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 to obtain insight into the mechanism that determines their 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 subsystems 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 study how excitability in different subsystems of the hippocampus changes in association with seizures. In one of these subsystems, 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 caused by 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-status epilepticus (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 this 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 excitability state of the septal DG in vivo in the epileptic rats. Because 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- to 3-mo-old Sprague-Dawley rats weighing 250–300 g at the beginning of the experiment were used in this study. The rats were individually housed under a controlled environment (21 ± 1°C, humidity, 60%; lights on from 8:00 AM to 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.

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Kainate-induced SE

Animals (n = 14, in the following N will denote the number of animals, and n will indicate the number of observations) were injected with kainate (10 mg/kg, ip) which induced an SE. After 10–30 min, 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, ip) 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 mo 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 (ip) with a mixture of ketamine (100 mg/kg, Woerden) and xylazine (20 mg/kg, Bayer AG, Leverkusen, 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, whereas the more dorsal electrode was placed in the molecular layer (MOL), 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 fixed to the skull with dental acrylic cement. After surgery all animals received topic application of Chloramphenicol (Sigma), xylocaine (5%, AstraZeneca), and buPROPrenorfine (Temgesic, 0.03 mg/kg ip, Schering-Plough) for pain relief. Animals were allowed to recover for at least 1 wk before chronic recordings started.

Electrophysiological recordings

The animals were handled ≥3 days before the experiments to get accustomed to the procedures. After that period, they were housed in the recording cage (40 × 40 × 80 cm) and connected to the equipment through a multistranded cable and electrical swivel. LFP signals were preamplified using a field-effect transistor (FET) located in the headset just above the connector. The signals were led to an amplifier and digitized (NI-6071E, National Instruments) at a sampling rate of 250 Hz (EEG, continuously) or 10 kHz (LFP, 600 ms perstimulus) 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 used biphasic square pulses (0.2-ms pulse duration; stimulation interval ≥ 10 s). 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 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 interpulse intervals (IPIs) varying from 20 to ≤500 ms and intensity was 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 ≥2 h 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 (IP: 20 ms, intensity: 30 or 80%) for periods of 4–20 h, at intervals of 15 or 30 s. Stimulation sessions were interrupted with periods (6–24 h) devoid of stimulation. Continuous recording of EEG and LFP was performed for ≥3 days 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 showed 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 postictal bursts. Seizure duration was the time difference between end and onset. When seizures had to be aligned to evaluate the time course of the LFP we defined the onset of the seizure as time point 0.

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 min before the seizure where the slope was not different from zero. The 6-min 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. To average over seizures, we 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), whereafter post hoc multiple comparisons were made using a Bonferroni correction. The trends in the amplitudes of fEPSP and PS in the 6 min before the seizure were analyzed with linear regression. Only seizures that lasted >30 s and that presented PS amplitudes >1 mV were used for statistical evaluation (n = 7; n = 84). All data are expressed as the mean ± SE. P < 0.05 was assumed to indicate a significant difference.
RESULTS

LFPs 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. 1D), as well as the excitability curves that plot PS as a function of fEPSP (Fig. 1B), were similar for control and epileptic rats. Epileptic excitability curves that plot PS as a function of fEPSP (Fig. 1B) and epileptic rats (29 ± 7 μA) followed. The maximum stimulation intensities ranged between 0 (threshold for the response) and 100% (saturation of the response). Only a subset of the recorded LFPs is shown. B: stimulus-response curves that relate paired-pulse extracellular postsynaptic potential (fEPSP; top) and population spike (PS; bottom) amplitudes as recorded in GCL of DG to normalized stimulation intensity. Open symbols give values for control rats (n = 16); closed symbols show 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 that plots PS amplitude as a function of fEPSP amplitude. Symbols as in B.

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 [fEPSP: F(1,26) = 6.23, P < 0.05; PS: F(1,26) = 1.49, not significant (NS)], but increasing intensity affected both groups similarly [group–intensity interaction: fEPSP, F(9,234) = 0.85, NS; PS, F(5,130) = 1.80, NS].

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 <100 ms [interval effect: F(1,8) = 138, P < 0.0001; Fig. 2B]. 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 <40 ms and >180 ms [interval effect: F(1,8) = 14.1, P < 0.0001]. For the intervals between 50 and 100 ms, PS ratio shifted toward facilitation (Fig. 2B and C). 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. 2C].

Continuous monitoring of LFPs in the presence of seizure activity

Figure 3 shows a typical session of continuous (14 h) monitoring of LFPs in an epileptic rat (80%, IPI = 70 ms). Although the fEPSP1 amplitude was relatively stable, the fEPSP2/fEPSP1 ratio showed characteristic changes when a seizure occurred: a strong rise during seizure activity that slowly recovered to baseline (=preictal period) values afterward with a time constant of ~60 min.

Spontaneous seizures from seven chronic epileptic animals were recorded in the period between 2 and 5 mo after the kainate-induced SE. In total, 337 seizures were recorded during the 978 h of EEG monitoring (mean recording time per animal: 140 ± 17 h). 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/h, 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:...
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63 ± 4 s vs. dark: 61 ± 6 s). Seizure duration was similar in all rats, except for one which had longer lasting seizures (85 ± 5 s). Figure 4A shows 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. Figure 4B shows the characteristicmorphologies of EEG seizures of different durations (note the different time scales).

About 70% of the seizures (236 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, NS) and mean seizure interval (159 ± 34 min with stimulation and 174 ± 48 min 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 preictal period and the subsequent duration of the following seizure (data not shown).

Peri-ictal LFPs

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 six LFPs, recorded over a period of 90 s in GCL and in MOL 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 an 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 preictal 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 preictal levels, which could take >50 min (Fig. 5C). 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 seven 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 s. Figure 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 before 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 (R² = 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 (Fig. 7, B4–C4), always after a period when it was difficult to evoke a LFP (Figs. 7C3 and 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 MOL indicates that these potentials were not locally generated (see Fig. 7C3). On the other hand, the spontaneous occurring spikes had opposite polarity in the MOL versus the GCL (Fig. 7C4), indicating that these were local events. Interestingly, during some seizures, such spontaneous events preceded the stimuli...
that evoked the LFP by <20 ms (Fig. 7C4,*). In these cases, there was PS depression in the following evoked response, similar to evoked PPD. Power spectrum analysis showed that these rapid discharges occurred at high frequencies (>60 Hz; Fig. 8B, white dashed square). We never observed these rapid discharges before a spontaneous seizure or during the first two thirds of a spontaneous seizure.

**Discussion**

The main findings of this study are 1) several months after kainate-induced SE, epileptic rats exhibit increased PPD in the DG (septal pole) during the interictal state; 2) a decrease of both fEPSP and PS amplitude can be regularly observed before the occurrence of a seizure; 3) spontaneous seizures >60 s were regularly accompanied by high-frequency local PS-like events and showed 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 s) than during and after brief seizures; 5) PPD was reduced during a long seizure and recovered slowly (>30 min) to preictal levels. Below we discuss these findings.

**Interictal local-evoked field potentials**

There is ample evidence now that PPD in the DG (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 this 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 that do not exclude each other: it is possible that the observed increased dentate depression in epileptic rats is caused by 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 Duke 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<sub>α</sub> receptor conductance. With respect to the latter option, there is evidence from in vivo and in vitro rat studies, (using bicuculline) that at shorter IPI intervals (<50 ms), depression of the second response reflects GABA<sub>α</sub>-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).

**LFP changes before a seizure**

Because 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 because 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 at 6 min 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, because in gerbils, seizures were triggered via exposure to a
novel environment, this is not unexpected (Buckmaster and Wong 2002; Buckmaster et al. 2000). 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 before 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

Because we positioned the recording electrodes in both the MOL and GCL, 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 showed 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 ≤20 s (Figs. 5, B2–C4, and 7, B3–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 s), PS-like high-frequency events started to appear in the GCL. These events showed opposite polarities in the GCL and in the MOL, 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 >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 is similar to the locally generated fast ripples observed in the DG during the interictal period in chronic epileptic rats (Bragin et al. 2002, 2004). However, in contrast to that study, we never observed these high-frequency events in the DG before 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 conclude that the phenomena described here—multiple PS-like events along with strong PPD—indicate that the DG 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 seems 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 (2005) in chronic epileptic pilocarpine-treated rats, and they concluded that the dentate gyrus cannot be the origin of spontaneous seizures. 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. 2005, 2007), perirhinal/insular cortex (McIntyre and 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 perisiezure events in these regions using multiple LFPs precisely situated to record from well-defined local neuronal populations.

Progressive decrease of PPD 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 often took >30 min (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 on 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 kainate-induced SE. However, the most frequent observed changes in LFP parameters several minutes before 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 DG (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 prediction needs to be determined by measuring EEG and LFPs simultaneously in associated limbic regions such as the entorhinal and peri-hippocampal cortices, which also have been implicated in temporal lobe epilepsy seizures (Bartolomei et al. 2005; Kobayashi et al. 2003; Tolner et al. 2005, 2007).

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REFERENCES

Andre V, Marescaux C, Nehlig A, Fritschi JM. Alterations of hippocampal GABAergic system contribute to development of spontaneous recurrent seizures in the rat lithium-pilocarpine model of temporal lobe epilepsy. 


Behr J, Lyson KJ, Mody I. Enhanced propagation of epileptiform activity through the kindled dentate gyrus. 


Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. 


Bower MR, Buckmaster PS. Changes in granule cell firing rates precede locally recorded spontaneous seizures by minutes in an animal model of temporal lobe epilepsy. 


Bragin A, Csicsvari J, Penttonen M, Buzsaki G. Functional regulation of the dentate gyrus by fiber axons in dentate gyrus following intrahippocampal kainate in the rat. 


Buckmaster PS, Dudek FE. Neuropil loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. 


Buckmaster PS, Jongen-Relo AL, Davarti SB, Wong EH. Testing the disinhibition hypothesis of epileptogenesis in vivo and during spontaneous seizures. 


Buckmaster PS, Wong EH. Evoked responses of the dentate gyrus during seizures in developing gerbils with inherited epilepsy. 


Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. 


Coulter DA, Carlson GC. Functional regulation of the dentate gyrus by GABA-mediated inhibition. 


Davenport CJ, Brown WJ, Babb TL. Sprouting of GABAergic and mossy fiber axons in dentate gyrus following intrahippocampal kainate in the rat. 


Dudek FE, Sutula TP. Epileptogenesis in the dentate gyrus: a critical perspective. 


Emori K, Katsumori H, Minabe Y. Changes in paired-pulse depression during the triggering of seizures by 2 Hz dentate gyrus stimulation: effect of the kindling. 


Esclapez M, Houser CR. Up-regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons in a model of temporal lobe epilepsy. 


Fountain NB, Bear J, Bertram EH III, Lothman EW. Responses of deep entorhinal cortex are epileptiform in an electrogene rat model of chronic temporal lobe epilepsy. 


Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Progression of spontaneous seizures after status epilepticus is associated with mossy fiber sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. 


Gorter JA, van Vliet EA, Aronica E, Lopes da Silva FH. Long-lasting increased excitability differs in dentate gyrus vs. CA1 in freely moving chronic epileptic rats after electrically induced status epilepticus. 


Gutierrez R. The GABAergic phenotype of the “glutamatergic” granule cells of the dentate gyrus. 


Harvey BD, Sloviter RS. Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. 


Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. 


Kobayashi M, Wen X, Buckmaster PS. Reduced inhibition and increased output of layer II neurons in the medial entorhinal cortex in a model of temporal lobe epilepsy. 


Leroy C, Poirot G, Keller AF, Nehlig A. Pharmacological plasticity of GABA(A) receptors at dentate gyrus synapses in a rat model of temporal lobe epilepsy. 


Lomo T. Potentiation of monosynaptic EPSPs in the perforant path-dentate granule cell synapse. 


Loscher W, Ebert U. The role of the piriform cortex in kindling. 


Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. 


McIntyre DC, Gibby KL. Mapping seizure pathways in the temporal lobe. 


Prince DA, Connors BW, Benardo LS. Mechanisms underlying interictal-ictal transitions. 


Sloviter RS. Feedforward and feedback inhibition of hippocampal principal cell activity evoked by perforant path stimulation: GABA-mediated mechanisms that regulate excitability in vivo. 


Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL. Basal expression and induction of glutamate decarboxylase and GABA in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. 


Taub RD, Wong RK. Cellular mechanism of neuronal synchronization in epilepsy. 


Williamson A, Telfian AE, Spencer DD. Increased GABA responses in dentate granule cells in slices isolated from patients with temporal lobe sclerosis. 


Wu K, Leung LS. Enhanced but fragile inhibition in the dentate gyrus in vivo in the kindled acidic model of temporal lobe epilepsy: a study using current source density analysis. 


Zappone CA, Sloviter RS. Translaminar disinhibition in the rat hippocampal dentate gyrus after seizure-induced degeneration of vulnerable hilar neurons. 