Mossy Fiber Sprouting as a Potential Therapeutic Target for Epilepsy

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Abstract: Hippocampal mossy fibers, axons of dentate granule cells, converge in the dentate hilus and run through a narrow area called the stratum lucidum to synapse with hilar and CA3 neurons. In the hippocampal formation of temporal lobe epilepsy patients, however, this stereotyped pattern of projection is often collapsed; the mossy fibers branch out of the dentate hilus and abnormally innervate the dentate inner molecular layer, a phenomenon that is termed mossy fiber sprouting. Experimental studies have replicated this sprouting in animal models of temporal lobe epilepsy, including kindling and pharmacological treatment with convulsants. Because these axon collaterals form recurrent excitatory inputs into dendrites of granule cells, the circuit reorganization is assumed to cause epileptiform activity in the hippocampus, whereas some recent studies indicate that the sprouting is not necessarily associated with early-life seizures. Here we review the mechanisms of mossy fiber sprouting and consider its potential contribution to epileptogenesis. Based on recent findings, we propose that the sprouting can be regarded as a result of disruption of the molecular mechanisms underlying the axon guidance. We finally focus on the possibility that prevention of the abnormal sprouting might be a new strategy for medical treatment with temporal lobe epilepsy.

Key Words: Temporal lobe epilepsy, hippocampus, granule cell, axon guidance, mossy fiber sprouting, fasciculation.

INTRODUCTION

Temporal lobe epilepsy (TLE) is one of the most common types of intractable epilepsy in adult humans and characterized clinically by the progressive development of spontaneous recurrent epileptic seizures originating from temporal lobe foci (Engel, 1998). From pathological viewpoints, TLE is characterized by several histological aberrations in the hippocampus. In the hippocampal tissues from TLE patients (de Lanerolle *et al.*, 1989; Sutula *et al.*, 1989; Houser, 1990; Houser *et al.*, 1990; Babb *et al.*, 1991; Isokawa *et al.*, 1993; Frank *et al.*, 1995; Masukawa *et al.*, 1995; Mathern *et al.*, 1995a, 1995b; Zhang and Houser, 1999) and experimental animal models (Cronin and Dudek, 1988; Sutula *et al.*, 1988, 1998; Mello *et al.*, 1993; Represa *et al.*, 1993; Okazaki *et al.*, 1995; Buckmaster and Dudek, 1997; Kotti *et al.*, 1997; Wenzel *et al.*, 2000), frequently observed is abnormal morphology of the axons of granule cells in the dentate gyrus, i.e., sprouting of hippocampal mossy fibers (MFs). MFs normally elongate within the dentate hilus and stratum lucidum and make synaptic connections with hilar cells and CA3 neurons (Henze *et al.*, 2000). In the TLE hippocampus, however, MF collaterals abnormally grow into the inner molecular layer of the dentate gyrus (Sutula *et al.*, 1995), in which the sprouted MFs offer excitatory recurrent inputs into dendrites of granule cells (Buckmaster *et al.*, 2002). This reorganized pathway is generally thought to contribute to hyperexcitability of the hippocampus, but the role of MF sprouting in TLE remains controversial. The skepticism is mainly based on observations that there are no or few relationships between MF sprouting and seizure development in terms of the onset, number, or duration of spontaneous seizures (Longo and Mello, 1997, 1998, 1999; Pitkanen *et al.*, 2000; Nissinen *et al.*, 2001). By summarizing recent literatures, this review aims to re-consider the contribution of MF sprouting to TLE and explores the possibility of MF sprouting as a therapeutic target for TLE. To achieve this goal, we will further focus on the nature of axonal MFs and propose that abnormalities of axon guidance could induce MF sprouting.

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MOSSY FIBER SPROUTING IN TEMPORAL LOBE EPILEPSY

Abnormally reorganized MF pathways are commonly observed in TLE hippocampal tissues (Sutula *et al.*, 1989; Represa *et al.*, 1989; Franck *et al.*, 1995; Houser, 1990; Houser *et al.*, 1990) although the degree varies depending on the stages of TLE (Mathern *et al.*, 1995a, 1995b). One of the simplest methods to detect MF sprouting is Timm silver staining, a histochemical technique that selectively labels synaptic terminals of the MFs because of their high Zn^{2+} content (Danscher and Zimmer, 1978; Henze *et al.*, 2000; Ueno *et al.*, 2002). This staining can reveal macroscopic distributions of MF terminals in fixed brain sections, fresh cryosections, and also hippocampal slice cultures. The further detailed morphology of MFs is obtained by intracellular labeling of granule cells with biocytin and other neurotracers (Sutula *et al.*, 1998; Wenzel *et al.*, 2000). These methods have revealed that in the TLE hippocampus, single granule cells bifurcate their MF axons in the hilar region, projecting the collaterals to both the CA3 and molecular layer (Fig. **1**). As a result, some of the granule cells are monosynaptically interconnected.

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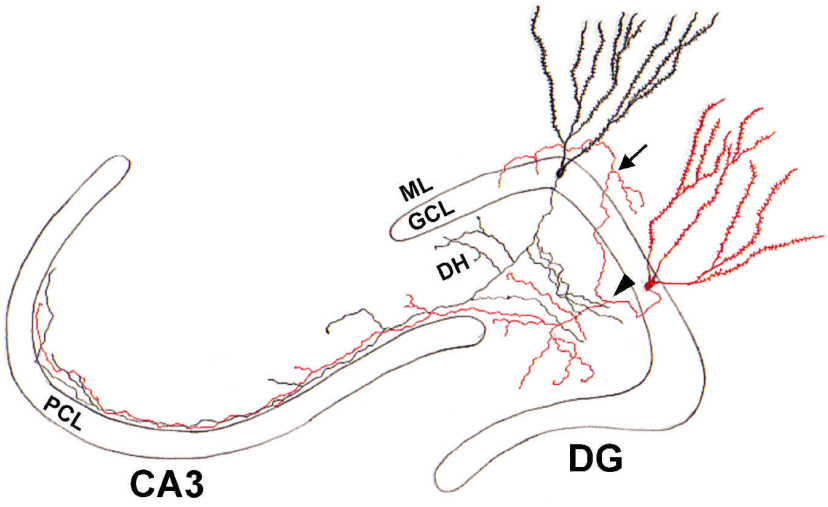

Fig. (1). Schematic drawing of normal (black) and sprouted MFs (red). Granule cells in the dentate gyrus (DG) normally project MF axons through dentate hilus (DH) to CA3 and make synaptic contacts with hilar cells, CA3 pyramidal cells and various types of interneurons. In TLE, new collaterals arise from the dentate hilus (arrowhead), run across the granule cell layer (GCL), project to the inner third molecular layer (ML) (arrow), and contact with dendrites of other granule cells, so-called MF sprouting. PCL, pyramidal cell layer.

Several types of experimental TLE models, including kainic acid-, pilocarpine- and kindling-induced epileptic rats, have been used in investigating the morphological and physiological properties in sprouted MFs (for review, see White, 2002). Most of these studies support a 'recurrent excitation' hypothesis; granule cells elaborate positive feedback MF projections. This recurrent circuit causes granule cells to excite one another and can be the focus of seizure activity. For instance, electron microscopic analyses revealed synaptic contacts between sprouted MF collaterals and granule cell dendrites (Wenzel *et al.*, 2000; Buckmaster *et al.*, 2002; Cavazos *et al.*, 2003). Physiological evidence has shown that epileptic activity was recorded from granule cells with MF sprouting. Studies using both animal models and TLE patients have brought about a large amount of knowledge of MF sprouting, however, elucidations are still underway. We review these details in the following sections.

MORPHOLOGICAL SUPPORTS FOR RECURRENT EXCITATION

In contrast to the prevailing recurrent excitation hypothesis, the opposite hypothesis, i.e., a 'recurrent inhibition' model has been also proposed. A portion of sprouted MFs make synaptic contacts with inhibitory interneurons in the dentate gyrus (Sloviter, 1992; Kotti *et al.*, 1997), which may in turn inhibit the excitability of granule cells. The hypothesis claims that the recurrent inhibition compensates enhanced hippocampal activity and suppresses the propagation of epileptiform activity. However, even in normal hippocampus, more than 90% of MF synapses terminate on GABAergic neurons (Acsady *et al.*, 1998). Because the MFs never innervate granule cells under normal conditions, the occurrence of excitatory recurrent could be more significant than a slight increase in innervation on interneurons.

While the MFs use glutamate as a neurotransmitter, they are also capable of releasing gamma-aminobutyric acid (GABA) (Walker *et al.*, 2002). Interestingly, seizure activity increases the expression of GABA and its synthesizing enzyme glutamic acid decarboxylase 67 (GAD_{67}) in MF terminals (Gutierrez, 2001; Gutierrez and Heinemann, 2001; Ramirez and Gutierrez, 2001; Gomez-Lira *et al.*, 2002, Gutierrez, 2002). This is another support for the recurrent inhibition hypothesis. However, inhibition of GABA receptors by Zn^{2+} released from MF terminals is also induced in epileptic hippocampus, probably via a molecular reorganization of $GABA_A$ receptor subunits (Buhl *et al.*, 1996; Shumate *et al.*, 1998; Coulter DA, 2000), and thus, whether GABAergic transmission is virtually functional at recurrent MF synapses remains unclear.

Recent electron microscopic works indicate that the vast majority of newly formed MF synapses are asymmetric (Cavazos *et al.*, 2003) and terminates on dendritic spines of granule cells (Buckmaster *et al.*, 2002), suggesting that they are mostly excitatory. In the pilocarpine model, Buckmaster *et al.* (2002) estimated that on average, one MF forms > 500 new synapses, a minority of which (< 25 synapses) contact with GABAergic interneurons. Therefore, the impact on excitatory neurons appears predominant. Interestingly, biocytin labeling has revealed that sprouted MFs make synapses at intervals of 7 μm in the granule cell layer and 3 μm in the molecular layer but do not overlap the dendrites of their original granule cells (Buckmaster *et al.*, 2002). Therefore, the newly formed synapses seem to terminate on other granule cells than their parent neurons, indicating the presence of robust control of circuit formation to avoid autapses.

PHYSIOLOGICAL SUPPORTS FOR RECURRENT EXCITATION

Prolonged (usually >30 min) continuous seizures or lack of recovery between discrete seizure for focal, complex partial, absence and other forms of convulsive seizures is termed status epilepticus. *In vitro* electrophysiological

experiments using rats that show status epilepticus have provided direct insights into the function of recurrent excitatory circuits. Focal application of glutamate to the molecular layer or granule cell layer evokes excitatory postsynaptic currents (EPSCs) (Molnar and Nadler, 1999) or excitatory postsynaptic potentials (EPSPs) (Lynch and Sutula, 2000) in granule cells far apart from the application loci in hippocampal slices from kainate-treated rats, which supports that the recurrent circuits are excitatory. Under pharmacological blockade of inhibitory network by bicuculline, a $GABA_A$ receptor antagonist, focal application of glutamate to the granule cell layer evokes trains of EPSPs and burst spike discharges. In kindled rats, the same phenomena are observed 1 week after seizure onsets, but not after 24 hours when no MF sprouting is yet established (Lynch and Sutula, 2000).

Studies using electric stimulation also suggest a relationship between MF spouting and the resultant hyperexcitation. In artificial CSF tuned to facilitate disinhibition by 10 μM bicuculline or elevated $[K^+]_o$ (6 mM), antidromic stimulation of granule cells in slices from kainic acid-induced epileptic rats evokes prolonged seizure-like bursts of population spikes in the inner blade of the granule cell layer, which are followed by long-lasting negative field potentials (sinks) (Wuarin and Dudek, 1996). By monitoring antidromically evoked EPSCs with whole-cell recording, Feng et al (2003) suggested that the recurrent excitatory pathway is functionally silent during baseline asynchronous activity of granule cells, whereas granule cells can fire spikes in synchrony after higher frequency stimulation. This form of short-term plasticity may underlie *in vivo* intermittent seizures that depend on behavioral states.

Stimulation of the perforant pathway, which innervates dendrites of granule cells in the molecular layer, also reveals the properties of MF recurrent circuits. In hippocampal slices from patients with TLE, the stimulation induces prolonged field sinks in the granule cell layer (Masukawa *et al.*, 1989; Isokawa and Fried, 1996). In the kainate models, single stimulation of the perforant path generates 3-12 successive population spikes, whereas one spike per stimulus is evoked in saline-treated control. These abnormal multiple population spikes were followed by prolonged field sinks (140-1500 ms of duration; 1-10 mV of amplitude) with several irregular population spikes superimposed, i.e., epileptiform activity. This was observed only when slices show robust Timm signals in the inner molecular layer (Patrylo *et al.*, 1999). These indicate that synaptic input from the entorhinal cortex, even if it is normal, is converted to epileptiform bursts through MF recurrent circuits, again suggesting an important role of the hippocampus in epileptogenesis.

In vivo studies using epileptic animal models suggest a correlation between development of MF sprouting and the progression of seizures. Sutula et al (1988) utilized histochemical Timm staining to investigate the effects of kindling on the spatial pattern of MF terminals. Timm-positive MF terminals gradually emerge in the supragranular layer of the dentate gyrus during the course of repeated kindling stimulation. Cavazos *et al.* (1991) found that progression of MF synaptic reorganization correlates with the evolution of behavioral kindled seizures, by referencing Timm scores as an index for the development of MF reorganization and the numbers of afterdischarges and the class V seizures evoked by perforant path stimulation as indices for seizure evolution. The authors elegantly proved that Timm scores correlate linearly with the number of afterdischarges (r = + 0.91) and weakly, but still significantly), with the number of class V seizures (r = + 0.74). The susceptibility to electric stimulation lasted as long as 8 months after the final seizures, suggesting a long lasting change in the network organization.

Whole-cell patch-clump recordings also revealed a correlation between the development of MF sprouting and the excitability of granule cells. Okazaki et al (1999) have indicated that antidromic stimulation of MFs evokes EPSCs in granule cells of pilocarpine-treated animals. The EPSCs were mediated by glutamatergic transmission. They were recorded in ~74% of granule cells in rats >10 weeks after status epilepticus, in which MF sprouting was robust, whereas only 5% of granule cells showed EPSCs 4-6 days after status epilepticus, a period when MF sprouting was hardly detected. Using the dual patch clamp technique, Scharfman *et al.* (2003) showed the formation of mutual synaptic connections among granule cells. Wuarin and Dudek (2001) also examined the time course of MF sprouting and the amplitude and frequency of EPSCs in granule cells evoked by flash photolysis of caged glutamate in the granule cell layer. Animals were studied 1-2, 2-4 and 10-51 weeks after injection of kainic acid. Timm scores increased gradually as a function of time after the treatment. Cumulative distribution of the EPSC amplitude was shifted toward larger amplitude, and the average frequency of EPSCs also increased with time after kainic acid treatment, although the effect was not observed in saline control. These observations have raised a possible conclusion that MF sprouting is causally related with seizure development. We discuss this in the next section.

IS MOSSY FIBER SPROUTING A CAUSE OR RESULT OF TLE?

Although many lines of evidence apparently support that MF sprouting reorganizes the dentate network and enhances hyperexcitation, it is clear that further investigations are still required to achieve a conclusion that the recurrent circuits cause systemic seizures. Some studies demonstrate little or no relationship between aberrant Timm staining and seizure frequency (Buckmaster and Dudek, 1997; Timofeeva and Peterson, 1999; Nissinen *et al.*, 2001). Moreover, despite the presence of extensive MF sprouting, the brain slices show no epileptic activity in granule cells unless inhibition is artificially reduced by application of $GABA_A$-receptor antagonists or elevation of extracellular K^+ levels (Wuarin and Dudek, 1996; Patrylo and Dudek, 1998; Lynch and Sutula, 2000). These data cast doubt on the potential importance of MF sprouting in epileptogenesis. Another line of skepticism arises from studies using cycloheximide, a protein synthesis inhibitor (Longo and Mello, 1997, 1998, 1999; Longo *et al.*, 2002, 2003). MF sprouting is thought to result, at least in part, from death of hilar mossy cells (Cavazos and Sutula, 1990; Houser, 1990, 1999; Babb *et al.*, 1991; Masukawa *et al.*, 1997, 1999). Cycloheximide prevents a loss of calcitonin gene related peptide (CGRP)-

positive cells (probably mossy cells) in the hilus and thereby inhibits seizure-induced MF sprouting (Longo *et al.*, 2003). It can hence be a pharmacological tool for investigating the consequence of MF sprouting. Cycloheximide does not affect the development and frequency of spontaneous recurrent epileptic seizures in pilocarpine- or kainic acid-induced epileptic rats. Other groups have challenged these observations. Williams et al (2002) reported that treatment with cycloheximide did not block either hilar cell loss or MF sprouting and that recurrent epileptic seizures can occur even in cycloheximide-treated rats.

We now face the question whether MF sprouting is a cause or result for TLE. To address this, Nissinen et al (2001) examined whether MF sprouting begins at the time of the first appearance of spontaneous seizure and at the stage of self-sustained status epilepticus, which was induced by electrical stimulation of the lateral nucleus of the amygdala. They found no correlation between MF sprouting and the onset of status epilepticus. As for early-life status epilepticus in febrile seizure models, the occurrence of MF sprouting is not necessarily required for epileptogenesis in the developing brain (Bender *et al.*, 2003; Raol *et al.*, 2003). Based on these observations, it is plausible to conclude that MF sprouting is a consequence of seizures at least at earlier stages of epilepsy.

However, because sprouted MFs make excitatory circuits, it is also feasible that MF sprouting aggravates or contributes to the chronic state of TLE. A recent report by Zhang et al (2002) supports this idea. The authors primed rats twice by kainate and treated them with kainate again. The primed rats showed no MF sprouting. Early in the process of chronic seizure development, these animals exhibited spontaneous recurrent seizures with similar degree with non-primed rats that showed MF sprouting. However, later in the process, the severity of seizures in the rats was less than that in non-primed animals, which suggest that MF sprouting may not be necessary for epileptogenesis but does contribute to the intensification of TLE seizures.

INDUCTION OF MOSSY FIBER SPROUTING BY ABNORMAL AXON GUIDANCE

We next consider the mechanism how MF sprouting occurs. MFs normally run through the dentate hilus and stratum lucidum (Henze *et al.*, 2000). This layer-specific axon growth is assumed to require a tightly controlled guidance system. We have recently proposed a two-step model for axon guidance of MFs i.e., developmental switch in MF guidance cues (Koyama *et al.*, 2002b). Early in development, MFs are guided mainly by chemoattractants from the CA3 region and chemorepellents from CA1, but thereafter, they can find their trajectories by contact cues alone, probably via fasciculation with pre-established MFs (Fig. **2**). The latter was attested using organotypic cocultures in which fresh microslices of the dentate gyrus are cocultured with chemically fixed slices of hippocampal Ammon's horn. In the fixed slices, diffusible chemoattractant is no longer produced; nonetheless, new MFs arising from the fresh dentate stumps are still capable of accessing their normal target area in the fixed tissues. This guidance was disturbed by treatment with *N*-glycopeptidase F, a glycosidase specific for *N*-glycans (GlcNAc-Asn bonds). The data imply a contribution of polysialylated neural cell adhesion molecule (PSA-NCAM) as a contact cue. Secreta-dependent guidance systems work mainly at earlier stages of development, while contact-mediated mechanisms are predominant in the mature hippocampus. MF sprouting in TLE may be explained, at least in part, by the aberration of this MF guidance system.

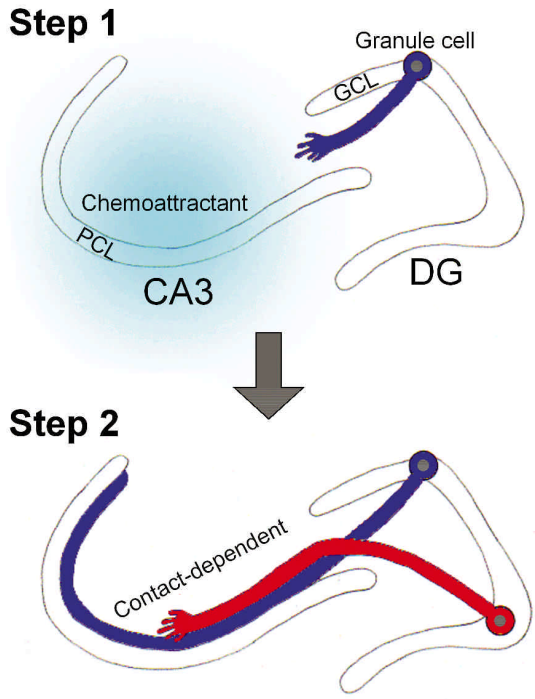

Fig. (2). Two-step model of MF guidance mechanisms. Early in development, pioneer MFs (blue) are guided to CA3 according to a gradient of chemoattractant in CA3 region (light blue) (Step 1). Late MFs (red) fasciculate with the pre-established MFs (blue), the adhesion being mediated probably by PSA-NCAM (Step 2). DG, dentate gyrus; GCL, granule cell layer; PCL, pyramidal cell layer.

MF sprouting can be divided into two processes (Fig. **3**). The first step is the formation of abnormal protrusions from MF axons, i.e., axon branching. The branching occurs in the hilar region (Fig. **3**), Step 1). The second step is backward guidance into the inner molecular layer (Fig. **3**, Step **2**).

The first step is probably triggered by epilepsy-related neural activity. Using rat hippocampal slice cultures, Ikegaya (1999) has indicated that treatment with picrotoxin, a $GABA_A$ receptor channel blocker, induces MF sprouting, an effect that is blocked by co-treatment with the voltage-sensitive Na^+ channel blocker tetrodotoxin. Therefore, excessive activity is sufficient to produce MF sprouting. One of candidate molecules linking between neural activity and the sprouting is brain-derived neurotrophic factor (BDNF). Expression levels of BDNF mRNA (Isackson *et al.*, 1991; Gall, 1993) and protein (Nawa *et al.*, 1995; Gall *et al.*, 1993; Elmer *et al.*, 1998; Rudge *et al.*, 1998; Vezzani *et al.*, 1999) are upregulated in epileptic animal models (for review, see Binder *et al.*, 2001). Because the induction of BDNF protein is most evident in the MF pathway, we hypothesize that

activity-dependent release of BDNF in the dentate hilus induces the formation of MF protrusions. Consistent with this, our preliminary study is revealing, indeed, that local application of exogenous BDNF into the hilus alone induces MF sprouting even under blockade of neural activity (Koyama *et al.*, 2003).

The second step in MF sprouting is the retrodirective growth of MF collaterals into the inner molecular layer. Why do the branched collaterals grow away from their proper targets in spite of the existence of chemoattractant that guides MFs to the CA3 region (Koyama *et al.*, 2002b)? Axon guidance is usually based on a balance of several guidance cues (Tessier-Lavigne, 1994; Tessier-Lavigne and Goodman, 1996). One possible explanation is, therefore, the aberration of guidance factors that normally co-act or counteract with the chemoattractant (Fig. **3**, Step **2a**). Sema3A, a member of the semaphorin family, is one of the candidates. Sema3A serves as a chemorepellent via a receptor complex composed of neuropilin-1 and plexinA (Kolodkin *et al.*, 1997; Takahashi *et al.*, 1999). Recently, Holtmaat et al (2003) have suggested a correlation between MF sprouting and downregulation of Sema3A mRNA expressed in stellate cells of the entorhinal cortex layer II in epileptic animals. They hypothesized that under normal conditions, stellate cells, projecting their axons to the dentate gyrus, provide a chemorepulsive gradient of Sema3A in the molecular layer, preventing MFs to invade the inner molecular layer, but in TLE, the disruption of this system may allow MF sprouting.

Another possible mechanism for the retrodirective growth of MFs is contact-dependent MF guidance (Fig. **3**, Step **2b**). As mentioned above, the contact-dependent mechanism is predominant in the mature hippocampus, and thus, the direction of the growth of newly sprouted MF collaterals would be mainly determined by the contact cue. The most probable candidate for the contact cue is PSA-NCAM expressed on MFs themselves. Unlike chemofactor gradients, contact cues do not involve the information of 'direction'. Therefore, the direction of growth is determined by chance, that is, it is identical for newly sprouted MF

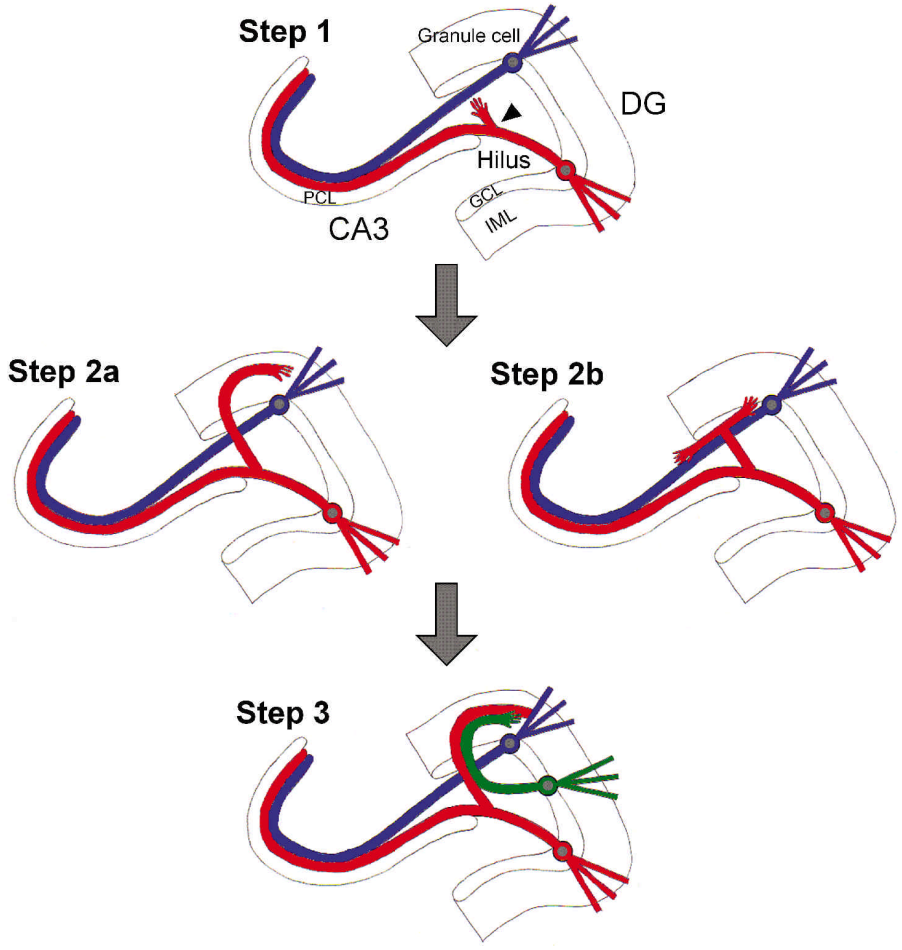

Fig. (3). Occurrence and maintenance of MF sprouting. Step 1: Hyperexcitability triggers MF branch-out in the hilus (arrowhead). Step 2: Disruption of the MF guidance system results in abnormal projection of the new collaterals (Step 2a), or fasciculation with pre-established MFs results in either anterograde or retrograde extension of the collaterals by chance (Step 2b). Step 3: Once the MF sprouting is developed, newly elongating MFs (green) fasciculate with the abnormal MFs (red) and are misguided into the inner molecular layer (IML). DG, dentate gyrus; GCL, granule cell layer; PCL, pyramidal cell layer.

collaterals to grow towards or away from CA3 by tracing previously developed MF track.

This idea is supported by a recent report by Kim *et al.* (2003). They established a slice overlay assay in which premature granule cells are dispersed over cultured hippocampal slices, and investigated their network formation in single cell resolution. These authors found that about 50% of granule cells ectopically growing on the dentate hilus project their axons towards inner molecular layer, even when neuronal activity is absent in the presence of tetrodotoxin. Therefore, the aberrant MF growth can be explained without assuming the presence of chemofactors. In this case, the MF branch-out is a critical step in aberrant MF growth. Once MF collaterals bud, 50% of them will grow into the molecular layer in a stochastic manner. In the younger hippocampus, however, secreta-dependent MF guidance also works, leading MFs to elongate towards CA3, independent of the contact-mediated system. Therefore, early in development, even if MF collaterals sprout, they would be preferentially guided towards CA3. This may explain a reason why younger epileptic patients or animals seldom display aberrant MF sprouting. Using the slice overlay method, Kim *et al.* also have indicated that almost all of granule cells growing in the molecular layer elongate their axons within the molecular layer but do not step out of this subregion. The molecular layer appears to be a region suitable for MF growth. Therefore, it is possible that MFs are inherently apt to grow towards the molecular layers even in the absence of the contact-dependent guidance system.

Dentate granule cells have the unusual property of prolonged postnatal neurogenesis that persists into adulthood (Altman and Das, 1965; Kaplan and Hinds, 1977), and thus, a portion of MFs may undergo a continuous turnover over a period of weeks (Gould *et al.*, 2001). Seki and Rutishauser (1998) demonstrated that neonatal exposure to PSA-specific endoneuraminidase induced a transient PSA-NCAM deficit, causing ectopic MF outgrowth. In spite of the continuous MF turnover in the mature hippocampus, surprisingly, this aberration is maintained until adulthood. Likewise, Pimenta *et al.* (1995) reported that functional blocking of limbic system-associated membrane protein in early postnatal rats results in aberrant MF growth, which is also retained until adulthood. Thus, the MF ectopia seems irreversible. This holds true in TLE. Epilepsy-related MF sprouting also appears irrecoverable (Ikegaya *et al.*, 2000).

The irreversibility of ectopic MFs is accounted for by the two-step model of MF development. In the presence of ectopic MFs, the axons of newly born granule cells fasciculate with the pre-existing, abnormal MFs and then are guided incorrectly (Fig. **3**, Step **3**). The MF ectopia is hence irreversible; replacement of granule cells cannot ameliorate aberrant MF sprouting. In light of this idea, it is understandable that TLE is often chronic and intractable. Expression of PSA-NCAM in the hippocampus is reported to increase in TLE patients (Mikkonen *et al.*, 1998; Proper *et al.*, 2000) and pilocarpine-induced epileptic rats (Shan *et al.*, 2002). This may make the MF ectopia more stubborn.

Based on our hypothesis, the strategy to treat aberrant MF sprouting is now clear; it is to block the function of contact-dependent cues and facilitate secreta-dependent mechanisms. This would lead MFs to grow properly, regardless of the presence of ectopic MFs, and may alleviate TLE seizures. At this time, unfortunately, the molecular profiles of contact or diffusible cues for MFs remain ill-defined, although guidance cues have been implicated so far, including diffusible and non-diffusible cues (Muller *et al.*, 1994; Pimenta *et al.*, 1995; Cremer *et al.*, 1997; Seki and Rutishauser, 1998; Chen *et al.*, 2000; Grimpe *et al.*, 2002; Koyama *et al.*, 2002a; Mizoguchi *et al.*, 2002). Further investigations would clarify therapeutic targets for TLE.

CONCLUSIONS

Intensive investigations on TLE and its model animals are bringing about a large body of information about MF sprouting. However, as mentioned above, there has not yet a consistent vision on the contributions of MF sprouting to TLE. We need to keep it in mind that MF sprouting may be one of many factors that are responsible for epileptogenesis. Although this review has focused on the MFs alone, the hippocampus seems to undergo various alterations in TLE, e.g., neuron loss, neurogenesis, dendritic changes and gliosis (for review, see Pitkanen and Sutula, 2002). In addition, some reports indicate that similar to MFs, the axons of CA1 pyramidal neurons display sprouting in epileptic animals (Esclapez *et al.*, 1999; Smith and Dudek, 2001). It is not admirable, therefore, to jump to a simple conclusion on whether or not only MF sprouting is causal in epileptogenesis. We should more comprehensively consider a role of the hippocampus in TLE.

Nevertheless, MF sprouting itself is still attractive. Besides TLE, clarifying its mechanism and consequence would lead to further understanding of CNS network reorganization, neural plasticity, circuit operation, and axon guidance, which, in turn, may again provide insights into TLE from different points of view.

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