Brain Research 1658 (2017) 1–10

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Dentate gyrus autonomous ictal activity in the status epilepticus rat model of epilepsy

Florian Weissinger [1], Matthias Wawra [*,1], Pawel Fidzinski, Mark Elsner, Hartmut Meierkord, Martin Holtkamp, Katharina Buchheim [2]

Epilepsy-Center Berlin-Brandenburg, Department of Neurology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

ARTICLE INFO

Article history:
Received 18 September 2016
Received in revised form 27 December 2016
Accepted 29 December 2016
Available online 3 January 2017

Keywords:
Intrinsic optical signal
Slice electrophysiology
Sustained stimulation
Low Mg^{++} model
Hippocampus

ABSTRACT

The dentate gyrus (DG) as part of the hippocampal formation is believed to serve as a gatekeeper with strong inhibitory properties against uncontrolled propagation of neuronal activity from the entorhinal cortex and neocortical structures. In temporal lobe epilepsy, the DG becomes hyperexcitable and loses its gate function, enabling propagation of ictal activity into downstream structures such as CA3 and CA1 areas. Furthermore, the DG, apart from facilitating propagation, may also be able to autonomously generate ictal activity, but this point has remained open so far. To tackle this question, we used intrinsic optical imaging in combination with electrophysiological recordings in brain slice preparations from rats in which status epilepticus had been induced electrically several weeks prior to measurements. Upon omission of Mg^{++} from the artificial cerebrospinal fluid, in 15 out of 33 slices (45.4%) from 9 out of 13 epileptic animals (69.2%), spontaneous and autonomous ictal activity, mostly seizure-like events (SLE), was observed in the DG. This activity manifested independently from SLE generated in adjacent cortices and never occurred in slices from control animals. SLE generated in the DG differed from those with origin in the entorhinal or temporal cortex by longer latency to the first event after Mg^{++} omission ($p < 0.001$), a higher SLE frequency ($p < 0.05$), higher amplitude ($p < 0.001$) and a longer SLE duration ($p < 0.05$). We conclude that in epilepsy, the DG, in addition to facilitated gating of activity from upstream structures, can serve as an independent generator of ictal activity.

1. Introduction

The dentate gyrus (DG), an archicortical structure located in the hippocampal formation, receives its major input from the entorhinal cortex (EC) via the perforant path and sends its main projections to the cornu ammonis 3 (CA3) area of the hippocampus proper (Amaral et al., 2007; Schultz and Engelhardt, 2014). The DG is believed to serve as a gatekeeper against uncontrolled propagation of neuronal activity into highly vulnerable downstream structures (Heinemann et al., 1992; Hsu, 2007; Lothman et al., 1992). The gatekeeper function is achieved by means of intrinsic granule cell properties preventing overexcitation, such as low resting membrane potential and strong spike-after hyperpolarisation (Scharfman and Schwartzkroin, 1990; Spruston and Johnston, 1992), but as well by DG network properties, i.e. a massive intrinsic inhibitory activity (Behr et al., 1998; Coulter and Carlson, 2007).

In temporal lobe epilepsy (TLE), the DG is considered to become hyperexcitable and to lose its gate function, enabling propagation of ictal activity into the hippocampal loop (Avoli et al., 2002; Behr et al., 1996; Brooks-Kayal et al., 1999; Lothman et al., 1992; Shirasaka and Wasterlain, 1994). It is still matter of debate whether this loss of inhibition after the initial epileptogenic event is persistent (Cohen et al., 2003; Sloviter, 1991) or rather transient and followed by gradual recovery to a normal level before seizures start to occur (Gorter et al., 2002; Holtkamp et al., 2005; Shirasaka and Wasterlain, 1994). In any case, a possible recovery of inhibition after initial loss is apparently not sufficient to prevent seizures.

Therefore, under the assumption of hyperexcitability in chronic epilepsy, the DG network theoretically should be able to generate ictal activity in an autonomous and input-independent manner. Bumanglag and Sloviter demonstrated in an *in vivo* model of

Abbreviations: ACSF, artificial cerebrospinal fluid; CA1, cornu ammonis area 1; CA3, cornu ammonis area 3; DG, dentate gyrus; EC, entorhinal cortex; fEPSP(s), field excitatory postsynaptic potential(s); GABA, gamma aminobutyric acid; NMDA, N-methyl D-aspartate; SE, status epilepticus; SLE, seizure-like event; TC, temporal cortex; TLE, temporal lobe epilepsy.

* Corresponding author.
E-mail address: matthias.wawra@charite.de (M. Wawra).

[1] These authors contributed equally.

[2] Deceased.

http://dx.doi.org/10.1016/j.brainres.2016.12.030
0006-8993/

chronic epilepsy (electrical stimulation of the perforant path) that epileptiform activity in the DG always precedes onset of spontaneous behavioral seizures (2008). However, it remained unclear if this activity aroused in the DG or in adjacent structures with secondary propagation into the DG. Apart from the abovementioned study and a report of ictal activity in the isolated DG in hippocampal slice cultures (Gutierrez et al., 1999), there is currently little evidence for the hypothesis of DG as an ictal generator. In various rodent models of epilepsy *in vitro* and *in vivo*, ictal activity originated in other structures within the temporal lobe such as in the CA3 and CA1 regions or in the EC (Dzhala and Staley, 2003; Le Duigou et al., 2008; Mody et al., 1987), but not in the DG. The DG displayed spontaneous activity only when connections to the EC were preserved (Jones and Heinemann, 1988; Walther et al., 1986; but see Meyer et al., 2016), suggesting that ictal activity recorded in the DG represented a propagation effect but that generation itself occurred in other regions. The aim of the present study was therefore to investigate the hypothesis that ictal epileptiform discharges could arise *de novo* from the DG.

As it is technically challenging to record simultaneously from the EC and the DG *in vivo*, we used a combined *in vivo* and *in vitro* approach. As a disease model, we used the electrically induced status epilepticus (SE) model of epilepsy *in vivo*. The investigation of ictal epileptiform discharges was performed *in vitro*. For this purpose, we employed intrinsic optical imaging in brain slice preparations. This method allows monitoring of temporal and spatial propagation of ictal activity in a non-invasive manner simultaneously covering various brain regions (Holtkamp et al., 2011; Meierkord, 2009; Weissinger et al., 2000). Consequently, this method is well suited to detect ictal generators. We used intrinsic optical imaging in combination with electrophysiological recordings in the low-Mg^{++} *in vitro* model inducing recurrent episodes of ictal activity. In brain slice preparations from epileptic animals exposed to low Mg^{++}, we observed spontaneous and autonomous ictal activity in the DG which comprised bursts and seizure-like events (SLE). Importantly, ictal activity in the DG was completely independent from SLE generated in adjacent entorhinal and temporal cortices. Thus, in a combined *in vivo* and *in vitro* model of TLE, our data suggest that the DG can serve as an independent generator of ictal activity and thus may give first hints to a novel pathophysiological concept of TLE.

2. Results

2.1. In vivo seizures

After recovery from electrode implantation, in 13 animals the perforant path was stimulated (for details, see Methods). This resulted in self-sustaining SE in all 13 animals. In the 11 control animals without perforant path stimulation, no SE was seen. All animals were video-monitored in several consecutive sessions, each lasting 48 h, for at least the following 8 weeks. Spontaneous recurrent seizures rated with a score $\geqslant 3$ (for the extent of motor activity) according to the Racine scale (Racine, 1972) occurred in the majority of SE animals (11 out of 13), but in none of the control animals ($p < 0.001$, Chi Square test).

2.2. Propagation patterns of seizure-like events

A total number of 33 slices from 13 SE treated animals and 25 slices from 11 control animals was used. Under near-physiological concentration of Mg^{++}, we never observed epileptiform events, neither in slices from healthy, nor in slices from epileptic animals. Upon omission of Mg^{++} from the ACSF, SLE were generated in the vast majority of slices from both SE treated and control animals. Fig. 1 illustrates the electrophysiological features of a typical *in vivo* seizure as recorded from the DG in contrast to those seen in *in vitro* SLEs as recorded from the DG and the EC.

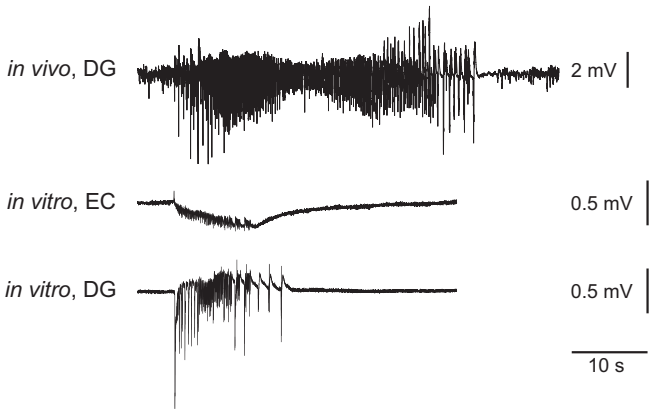

Fig. 1. Typical electrophysiological recordings of an *in vivo* seizure and *in vitro* seizure-like events. Example recordings from the same animal. The trace at the top shows the electrophysiological recording of a spontaneous seizure in the DG *in vivo*. The two other traces show electrophysiological recordings from the EC (middle) and the DG (bottom) *in vitro*. The SLE are recorded from one slice at the same time point. The ictal discharges are propagating from EC to DG. Note the discrepancies in amplitudes and durations.

As observed before (Holtkamp et al., 2011), intrinsic optical signal recordings demonstrated that onset of ictal events was located in the EC in most slices from both SE-treated (28/33 slices, 84.8%) and control animals (22/25 slices, 88.0%), and that ictal activity propagated into adjacent regions (Fig. 2A). In fewer slices, onset of ictal events was also located in TC (SE-treated: 9/33, 27.3%; control: 8/25, 32.0%). A subset of slices from SE treated animals also showed a hypersynchronous/multiregional onset of ictal events (6/33 slices, 18.2%) with rapid secondary involvement of DG and hippocampus proper in 2/33 (6.1%) slices (Fig. 2B). Such hypersynchronous activity was observed in 3/25 (12.0%) slices from control animals (n.s., Chi square test). The onset of ictal activity did not always remain consistent in the course of the experiment and occasionally changed to a second onset location. Such slices were assigned to both onset location groups.

In addition to the events described above, in slices from epileptic animals we noticed also a paroxysmal change in light transmittance in the DG that was independent from the onset in and propagation from the EC (Fig. 2C). Due to the electrode position in the EC and triggering IOS detection by electrophysiological events, so far we mainly detected events with onset in the EC. Now, the occasional independent change of optical signal in the DG suggested a possible additional onset region. To investigate this phenomenon in more detail and to increase the detection rate, we positioned a second electrode in the DG. We then observed that in a large subset of slices from SE treated animals (9 out of 13 animals, 69.2%; 15 out of 33 slices, 45.5%) generation of ictal activity in the DG occurred independently from the EC. In 9 out of these 15 slices, the activity fulfilled electrophysiological criteria for SLE (7 out of 9 animals). Strikingly, such autonomous DG activity was never observed in slices from control animals.

2.3. Properties of autonomous dentate gyrus activity

In the majority of slices (14 out of 15) with autonomous DG activity, ictal activity did not occur exclusively but in addition to SLE generated in non-DG regions like the EC. However, SLE generated in the DG were different from those generated in the EC or TC as they typically lacked the negative DC shift and displayed much larger amplitude of discharges within each SLE (Fig. 3).