Does Mossy Fiber Sprouting Give Rise to the Epileptic State?

13

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Abstract

Many patients with temporal lobe epilepsy display structural changes in the seizure initiating zone, which includes the hippocampus. Structural changes in the hippocampus include granule cell axon (mossy fiber) sprouting. The role of mossy fiber sprouting in epileptogenesis is controversial. A popular view of temporal lobe epileptogenesis contends that precipitating brain insults trigger transient cascades of molecular and cellular events that permanently enhance excitability of neuronal networks through mechanisms including mossy fiber sprouting. However, recent evidence suggests there is no critical period for mossy fiber sprouting after an epileptogenic brain injury. Instead, findings from stereological electron microscopy and rapamycin-delayed mossy fiber sprouting in rodent models of temporal lobe epilepsy suggest a persistent, homeostatic mechanism exists to maintain a set level of excitatory synaptic input to granule cells. If so, a target level of mossy fiber sprouting might be determined shortly after a brain injury and then remain constant. Despite the static appearance of synaptic reorganization after its development, work by other investigators suggests there might be continual turnover of sprouted mossy fibers in epileptic patients and animal models. If so, there may be opportunities to reverse established mossy fiber sprouting. However, reversal of mossy fiber sprouting is unlikely to be antiepileptogenic, because blocking its development does not reduce seizure frequency in pilocarpine-treated mice. The challenge remains to identify which, if any, of the many other structural changes in the hippocampus are epileptogenic.

Keywords

Dentate gyrus • Granule cell • Epilepsy • Epileptogenesis • Hilus • Seizure • Pilocarpine

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13.1 Introduction

Temporal lobe epilepsy is common and its underlying mechanisms remain unclear [12]. Many patients have a history of an initial brain insult followed by a latent period [30]. A common view of temporal lobe epileptogenesis is that during the latent period, cascades of molecular and cellular events together alter the excitability of neuronal networks, ultimately causing spontaneous seizures [38]. According to this view, following an injury there is a critical period when temporary treatment might permanently prevent network reorganization. Substantial network reorganization occurs in the hippocampus of many patients with temporal lobe epilepsy [29]. The hippocampus is prone to epileptic activity [40] and is a site of seizure initiation in patients [37, 45]. Therefore, it is logical to ask whether structural changes in the hippocampus give rise to the epileptic state and whether blocking the development of structural changes during a critical period would prevent epileptogenesis. Structural changes in the hippocampus of patients with temporal lobe epilepsy include specific patterns of neuron loss [29], including inhibitory interneurons [11], hypertrophy of some surviving interneurons [28], GABAergic axon sprouting [1], dispersion of granule cells to ectopic locations [19], excessive development of hilar basal dendrites on granule cells [53], and mossy fiber sprouting (reviewed in [2]).

Philip Schwartzkroin's research included work on mossy fiber sprouting. His laboratory's slice experiments on tissue resected to treat patients revealed a general correlation between mossy fiber sprouting and hyperexcitability [13]. In those experiments, intracellular labeling and electron microscopy showed sprouted mossy fibers synapsing with dendrites of granule cells and interneurons. Those findings were supported and extended by experiments with kainate-treated rats that included evidence of autaptic synapses by sprouted mossy fibers [58]. However, Phil and colleagues cautioned that the results provided no direct evidence that mossy fiber sprouting was either necessary or sufficient for hyperexcitability [13].

Phil and colleagues characterized mossy fiber sprouting in other animal models, including p35-deficient mice with cortical dysplasia [57], different mouse strains treated with kainic acid [31], and infant monkeys after limbic status epilepticus [16, 56]. Phil's laboratory also helped localize the zinc transporter-3 to mossy fiber synaptic vesicle membranes [55], which has become a useful marker for visualizing mossy fiber sprouting.

Despite much investigation, the role of mossy fiber sprouting in epileptogenesis remains unclear and controversial. It has been proposed to be proepileptogenic [48], antiepileptogenic [44], and an epiphenomenon [15]. Recent evidence reviewed here raises questions about whether there is a critical period for mossy fiber sprouting after epileptogenic injuries and whether mossy fiber sprouting contributes to the generation of spontaneous seizures.

13.2 Is Mossy Fiber Sprouting a Homeostatic Mechanism?

Neuron loss in the hilus of the dentate gyrus is a common structural change in patients with temporal lobe epilepsy [29] that is replicated in animal models. Nadler et al. (1980) first showed that the excitotoxin kainic acid kills hilar neurons, whose axons degenerate in the inner third of the molecular layer into which mossy fibers later sprout. To quantify the initial loss of synapses onto granule cell proximal dendrites in the inner molecular layer and the later restoration of excitatory synaptic input from sprouted mossy fibers, we used stereological electron microscopy to evaluate a rat model of temporal lobe epilepsy [49]. Tissue was obtained: (1) from rats 5 days after pilocarpine-induced status epilepticus to measure loss of synaptic input to granule cells before axon sprouting had occurred and (2) after mossy fiber sprouting was well established 3–6 months after status epilepticus. Numbers of granule cells were estimated from Nissl stained sections. Numbers of excitatory synapses in the molecular layer, where granule cell dendrites extend, were estimated in serial electron micrographs

that had been processed by post-embedding immunocytochemistry for GABA to avoid counting inhibitory synapses. Subsequently, numbers of excitatory synapses per granule cell were calculated for each rat (Fig. 13.1a). Analysis of the inner third of the molecular layer revealed that the number of excitatory synapses per granule cell decreased to only 38 % of controls by 5 days after pilocarpine-induced status epilepticus (Fig. 13.1b). This substantial loss of synapses probably is attributable primarily to loss of hilar mossy cells. Mossy cells, which were first characterized electrophysiologically by intracellular recording and anatomical labeling techniques in Phil's laboratory [39], project most of their axon collaterals to the inner molecular layer of the dentate gyrus where they form glutamatergic synapses with proximal dendrites of granule cells [6, 8, 54]. Epileptogenic injuries, like status epilepticus, kill mossy cells [4] and thereby denervate proximal dendrites of granule cells. The extent of mossy cell loss correlates with the extent of mossy fiber sprouting [23]. However, mossy cell loss alone is insufficient to cause mossy fiber sprouting [24], and the molecular signals necessary for triggering mossy fiber sprouting are not yet known.

After initial loss, numbers of excitatory synapses per granule cell in the inner molecular layer partially rebound to 84 % of controls in rats 3–6 months after status epilepticus (Fig. 13.1b). This recovery probably is attributable primarily to mossy fiber sprouting [9], but other sources of excitatory synaptic input to the proximal dendrites of granule cells include surviving mossy cells and proximal CA3 pyramidal cells [60]. Synapses with proximal dendrites of granule cells at 3–6 months after status epilepticus are 1.3-times larger than in controls and twice as likely to be perforated [49]. Large, perforated synapses are likely to be functionally stronger than small, nonperforated synapses [14, 33, 35]. To maintain functional stability in the face of change, brains use an array of homeostatic mechanisms, including synaptic scaling [50]. Larger, stronger mossy fiber synapses in the inner molecular layer of epileptic rats might be a homeostatic mechanism to compensate for fewer synapses (84 % of controls, in this case).

Similarly, on granule cell distal dendrites in the outer two-thirds of the molecular layer, numbers of excitatory synapses decrease to 69 % of controls by 5 days after status epilepticus, but rebound to 101 % of controls by 3–6 months (Fig. 13.1b). With more complete recovery of synapse numbers, synapse size in the outer molecular does not change significantly [49]. Initial loss of synapses with distal dendrites of granule cells probably is attributable to partial loss of layer II entorhinal cortical neurons caused by status epilepticus [26]. And recovery of synapses probably is attributable to sprouting of axons of surviving layer II neurons [46]. Together, findings from the inner and outer molecular layer suggest a homeostatic mechanism maintains excitatory synaptic input to granule cells in response to synapse loss after an epileptogenic injury.

13.3 No Critical Period for Mossy Fiber Sprouting

If a homeostatic mechanism controls the number of excitatory synapses with granule cells, signals underlying that control might persist as long as a synaptic deficit continues. Persistent signals contrast with the view of a transient cascade of molecular and cellular events that peak and then diminish after a critical period following a brain injury. To address these issues, we determined whether mossy fiber sprouting would occur after a 2 month delay [27]. Rapamycin, which inhibits mossy fiber spouting [3], was administered to mice daily beginning 24 h after pilocarpine-induced status epilepticus. After 2 months, mossy fiber sprouting was suppressed almost by half in the rapamycin group compared to vehicle-treated controls (Fig. 13.1c). Another cohort was evaluated 6 months after the end of treatment, which was 8 months after status epilepticus. Mossy fiber sprouting was well developed in both vehicle- and rapamycin-treated mice, indicating that signals stimulating mossy fiber sprouting must have persisted for more than 2 months. These findings suggest there is no transient critical period for mossy fiber sprouting after an epileptogenic
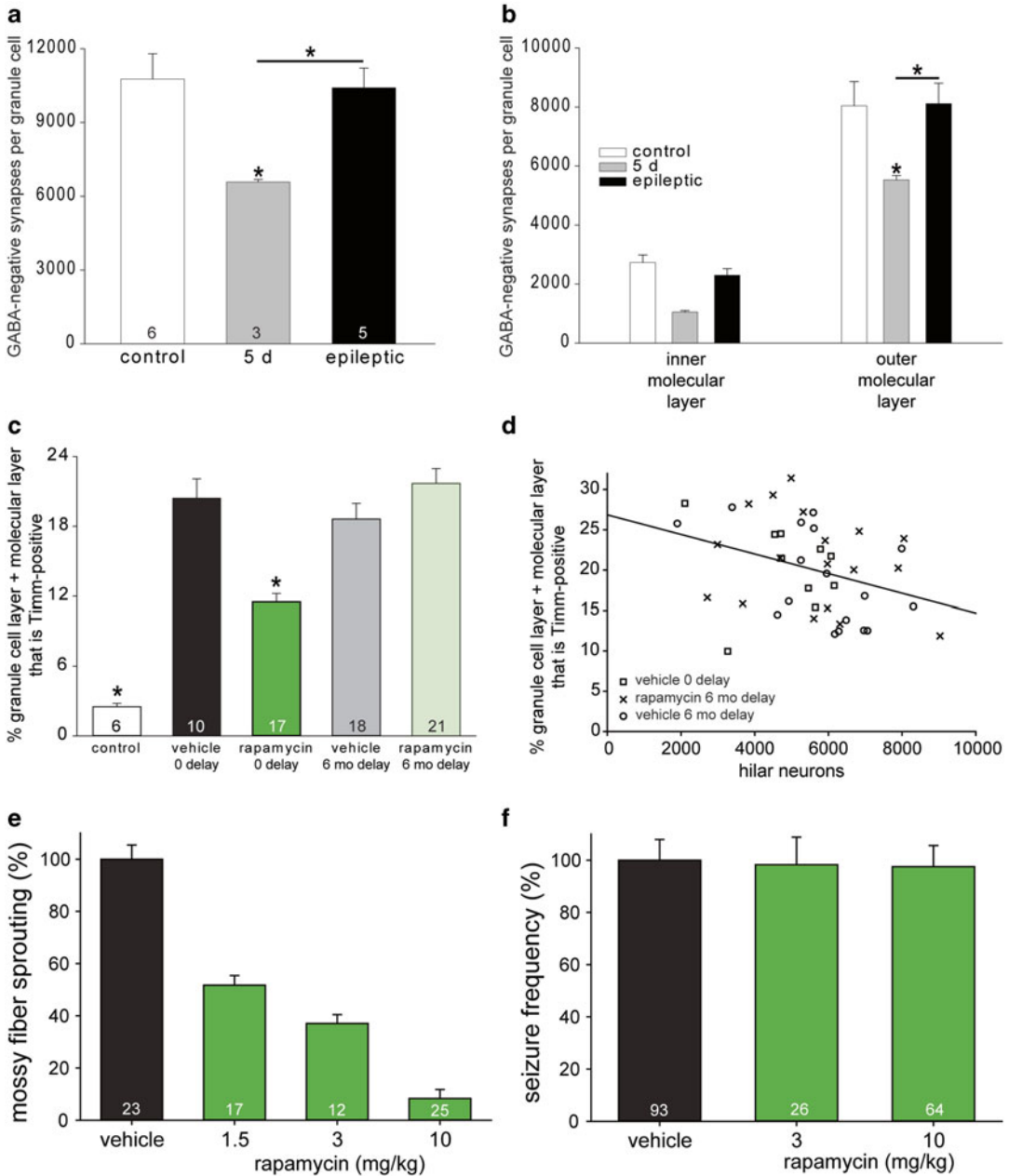
Fig. 13.1 Excitatory synapse loss and mossy fiber sprouting of granule cells in pilocarpine-treated rodent models of temporal lobe epilepsy. (**a**) Number of putative excitatory synapses per granule cell in control rats and in rats 5 days and 3–6 months (epileptic) after pilocarpine-induced status epilepticus. (**b**) Number of synapses with granule cell dendrites in the inner one-third and outer two-thirds of the molecular layer. Values represent mean ± sem. Sample size indicated at base of bars. Asterisks indicate differences from the control value unless specified by a horizontal line (p<0.05, ANOVA, Student-Newman Keuls method). (**a**) and (**b**) from Thind et al. [49]. (**c**) Extent of mossy fiber sprouting in control mice and mice that experienced status epilepticus and were treated with vehicle or 1.5 mg/kg rapamycin every day for 2 months and then were perfused with no delay (0 delay) or after a 6 month delay (6 month delay) (From Lew and Buckmaster [27]). (**d**) Number of large hilar neurons (>12 μm soma diameter) per hippocampus versus extent of mossy fiber sprouting in mice that experienced pilocarpine-induced status epilepticus and were treated with vehicle or rapamycin for 2 months and then evaluated immediately (vehicle 0 months) or after another 6 months (vehicle or rapamycin 6 months). A linear regression line is plotted (R=0.34, p=0.021, ANOVA). (**e**) Percent mossy fiber sprouting was calculated by subtracting the average percentage of the molecular layer plus granule cell layer that was Timm-positive in control mice and normalizing by the average value of mice that had experienced status epilepticus and were treated with vehicle for 2 months. Averages of all groups are significantly different from others (p<0.05, ANOVA, Student-Newman-Keuls method). (**f**) Percent seizure frequency was calculated by normalizing by the average of the vehicle-treated group. (**e**) and (**f**) from Heng et al. [18]

brain injury. Instead, preventing mossy fiber sprouting might require long-term or continuous treatment. This scenario challenges the view of transient signaling cascades whose consequences could be permanently blocked by temporary treatment during a critical period.

One might question whether the precipitating injury in the mouse model was so severe that it maximally stimulated mossy fiber sprouting toward saturation levels despite the delay caused by rapamycin. However, an all-or-none "toggle-like" signal and saturation effect is inconsistent with the graded degree of mossy fiber sprouting among individual mice, which ranged over a factor of three and was correlated with the extent of hilar neuron loss (Fig. 13.1d). Wide ranges in mossy fiber sprouting between individuals were evident in vehicle-treated mice 2 months after status epilepticus and vehicle- and rapamycin-treated mice 8 months after status epilepticus, indicating that sprouting did not progressively develop toward saturated levels. These findings suggest that a target level of mossy fiber sprouting in an individual might be determined shortly after a brain injury and then remain constant.

Together, findings from stereological electron microscopy and rapamycin-delayed mossy fiber sprouting suggest a persistent, homeostatic mechanism exists to maintain a set level of excitatory synaptic input to granule cells. If mossy fiber sprouting were epileptogenic, this might be an example of a normally adaptive homeostatic mechanism that became pathogenic in response to an injury, which has been proposed previously as a theoretical possibility [10]. More generally, epileptogenesis might be an unintended side-effect of the brain's homeostatic mechanisms, which evolved to maintain function in the face of plasticity. Epileptogenic injuries might trigger changes so much more extensive than normal plasticity that they push homeostatic responses into a range that creates a network that generates spontaneous seizures. Phil proposed a similar idea [41]: "I believe that the brain has been designed to operate at a knife's edge. The evolutionary demand for plasticity – a key attribute of higher order learning, memory, and all those complex functions that are characteristic of the mammalian CNS – has necessitated a sacrifice in stability of neuronal function."

On the other hand, if epileptogenesis is maintained by homeostatic mechanisms gone awry, there may be opportunities to reverse established epilepsy-related structural abnormalities. Although mossy fiber sprouting appears to develop gradually, plateau, and then cease, there might instead be continual turnover. Mossy fibers in tissue from patients with temporal lobe epilepsy display evidence of continuing synaptic reorganization years after precipitating injuries [21, 32, 36]. At least some sprouted mossy fibers arise from adult generated granule cells [22, 25], which might continue to be generated long after precipitating injuries (but see [17]). To test whether mossy fiber sprouting could be reversed after it had established, we infused rapamycin focally into the dentate gyrus for 1 month beginning 2 months after pilocarpine-induced status epilepticus in rats, but there was no effect [3]. However, Huang et al. [20] reported that in chronically epileptic pilocarpine-treated rats, systemically administered rapamycin partially reversed already established mossy fiber sprouting. Moreover, grafts of CA3 pyramidal cells reduce mossy fiber sprouting even when implanted 45 days after kainate-treatment, during which time considerable mossy fiber sprouting is likely to have developed [42]. In addition, mild mossy sprouting generated by electroconvulsive shock was reported to decline over time [51]. Thus, more work is needed to test the reversibility of mossy fiber sprouting.

13.4   Mossy Fiber Sprouting Is Not Epileptogenic

Rapamycin also was used to test whether mossy fiber sprouting was epileptogenic. Systemic treatment with rapamycin at increasing doses to inhibit mossy fiber sprouting to increasing degrees had no effect on the frequency of spontaneous seizures in mice that had experienced pilocarpine-induced status epilepticus (Fig. 13.1e, f) [5, 18]. These findings suggest that mossy fiber sprouting is neither pro- nor antiepileptogenic, but instead is

an epiphenomenon unrelated to seizure genesis. There are caveats with this conclusion, because rapamycin has side-effects [47], including suppression of axon sprouting by inhibitory GABAergic interneurons [7]. And rapamycin reduces seizure frequency in some rat models of temporal lobe epilepsy [20, 52, 59] but not all [43]. It remains unclear whether rapamycin's action in rats is antiseizure or antiepileptogenic. Nevertheless, the findings from the mouse studies suggest mossy fiber sprouting is not epileptogenic.

13.5 Conclusions

Patients with temporal lobe epilepsy display many structural changes, especially in the hippocampus. One possibility is that together numerous structural changes and other abnormalities all contribute partially to seizure generation. In that scenario, blocking the development of any one change, like mossy fiber sprouting, might have negligible effects on epileptogenesis. Another possibility is that some or perhaps even many epilepsy-related structural changes are not epileptogenic, including mossy fiber sprouting, and that seizure generation is attributable to one or two critical abnormalities whose importance has not yet been recognized. These alternate possibilities – many abnormalities each contributing partially versus one or two abnormalities primarily responsible for seizure generation–might require different therapeutic approaches, so it is important to distinguish between them. To do so, it will be useful to tap the ever-increasing knowledge base of molecular and cellular mechanisms underlying brain developmental processes and responses to injury. Creative application of ideas and reagents (for example, rapamycin), even from fields outside of epilepsy research, might yield useful approaches for specifically inhibiting or exacerbating individual epilepsy-related structural changes. Experimental manipulation of specific structural changes, one at a time, and rigorous measurement of effects on spontaneous seizures, might eventually reveal which, if any, are epileptogenic. If no single change alone appears to be responsible, then blockade of many or all could be

used to test whether together they make the brain epileptic or if the cause of seizures is unrelated to structural changes in the hippocampus.

References

1. Babb TL, Pretorius JK, Kupfer WR, Crandall PH (1989) Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. J Neurosci 9:2562–2574
2. Buckmaster PS (2012) Mossy fiber sprouting in the dentate gyrus. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds) Japser's basic mechanisms of the epilepsies, 4th edn. Oxford University Press, New York, pp 416–431
3. Buckmaster PS, Ingram EA, Wen X (2009) Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. J Neurosci 29:8259–8269
4. Buckmaster PS, Jongen-Rêlo AL (1999) Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. J Neurosci 19:9519–9529
5. Buckmaster PS, Lew FH (2011) Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy. J Neurosci 31:2337–2347
6. Buckmaster PS, Strowbridge BW, Kunkel DD, Schmiege DL, Schwartzkroin PA (1992) Mossy cell axonal projections to the dentate gyrus molecular layer in the rat hippocampal slice. Hippocampus 2:349–362
7. Buckmaster PS, Wen X (2011) Rapamycin suppresses axon sprouting by somatostatin interneurons in a mouse model of temporal lobe epilepsy. Epilepsia 52:2057–2064
8. Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA (1996) Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. J Comp Neurol 366:270–292
9. Buckmaster PS, Zhang G, Yamawaki R (2002) Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. J Neurosci 22:6650–6658
10. Davis GW, Goodman CS (1998) Genetic analysis of synaptic development and plasticity: homeostatic regulation of synaptic efficacy. Curr Opin Neurobiol 8:149–156
11. de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD (1989) Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 495:387–395

12. Engel J Jr, Williamson PD, Wieser H-G (1997) Mesial temporal lobe epilepsy. In: Engel J Jr, Pedley TA (eds) Epilepsy: a comprehensive textbook. Lippincott-Raven, Philadelphia, pp 2417–2426

13. Franck JE, Pokorny J, Kunkel DD, Schwartzkroin PA (1995) Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. Epilepsia 36:543–558

14. Ganeshina O, Berry RW, Petralia RS, Nicholson DA, Geinesman Y (2004) Differences in the expression of AMPA and NMDA receptors between axospinous perforated and nonperforated synapses are related to the configuration and size of postsynaptic densities. J Comp Neurol 468:86–95

15. Gloor P (1997) The temporal lobe and limbic system. Oxford University Press, New York, pp 677–691

16. Gunderson VM, Dubach M, Szot P, Born DE, Wenzel JH, Maravilla KR, Zierath DK, Robbins CA, Schwartzkroin PA (1999) Development of a model of status epilepticus in pigtailed macaque infant monkeys. Dev Neurosci 21:352–364

17. Hattiangady B, Rao MS, Shetty AK (2004) Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. Neurobiol Dis 17:473–490

18. Heng K, Haney MM, Buckmaster PS (2013) High-dose rapamycin blocks mossy fiber sprouting but not seizures in a mouse model of temporal lobe epilepsy. Epilepsia 54:1535–1541

19. Houser CR (1990) Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res 535:195–204

20. Huang X, Zhang H, Yang J, Wu J, McMahon J, Lin Y, Cao Z, Gruenthal M, Huang Y (2010) Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. Neurobiol Dis 40:193–199

21. Isokawa M, Levesque MF, Babb TL, Engel JE Jr (1993) Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. J Neurosci 13:1511–1522

22. Jessberger S, Zhao C, Toni N, Clemenson GD Jr, Li Y, Gage FH (2007) Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. J Neurosci 27:9400–9407

23. Jiao Y, Nadler JV (2007) Stereological analysis of GluR2-immunoreactive hilar neurons in the pilocarpine model of temporal lobe epilepsy: correlation of cell loss with mossy fiber sprouting. Exp Neurol 205:569–582

24. Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K (2012) Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 76:1189–1200

25. Kron MM, Zhang H, Parent JM (2010) The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. J Neurosci 30:2051–2059

26. Kumar SS, Buckmaster PS (2006) Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. J Neurosci 26:4613–4623

27. Lew F, Buckmaster PS (2011) Is there a critical period for mossy fiber sprouting in a mouse model of temporal lobe epilepsy? Epilepsia 52:2326–2332

28. Maglóczky Z, Wittner L, Borhegyi Z, Halász P, Vajda J, Czirják S, Freund TF (2000) Changes in the distribution and connectivity of interneurons in the epileptic human dentate gyrus. Neuroscience 96:7–25

29. Margerison JH, Corsellis JAN (1966) Epilepsy and the temporal lobes. Brain 89:499–530

30. Mathern GW, Pretorius JK, Babb TL (1995) Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. J Neurosurg 82:220–227

31. McKhann GM 2nd, Wenzel HJ, Robbins CA, Sosunov AA, Schwartzkroin PA (2003) Mouse strain differences in kainic acid sensitivity, seizure behavior, mortality, and hippocampal pathology. Neuroscience 122:551–561

32. Mikkonen M, Soininen H, Kälviäinen R, Tapiola T, Ylinen A, Vapalahti M, Paljärvi L, Pitkänen A (1998) Remodeling of neuronal circuitries in human temporal lobe epilepsy: increased expression of highly polysialylated neural cell adhesion molecular in the hippocampus and entorhinal cortex. Ann Neurol 44:923–934

33. Murthy VN, Schikorski T, Stevens CF, Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. Neuron 32:673–682

34. Nadler JV, Perry BW, Cotman CW (1980) Selective reinnervation of hippocampal area CA1 and the fascia dentata after destruction of CA3-CA4 afferents with kainic acid. Brain Res 182:1–9

35. Nusser Z, Lujan R, Laube G, Roberts JDB, Molnar E, Somogyi P (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron 21:545–559

36. Proper EA, Oestreicher AB, Jansen GH, Veelen CWM, van Rijen PC, Gispen WH, de Graan PNE (2000) Immunohistochemical characterization of mossy fibre sprouting in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. Brain 123:19–30

37. Quesney LF (1986) Clinical and EEG features of complex partial seizures of temporal lobe origin. Epilepsia 27(Suppl 2):S27–S45

38. Rakhade SN, Jensen FE (2009) Epileptogenesis in the immature brain: emerging mechanisms. Nat Rev Neurol 5:380–391

39. Scharfman HE, Schwartzkroin PA (1988) Electrophysiology of morphologically identified mossy cells of the dentate hilus recorded in guinea pig hippocampal slices. J Neurosci 8:3812–3821

40. Schwartzkroin PA (1994) Role of the hippocampus in epilepsy. Hippocampus 4:239–242

41. Schwartzkroin PA (1997) Origins of the epileptic state. Epilepsia 38:853–858

42. Shetty AK, Zaman V, Hattiangady B (2005) Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. J Neurosci 25:8391–8401

43. Sliwa A, Plucinska G, Bednarczyk J, Lukasiuk K (2012) Post-treatment with rapamycin does not prevent epileptogenesis in the amygdala stimulation model of temporal lobe epilepsy. Neurosci Lett 509:105–109

44. Sloviter RS (1992) Possible functional consequences of synaptic reorganization in the dentate gyrus of kainate-treated rats. Neurosci Lett 137:91–96

45. Spencer SS, Williamson PD, Spencer DD, Mattson RH (1987) Human hippocampal seizure spread studied by depth and subdural recording: the hippocampal commissure. Epilepsia 28:479–489

46. Steward O (1976) Reinnervation of dentate gyrus by homologous afferents following entorhinal cortical lesions in adult rats. Science 194:426–428

47. Swiech L, Perycz M, Malik A, Jaworski J (2008) Role of mTOR in physiology and pathology of the nervous system. Biochim Biophys Acta 1784:116–132

48. Tauck DL, Nadler JV (1985) Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. J Neurosci 5:1016–1022

49. Thind KK, Yamawaki R, Phanwar I, Zhang G, Wen X, Buckmaster PS (2010) Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. J Comp Neurol 518:647–667

50. Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135:422–435

51. Vaidya VA, Siuciak JA, Du F, Duman RS (1999) Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. Neuroscience 89:157–166

52. van Vliet EA, Forte G, Holtman L, den Buerger JCG, Sinjewel A, de Vries HE, Aronica E, Gorter JA (2012) Inhibition of mammalian target of rapamycin reduces epileptogenesis and blood-brain barrier leakage but not microglia activation. Epilepsia 43:1254–1263

53. von Campe G, Spencer DD, de Lanerolle NC (1997) Morphology of dentate granule cells in the human hippocampus. Hippocampus 7:472–488

54. Wenzel HJ, Buckmaster PS, Anderson NL, Wenzel ME, Schwartzkroin PA (1997) Ultrastructural localization of neurotransmitter immunoreactivity in mossy cell axons and their synaptic targets in the rat dentate gyrus. Hippocampus 7:559–570

55. Wenzel HJ, Cole TB, Born DE, Schwartzkroin PA, Palmiter RD (1997) Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. Proc Natl Acad Sci U S A 94:12676–12681

56. Wenzel HJ, Born DE, Dubach MF, Gunderson VM, Maravilla KR, Robbins CA, Szot P, Zierath D, Schwartzkroin PA (2000) Morphological plasticity in an infant monkey model of temporal lobe epilepsy. Epilepsia 41(Suppl 6):S70–S75

57. Wenzel HJ, Robbins CA, Tsai LH, Schwartzkroin PA (2001) Abnormal morphological and functional organization of the hippocampus in a p35 mutant model of cortical dysplasia with spontaneous seizures. J Neurosci 21:983–998

58. Wenzel HJ, Woolley CS, Robbins CA, Schwartzkroin PA (2000) Kainic acid-induced mossy fiber sprouting and synapse formation in the dentate gyrus of rats. Hippocampus 10:244–260

59. Zeng L-H, Rensing NR, Wong M (2009) The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J Neurosci 29:6964–6972

60. Zhang W, Huguenard JR, Buckmaster PS (2012) Increased positive-feedback from hilar and CA3 neurons to granule cells in a rat model of temporal lobe epilepsy. J Neurosci 32:1183–1196