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Survival of Dentate Hilar Mossy Cells after Pilocarpine-Induced Seizures and their Synchronized Burst Discharges with Area CA3 Pyramidal Cells

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Abstract

The clinical and basic literature suggest that hilar cells of the dentate gyrus are damaged after seizures, particularly prolonged and repetitive seizures. Of the cell types within the hilus, it appears that the mossy cell is one of the most vulnerable. Nevertheless, hilar neurons which resemble mossy cells appear in some published reports of animal models of epilepsy, and in some cases of human temporal lobe epilepsy. Therefore, mossy cells may not always be killed after severe, repeated seizures. However, mossy cell survival in these studies was not completely clear because the methods did allow discrimination between mossy cells and other hilar cell types. Furthermore, whether surviving mossy cells might have altered physiology after seizures was not examined. Therefore, intracellular recording and intracellular dye injection were used to characterize hilar cells in hippocampal slices from pilocarpine-treated rats that had status epilepticus and recurrent seizures (‘epileptic’ rats). For comparison, mossy cells were also recorded from age-matched, saline-injected controls, and pilocarpine-treated rats that failed to develop status epilepticus.

Numerous hilar cells with the morphology, axon projection, and membrane properties of mossy cells were recorded in all three experimental groups. Thus, mossy cells can survive severe seizures, and those that survive retain many of their normal characteristics. However, mossy cells from epileptic tissue were distinct from mossy cells of control rats in that they generated spontaneous and evoked epileptiform burst discharges. Area CA3 pyramidal cells also exhibited spontaneous and evoked bursts. Simultaneous intracellular recordings from mossy cells and pyramidal cells demonstrated that their burst discharges were synchronized, with pyramidal cell discharges typically beginning first.

From these data we suggest that hilar mossy cells can survive status epilepticus and chronic seizures. The fact that mossy cells have epileptiform bursts, and that they are synchronized with area CA3, suggest a previously unappreciated substrate for hyperexcitability in this animal model.

Keywords

dentate gyrus; epilepsy; excitotoxicity; hyperexcitability; synchronization; status epilepticus

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1. UNCITED REFERENCES: Slomianka et al., 1997

The hilus of the rat dentate gyrus contains a diverse group of excitatory and inhibitory neurons (Amaral, 1978; Freund and Buzsaki, 1996). On the basis of many *in vivo* and *in vitro* studies of the rat dentate gyrus, two major classes of hilar neurons have been demonstrated. There are glutamatergic neurons called 'mossy' cells, and a heterogeneous group of GABAergic inhibitory neurons, which are often referred to as 'interneurons'.

Mossy cells can be distinguished anatomically from interneurons by their large, complex spines on proximal dendrites called 'thorny excrescences' (Amaral, 1978; Frotscher et al., 1991; Fujise et al., 1998; Ribak et al., 1985; Seress and Ribak, 1995). In addition, mossy cells are excitatory (Scharfman, 1995b; Soriano and Frotscher, 1994) and can be identified by immunoreactivity for calcitonin gene-related peptide (Bulloch et al., 1996; Freund et al., 1997) and the glutamate receptor subunit GluR2/3 (Leranth et al., 1996). They have a unique axonal arbor that includes a distant projection to the ipsilateral and contralateral inner molecular layer, as well as local collaterals in the hilus, and to a lesser extent, local collaterals to the inner molecular layer (Buckmaster et al., 1992, 1996; Laurberg and Sorensen, 1981; Ribak et al., 1985; Swanson et al., 1978; Zimmer, 1971). Many of the other characteristics of mossy cells have been reviewed elsewhere (Frotscher et al., 1991; Scharfman, 1999).

Studies of human disease and animal models of disease have shown that in several pathological states there is hilar cell loss, and mossy cells are one of the vulnerable cell types. Regarding hilar cell loss in general, studies of temporal lobe epilepsy (TLE) show that there can be dramatic and selective hilar cell loss, a condition termed 'endfolium sclerosis' (Babb and Brown, 1987; Margerison and Corsellis, 1966; Mathern et al., 1997; Meldrum and Bruton, 1992). In such individuals, there may be little indication of other pathology. In other cases of TLE with multiple areas of cell loss, the hilus is consistently one of the damaged sites (deLanerolle et al., 1989; Margerison and Corsellis, 1966). A similar correlation between hilar cell damage and hippocampal hyperexcitability has been reported in animal studies *in vivo* (Sloviter, 1991) and *in vitro* (Scharfman and Schwartzkroin, 1989, 1990a,b), and mossy cells specifically were identified as a vulnerable cell type. Indeed, the 'dormant basket cell' hypothesis holds that mossy cell loss is a critical factor in the development of hyperexcitability (Sloviter, 1991). This hypothesis proposes that inhibitory 'basket' cells lose a major source of excitatory input when mossy cells are damaged, leading to disinhibition of basket cell targets, i.e. granule cells. Mossy cells also appear vulnerable in animal models of injury (Lowenstein et al., 1992; Toth et al., 1997), after ischemia (Crain et al., 1988; Hong et al., 1993; Hsu and Buzsaki, 1993), and possibly aging (Shetty and Turner, 1999).

These studies contribute to the general assumption that mossy cells are damaged or killed under conditions that other hippocampal neurons survive. However, after seizures produced by the muscarinic agonist pilocarpine, we and others have noticed that a large number of hilar neurons appear to survive, as shown by Nissl or other stains (Liu et al., 1994; Motte et al., 1998; Obenaus et al., 1993; Scharfman et al., 2000). One reason to suspect that some of these hilar cells are mossy cells comes from recent studies of TLE specimens, in which large hilar neurons were demonstrated, although their immunoreactivity differed from rat mossy cells (Magloczky et al., 2000). Other studies of TLE tissue have shown that cells with anatomical characteristics of mossy cells survived, but not in specimens with classic Ammon's horn sclerosis (Blumcke et al., 1999). In support of the possibility that mossy cells can survive at least some degree of insult or injury, a recent study showed that mossy cells can survive experimental trauma (Santhakumar et al., 2000).

To determine if some hilar neurons that survive severe seizures might indeed be mossy cells, we recorded and labeled hilar neurons in slices of rats after pilocarpine-induced status epilepticus. We chose to study the dentate gyrus after animals had both status epilepticus as

well as recurrent spontaneous seizures to identify whether mossy cells could survive both an initial, intense period of seizures, as well as repetitive but intermittent seizures.

To distinguish mossy cells from the interneurons, even with intracellular recordings, is not trivial. Although some subtypes of dentate interneurons are distinct in many ways, others can resemble mossy cells. For example, there are some interneurons that have large cell bodies and spines, like mossy cells. A specific subtype of interneuron targets the inner molecular layer, like mossy cells (Han et al., 1993). Perhaps the most useful morphological characteristic to distinguish mossy cells is thorny excrescences. Physiologically it is also not trivial to differentiate interneurons and mossy cells. Thus, mossy cells have 'regular spiking' action potentials (APs) (McCormick et al., 1985) (i.e. broad duration, like cortical pyramidal cells), but some dentate interneurons also do (Freund and Buzsaki, 1996; Lübke et al., 1998; Scharfman, 1995a). Therefore, to unequivocally identify mossy cells, we used a combination of both morphological (thorny excrescences) and physiological characteristics (Scharfman, 1993; Scharfman and Schwartzkroin, 1988) to identify mossy cells.

Experimental Procedures

Animal care and use met the guidelines set by the National Institutes of Health and the New York State Department of Health. All efforts were made to minimize the number of animals used and their suffering. All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise noted.

Pilocarpine treatment

Adult male Sprague–Dawley rats (180–240 g) were obtained from Taconic (Germantown, NY, USA), injected with atropine methylbromide (1 mg/kg subcutane (s.c.)) and 30 min later with pilocarpine hydrochloride (380 mg/kg intraperitoneal (i.p.)) or an equivalent volume of 0.9% saline. Diazepam (5 mg/kg i.p., Wyeth-Ayerst, Philadelphia, PA, USA) was injected after 1 h of status, and saline controls were injected with the same dose of diazepam at approximately the same time. The onset of status was defined as the first stage 5 seizure (Racine, 1972) that did not abate after 2 min. After diazepam, some behavioral (motor) manifestations of seizures persisted, but rarely reached stage 5. After approximately 5 h, animals were injected with 2.5 ml 5% dextrose in lactate-Ringer's s.c. For approximately 7 days, the standard rat chow diet was supplemented with apples that were cut open and left at the bottom of the cage. Diet supplementation was employed because it appeared, in the first days after status epilepticus, that some of the rats did not eat or drink.

Rats were observed for spontaneous, recurrent, behavioral seizures (stage 5) at random times between 07:00 and 20:00 h. After observing at least three spontaneous seizures, animals were considered 'epileptic'.

Hippocampal slice preparation

Hippocampal slices (400 μm thick) were prepared from ether-anesthetized rats after decapitation. After one hemisphere of the brain was immersed in ice-cold buffer ('sucrose buffer', containing, in mM, 126 sucrose, 5 KCl, 2.0 $CaCl_2$, 2.0 $MgS0_4$, 26 $NaHC0_3$, 1.25 NaH_2PO_4, and 10 D-glucose), it was blocked to remove the rostral pole, and sliced in the horizontal plane using a Vibroslice (Stoelting Instruments, Wood Dale, IL, USA). Slices were immediately placed on a nylon net at an interface of sucrose buffer and warm (32–33°C), humidified (95% O_2, 5% CO_2) air. The slice chamber (Fine Science Tools, Foster City, CA, USA) was modified in two ways: (1) buffer approached the slices from their undersurface and was directed up and over them and then to a distant exterior port; (2) more air vents were made to allow more humidified air to the area where slices were located. All slices from a given

animal were placed in the recording chamber immediately after the dissection. Thirty min after slices were placed in the chamber, buffer was switched to one containing NaCl substituted equimolar for sucrose ('NaCl buffer'). Recordings began 30 min thereafter until approximately 7 h after the dissection. Flow rate was approximately 1 ml/min.

Recording and stimulation

Intracellular and extracellular recordings were made as previously described (Scharfman, 1994c, 1995a). Recordings were made with intracellular glass electrodes (0.59 or 0.75 mm inner diameter, 1.0 mm outer diameter) filled with 4% Neurobiotin (Vector Laboratories, Burlingame, CA, USA) in 1 M potassium acetate (60–140 MΩ). Intracellular data were collected using an intracellular amplifier with a bridge circuit (Axoclamp 2B, Axon Instruments, Foster City, CA, USA) and the bridge was balanced whenever current was passed. Extracellular electrodes were filled with NaCl buffer (5–10 MΩ). Data were collected using a digital oscilloscope (Nicolet Instruments, Madison, WI, USA) and analyzed with accompanying software. Data were also digitized and saved on tape (Neurocorder DR-484, Cygnus Technology, Delaware Water Gap, PA, USA) for analysis offline.

Cells that were impaled were first screened to ensure that they were healthy (stable resting potential more hyperpolarized than −50 mV, input resistance over 50 MΩ, and overshooting AP; Table 1). Their intrinsic (membrane) properties were then characterized using intracellularly injected current steps (0.05–1.5 nA, 150 ms). The outer molecular layer was stimulated by placing a monopolar, Teflon-coated stainless steel wire (75 μm outer diameter) on the border of the outer molecular layer and the fissure, just ventral to the subiculum, over the white striations comprising the perforant path as they enter the dentate gyrus (see diagram in Fig. 10). Stimuli were square pulses (10–200 μA, 10–20 μs), triggered at 0.02–0.05 Hz (Pulsemaster, World Precision Instruments, Sarasota, FL, USA) using a stimulus isolator (Isoflex, A.M.P.I. Products, Jerusalem, Israel).

Data analysis

Intrinsic properties—Analysis of intrinsic properties was made as previously described (Scharfman, 1994c, 1995a). Resting potential was defined as the difference between the potential while intracellular and that recorded after withdrawing the microelectrode from the cell. Input resistance was defined by the steepest slope of the *I–V* curve based on steady state responses to a family of current pulses (0.05–1.0 nA, 150 ms). Time constant was defined as the time to reach 63% of the steady state response to a minimal current step (0.1 nA), i.e. one that did not activate rectifying currents.

AP characteristics were based on a single AP at threshold, evoked by current injected intracellularly (a 0.1–0.5 nA, 150 ms pulse) at resting potential. AP 'total' amplitude was measured from resting potential to peak, and 'threshold' amplitude was from the membrane potential where the AP was triggered; it was also measured to the peak. Total AP duration was the time interval between the start of the rising phase of the AP until the point during the repolarization phase when the AP had repolarized to the membrane potential at which the rising phase began. Half-width was the width of the AP at half-amplitude (amplitude measured from the start of the rising phase to the peak). AP rising and decay slopes were defined by the maximum d*v*/d*t* using a resolution of 50 kHz. d*v*/d*t* ratio was defined as the ratio of slope/decay.

Afterhyperpolarization (AHP) amplitude was measured from the membrane potential where the AP started to the peak of the AHP. AHP half-duration was measured from the point when the AHP started to the point on its decay when it had decreased to half its peak amplitude.

'Sag' refers to rectification following a hyperpolarizing current pulse which makes the initial voltage response greater than the one at steady state. It was defined as a difference between peak and steady state that was more than 1 mV. Test pulses were up to −0.5 nA (150 ms) and were tested from resting potential as well as a hyperpolarized potential (between −70 and −80 mV), to ensure that it would be detected if it were present. Pulses up to −0.5 nA were used because when sag was present it was always detected using these current commands.

Synaptic responses—Threshold for responses to outer molecular layer stimulation was defined as the stimulus that produced APs in 50% of trials and excitatory postsynaptic potentials (EPSPs) in the other 50% of trials. Responses were included only if spontaneous activity, i.e. spontaneous postsynaptic potentials (PSPs), was absent when the stimulus was triggered. Amplitudes of responses were measured from baseline to peak. Latency to onset of the EPSP was measured from the stimulus artifact to the start of the EPSP. Latency to peak was measured from the artifact to the EPSP peak. Half-duration 'from the stimulus' was defined as the time from the stimulus artifact to the point on the EPSP's decay that was half the peak amplitude. Total duration 'from the stimulus' was measured from the stimulus to the point when the EPSP repolarized completely. Half-duration and total duration were also measured from the onset of the EPSP. The latter were calculated because in some cases there was a substantial latency to onset of EPSPs that could potentially confound the measurement of duration.

Statistics—Statistical comparisons were made using Student's *t*-tests or one-way analysis of variance (ANOVA) (PSI-plot version 4.5, Poly Software International, Salt Lake City, UT, USA) or Chi-square analysis. Statistical significance was set $P<0.05$.

Intracellular labeling and processing

Neurobiotin was injected from the recording electrode using repetitive depolarizing current pulses (+0.3–1.0 nA, 20 ms, 30 Hz, 5–15 min) after electrophysiological data were collected. Immediately after the experiment, slices were immersed in fixative (4% paraformaldehyde, pH 7.4) and refrigerated for up to 2 weeks. They were then sliced into 50 μm sections using a vibratome (Ted Pella, Redding, CA, USA). Following incubation overnight in 0.4% Triton X-100, sections were washed in Tris buffer (3×5 min), incubated in 0.3% H_2O_2 in 10% methanol for 30 min, washed, incubated in avidin–biotin–peroxidase complex (ABC standard kit, Vector Laboratories, Burlingame, CA, USA), washed, incubated in 3,3′-diaminobenzidine (Polysciences, Warrington, PA, USA; 50 mg/100 ml Tris) and 0.1% $NiNH_3SO_4$ until the cell could be fully visualized (10–30 min), washed, dehydrated in a series of graded alcohols (10 min each: 70%, 90%, 95% then 10 min in 100% twice), cleared in xylene, and coverslipped in Permount (Fisher Scientific, Pittsburgh, PA, USA). Slides were examined using an Olympus BH-2 light microscope and photographed with 35 mm camera attachment using Tmax film (100ASA; Kodak).

Immunocytochemistry

The hemisphere contralateral to the one used for slicing was placed in ice-cold sucrose-artificial cerebrospinal fluid immediately after the brain was hemisected, and immersion-fixed (4% paraformaldehyde, pH 7.4) immediately after slices from the contralateral hemisphere were cut. It was placed on a rotator at room temperature for at least 6 h, and then refrigerated in fixative for at least 3 days. The tissue was sectioned (50 μm) using a vibratome (Ted Pella) and subsequently processed immunocytochemically. Neuronal loss was examined using an antibody to a neuronal specific nuclear marker (neuronal specific nuclear protein (NeuN); monoclonal, 1:5000, Chemicon International, Temecula, CA, USA). NeuN immunocytochemistry allowed a clearer picture of neuronal loss because it preferentially stains neurons relative to glia. By examining neuronal distribution in the absence of glia (e.g. reactive

glia), neuronal loss is easier to appreciate. Sprouting was detected using an antibody to neuropeptide Y (polyclonal, 1:2000, Peninsula Labs, Belmont, CA, USA). Neuropeptide Y is a robust marker of mossy fibers after seizures (Lurton and Cavalheiro, 1997; Sperk et al., 1996) and previous studies showed that it consistently labeled sprouted mossy fibers in the inner molecular layer, i.e. to the same extent as the other commonly used stain for mossy fibers, Timm stain (Scharfman et al., 2000). Detailed immunocytochemical methods have been described previously (Scharfman et al., 1999; Sloviter, 1991).

Results

This study is based on 42 mossy cells recorded from 21 animals. Eight were injected with pilocarpine and had status epilepticus followed by recurrent seizures. The number of seizures that were witnessed ranged from three to 42, but this is quite likely to be an underestimate because observations were not made continuously (see Experimental procedures). These rats are referred to below as ‘epileptic’. Slices were made 1–6 months after status, and 20 mossy cells were recorded.

Six rats were injected with pilocarpine and did not have status epilepticus, but they showed behavioral signs of mild seizures, such as facial automatisms. They resumed normal behavior within 2 h of pilocarpine injection, and spontaneous motor seizures were never observed. They are referred to below as the ‘status control’ group. These animals were used for slice experiments from 1.25 to 6.75 months after pilocarpine administration, and nine mossy cells were sampled.

In addition to the pilocarpine-treated rats, seven rats were injected with saline instead of pilocarpine. Otherwise they were treated the same as pilocarpine-injected rats (i.e. they received atropine before saline, diazepam, etc.; see Experimental procedures). These rats are referred to as ‘saline controls’, and 13 mossy cells were recorded from these rats between 1.25 and 6 months after saline injection.

Anatomy

General observations

The neuronal distributions in a saline control, status control, and epileptic rat are shown in Fig. 1. An antibody to a neuronal nuclear protein (NeuN) was used as a marker of neurons. Immunocytochemistry was performed using the hemisphere opposite to the one used to prepare slices (see Experimental procedures). Fig. 1 shows that there was preservation of a large number of neurons in the hilus in all experimental groups (Fig. 1A–C). In all epileptic rats ($n = 8$), there was substantial cell loss in the entorhinal cortex (Fig. 1C, arrows). In three of six status controls, a small degree of cell loss occurred in the entorhinal cortex (Fig. 1B, arrows). Neuronal loss was not detected in any saline controls ($n = 7$; Fig. 1A).

Fig. 1D shows a section from the same epileptic rat as Fig. 1C, but this section was stained with an antibody to neuropeptide Y, to stain mossy fibers. There is evidence of ‘mossy fiber sprouting’ in the inner molecular layer. Mossy fiber sprouting refers to the growth of new collaterals of the ‘mossy fiber’ axons of dentate granule cells. Mossy fiber sprouting occurs in various animal models of epilepsy, as well as human TLE (Babb et al., 1991;Sutula et al., 1989,1988;Tauck and Nadler, 1985;Turski et al., 1989). Previous studies have shown that neuropeptide Y can be used to stain the sprouted mossy fibers (Lurton and Cavalheiro, 1997;Sperk et al., 1996).

Fig. 2 compares the extent of mossy fiber sprouting in all three groups. Although neuropeptide Y-immunoreactive fibers and hilar neurons are present in all groups, sprouting was not evident

in any controls (n = 7; Fig. 2A), but was evident in all epileptic rats (n = 8; Fig. 2C). Often the neuropeptide Y staining of the sprouted axon plexus appeared relatively diffuse (Fig. 2C). Interestingly, one of the six status control rats demonstrated sprouting (data not shown).

Fig. 3 illustrates the positions of mossy cells that were recorded in epileptic and control slices. Mossy cells were located throughout the hilus, indicating that there was no single area of the hilus that exhibited greater mossy cell survival. However, the extreme septal hippocampus was not sampled. Also, electrode tracks were made only in areas of the hilus that were approximately 100–200 μm from the hilar/CA3c border (to be sure that CA3c pyramidal cells were not included in the sample).

Morphological characteristics of mossy cells

The basic morphology of mossy cells in epileptic and control rats was similar to previously described mossy cells (Amaral, 1978; Frotscher et al., 1991; Fujise et al., 1998; Ribak et al., 1985; Seress and Mrzljak, 1992). A defining feature of mossy cells, their complex spines or 'thorny excrescences', was present on proximal dendrites of all cells (Fig. 4). Interestingly, two cells from epileptic tissue (Fig. 4A,B) had excrescences in an unusual location, the initial portion of the axon. This was not an overlay effect, because focusing through the section did not reveal dendrites that could be associated with these excrescences. There were no clear differences in the number or complexity of excrescences on mossy cells from either epileptic or control tissue, but this will clearly need quantitative measures to be definitive.

Regarding their axon projection, mossy cells in epileptic and control rats were also similar to mossy cells that have previously been described (Buckmaster et al., 1992, 1996; Laurberg and Sorensen, 1981; Ribak et al., 1985; Swanson et al., 1978; Zimmer, 1971). The main axon descended into stratum oriens of CA3b, presumably destined for the fimbria, and collaterals were located throughout the hilus and inner molecular layer (Fig. 5). Spines were located throughout the dendritic tree on all sampled cells.

Characteristics of mossy cell dendrites were comparable to previous descriptions of mossy cells from normal rats as well. For example, hilar dendrites of mossy cells were thick and spiny proximally, and their reach could be extensive, e.g. from one end of the hilus to the other (Fig. 5A,B). In addition, mossy cell dendrites could permeate the granule cell layer (Fig. 5A–C). However, all of these characteristics were not demonstrated in every cell, i.e. there was morphological heterogeneity, as previously reported for mossy cells in normal tissue (Amaral, 1978; Frotscher et al., 1991; Fujise et al., 1998; Scharfman and Schwartzkroin, 1988; Seress and Ribak, 1995; Seress and Mrzljak, 1992).

Examples of intracellularly labeled mossy cells are shown in Figs. 4 and 5. Fig. 4A,B illustrates two different cells from epileptic rats. Reconstructions of these cells are shown in Fig. 5A,B. Fig. 4C is a mossy cell from another epileptic rat that was recorded simultaneous to a pyramidal cell (recordings from these cells are shown in Fig. 12). Figs. 4D and 5D illustrate a mossy cell from a status control, and Fig. 5C illustrates a mossy cell from a saline control. Recordings of the cells in Figs. 4D and 5C,D are shown in Fig. 9.

Electrophysiology

Intrinsic properties

Intrinsic properties of mossy cells from epileptic and control rats were not statistically different (Table 1; oneway ANOVA). Resting potentials were close to –60 mV (range, epileptic: –57 to –67; controls: –50 to –63). Input resistance was approximately 70 MΩ (range, epileptic: 50–100; control: 50–120), and time constants were usually long (up to 45 ms; Table 1).

Mossy cells from epileptic and control rats were heterogeneous with respect to rectification in response to a hyperpolarizing current command (i.e. 'sag'). Thus, in both epileptic and control groups, some mossy cells demonstrated sag and others did not (epileptic, 5/10; status controls, 6/9; saline controls, 2/7). The proportion of cells with sag from epileptic tissue was not statistically different from the proportion of cells with sag from status controls ($\chi^2 = 0.55$; $P>0.20$) or saline controls ($\chi^2 = 1.15$; $P>0.20$).

APs of mossy cells from epileptic and control rats were not statistically different (Table 1; one-way ANOVA). AP slopes and durations of mossy cells were consistent with 'regular spiking' cells (McCormick et al., 1985;Scharfman, 1992b,1995a;Smith et al., 1995) (Table 1). Thus, AP duration was broad (range, epileptic: 2.0–3.0 total duration, 0.22–0.32 half-width; controls: 2.0–3.5 total duration, 0.20–0.35 half-width) and AP slopes showed a large d*v*/d*t* ratio (range, epileptics: 1.50–3.52; controls: 1.75–3.83; Table 1).

AHPs following single APs were rare, similar to previous studies of mossy cells in normal rats using sharp microelectrodes (Scharfman and Schwartzkroin, 1990a); AHPs of mossy cells appear more common using patch electrodes (Lübke et al., 1998). Thus, AHPs occurred in only four cells (three from epileptic tissue, one from control tissue), and these were small in amplitude (1–4 mV, 16–60 ms duration). Firing behavior in mossy cells was highly variable from trial to trial, regardless of the experimental group. Thus, a fixed amplitude current pulse could evoke a train of adapting APs, a train of irregular discharge, or a single AP (data not shown), as has previously been shown for normal rats (Scharfman and Schwartzkroin, 1988). The frequent spontaneous depolarizing potentials in mossy cells (Figs. 6, 7 and 9), which are a hallmark of mossy cells (Livsey and Vicini, 1992; Scharfman, 1993; Scharfman and Schwartzkroin, 1988; Soltesz and Mody, 1994; Strowbridge et al., 1992), were also present in each experimental group, and presumably contributed to the variability in firing behavior.

Synaptic responses

Responses to subthreshold stimul—Subthreshold stimulation of the outer molecular layer produced depolarizing PSPs in both epileptic and control tissue, similar to previous reports of normal rats (Scharfman, 1993). Table 2 shows the peak amplitude, latency to onset, and durations of PSPs evoked at threshold for AP generation ('threshold EPSPs'). These PSPs were assumed to be excitatory (i.e. EPSPs) because they evoked APs, and were often evoked at potentials close to the reversal potential of $GABA_A$ receptor-mediated inhibitory PSPs (IPSPs) (−70 mV). In addition, previous experiments in normal rats showed that PSPs evoked by outer molecular layer stimulation were blocked by glutamate antagonists (Scharfman, 1992a). Only after blockade, and increased current, could IPSPs be evoked (Scharfman, 1992a). However, we cannot rule out the possible contribution of IPSPs in the present experiments (Soltesz and Mody, 1994).

EPSPs of mossy cells in epileptic and control tissue could not be distinguished statistically in latency to onset and peak amplitude (Table 2; one-way ANOVA, $P<0.05$), and also were similar in other ways. There was a steep input-output (I–O) function because the difference between the minimal stimulus strength that could evoke a response and the threshold stimulus strength (the stimulus strength that evoked an AP in 50% of trials) was very small (often 2–10 μA). In four cells the function was so steep that there was no detectable difference at all; i.e. a stimulus that was too weak to evoke a response in some trials could evoke a burst of APs superimposed on an EPSP in other trials (Fig. 8B). Three of these cells were from epileptic tissue and one was from a saline control rat, so the steep I–O function was not necessarily due to the epileptic nature of the tissue.

There was only one difference between epileptic and control tissue that was detected in threshold EPSPs. When half-duration was used to measure EPSP duration, the durations were

significantly longer in mossy cells from epileptic rats (Table 2; one-way ANOVA, $P<0.05$). This difference was apparent whether half-duration was measured from the stimulus artifact or from the onset of the EPSP (Table 2; one-way ANOVA, $P<0.05$). However, total duration was not different (Table 2). One reason that half-duration was longer might be that polysynaptic PSPs contributed to the evoked responses of mossy cells in epileptic slices. If they occurred before the late decay phase of the EPSP, half-duration might be affected, but not total duration. It is also possible that the difficulty in measuring total duration made that measurement less accurate. The difficulty lies in the fact that the late phase of the EPSP repolarizes very slowly and hence cannot be distinguished readily from baseline and spontaneous PSPs; indeed, in some cases duration could not be measured at all because of confounding spontaneous events (Table 2).

Spontaneous and evoked bursts of APs in mossy cells from epileptic rats—All mossy cells from epileptic rats had periodic spontaneous bursts (0.1–0.25 Hz), many of which resembled paroxysmal depolarization shifts (PDSs; Fig. 6). The bursts were like PDSs because their onset could be sudden (paroxysmal), and they were composed of a large (>30 mV) depolarization, accompanied by repetitive APs, and followed by after-discharges (Fig. 6). However, some cells had bursts that were brief, and these bursts did not resemble PDSs very well (Fig. 7). Brief bursts were composed of a large depolarization and repetitive APs, like PDSs, but the duration of these events and number of APs were relatively small (compare Figs. 6 and 7).

Bursts could be evoked by stimulation of the outer molecular layer either at threshold stimulus strength or using suprathreshold currents. For a given cell, evoked bursts were similar to spontaneous bursts in the underlying depolarization, number of APs elicited, and the burst duration (Fig. 7). Evoked bursts were similar to previously described epileptiform bursts in slices exposed to convulsants because they were all-or-none, and hyper-polarization of the cell revealed a ‘giant’ EPSP (Johnston and Brown, 1986) (Fig. 8A; see also Fig. 11).

Burst frequency could vary while recording from a particular cell, particularly in those slices which had brief epileptiform discharges. Changes were slow when they occurred; i.e. burst frequency did not change over a 10–15 min period, but could vary if a cell was recorded for a longer period of time. If spontaneous discharges had become infrequent (<0.03 Hz), one to five stimuli to the molecular layer at 0.02–0.03 Hz could increase burst frequency. In four of 10 cells, spontaneous bursts were detected only after an initial one to two stimuli to the molecular layer had been tested. Thereafter, spontaneous bursts occurred for at least 10 min.

None of the mossy cells from saline controls had bursts that were spontaneous or evoked (n = 11; Fig. 9A). The greatest evoked response had two APs (Fig. 9A). These responses are not referred to as ‘bursts’ because the number of APs was limited, there was no underlying ‘giant’ EPSP (i.e. > 30 mV) and the duration of the response was brief (i.e. < 100 ms; compare Fig. 9 with Figs. 6–8).

None of the mossy cells from status controls had spontaneous bursts, but molecular layer stimulation evoked a burst in one cell. The underlying EPSP was large (> 30 mV), four APs were triggered at its peak, and it was otherwise similar to the brief bursts of some mossy cells in epileptic tissue. Interestingly, in this animal there was some hilar cell loss evident by NeuN staining, and there was mossy fiber sprouting (data not shown). The animal had facial automatisms immediately after pilocarpine injection, but did not have status epilepticus and no spontaneous motor seizures were ever observed. No other status control tissue demonstrated sprouting. Thus, abnormalities in mossy cell function may arise without status epilepticus, and may be related to mossy fiber sprouting. However, without a greater sample of abnormal mossy cells in status controls, it is not possible to draw conclusions.

Mossy cell vs. granule cell threshold—Mossy cells required a very small stimulus to evoke a response at threshold compared with granule cells in the same slice, as has previously been described for some of the mossy cells in normal rats (Scharfman, 1991). Therefore, we compared mossy cell and granule cell thresholds by sequential impalement of these cell types in the same slice, using the same site in the outer molecular layer for stimulation (Fig. 10). In all five slices from epileptic rats that were examined in this manner, the stimulus strength that evoked APs in mossy cells was too weak a stimulus to evoke APs in granule cells (Fig. 10), similar to previous studies in normal juvenile rats (Scharfman, 1991). In each slice, a single mossy cell was compared with three to six granule cells. The granule cells were located 50–250 μm from the stimulating electrode, much closer to the stimulation site than the mossy cells. Yet the mossy cell threshold was lower than granule cells in the same slice. This could be a reflection of the high resting potential of granule cells relative to mossy cells (approximately −75 mV for granule cells, −60 mV for mossy cells), because when granule cells were depolarized by injected current, the same stimulus that evoked APs in mossy cells evoked APs in granule cells (Fig. 10).

Simultaneous recordings from mossy cells and pyramidal cells

Our recent studies in pilocarpine-treated rats demonstrated that CA3 pyramidal cells often have spontaneous, rhythmic epileptiform bursts in pilocarpine-treated rats after status epilepticus and recurrent seizures (Scharfman et al., 2000). Given that pyramidal cells innervate mossy cells (Kunkel et al., 1993; Scharfman, 1994c), and that pyramidal cell epileptiform discharges precede mossy cell epileptiform bursts in convulsant-treated slices from normal rats (Scharfman, 1994), we hypothesized that mossy cell bursts could be due to spontaneous discharges of pyramidal cells. This was tested by simultaneous recordings of mossy cells and pyramidal cells.

In initial studies, simultaneous intracellular recordings from mossy cells and extracellular recordings of population discharges in the CA3 cell layer were made. In all cases where mossy cell bursts were recorded, there also were spontaneous discharges in the CA3 cell layer (n = 6; Fig. 11). Extracellularly recorded bursts were largest in amplitude in area CA3b than area CA3a or c. These recordings showed that mossy cell bursts were synchronized with the CA3 population.

To determine relative timing of mossy cell and pyramidal cell burst discharges more accurately, recordings were made using intracellular electrodes for both mossy cells and pyramidal cells. Pyramidal cells were recorded in area CA3b (n = 2) and CA3c (n = 1), and at least 20 bursts were recorded for each pair of CA3 and mossy cells (three pairs in three different animals). Fig. 12 shows an example in which the first AP of each CA3 cell burst immediately preceded the depolarization of the mossy cell. This timing is similar to monosynaptically connected pyramidal cells that innervate mossy cells in normal rats (Scharfman, 1994c). On the basis of these recordings, one would predict that pyramidal cells initiate bursts in mossy cells, and the most parsimonious explanation would be that this occurs via their normal innervation of mossy cells (Kunkel et al., 1993;Scharfman, 1994c). Interestingly, there was no evidence for the converse, i.e. that mossy cells drive bursts in pyramidal cells, although evidence for reciprocal innervation in normal rats exists (Kunkel et al., 1993). Also noteworthy is that, in one pair, there was variation in the delay between the pyramidal cell's first AP and the onset of the mossy cell depolarization. There could be no delay, as described above, or up to 5 ms delay, indicating polysynaptic circuits could play a role. In one instance the depolarization of each cell appeared to occur at the same time, which would suggest that gap junctions were involved or the cells received a common input.

Discussion

Summary

The results suggest that mossy cells can survive status epilepticus and chronic seizures in rats. The cells that survived had similar morphological and intrinsic electrophysiological properties as mossy cells from saline controls, but had spontaneous and evoked epileptiform discharges. These discharges were synchronized with epileptiform bursts of area CA3 pyramidal cells. Simultaneous intracellular recording showed that the majority of pyramidal cell bursts occurred prior to mossy cell burst onset, suggesting that pyramidal cells initiated burst discharges.

Survival of mossy cells after seizures

The results were surprising in light of the assumption that much of the hilus, and mossy cells in particular, die or are damaged after long periods of excitation, severe seizures, severe injury, and other pathological conditions. Therefore, one question we asked after our initial observations was whether the survival of mossy cells might be due to a relatively short episode of status, because we administered diazepam after just 1 h of status epilepticus. We also questioned whether recurrent seizures in our animals might have been relatively limited. Either of these factors could have increased the chance of mossy cell survival.

While it is true that in our studies diazepam was injected after 1 h of status epilepticus, this did not necessarily make status epilepticus benign, since it had already lasted a full hour, and diazepam did not stop seizures (it merely decreased their severity). Behaviors associated with seizures, such as a frozen posture, head nodding, and occasional seizures that reached stage 5, continued for hours after diazepam injection. Only by the next day did normal behavior resume. In addition, recent studies in our laboratory without diazepam showed a substantial number of hilar cells survive without any anticonvulsant treatment (Goodman et al. 2000), indicating that the 1 h duration of status epilepticus may not be a factor in our results. In all of these rats, spontaneous seizures in subsequent weeks and months were quite severe (stage 5), sometimes including vocalization and wild running. In one case, status epilepticus occurred several months after pilocarpine administration. Thus, status epilepticus and chronic seizures were not modest in our studies.

Thus, it is quite likely that at least some mossy cells die after pilocarpine-induced status epilepticus and recurrent seizures. However, it is unclear from the present results whether mossy cells survive in other animal models besides the pilocarpine model. After kainic acid, it has been reported that there is some mossy cell survival (Buckmaster and Jongen-Relo, 1999), but usually hilar and CA3 neurons are more susceptible after kainic acid than pilocarpine (Ben-Ari, 1985; Nadler, 1981; Sperk, 1994; Wuarin and Dudek, 1996), so the present results may not necessarily be generalized to the kainic acid model. Each model will need to be evaluated separately. However, the tacit assumption that all mossy cells are killed after status epilepticus or recurrent limbic seizures is no longer tenable.

Which mossy cells survive? It is possible that cells which die differ in some way from those that survive, and this difference explains their resistance. Implicit in this suggestion is the assumption that there are subtypes of mossy cells in the normal rat, some of which are more vulnerable than others. There is no clear evidence for this, although there is evidence of heterogeneity, and whenever there is heterogeneity the possibility of subtypes arises. For example, mossy cells in the normal rat with dendrites in the molecular layer have a low threshold for molecular layer-evoked responses compared with those that have only hilar dendrites (Scharfman, 1991). Mossy cells that are located close to area CA3 have greater evidence of inhibitory input than mossy cells located close to the granule cell layer (Scharfman, 2000). The cells with the lowest threshold and weakest inhibitory input may be most vulnerable

to seizure activity. However, several of the mossy cells from epileptic rats had a low threshold, and some had dendrites in the molecular layer (albeit few). Mossy cells were evident near and far from the granule cell layer. Therefore, a low threshold, dendrites in the molecular layer and weak inhibitory input do not necessarily explain vulnerability. Another argument against the idea that subtypes explain the results is that the same range of morphological and electrophysiological characteristics that were present in mossy cells from control rats were present in epileptic rats. For example, some mossy cells from epileptic tissue were bipolar and some multipolar, similar to a normal rat. There were overlapping ranges of resting membrane potential, input resistance, time constant, etc. for mossy cells in epileptic and control rats. In summary, there is no experimental support for the hypothesis that one subtype of mossy cell is more vulnerable and was lost after status epilepticus and recurrent seizures.

The fact that mossy cells can survive status epilepticus and recurrent seizures is consistent with studies of traumatic brain injury, because it was shown recently that mossy cells can survive trauma (Santhakumar et al., 2000). Interestingly, mossy cell burst discharges were also evident after trauma (Santhakumar et al., 2000), comparable to the situation after pilocarpine described here. Thus, survival of mossy cells after severe seizures or injury is possible, and appears to predispose these cells to prolonged periods of excitation.

Differences between mossy cells from epileptic and control rats

There were many morphological and electrophysiological similarities of mossy cells from epileptic and control rats. Indeed, the classic morphology of mossy cells in normal rats could not be distinguished from mossy cells in epileptic tissue. The thorny excrescences, in combination with the thick and spiny dendrites, inner molecular layer axon projections, and electrophysiology, would be difficult to associate with any other cell type, even given the substantial plasticity in the epileptic brain. Although we cannot rule out that quantitative measures of axonal length, number of spines, dendritic tree, or thorns would reveal differences, our data do not provide any indications of differences. Differences between mossy cells in control and epileptic slices were only apparent in synaptic responses and abnormal burst discharges. The results suggest that severe seizures do not change fundamental intrinsic electrophysiological or morphological characteristics, but do modify mossy cell behavior.

One difference in mossy cells from epileptic tissue was detected by measurement of EPSPs evoked by stimulation of the outer molecular layer at threshold. This stimulating electrode could have activated many cell types, so it served merely as a tool to assess afferent input to mossy cells, rather than perforant path fibers selectively. Interestingly, the same range of latencies and peak amplitudes was found in epileptic and control rats, suggesting that these aspects of synaptic depolarizations (EPSPs) of mossy cells by molecular layer stimulation were not altered by status or recurrent seizures.

The one difference was in the half-duration of threshold EPSPs, which were longer in mossy cells from epileptic tissue. The results suggest that either the factors contributing to EPSP decay were altered, or additional, long latency pathways could have been recruited in epileptic rats that contributed to the late phases of EPSPs.

One possibility is that the increased duration EPSPs were due to long latency excitatory pathways. For example, CA3 could be activated by the molecular layer stimulus because the stimulus could activate granule cells that innervate CA3, and it could also backfire perforant path axons that innervate CA3 pyramidal cell apical dendrites (Yeckel and Berger, 1990). CA3 excitation could lead to subsequent activation of mossy cells (Kunkel et al., 1993; Scharfman, 1994c). However, these polysynaptic circuits are unlikely to explain the long EPSP duration in mossy cells from epileptic rats, because they are also present in normal tissue (Kunkel et al., 1993; Penttonen et al., 1997; Scharfman, 1994c; Wu et al., 1998).

Another pathway that could lead to long latency excitation of mossy cells involves sprouted granule cells. Thus, the molecular layer stimulus could have activated granule cells directly, and they may have then excited other granule cells via their sprouted axon collaterals; all of these granule cells could potentially activate a given mossy cell, but would do so at different latencies. Another possibility is that mossy cells which are initially activated could excite granule cells, which in turn could lead to activation of other granule cells via sprouted axons, and those granule cells could then re-excite the same mossy cell. However, this assumes that the neurons that are initially activated can activate downstream neurons over threshold, and it is not clear that this is the case (particularly given the high threshold of granule cells). Furthermore, any positive feedback loop would also be influenced by concurrently activated inhibitory inputs.

GABAergic pathways are unlikely to play a direct role in prolonged EPSPs of mossy cells because recordings were made at membrane potentials similar to the equilibrium potential of $GABA_A$ receptor-mediated IPSPs (i.e. ~ −70 mV), and depolarizing to the equilibrium potential for $GABA_B$ receptor-mediated IPSPs.

Mossy cell and pyramidal cell burst discharges

A clear abnormality in epileptic tissue was the spontaneous, rhythmic burst discharges in mossy cells and CA3 pyramidal cells. These burst discharges were similar in frequency to those that occur in normal slices after pharmacological disinhibition (Chestnut and Swann, 1988; Müller and Misgeld, 1991; Perreault and Avoli, 1991; Rutecki and Yan, 1998; Scharfman, 1994b; Swann and Brady, 1984; Wong and Traub, 1983). Therefore, mossy cell and pyramidal cell burst discharges may simply be due to seizure-related loss of some inhibitory neurons. Indeed, somatostatin-immunoreactive interneurons in the dentate gyrus, a subpopulation of hilar GABAergic neurons, are substantially reduced in our epileptic rats (Scharfman et al., 2000). Others have shown that GABAergic neurons can be lost in the pilocarpine model (Obenaus et al., 1993). However, other factors besides interneuronal loss may also contribute to mossy cell and pyramidal cell burst discharges, such as altered expression of glutamate receptors, or enhanced recurrent excitatory circuitry because of sprouting.

The results of simultaneous intracellular recordings suggest another similarity between burst discharges in epileptic rats and disinhibited normal slices. In both situations, the first AP of a given epileptiform burst usually occurred in pyramidal cells first (Scharfman, 1994). Taken together with the known propensity for CA3 to generate burst discharges (Swann and Brady, 1984; Wong and Traub, 1983) (but not isolated mossy cells (Scharfman et al., 1999)), and the known projection of pyramidal cells to mossy cells (Kunkel et al., 1993; Scharfman, 1994c), the most parsimonious explanation that bursts are initially generated in pyramidal cells and then are propagated monosynaptically to mossy cells. However, there was variability in interval between pyramidal cell and mossy cell burst onsets. Therefore, multiple factors could be involved in synchronizing these cells, ranging from normal to abnormal synaptic pathways, or even non-synaptic mechanisms.

Implications – previous studies

Our results suggest that it may be necessary to reinterpret previous studies of dentate gyrus function after seizures in which it was assumed that mossy cells were dead. For studies involving hilar stimulation, for example, effects of hilar stimulation on granule cells that were assumed to be primarily antidromic could actually have involved stimulation of hilar cells. For studies of mossy fiber sprouting, the assumption that excitatory, zinc-labeled terminals in the inner molecular layer are solely due to granule cell axons needs reconsideration, because mossy cell terminals are also excitatory and contain zinc, albeit far less than mossy fibers (Haug, 1974).

For the ‘dormant basket cell’ hypothesis (Soltesz and Mody, 1994), which proposes that the death of mossy cells leads to increased network excitability because inhibitory ‘basket’ cells lose afferent drive, it is important to reconsider the extent that mossy cells actually die.

Implications – epileptogenesis

Our data also provide new insight into factors that could contribute to seizure activity in the pilocarpine model. The fact that mossy cells have repetitive burst discharges is potentially significant because they could provide a source of excitatory drive to granule cells. This would have a greater effect on the granule cell network than in normal conditions, because each granule cell that a mossy cell innervates may in turn excite many other granule cells due to mossy fiber sprouting (Okazaki et al., 1995) (Fig. 13). Normally granule cell excitation would be decreased by concurrent activation of interneurons. However, inhibitory input to granule cells may be decreased after pilocarpine-induced status epilepticus because some interneurons die (Obenaus et al., 1993). Under these conditions, surviving mossy cells could become a potentially powerful ‘trigger’ for the sprouted granule cell network.

Substantial granule cell activation could have important implications, particularly if activation were synchronous, which is likely because input from mossy cells would be synchronous burst discharges. Synchronization of granule cells would also follow from the fact that the sprouted axons of granule cells can have extensive terminal fields (Isokawa et al., 1993; Okazaki et al., 1995; Sutula et al., 1998). Synchronous activation of large numbers of granule cells could potentially ‘detonate’ CA3 (Fig. 13). Strong CA3 activation, and subsequent re-activation of granule cells due to the pyramidal cell–mossy cell–granule cell pathway, might result in reverberatory activity and an eventual transition from brief, ‘interictal’ burst discharges in mossy cells and CA3 to longer, possibly ‘ictal’ episodes.

Thus, the survival of mossy cells might contribute to limbic seizures in pilocarpine-treated rats. In essence, we hypothesize that mossy cells and CA3 neurons could act as a ‘focus’. This perspective is almost exactly opposite to previous conceptions, which held that hilar death contributed to seizures. We now hypothesize that it is the survival of mossy cells, not their death, that, taken together with the development of their spontaneous burst discharges with CA3, may contribute to seizures in the pilocarpine model.

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Abbreviations

AP

action potential

AHP

afterhyperpolarization

ANOVA

	analysis of variance
EPSP	
	excitatory postsynaptic potential
IPSP	
	inhibitory postsynaptic potential
NeuN	
	neuronal specific nuclear protein
PDS	
	paroxysmal depolarization shift
PSP	
	postsynaptic potential
TLE	
	temporal lobe epilepsy

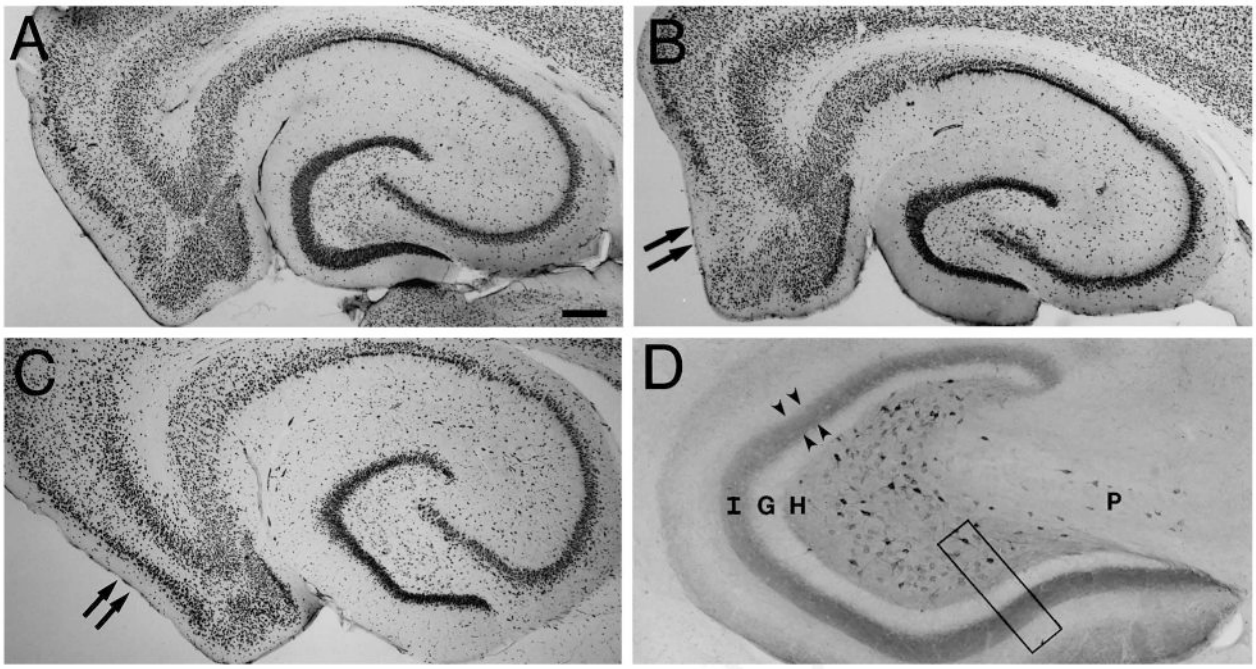

Fig. 1.

Neuronal loss and mossy fiber sprouting after saline or pilocarpine treatment. A: A section through the middle of the hippocampus of a saline-injected rat ('saline control') that was stained with an antibody to a neuronal marker (NeuN) shows no evidence of neuronal loss 2 months after saline injection. A mossy cell from this animal, and recordings, are shown in Figs. 5C and 9A, respectively. B: A NeuN-stained section through the middle of the hippocampus of a pilocarpine-treated rat that failed to exhibit status epilepticus ('status control') demonstrates a small degree of cell loss in medial entorhinal neurons (arrows; as compared with C). This animal exhibited facial automatisms immediately after pilocarpine injection but no subsequent evidence of abnormal behavior; slices were prepared from one hemisphere 1.25 months later, and the opposite hemisphere was immersion-fixed for immunocytochemistry. A mossy cell from this animal and recordings are shown in Figs. 4D and 5D (morphology) and Fig. 9B (recordings). C: A NeuN-stained section through the middle of the hippocampus in a pilocarpine-treated rat that had status epilepticus and recurrent seizures. Neuronal loss was substantial in the entorhinal cortex (arrows). In the dentate gyrus, hilar neurons survived. This rat had nine observed motor seizures in 5.5 months after status epilepticus; more are likely to have occurred because rats were not observed at all times (see Experimental procedures). A mossy cell from this rat is shown in Figs. 4B and 5B. D: A tissue section from the same animal as shown in C, stained with an antibody to neuropeptide Y to show mossy fiber sprouting (arrowheads) in the inner molecular layer (I). Neuropeptide Y staining was used to demonstrate mossy fiber sprouting because the mossy fibers make neuropeptide Y after seizures (Lurton and Cavalheiro, 1997;Sperk et al., 1996). G = granule cell layer; H = hilus; P = pyramidal cell layer. The area enclosed in the box is shown at higher power in Fig. 2C. Scale bar (in A) = 200 μm (D); 400 μm (A–C).

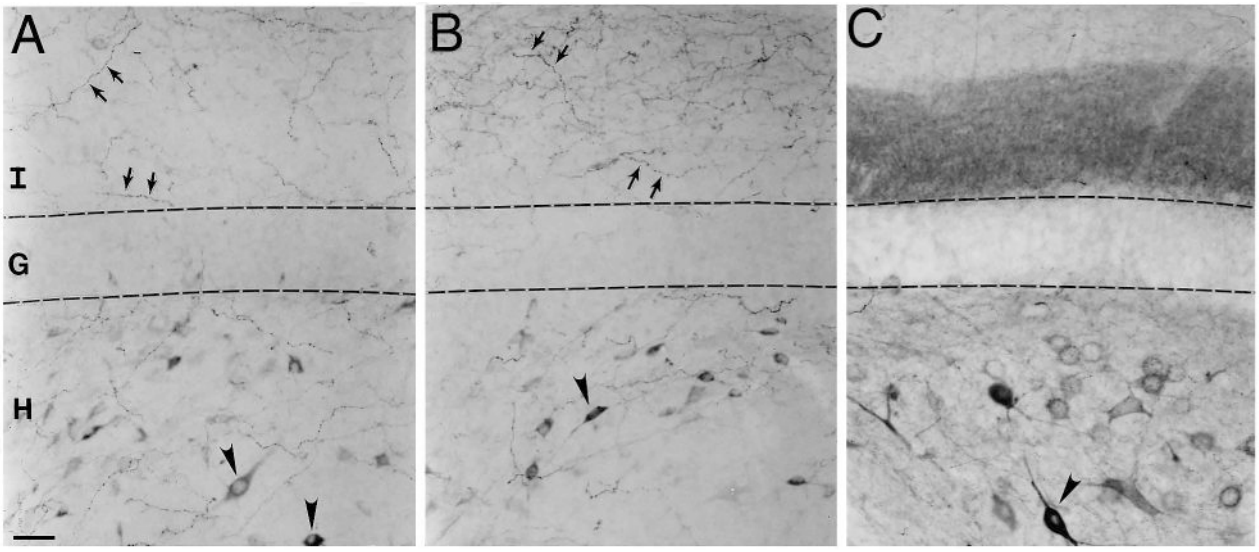

Fig. 2.

Mossy fiber sprouting in pilocarpine- and saline-treated rats. A: A section through the lower blade of the dentate gyrus from a saline control rat that was stained using an antibody to neuropeptide Y. The approximate position of this section within the dentate gyrus is indicated by the box in Fig. 1D. A similar location was chosen for A, B, and C of this figure. Note that hilar cells are neuropeptide Y-immunoreactive (arrowheads), as are fibers in the molecular layer (arrows). B: A section from a similar area of the dentate gyrus as A, but the section was from a status control rat (same animal as Fig. 1B). The neuropeptide Y immunoreactivity has a similar pattern as in A. C: A section from the same pilocarpine-treated rat as in Fig. 1C illustrates increased neuropeptide Y staining in hilar neurons (arrowhead) and the inner molecular layer relative to control tissue. H = hilus, G = granule cell layer, I = inner molecular layer. Calibration (in A) = 50 μm.

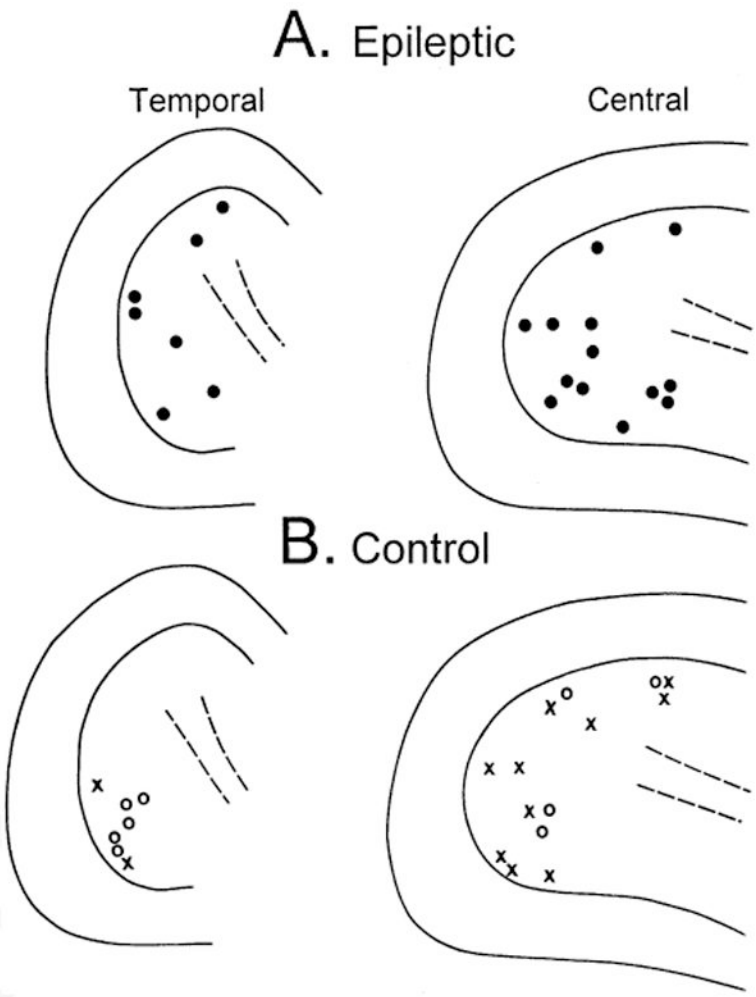

Fig. 3.

The distribution of recorded mossy cells in epileptic and control rats. A: The location of somata of all mossy cells that were recorded in epileptic rats are shown by filled circles in schematics of temporal hippocampus and central hippocampus. The one cell recorded from dorsal hippocampus is included in the central hippocampus schematic for the purposes of this figure. Note that the locations of somata are widely distributed. B: The locations of somata of all mossy cells that were recorded in control rats are shown. Mossy cells from saline controls are designated by the crosses and cells from status control rats are indicated by open circles. There were no dorsal hippocampal cells sampled from control rats.

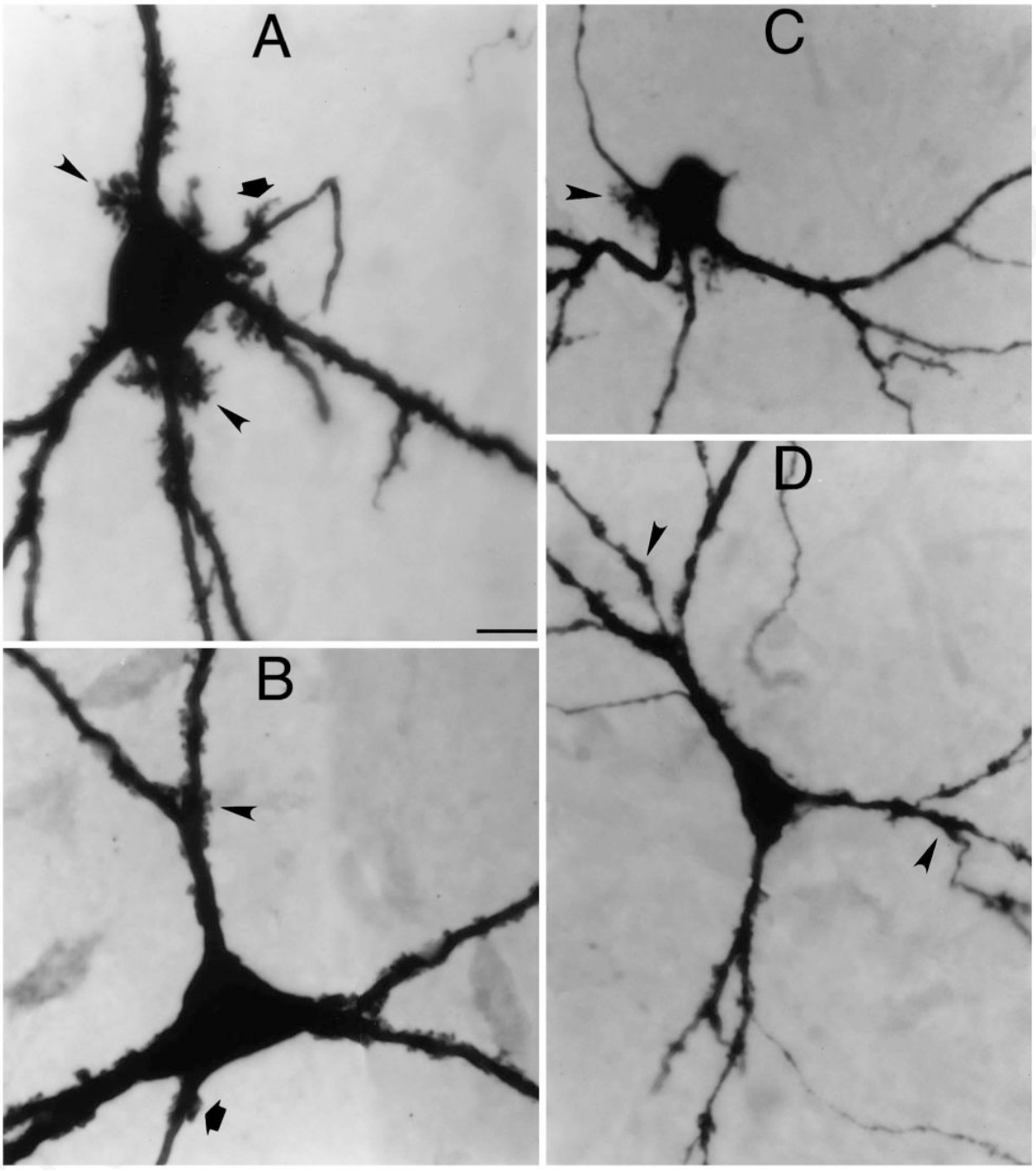

Fig. 4.

Thorny excrescences on mossy cells from epileptic and control rats. A: A mossy cell that was recorded from an epileptic rat and filled with Neurobiotin is shown, illustrating one of the identifying features of mossy cells, thorny excrescences on proximal dendrites (arrowheads). This cell, and the one in B, had excrescences on the initial segment of the axon (thick arrow). A drawing of this cell is shown in Fig. 5A. The rat was killed 1.5 months after status. Dorsal is up and area CA3 is to the right. B: A mossy cell from a different epileptic rat (same rat as for Figs. 1C and 2C). The dendrites and axon of this cell are shown in Fig. 5B. Dorsal is to the right and area CA3 is down. C: A mossy cell from an epileptic rat that was killed 2.25 months after status. The cell was located approximately 200 μm from the crest of the dentate gyrus.

Recordings from this cell are shown in Fig. 12. Dorsal is to the left and area CA3 is up. D: A mossy cell from a status control. A drawing of this cell is shown in Fig. 5D. It was from the same animal used for Figs. 1B, 2B, and 9B. Dorsal is up and area CA3 is to the left. Scale bar (in A) = 20 μm (A,B); 35 μm (C,D).

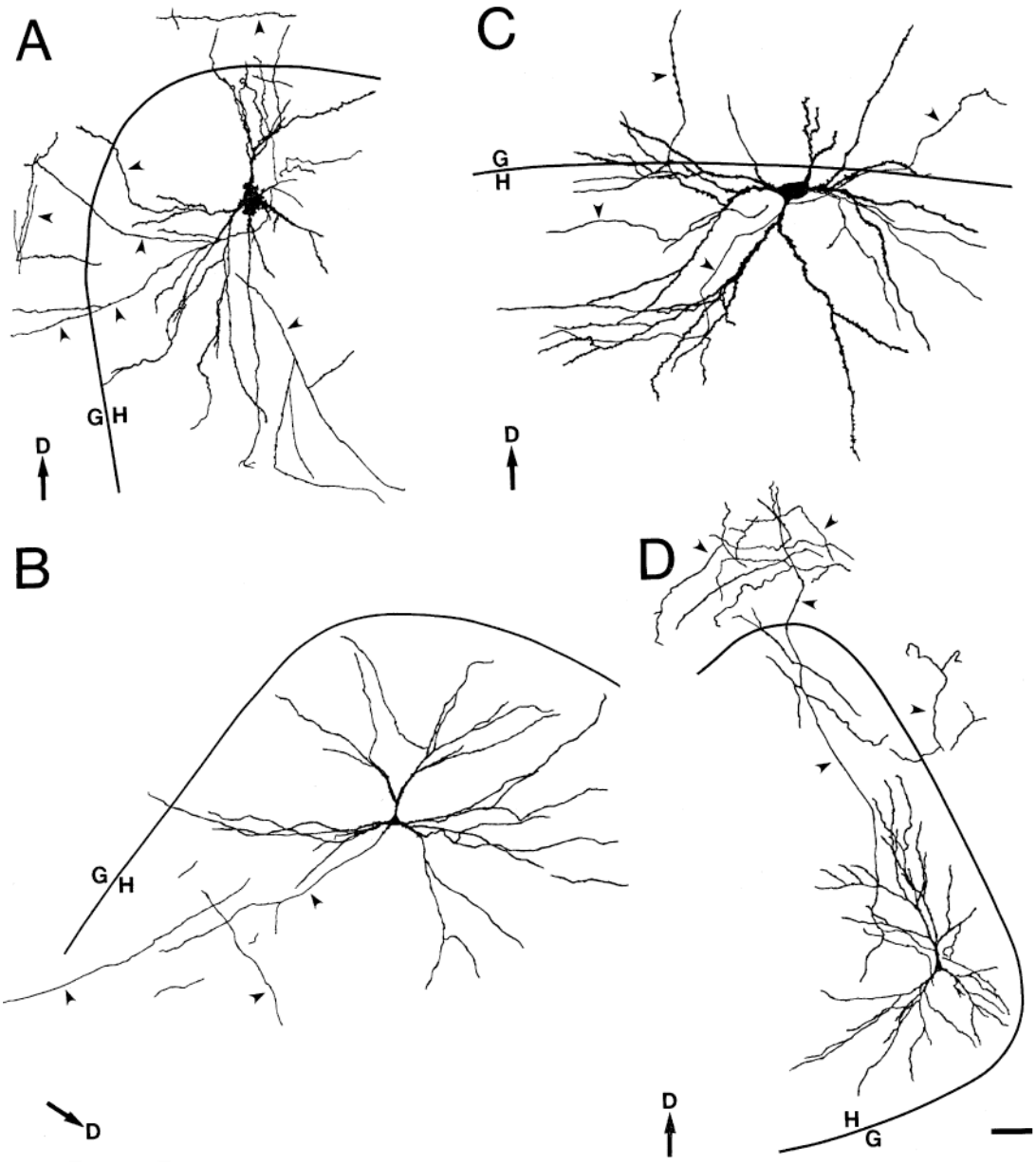

Fig. 5.

Illustrations of the dendrites and axons of mossy cells labeled with Neurobiotin. A: A mossy cell from an epileptic rat (same cell as Fig. 4A) is illustrated. Arrowheads point to axon collaterals. Calibration (in D) = 75 μm. B: Another mossy cell from an epileptic rat (same cell as Fig. 4B). Calibration (in D) = 150 μm. C: A mossy cell from a saline control. Immunocytochemically stained sections from this rat are shown in Figs. 1A and 2A, and recordings from this cell are shown in Fig. 9A. Calibration (in D) = 75 μm. D: A mossy cell from a status control (same cell as Fig. 4D). Recordings from this cell are shown in Fig. 9B. Calibration = 100 μm. G = granule cell layer; H = hilus. The dorsal direction is indicated by the direction of the arrow labeled ‘D’. Scale bar (in D) = 75 μm (A,C), 100 μm (D), 150 μm (B).

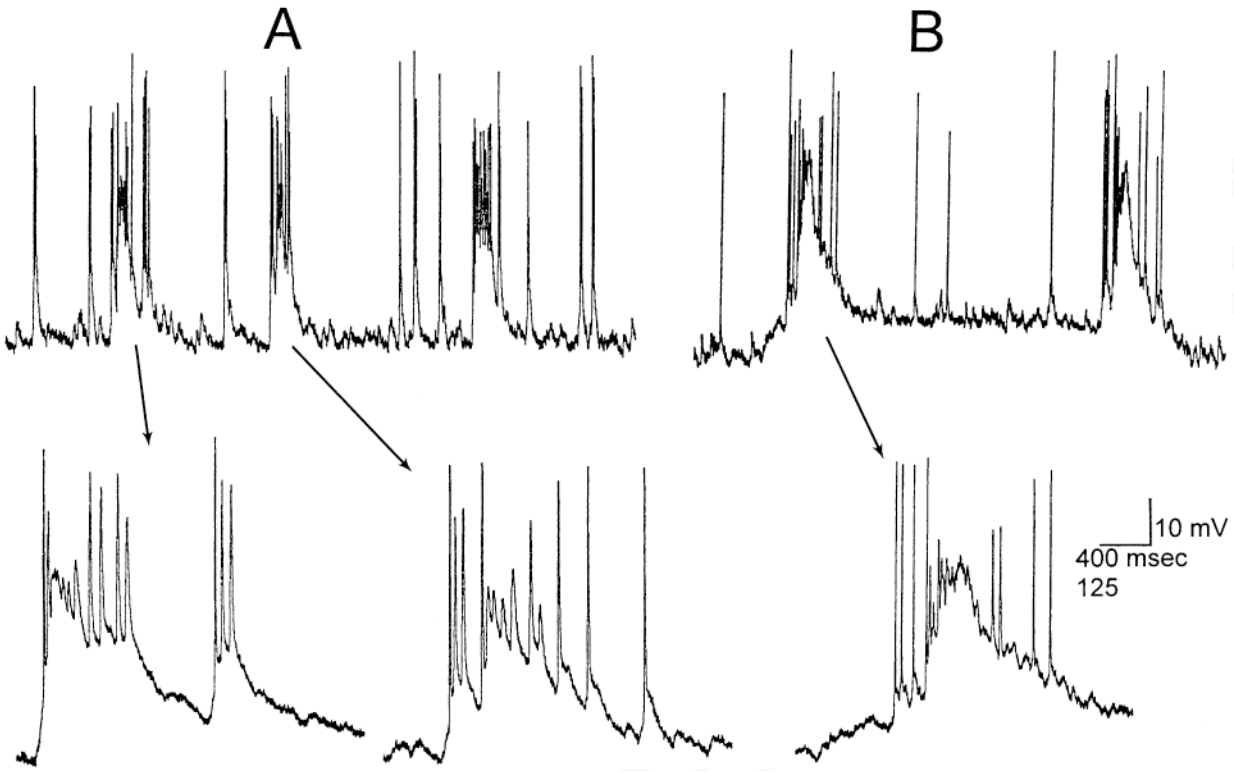

Fig. 6.

Spontaneous epileptiform discharges in mossy cells from epileptic rats. A: Spontaneous discharges in an epileptic rat (top). The first bursts are shown with a slower time base below, as indicated by the arrows. For both A and B, the temporal calibration for top traces is 400 ms and for lower traces it is 125 ms. B: Spontaneous discharges of a mossy cell in a different epileptic rat show a different burst frequency and burst morphology, indicating the variation across mossy cells.

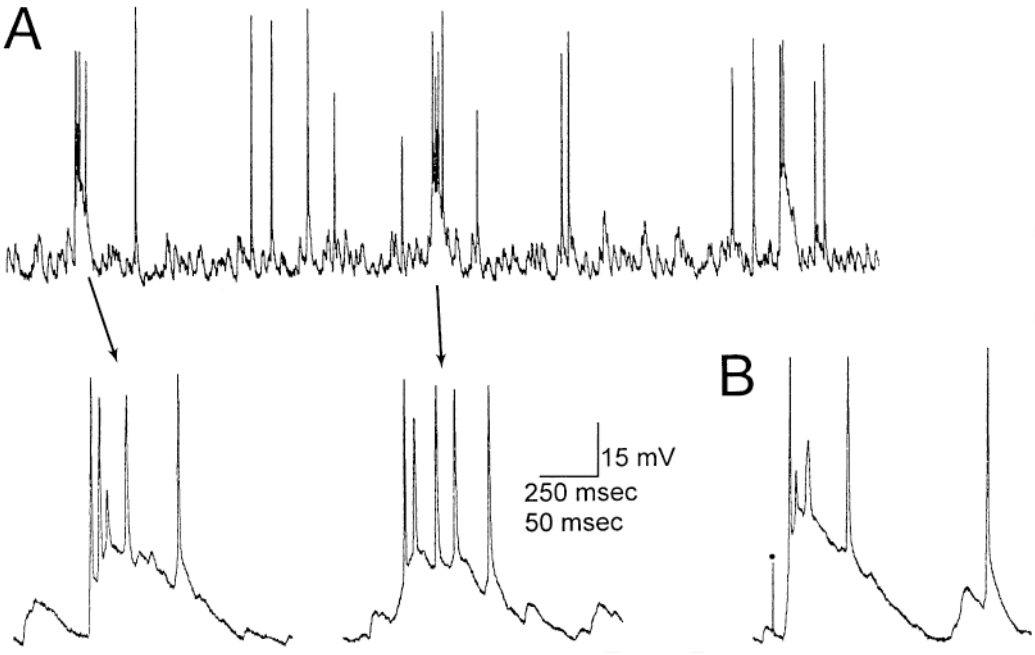

Fig. 7.

Brief burst discharges in a mossy cell from an epileptic rat. A: Spontaneous burst discharges from a mossy cell of an epileptic rat are shown. This rat was different from the ones used for recordings in Fig. 6, and the burst discharges were different also. Each burst was relatively short. Calibration, top = 250 ms; bottom = 50 ms. B: A response to outer molecular layer stimulation in the same cell shows a similar duration of an evoked burst as the spontaneous bursts shown in A. Stimulation occurred at the dot. Calibration the same as bottom traces in A.

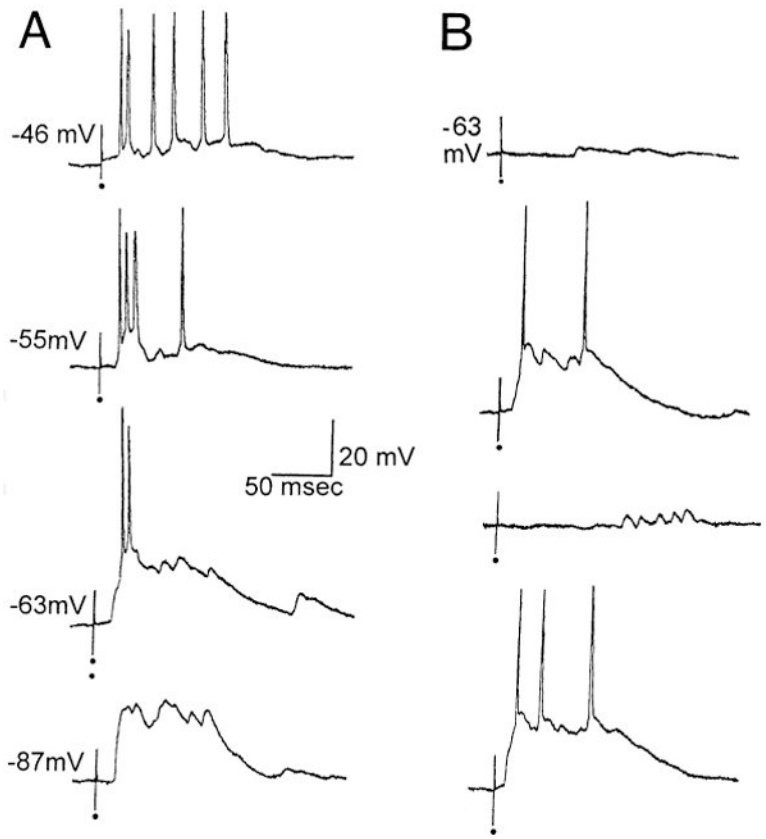

Fig. 8.

Evoked bursts of mossy cells in response to outer molecular layer stimulation. A: Recordings of a mossy cell from an epileptic rat are shown. The same stimulus was triggered at several membrane potentials. At the most hyperpolarized membrane potential (−87 mV), the stimulus evoked a complex EPSP. The dots mark stimulus artifacts, which are truncated. B: Responses to stimulation of the same cell as A. Four consecutive responses to the same stimulus are shown, using the same stimulus as for A, delivered at 0.05 Hz and at resting potential (−63 mV). This stimulus either produced no response (e.g. top trace) or a burst (e.g. second trace from the top), illustrating its all-or-none nature. Lower stimulus strengths evoked no response at all (data not shown). Same calibration as A.

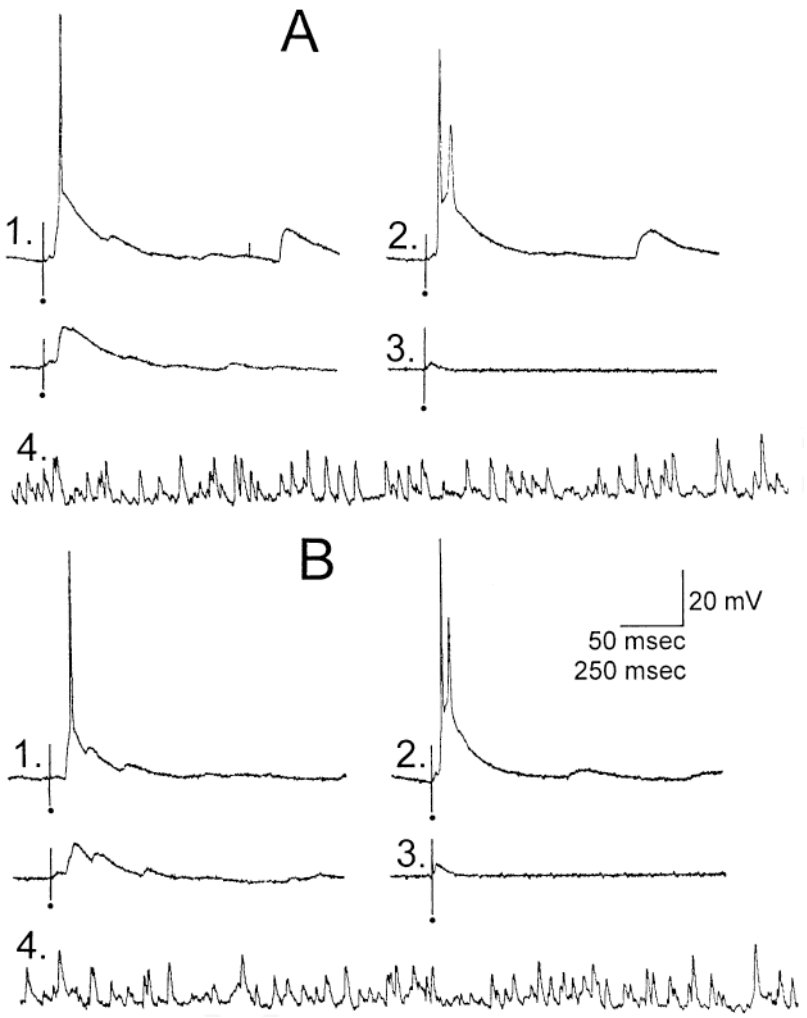

Fig. 9.

Recordings of mossy cells in age-matched controls. A: Recordings of a mossy cell from a saline control are shown. Calibrations are in B. The cell is shown in Fig. 5C. Immunocytochemical staining of sections from this rat is shown in Figs. 1A and 2A. In both A and B, the temporal calibration for #1–3 is 50 ms, and for #4 it is 250 ms. 1: Stimulation of the outer molecular layer at threshold evoked a relatively simple EPSP or AP. 2: Stimulation over threshold produced more than one AP, which is common for mossy cells in normal rats (Scharfman, 1993). 3: The response to the same stimulus, recorded just after exiting the cell. A small field potential was evoked. 4: Spontaneous activity recorded from the same cell illustrates no spontaneous bursts. B: Analogous recordings of a mossy cell from a status control. This cell is shown in Figs. 4D and 5D, and stained sections from the same animal are shown in Figs. 1B and 2B.

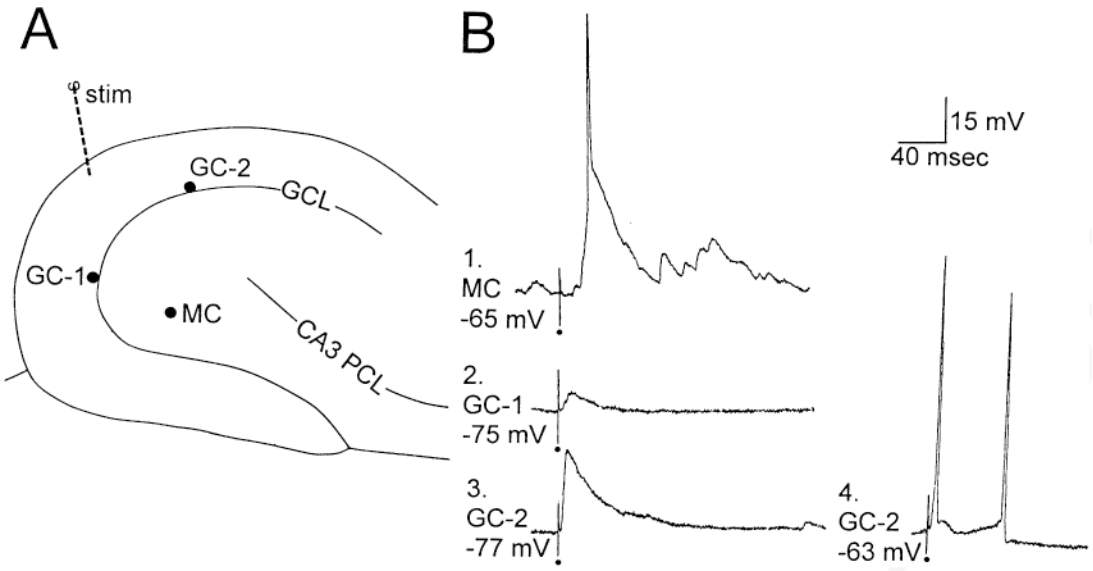

Fig. 10.

A mossy cell from an epileptic rat with a low threshold to outer molecular layer stimulation relative to granule cells. A: A diagram of the experimental preparation for B. Stim = stimulating electrode, placed in the outer molecular layer near the fissure. A mossy cell (MC) and two granule cells (GC-1, GC-2) were recorded sequentially. GCL = granule cell layer; PCL = pyramidal cell layer. B: 1: A stimulus to the mossy cell evoked a suprathreshold response. The cell was recorded at −65 mV, its resting potential. 2: The same stimulus evoked a subthreshold response in a granule cell located at the crest (GC-1) at resting potential, −75 mV. 3: The same stimulus evoked a subthreshold response in a second granule cell located in the upper blade (GC-2). 4: The same stimulus evoked APs in the second granule cell when it was depolarized to −63 mV with injected current.

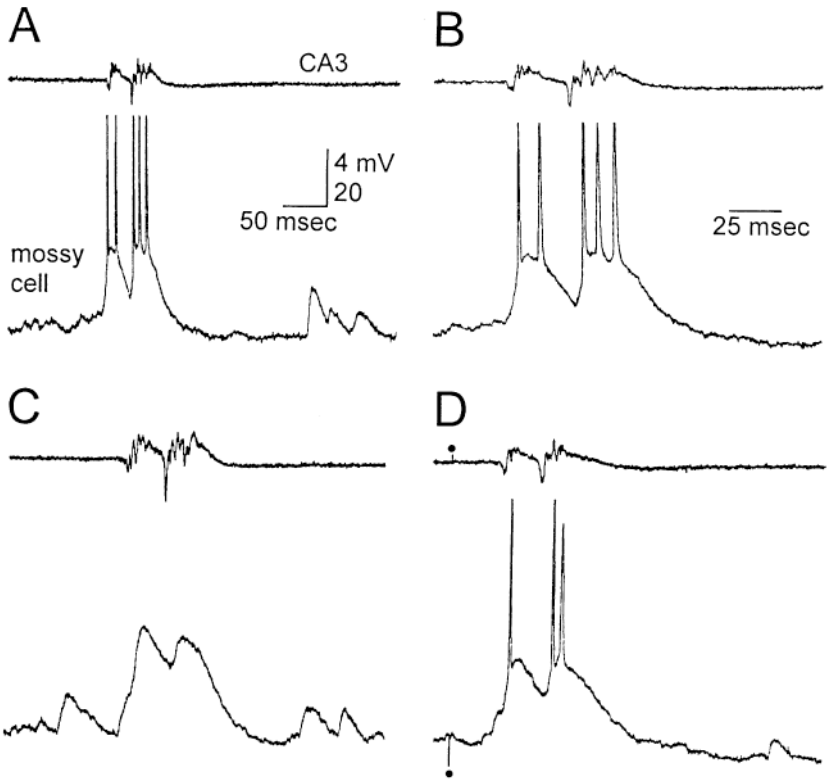

Fig. 11.

Simultaneous burst discharges in a mossy cell from an epileptic rat and the pyramidal cell layer. A: A mossy cell from an epileptic rat was recorded simultaneous to an extracellular recording from the CA3b pyramidal cell layer in the same slice. Synchronous burst discharge occurred spontaneously in both the cell and the pyramidal cell population. Calibration, top (CA3) = 4 mV, 35 ms; bottom (mossy cell), 20 mV, 35 ms. B: The recordings in A are shown with a different time base. Calibration, top (CA3) = 4 mV, 25 ms; bottom (mossy cell), 20 mV, 25 ms. C: Another spontaneous event, recorded from the same cell and pyramidal cell layer locations, with the mossy cell hyperpolarized using injected current, reveals a large EPSP underlying the burst discharge. Same calibration as in B. D: Outer molecular layer stimulation (at the dot) evoked burst discharges that were similar to the spontaneous burst discharges. Same calibration as in B.

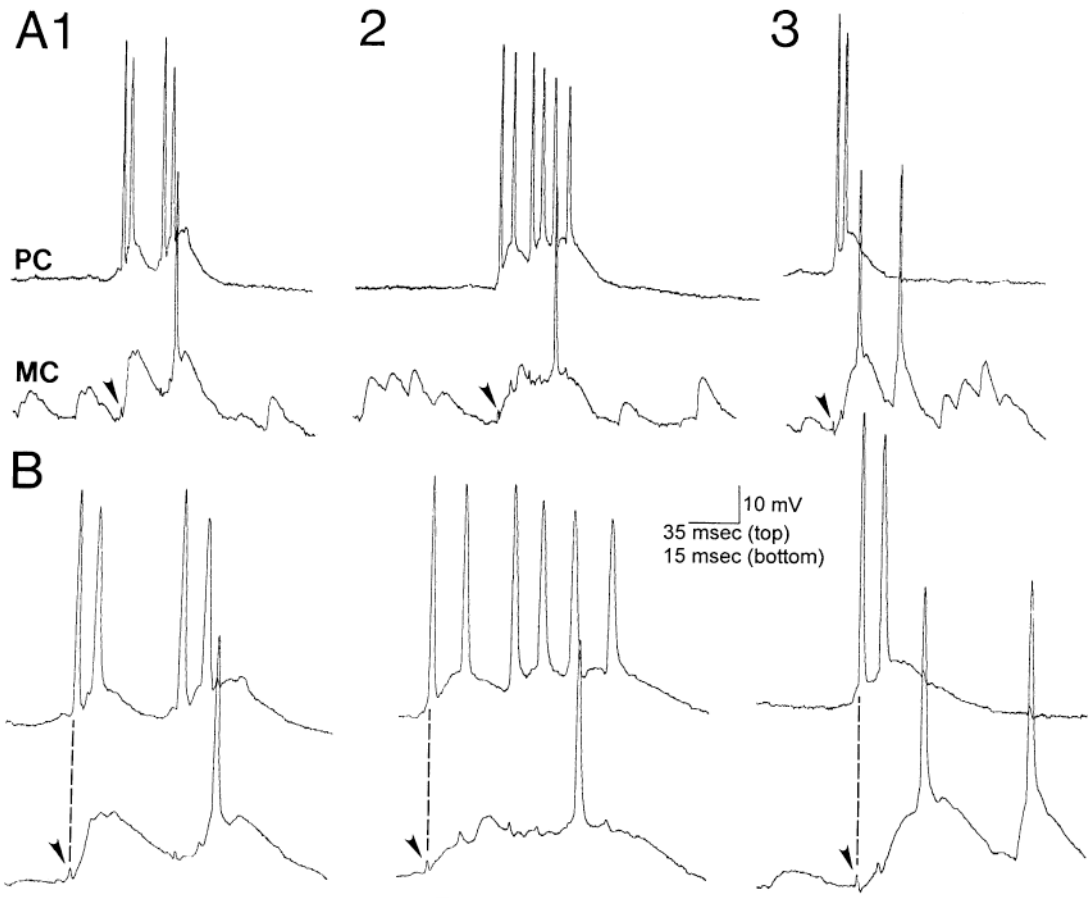

Fig. 12.

Simultaneous intracellular recordings of mossy cells and pyramidal cells in slices from epileptic rats. A: Simultaneously recorded burst discharges of a CA3b pyramidal cell (PC, top) and mossy cell (MC, bottom) are shown. The mossy cell is shown in Figs. 4C and 5C. The mossy cell was hyperpolarized with −0.3 nA DC current. The animal was killed 2.25 months after status epilepticus. B: The onset of the burst discharges is shown at a different scale to demonstrate the close temporal proximity of pyramidal cell APs and the initial depolarizations of the mossy cell (dotted line). The capacitative artifact (arrowheads) marks the rising phase of the pyramidal cell's AP. Note that mossy cell depolarizations begin immediately after the capacitative artifacts.

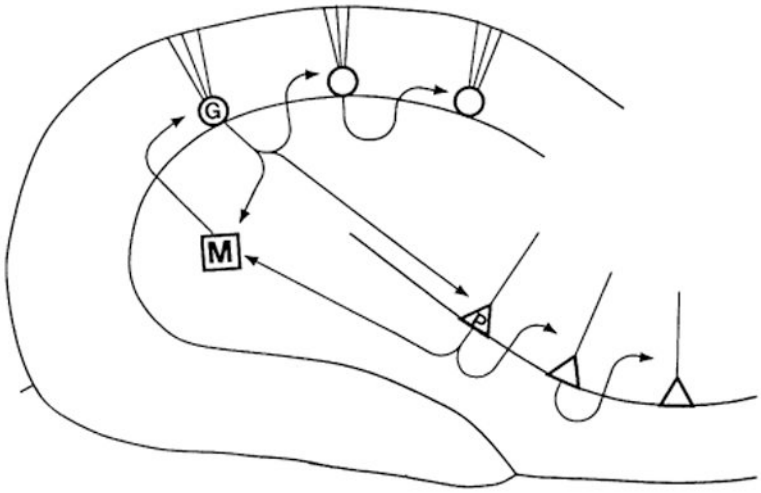

Fig. 13.

A circuit diagram of dentate gyrus/CA3 circuitry in epileptic rats illustrates potential excitatory feedback pathways. A simplified diagram of granule cell, mossy cell, and CA3 circuitry in epileptic rats illustrates that there are many potential recurrent excitatory pathways. Additional pathways are discussed in the text (see Discussion). G = granule cell, M = mossy cell, P = pyramidal cell. Only some of the dendrites and axons are shown in order to simplify the diagram. Other cell types are not shown.

Table 1

Membrane properties of mossy cells in pilocarpine-treated rats and controls

	RMP (mV)	R_{in} (MΩ)	τ (ms)	AP amplitude		AP slope		AP	AP duration	
				total (mV)	from threshold (mV)	max rise (V/S)	max fall (V/S)	dv/dt ratio	total duration (ms)	half-width (ms)
Epileptic										
Mean	−60.8	68.0	22.8	82.4	65.2	183.0	78.7	2.47	2.44	0.277
S.E.M.	1.85	4.84	4.29	2.23	2.09	16.80	8.61	0.18	0.21	0.026
n	5	10	10	10	10	10	10	10	7	9
Status controls										
Mean	−59.8	85.1	17.4	84.1	71.2	177.4	74.1	2.47	2.50	0.254
S.E.M.	0.79	9.08	3.80	2.72	2.59	17.72	6.00	0.25	0.18	0.034
n	6	8	8	8	8	8	8	8	5	8
Saline controls										
Mean	−56.8	72.2	15.3	83.6	68.8	174.9	63.4	2.79	2.42	0.251
S.E.M.	1.30	5.31	2.22	3.81	1.83	15.90	4.33	0.24	0.58	0.009
n	8	9	9	7	7	9	9	9	9	9

Membrane properties of mossy cells from epileptic, status control, and saline control rats. RMP = resting membrane potential. R_{in} = input resistance. τ = membrane time constant. Measurements are from an AP at threshold using injected current (see Experimental procedures). AP amplitude was measured from resting potential to peak (AP amplitude, total) or from its threshold (AP amplitude, from threshold). AP max rise refers to the maximum slope of the rising phase of an AP; AP max decay refers to the decay of the same AP; their ratio is the d*v*/d*t* ratio. AP duration was measured from its onset to the point it repolarized (AP duration, total duration), and AP half-width was also determined (for further definition see Experimental procedures). There were no significant differences (one-way ANOVA, $P>0.05$).

Table 2

Characteristics of EPSPs evoked by outer molecular layer stimulation at threshold

	Amplitude to peak (mV)	Latency to onset (ms)	Latency to peak (ms)	Half-duration (from onset) (ms)	Total duration (from onset) (ms)	Half-duration (from stimulus) (ms)	Total duration (from stimulus) (ms)	Interval onset to peak (ms)
Epileptic								
Mean	13.0	10.4	24.7	57.6[a]	100.3	68.0[a]	108.3	14.3
S.E.M.	2.4	1.6	14.6	11.0	14.6	10.4	16.2	3.7
n	6	7	7	7	7	7	7	7
Status control								
Mean	12.9	7.1	19.0	32.3	110.0[b]	39.0	103.5[b]	32.3
S.E.M.	2.4	1.6	4.3	4.7	—	6.6	—	4.7
n	4	4	3	3	1	3	1	3
Saline control								
Mean	8.4	10.6	16.4	24.4[a]	67.0	33.9[a]	76.0	7.0
S.E.M.	2.4	2.6	2.3	2.9	15.2	2.6	15.3	1.3
n	6	6	6	6	6	6	6	6

Measurements of EPSPs evoked at threshold by outer molecular layer stimulation are shown for all experimental groups. Duration was measured from the onset of the EPSP (from onset) or the stimulus artifact (from stimulus). Some cells are not included because they were not tested at threshold, or the threshold response included no detectable PSP in response to stimuli that could evoke APs in subsequent trials (Fig. 8B).

[a]Differences between the three groups were statistically significant by one-way ANOVA ($P>0.05$), and the epileptic vs. saline control means were significantly different by a Student's *t*-test ($P<0.05$) conducted subsequently.

[b]Total duration was not measured in all cases because spontaneous activity often interrupted the late phase of EPSPs. Therefore, total durations of epileptic and saline control groups were compared statistically using Student's *t*-tests. Differences were not significant ($P>0.05$).