Newly Formed Excitatory Pathways Provide a Substrate for Hyperexcitability in Experimental Temporal Lobe Epilepsy

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ABSTRACT

Temporal lobe epilepsy (TLE) in humans and animals is associated with axonal sprouting of glutamatergic neurons and neosynaptogenesis in the hippocampal formation. We examined whether this plasticity of excitatory pathways contributes to an increased level of glutamatergic excitation in the CA1 region of rats experiencing chronic spontaneous limbic seizures following kainic acid or pilocarpine treatment. In chronic cases, we report an extensive axonal sprouting of CA1 pyramidal neurons, with many axonal branches entering the pyramidal cell layer and stratum radiatum, regions that are not innervated by axonal collaterals of CA1 pyramidal neurons in control animals. Concurrently with this anatomical reorganization, a large increase of the spontaneous glutamatergic drive is observed in the dendrites and somata of CA1 pyramidal cells. Furthermore, electrical activation of the reorganized CA1 associational pathway evokes epileptiform bursts in CA1 pyramidal cells. These findings suggest that reactive plasticity could contribute to the hyperexcitability of CA1 pyramidal neurons and to the propagation of seizures in these two models of TLE. J. Comp. Neurol. 408:449–460, 1999. (*) 1999 Wiley-Liss, Inc.

Indexing terms: rat; hippocampus; CA1; pilocarpine; kainate; plasticity

Pyramidal cells in the hippocampus and neocortex give rise to networks of axonal recurrent collaterals (associational pathways) that spread excitation to neighboring neurons. Based on this morphological specificity, a permanent increase of recurrent glutamatergic excitation on other excitatory cells has been proposed as one of the key factors responsible for hyperexcitability in various forms of epilepsy and particularly in temporal lobe epilepsy (TLE). Increase of recurrent excitation can be achieved by several factors: (1) A deficit of gamma-aminobutyric acid (GABA)-ergic inhibition: although pharmacological blockade of fast inhibition results in hyperexcitability spreading through associational pathways in control tissue (Traub and Wong, 1982; Miles and Wong, 1983; Chagnac-Amitai and Connors, 1989; Jefferys and Whittington, 1996, Meier and Dudek, 1996), recent studies have indicated that fast inhibition is still operative (Esclapez et al., 1997a,b; Rempe et al., 1997) or even increased (Buckmaster and Dudek, 1997; Prince et al., 1997; Nusser et al., 1998) in epilepsy. (2) The formation of new recurrent excitatory connections: morphological evidence has indicated that glutamatergic axons sprout and establish new, sometimes aberrant, synapses in human TLE and its animal models. Although best documented for the hippocampal mossy fibers (Nadler et al., 1980a,b; Tauck and Nadler, 1985; Represa et al., 1987; DeLanerolle et al., 1989; Represa and Ben-Ari, 1992; Cronin et al., 1992), this has also been described for the axons of CA1 pyramidal neurons (Perez et al., 1996), suggesting that axonal sprouting and glutamatergic neosynapse formation may be a general property of epileptic circuits (see also Salin et al., 1995; McKinney et al., 1997). However, the link between epilepsy and the "too-well-connected brain" (Prince, 1997) is not known because the functionality of the newly formed glutamatergic synapses and their contribution to seizure generation have not been established.

To address these issues, we performed patch clamp recordings and morphological analysis of CA1 pyramidal neurons in hippocampal slices from kainic acid- or pilocarpine-treated rats with spontaneous chronic limbic seizures, two models of human TLE. Because of the crucial role of dendrites in controlling cell excitability, we also

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examined the long-term consequences of seizures in the dendrites in addition to more conventional recordings of somata of pyramidal neurons. Preliminary data of this study have been presented in abstract form (Esclapez et al., 1997a,b).

MATERIALS AND METHODS Animals

Young adult male Wistar rats (180-200 g) were treated with pilocarpine or kainic acid (KA) according to established procedures (Nadler et al., 1980c; Turski et al., 1983; Obenaus et al., 1993; Bernard and Wheal, 1996). Briefly. pilocarpine (325–340 mg/kg) was injected intraperitonealy 30 minutes after the administration of a low dose of the cholinergic antagonist methyl scopolamine nitrate (1 mg/ kg, i.p.) to minimize peripheral cholinergic effects. KA (0.5 μ g in a volume of 0.5 μ l of phosphate buffer, pH 7.4) was infused, over a 30-minute period, into the left lateral ventricule of the rats under chloral hydrate (350 mg/kg) anesthesia. Following drug injection, 80% of the animals in both models showed robust behavioral seizures for 3-4 hours or longer. Sixty percent of the pilocarpine-treated animals and more than 90% of the KA-treated rats survived this period of acute seizures. These rats were then observed periodically (at least three hours a day) in the vivarium for the occurrence of spontaneous seizures. Among these animals, 90% of the pilocarpine-treated and 70% of the KA-treated rats developed spontaneous seizures, with a frequency of one to four seizures per week. Only seizures of grade 3 or greater on the Racine (1972) scale were scored (i.e., forelimb clonus \pm rearing \pm falling). The onset of the spontaneous seizure occurrence was 4-6 weeks for pilocarpine rats and 2-8 months for KA animals. Twenty KAtreated and 14 pilocarpine-treated rats which had displayed spontaneous limbic seizures were included in this study. The time between drug treatment and perfusion for electrophysiological and morphological studies was 2-3 months for pilocarpine animals and 2-12 months for KA rats. Any animal that had displayed a seizure within the last 24 hours before the final experiment was excluded. Fifteen age-matched rats from the same litters were used for control experiments. All animal-use protocols conformed to NIH guidelines and the French Public Health Service Policy on the use of laboratory animals.

Electrophysiology

The animals were intracardially perfused, under chloral hydrate (350 mg/kg, i.p.) anesthesia, with modified artificial cerebrospinal fluid (ACSF), and 400-µm-thick hippocampal slices were prepared with a Leica VT 1000E tissue slicer as previously described (Hirsch et al., 1996). ACSF contained (in mM) 124 NaCl, 3 KCl, 1.25 KH₂PO₄, 26 NaHCO₃, 1.3 MgSO₄-7H₂O, 2 CaCl₂, and 10 D-glucose and was continuously aerated with 95% O2 and 5% CO2. The temperature in the submerged recording chamber was maintained at 30-32°C. In all experiments, before electrophysiological recordings, the slices were recut at the level of the subiculum to isolate the hippocampal formation (the dentate gyrus and the hippocampus) from adjacent cortical regions. In addition, in some experiments the CA1 area was surgically isolated from the CA3 area by a knife cut performed in the CA1 area close to the CA3 border. Evoked and spontaneous postsynaptic currents and potentials were recorded from somata or dendrites of CA1 pyramidal

TABLE 1. Number of CA1 Pyramidal Neurons Detected With Each Morphological Method for Each Animal Group*

	ABC on resectioned slices ¹	ABC on unsectioned slices	FITC-avidine D on unsectioned slices	Total number of cells
Control	37 (19)	8	22	67
Pilocarpine	25 (15)	8	36	69
KA ipsilateral side	26 (14)	4	15	45
KA contralateral side	32 (17)	5	17	54

 \ast FITC, fluorescein isothiocyanate; KA, kainic acid; ABC, avidin-biotinylated peroxidase complex.

¹The number of pyramidal neurons that exhibited, in addition to somatic and dendritic staining, a complete and uniform labeling of the axon is indicated in parentheses.

neurons with tight-seal whole-cell patch clamp pipettes. Microelectrodes had a resistance of $4-12 \text{ M}\Omega$, and internal solutions of the following compositions were used (in mM): (1) for current clamp recordings: 135 K-gluconate, 10 MgCl₂, 0.1 CaCl₂, 1 EGTA, 2 Na₂ adenosine triphosphate, 10 HEPES, 0.5% biocytin, pH 7.25; (2) for voltage clamp recordings: K-gluconate was replaced by Cs-gluconate. The osmolarity of the internal solutions was 265-275 mOsm. Each of the 235 recorded pyramidal cells was identified post hoc. Spontaneous excitatory currents were measured at the reversal potential for GABAergic events (around -60 mV) according to established procedure (Esclapez et al., 1997a,b). D-2-amino-5phosphonovaleric acid (D-APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were applied at the end of the experiments to verify that the currents were indeed glutamatergic. CNQX and D-APV were a gift from Novartis (Basel, Switzerland). Bicuculline, KA, pilocarpine, methyl scopolamine nitrate, and biocytin were obtained from Sigma (St. Louis, MO), and tetrodotoxin (TTX) was obtained from Latoxan (Rosans, France). Signals were fed to an Axopatch 200A,B amplifier (Axon Instruments, Inc., Foster City, CA). All data were digitized (10 kHz) with a Labmaster interface card attached to a personal computer and analyzed with Acquis1 program (G. Sadoc, 1994, Biologic, Grenoble, France). The mean values and corresponding standard errors (S.E.M.s) were calculated for all the experimental parameters (measures) and for each animal group. For each parameter, the mean values obtained in control, pilocarpine-, and KA-treated rats were compared with a mixed model analysis of variance (ANOVA) and Student's t-test.

Morphology

Techniques for the detection of biocytin-filled neu-After electrophysiological recordings, slices were rons. fixed overnight at 4°C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). After fixation, slices were rinsed in PB, cryoprotected in sucrose, and quickly frozen on dry ice. After these steps, three protocols were used to visualize the biocytin-filled neurons. (1) To study the morphological features of CA1 pyramidal cells, detection of labeled neurons was performed on resectioned slices. Briefly, 60-µm-thick sections were cut on a cryostat. To neutralize endogenous peroxidase, sections were pretreated for 30 minutes in 1% H₂O₂. After several rinses in 0.1 M phosphate buffered saline (pH 7.4; PBS), sections were incubated overnight at 4°C in avidin-biotinylated peroxidase complex (1:200; Vector Laboratories, Burlingame, CA) diluted in PBS. After 30-minute rinses in PBS, sections were processed with 0.06% 3,3'diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.006% H₂O₂ diluted in PBS. Biocytin-labeled neurons that displayed complete dendritic and axonal arboriza-



Fig. 1. Neuronal cell loss in the hippocampus of chronic pilocarpine (B) and kainic acid (KA)-treated (C,D) animals illustrated by sections stained with cresyl violet. **A**: In control specimens, neuronal cell bodies of pyramidal neurons are highly concentrated and form a continous band in the CA3 and CA1 fields, and numerous neurons are present in the hilus (H) of the dentate gyrus. **B**: In a pilocarpine-treated rat with spontaneous seizures (three months after injection), a marked reduction in the number of neurons is present in the hilus. In addition, a slight neuronal loss is observed in some regions of CA3 (arrows). In contrast, the CA1 pyramidal cell layer is well preserved. **C**: In a chronic KA-treated animal (three months after injection) of KA displays a

very extensive cell loss of hilar neurons and of CA3 pyramidal cells. A reduction in the number of CA1 pyramidal neurons is also observed. **D**: In contrast, the hippocampus contralateral to the KA injection exhibits very little neuronal loss in the CA3 and CA1 regions. **E-F**: High magnification photomicrographs of CA1 pyramidal cell layers from the same specimens illustrated in C and B, respectively. E: Despite extensive neuronal loss (see C), many CA1 pyramidal cell bodies remain in the hippocampus ipsilateral to the cerebroventricular injection of a chronic KA-treated animal (three months after injection). F: In a pilocarpine-treated rat (three months after injection), numerous well-preserved CA1 pyramidal cells are observed in the hippocampus. Scale bars = 400 µm in A–D, 200 µm in E,F.

tions were reconstructed from serial adjacent sections with the Neurolucida system (Microbrightfield, Inc., Colchester, VT). (2) To investigate putative synaptic contacts between CA1 pyramidal cells, we conducted experiments in which three to five neurons were injected with biocytin in the same 250-µm-thick slice (two slices for control animals and four slices for pilocarpine- and KA-treated rats). The detection of the biocytin-filled neurons was performed on unsectioned slices. After the pretreatement step in 1% H_2O_2 and several rinses in PBS, slices were incubated for 24 hours at 4°C in 1:100 avidin-biotinylated peroxidase complex diluted in PBS containing 0.3% Triton X-100.



Figure 2

DAB and H_2O_2 were used as chromogens. (3) Some slices were processed for detection of biocytin with a fluorescent marker to find the trace of the patch electrode. The slices were incubated for 24 hours in fluorescein isothiocyanate and avidin-D (Vector Laboratories, Inc.) diluted in PBS containing 0.3% Triton X-100. All sections and slices were mounted on gelatin-coated slides and coverslipped in aqueous media (Crystal or Gel/Mount, Biomeda Corp., Foster City, CA). Table 1 summarizes the number of CA1 pyramidal neurons detected with each method for each animal group and indicates (in parentheses) the number of pyramidal neurons that exhibited, in addition to somatic and dendritic staining, a complete and uniform labeling of the axon.

Quantitative analysis of the axonal arborization and associated varicosities. Among the 65 biocytinfilled CA1 pyramidal neurons that displayed uniform labeling of the axonal and dendritic trees (Table 1), 14 neurons from control, KA-, and pilocarpine-treated animals were selected for quantitative analysis of axonal arborization. These neurons were selected according to the following criteria. Neurons had to exhibit extensive labeling of the axon. We were able to reconstruct the entire course of the axon from the cell body. For these neurons, in addition to the dendritic and axon arbors, the varicosities (putative boutons) along axonal processes were marked during the acquisition with the computer software program Neurolucida. The total length of the axon running in the alveus, stratum oriens, and stratum radiatum (KA and pilocarpine rats; see Results) in the CA1 region, the numbers of axonal branches, and varicosites per branches were automatically calculated by the Neurolucida software. The axonal branch originating from the soma or a proximal dendrite was called the first-order axonal segment and ended at the first branching point. From the first branching point emerged two second-order axonal segments, and so on. Thus, an nth-order axonal branch is bounded by a (n-1)th (or the soma) and an nth (or the end tip of the axonal branch) branching point.

Quantitative estimation of the complexity of the axonal arborization and putative synaptic contacts was determined by calculating the mean numbers of total axonal length, of segment order per axon, and of varicosities per 100 μ m of axon for each nth-order axonal branch. All

values are given as means \pm S.E.M. Statistical analysis of differences in the number of nth-order axonal segment, the total axonal length, and the number of varicosities among control and pilocarpine- or KA-treated animals were performed with a mixed model ANOVA and Student's t-test.

Digital processing of figures

Figures 2-6 were assembled digitally. The computergenerated images of the four-color photomicrographs (Fig. 3) and of the black-and-white photomicrographs associated with electrophysiological traces (Fig. 4) or with neuron drawings (Fig. 2) were performed as follows. Color positive (Fig. 3: Ektachrome 160T, Kodak, Rochester, NY) or black-and-white negative (Fig. 4: TMAX 3200 ASA, Kodak; Fig. 2: AGFAPAN 25 ASA, AGFA, Mortsel, Belgium) films of histological preparations were taken with a Nikon photomicroscope. Each film was digitized with a Nikon Film Scanner LS-1000 (300 dpi) driven by Adobe Photoshop, version 4.0, running on a Power Macintosh 7500/100. No modification or alteration of the initial images was done. The electrophysiological traces (Fig. 4) were obtained after acquisition of the data with Acquis 1 software (G. Sadoc) installed on a PC and were transferred as HPLG or ASCII files to a Macintosh using Claris Draw, version 1.03. The drawings of biocytin-filled pyramidal neurons and axograms were generated with Neurolucida, version 3, on a PC and were exported as EPS files to a Macintosh using Adobe Photoshop. The montages of the digitized images were made with Quark XPress, version 3.31, for Macintosh and printed with a Tektronic Phaser II SDX printer.

RESULTS

General neuronal loss within the hippocampal formation of pilocarpineor KA-treated animals

Cresyl-violet-stained sections were studied to evaluate the extent of the neuronal loss in the two models of chronic limbic seizures. In pilocarpine-treated animals with spontaneous recurrent seizures (2-3 months after drug injection), a marked and consistent cell loss was observed in the hilus of the dendate gyrus. In addition, these animals displayed variable amounts of neuronal loss in the CA3 pyramidal cell layer, but this loss was seldom extensive and many neuronal cell bodies remained in this layer (Fig. 1B; compare with the control shown in Fig. 1A). The dentate granule cell and the CA1 pyramidal cell layers were relatively well preserved (Fig. 1B,F). A similar pattern of cell loss has been described previously in this model (Turski et al., 1986; Mello et al., 1993; Obenaus et al., 1993; Liu et al., 1994). In all KA-treated animals, a massive cell loss of hilar neurons and CA3 pyramidal cells was observed in the hippocampus ipsilateral to the intracerobroventricular injection of KA (Fig. 1C; compare with the control shown in Fig. 1A). A variable amount of neuronal loss was observed in the CA1 pyramidal cell layer in the ipsilateral hippocampus; the extent of loss increased with time after drug injection. Whereas many neuronal cell bodies remained in the CA1 pyramidal cell layer three months after drug injection (Fig. 1E), almost all of them had degenerated 12 months after KA treatment. In contrast, the hippocampus contralateral to the KA injection displayed only little damage that was observed mainly in

Fig. 2. Pyramidal neuron with exuberant axonal sprouting in the CA1 region of pilocarpine-treated animals. A,B: Neurolucida reconstruction of the axonal (gray) and dendritic arborizations of biocytinfilled pyramidal neurons from control (A) and pilocarpine-treated (B) rats. A: In the control animal, the axon of a CA1 pyramidal cell arises from the soma and gives off one axonal branch running toward the fimbria and four branches directed toward the subiculum (S). These axonal branches running in stratum oriens and the alveus display only few collaterals within the CA1 region. B: In a pilocarpine-treated rat, the axon of the CA1 pyramidal neurons originates from a proximal basal dendrite, ascends to the alveus, but gives rise to an aberrant dense axonal plexus in stratum oriens (O), with many branches entering the pyramidal layer (P) and stratum radiatum (R). C: Photograph of an axonal collateral running throughout the pyramidal cell layer of the CA1 region outlined in B. This axonal collateral displays one or two varicosities (arrowheads) making putative contacts on an unlabeled proximal dendrite and somata of pyramidal cells (*); somata are outlined by dashed lines. D: Axogram of the pyramidal neuron showed in A. E: Axogram of the pyramidal neuron shown in B. Note the numerous branching points and axonal segments of the axon from the pyramidal cell in the pilocarpine-treated rat. Scale bars = 100 µm in A,B, 10 µm in C.



Fig. 3. Photomicrographs of biocytin-labeled CA1 pyramidal cells detected in 250-μm-thick slices from a kainic-acid-treated rat with spontaneous seizures. **A:** The main axonal branch of a CA1 pyramidal neuron gives rise to many thin collaterals (arrowheads), with numerous varicosities that correspond to putative en passant boutons. **B:** High magnification of A, showing two varicosities (arrowheads) of the thin collateral making putative synaptic contacts with a basal

dendrite of the same pyramid cell. **C:** An axonal collateral (arrowheads) of the biocytin-labeled pyramid shown in A surrounds a basal dendrite of a neighboring labeled CA1 pyramid. At least four varicosities are in close apposition with this dendrite. **D:** An axonal collateral of the CA1 pyramidal neurons shown in A, running through stratum pyramidale (P) and stratum radiatum (R), displays many varicosities (arrowheads). Scale bars = 10 μ m in B,C, 50 μ m in A,D.

the CA3 pyramidal layer, whereas dentate granule cells and pyramidal neurons of CA1 were well preserved (Fig. 1D). A similar pattern of cell loss has been reported previously in this model (Nadler et al., 1980c; Lancaster and Wheal, 1982).

Morphological alterations of CA1 pyramidal cells

In slices from control animals, the main axonal branches of CA1 pyramidal cells (n = 67) displayed rare, thin, and short axonal collaterals in the stratum oriens (Fig. 2A) that did not enter the stratum radiatum (Tamamaki and Nojyo, 1990; Perez et al., 1996). In contrast, in one-fourth of the CA1 pyramidal cells (42 of 168) from animals with spontaneous seizures, the axon gave rise very quickly to a large number of thin collaterals that spread throughout the basal dendrites field (Figs. 2B, 3A). In keeping with the observations of Perez et al. (1996), the main axonal branches running in stratum oriens and the alveus displayed numerous collaterals in stratum oriens. In addition, we now report that collaterals often invaded strata pyramidale and radiatum in the CA1 region (Figs. 2A,C, 3D). Quantitative analysis of the numbers of axonal branch order and total length of the axonal arborization measured in all strata including the alveus further demonstrated the increase in the complexity of the axon in epileptic versus control animals. The mean number of segment order per axon was increased from 3.5 ± 2.5 in control (n = 4; Fig. 2D) to 14.0 ± 5.3 in chronic (n = 10; P < 0.001; Fig. 2E) animals. The mean total axon length was significantly longer in epileptic animals (7,511 \pm 850 μm vs. 3,732 \pm 963 μ m in control animals; P < 0.001). CA1 pyramidal neurons from pilocarpine and KA-treated animals exhibited small varicosities along all axonal branches including collaterals entering in stratum radiatum (Fig. 3D). These varicosities seemed to establish synaptic contacts with somata and dendritic processes of pyramidal neurons (Figs. 2C, 3A-C), including putative autapses (Fig. 3C). The mean number of varicosities per 100 µm of axon for each segment order was not significantly different between control and epileptic animals (P > 0.05): 4.02 \pm 1.5 and 4.31 \pm 1.33, respectively, for first-order segments; 10.04 \pm 2.98 and 9.15 \pm 2.56 for second-order segments; 14.78 \pm 3.42 and 13.51 \pm 5.36 for third-order segments; 12.34 \pm 2.6 and 10.04 \pm 3.57 for fourth-order segments. The mean density of varicosities for the following order segments in pilocarpine- and KA-treated rats was 10.62 \pm 5.44. Despite similar linear densities of varicosities in epileptic and control animals, the total number of these putative en passant boutons was higher in chronic animals because of the increased number of axonal branches.

We then determined whether these pathological alterations could represent a morphological substrate for electrophysiological modifications in the apical dendrites and somata of CA1 pyramidal cells. In particular, we examined whether the reorganized CA1 associational pathway (1) could trigger epileptiform activity when activated and (2) results in an enhanced glutamatergic excitatory drive.

Epileptiform activity in the CA1 area

Patch clamp recordings from somata and apical dendrites of CA1 pyramidal cells were performed (control, n = 67; pilocarpine, n = 69; KAipsi, n = 45; KAcontra, n = 54). A dent on the biocytin-filled apical dendrite (Fig. 4A) indicated that the recording site was 375 \pm 55 μm from the soma (KA, n = 21; pilocarpine, n = 24; control, n = 13; same average distance for each group). Bulk electrical stimulations applied to stratum radiatum evoked graded epileptiform discharges in the apical dendrites (Fig. 4B-C) and the somata (Fig. 4E-F) of 98% of the pyramidal neurons (n = 164) in chronic animals. Spontaneous paroxysmal discharges (Fig. 4D) also occurred in somata (n = 67) and apical dendrites (n = 20). In contrast, suprathreshold stimulations applied to control slices evoked only a single action potential in somata, as described by Esclapez et al. (1997a,b), and apical dendrites (not shown). Spontaneous and evoked discharges were blocked by the glutamatergic antagonists CNQX (10 μ M) and D-APV (50 μ M; Fig. 4E). Therefore, in slices from chronic animals, epileptiform discharges are present in somata and apical dendrites of CA1 pyramidal cells.

Selective activation of the CA1 associational pathway by focal electrical stimulation applied to stratum oriens (n = 2), the alveus close to the subiculum (n = 2), or the subiculum itself (n = 8) evoked glutamatergic graded bursts of action potentials in both somata and apical dendrites of CA1 pyramidal cells in chronic animals (Fig. 4F). These bursts were blocked by CNQX (10 μ M) and D-APV (50 μ M; not shown). In contrast, stimulations applied at similar locations in control slices evoked an excitatory/inhibitory sequence or an antidromic action potential (Fig. 4G; n = 5). Therefore, activation of the reorganized CA1 associational pathway triggers epileptiform discharges in chronic animals.

To estimate the frequency of connections between cells, we made paired pyramidal cell recordings in the presence of bicuculline (10 µM) in the surgically isolated CA1 area in slices of control (n = 10 pairs) and chronic (n = 18 pairs) animals. None of these 28 pairs was monosynaptically connected. In the CA3 area, which possesses abundant recurrent collateral fibers, when GABAA receptor-mediated inhibition is blocked, a high-frequency train of action potentials generated in a single pyramidal neuron by depolarizing steps triggers epileptiform activity in another simultaneously recorded pyramidal cell and in the entire CA3 network (Miles and Wong, 1983). However, a similar paradigm applied to the CA1 area did not trigger polysynaptic epileptiform discharges in the other cells in control (n = 10, not shown) or chronic (n = 18, not shown) animals. Therefore, although the reorganized CA1 associational pathway in epileptic animals shares morphological features with that of CA3, the amount of functional interconnectivity between CA1 pyramidal cells in chronic animals remains below that of control CA3 neurons.

Increased spontaneous glutamatergic activity in somata and apical dendrites in chronic animals

If the sprouting process gives rise to new functional glutamatergic synapses, the excitatory drive provided by spontaneous postsynaptic excitatory currents (sEPSCs) should be increased because many slices from chronic animals displayed spontaneous epileptiform discharges. In control pyramidal cells, somatic and dendritic sEPSCs were composed of small amplitude currents (range = 50 pA; Table 2, Fig. 5A), with means \pm S.E.M. of 16 \pm 9 pA and 15 ± 8 pA, respectively. The frequency of sEPSCs was statistically lower in the soma than in the apical dendrites (Table 2; P < 0.001). The frequency of sEPSCs was similar in pilocarpine- and KA-treated rats (3.34 \pm 0.6, n = 13; 3.51 ± 0.73 , n = 18; respectively). However, in these chronic animals, the frequency of sEPSCs was dramatically increased in both somata (520% of control; P < 0.001; Table 2, Fig. 5) and apical dendrites (306% of control; P <0.001; Table 2, Fig. 5) as compared with control animals. The difference between dendrites and somata in chronic animals was also statistically significant (P < 0.001). In chronic animals, the amplitude distribution of sEPSCs was similar to that of controls (mean \pm S.E.M.: 15 \pm 10 pA for somata, 16 ± 9 pA for dendrites; Fig. 6A), with the exception of a small population of very large sEPSCs that was not observed in control animals (Fig. 6A-B, Table 2). These large amplitude sEPSCs had the same time course as the smaller ones, suggesting their monosynaptic origin (Fig. 6B). In chronic and control rats, the Na⁺ channel blocker TTX (1 µM) abolished most of the sEPSCs in the somata and apical dendrites (>80%; Table 2, Fig. 5), suggesting that sEPSC activity is mainly action potential dependent. The increased sEPSC activity is not due to a tonic reduction of the inhibitory drive because, when bicuculline (10 μ M) was applied to the surgically isolated CA1 area of slices from control and chronic animals, the frequency of sEPSCs in pyramidal cells was not increased (Table 2). Therefore, in epileptic animals the axonal sprouting of CA1 pyramidal neurons could subserve a substrate for the dramatically increased frequency of action-potentialdependent sEPSCs and the occurrence of large amplitude sEPSCs in both somata and dendrites.



NEW FUNCTIONAL GLUTAMATERGIC NETWORK IN EPILEPSY

of CA1 Pyramidal Cells From Control and Epileptic Animals*						
Amplitudes (%)	Recording site (n)	0–50 pA	51–60 pA	61–90 pA		
Control	Soma (16)	99.5	0.5	0.0		
Epileptic	Soma (31)	98.5	0.5	1.5		
Control	Dendrite (13)	99.4	0.6	0.0		
Epileptic	Dendrite (9)	98.0	1.5	0.0		
Frequencies (Hz)	Soma (n)	Soma $+$ TTX (n)	Dendrite (n)	Dendrite + TTX (n)		
Control	0.66 ± 0.26 (16)	0.13 ± 0.04 (5)	1.79 ± 0.37 (13)	0.30 ± 0.03 (11)		
Epileptic	3.43 ± 0.52 (31)	0.17 ± 0.03 (8)	5.48 ± 1.71 (9)	0.66 ± 0.12 (8)		
Frequencies (Hz)	Soma in cut CA1 (n)	Bicuculline (n)				
Control	0.77 ± 0.17 (10)	0.57 ± 0.16 (10)				
Epileptic	5.49 ± 1.29 (10)	3.48 ± 0.75 (10)				

TABLE 2. Distribution of Spontaneous Postsynaptic Excitatory Current (sEPSC) Peak Amplitudes and Frequencies in Somata and Dendrites of CA1 Pyramidal Cells From Control and Epileptic Animals*

*Dramatic change in the frequency but not the amplitude of sEPSCs in the somata and dendrites of CA1 pyramidal neurons in experimental animals. The sEPSCs were measured at the reversal potential for GABAergic events (around -60 mV) according to a well-established procedure (see Materials and Methods for details) and divided arbitrarily into three amplitude groups. For each cell, the frequency of sEPSCs was estimated in 60-second epochs. The effects of tetrodotoxin (TTX) in controls and temporal lobe epilepsy (TLE) neurons were similar: a decrease of the frequency of sEPSCs. in the sufficient sEPSCs are generated by action potentials. Adding bicuculline, to reduce tonic inhibition, did not modify the frequency of sEPSCs in the somata of pyramidal neurons recorded in the surgically isolated CA1 area of control and epileptic rats.

DISCUSSION

Our results suggest that, in two animal models of TLE, (1) glutamatergic axons sprout and establish novel connections on the somata and dendrites of pyramidal neurons and (2) these new connections (putative synapses) are functional and could participate in the propagation of paroxysmal activities.

Morphological reorganization of the CA1 area

Intracerebroventricular administration of KA leads to massive death of CA3 pyramidal cells in the hippocampus ipsilateral to the lesion (Nadler et al., 1980a-c), which is followed by a rapid decrease of the number of asymmetric synapses in stratum radiatum of the CA1 area (Nadler et al., 1980b; Phelps et al., 1991). The number of asymmetric synapses then slowly recovers, suggesting that a reactive synaptogenesis has taken place (Nadler et al., 1980b; Phelps et al., 1991). These newly formed synapses may originate from surviving CA3 (mainly commissural fibers), CA1 (associational pathway) pyramidal cells, entorhinal cortex (perforant path), or the septum but not from mossy fibers, which sprout in the dentate gyrus and the CA3 area but do not invade the CA1 area (Nadler et al., 1980a,b; Represa and Ben-Ari, 1992). The favored hypothesis is that the CA1 associational pathway sprouts. In experimental models of epilepsy, morphologically identified reactive synaptogenesis often involves local recurrent pathways in

the dentate gyrus (Nadler et al., 1980a,b), the CA3 area (McKinney et al., 1997), or the neocortex (Salin et al., 1995). In the CA1 area, two indirect electrophysiological data support this hypothesis. (1) In chronic but not in control animals, N-methyl-D-aspartic acid (NMDA) receptors directly participate in normal synaptic transmission (Turner and Wheal, 1991; Hirsch et al., 1996). In control rats, this NMDA component does not exist in the Schaffer/ commissural pathway (Andreasen et al., 1989) but is present in the CA1 associational pathway (Thomson and Radpour, 1991; Deuchars and Thomson, 1996). Therefore, it has been hypothetized that the NMDA receptor component seen in epileptic animals originates from the sprouted CA1 associational pathway (Turner and Wheal, 1991; Perez et al., 1996). (2) Epileptiform discharges are more easily triggered in the isolated CA1 area of KA-treated rats than of control animals (Meier and Dudek, 1996). More direct evidence has been presented in the report that the CA1 associational pathway sprouts in KA-treated rats (Perez et al., 1996). However, new axonal collaterals were described in stratum oriens only, leaving unresolved the origin of the newly formed synapses along the apical dendrites. In the present study, we alleviated this contradiction in part because we report a massive sprouting, not only in stratum oriens but also in strata pyramidale and radiatum. The quantitative data reflect the profuse axonal sprouting because the number of branching order was considerably increased in chronic animals (14 vs. 3.5 in control) and the total axonal length increased from 3,700 μm to 7,500 μm . These values, including the control ones, are higher than those reported previously (Perez et al., 1996). In the study by Perez et al., the number of branches increased from 1.5 to 3 and the axonal length from 1,500 µm to 2,000 µm in control and chronic animals, respectively. The discrepancies with the study by Perez et al. (1996) may stem from the fact that our animals were killed after they had developed spontaneous recurrent seizures.

There was no clear-cut relationship between the hippocampal damage and the extent of the sprouting of the CA1 associational pathway. Whereas in KA-treated rats the CA3 pyramidal neurons were totally destroyed in the hippocampus ipsilateral to the KA injection, the amount of axonal sprouting was comparable to that observed in the contralateral side and in the pilocarpine model, in which few CA3 pyramidal cells disappeared. Therefore, the sprouting of the CA1 associational pathway alone does not seem to account for the recovery of 72% of asymmetric synapses reported in the KA model (Nadler et al., 1980b),

Fig. 4. Graded bursts in apical dendrites from pyramidal neurons in the CA1 region of epileptic rats. A: Identified dendritic recording site in a pyramidal neuron filled with biocytin (detected by fluorescence). Inset at higher magnification shows the trace left by the patch electrode on the apical dendrite (pilocarpine-treated rat, 2.5 months after injection). B,C: Graded bursts recorded in the dendrites of kainic acid (KA)-treated rats in the cell-attached (B, 12 months postinjection) and the whole cell (C, three months postinjection) configurations. D: Spontaneous bursts of action potentials recorded simultaneously in the apical dendrite and the soma of two pyramidal neurons. The last burst is shown expanded on the right (12 months after KA injection). E-G: Somatic recordings. E: The evoked epileptiform burst is suppressed by glutamate antagonists (KA-treated rat, three months). F: This neuron responded with a burst to different loci of stimulation (KA-treated rat, three months). G: Control pyramidal neuron that responded with an antidromic action potential to stimulation of the subiculum. All recordings were obtained in the current clamp mode at resting membrane potential. D-APV, D-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione. Scale bars = 100 μ m in A, 1 μ m in inset.



 10_{10} Somata 8 Frequency (%) 6 4 2 TLE Control 0 0 20 40 60 80 Peak amplitude (pA) 10_{-} Apical dendrites Frequency (%) 8 6 4 2 TLE Control 0 0 40 80 20 60 Peak amplitude (pA) B 20pA ∟ 100 ms 2 10 ms

Α

Fig. 5. The frequency of glutamatergic spontaneous postsynaptic excitatory currents recorded from the somata and apical dendrites of pyramidal neurons is considerably increased in slices from experimental animals with temporal lobe epilepsy (Exp TLE) as opposed to that of naive rats. Somatic recording from a kainic-acid-treated rat (five months). Dendritic recording from the neuron illustrated in Figure 4A. In both soma and the apical dendrite of the pyramidal neuron from the epileptic animals, the frequency of spontaneous currents is higher than that in the control animals. For all the recordings, the membrane was voltage clamped at the reversal potential for GABAergic currents (around -60 mV). Tetrodotoxin (TTX) blocked all spontaneous currents from control and epileptic animals. ACSF, modified artificial cerebrospinal fluid.

suggesting that other glutamatergic fibers yet to be characterized also contribute to the reactive synaptogenesis.

Functionality of the new excitatory connections in CA1

In keeping with previous studies (Tamamaki and Nojyo, 1990; Deuchars and Thomson, 1996; Perez et al., 1996), we

Fig. 6. Distribution of spontaneous postsynaptic excitatory current (sEPSC) amplitude in somata and dendrites of pyramidal neurons from control and experimental animals. A: Superimposed frequency histograms of peak amplitudes (bin 1 pA) of sEPSCs recorded in the somata of neurons from control (dotted line) and epileptic (solid line) animals. There was no significant difference between neurons from control and epileptic animals for either the somata or the dendrites. However, for pyramidal neurons in experimental rats, the longer tail of the histogram leaned toward large amplitude sEPSCs in the somata and to a lesser degree in the dendrites. B: Somatic recording of sEPSCs. Averages of 15 large (*) and small (x) EPSCs are shown together scaled to the same amplitude (1 + 2), demonstrating identical kinetics (kainic acid, six months after injection). TLE, temporal lobe epilepsy.

report that in control animals the CA1 associational pathway is restricted to stratum oriens. In chronic animals, because it is not yet possible to distinguish between

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old and new branches in stratum oriens, newly formed synapses cannot be identified in this region. In contrast, the axonal collaterals that enter strata pyramidale and radiatum in chronic animals are newly formed. All CA1 pyramidal cell axonal recurrent collaterals were covered with numerous varicosities in chronic animals, including the branches crossing the pyramidal cell layer and entering stratum radiatum. Because varicosities are generally associated with synaptic contacts (Sik et al., 1993; Deuchars and Thomson, 1996; Vida et al., 1998), it is likely that the sprouted CA1 associational pathway forms new synapses along the apical dendrites of CA1 pyramidal cells. Although paired recordings associated with electron microscopy studies are required, the following observations obtained in hippocampal slices isolated from surrounding cortical inputs suggest that the increased glutamatergic activity observed in epileptic animals is generated within the CA1 region by the newly formed glutamatergic axon collaterals: (1) The glutamatergic activity is mainly action potential dependent because TTX abolished most sEPSCs in the somata and dendrites. (2) The neurons generating this activity, i.e., the pyramidal neurons (provided that there are no excitatory interneurons) are located within the CA1 area because this glutamatergic activity remained the same when the CA1 area was surgically isolated from the CA3 area in epileptic animals. (3) As a consequence, the increased frequency of sEPSCs in the apical dendrites of CA1 pyramidal neurons from chronic animals originates from the newly formed recurrent collaterals of pyramidal cells that now enter stratum radiatum because in control tissue CA1 recurrent collaterals do not contact the apical dendrites. (4) Due to cable properties, the sEPSCs recorded in somata and apical dendrites are probably generated in the vicinity of the recording site (Hirsch et al., 1998).

Increased interconnectivity and hyperexcitability

Extensive physiological data suggest that brain areas in which principal cells are interconnected by a dense network of excitatory recurrent colaterals are particularly prone to seizure. In lesioned animals, the sprouting of associational pathways should increase the excitatory connections between principal cells (McKinney et al., 1997). As a consequence of this hypothesis, the excitatory drive should be increased in principal cells and its propagation facilitated. Our results clearly showed an increase in the baseline excitatory synaptic drive in CA1 pyramidal cells (frequency and amplitude of sEPSCs) in both KA- and pilocarpine-treated animals. Similar findings have been reported in granule cells of the dentate gyrus of KAtreated animals (Wuarin and Dudek, 1998). Furthermore, we report that, in epileptic slices recorded in physiological conditions, stimulation of the associational pathway triggers epileptiform activity in the CA1 region, demonstrating that the reorganized and more extensive associational pathway can participate in the generation and spread of paroxysmal discharges. These results directly support the hypothesis that the connectivity between principal cells is functionally increased in reorganized networks (Salin et al., 1995; Meier and Dudek, 1996; McKinney et al., 1997; Wuarin and Dudek, 1998). The extent of this increased connectivity remains to be quantified. For example, the probability of finding connected pyramidal pairs should be higher in chronic animals because our morphological data demonstrated in pyramidal cells a large increase in the number of axonal recurrent collaterals. However, in keeping with a previous study (Nakajima et al., 1991), such an increase was not shown by paired recordings of CA1 pyramidal cells. The reason may be statistical because the interconnectivity between CA1 pyramidal cells in the control animals was very low, less than 1% (Deuchars and Thomson, 1996), suggesting that recording from hundreds of pairs will be required to estimate the number of newly formed synaptic contacts.

Epilepsy and the "too-well-connected brain"

The relationship between sprouting and hyperexcitability does not seem straightforward in epilepsy. Sprouted mossy fibers in the dentate gyrus in experimental models (but see Longo and Mello, 1997) can provide a morphological substrate for hyperexcitability when inhibition is removed (Patrylo and Dudek, 1998; Wuarin and Dudek, 1998). However, in chronic animals recorded in physiological conditions, inhibition in the dentate gyrus is enhanced by (1) a long-term potentiation at GABAergic synapses on granule cells (Nusser et al., 1998) and (2) an increased excitatory drive on inhibitory interneurons (Buhl at al., 1996; Buckmaster and Dudek, 1997). In contrast to the dentate gyrus, the CA1 area is hyperexcitable in TLE and in its experimental models, and inhibition does not seem to be functionally affected in this region (Esclapez et al., 1997a,b), thus supporting the hypothesis that sprouting of the associational pathway plays an active role. Although it is not yet possible to determine whether sprouting is epileptogenic per se, the remodeled CA1 area will most likely facilitate the propagation of epileptiform activities to the subiculum and from there to the neocortex. The critical issue for temporal lobe epilepsy may not be where the epileptogenic area is located but how epileptic discharges propagate from the hippocampus to the neocortex. The CA1 and CA3 areas, because they can easily propagate epileptiform activity in experimental models (Meier and Dudek, 1996; Smith et al., 1998), are ideally located for that purpose. It remains to be determined whether the sprouting of the CA1 and CA3 associational pathways also occurs in human TLE and results in an increased excitatory drive and hyperexcitability through the formation of new functional glutamatergic connections as our experimental data suggest. Our results also provide the experimental basis for an alternative to the classic boost of inhibition in the drug treatment of TLE. Glutamatergic synapses seem to be a promising target.

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LITERATURE CITED

- Andreasen M, Lambert J, Jensen M. 1989. Effects of the new non-N-methyl-D-aspartate antagonists on synaptic transmission in the in vitro rat hippocampus. J Physiol (Lond) 414:317–336.
- Bernard C, Wheal HV. 1996. A role for synaptic and network plasticity in controlling epileptiform activity in CA1 in the kainic acid-lesioned rat hippocampus in vitro. J Physiol (Lond) 495:127–142.
- Buckmaster PS, Dudek FE. 1997. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainatetreated rats. J Comp Neurol 385:385–404.

- Buhl EH, Otis TS, Mody I. 1996. Zinc-induced collapse of augmented inhibition by GABA in temporal lobe epilepsy model. Science 271:369– 373.
- Chagnac-Amitai Y, Connors BW. 1989. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J Neurophysiol 61:747–758.
- Cronin J, Obenaus A, Houser CR, Dudek FE. 1992. Electrophysiology of dentate granule cells after kainate-induced synaptic reorganization of the mossy fibers. Brain Res 573:305–310.
- DeLanerolle N, Kim J, Robbins R, Spencer D. 1989. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 495:387–395.
- Deuchars J, Thomson AM. 1996. CA1 pyramid–pyramid connections in rat hippocampus in vitro: dual intracellular recordings with biocytin filling. Neuroscience 74:1009–1018.
- Esclapez M, Hirsch JC, Agassandian C, Ben-Ari Y, Bernard C. 1997a. Enhanced glutamatergic excitatory drive in the dendrites and somata of CA1 pyramidal neurons in epilepsy. Soc Neurosci Abstr 2158:838.
- Esclapez M, Hirsch JC, Khazipov R, Ben-Ari Y, Bernard C. 1997. Operative GABAergic inhibition in hippocampal CA1 pyramidal neurons in experimental epilepsy. Proc Natl Acad Sci USA 94:1251–1256.
- Hirsch JC, Quesada O, Esclapez M, Gozlan H, Ben-Ari Y, Bernard C. 1996. Enhanced NMDAR-dependent epileptiform activity is controlled by oxidizing agents in a chronic model of temporal lobe epilepsy. J Neurophysiol 76:4185–4189.
- Hirsch JC, Esclapez M, Cannon RC, Wheal HV, Ben-Ari Y, Bernard C. 1998. Heterogeneous distribution of spontaneous and miniature GABAergic and glutamatergic synaptic currents along the somato-dendritic axis of neurones. Soc Neurosci Abstr 919:362.
- Jefferys JGR, Whittington MA. 1996. Review of the role of inhibitory neurons in chronic epileptic foci induced by intracerebral tetanus toxin. Epilepsy Res 26:59–66.
- Lancaster B, Wheal HV. 1982. A comparative histological and electrophysiological study of some neurotoxins in the rat hippocampus. J Comp Neurol 211:105–114.
- Liu Z, Nagao T, Desjardins GC, Gloor P, Avoli M. 1994. Quantitative evaluation of neuronal loss in the dorsal hippocampus in rats with long-term pilocarpine seizures. Epilepsy Res 17:237–247.
- Longo BM, Mello LEAM. 1997. Blockade of pilocarpine- or kainate-induced mossy fiber sprouting by cycloheximide does not prevent subsequent epileptogenesis in rats. Neurosci Lett 226:163–166.
- McKinney RA, Debanne D, Gahwiler BH, Thompson SM. 1997. Lesioninduced axonal spouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of post-traumatic epilepsy. Nature Med 3:990–996.
- Meier CL, Dudek FE. 1996. Spontaneous and stimulation-induced synchronized burst afterdischarges in the isolated CA1 of kainate-treated rats. J Neurophysiol 76:2231–2239.
- Mello LEAM, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. 1993. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia 34:985–995.
- Miles R, Wong RKS. 1983. Single neurones can initiate synchronized population discharges in the hippocampus. Nature 306:371–373.
- Nadler JV, Perry BW, Gentry C, Cotman CW. 1980a. Loss and reacquisition of hippocampal synapses after selective destruction of CA3–CA4 afferents with kainic acid. Brain Res 191:387–403.
- Nadler JV, Perry BW, Cotman CW. 1980b. Selective reinnervation of hippocampal area CA1 and fascia dentata after destruction of CA3– CA4 afferents with kainic acid. Brain Res 182:1–9.
- Nadler JV, Perry BW, Gentry C, Cotman CW. 1980c. Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. J Comp Neurol 192:333–359.
- Nakajima S, Franck JE, Bilkey D, Schwartzkroin PA. 1991. Local circuit synaptic interactions between CA1 pyramidal cells and interneurons in the kainate-lesioned hyperexcitable hippocampus. Hippocampus 1: 67–78.
- Nusser Z, Hajos N, Somogyi P, Mody I. 1998. Increased number of synaptic GABA (A) receptors underlies potentiation at hippocampal inhibitory synapses. Nature 395:172–177.

- Obenaus A, Esclapez M, Houser CR. 1993. Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. J Neurosci 13:4470–4485.
- Patrylo PR, Dudek FE. 1998. Physiological unmasking of new glutamatergic pathways in the dentate gyrus of hippocampal slices from kainateinduced epileptic rats. J Neurophysiol 79:418–429.
- Perez Y, Morin F, Jutras I, Beaulieu C, Lacaille J-C. 1996. Axonal sprouting of CA1 pyramidal cells in hyperexcitable hippocampal slices of kainate-treated rats. Eur J Neurosci 8:736–748.
- Phelps S, Mitchell J, Wheal HV. 1991. changes to synaptic ultrastruture in field CA1 of the rat hippocampus following intracerebroventricular injection of kainic acid. Neuroscience 40:687–699.
- Prince DA. 1997. Epilepsy and the too-well-connected brain. Nature Med 3:957–958.
- Prince DA, Jacobs KM, Salin PA, Hoffman S, Parada I. 1997. Chronic focal neocortical epileptogenesis: does disinhibition play a role? Can J Physiol Pharmacol 75:500–507.
- Racine RJ. 1972. Modification of seizure activity by electrical simulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281–294.
- Rempe DA, Bertram EH, Williamson JM, Lothman EW. 1997. Interneurons in area CA1 stratum radiatum and stratum oriens remain functionally connected to excitatory synaptic input in chronically epileptic animals. J Neurophysiol 78:1504–1515.
- Represa A, Ben-Ari Y. 1992. Epilepsy is associated with the formation of novel mossy fiber synapses in the CA3 region. Exp Brain Res 92:69–78.
- Repressa A, Tremblay E, Ben-Ari Y. 1987. Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. Neuroscience 20:739–748.
- Salin P, Tseng GF, Hoffman S, Parada I, Prince DA. 1995. Axonal sprouting in layer V pyramidal neurons of chronically injured cerebral cortex. J Neurosci 15:8234–8245.
- Sik A, Tamamaki N, Freund TF. 1993. Complete axon arborization of a single CA3 pyramidal cell in the rat hippocampus, and its relationship with postsynaptic parvalbumin-containing interneurons. Eur J Neurosci 5:1719–1728.
- Smith KL, Lee CL, Swann JW. 1998. Local circuit abnormalities in chronically epileptic rats after intrahippocampal tetanus toxin injection in infancy. J Neurophysiol 79:106–116.
- Tamamaki N, Nojyo Y. 1990. Disposition of slab-like modules formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. J Comp Neurol 291:509–519.
- Tauck DL, Nadler JV. 1985. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid treated rats. J Neurosci 5:1016–1022.
- Thomson A, Radpour S. 1991. Excitatory connections between CA1 pyramidal cells revealed by spike triggered averaging in slices of rat hippocampus are partially NMDA receptor mediated. Eur J Neurosci 3:587–601.
- Traub RD, Wong RK. 1982. Cellular mechanism of neuronal synchronization in epilepsy. Science 216:745–747.
- Turner DA, Wheal HV. 1991. Excitatory synaptic potentials in kainic acid-denervated rat CA1 pyramidal neurons. J Neurosci 11:2786–2794.
- Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. 1983. Limbic seizures produced by pilocarpine in rats: a behavioural, electroencephalographic and neuropathological study. Behav Brain Res 9:315–336.
- Turski L, Cavalheiro EA, Sieklucka-Dziuba M, Ikonomidou-Turski C, Czuczwar SJ, Turski WA. 1986. Seizures produced by pilocarpine: neuropathological sequelae and activity of glutamate decarboxylase in the rat forebrain. Brain Res 398:37–48.
- Vida I, Halasy K, Szinyei C, Somogyi P, Buhl EH. 1998. Unitary IPSPs evoked by interneurons at stratum radiatum-stratum lacunosummoleculare border in the CA1 area of the rat hippocampus in vitro. J Physiol (Lond) 506:755–773.
- Wuarin JP, Dudek FE. 1998. Evidence from in vitro whole-cell patch-clamp recordings that baseline excitatory synaptic input is abnormally high in granule cells with mossy fiber sprouting from kainate-induced epileptic rats. Soc Neurosci Abstr 851:2141.