effect was stimulation-site specific: stimulation of the VPM induced a large number of AD whereas AD were only rarely seen following ATN stimulation. AD could be recorded in both cortical and thalamic recording sites, showing that the above stated hypothesis (Chapter 3) that AD like SWD are cortico-thalamo-cortical phenomena, is valid. Next to a similar morphological structure, frequency and cortico-thalamic synchrony of SWD and AD, both oscillations were also found to react in the same way to experimental manipulation: stimulation of the ATN but not the VPM led to an increase of SWD and AD duration over time. Since the ATN does not possess any direct connections to the somatosensory cortex this effect is thought to be mediated via its connection to the rostral RTN, and therefore might indicates an opposite or differential role of caudal (receiving
connections from the VPM) and rostral RTN in the control of SWD/AD duration. In sum, these results led to the conclusion that AD are stimulation induced SWD.

Using the above, (Chapter 4) evaluated online SWD detection algorithm, the second part of Chapter 5 investigates the effect and brain-site specificity of high frequency, closed loop DBS (i.e. a short lasting stimulation of 1sec duration in response to each SWD) to disrupt SWD. Interestingly, whereas stimulation-site specific effects were found for the open loop (i.e. continuous) low frequency stimulation for the induction of seizures, as described above, no differences between ATN and VPM stimulation were found for the disruption of SWD (in terms of required intensity and percentage of disrupted SWD) using high frequency closed loop stimulation. It was speculated that the absence of this brain-site specificity might be attributable to the relatively late onset of stimulation (1 sec following SWD onset) when the SWD is already fully generalized. Brain-site specific effect, by contrast might only be visible in the very early stage of SWD generation.

To get a more detailed insight into the type and time point of network changes (interactions) that go along with SWD generation a switch from the experimental to the network analytical investigation is made (Chapter 6). Here the dynamic changes in coupling strength, coupling direction and time-shifts between different layers of the somatosensory cortex and different thalamic nuclei (VPM, caudal RTN, rostral RTN, Po and ATN) were sketched using non-linear association analysis. It was found that earliest and strongest increases in coupling strength (as compared to non-epileptic control periods) was seen between the somatosensory cortex and the posterior thalamic nucleus. These were seen as early as 1.25 secs prior to the onset of a full blown cortico-thalamic SWD (FCTS). Other thalamic nuclei, by contrast, only showed significant increases of coupling strength at FCTS. They became driven by the cortex during the first 500-750 ms of the SWD, whereas the Po stayed in a bidirectional crosstalk with the cortex throughout the whole SWD. Therefore the Po is the only nucleus which can function as a reverberator to the cortex within the first 500ms of SWD, which might be crucial for SWD generation. The finding that most other thalamic nuclei started to send input to the Po during SWD might additionally point to the possibility that the Po is involved in synchronizing and channeling thalamic output to the cortex and therefore supporting the generalization and maintenance of SWD. Lastly, again, evidence for a differential role of rostral and caudal RTN was revealed, since the caudal RTN was the only nucleus, which showed a pre-SWD decrease in coupling strength with the VPM, the Po and layer 4 of the somatosensory cortex.

The same dataset was used in Chapter 7, now two frequency domain analyses methods were employed: Time frequency (TFA) and paired phase consistency (PPC). TFA analyses revealed strong spectral precursor activity in the delta and theta frequency range which was
earliest (about 2.5 secs prior to SWD onset) and most pronounced in the somatosensory cortex. PPC analysis showed pre-SWD decrease in PPC in 4 out of 28 channel pairs. Here the caudal RTN came under the spotlight: it decoupled from Po, VPM and cortical layer 4 of the somatosensory cortex. A pre-ictal desynchronization was also reported in patients with absence seizures (Amor et al., 2009). Following the interpretation/proposal of these authors (Amor et al., 2009), it is proposed that this decoupling prepares the cortico-thalamo-cortical system towards a pro-epileptic state.

Strong increases in PPC were seen between cortex and thalamus, during SWD (starting at SWD onset). Intrathalamically all thalamic nuclei showed increases in PPC with the Po but not with VPM, which again was interpreted as indicating that the PO might be engaged in “channeling and synchronization” the thalamic output to the cortex.

In the last study of this thesis, presented in Chapter 8, frequency resolved Granger Causality was applied to pre-SWD -> SWD transition periods as well as to SWD -> post SWD transition periods, again recorded in layer 4,5 and 6 of the somatosensory cortex, VPM, Po, caudal and rostral RTN and ATN. This was done in order to verify earlier network results on SWD generation with another directed connectivity analysis and to investigate dynamics of network interactions relevant for the spontaneous termination of SWD. In line with earlier results on SWD generation, it could be verified that during the first 500ms the deep somatosensory cortex drives most thalamic nuclei while cortex and Po show an increased ‘bidirectional’ crosstalk and that following the first 500 ms other cortico-thalamic channel pairs also turned into a bidirectional interaction. One exception to this was seen in channel pair ctx4-rRTN, where a continuous cortical drive in the SWD relevant 8-10Hz band drive remained. This cortical drive was found to stop 1.5 seconds prior to SWD termination, representing the earliest change in coupling shortly prior to SWD termination, shortly followed by an increase in directional from the caudal onto the rostral RTN 1 second prior to SWD termination. At SWD offset most channel-pairs returned to baseline coupling values, while channel-pairs ctx5-Po and ctx6-Po kept a heightened cortex->thalamus coupling until 1.5 secs following SWD offset. It was concluded that SWD termination is a gradual process which involves both cortico-thalamic as well as intra-thalamic processes. The heightened drive of the cortex onto the Po following SWD termination was interpreted as a SWD re-initiation attempt. Lastly, the rostral RTN was proposed to be an important resonator for SWD maintenance, while the caudal RTN might be involved in breaking/ inhibit SWD oscillations.

The last chapter of this thesis, Chapter 9, contains the general discussion. In this discussion all results of this thesis were evaluated in the light of the three above outlined main aims of the thesis. This evaluation revealed the following conclusions: Aim 1: In line with the cortical focus theory it could be established that the deep somatosensory cortex of absence epileptic
rats contains a local region, which possesses multiple characteristics of an epileptic focus: it is more excitable than other cortical areas, it can generate local epileptic discharges, generalized SWD can be seen earlier in this region than in the thalamus, and electrical stimulation of this region can induce self-sustained afterdischarges. Aim 2: Based on the network analytical studies performed in Chapter 6 to 8 a SWD generation and an SWD termination scenario was proposed, showing that both the generation of SWD as well as the termination of SWD are not sudden and unpredictable events. Aim 3: The thalamus is not a homogeneous structure and that it is more than a ‘passive’ resonator. Some nuclei help to bring the cortico-thalamic system into a pro-epileptic state. In particular the following functional roles could be assigned to the different nuclei: the Po functions as a ‘reverberator’, it is important for SWD generation. It is the only thalamic nucleus that responds and gives feedback to the cortical focal area at SWD onset. The VPM was given a role as an ‘input relay station’, which relays crucial initializing SWD triggering stimuli to the epileptic focus. The rostral RTN was given the role of a ‘resonator’, important for SWD sustainment, while the caudal RTN was found to break or hamper SWD oscillations. Lastly, scientific and clinical implications resulting from the outcomes of this thesis are discussed and suggestions and hypothesis for future studies are proposed.
Nederlandse Samenvatting

Bilateraal gegeneraliseerde piek-golf ontladingen (SWD) zijn het elektroencephalografische kenmerk van absence epilepsie (AE), een niet convulsieve vorm van epilepsie die voornamelijk bij kinderen voorkomt. De opvatting dat SWD binnen het thalamo-corticale netwerk worden gegenereerd is wijd verspreid, echter onderzoekers zijn het oneens over de relatie tussen de bijdrage van cortex en thalamus. Dit is de aanleiding voor meerdere theorieën over het ontstaan van SWD, die ofwel een thalamische dan wel een corticale oorsprong veronderstellen. Daarnaast is het onduidelijk hoe de communicatie tussen deze beide delen van het brein, die samen het cortico-thalamo-corticale netwerk vormen, verandert zowel tijdens het ontstaan als tijdens het stoppen van SWD. De meest recente theorie over het ontstaan van SWD is de in Nijmegen geformuleerde corticale-focus theorie. Deze theorie staat tegenwoordig in het brandpunt van de belangstelling bij onderzoekingen naar de oorzaak van deze vorm van epilepsie. De theorie stelt, dat de diepe lagen van het peri-orale gebied van de somatosensorische cortex van absence epileptische ratten een hyperexcitabel gebied bevat wat de bron/generator van de SWD is, terwijl de thalamus gezien wordt als een noodzakelijke, maar ‘passieve’ resonator, die verantwoordelijk is voor het onderhouden van SWD.

Het doel van dit proefschrift is om de functie van het cortico-thalamo-corticale systeem betrokken bij het genereren, onderhouden en stoppen van SWD in kaart te brengen door
a) de corticale focus theorie experimenteel te toetsen;
b) dynamische veranderingen in de communicatie tussen het potentiële corticale, epileptisch focus en verschillende thalamische kernen te beschrijven in periodes voor, tijdens en na de aanwezigheid van SWD (preSWD->SWD en SWD->post-SWD transitie-periodes) en daarmee
c) te onderzoeken of er verschillen zijn in de bijdrage van verschillende thalamische kernen en de cortex voor het genereren, onderhouden en stoppen van SWD.

Om dit te doen zijn er experimentele, elektrische stimulatie - en signaal analytische studies gedaan, welke in hoofdstuk 1 van dit proefschrift, de algemene inleiding, in detail worden uitgelegd. Daarnaast wordt in hoofdstuk 1 het klinisch beeld van absence epilepsie, de anatomie en fysiologie van het cortico-thalamo-corticale systeem en gedachten/theorieën over het ontstaan van SWD nader toegelicht.

In hoofdstuk 2 wordt allereerst met behulp van een literatuurreview een overzicht gegeven wat er aan het begin van het proefschrift al bekend was over netwerk interacties die betrokken zijn bij het tot stand komen van SWD. Hierbij is vastgesteld dat in alle eerdere studies nooit interacties tussen het veronderstelde corticale focus (in de diepe lagen van de
somatosensorische cortex) en multiple thalamische kernen onderzocht zijn. Daarnaast werd in dit hoofdstuk een nieuwe door ons ontwikkeld multiple-elektrode systeem geïntroduceerd; dit stelt ons in staat om tegelijkertijd van verschillende breinstructuren lokale veld potentialen (local field potentials, LFP) te meten en daarmee netwerk interacties tussen de structuren te bestuderen.

De eerste metingen met behulp van dit systeem lieten zien dat er soms gelokaliseerde SWD activiteit in de somatosensorische cortex te zien valt terwijl deze in de thalamische afleidingen ontbreekt. Daarnaast werd opgemerkt dat voor sommige gegeneraliseerde ('full-blown') SWD, dat wil zeggen met SWD activiteit in cortex en thalamus, deze activiteit eerder in de cortex dan in de thalamus te zien is en aan het einde van de SWD ook in de cortex langer doorgaat dan in de thalamus.

In hoofdstuk 3 begint het experimentele gedeelte van dit proefschrift. In dit hoofdstuk wordt de idee van een lokaal hyperexcitabel gebied met behulp van een dubbel pulst stimulatie protocol getoetst: in twee groepen epileptische WAG/Rij ratten werd of in de diepe lagen van de somatosensorische cortex of in de diepe lagen van de motor cortex, elektrische prikkels met een lage stimulatie intensiteit toegediend. Door middel van een derde elektrode, die zich precies naast de stimulatie elektrodes bevond, werd de reactie van het hersengebied op de elektrische stimulatie gemeten. De grootte van de reactie is een goede maat voor excitabiliteit. Stimulatie van de somatosensorische cortex wekte sterkere (groteere amplitude) reacties op dan stimulatie van de motor cortex en dat dit verschil werd niet gevonden tussen somatosensorisch- en motor cortex stimulatie in niet-epileptische ratten. Daarom kan worden geconcludeerd, dat de somatosensorische cortex van absence epileptische ratten inderdaad excitabeler is dan een ander (aangrenzend) corticaal gebied. Daarnaast werd geconstateerd dat door stimulatie van de somatosensorische cortex van absence epileptische ratten soms epileptische na-ontladingen werden opgewekt, die qua morfologie en frequentie sterk op SWD lijken. Net als SWD, treden deze na-ontladingen voornamelijk op tijdens een toestand van lichte slaperigheid. Gezien deze overeenkomsten tussen na-ontladingen en SWD werd aangenomen dat dit soort na-ontladingen, net als SWD, cortico-thalamo-corticale, epileptische ontladingen zijn, ondanks dat er in dit experiment geen metingen in de thalamus gedaan zijn.

Om ook elektrische stimulatie van het brein in onmiddellijke reactie op een SWD te kunnen geven, wordt in hoofdstuk 4 van dit proefschrift een automatische SWD detectie programma ontwikkeld en gevalideerd. Dit programma, ontwikkeld door onderzoekers van de universiteit van Saratov, analyseert de power in het EEG in het frequentiegebied tussen 30-80 Hz met behulp van een wavelet algoritme: geconstateerd werd dat het algoritme in staat is om
binnen een halve seconde een SWD te kunnen detecteren met een sensitiviteit van 100% en een specificiteit van 96%.

Als vervolg op de experimenten zoals beschreven in hoofdstuk 3, wordt in het eerste gedeelte van hoofdstuk 5 hetzelfde type dubbel puls stimulatie als gebruikt is in hoofdstuk 3 toegepast aan twee thalamische kernen (VPM en ATN) en werd de reactie op de stimulatie in de cortex en in de thalamus gemeten. Geconstateerd werd dat ook thalamische stimulatie SWD-achtige na-ontladingen kan opwekken en dat de na-ontladingen zowel in de cortex als in de thalamus synchroon aanwezig zijn. De na-ontladingen worden vaker gezien na stimulatie van de VPM, een kern die rechtstreekse verbindingen heeft met het gebied waar het epileptisch focus zich bevindt. ATN stimulatie wekte echter nauwelijks na-ontladingen op, wat te verklaren zou kunnen zijn door het feit dat de ATN geen rechtstreekse verbindingen naar het epileptische focus heeft. Aangezien de na-ontladingen synchroon in cortex en thalamus (VPM) werden gemeten, kon de in hoofdstuk 3 geformuleerde hypothese, dat deze na-ontladingen van cortico-thalamo-corticale natuur zijn, worden bevestigd. Naast de optische gemeenschappelijkheden (frequentie, morfologie) tussen na-ontladingen en SWD werd ook ontdekt dat beide types oscillaties op dezelfde manier op experimentele manipulaties reageren: langdurige (gedurende 5 uur), laag frequentie stimulatie van de ATN, maar niet van de VPM, verlengde de duur van na-ontladingen en SWD over de tijd. Gezien de ATN geen rechtstreekse verbindingen naar het corticale focus heeft, werd aangenomen dat dit stimulatie effect veroorzaakt werd via de rostrale RTN, die projecties van de ATN ontvangt. Ook de VPM heeft projecties naar de RTN maar in tegenstelling tot de ATN, projecteert de VPM niet naar het rostrale gedeelte maar naar het caudale gedeelte van de RTN. De verschillende effecten van stimulatie van de caudale en de rostrale RTN op de duur van SWD suggereren een differentiële rol van de beide delen in het controleren ofwel beïnvloeden van de duur van SWD. Specifieker geformuleerd: het lijkt er vooral op dat de rostrale RTN een rol speelt in het controleren van de duur van SWD ofwel een bijdrage lijkt te hebben in het onderhouden van SWD.

Op basis van de verschillende overeenkomsten tussen na-ontladingen en SWD kan worden geconcludeerd, dat dit soort na-ontladingen stimulatie geïnduceerde SWD zijn.

Naast deze ‘open loop’, laag frequentie stimulatie, wordt in het tweede gedeelte van hoofdstuk 5, ook het effect van een ‘closed loop’, hoog frequent stimulatie (130 Hz, bifasische pulsjes 0.4 ms, duur stimulatie 1 sec) onderzocht. Deze werd met behulp van het in hoofdstuk 4 ontwikkelde SWD detectie algoritme meteen na detectie van een SWD (circa een seconde na het begin van de SWD) aan de VPM of de ATN toegepast. Dit werd gedaan om te onderzoeken of dit soort stimulatie in staat is om SWD af te breken en om te
kijken of de effectiviteit om SWD af te breken afhankelijk is van de gestimuleerde thalamische kern.

Dit werd gemeten aan de hand van de intensiteit die nodig was om SWD af te breken (zoals bepaald in een drempelwaarde test) en het percentage afgebroken SWD gedurende een stimulatie sessie van 3 uur. Verwacht werd, dat net als bij de laag frequente stimulatie, hoog frequente stimulatie van de VPM, met directe verbindingen naar het epileptische focus, effectiever zou zijn om SWD af te breken, dan stimulatie van de ATN. Er werden in tegenstelling tot onze verwachtingen geen verschillen tussen ATN en VPM stimulatie gevonden. Hoog frequente, closed loop stimulatie van beide structuren was uitermate efficiënt om SWD af te breken. Gespeculeerd werd dat het ontbreken van verschillen tussen ATN en VPM stimulatie misschien te wijzen valt aan het feit dat de stimulatie pas 1 seconde na begin van het SWD werd toegediend, een tijdstip waar de SWD al gegeneraliseerd zijn. Vroegere stimulatie zou echter nog steeds verschillen kunnen opleveren tussen beide thalamische kernen.

Om een nauwkeuriger beeld te krijgen over de aard en tijdstip van netwerkveranderingen die gepaard gaan met het ontstaan van een SWD, wordt in hoofdstuk 6 de eerste signaal analytische studie gedaan. Hiervoor werd in 16 absence epileptische WAG/Rij ratten in de diepe lagen van de somatosensorische cortex en meerder thalamische kernen (VPM, ATN, Posterior thalamus (Po), rostrale en caudale RTN LPF gemeten. Deze werden geanalyceerd met behulp van een niet-lineaire associatie analyse: in een data-window van 500 milliseconden dat langs zes seconden durend pre-SWD->SWD transitie periodes werd geschoven, werden de veranderingen in de sterkte van koppeling (communicatie) tussen kanaalparen, de richting van koppeling (is er een structuur die een andere structuur aanstuurt) en de duur van signaal transductie (de tijd die nodig is om informatie van structuur A naar structuur B te sturen) met een hoge temporele resolutie in kaart gebracht.

Ontdekt werd dat de vroegste toenames in koppelindexstrenkte (vergelijken met niet-epileptische controle periodes) te vinden zijn tussen de diepe lagen van de somatosensorische cortex en de Po. Deze toenames waren al 1.25 seconden voor het ontstaan van een ‘full blown’ cortico-thalamo-corticale SWD (met SWD activiteit in cortex en thalamus) te zien. Alle andere thalamische kernen lieten deze toename in koppelindexstrenkte met de cortex pas aan het begin van de SWD zien. Terwijl de thalamische kernen, die de toename in koppelindex pas aan het begin van de ‘full blown’ SWD lieten zien, door de somatosensorische cortex gedurende de eerste 500 tot 750 milliseconden na begin van het SWD werden gestuurd, onderhield de Po altijd een bidirectionele communicatie met de cortex. Met andere woorden, de Po is de enige thalamische kern, die op de cortex kan
blijven reageren/antwoorden gedurende de hele SWD periode. Gespeculeerd werd dat deze bi-directionele communicatie belangrijk is voor het ontstaan van SWD. Daarnaast was te zien, dat de andere thalamische kernen informatie naar de Po begonnen te sturen. Dat werd geïnterpreteerd als dat de Po geïncludeerd is in het verzamelen van de thalamische activiteit om een gehele (synchrone) thalamische, output-reactie naar de cortex te kunnen sturen. 

Ten slotte werden er ook in deze studie indicaties voor een differentiële rol van de caudale en rostrale RTN gevonden. Vastgesteld werd dat de caudale RTN als enige thalamische kern een afname in koppelingsterkte liet zien; dit gebeurde in een periode vlak voor het begin van een SWD en het betrof een ontkoppeling van de caudale RTN naar de Po.

In hoofdstuk 7 wordt dezelfde dataset als die van hoofdstuk 6 opnieuw geanalyseerd om signaal karakteristieken in the frequentie domein te bekijken en om pairwise phase consistency (PPC, faseconsistenties) tussen kanalen te kunnen bestuderen. Allereerst is met behulp van een time-frequency analyse de spectraal-inhoud (power) van de LFP in de verschillende gebieden bepaald. Ontdekt werd, dat er in de diepe lagen van de somatosensorische cortex al heel vroeg (2.5 seconden voor de start van de SWD) precursor activiteit (toename in power groter dan twee keer de power in controle periodes) te zien valt. Deze precursor activiteit was te vinden in het delta en theta frequentie bereik. Met behulp van PPC analyse werd vastgesteld dat 4 van de 28 kanaalparen al veranderingen lieten zien voor de start van de SWD. In alle gevallen was de pre-SWD verandering een verlaging van de PPC vergeleken met niet epileptische controle fragmenten. Daarnaast is opmerkelijk dat in 3 van de 4 gevallen de caudale RTN geïncludeerd was. Deze kern ontkoppelde van de Po, de VPM en laag 4 van de somatosensorische cortex. Interessant is dat er ook in een EEG studie met juvenile absence epilepsie patiënten (Amor en collega’s, 2009) een pre-SWD desynchronisatie werd gevonden. In overeenstemming met bevindingen interpreteren ook wij onze pre-SWD phase desynchronisatie als een voorbereidingsproces bij het ontstaan van een “full blown” SWD, waarbij sprake is van massale synchronisatie; het is een voorbereidingsfase van het cortico-thalamo-corticale systeem dat dan in een toestand gebracht kan worden waarin massale synchronisatie mogelijk is. De ontkoppeling van de caudale RTN lijkt daarbij een centrale voorwaarde te zijn.

Aan het begin van de ictale fase lieten alle cortico-thalamische kanaalparen sterke toenames in PPC zien. Intrathalamisch werd, net als in hoofdstuk 6, vastgesteld dat alle thalamische kernen een verhoogde koppeling met de Po lieten zien, maar niet met de VPM. Dit werd opnieuw geïnterpreteerd als een teken, dat de Po geïncludeerd is in het verzamelen van thalamische informatie om deze vervolgens gezamenlijk naar de cortex terug te kunnen sturen.
In het laatste experimentele hoofdstuk van dit proefschrift, hoofdstuk 8, zijn naast pre-SWD ->SWD transitie periodes ook SWD -> post-SWD transitie periodes geanalyseerd. Dit werd gedaan met een (lineaire), frequentie domein gevoelige Granger Causality analyse, die net als de niet-lineaire associatie analyse de richting van koppeling tussen twee kanaalparen beschrijft. Doel hiervan was om eerder opgedaan netwerk resultaten over het initiëren van SWD te valideren met behulp van een andere analysemethode die ook in staat is de richting van koppeling te beschrijven, en om inzicht te krijgen in netwerk interacties die samengaan met het stoppen van SWD. In overeenstemming met de resultaten van hoofdstuk 6 werd ook hier gevonden dat de cortex de meest thalamische kernen in de eerste 500 milliseconden na het opstarten van de SWD stuurt, terwijl cortex en Po een bidirectionele communicatie blijven onderhouden. Ook in overeenstemming met hoofdstuk 6 was dat alle cortico-thalamische paren na de eerste 500ms net als cortex-Po in een bidirectionele wijze communiceren. Een uitzondering was kanaalpaar cortex - rostrale RTN, waar de sturing van de cortex in het voor SWD relevante 8-10Hz frequentie geschiedde.

Deze corticale sturing van de rostrale RTN bleek 1.5 seconden voor het stoppen van de SWD te eindigen. Het kan worden opgemerkt dat dit de vroegste verandering in netwerk communicatie representeert die geassocieerd zou kunnen worden met het stoppen van SWD. Dit werd gevolgd, 1 seconde voor het stoppen van de SWD, door een toename van directionele sturing van de caudale naar de rostrale RTN. De meeste andere cortico-thalamische paren en alle intra-thalamische paren lieten echter een plotselinge daling van hun koppeling waardes aan het einde van de SWD zien. Slechts twee cortico-thalamische paren (somatosensorische cortex laag 5 -> Po en somatosensorische cortex laag 6 -> Po) vormden een uitzondering. Voor deze twee paren werd vastgesteld dat de cortex opnieuw de Po ging sturen. Dit begon aan het einde van de SWD en bleef bestaan tot 1.5 seconden na afloop van de SWD. In tegenstelling tot de SWD initiatie en onderhoudsfase werd voor deze twee kanaalparen echter gezien dat de Po niet op de cortex reageerde, er was dan geen sprake meer van een bi-directionele communicatie. Geconcludeerd werd dat het stoppen van SWD een geleidelijk proces is dat cortico-thalamische en intra-thalamische processen bevat. De sturing van de Po door de cortex na afloop van de SWD werd als een SWD re-initiatie poging geïnterpreteerd, welke mislukt gezien de Po niet meer op de cortex reageert met een poging tot beïnvloeding van de cortex. Daarnaast werd voorgesteld dat de rostrale RTN de functie van een resonator, belangrijk voor het onderhouden van SWD, heeft, terwijl de caudale RTN de functie van een rem voor SWD activiteit zou kunnen vervullen.
Hoofdstuk 9 van dit proefschrift bevat de algemene discussie van de proefschrift, waar alle resultaten van deze proefschrift worden geëvalueerd in het licht van de drie bovengenoemde ‘hoofddoelen’ van de proefschrift. Deze evaluatie leidde tot de volgende conclusies:

Doel 1: In overeenstemming met een hypothese, die rechtstreeks voortvloeit uit de corticale focus theorie, kan worden geconstateerd, dat de diepe lagen van de somatosensorische cortex van absence epileptische WAG/Rij ratten inderdaad een lokaal gebied bevatten, dat meerdere karakteristieken van een epileptische bron heeft. Het gebied is meer excitabel dan andere corticale gebieden, er kan soms lokale epileptische activiteit worden gezien, SWD activiteit begint eerder in dit gebied dan in de thalamus en de cortex stuurt de thalamus in het begin van een aanval, en elektrische stimulatie van dit gebied kan SWD-achtige na-ontladingen opwekken.

Doel 2: Baserend op de netwerk-analytische studies uit hoofdstuk 6 t/m 8 werd er een gedetailleerd scenario over het starten en stoppen van SWD geformuleerd. Voor beide gevallen kan worden geconcludeerd dat het geleidelijke in plaats van plotseling mechanisms zijn. In totaal konden er vijf fases worden geïdentificeerd: een voorbereidingsfase, die het thalamo-corticale systeem in een pro-epileptische toestand zet (een tot twee seconden voor de start van de SWD), een initiatiefase (de eerste 500 seconden), een onderhoudsfase, een geleidelijke stopfase (beginnend 1,5 seconden voor het stoppen van de SWD) en een reinitialisatie-poging fase.

Doel 3: Geconcludeerd kan worden dat de thalamus geen homogene structuur is. Sommige thalamische kernen helpen mee om het cortico-thalamische systeem in een pro-epileptisch stadium te brengen. Concreet zijn de volgende functies aan de verschillende thalamische kernen toegekend: De Po krijgt de functie van een ‘reverberator’, gezien het de enig thalamische kern is die binnen de eerst 500ms een bidirectionele communicatie met het focale gebied onderhoudt. Deze bidirectionele communicatie lijkt heel belangrijk voor het opstarten van een SWD, dat blijkt ook uit het feit dat vlak naar afloop van de SWD de unidirectionele sturing van de cortex naar de thalamus (Po) niet voldoende is om een nieuwe SWD te kunnen starten. De VPM krijgt de functie van een ‘input relay station’ die initialiserende trigger-input stimuli naar het epileptische focus kan sturen; de rostrale RTN krijgt de functie van een ‘resonator’, die belangrijk is voor het onderhouden van SWD terwijl de caudale RTN de functie van een rem voor SWD activiteit toebedeeld krijgt. Aan het begin van een SWD moet de cRTN ontkoppelen (de rem loslaten) en aan het eind van een SWD moet de caudale RTN de rostrale RTN remmen.

Ter afsluiting van dit laatste hoofdstuk worden wetenschappelijke en klinische implicaties van deze resultaten en ideeën voor mogelijk vervolg onderzoek besproken.
Curriculum Vitae
Annika Katharina Lüttjohann was born on the 17th of August 1983 in Emmerich, Germany. After finishing her secondary school at Willibrord Gymnasium Emmerich in 2003, she continued her education in the Netherlands with the study of psychology at the Radboud University Nijmegen. During this study she specialized on the sub-discipline of biological psychology, obtaining her Bachelor degree in 2007 with her thesis entitled “The relationship between HPA-axis dysfunction and serotonin depletion in unipolar depression”. During her Master internship under the supervision of Prof. Dr. van Luijtenaar at the department of biological psychology she gathered first experiences in experimental epilepsy research resulting in her Master degree in 2008 with the thesis entitled “A Revised Racine’s scale for PTZ induced seizures in rats”. The PhD-project “The role of the cortico-thalamo cortical system in absence epilepsy” finalized and presented in the current thesis was offered to her in 2008. Since August 2013 she continues to study the cortico-thalamo-cortical system in absence epilepsy as a post-doctoral fellow at the Institute of Physiology I, Westfälische Wilhelms Universität, Münster, Germany.

Awards and Sponsorships
2008  Nominated as Eligible Master student by Dutch Neurofederation
2008  Sponsoring of the International League Against Epilepsy (ILAE) for the attendance of the SanServolo Advanced International Summer School: Bridging Basic with Clinical Epileptology
2011  Young Investigator Travel Grand Award of the American Epilepsy Society granted to poster abstract: "The role of the posterior thalamic nucleus revealed by signal analytical network analysis"

Attended Conferences
2009  28th International Epilepsy Congress, Budapest, Hungary
2010  9th European Congress on Epileptology, Rhodes, Greece
2010  7th FENS Forum of European Neuroscience, Amsterdam, The Netherlands
2011  AES 2011 (Annual Meeting of the American Epilepsy Society)
2012  8th FENS Forum of European Neuroscience, Barcelona, Spain
List of Publications

International peer reviewed journals


Patient oriented PR

An interview with Annika Lüttjohann and Gilles van Luijtelaar about their work on DBS in epilepsy was published in the patient magazine of the Dutch League Against Epilepsy: “Stroom tegen Stroom” In Episcoop (June 2012)

Annika Lüttjohann Nieuwe mogelijkheden voor lokale, niet invasieve behandeling van absences, published in the patient magazine ‘Transmissie’ (Oktober 2012) http://www.epilepsievereniging.nl/alles-over-epilepsie/uit-de-wetenschap/absences-nieuwe mogelijkheden/
Book chapters

Contribution in Dutch, scientific journals
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