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DYNAMICS OF DIRECTIONAL COUPLING UNDERLYING SPIKE-WAVE DISCHARGES

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Abstract—*Purpose:* Spike and wave discharges (SWDs), generated within cortico-thalamo-cortical networks, are the electroencephalographic biomarker of absence epilepsy. The current work aims to identify mechanisms of SWD initiation, maintenance and termination by the analyses of dynamics and directionality of mutual interactions between the neocortex and various functionally different thalamic nuclei. *Methods:* Local-field potential recordings of 16 male Wistar Albino Glaxo from Rijswijk (WAG/Rij) rats, equipped with electrodes targeting layer 4–6 of the somatosensory cortex, rostral and caudal reticular thalamic nuclei (rRTN and cRTN), ventro-posteromedial (VPM), anterior (ATN) and posterior (PO) thalamic nuclei, were obtained. 3 s epochs prior to SWD onset, after SWD onset, prior to SWD offset and after SWD offset were analyzed with newly developed time-variant adapted nonlinear Granger causality. *Results*: A gradual increase in coupling toward SWD onset between cortico-cortical pairs appears as early as 2 s preictally. Next first unidirectional increase in coupling is noticed in a restricted number of cortico-thalamic and thalamo-cortical channel pairs, which turn into bidirectional coupling approaching SWD onset, and a gradual increase of intra-thalamic coupling. Seizure onset is characterized by a coupling decrease for more than a second in a majority of channel pairs, only the cortex kept driving the cRTN. Intrathalamically the cRTN drives the PO, VPM and ATN. Most channel pairs no longer show differences in coupling with baseline during SWD maintenance, a major exception is the unidirectional coupling between cortex and cRTN. Toward the end of SWDs, more and more channel pairs show an increase in often bidirectional coupling, this increase suddenly vanishes at SWD offset. *Conclusion:* The initiation of SWD is due to a gradual increase in intracortical coupling, followed by a selective increase in first unidirectional and later bidirectional coupling between the cortex and thalamus and also intrathalamically. Once the network is oscillating, coupling decreases in most of the channel pairs, although the cortex keeps its influence on the cRTN. The SWD is dampened by a gradual increase in coupling strength and in the number of channel pairs that influence each other; the latter might represent an endogenous brake of SWDs.

Key words: WAG/Rij rats, network analysis, absence epilepsy, time-variant adapted nonlinear Granger causality, cortex, thalamus.

INTRODUCTION

Absence epilepsy is classically considered as a nonconvulsive generalized epilepsy (classification of the International League Against Epilepsy, ILAE; Berg et al., 2010) of unknown etiology. Its clinical symptoms are rather small and might even go unnoticed especially when they last only several seconds, facial automatisms might be lacking and the brief decrease of responsiveness, the interruption of ongoing behavior, and the impaired mental functioning are difficult to notice. In contrast, the electroencephalogram (EEG) during typical absence seizures is an archetypical clear and easily recognized 3–4 Hz pattern of spike-and-wave discharges (SWDs). The SWDs are conceptualized to originate at some point within the cortico-thalamic system, and rapidly engage other parts of a bilaterally distributed circuit. Sites of origin can be visualized with imaging and signal analysis techniques and nowadays most often cortical origin sites are reported (Holmes et al., 2004; Westmijse et al., 2009; Tenney et al., 2013; Ossenblok et al., 2013).

Similar EEG paroxysms, SWDs, appear in rat strains with a genetic predisposition to develop absence epilepsy, such as GAERS (Genetic Absence Epilepsy Rats from Strasbourg – (Vergnes et al., 1987)) and WAG/Rij (Wistar Albino Glaxo from Rijswijk – (Sitnikova and van Luijtelaar, 2006)). The EEG waveform and duration (1–30 s, mean 5–6 s) of SWD in rats and in humans are comparable, but the frequency of SWD in rats is higher 7–11 Hz (van Luijtelaar and Coenen, 1986; Sitnikova and van Luijtelaar, 2007). In WAG/Rij rats a consistent initiation zone in the perioral region of the

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Abbreviations: ATN, anterior thalamic nucleus; cRTN, caudal reticular thalamic nucleus; EEG, electroencephalogram; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; GC, Granger causality; LPFs, local field potentials; MI, mutual information; PO, posterior thalamic nucleus; rRTN, rostral reticular thalamic nucleus; SWDs, spike and wave discharges; VPM, ventro-posteromedial thalamic nucleus; WAG/Rij, Wistar Albino Glaxo from Rijswijk.

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somatosensory cortex was found (Meeren et al., 2002). This leading role of a cortical initiation zone in spreading of SWD in humans and in WAG/Rij rats was established with signal analytical methods that did take into account simultaneous processes in different locations (network analyses). Time resolved methods of coupling analysis applied for multichannel time series were used such as nonlinear association analyses, but also linear Granger methods (Granger, 1969) and phase synchronisation (Pijn et al., 1989; Westmijse et al., 2009; Sitnikova et al., 2006; Lüttjohann and van Luijtelaar, 2012; Lüttjohann et al., 2013). While earlier studies focused on cortical spreading and confirmed a leading role of the somatosensory cortex in the initiation of SWD (Meeren et al., 2002), subsequent studies found strong and reciprocal coupling between the frontal cortex and thalamus or increase in SWD-related increase of intra- and interhemispheric and intrathalamic coherence during SWD (Sitnikova et al. 2006, 2008); the role of subparts of the thalamus was less well investigated. Classically the ventro-posteromedial (VPM) and rostral reticular (rRTN) thalamic nuclei were thought to be the primary nuclei in SWD occurrence and maintenance. The thalamus is a collection of functionally heterogeneous nuclei (sensory, limbic, motor, arousal) reciprocally connected with different parts of the cortex. Recent studies toward interactions of the assumed cortical site of origin of the SWD with various thalamic nuclei with time resolved methods revealed an additional role for the posterior nucleus (PO) of the thalamus, and for the caudal part of the reticular nucleus (cRTN) in SWD initiation and maintenance (Lüttjohann and van Luijtelaar, 2012; Lüttjohann et al., 2013).

In comparison with traditional methods of network analysis, such as cross-correlation, coherence, phase synchronisation, Granger causality (GC) may detect weak or hidden coupling, which not necessarily lead to synchronisation. It defines next to changes in coupling strength also changes in the direction of coupling within a network. Granger used only linear predictive (autoregressive) models; new nonlinear models were successfully developed and applied (Wang, 2007; Bezruchko and Smirnov, 2010). This is not trivial for its application in EEG paroxysms considering that seizure activity has nonlinear properties (Le van Quyen et al., 1999; Lehnertz, 1999; Lopes da Silva et al., 2003) and linear methods may capture only part of the coupling.

Here a recently developed new nonlinear approach called time-variant adapted GC was used and applied to a previously published data set (Lüttjohann and van Luijtelaar, 2012; Lüttjohann et al., 2013, 2014) of *in vivo* local field potentials (LPFs) data recorded by means of intracranial electrodes implanted in the deep somatosensory cortex and in five different parts of the thalamus in WAG/Rij rats. Recent data in humans and in these genetic absence epileptic rats suggest that SWD do not arise out of the blue (Holmes et al., 2004) but are preceded by precursor and network activity in and between cortex and thalamus (Gupta et al., 2011). Also the end of SWD was initiated by a decrease of linear coupling from the somatosensory cortex to the rRTN, as well as increased coupling from the caudal to the rRTN (Lüttjohann et al., 2014). Here the dynamics of cortico-cortical, cortico-thalamic and thalamo-thalamic network interactions will be investigated in this absence model in three stages using this new nonlinear method (Sysoeva et al., 2014): the transition from preictal to ictal phase, the ictal phase and the transition from the ictal to the post-ictal state.

EXPERIMENTAL PROCEDURES

Subjects

Sixteen male WAG/Rij rats, 6–9 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 (light off at 8.30 AM). After surgery rats were housed individually. The experiment was approved by the Ethics 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: $A/P = 0.0$, $M/L = -4.6$ depth $= -2.8$ (layer 4), -3.1 (layer 5), $-$ 3.6 (layer 6); anterior thalamus: $A/P = -1.4$ $M/L = -1$, depth $= -6.2$; rRTN: $A/P = -1.4$, $M/L = -1.9$, depth $= -6.6$; PO: $A/P = -3.6$, $M/L = -2$, depth $= -5.4$; VPM: $A/P = -4.16$, $M/L = -2.8$, depth $= -6$ and cRTN: $A/P = -3.1$, $M/L = -3.5$, depth $= -6.6$. All coordinates were determined relative to bregma according to the rat-brain atlas of Paxinos and Watson, 2006. Electrode wires, assembled in a self-constructed electrode system (Lüttjohann and van Luijtelaar, 2012) 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.

Recording of local field potentials

Two weeks after surgery rats were placed individually in a $20 \times 35 \times 25$ inch Plexiglas registration boxes and connected to the recording leads for multichannel LFP recordings. These were attached to a swivel-contact,