Dynamics of evoked local field potentials in the hippocampus of epileptic rats with spontaneous seizures

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Abstract

A change in neuronal network excitability within the hippocampus is one of the hallmarks of temporal lobe epilepsy (TLE). In the dentate gyrus (DG), however, neuronal loss and mossy fiber sprouting are associated with enhanced inhibition rather than progressive hyperexcitability. The aim of this study was to investigate how alterations in excitability take place in association with spontaneous seizures expressed in the DG, before, during and after a seizure. For this purpose, we used freely moving rats that had developed spontaneous seizures after a kainate induced status epilepticus (SE). Continuous EEG was recorded in the DG during several days along with local field potentials (LFP) that were evoked every 15-30 seconds, by applying paired-pulse stimuli to the angular bundle. Input-output relations showed increased paired pulse depression in epileptic compared to control rats, suggesting a rather strong inhibition in the DG during the interictal state. A characteristic pattern of changes in intrinsic excitability was observed during the ictal period: an increase in the population spike (PS) amplitude, mostly during the early phase of a seizure and often followed by a decrease of the main evoked potential amplitude. The paired-pulse extracellular postsynaptic potential (fEPSP) ratio increased during the seizure and did slowly recover to pre-ictal levels after the seizure ended. Although, clear changes in excitability occurred during and after seizure activity, changes of LFP parameters were more subtle before seizure onset; a significant reduction of LFP and PS amplitudes was observed that started 1-2 minutes in advance in ~33% of the cases; in ~18% an increase of LFP/PS amplitude was observed; in the other cases no significant change was observed. Taken together, the present results provide evidence that in this experimental model, DG physiology is more likely to follow the already ongoing seizure activity rather than to contribute to its generation.

Keywords: EEG, inhibition, excitability, dentate gyrus, kainate, epilepsy.
Introduction

Epilepsy is a syndrome characterized by recurrent and spontaneous seizures produced by abnormal paroxysmal activities of neuronal ensembles. It is commonly assumed that spontaneous seizures appear when enhanced neuronal excitability reaches a threshold combined with a high level of synchrony between neurons (Prince et al. 1983; Traub and Wong 1982). It is important to understand the changes in the excitability of neuronal networks in relation to the occurrence of seizures in order to obtain insight into the mechanism that determines its occurrence. This is particularly important in the case of Temporal Lobe Epilepsy (TLE) where a number of changes at the cellular level are now known although the contributions of different sub-systems within the hippocampal formation are not yet precisely understood.

An appropriate experimental model of TLE can be produced in the rat by systemic injection of kainic acid (Ben-Ari 1985; Buckmaster and Dudek 1997). This model offers the opportunity to investigate how excitability in different sub-systems of the hippocampus changes in association with seizures. In one of these sub-systems, the dentate gyrus (DG), the changes that occur at the cellular level have been well characterized and two of them are prominent: (1) selective loss of vulnerable interneurons in the hilus and (2) the formation of new recurrent excitatory circuits due to mossy fiber sprouting (Dudek and Sutula 2007). These cellular and synaptic changes are accompanied by enhanced granule cell inhibition rather than hyperexcitability as one might have expected in view to the association of these changes with the occurrence of seizures (Gorter et al. 2002; Harvey and Sloviter 2005; Wu and Leung 2001). Furthermore, several studies in post-SE models have shown that inhibition impinging on granule cells of the septal DG is still present but in a much more fragile condition (Buhl et al. 1996; Coulter and Carlson 2007; Wu and Leung 2001).

In the present study we formulated the question of how the changes of excitability of the septal part of the DG develop before, during and after a seizure with expression in the DG. To answer this question we adopted the experimental
model of spontaneous seizures induced in the rat by systemic injection of kainate. We used the paired pulse paradigm to evoke Local Field Potentials (LFPs) by means of which we could assess local changes of the excitability state of the septal DG *in vivo* in the epileptic rats. Since LFPs were evoked at a very regular basis (15-30 s), we were able to assess the dynamics of DG excitability before and along spontaneous seizures.
Methods

Experimental animals

Male 2-3 months old Sprague-Dawley rats weighing 250-300 g at the beginning of the experiment were used in the present study. The rats were individually housed under controlled environment (21±1°C; humidity 60%; lights on from 8:00 am -- 8:00 pm). Food and tap water were available ad libitum. The experimental procedures were in agreement with the Dutch Experiments on Animal Act (1997), and were approved by the animal welfare committee of the University of Amsterdam.

Kainate-induced status epilepticus

Animals (N=14, in the following “N” will denote the number of animals, “n” will indicate the number of observations) were injected with kainate (10 mg/kg, i.p.) which induced a status epilepticus (SE). After 10-30 minutes, 75% of all animals presented at least one behavioral seizure. The animals that did not present behavioral seizures were injected again with kainate (5 mg/kg, i.p.) up to a maximum of three injections. Animals that did not show SE after the third injection were not included in the study (N=4). The other animals (N=10) presented a mild SE but they did not need drugs to arrest the ongoing seizure activity after SE.

Electrodes implantation

Approximately 1 month after SE the animals were implanted with electrodes as previously described (Gorter et al. 2001). Briefly, control (N=21) and SE animals (N=10) were anesthetized (i.p.) with a mixture of ketamine (100 mg/kg, Woerden, The Netherlands) and xylazine (20 mg/kg, Bayer AG, Leverkussen, Germany) and placed in a stereotaxic apparatus (Paxinos and Watson 1986). Body temperature was maintained at 37°C with an electric blanket and the eyes were covered with soft paper soaked with saline. A pair of insulated stainless steel
stimulation electrodes (diameter 60 µm, tip separation 500 µm) was placed in the angular bundle (from bregma, AP: 7.2; ML: 4.5 mm) aimed to stimulate the major afferents of DG. LFP were recorded from the hippocampus with a pair of insulated stainless steel electrodes (diameter 60 µm, tip separation 800 µm) placed in the left DG (AP: 3.6; ML: 1.9 mm). The two pairs of electrodes were finely adjusted in the dorsoventral plane using electrical stimulation of the angular bundle and recording from the granule cell layer (GCL). The LFP recorded from the deepest electrode consisted of a positive inflection (field extracellular postsynaptic potential: fEPSP) with a fast transient negative component (population spike: PS) superimposed on the rising phase of the fEPSP, while the more dorsal electrode was placed in the molecular layer (ML), and recorded a fEPSP of opposite polarity. A reference electrode was placed in the skull. After optimizing the evoked responses, electrodes were fixed and attached to a connector. The whole assembly was then fixed to the skull with dental acrylic cement. After surgery all animals received topic application of Chloramphenicol (Sigma), xylocaine (5%, AstraZeneca) and Temgesic (0.03 mg/kg i.p., Schering-Plough, UK) for pain relief. Animals were allowed to recover for at least one week before chronic recordings started.

**Electrophysiological recordings**

The animals were handled at least 3 days prior to the experiments to get accustomed to the procedures. After that period, they were housed in the recording cage (40 x 40 x 80 cm) and connected to the equipment via a multistranded cable and electrical swivel. LFP signals were pre-amplified using a field-effect transistor (FET) located in the headset just above the connector. The signals were then lead to an amplifier and digitized (NI-6071E, National Instruments) at a sampling rate of 250 Hz (EEG, continuously) or 10 kHz (LFP, 600 ms peri-stimulus) and stored for further off-line analysis.
Stimulation protocols

After the rats had accustomed to the recording cage, several stimulation protocols were used to assess basic properties of the DG neuronal network. They all employed biphasic square pulses (0.2 ms pulse duration; stimulation interval at least 10 seconds). For each animal the minimum stimulation intensity capable of evoking a response (threshold) and the maximum stimulation intensity where the response saturated, were determined and intensities were then set using these two values as 0% and 100%, respectively. Stimulus response curves were constructed using stimulation at current intensities of 0, 5, 10, 15, 20, 30, 40, 50, 80 and 100%. Double pulse protocols were constructed using inter-pulse intervals (IPIs) varying from 20 ms up to 500 ms and intensity set at 80%. All measurements took place during the interictal period. If a spontaneous seizure occurred in the course of such a recording, the stimulation protocol was interrupted and restarted at least 2 hours after the seizure. Once baseline LFPs were acquired to define DG properties during the interictal period, a selected set of double-pulses was continuously applied (IPI: 20 ms, intensity: 30% or 80%) for periods of 4-20 hours, at intervals of 15 or 30 seconds. Stimulation sessions were interrupted with periods (6–24 hours) devoid of stimulation. Continuous recording of EEG and LFP was performed for at least 3 days in order to determine seizure frequency. Between recording sessions, stimulus-response curves were again recorded and threshold and maximum intensities were adjusted if necessary.

After the final recording session, the animal was perfused with paraformaldehyde (PFA) 4% and histological analysis was performed to confirm electrode locations. All animals in this study displayed LFP morphology reflecting DG activation and had confirmed correct electrode position.

Data analysis

EEGs were visually inspected to detect the occurrence of spontaneous seizures by two experienced investigators. Seizure initiation was defined as the first spike
before the flattening of the EEG that precedes the paroxysmal activity. The end of the paroxysmal activity was characterized by its disappearance, by depression of the EEG amplitude and occasionally by low frequency post-ictal bursts. Seizure duration was the time difference between end and onset. When seizures had to be aligned in order to evaluate the time course of the LFP we defined the onset of the seizure as time point zero.

The evoked LFPs were analyzed with a computer algorithm. The amplitude of the fEPSP was calculated as the difference between baseline (mean amplitude during 2 ms preceding the stimulus) and the maximum amplitude within the time window 2 -- 12 ms after the stimulation (Fig 1C). The population spike (PS) amplitude was the amplitude of the fast negative transient superimposed on the fEPSP (for details see Fig 1C). Double-pulse ratios were calculated by dividing the amplitude of the second response by that of the first one, resulting in either depression (ratio<1) or facilitation (ratio>1). The time of occurrence of a LFP was defined as the time difference with the starting point of the seizure. The absolute value of the amplitude of fEPSP and PS is dependent on the electrode configuration and the precise location and orientation and thus needs to be normalized over animals. When evaluating trends in fEPSP and PS amplitude in the period before the seizure, we expressed the amplitude as the absolute difference with its mean value in the period 6-12 minutes before the seizure where the slope was not different from zero. The 6 minutes time period was chosen because this time point created the largest statistical contrast between the constant amplitude far before the seizure and the negative trend just before the seizure. In order to average over seizures we then interpolated the values onto a fixed time raster (10 s spacing) in respect to seizure onset.

Statistical analysis

Only epileptic animals with spontaneous seizures (N=7) were used for statistical analysis during the interictal period. Comparison of parameters between control and epileptic animals was made using ANOVA (Statview), where after post-hoc
multiple comparisons were made using a Bonferroni correction. The trends in the amplitudes of fEPSP and PS in the 6 minutes before the seizure were analyzed with linear regression. Only seizures that lasted more than 30 s and that presented PS amplitudes higher than 1 mV were used for statistical evaluation (N=7; n=84). All data are expressed as the mean ± the standard error of the mean (S.E.M.). P<0.05 was assumed to indicate a significant difference.
Results

Local field potentials during the interictal period.

Stimulus-response relations after angular bundle stimulation are shown in Fig. 1. Stimulation threshold was not different between control (47±8 µA, N=16 for all control values that follow) and epileptic rats (29±7 µA; N=7 for all values of epileptic rats that follow). The maximum stimulation intensities were also comparable in controls (474±56 µA) and in epileptic rats (457±127 µA). The stimulus-response relation of fEPSP amplitude and PS amplitude (Fig.1B) as well as the excitability curves which plot PS as a function of fEPSP (Fig.1D) were similar for control and epileptic rats. Epileptic animals showed a smaller latency to the peak of the PS (3.3±0.1 ms) when compared with controls (3.7±0.1 ms, P<0.05), but the changes in the latency to the peak of the fEPSP (epileptic: 5.6±0.2 ms, versus controls 5.9±0.1 ms) did not reach significance. The shorter PS latency in epileptic rats suggests an increased synchrony of the cellular responses contributing to the LFP. The present study does not explicitly address mechanisms that contribute to PS latency in the DG after angular bundle stimulation, but it is worth noting that the same feature was described in the chronic phase of electrically stimulated temporal lobe epilepsy (Matzen et al. 2007).

Double-pulse interactions during the interictal period

Double-pulse stimulations were used to assess the short-term dynamics of DG circuitry after angular bundle stimulation (Fig. 2). This protocol is used to assess the ability of a population of granule cells to suppress subsequent population responses and is often interpreted as reflecting the influence of GABA mediated inhibition within the dentate network (Andersen et al. 1966; Lomo 1971; see discussion). Double stimulation with 20 ms intervals showed intensity-dependent
depression of the second response: higher intensity leading to stronger depression (Fig. 2A). This depression was more pronounced in epileptic animals than in controls (for fEPSP: $F[1,26]=6.23$, $P<0.05$ and for PS $F[1,26]=1.49$, n.s.), but increasing intensity affected both groups similarly (group-intensity interaction for fEPSP: $F[9,234]=0.85$, n.s. and for PS: $F[5,130]=1.80$, n.s.).

< INSERT FIGURE 2 HERE >

To evaluate the kinetics of double-pulse depression we used different IPIs and high stimulation intensity (80%) (Fig. 2B). In control animals, a characteristic fEPSP paired-pulse depression (PPD) was observed for intervals shorter than 100 ms (interval effect: $F[1,8]=138$, $P<0.0001$; Fig. 2B1). In epileptic animals, PPD was significantly enhanced for those intervals (group effect: $F[1,21]=13.3$, $P<0.005$; group-interval interaction: $F[8,168]=6.5$, $P<0.0001$). In control animals, PS ratio showed PPD for intervals shorter than 40 ms and larger than 180 ms (interval effect: $F[1,8]=14.1$, $P<0.0001$). For the intervals between 50 and 100 ms, PS ratio shifted towards facilitation (Figs. 2B2 and 2C1). This pattern of facilitation was not observed in the epileptic group, in which only PPD was observed irrespective of IPI (group effect: $F[1,21]=8.4$, $P<0.01$; group-interval interaction: $F[8,168]=1.95$, $P=0.055$; Fig 2C2).

Continuous monitoring of LFPs in the presence of seizure activity.

Fig. 3 illustrates a typical session of continuous (14 hours) monitoring of LFPs in an epileptic rat (80%, IPI = 70 ms). While the fEPSP1 amplitude was relatively stable, the fEPSP2/fEPSP1 ratio showed characteristics changes when a seizure occurred: a strong rise during seizure activity that slowly recovered to baseline (= pre-ictal period) values afterwards with a time constant of ~60 minutes.

< INSERT FIGURE 3 HERE >
Spontaneous seizures from 7 chronic epileptic animals were recorded in the period between 2 to 5 months after the kainate-induced SE. In total, 337 seizures were recorded during the 978 hours of EEG monitoring (mean recording time per animal: 140±17 hours). Spontaneous seizures were characterized by an initial immobilization followed by unilateral forepaw clonus, rearing, bilateral eyelid closure, and bilateral forepaw clonus. The mean seizure frequency was 0.45±0.16 seizures/hour and the mean duration was 62±4 s (range: 14 -229 s). Seizures were more prevalent during the light period (61±4 %) than in the dark period (light and dark recordings were equally well represented) but there was no difference in duration (light: 63±4 s versus dark: 61±6 s). Seizure duration was similar in all rats, except for one which had longer lasting seizures (85±5 s). Fig. 4A illustrates the distribution of seizure durations obtained from the seven epileptic rats, where besides the main peak around 40-45 s, two additional shoulders/peaks can be distinguished, one around 60-65 s and another one around 95-100 s. Fig. 4B illustrates the characteristic morphologies of EEG seizures of different durations (note the different time scales).

About 70% of the seizures (236 out of 337 seizures) were recorded during angular bundle LFP stimulation. The other 101 seizures occurred during stimulation-free periods. Mean seizure duration (60±2 s with stimulation and 60±3 s without stimulation, N=7, n.s.) and mean seizure interval (159±34 minutes with stimulation and 174±48 minutes without stimulation, N=7, $P>0.05$) were not different in both groups and we conclude that stimulation at this rate and intensity has no direct effect on seizures per se. We did not detect a relation between fEPSP or PS amplitude in the pre-ictal period and the subsequent duration of the following seizure (data not shown).
Peri-ictal local field potentials

LFPs were evoked continuously with intervals of 15 or 30 s, and could thus be evaluated in the period before, during and after an epileptic seizure (Figs. 5 and 7). Figure 5 shows a set of 6 LFPs, recorded over a period of 90 s in GCL and in ML of DG. The first LFP is given a few seconds before seizure onset (indicated by the asterisk); the entire set of LFPs covers the whole duration of the seizure. The first LFP (marker 1 in Fig. 5A, extended time scale in Fig. 5C1) has the typical DG characteristics with a pronounced PPD. The second LFP was evoked 11.4 s after the seizure onset in the initial phase (marker 2 in Fig. 5A, extended time scale in Fig. 5C2); in respect to the LFP before the seizure (Fig. 5C1) the PS amplitude was larger. As the seizure progressed, the PS amplitude increased even more (Fig. 5C3) but 15 s later only a LFP of very low amplitude was recorded with no evident PS (Fig. 5C4). The next stimulus was given as the seizure was waning; the LFP recovered and displayed an additional characteristic in the form of multiple population spikes with a high frequency of ~350 Hz (Fig. 5C5). The PPD also followed a typical pattern of evolution in relation to seizure timing. All LFPs in GCL of DG showed a strong PPD characterized by a low amplitude ratio in the interictal period (Fig 5C1 and Fig. 6B); the fEPSP ratio increased in the initial phase of the seizure and could sometimes be close to 1 (Fig 6B). After seizure termination the first evoked LFP quickly recovered to its pre-ictal amplitude (Fig. 5C6), although often with a reduced PS amplitude and an increased paired-pulse ratio (PPR) than during baseline. The PPR slowly recovered to pre-ictal levels which could take more than 50 minutes (Fig. 3). The sequence of changes in LFP, PS and PPR described above was more pronounced during seizures of longer duration.
Quantification of fEPSP and PS before, during and after seizures from 7 different epileptic animals is presented in Fig. 6. As described above, LFPs, aligned according to seizure onset (time 0) were evoked applying paired-pulse stimuli (IPI = 20 ms) every 15-30 seconds. Fig. 6A shows the averaged fEPSP (top) and PS (bottom) amplitudes (N=7) and Fig. 6B represents the averaged paired-pulse ratios (fEPSP2/fEPSP1 and PS2/PS1, N=4).

In total we analyzed 84 seizures (Fig. 6C). Thirty three percent was preceded by a significant decrease of the fEPSP (38% for PS) and 18 % of the seizures were preceded by an increase of fEPSP (14% PS) starting several minutes prior to the seizure. However, 49% of the seizures were not preceded by any significant change of fEPSP (46% for PS). Linear regression analysis of the 6 min preceding seizure onset (Fig. 6D) showed a significant decrease of both fEPSP (slope = -0.106 mV/min) and PS (slope = -0.095 mV/min) amplitudes ($R^2$=0.60 and 0.51, respectively; $P<0.005$).

Occasionally long seizures (> 60 s) showed high frequency spontaneous PS-like events during the last third of the seizure (Figs. 7B4-C4), always after a period when it was difficult to evoke a LFP (Fig. 7C3, and also Fig. 5C4). This “refractory” period (to stimulation) appeared in the spectrogram with frequency bands between 10 and 20 Hz (Fig. 8B). The fact that, at this moment, the spontaneous LFPs had the same polarity in GCL and ML indicates that these potentials were not locally generated (see Fig. 7C3). On the other hand, the spontaneous occurring spikes had opposite polarity in the ML versus the GCL (Fig. 7C4), indicating that these were local events. Interestingly during some seizures such spontaneous events preceded the stimulus that evoked the LFP by
less than 20 ms (bold star ✴ in Fig. 7C4). In these cases there was PS depression in the following evoked response, similar to evoked PPD. Power spectrum analysis revealed that these rapid discharges occurred at high frequencies (above 60 Hz; Fig. 8B, white dashed square). We never observed these rapid discharges before a spontaneous seizure or during the first 2/3 of a spontaneous seizure.

< INSERT FIGURE 8 HERE >
Discussion

The main findings of the present study are: (1) Several months after kainate induced SE, epileptic rats exhibit increased paired pulse depression (PPD) in the dentate gyrus (septal pole) during the interictal state; (2) A decrease of both fEPSP and PS amplitude can be regularly observed prior to the occurrence of a seizure; (3) Spontaneous seizures longer than 60 seconds were regularly accompanied by high frequency local PS-like events and displayed LFPs that tended to present multiple PS, particularly during the last part of the seizure; (4) Changes in evoked LFP characteristics are much more pronounced during and after a seizure of long duration (>60 seconds) than during and after brief seizures; (5) PPD was reduced during a long seizure and recovered slowly (>30 minutes) to pre-ictal levels. Below we discuss these findings.

Interictal local evoked field potentials

There is ample evidence now that PPD in the dentate gyrus (septal pole) is increased in different post-SE models during the interictal state (Buckmaster and Dudek 1997; Gorter et al. 2002; Harvey and Sloviter 2005; Wu and Leung 2001). In the present study we also observed an increase in PPD in chronic epileptic rats. The paired-pulse protocol is used to assess the ability of a population of granule cells to suppress subsequent population responses and is often interpreted as reflecting the influence of GABA-mediated depression within the dentate network (Andersen et al. 1966; Lomo 1971). There can be several explanations for the change in paired pulse ratio which do not exclude each other: it is possible that the observed increased dentate depression in epileptic rats is due to preferential reinnervation of GABAergic neurons that survive in the inner molecular layer (Gorter et al. 2001; Harvey and Sloviter 2005), sprouting of GABAergic neurons (Andre et al. 2001; Buckmaster and Dudek 1997; Davenport et al. 1990; Esclapez and Houser 1999), an upregulation of granule cell GABAergic function (Gutierrez 2003; Sloviter et al. 1996) or a change in GABA-A
receptor conductance. With respect to the latter option, there is evidence from in vivo as well as in vitro rat studies, (using bicuculline) that at shorter IPI intervals (<50 ms) depression of the second response reflects γ-aminobutyric acid (GABA)-A mediated depression in the dentate gyrus (Leroy et al. 2004; Sloviter 1991; Wu and Leung 2001; Zappone and Sloviter 2004). Finally, although this question has not been specifically addressed, there is evidence that functional differences exist in the septal versus the ventral pole with the latter showing more loss of PPD and loss of inhibitory neurons (Kobayashi and Buckmaster 2003; Williamson et al. 1995).

Local field potential changes prior to a seizure

Since the dentate gyrus is often considered as an important gate to the CA fields of the hippocampus (Behr et al. 1998; Heinemann et al. 1992; Lothman et al. 1992) and since this structure undergoes consistent pathological changes, it was hypothesized that changes in network dynamics in this region might eventually lead to a seizure. If in this experimental model seizures would be generated in the dentate gyrus by a build-up of changes such as a gradual loss of depression, one might be able to anticipate the occurrence of a seizure using the repeated paired pulse LFP paradigm. Although no clear changes in PPD were observed before a seizure started, we did observe a decrease in LFP and PS amplitude that started ~6 minutes in advance. However, the changes were often in the direction of a decrease of local excitability (smaller amplitude fEPSP or PS) and less frequent in the opposite direction of the expected increase. Using a similar kind of stimulation protocol to elicit LFPs in epileptic gerbils, Buckmaster and colleagues also did not find changes in PPD before seizures. However, since in these animals seizures were triggered via exposure to a novel environment this is not unexpected (Buckmaster et al. 2000; Buckmaster and Wong 2002). More recently, the same group used tetrode recordings and showed that an increase in granule cell action potentials could be recorded minutes in advance of a spontaneous seizure. Although, the authors emphasized the increased firing prior
to a seizure, a decrease or no change in granule cell firing was observed in 66% of the recordings (Bower and Buckmaster 2008).

High frequency firing dynamics during seizures

Since we had positioned the recording electrodes in both the molecular layer and the granule cell layer, the inverse polarity of the laminar field potentials enabled us to estimate whether an event is local or not. Indeed the responses evoked by perforant path stimulation displayed opposite polarity in the two layers. However, during a seizure, most of the EEG potentials had the same polarity in both layers indicating that the activity was not locally generated. Similarly as observed in gerbils during long seizures (Buckmaster and Wong 2002) we observed decreased responsiveness in the dentate for a period that could last up to 20 seconds (Figs. 5B2-C4 and 7B3-C3). They suggested that the decrease in responsiveness may reflect the operation of homeostatic mechanisms that help to terminate a seizure. Nevertheless, we found that the seizure may continue after this unresponsive period. Moreover, occasionally at the end of a seizure of long duration (>60 seconds) PS-like high frequency events started to appear in the GCL. These events displayed opposite polarities in the GCL and in the ML, so that we can assume that these events were locally generated and that they represent firing of granule cells. Most of the time, the frequency of these events were above 60 Hz (Fig. 8; see also Bragin et al. 1997). Sometimes at the end of seizure activity, stimulation-evoked potentials showed multiple population spikes with a frequency of 350 Hz, which reminds of the locally generated fast ripples observed in the DG during the interictal period in chronic epileptic rats (Bragin et al. 2002; Bragin et al. 2004). However, in contrast to that study, we never observed these high frequency events in the DG prior to or during the early phase of a spontaneous seizure which suggests that the origin of these events could be different. The observation that such PS-like events appeared concomitantly with strong PPD suggests that a strong feedback inhibition is compatible with a state of high excitability reflected in the appearance of PS-like events during this phase of the seizure. Assuming that the onset of the
spontaneous seizures was outside the granule cell layer of the dentate gyrus, as indicated by the lack of polarity reversal of the laminar LFPs, we may conclude that the phenomena described here – multiple PS-like events along with strong PPD – indicate that the dentate gyrus neuronal population is in a rather labile excitability state, as if it was trying to control a barrage of incoming seizure oscillations.

The use of LFPs appears to be a valuable tool in assessing the state of network excitability in epilepsy, and it can yield new insights concerning the physiological role played by the DG with respect to seizure activity. A similar observation was recently made by Harvey and Sloviter in chronic epileptic pilocarpine-treated rats and they concluded that the dentate gyrus cannot be the origin of spontaneous seizures (Harvey and Sloviter 2005). Although our data do not contradict these findings, multiple electrode measurements and current source density analysis of (evoked) responses at different sites of the hippocampal-entorhinal circuitry should resolve this issue more definitively. There is growing evidence that temporal lobe epilepsy seizures can be easily generated in superficial entorhinal cortex (Bertram et al. 1998; Fountain et al. 1998; Jones and Heinemann 1988; Kobayashi et al. 2003; Tolner et al. 2007; Tolner et al. 2005), perirhinal/insular cortex (McIntyre & Gilby, 2008) or piriform cortex (Loscher and Ebert 1996), such that it is necessary to study the evolution in time of the excitability state of these associated brain structures, by simultaneous recordings of peri-seizure events in these regions using multiple LFPs precisely situated to record from well defined local neuronal populations.
Progressive decrease of paired pulse depression during a seizure

We found progressively diminishing PPD after the seizure had begun, indicating that the technique used, is sensitive enough to detect local changes in field potentials. The fact that evoked inhibition progressively decreased during the seizure, suggests that this could contribute to seizure spread to connected brain regions. Long seizures (>60 s) had much more impact on the characteristics of LFPs than brief seizures and full recovery of PPD took often more than 30 minutes (Fig. 3). Thus the next seizure appeared again while dentate paired pulse inhibition was growing stronger which also supports the notion that the origin of the seizure lies elsewhere. The mechanism of this decrease in dentate paired-pulse inhibition during the seizure is not known and could depend upon changes in local ionic concentrations (potassium, calcium or chloride), alterations in neurotransmitter release, or desensitization of GABA receptors or a combination of these alterations. Similar observations of failure of inhibition after seizure onset have been reported in epileptic gerbils (Buckmaster and Wong 2002) and during afterdischarges triggered by tetanic kindling stimulations in rats (Emori et al. 1997).

Conclusion

The data show that dentate LFP parameters can change in anticipation of an upcoming seizure in the experimental model in which spontaneous seizures were elicited after a kainate induced SE. However the most frequent observed changes in LFP parameters several minutes prior to a seizure suggest decreased or unchanged dentate excitability instead of the hypothesized increased excitability. Thus we conclude that it is not very likely that these seizures originate in the dentate gyrus (septal pole) by a local gradual loss of inhibition or increase in excitation. Whether this paired-pulse stimulation paradigm might be a useful technique for seizure anticipation needs to be determined by measuring EEG and LFPs simultaneously in associated limbic regions such as the entorhinal and peri-rhinal cortices which also have been
implicated in temporal lobe epilepsy seizures (Bartolomei et al. 2005; Kobayashi et al. 2003; Tolner et al. 2007; Tolner et al. 2005).
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Disclosures

Authors state that they have no conflicts of interest.
References


Figure legends

Figure 1 – Properties of LFPs evoked by angular bundle stimulation and recorded from GCL in DG. (A) Typical LFP recorded in a control animal (left example) and in two different epileptic rats (right examples). Stimulus intensities ranged between 0% (threshold for the response) and 100% (saturation of the response). Only a subset of the recorded LFPs is illustrated. (B) Stimulus-response curves that relate fEPSP (top panel) and PS (lower panel) amplitudes as recorded in GCL of DG to normalized stimulus intensity. Open symbols give values for control rats (N=16), closed symbols illustrate values for epileptic rats (N=7). (C) Graphic illustration of the algorithm that was used to calculate the amplitudes of fEPSP and PS. (D) Excitability curve which plots PS amplitude as a function of fEPSP amplitude. Symbols as in B. There were no differences between control and epileptic groups in 1B and 1D suggesting that the populations that generate them are not fundamentally changed. Data are given as mean±S.E.M.. Error bars show S.E.M..
Figure 2 – Properties of double pulse LFPs evoked by angular bundle stimulation and recorded from GCL in DG. (A) Stimulus-response curves that relate the value of fEPSP2/fEPSP1 (A1) or PS2/PS1 (A2) to relative stimulus intensity. Open symbols represent control rats (N=16), closed symbols represent epileptic rats (N=7), IPI=20 ms. Note the increasing paired-pulse depression (PPD) of fEPSP with increasing stimulation intensity in both groups. (B) PPD as a function of IPI (Intensity: 80%). The value of fEPSP2/fEPSP1 (B1) shows stronger PPD for intervals smaller than 100 ms in epileptic animals than in control animals. Symbols as in 2A. All data expressed as mean ± S.E.M. ★ indicates \( P<0.05 \), ANOVA followed by post-hoc test with Bonferroni correction. (C) Typical example of traces illustrating evoked LFPs from a control (C1) and an epileptic rat (C2) showing the morphology of the evoked potentials at different IPIs.
Figure 3 – Typical example of analysis of 14 hour LFP recording in DG (angular bundle stimulation) in a rat 3 months after kainate-induced SE. The amplitude of fEPSP1 (left axis, open symbols) is quite stable during the whole period. The value of fEPSP2/fEPSP1 (80%, IPI=70ms, right axis, closed symbols) is strongly affected by seizure activity. The horizontal dotted line indicates fEPSP ratio =1. The vertical dotted lines indicate the onset moments of individual seizures; their duration is indicated by the number in the top of the graph.
**Figure 4** – Distribution of seizure duration. (A) Histogram illustrating the distribution of the durations of the 337 seizures recorded in GCL in DG of 7 epileptic animals. The mean value of seizure duration was 60±2 s; (B) Six examples of typical seizures of different duration (5 animals). The top trace shows a brief seizure in the EEG that lasts 28 seconds. The bottom trace shows a seizure that lasts 135 seconds. The two vertical dotted lines in each trace mark the onset and the end of seizure activity. Note the differences in time scale between the traces.
Figure 5 – EEG and LFP in DG, before, during and after a seizure in an epileptic rat at three months after kainate-induced SE. (A) Typical seizure recorded in the ML (top) and GCL (bottom) of DG. Big rectangles indicate the time period expanded in B1-B2. Arrows mark the moments in time where a double-pulse stimulus evoked a LFP; numbers indicate the LFPs that are expanded in C1-C6. (B) Expanded parts of traces indicated by rectangles in A. B1 illustrates seizure onset marked by asterisk. B2 illustrates the period of rhythmic and synchronous activity in both channels. (C) LFPs recorded every 15 seconds at moments indicated by marked arrows in A. (80%; IPI=70ms, stimuli given 3.6 s before and 11.4, 26.4, 41.4, 56.4 and 71.4 s after seizure onset). Compared to the pre-ictal values the PS amplitude recorded in GCL initially increased (C3, arrow), followed by a period with almost complete failure to evoke a response (C4). Finally a period of enhanced excitability was reflected by the occurrence of high frequency multiple PSs (C5). fEPSP amplitude was also strongly reduced during the mid-phase of the seizure (C4). After the end of the seizure, fEPSP amplitude recovered to pre-ictal levels relatively quick, but it took often more than 30 minutes for the characteristic PPD to recover to its original value (see Fig. 3).
Figure 6 – The time course of changes in fEPSP and PS in DG in relation to seizure activity. (A) The mean value of fEPSP and PS amplitude (N=7, n=84) in the period from 12 to 6 minutes before seizure onset (at time point 0) was used as reference and changes in amplitude were expressed in relation to this level. Peri-ictal fEPSP (top) and PS (bottom) amplitude were determined for 84 seizures that lasted more than 30 seconds and had average PS amplitude larger than 1 mV in the reference period. As the stimulation rate was low and not synchronized with the start of the seizure, we interpolated the amplitude values onto a time grid linked to seizure onset and then averaged the values over all seizures. Mean values are given by the thick line, the thin lines indicate S.E.M. During seizure activity fEPSP amplitude (top panel) decreased while PS amplitude (bottom panel) increased. (B) The same procedure as described above was used to average peri-ictal fEPSP ratio (top panel) and PS ratio (bottom panel) as a function of time related to seizure onset (time point 0) (80%; IPI=20 ms, N=4, n=14). Paired-pulse ratios for fEPSP and PS are stable before seizure onset, and both rise strongly in the beginning of the seizure; they recover more slowly than fEPSP and PS. (C) Raster plots of fEPSP (top panel) and PS (bottom panel) amplitude as a function of time (time scale aligned to seizure onset at time point 0) for all 84 seizures used in A. Colors code the difference in amplitude with the mean in the reference period (-12 to -6 minutes, see color bar). Based on the statistics of the trend in fEPSP and PS amplitude in the 6 minutes before seizure onset we defined three possible situations: (a) a significant negative trend (decrease), (b) no significant trend, (c) a significant positive trend (increase). The horizontal lines separate the three ‘classes’ of recordings (N=7; n=84) (D) Using the procedure described in C we determined for each animal (N=7) the mean of fEPSP and PS change in the 6 minutes before the seizure. In the top panel the mean trend over the seven animals is given for the fEPSP amplitude (slope = -0.106 mV/min; R²=0.60; P<0.005), in the bottom panel the same analysis is given for PS amplitude (slope = -0.095 mV/min; R²=0.51; P<0.005). Both trends indicate a small but significant decline of the amplitude in the 6 minutes before the seizure.
Figure 7 – Example of simultaneous recording of spontaneous and evoked activity in the dentate gyrus, before, during and after a spontaneous seizure with spontaneous occurring spikes during the late phase of the seizure. (A) As in Fig. 5A, the numbers above the arrows indicate the LFP profile in the evoked response (in this example, one every 30 s). (B) Expanded EEG traces depict the different phases of the spontaneous seizure recorded in the dentate gyrus. The asterisk (B1) marks the positive sharp event that indicates the beginning of the seizure activity. Synchronous activity with same polarity was also observed (B3) and it was followed by spontaneous high frequency events (B4). (C) Evoked LFP (IPI=70ms; I=80%) evolves similarly as in Fig. 5, i.e., increased PS in the initial phase (C1), followed by disappearance of evoked response (C3). In C4 we did not observe evoked multiple PS as in Fig. 5 inset B5. However, we recorded fast spikes (*) that resemble evoked LFP with inversed polarity (double arrow in inset C4) between molecular layer (ML) and granule cell layer (GCL). One of these spontaneous events (**) occurred before (16 ms) the stimulation. Interestingly, this spontaneous event was able to produce PPD in the first following evoked LFP. As a consequence, stimulation-induced multiple population spikes were only observed in the second stimulation, after 70 ms. This observation suggests that the ensemble of granule cells (seen as PS-like potentials) massively fires during late phase of the spontaneous seizures, mainly after a period of “refractoriness” (i.e., where it was difficult to evoke a response). T = time of seizure onset.
Figure 8 – Representative example of the frequency components of a spontaneous seizure. (A) Compressed representation (~ 18 min) showing the activity recorded in the granular cell layer (GCL) of the septal dentate gyrus (A1) and its frequency components (A2) that precedes and follows the spontaneous seizure (rectangle). Because the rat had an episode of rapid eye movement sleep, the spontaneous seizure was preceded by a theta oscillation in this example (dashed line above the spectrogram in A2). (B) Enlarged view of the seizure (rectangle in A) and its frequency representation. Seizure onset is depicted by an arrow (B1) and a vertical dotted line (B2). Spectrograms were calculated using a Hanning sliding window of 2 seconds with 50% overlap. Spectrogram was smoothed using 2D filter (average of 3 points) and the color bars represent power. Note the increase of frequency (60 -120 Hz) at the end of the seizure (white dashed square).