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Thalamic lesions in a genetic rat model of absence epilepsy: Dissociation between spike-wave discharges and sleep spindles

Hanneke K.M. Meeren [a], Jan G. Veening [b], Tanja A.E. Möderscheim [c], Anton M.L. Coenen [c], Gilles van Luijtelaar [c,*]

[a] Cognitive and Affective Neuroscience, Tilburg University, Tilburg, The Netherlands
[b] Donders Center for Neuroscience, Radboud University Nijmegen, Nijmegen, The Netherlands
[c] Donders Center for Cognition, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands

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ABSTRACT

Recent findings have challenged the traditional view that the thalamus is the primary driving source of generalized spike-wave discharges (SWDs) characteristic for absence seizures, and indicate a leading role for the cortex instead. In light of this we investigated the effects of thalamic lesions on SWDs and sleep spindles in the WAG/Rij rat, a genetic model of absence epilepsy. EEG was recorded from neocortex and thalamus in freely moving rats, both before and after unilateral thalamic ibotenic acid lesions. Complete unilateral destruction of the reticular thalamic nucleus (RTN) combined with extensive destruction of the thalamocortical relay (TCR) nuclei, resulted in the bilateral abolishment of SWDs and ipsilateral abolishment of sleep spindles. A suppression of both types of thalamocortical oscillations was found when complete or extensive damage to the RTN was combined with minor to moderate damage to the TCR nuclei. Lesions that left the rostral pole of the RTN and part of the TCR nuclei intact, resulted in an ipsilateral suppression of sleep spindles, but a large increase of bilateral SWDs. These findings demonstrate that the thalamus in general and the RTN in particular are a prerequisite for both the typical bilateral 7–11 Hz SWDs and natural occurring sleep spindles in the WAG/Rij rat, but suggest that different intrathalamic subcircuits are involved in the two types of thalamocortical oscillations. Whereas the whole RTN appears to be critical for the generation of sleep spindles, the rostral pole of the RTN seems to be the most likely part that generates SWDs.

Introduction

Bilaterally synchronous generalized spike-wave discharges (SWDs) in the electroencephalogram (EEG), characteristic for absence epilepsy, reflect high synchronized oscillations in the cortico-thalamo-cortical network (e.g., Gloor, 1968; Niedermeyer, 1996; Penfield and Jasper, 1954). It is commonly assumed that the generation of these paroxysmal oscillations, just like the normal physiological spindle oscillations, critically depends on the intrathalamic circuitry, through synaptic interactions between the GABAergic neurons of the reticular thalamic nucleus (RTN) and the glutamatergic neurons of the thalamocortical relay (TCR) nuclei. More specifically, the RTN is thought to act as pacemaker of the oscillations and considered to be an important source for the synchronization between different TCR nuclei (Avanzini et al., 1993, 2000; Buzsáki, 1991; Huguenard and McCormick, 2007; Kostopoulos, 2000; McCormick and Bal, 1997; Steriade et al., 1985, 1987; Steriade and Llinas, 1988).

The thalamus has indeed been demonstrated to be a prerequisite for the generation of typical bilaterally synchronous SWDs (Avanzini et al., 1992; Avoli and Gloor, 1981; Buzsáki et al., 1988; Pellegrini et al., 1979; Vergnes and Marescaux, 1992). More specifically, the RTN proved to be necessary for the occurrence of spontaneous genetically determined SWDs in three different rat strains. In the Genetic Absence Epilepsy Rats from Strasbourg (GAERS; Danober et al., 1998), a well established genetic model of absence epilepsy, restricted ibotenic acid lesions of the RTN completely suppressed the occurrence of SWDs (Avanzini et al., 1992), and local RTN injections of cadmium, which blocks calcium channels and calcium-activated potassium channels, attenuated SWDs (Avanzini et al., 1993). Ibotenic acid lesions of the RTN in Sprague–Dawley and "Fisher 344" rats highly attenuated the so-called "high-voltage spindles" (Buzsáki et al., 1988), which appear to be morphologically identical to SWDs as they occur in absence epileptic WAG/Rij rats (Coenen and van Luijtelaar, 2003) and GAERS.

Recent findings however have challenged the traditional view that the thalamus is the primary driving source of SWDs (Meeren et al., 2005). In cat, experimental and spontaneous SWDs have been found that arise in the neocortex and do not depend on the thalamic circuitry (Steriade and Contreras, 1998). Moreover, a cortical focus within the peri-oral region of the primary somatosensory (S1) cortex was found to be the leading structure in the initiation of spontaneous generalized SWDs in the WAG/Rij rat model of absence epilepsy (Meeren et al., 2002). Other cortical sites and thalamic sites were found to lag behind this cortical focal zone during the first 500 ms of the seizure. Additional and converging experimental evidence for a

* Corresponding author. Fax: +31 24 3616066.
E-mail addresses: g.vanluijtelaar@donders.ru.nl, gillesnijmegen@gmail.com (G. van Luijtelaar).

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leading role of a cortical focus in absence-epileptic rats has accumulated since in WAG/Rij rats and GAERS (D'Antuono et al., 2006; D'Arcangelo et al., 2006; Gurbanova et al., 2006; Karpova et al., 2005; Klein et al., 2004; Kole et al., 2007; Manning et al., 2004; Merlo et al., 2007; Pinault, 2003; Polack et al., 2007; Pumain et al., 1992; Sitnikova and van Luijtelaar, 2004; Strauss et al., 2004).

The present study was undertaken for two reasons. First, in light of recent findings on the primary role of the cortex in the WAG/Rij rat, we wanted to verify the assumption that an intact thalamus in general and the RTN in particular are also a prerequisite for the generation of SWDs in this well established and commonly used genetic model (for reviews see Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006), as this has not been explicitly investigated before. Recently, evidence was found that the RTN is indeed involved in the occurrence of SWDs as small lesions of the rostral pole of the RTN with the specific cholinotoxin AF64A resulted in a two-fold reduction of SWDs (Berdiev et al., 2007). The question whether the RTN is essential in the generation of SWDs or is merely modulating their occurrence could however not be answered. It remains elusive whether larger thalamic/RTN lesions would completely abolish the occurrence of cortical SWD, or whether the cortex would still be able to generate and sustain SWDs without concomitant activity in the thalamus.

Second, we wanted to study the relationship between spontaneous genetically determined SWDs and naturally occurring sleep spindles and their respective thalamic dependencies. It is generally assumed that SWDs develop in the same neuronal circuits that normally generate sleep spindles (Avanzini et al., 2000; Gloor et al., 1977; Kostopoulos, 2000; Quesney et al., 1977). The assumption is based on extensive investigation of the feline penicillin generalized epilepsy (FPGE) model (Fisher and Prince, 1977; Gloor et al., 1977, 1990; Kostopoulos et al., 1981a,b; Quesney et al., 1977), and was later confirmed by findings in the thalamic slice preparation (von Krosigk et al., 1993), and spontaneous SWDs in cat (Steriade et al., 1994; Steriade and Contreras, 1995). The SW seizures in the FPGE model however, are pharmacologically induced while spontaneous SWDs in the cat develop from sleep-related oscillations during ketamine–xylazine anesthesia (Steriade et al., 1994; Steriade and Contreras, 1995). The validity of these findings may not pertain to a genetic model in which both phenomena occur spontaneously, without any drug. Also, there is an important species difference between cat and rat with respect to the cytoarchitectonics of thalamic relay nuclei. Whereas a significant number of GABAergic interneurons exists in the feline thalamus, in rats these are almost absent (Jones, 1985).

Caution should indeed be taken to extrapolate concepts and findings to other models. The genetically determined SWDs in GAERS do not arise from naturally occurring sleep spindles of 7–15 Hz, but from normal physiological wake-related thalamocortical 5–9 Hz oscillations (Pinault et al., 2001, 2006). Furthermore, SWDs and sleep spindles in the WAG/Rij rat show opposite sensitivity for drugs such as diazepam, phenobarbital and clonidine (van Luijtelaar, 1997). Whether sleep spindles and SWDs depend on the same local thalamic circuitries has never been explicitly investigated; a genetic animal model of absence epilepsy, in which both phenomena occur spontaneously, is well suited for this purpose. We therefore addressed this question in the WAG/Rij rat by making unilateral ibotenic acid lesions in the thalamus and analyzing the cortical and thalamic EEG recorded in freely moving and naturally sleeping animals both before and after the lesion.

Materials and methods

Animals

Male WAG/Rij rats born and raised in our laboratory were used. They were housed in groups of 2–3 animals and had free access to water and food. At the time of surgery they were 10–20 months of age and weighted 275–450 g. After surgery they were individually housed and maintained on a 12–12 h light–dark regime, with white lights on at 7:00 am. All procedures were approved by the Ethical Committee on Animal Experimentation of the Radboud University Nijmegen. The study was performed in accordance with the European Communities Council Directive 86/609/EEC.

Experimental design

Baseline EEG was recorded 7–11 days after the implantation of electrodes and cannulas. Lesioning took place four days after baseline registrations. The postlesion EEG was recorded on day three after lesioning, as the histological damage then reaches its full extent with little functional recovery (Markowska et al., 1985). Fourteen days after lesioning animals were perfused for histological verification of the lesion.

Implantation of electrodes and cannulas

Chronic stainless steel electrodes (Plastics One Inc., MS303/2) and cannulas were implanted during stereotactic surgery under isoflurane anesthesia complemented with 2% lidocaine for local analgesia of the periost and wound edges. All stereotactic coordinates are relative to Bregma and according to Paxinos and Watson (1986). Each rat received one epidural electrode placed on the surface of the frontal cortex of each hemisphere (AP +2.0 mm, ML ±3.5 mm) and two electrodes on the cerebellum, which served as reference and ground. In each hemisphere one depth electrode was placed in the ventroposterior medial nucleus (VPM) of the thalamus (AP −3.3 mm and ML ±2.5 mm, 7.0 mm under skull surface). Two cannulas made of polyethylene tubing (ID 0.5 mm, OD 1.0 mm, length 5 mm) were implanted 2 mm under the skull surface on the right hemisphere to enable needle penetration for ibotenic acid injection later in the experiment. The whole assembly was fixed to the skull with four stainless steel screws and dental cement.

EEG registration

Bilateral cortical and thalamic EEG registrations were made in freely moving rats for a period of 3.75 h (1:00–4:45 pm) both before (=baseline EEG) and after the lesion (=postlesion EEG). Simultaneous referential registrations were made from the cortex and thalamus of both hemispheres. Signals were amplified, band-pass filtered between 1 and 100 Hz, digitized with 512 samples/second and stored onto optical disk (CODAS hardware and software). The animals were observed throughout the registration period and their behavior (active vs passive) was observed and encoded on disk along with the EEG (van Luijtelaar and Coenen, 1986).

Lesioning

Unilateral ibotenic acid lesions were made in the right hemisphere under general isoflurane anesthesia. Ibotenic acid (IBO, Research Biochemicals International, I-116), dissolved in a phosphate buffer (pH 7.3), was delivered by a stereotactically guided stainless steel needle (OD 0.4 mm) connected to a 1 μl Hamilton microsyringe at two different rostrocaudal locations in the RTN. At the rostral injection site (AP −1.4 mm, ML −2.4 mm) 0.5 μl was infused at two different depths (6.5 and 7.5 mm under skull surface; total injection volume 1 μl). At the caudal injection site (AP −3.15 mm, ML −3.9 mm) 0.33 μl was infused at three different depths (6.0, 6.8, and 7.6 mm under skull surface; total injection volume 1 μl). To obtain lesions of different sizes the concentration of IBO was varied between subjects (7, 6 or 5 μg/μl, see Table 1). In addition, four rats only received IBO at the caudal site consisting of 0.15 μl (10 μg/μl) at the three depths (0.45 μl total injection volume).