Optical Recording Study of Granule Cell Activities in the Hippocampal Dentate Gyrus of Kainate-Treated Rats

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INTRODUCTION

Hippocampal tissue from patients with temporal lobe epilepsy (Babb et al. 1991; de Lanerolle et al. 1989; Franck et al. 1995; Masukawa et al. 1992; Sutula et al. 1989) and from animal models of epilepsy (Cavazos et al. 1991; Cronin and Dudek 1988; Sutula et al. 1988) is characterized by aberrant sprouting of dentate granule cell axons (mossy fibers). Histological studies using Timm staining (Cavazos et al. 1991, 1992) and electron microscopic techniques (Franck et al. 1995; Okazaki et al. 1995) demonstrated that sprouted mossy fibers project into the inner molecular layer (the proximal dendritic layer) of the dentate gyrus and that their terminals make asymmetric excitatory synapses on granule cell dendrites. It has been hypothesized that the recurrent excitation connections formed by sprouted mossy fibers increase the seizure susceptibility of granule cells (for review see Dudek et al. 1994; McNamara 1994).

Although several studies attempted to provide electrophysiological evidence for this hypothesis (Golarai and Sutula 1996; Masukawa et al. 1992; Tauck and Nadler 1985), the exact spatial relationship between the distribution of synaptic excitation along the apical dendrites of granule cells and the terminal fields of sprouted mossy fibers is not well known.

High-speed optical imaging of neural activity using voltage-sensitive dye provides spatiotemporal information on the propagation of neural activity in the imaged area of a slice preparation. The hippocampal slice preparation, with its clear laminar structure, is particularly well-suited to this procedure. Therefore, in the present study, we employed this technique, along with Timm staining, to examine the distribution of sprouted mossy fibers in the dentate gyrus and their contributions to excitation in individual laminae of the dentate gyrus.

Mossy fiber sprouting seems to perform both excitatory and inhibitory actions on granule cells. Tauck and Nadler (1985) observed that antidromic stimulation of mossy fibers evokes synchronized burst action potentials in the dentate gyrus of slices from a kainate-treated epileptic rat (KA rat). However, subsequent electrophysiological studies failed to confirm the hyperexcitability of granule cells in slices with mossy fiber sprouting (Cronin et al. 1992; Franck et al. 1995), although it was observed when GABAergic inhibition was blocked by bicuculline. In addition, Sloviter (1992) concluded that the function of sprouted mossy fibers may be inhibitory because they densely innervate inhibitory neurons of KA rats. Thus it remains unclear whether the synaptic reorganization caused by mossy fiber sprouting plays a critical role in epileptogenesis.

METHODS

KA treatment rat

Male Wistar rats (350–450g) were injected subcutaneously with kainate (KA, 12 mg/kg in saline). The rats treated with KA were...
monitored for the presence of seizure behavior for at least 3 h after KA injection. Only animals showing limbic seizures such as facial twitches, forelimb clonus, head nodding, and wet-dog shakes were used in this study.

### Slice preparation

Two to three months after KA injection, transverse slices (400 μm thick) were taken from the hippocampal formation and incubated in normal perfusion medium at 30°C for at least 1 h. There was regional variation in the density of sprouted mossy fiber terminals in the dentate gyrus along the septotemporal axis of hippocampus from KA rats, as detected by Timm staining methods (see Scoring of Timm granule density). Mossy fiber sprouting was more prominent on the temporal side than on the septal side (data not shown). To clearly see the effects of sprouted mossy fibers on neural activity of the dentate gyrus, slices prepared from the temporal half (from the middle portion to the temporal end) of the hippocampus were used. Control slices were prepared from rats that were not injected with KA. The composition of the normal perfusion medium was (in mM) 124 NaCl, 5 KCl, 2 CaCl₂, 1.25 MgSO₄, 1.25 NaH₂PO₄, 22 NaHCO₃, and 10 glucose (pH 7.4 when bubbled with 95% O₂-5% CO₂). Ca²⁺-free medium was prepared by replacing CaCl₂ with the same concentration of MgCl₂ (final Mg²⁺ concentration of 3.25 mM). In some experiments, 10 μM bicuculline methiodide (Sigma), an antagonist of GABAₐ receptors, was added to the perfusate.

### Field potential recording

After a slice was transferred to a submerged recording chamber, a tungsten bipolar stimulating electrode with a tip separation of ~150 μm and a glass recording electrode filled with normal medium (5–10 MΩ) were positioned in the hilus and in the dentate granule cell layer, respectively. Field potential responses to hilar stimulation were recorded in the granule cell layer. The stimulus intensity was adjusted to evoke a submaximal antidromic population spike of 1–5 mV. The threshold intensity used to evoke an antidromic population spike ranged from 100 to 300 μA for 300 μs.

### Optical recording

Optical recording methods have been described in detail in previous publications (Barish et al. 1996; Ichikawa et al. 1993; Iijima et al. 1996). The absorption associated with membrane potential changes was measured with a high-speed imaging photodetector, a metal-oxide semiconductor-based solid-state camera (128 × 128 elements). The camera was mounted on a Zeiss Axioplan upright microscope. The imaged area was ~2 × 2 mm² or 1 × 1 mm², depending on the objective used. Pixels were binned in 2 × 2 blocks to enhance sensitivity and images of 64 × 64 pixels were acquired at 0.6 ms/image in blocks of 256 images. The total recording time was 151.2 ms/block. For each experiment, eight blocks of 256 frames were acquired in rapid succession and the images were averaged; each trace (optical signals in Figs. 1–3 and 6) represents an averaged response to 8 identical stimulations at an interval of 5 s.

For staining, a slice was perfused for 3–4 min with a solution of RH-155 (2.5 mg/ml; Molecular Probe) in normal medium. After staining, the dye was washed away by perfusing the slice with normal medium for >10 min. RH-155 enhances absorption of 720-nm light as transmembrane voltages become more positive, therefore illumination by a tungsten-halogen lamp was filtered at 720 nm (±15 nm band pass) and optically recorded changes in membrane voltage are presented as the fractional change in light transmittance, fΔT.

### Evaluation of the late component

Figure 1B shows the changes in optical signal and field potential evoked by hilar stimulation in the inner molecular layer of a KA rat slice under three different conditions: normal, Ca²⁺-free, and bicuculline-containing media. The field potential obtained in normal medium had at least two components, a fast negative-going signal followed by a slower signal with a separate peak. The fast component, obtained even in Ca²⁺-free medium, which blocks synaptic connection, represents the granule cell population spike evoked by antidromic stimulation of the mossy fibers. The slow component, which appeared upon the return of Ca²⁺-free medium to normal medium, is thought to originate in the granule cell responses to synaptic inputs, which probably come from sprouted axon collaterals and interneurons in the hilus (Fig. 1A and see RESULTS). This slow component seemed to be composed of excitatory postsynaptic potentials (EPSPs) and a portion of the electrotonic potentials of granule cell action potentials that were evoked by the synaptic inputs, which was apparent in the transient negative-going signal in the slow component obtained in bicuculline-containing medium.

The optical signal obtained in normal medium was also composed of two components, a fast-rising transient signal followed by a larger and slower positive signal. Upon consideration of the correspondence of the optical signal to the electrical signal, the fast component seems to reflect the population spikes of granule cells evoked by antidromic stimulation, and the late component seems to reflect the responses of the granule cells to synaptic inputs. The late component involves a glial cell component (Barish et al. 1996). After bicuculline was added to the external medium, the amplitude and duration of the late component of both the electrical and optical signals became larger and longer, although the fast components of both signals were almost unchanged. This observation is consistent with the interpretation that
the late component reflects newly developed synaptic inputs on granule cells.

To evaluate the effects of synaptic input on granule cells, we measured the size of the late component. In optical recordings of changes in transmitted light absorbance, each pixel records the sum of the membrane potential changes of every membranous structure projected onto the pixel. Thus, fluctuations of the optical signal from baseline represent the sum of membrane potential changes (Grinvald et al. 1988). To evaluate only the contribution of the late component in normal medium, we subtracted the area of optical signal above the baseline in Ca\(^{2+}\)-free medium (Fig. 1B, area c) from the optical signal in normal medium. The difference is shown as area a in Fig. 1B. The late component was increased in bicuculline-containing medium; this increase implies that synaptic excitation in normal medium is partially masked by GABAergic innervation. To evaluate the extent of excitation drive masked by GABAergic inhibition, the late component in normal medium was subtracted from the late component in bicuculline-containing medium (Fig. 1B, area b). Data are expressed as means ± SD and the relationship between response characteristics was evaluated using Spearman’s correlation coefficients.

**Histology**

After the field potential and optical recordings, Timm staining was performed to detect mossy fiber terminals. Slices were placed into 0.37% sulfide solution for 10–15 min followed by 10% neutral buffered formalin for 14–16 h. Sections 40 μm thick were cut on a freezing microtome and were mounted on slides. The slides were dried and developed in the dark for 30–40 min in the following solution: gum arabic (about 30% wt/vol), citrate buffer (2 M), hydroquinone (5.7% wt/vol), and silver nitrate (17% wt/vol) in a ratio of 6:1:3:0.05, respectively. After development, sections were rinsed and then stained with cresyl violet using standard histological procedures (Sloviter 1982). The stained granules (Timm granules) indicated the zinc-containing mossy fiber terminals.

**Scoring of Timm granule density**

The density of Timm granules in the inner molecular layer was evaluated by two independent observers using the subjective rating scale suggested by Tauck and Nadler (1985). Timm scores were rated according to the following criteria: 0, no sprouting; 1, scattered light sprouting; 2, continuous band of light sprouting; 3, continuous band of dense sprouting. In some cases the density of Timm granules was evaluated on a personal computer. Histological photomicrographs were digitized on a color imaging scanner and the color representing Nissl-stained cell bodies was removed by filtering. The luminance values (0–256) of the resulting images were measured using the program NIH Image (http://zippy.nimh.nih.gov/pub/nih-image) along an axis passing through the areas of interest that were used to measure

**FIG 2.** Relationship between the distribution pattern of Timm granules and the optical signals generated by hilar stimulation in a control slice. A: photomicrograph of the dentate gyrus (1 × 1 mm area) stained using Timm and Nissl methods. The 7 squares indicate the position of the selected photodetectors measuring the absorption intensity. GCL, granule cell layer; ML, molecular layer; Stim, position of stimulating electrode. B: distribution pattern of Timm granule density along the axis through the photodetectors. C: hilar stimulation–evoked optical signals recorded with the photodetectors whose locations are shown in A. Blue and red traces, optical signals in normal and Ca\(^{2+}\)-free medium, respectively. Arrow and vertical dashed line, time of hilar stimulation.
depolarizations (Figs. 2B and 3B). Similar to the method of Van der Zee et al. (1995), the density of Timm granules was evaluated by subtracting background values, which were measured in the middle molecular layer (pixel 6 in Figs. 2A and 3A) in which there were no Timm granules.

RESULTS

Optical signals at mossy fiber stimulation in control and KA rats

Optical recordings and associated anatomic studies were made in slices from control and KA rats. Figures 2 and 3 are typical examples of results obtained. Figures 2C and 3C show the optical signals recorded after mossy fiber stimulations. In both figures, the numeral on each optical signal represents the site (pixel) of recording, which is shown in Figs. 2A and 3A. Each optical density signal in Figs. 2B and 3B was acquired along a line of these pixels (~120 μm separation) perpendicular to the granule cell layer.

Optical signals obtained in Ca^{2+}-free medium are represented by a red line in Figs. 2C and 3C. As described in METHODS, these optical signals mainly reflect membrane potential changes of granule cells activated directly by mossy fiber stimulation (antidromic stimulation). The origin of the signal change differs depending on the recording site. Signals from the hilus reflect the excitation of mossy fibers, signals from the granule cell layer reflect the action potentials of the granule cell somata, and signals from the molecular layer reflect the electrotonic potential change in granule cell apical dendrites induced by somatic action potentials.

In control slices, after the external medium was changed from Ca^{2+}-free to normal, the optical signals (blue line) did not change markedly in the granule cell layer or the molecular layer (Fig. 2C, 4–7), although the late component appeared slightly in the hilus (Fig. 2C, 1–3). The mossy fibers make synaptic connections on mossy cells and other types of interneurons in the hilus (Han et al. 1993; Scharfman 1995), and these interneurons send their fibers to granule cells (recurrent circuit of granule cells; see Fig. 1A). The appearance of the late component in the hilus may reflect the recovery of the synaptic responses of the interneurons. In KA rats, in contrast with control rats, the optical signals were changed dramatically after the external medium was changed from Ca^{2+}-free to normal (Fig. 3C). Huge increases of the late component were observed in signals obtained from the granule cell layer and the inner molecular layer. These results may imply that in KA rats the granule cell layer and the inner molecular layer receive much larger synaptic inputs than in control rats, and that these inputs originate from the newly developed synaptic connections. The signals in the inner molecular layer had peak latencies of 12.8 ± 6.3 ms (n = 14) (control rat, 5.0 ± 5.2 ms; n = 14) after antidromic stimulation and lasted for 91.7 ± 18.5 ms (n =
14) (control rat, 56.1 ± 38.6 ms; n = 14). Despite the large and long-lasting synaptic depolarization, repetitive firing in granule cells was not induced as reported by Tauck and Nadler (1985), who stated that antidromic stimulation of mossy fibers evoked synchronized burst action potentials in the dentate gyrus of slices from a KA rat.

Real-time imaging of the spread of neural activity

In Fig. 4, the results of optical recordings from the slices used in Figs. 2 and 3 are shown as real-time images. Membrane potential changes were imaged with different pseudocolors, depending on their sizes. In slices from control rats in Ca\(^{2+}\)-free medium, only a transient activation was observed in the hilus, the granule cell layer, and the inner molecular layer, as seen in the waveforms in Fig. 2C. In normal medium following Ca\(^{2+}\)-free medium, activation continues for a longer period of time (compare both 20-ms images in Fig. 4A). KA rats in Ca\(^{2+}\)-free medium showed a pattern similar to control rats, but large differences were observed in normal medium. The differences were particularly apparent in the images obtained 6 and 20 ms after stimulation. In the KA rat, large membrane depolarizations were imaged in the granule cell layer and the molecular layer.

Relationship between Timm granule density and optical signals

Differences in images showing the spread of neural activity in slices from control rats and KA rats may originate in newly developed synaptic connections of sprouted mossy fibers in the KA rat (Masukawa et al. 1992; Tauck and Nadler 1985). Therefore, in each slice preparation, we compared the distribution of mossy fibers with the distribution of their terminals by using the Timm staining method. Typical results for control and KA rats are shown in Figs. 2A and 3A, respectively. In the slice from a control rat, most of the hilus was stained and staining was not observed in other regions. In contrast, the slice from a KA rat showed, in addition to hilus staining, extensive staining in the inner molecular layer and parts of the granule cell layer.

To evaluate the distributions of stained mossy fibers and their terminals more quantitatively, we measured the density of Timm granules (see methods). A density profile along an
axis perpendicular to the granule cell layer in a control rat is shown in Fig. 2B. This profile shows a dense distribution of mossy fibers and their terminals restricted to the hilus, which is in parallel with the image of the Timm-stained slice in Fig. 2A. The density profile showed considerable differences in KA rats (Fig. 3B). The density of Timm-reacted granules was markedly increased in the granule cell layer adjacent to the molecular layer and also in part of the inner molecular layer. The density of Timm granules was slightly higher in these layers than in the hilus. This might reflect a decrease in mossy fiber terminals associated with cell loss in the hilus (Masukawa et al. 1992). The peak density (~165) was detected in the transitional region between the granule cell layer and the molecular layer. The appearance of this additional distribution of Timm granules in the transitional region colocalized with the appearance of the late component in optical signals from KA rats. This evidence is consistent with the interpretation that the huge late component in the inner molecular layer of the KA slice originates from the synaptic inputs of sprouted mossy fibers.

We investigated the quantitative relationship between the density of the additional Timm granules and the size of depolarization signals in the inner molecular layer. Figure 5 shows the relationship between Timm scores and the ratio of late components (area alarea c) in the inner molecular layers of slices from control and KA rats. In most of the control slices, no Timm granules were observed in the inner molecular layer. It was therefore supposed that almost no mossy fiber projections into the inner molecular layer were formed in control slices. A scatter of Timm granules was observed in only two control slices (Timm score 1; Fig. 5, bottom). One of these slices showed a relatively large excitatory synaptic response (ratio ~10) despite a Timm score of 1. This slice was prepared from close to the temporal end of the hippocampus, in which a prominent projection of mossy fibers into the inner molecular layer has been observed, even in control animals (Cavazos et al. 1992). In KA slices, a significant positive correlation was observed between Timm scores and the ratio of late components ($r = 0.67, P < 0.01, n = 24$). These results indicate that denser synaptic connections in the inner molecular layers of slices are associated with the larger synaptic component in KA rats.

**Increases in both excitatory and inhibitory synaptic responses in the inner molecular layers of KA rats**

Despite the large depolarization in the inner molecular layer caused by sprouted mossy fiber inputs, we could not observe repetitive firing in granule cells from KA rat slices. Sloviter (1992) observed that sprouted mossy fibers densely innervate the inhibitory neurons of KA rats and concluded that the function of sprouted mossy fibers may be inhibitory. Therefore, we used a GABA$_A$ receptor antagonist to examine the extent to which sprouted mossy fiber–mediated synaptic excitation is masked by GABAergic inhibition. In Fig. 6, optical signals measured in a KA rat slice in Ca$^{2+}$-free (red trace), normal (blue trace), and bicuculline-containing (green trace) media are shown. The signals were collected from the granule cell layer (GCL), the inner molecular layer (iML), the middle molecular layer (mML), and the outer molecular layer (oML). The numerals on the traces represent the distance (in μm) of the recording site from the center of the GCL. In a KA rat slice, large late components appeared in the signals obtained from the GCL and the molecular layer when Ca$^{2+}$-free medium was changed to normal medium, which is similar to the observations shown in Fig. 3C. When normal medium was replaced by bicuculline-containing medium, late components increased twice as much as in normal medium at the peak level (Fig. 6). In control slices, the effect of bicuculline on the late component was not as significant as in KA rat slices (a typical record is shown in Fig. 6, inset). From these observations it was supposed that in KA rat slices GABAergic inhibitory synaptic inputs greatly suppress the excitatory responses in the iML caused by sprouted mossy fiber inputs.

To determine the relationship between GABAergic inhibition and excitatory synaptic responses in the inner molecular layer, the size of the excitatory responses masked by GABAergic inhibition and the size of the excitatory response, which are equivalent to area b and area a, respectively (as described in METHODS), are compared in Fig. 7. In 17 slices from the five KA rats examined, a linear relationship was obtained between the two components. This implies that the extent of excitation masked by GABAergic inhibition (area b) in the iML is proportional to that of depolarization in the same layer in normal medium (area a) ($r = 0.66, P < 0.01, n = 17$).
means that the inhibitory synaptic inputs develop in proportion to the degree of the excitatory synaptic inputs of sprouted mossy fibers.

We examined the relationship between Timm granule density (mossy fiber inputs) and GABAergic inhibition in the iML. In KA rat slices with Timm scores of 0 and 1, the degree of GABAergic inhibition (ratio of area $b$ to area $c$) was $1.41 \pm 2.2$ ($n = 16$). On the other hand, in KA rat slices with Timm scores of 2 and 3, the degree of GABAergic inhibition was significantly larger ($6.39 \pm 6.0$; $n = 15$) (Mann-Whitney $U$ test; $P < 0.01$). The parallel increase in the degree of GABAergic inhibition and the Timm score in the iML is also consistent with the conclusion stated in the final sentence of the preceding paragraph. The relative size of the synaptic component masked by GABAergic inhibition was $40.1 \pm 22.3\%$ ($n = 17$) of the total synaptic components evoked in the inner molecular layer of KA rat slices.

Despite the fairly large synaptic depolarization evoked in KA rat slices in bicuculline-containing medium, seizurelike spiking activity was not observed in the electrical or optical signals ($n = 12$). However, small double or triple spikes were occasionally evoked by hilar stimulation in a few KA rat slices in bicuculline-containing medium.

**Figure 6.** Spatial distribution of optical signals in the dentate gyrus in a KA and a control rat in 3 different media. Time course of optical signals was obtained from the regions and the distance (μm) was measured from the middle of the GCL along a straight line perpendicular to the GCL. The optical signal changes measured in Ca$^{2+}$-free (red trace), normal (blue trace), and bicuculline-containing (green trace) media are shown. Arrow and vertical dashed line, time of hilar stimulation. Inset: optical signals in the iML in a control rat.

**Figure 7.** Relationship between postsynaptic responses evoked in the iML in normal medium and postsynaptic responses enhanced by adding bicuculline to the medium. Ratio of postsynaptic responses in the iML in the bicuculline-containing medium (area $b$/area $c$, see METHODS) is plotted against the ratio of postsynaptic responses in the same layer in normal medium (area $a$/area $c$). Circles, values obtained in slices prepared from KA rats; dashed line, standard linear regression line.

**Discussion**

**Excitation of granule cells by recurrent mossy fiber synapses**

It has been inferred that mossy fiber sprouting produces a large synaptic excitation in the proximal dendrites of granule cells and that this excitation triggers repetitive firing and facilitates the development of epileptogenesis in the dentate gyrus (Babb et al. 1991; Sutula et al. 1988, 1989). Previous studies using extracellular or intracellular recording techniques in KA rats or in epileptic patients with massive mossy fiber sprouting have shown that a single antidromic or orthodromic stimulation can evoke repetitive firing of granule cells (Masukawa et al. 1992; Tauck and Nadler 1985) and a large excitatory synaptic response (Cronin et al. 1992; Franck et al. 1995). However, these studies failed to reveal the exact site of the synaptic input. Golarai and Sutula (1996) used current-source density (CSD) analysis to show that the peak amplitude of the late synaptic sink current following a synchronized discharge of granule cells colocalized with the laminar distribution of sprouted mossy fiber terminals. Similarly, in the present study, huge synaptic depolarizations evoked by hilar stimulation were observed in the iMLs of KA rats. We also show that the synaptic response in the iML tends to increase along with the increase in Timm score in the iML. These results indicate that the large synaptic response is evoked through sprouted mossy fibers.

A small excitatory synaptic response was even observed in the iMLs of control slices after hilar stimulation in normal medium (Figs. 2C and 6, inset). It is likely that the synaptic response is mainly caused by the activation of mossy cells and excitatory hilar neurons (Buckmaster et al. 1992; Jackson and Scharfman 1996; Ribak et al. 1985; Scharfman 1995). It has been reported that mossy cells are lost in the dentate gyrus of epileptic rats (Sloviter 1987). Therefore, in KA rats, synaptic excitation through mossy cells does not appear to contribute to synaptic depolarization.
Spatiotemporal pattern of synaptic depolarization evoked through sprouted mossy fibers

We observed that a long-lasting synaptic depolarization was evoked in KA rats along the apical dendrites of granule cells up to the oML. At the same time, a short-lasting antidromic spike invaded back toward the dendrites of granule cells (Figs. 3C and 6). This far-reaching synaptic depolarization is probably caused by passive and electrotonical propagation of the large depolarization in the iML because there are no Timm granules from the mML to the oML, which means that there are no mossy fiber inputs in these regions. However, it is possible that new excitatory synaptic inputs, which are not detectable with Timm staining, developed from the hilus to these regions. Because synaptic depolarization was poorly evoked by hilar stimulation in the GCL of control rats (Fig. 2C), and because the density of Timm granules in the granule cell layer of KA rats was much less than that in the iML (Fig. 2B), the comparatively large depolarization in the GCL of KA rats seems to result from the passive spread of synaptic excitation evoked in the iML.

In hippocampal CA1 pyramidal cells, dendritic depolarization produced by back-propagating action potentials can enhance the excitatory synaptic response by releasing the Mg$^{2+}$ block of N-methyl-d-aspartate (NMDA) receptors (Magee and Johnston 1997). The perforant path fiber forms excitatory synapses partially composed of NMDA receptors on the middle and distal parts of granule cell dendrites in the dentate gyrus. Therefore, it seems likely that the widespread and long-lasting depolarization of granule cell dendrites in the dentate gyrus of KA rats can also enhance perforant path inputs by a similar mechanism. The enhancement of perforant path inputs to granule cells may increase the seizure susceptibility of granule cells.

Despite widespread and long-lasting excitation in the granule cell dendrites of KA rats, we did not observe the seizure-like spiking activity in normal medium that was seen by Tauck and Nadler (1985). Cronin et al. (1992) observed that hilar stimulation evoked relatively normal responses in granule cells in KA slices, but that epileptic burst discharges occurred in the presence of bicuculline. Thus, their findings suggest that enhanced GABAergic inhibition may suppress the hyperexcitability of granule cells in mossy fiber–sprouted KA rats.

Enhancement of synaptic inhibition in the dentate gyri of KA rats

The present study demonstrates that the amount of the excitatory synaptic component disinhibited by bicuculline is closely correlated with the size of the excitatory synaptic response in normal medium and the density of Timm granules in the iML. In accordance with previous findings (Cronin et al. 1992; Franck et al. 1995), our results indicate that greater mossy fiber sprouting is associated with enhanced GABAergic inhibition to granule cells in KA rats.

The enhancement of GABAergic inhibition in the dentate gyrus of KA rats appears to be produced by several different mechanisms. For example, 1) the sprouting of GABAergic fibers and/or facilitation of the synthesis and release of GABA in the iML (Davenport et al. 1990), which can be detected as an increase in the number of glutamate decarboxylase (GAD) immunoreactive puncta in the iML; 2) increasing the numbers or changing the subunits of the postsynaptic GABA$_A$ receptor, which results in potentiation of GABA$_A$ receptor–mediated responses (Gibbs et al. 1997; McNamara et al. 1980; Otis et al. 1994; Shin et al. 1985; Titulaer et al. 1994; Valdes et al. 1982); and 3) downregulation of presynaptic GABA$_B$ receptors, which causes decreased autoinhibition of GABA release (Hass et al. 1996). Buhl et al. (1996) concluded that the enhancement of GABAergic inhibition might result from an increased excitatory drive on inhibitory interneurons and from decreased autoinhibition of GABA release. Moreover, Kotti et al. (1997) demonstrated that sprouted mossy fibers surround parvalbumin-containing interneurons (probably GABAergic neurons) and create excitatory synapses, which suggests that the activation of GABAergic interneurons is potentiated by increased innervation of sprouted mossy fibers. Together, these changes in the GABAergic recurrent circuit may lead to long-lasting enhancement of GABAergic inhibition in the dentate gyrus of KA rats, masking the hyperexcitability of granule cells in KA rats.

Mossy fiber sprouting and epileptogenesis

One of the leading hypotheses of epileptogenesis in temporal lobe epilepsy is that mossy fiber sprouting causes recurrent excitation of dentate granule cells and increases seizure susceptibility (Babb et al. 1991; Dudek et al. 1994; Sutula et al. 1988, 1989). Cavazos et al. (1991) showed that there is a close correlation between the time course for the development of mossy fiber sprouting and kindling development. Electrophysiological studies have shown that epileptic burst discharges can be evoked in granule cells with massive mossy fiber sprouting in normal or bicuculline-containing media (Cronin et al. 1992; Franck et al. 1995; Isokawa et al. 1993; Masukawa et al. 1992; Tauck and Nadler 1985; Uruno et al. 1994; Wuarin and Dudek 1996). This hypothesis is further supported by recent findings that intraventricular administration of antibodies against nerve growth factor (NGF) or a synthetic NGF peptide sequence that interferes with the binding of NGF to its receptors (Rashid et al. 1995; Van der Zee et al. 1995), or a null mutation of c-fos (Watanabe et al. 1996), suppressed both kindling development and mossy fiber sprouting.

In contrast, we found that hilar stimulation did not evoke epileptic burst discharge, even in the presence of bicuculline, despite the large and long-lasting depolarization observed in granule cell dendrites. Our work thus suggests that mossy fiber sprouting dose not in itself cause seizures. Support for this conclusion comes from work that has shown that the amount of mossy fiber sprouting in the iML does not correlate with kindling development (Elmer et al. 1997; Inosaka et al. 1993). A similar suggestion was made by Larmet et al. (1995) who showed that chronic intrahippocampal perfusion of brain-derived neurotrophic factor allowed mossy fibers to sprout but significantly retarded kindling development. Other evidence was provided by Longo and Mello (1997) who demonstrated that a protein synthesis inhibitor almost completely blocked mossy fiber sprouting but did not prevent the subsequent development of spontaneous seizures. Our study using CSD analysis (Ohata et al. 1996) showed that perforant path stimulation evoked a late excitatory synaptic current in the iML but did not produce recurrent discharges of granule cells in kindled

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rats with robust mossy fiber sprouting. The late excitatory synaptic current was also shown to decrease in amplitude during 5-Hz stimulation of the perforant path and to disappear before seizure induction. These findings, together with the present study, suggest that mossy fiber sprouting does not play a critical role in epileptogenesis.

In dentate granule cells of control rats, recurrent excitatory inputs are almost negligible. KA treatment results in massive sprouting of mossy fibers, and the sprouted mossy fibers recur- currently innervate the dentate granule cells, making them hyperexcitable. However, at the same time, inhibitory inputs are strengthened by the mechanisms discussed in Enhancement of synaptic inhibition in the dentate gyri of KA rats, and the cells are thereby stabilized. This reaction appears to be a normalization mechanism by which the dentate gyrus maintains stable functioning.

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