Dysfunction of the Dentate Basket Cell Circuit in a Rat Model of Temporal Lobe Epilepsy

Wei Zhang¹ and Paul S. Buckmaster^{1,2}

Departments of ¹Comparative Medicine and ²Neurology and Neurological Sciences, Stanford University, Stanford, California 94305-5342

Temporal lobe epilepsy is common and difficult to treat. Reduced inhibition of dentate granule cells may contribute. Basket cells are important inhibitors of granule cells. Excitatory synaptic input to basket cells and unitary IPSCs (uIPSCs) from basket cells to granule cells were evaluated in hippocampal slices from a rat model of temporal lobe epilepsy. Basket cells were identified by electrophysiological and morphological criteria. Excitatory synaptic drive to basket cells, measured by mean charge transfer and frequency of miniature EPSCs, was significantly reduced after pilocarpine-induced status epilepticus and remained low in epileptic rats, despite mossy fiber sprouting. Paired recordings revealed higher failure rates and a trend toward lower amplitude uIPSCs at basket cell-to-granule cell synapses in epileptic rats. Higher failure rates were not attributable to excessive presynaptic inhibition of GABA release by activation of muscarinic acetylcholine or GABA_B receptors. High-frequency trains of action potentials in basket cells generated uIPSCs in granule cells to evaluate readily releasable pool (RRP) size and resupply rate of recycling vesicles. Recycling rate was similar in control and epileptic rats. However, quantal size at basket cell-to-granule cell synapses was larger and RRP size smaller in epileptic rats. Therefore, in epileptic animals, basket cells receive less excitatory synaptic drive, their pools of readily releasable vesicles are smaller, and transmission failure at basket cell-to-granule cell synapses is increased. These findings suggest dysfunction of the dentate basket cell circuit could contribute to hyperexcitability and seizures.

Introduction

Epilepsy is a common neurological disorder (Hirtz et al., 2007). In temporal lobe epilepsy, the most common type of epilepsy in adults, seizures initiate in the mesial temporal lobe (Engel et al., 1997). The mesial temporal lobe includes the dentate gyrus, which is suspected to play a role in epileptogenesis, in part, because it displays neuropathological changes (Margerison and Corsellis, 1966). In tissue from patients, dentate granule cells are hyperexcitable (Williamson et al., 1995, 1999; Gabriel et al., 2004). In laboratory animal models of temporal lobe epilepsy, granule cells receive less inhibitory synaptic input [for example, reduced frequency of miniature IPSCs (mIPSCs)], which has been attributed to loss of some interneurons (Kobayashi and Buckmaster, 2003; Sayin et al., 2003; Shao and Dudek, 2005; Sun et al., 2007), but additional factors may contribute.

Basket cells are GABAergic, form perisomatic synapses with principal cells, and control action potential discharge of many excitatory neurons (Cobb et al., 1995; Miles et al., 1996). Dentate basket cells efficiently inhibit granule cells, mainly because of large quantal sizes and high release probabilities at multiple synapses (Kraushaar and Jonas, 2000). A subset of dentate basket cells expresses the calcium-binding protein parvalbumin (Ribak et al., 1990) and accounts for 8–9% of all GABAergic interneu-

rons in the dentate gyrus (Austin and Buckmaster, 2004). Numbers of parvalbumin-immunoreactive interneurons appear to be reduced in the epileptic dentate gyrus (Zhu et al., 1997; Andrioli et al., 2007), but they are relatively more enduring than other interneuron subtypes (Sloviter et al., 1991; Buckmaster and Dudek, 1997; Wittner et al., 2001).

It is unclear whether surviving dentate basket cells function normally in epileptic tissue. It has been proposed they lose excitatory synaptic input and become inactive or dormant (Sloviter, 1994; Sloviter et al., 2003), but this hypothesis is controversial (Bernard et al., 1998). Many questions persist about synaptic inputs to and outputs from basket cells. For example, direct methods have not been used to evaluate spontaneous excitatory synaptic input to basket cells. Unitary IPSCs (uIPSCs) of basket cell-to-granule cell synapses have been evaluated in control (Kraushaar and Jonas, 2000; Harney and Jones, 2002) but not epileptic animals. Furthermore, it is unclear to what extent reduced frequency of mIPSCs in granule cells in epileptic tissue (see above) is attributable to loss of GABAergic synapses versus reduced probability of GABA release. It is important to distinguish between these possible underlying mechanisms, because each calls for different therapeutic strategies.

In the present study, excitatory synaptic input to dentate basket cells and uIPSCs at basket cell-to-granule cell synapses were examined in a rat model of temporal lobe epilepsy. We asked the following: (1) Do basket cells receive less excitatory synaptic input after an epileptogenic injury? (2) If so, is excitatory synaptic input restored as granule cell axons sprout? (3) Is reliability of GABAergic transmission reduced at basket cell-to-granule cell synapses in epileptic animals? (4) If so, what is the underlying mechanism?

Received Dec. 31, 2008; revised April 22, 2009; accepted May 12, 2009.

This work was supported by National Institutes of Health—National Institute of Neurological Disorders and Stroke. We are grateful to Dr. Masayuki Kobayashi for advice on paired recordings.

Correspondence should be addressed to Paul S. Buckmaster, Department of Comparative Medicine, 300 Pasteur Drive, R321 Edwards Building, Stanford University, Stanford, CA 94305-5342. E-mail: psb@stanford.edu.

DOI:10.1523/JNEUROSCI.6199-08.2009

Copyright © 2009 Society for Neuroscience 0270-6474/09/297846-11\$15.00/0

Materials and Methods

Animals. All experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the Stanford University Institutional Animal Care and Use Committee. Male Sprague Dawley rats (Harlan) were treated with pilocarpine when they were 24–63 d old, as described previously (Buckmaster, 2004). Briefly, pilocarpine (380 mg/kg, i.p.) was administered 20 min after atropine methyl bromide (5 mg/kg, i.p.). Diazepam (Hospira) was administered (10 mg/kg, i.p.) 2 h after onset of stage 3 or greater seizures (Racine, 1972) and repeated as necessary to suppress convulsions. Control rats included animals that were treated identically but did not develop status epilepticus ($n = 9$) and age-matched naive rats ($n = 23$). Control rats were 51 ± 4 d old (range 26–118 d) when slices were prepared. Control animals were compared with rats 3–7 d after status epilepticus ($n = 23$) to test for changes early after the epileptogenic treatment and epileptic rats ($n = 47$). At the time of slice experiments, 3–7 d poststatus epilepticus rats were 42 ± 2 d old (range 28–55 d), epileptic rats were 69 ± 3 d old (range 40–145 d), which was 36 ± 3 d (range 13–111 d) after pilocarpine treatment. Video monitoring, which began at least 10 d after pilocarpine treatment, verified spontaneous motor convulsions in all epileptic rats. No controls were observed to have spontaneous seizures.

Slice preparation. Animals were deeply anesthetized with urethane (1.5 g/kg, i.p.) and decapitated. Tissue blocks including the dentate gyrus were removed rapidly and stored for 3 min in modified ice-cold artificial CSF (mACSF) containing (in mM) the following: 230 sucrose, 2.5 KCl, 10 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 2.5 CaCl₂, and 10 D-glucose. Horizontal slices (300 μ m) were prepared with a microslicer (Leica; VT1000S). The most dorsal slice was used for histology. Slices were incubated at 32°C for 30 min in a submersion-type holding chamber that contained 50% mACSF and 50% normal ACSF, which consisted of (in mM) the following: 126 NaCl, 3 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 2 CaCl₂, and 10 D-glucose. After that, slices were transferred to normal ACSF at 32°C for 1 h. ACSF was aerated continuously with a mixture of 95% O₂ and 5% CO₂. Slices were maintained at room temperature until used for recording.

Recording. Cells were visualized with Nomarski optics (40 $\times$; Nikon) and an infrared-sensitive video camera (Hamamatsu Photonics). Whole-cell patch-clamp recordings were obtained at $32 \pm 1^\circ\text{C}$. Interneurons were recorded in either voltage-clamp mode or current-clamp mode (Axopatch 1D; Molecular Devices). Interneuron pipette solution contained (in mM) the following: 100 potassium gluconate, 40 HEPES, 20 biocytin, 10 EGTA, 5 MgCl₂, 2 disodium ATP, and 0.3 sodium GTP. Granule cells were recorded in voltage-clamp mode (Axopatch 200B; Molecular Devices). Granule cell pipette solution contained (in mM) the following: 120 cesium methanesulfonate, 20 biocytin, 10 HEPES, 5 NaCl, 5 QX-314, 2 magnesium ATP, 0.3 sodium GTP, and 0.1 BAPTA. The measured liquid junction potential was 7 mV, and all membrane potentials were corrected accordingly. Patch electrodes were pulled from borosilicate glass (1.5 mm outer diameter, 0.75 mm inner diameter, 3–4 M Ω). Seal resistance was >5 G Ω , and only data obtained from electrodes with access resistance <20 M Ω and $<20\%$ change during recordings were included. Series resistance was 80% compensated, and compensation was readjusted during experiments, when necessary. Drugs were bath applied. Data were acquired (pCLAMP; Molecular Devices) and stored on computer for off-line analysis. Membrane currents and potentials were low-pass filtered at 2 kHz and digitized at 10 kHz.

EPSCs were analyzed using Mini Analysis (Synaptosoft). Threshold for event detection was 3 times root mean square (rms) noise level, which was not significantly different between experimental groups. Epochs for analysis were at least 2 min. Rise time was measured as the interval between points corresponding to 10 and 90% of peak amplitude during the rising phase. Amplitude was measured as the difference between the peak amplitude and baseline. Decay time (τ) was measured as the interval between points corresponding to 100% and 37% of peak amplitude during the falling phase. Charge transfer/event was measured as area under individual detected events. Mean charge transfer was measured as cumulative charge transfer per minute.

uIPSCs were measured similarly to EPSCs. Latencies of uIPSCs were measured from peak of presynaptic action potentials to 5% of uIPSC amplitude. Failures were defined as events with amplitudes <3 times rms noise level. To estimate vesicle numbers in readily releasable pools (RRPs), quantal size was measured at basket cell-to-granule cell synapses using an established approach (Kraushaar and Jonas, 2000). Briefly, uIPSCs were recorded in slices bathed in ACSF containing 0.5 mM Ca²⁺ and 3.5 mM Mg²⁺. At the reduced Ca²⁺/Mg²⁺ ratio, failure rates increased to $>90\%$, and any uIPSCs that occurred were likely to be quantal events.

Histology. The most dorsal slice was immediately fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) at 4°C at least overnight, before storing in preservation solution (30% ethylene glycol and 25% glycerol in 50 mM PB) at -20°C . In some cases, the most dorsal slice was prepared for Timm staining by placing in 0.37% sodium sulfide at least 10 min before fixation. Fixed slices were cryoprotected in 30% sucrose in 0.1 M PB and sectioned with a microtome set at 40 μ m. Sections were mounted on slides and processed for Timm or Nissl stain, as described previously (Buckmaster and Dudek, 1997). The number of Nissl-stained neuron profiles per hilus was counted by an investigator blind to experimental group using a NeuroLucida system (MBF Bioscience).

Biocytin labeling. After recording, slices were placed in 4°C 4% paraformaldehyde in 0.1 M PB at least overnight and then stored at -20°C in preservation solution. Slices were rinsed in 0.5% Triton X-100 and 0.1 M glycine in 0.1 M PB and then placed in blocking solution containing 0.5% Triton X-100, 2% goat serum (Vector Laboratories), and 2% bovine serum albumin in 0.1 M PB for 4 h. Slices were incubated in mouse anti-NeuN serum (1:1000, MAB377; Millipore Bioscience Research Reagents) in blocking solution overnight. After a rinsing step, slices incubated with Alexa Fluor 594 streptavidin (5 μ g/ml) and Alexa Fluor 488 goat anti-mouse (10 μ g/ml; Invitrogen) in blocking solution overnight. Slices were rinsed, mounted on slides, and coverslipped with Vectashield (Vector Laboratories). For reconstruction, labeled basket cells were scanned with a confocal microscope (LSM 5 Pascal; Zeiss) at a magnification just large enough to include the entire dendritic arbor. Stack height was adjusted to include all dendritic processes. Optical section interval was 3 μ m. Basket cells were reconstructed three-dimensionally using a NeuroLucida system and measured using Neuroexplorer (MBF Bioscience).

Synaptophysin immunocytochemistry. Some slices that contained biocytin-labeled basket cells were processed for synaptophysin immunocytochemistry. After fixation as described above, slices were cryoprotected in 30% sucrose in PB and sectioned with a sliding microtome set to 20 μ m. Free-floating sections rinsed in 0.1 M Tris-buffered saline (TBS) before 2 h exposure to blocking solution containing 3% goat serum, 2% bovine serum albumin, and 0.3% Triton X-100 in 0.05 M TBS. After rinsing in TBS, sections incubated overnight in mouse anti-synaptophysin serum (1:1000; Millipore Bioscience Research Reagents; MAB5258) in 1% goat serum, 0.2% bovine serum albumin, and 0.3% Triton X-100 in 0.05 M TBS at 4°C. After rinsing in TBS, sections were exposed for 3 h to 10 μ g/ml goat anti-mouse Alexa 498 and 5 μ g/ml streptavidin Alexa 594 (Invitrogen) in 2% bovine serum albumin and 0.3% Triton X-100 in 0.05 M TBS. After rinsing in TBS, sections were mounted on slides and coverslipped with Vectashield (Vector Laboratories). For analysis of synaptophysin-positive punctae apposed to basket cell membranes, sections were scanned with a 63 $\times$ oil objective and confocal microscope (Zeiss). Stacks of images were collected through entire depth of tissue sections at 0.5 μ m intervals. Most synaptophysin immunoreactivity was limited to 4–5 μ m depth from section surfaces, and analysis was limited to those regions. Synaptophysin-positive punctae were checked for direct apposition to basket cell membranes using orthogonal views (Zeiss; LSM Image Browser). Basket cell membrane surface areas were measured by reconstructing soma and dendrites with NeuroLucida (MBF Bioscience). Three cell regions were analyzed: soma; proximal dendrites, which were within hilus, granule cell layer, and inner third of molecular layer; and distal dendrites, which were within outer two-thirds of molecular layer. In each region, number of synaptophysin-positive punctae per μm^2 of basket cell membrane was calculated.

All chemicals and drugs were obtained from Sigma, unless specified

otherwise. Results are reported as mean $\pm$ SEM. Statistics were performed using Sigma Stat (Systat). $p < 0.05$ was considered significant.

Results

Hilar neuron loss and mossy fiber sprouting in epileptic rats

Previous studies found fewer hilar neurons and aberrant mossy fiber sprouting in epileptic pilocarpine-treated rats (Mello et al., 1993), similar to patients with temporal lobe epilepsy (Babb et al., 1991). To determine whether similar changes occurred in rats of the present study, Nissl-stained hilar neuron profiles were counted in sections from slices adjacent to those used for recording. Hilar neuron loss was evident in rats 3–7 d after status epilepticus and in epileptics (Fig. 1*A–C*). Average numbers of hilar neuron profiles per section were significantly reduced in rats 3–7 d after status epilepticus (111 ± 10 , $n = 22$) and epileptics (89 ± 6 , $n = 17$) compared with controls (179 ± 6 , $n = 20$, $p < 0.05$, Kruskal–Wallis ANOVA on ranks with Dunn's method). The difference between rats 3–7 d after status epilepticus and epileptics was not significant. Slices from a subset of control ($n = 2$) and epileptic rats ($n = 6$) were prepared for Timm staining. Mossy fiber sprouting was evident in all epileptic rats and no controls (Fig. 1*D,E*). These findings verified typical temporal lobe epilepsy-related neuropathological changes in dentate gyrus of epileptic rats used in the present study.

Basket cell identification

Neurons with a relatively large soma at the border of the granule cell layer and hilus were selected for recording and tentatively identified as basket cells during experiments, based on previously described electrophysiological criteria (Lübke et al., 1998; Kraushaar and Jonas, 2000; Harney and Jones, 2002), including brief action potentials, non-adapting high-frequency firing in response to depolarizing current injection, and frequent spontaneous EPSPs (Fig. 2*A*). However, other types of interneurons are found at the hilar/granule cell layer border, and their electrophysiological properties may overlap to some degree with those of basket cells (Scharfman, 1995; Mott et al., 1997). Therefore, all recorded cells were labeled with biocytin to determine whether their axon collaterals concentrated in the granule cell layer, which is a defining feature of basket cells (Freund and Katona, 2007). Of 181 interneurons, 153 displayed anatomical features of basket cells. Their firing frequency in response to a 750 pA depolarizing current injection was 140 ± 5 Hz, and spike width was 0.68 ± 0.03 ms. In addition to basket cells, 11 interneurons were identified whose axon collaterals concentrated in the inner molecular layer (Fig. 2*B*). They have been called hilar cells forming a dense axonal plexus in the commissural and associational pathway terminal field (HICAP cells) (Han et al., 1993) and are likely to be immunoreactive for cholecystokinin (Hefft and Jonas, 2005) with axon terminals expressing cannabinoid receptors, unlike parvalbumin-positive basket cells (Morozov and Freund, 2003). Another type of interneuron ($n = 2$) concentrated its axon collaterals in the outer molecular layer (Fig. 2*B*). They have been called hilar cells with their axon ramifying in the perforant path terminal field (HIPPA cells) (Han et al., 1993) and are likely to be somatostatin-immunoreactive (Leranth et al., 1990). The most frequently encountered type of pyramidal-shaped neuron at the hilar/granule cell border was regular spiking and had a prominent apical dendrite. Many of these nonbasket cells were morphologically identified, and 15 had well labeled axon collaterals that extended diffusely throughout the entire width of the molecular layer (Fig. 2*B*). Cells with similar morphology have been identified by Golgi staining and are GABAergic (Soriano and

Figure 1. Hilar neuron loss and mossy fiber sprouting in epileptic rats. *A–C*, Nissl-stained sections of dentate gyrus from a naive control (*A*), a rat 4 d after status epilepticus (*B*), and an epileptic rat (*C*). m, Molecular layer; g, granule cell layer; h, hilus; CA3, CA3 pyramidal cell layer. *D, E*, Timm-stained sections of dentate gyrus from a pilocarpine-treated control (*D*) and an epileptic rat (*E*). Arrow indicates black, aberrant Timm-staining in the inner molecular layer of the epileptic rat.

Figure 2. Identification of dentate basket cells. **A**, Dentate basket cells were identified initially based on electrophysiological characteristics, including action potential duration <1 ms, non-adapting high-frequency spike firing, and frequent spontaneous EPSPs (arrows). Responses to injected current, -100 pA and $+800$ pA. Subsequently, basket cell identity was verified based on the presence of biocytin-labeled axon concentrated in the granule cell layer (g) (arrowheads). m, Molecular layer; h, hilus. **B**, Other interneurons (HICAP, HIPP, and regular-spiking interneurons) were found at the border of the granule cell layer and hilus but concentrated their axon projections in the molecular layer (arrowheads) and were excluded from further analysis.

Frotscher, 1993). Data presented below were obtained only from neurons identified as basket cells by both electrophysiological and morphological characteristics. However, although no cells displayed obvious vertical aggregations of axonal varicosities (Soriano et al., 1990), without electron microscopic identification of their synaptic targets it is possible that some neurons identified as basket cells in the present study actually were axo-axonic cells (Somogyi and Klausberger, 2005).

Excitatory synaptic input to basket cells

Excitatory synaptic input to basket cells was evaluated by recording EPSCs at a holding potential of -60 mV (Fig. 3). Spontaneous EPSCs (sEPSCs) were recorded in normal ACSF, miniature EPSCs (mEPSCs) after addition of $1 \mu\text{M}$ tetrodotoxin (Tocris Bioscience). For sEPSCs, amplitudes and rise times were similar among controls ($n = 26$ cells, 16 rats), rats 3–7 d after status epilepticus ($n = 20$ cells, 16 rats), and epileptics ($n = 37$ cells, 23 rats) (Fig. 3C). Average decay time was significantly shorter in rats 3–7 d after status epilepticus. In epileptic and 3–7 d poststatus epilepticus rats, average frequencies and mean charge transfers of sEPSCs were reduced to 62–68% and 46–50% of control values, respectively, but variability was high and differences were not significant. For mEPSCs, amplitudes, rise times, and decay times were similar among controls ($n = 17$ cells, 8 rats), rats 3–7 d after status epilepticus ($n = 8$ cells, 5 rats), and epileptics ($n =$

23 cells, 11 rats). In epileptic and 3–7 d poststatus epilepticus rats, average frequencies and mean charge transfers were reduced to 48–51% and 44–48% of control levels, respectively, and differences were significant for the epileptic group. Adjacent slices from a subset of epileptic rats ($n = 6$ cells, 4 rats) were processed for Timms staining. All displayed aberrant mossy fiber sprouting (Fig. 1E). Average frequency of mEPSCs of this subset (5.0 ± 1.0 Hz) was similar to that of the entire sample of epileptic rats (6.8 ± 1.1 Hz). These findings suggest excitatory synaptic input to basket cells decreases immediately or shortly after pilocarpine-induced status epilepticus and remains low in epileptic rats.

Pilocarpine-induced status epilepticus causes excitotoxicity, and distal dendrites of some dentate interneurons are particularly susceptible to excitotoxicity (Zhu et al., 1997; Oliva et al., 2002). To test the possibility that mEPSC frequency might be reduced in basket cells because their dendrites retract or branches are lost after status epilepticus, biocytin-labeled basket cells in control ($n = 9$ cells, 7 rats) and epileptic rats ($n = 8$ cells, 7 rats) were three-dimensionally reconstructed and measured (Fig. 4A,B). Qualitatively, basket cells in both groups looked similar. Somata were triangular, round, or polygonal. Soma area was similar in control ($429 \pm 65 \mu\text{m}^2$) and epileptic rats ($399 \pm 41 \mu\text{m}^2$) (Fig. 4D). Dendrites extended into the molecular layer and hilus. Control and epileptic rats had similar numbers of primary dendrites (4.2 ± 0.4 vs 4.4 ± 0.4) and dendritic ends (40 ± 3 vs 40 ± 4).

Figure 3. Reduced excitatory synaptic input to dentate basket cells in pilocarpine-treated epileptic rats. *A, B*, Spontaneous (*A*) and miniature EPSCs (*B*) recorded in basket cells from controls, rats 3–7 d after status epilepticus, and epileptics. Expanded views indicated by bars. *C*, Amplitude (number of recorded basket cells indicated), rise time (10–90%), τ (100–37% decay time), mean charge transfer per minute, and frequency of spontaneous and miniature EPSCs. Error bars indicate SEM. * $p < 0.05$, compared with control and epileptic sEPSCs; ** $p < 0.05$, compared with control mEPSCs (ANOVA).

Cumulative dendritic length/cell in epileptic rats ($5376 \pm 528 \mu\text{m}$) was 119% of controls ($4530 \pm 393 \mu\text{m}$), but the difference was not significant (Fig. 4*D*). These findings suggest dendritic length was not reduced in epileptic rats, and, therefore, reduced

frequency of mEPSCs was not likely caused by loss or retraction of basket cell dendrites.

To test the possibility that mEPSC frequency might be reduced in basket cells because they receive less excitatory synaptic input in epileptic rats, biocytin-labeled basket cells in control ($n = 7$ cells, 6 rats) and epileptic rats ($n = 10$ cells, 7 rats) were evaluated for apposed synaptophysin-immunoreactive puncta (Fig. 4*C*). Three basket cell regions were analyzed: somata, proximal dendrites, and distal dendrites. Proximal dendrites were in granule cell layer, inner molecular layer, or hilus. In all three subregions, synaptophysin-positive puncta densities were similar, so results were combined as a single “proximal dendrite” group. In both control and epileptic rats, densities of synaptophysin-positive puncta were similar on somata and proximal dendrites and higher on distal dendrites ($p < 0.05$, ANOVA, Student–Neuman–Keuls method). However, there were no significant differences between control and epileptic rats (Fig. 4*E*). These findings suggest basket cells in epileptic rats might receive similar numbers of synapses as in controls.

Basket cell-to-granule cell uIPSCs

To evaluate synaptic input from basket cells to granule cells, recordings were obtained from monosynaptically coupled pairs (Fig. 5*A–D*). Basket cells were recorded with potassium gluconate-based pipette solution, granule cells with cesium methanesulfonate-based solution that included QX-314. IPSCs were enhanced and EPSCs minimized by holding granule cells at 0 mV. Recordings were obtained from somata $<200 \mu\text{m}$ apart. Action potentials were evoked in basket cells by brief (1.2 ms) depolarizing (1.5 nA) current injection (0.1 Hz), while uIPSCs were recorded in granule cells at consistent and short latencies after basket cell action potentials (1.14 ± 0.03 ms), similar to previous reports (Kraushaar and Jonas, 2000; Harney and Jones, 2002). Average latencies were similar among experimental groups. Probability of detecting synaptically coupled cells was similar in controls (68%, 70 of 103 pairs tested), rats 3–7 d after status epilepticus (69%, 53 of 77), and epileptics (64%, 98 of 153). Reliability of transmission at basket cell synapses in control animals ($n = 28$ pairs, 16 basket cells, 16 rats) was high (Fig. 5*E*), as reported previously (Kraushaar and Jonas, 2000; Bartos et al., 2001). However, failures of uIPSCs were 2.3 times more likely in epileptic rats ($n = 64$ pairs, 39 basket cells, 31 rats) compared with controls. Failure rate in rats 3–7 d after status epilepticus ($n = 32$ pairs, 15 basket cells, 13 rats) was 1.8 times control levels, but the difference was not significant. At 3–7 d after status epilepticus, rise times were shorter (84–91% of epileptic and control values, respectively), decay times were shorter (81–83% of epileptic and control values, respectively), and amplitudes were higher (1.5–2.1 times control and epileptic values, respectively), suggesting possible changes in GABA_A receptors by granule cells (Brooks-Kayal et al., 1998). In epileptic rats, uIPSC amplitude and charge transfer (excluding failures) were 67–71% of control levels, but differences were not significant.

Testing possible mechanisms of failure at basket cell-to-granule cell synapses

Several possible mechanisms were tested that might account for increased failure rate of uIPSCs at basket cell-to-granule cell synapses in epileptic rats. Previous studies found that in control rats, failure rates at basket cell-to-granule cell synapses increase when muscarinic acetylcholine (mACh) receptors are activated (Harney and Jones, 2002) (but see Hefft et al., 2002). Cholinergic fibers sprout near the granule cell layer in epileptic pilocarpine-

Figure 4. Similar dentate basket cell somatic and dendritic morphology and synaptophysin-immunoreactive punctae density in control and epileptic rats. **A**, Examples of basket cells recorded and biocytin-labeled in control and epileptic rats. m, Molecular layer; g, granule cell layer; h, hilus. **B**, Reconstructions of basket cells from control (left) and epileptic rats (right). *Same basket cells shown in **A**. **C**, Biocytin-labeled basket cells (red) and synaptophysin immunoreactivity (green) in a control (left) and epileptic rat (right). **D**, Soma area and total dendritic length per basket cell were similar in control and epileptic rats. **E**, Densities of

treated rats (Holtzman and Lowenstein, 1995), making excessive cholinergic activation a reasonable possibility. To test whether increased mACh receptor activation in epileptic rats might account for increased failure rate, uIPSCs were recorded before and during application of $5 \mu\text{M}$ atropine (Fig. 6). Failure rate was 0.32 ± 0.05 before and 0.27 ± 0.06 after applying atropine, which was not significantly different ($n = 6$ pairs, 5 rats, $p = 0.22$, paired t test). These findings suggest excessive mACh receptor activation is not likely to cause failure at basket cell-to-granule cell synapses in epileptic rats.

Previous studies have found that in control rats activation of GABA_B receptors increases failure rates at basket cell-to-granule cell synapses (Hefft et al., 2002). In a mouse model of temporal lobe epilepsy, GABA_B receptor expression increases in the granule cell layer (Straessle et al., 2003) where basket cell axon terminals are located. To test whether increased GABA_B receptor activation might account for increased failure rate in epileptic rats, uIPSCs were recorded before and during application of $10 \mu\text{M}$ CGP55845 (Tocris Bioscience), a GABA_B receptor antagonist (Fig. 6). Previous studies have shown that blocking GABA_B receptors increases paired-pulse ratios of IPSCs in granule cells (Mott et al., 1993). Similarly in the present study, application of $10 \mu\text{M}$ CGP55845 in epileptic rats increased paired-pulse ratios (second/first uIPSC peak amplitude) evoked at 20 ms interstimulus intervals (0.62 ± 0.10 vs 0.78 ± 0.06 , $p < 0.05$, paired t test, $n = 3$ pairs, 3 rats), suggesting effective blockade of GABA_B receptors. However, uIPSC failure rates did not decrease: 0.20 ± 0.07 before and 0.30 ± 0.09 after applying CGP55845, which was not significantly different. These findings suggest excessive GABA_B receptor activation is not likely to cause uIPSC failure at basket cell-to-granule cell synapses in epileptic rats.

High-frequency transmission at basket cell-to-granule cell synapses

At some synapses, probability of release correlates with size of the RRP of synaptic vesicles (Schikorski and Stevens, 1997). RRP size can be estimated functionally by evaluating postsynaptic responses to trains of presynaptic action

←

synaptophysin-positive punctae were higher on distal dendrites versus somata and proximal dendrites. There were no significant differences between control and epileptic rats.

potentials (Schneggenburger et al., 1999), including action potentials of GABAergic hippocampal interneurons (Moulder and Mennerick, 2005). Differences in frequency-dependent depression of GABAergic versus glutamatergic synapses are important for functional stability of neocortical circuits (Galarreta and Hestrin, 1998; Varela et al., 1999). In the present study, trains of 20 action potentials in basket cells were evoked at 50 Hz, uIPSCs were recorded in granule cells, and 10–50 traces were averaged (Fig. 7A). As reported previously for basket cells in control animals, synaptic transmission showed marked depression in early phases of trains, then stabilized during later phases (Kraushaar and Jonas, 2000; Bartos et al., 2001). After ~100 ms (5–6 basket cell action potentials), cumulative amplitudes of granule cell uIPSCs increased linearly at steady-state levels (Fig. 7B), which reflect resupply rates of recycling vesicles. To estimate RRP cumulative amplitude, vesicle resupply rate was factored out by extrapolating regression lines, calculated from values ≥ 200 ms (last 10 basket cell action potentials of a train), back to time 0. Average RRP cumulative amplitude in epileptic rats ($n = 41$ pairs, 21 basket cells, 18 rats) was 84% of control values (26 pairs, 15 basket cells, 15 rats), but the difference was not statistically significant (Fig. 7D). Average slopes of regression lines were similar in control and epileptic rats. These findings suggest a trend toward reduced RRP cumulative amplitude in epileptic rats but similar vesicle recycling rates at basket cell-to-granule cell synapses.

Number of vesicles in RRP can be estimated by dividing RRP cumulative amplitude by quantal size. In a subset of uIPSC recordings, quantal size at basket cell-to-granule cell synapses was measured under conditions of very low release probability ($0.5 \text{ mM Ca}^{2+}/3.5 \text{ mM Mg}^{2+}$) (Fig. 7C). Average amplitude of putative quantal IPSCs (failures excluded) in epileptic rats ($n = 24$ pairs, 12 basket cells, 9 rats) was significantly increased to 1.4 times control values ($n = 10$ pairs, 5 basket cells, 5 rats) (Fig. 7E). Numbers of vesicles in RRP were estimated, and epileptic values were significantly reduced to 67% of control levels.

Discussion

The principal findings of this study are that dentate basket cells receive less excitatory synaptic input, readily releasable pool size of basket cell synapses is reduced, and transmission at basket cell-to-granule cell synapses is more likely to fail in a rat model of temporal lobe epilepsy. These findings reveal dysfunction of the dentate basket cell circuit, which could contribute to hyperexcitability and seizures.

Figure 5. Higher failure rate of unitary IPSCs at dentate basket cell-to-granule cell synapses in epileptic rats. **A–D**, uIPSCs were evoked in granule cells by discharging action potentials in presynaptic basket cells in controls (**A**, **C**), rats 3–7 d after status epilepticus, and epileptics (**B**, **D**). Biocytin labeling revealed recorded granule cells (arrowheads) surrounded by axons concentrated in the granule cell layer (g) from recorded basket cells (arrows). There are two closely apposed labeled granule cells in the control rat. m, Molecular layer; h, hilus. **C**, **D**, Failures of uIPSCs indicated by red traces. **E**, Failure rate, amplitude, 10–90% rise time, 100–37% decay time, and charge transfer/event of uIPSCs. Failures were excluded for all parameters except failure rate. Error bars indicate SEM. * $p < 0.05$ (Kruskal–Wallis ANOVA on ranks).

Basket cells receive less excitatory input in epileptic animals

In epileptic rats, frequencies of spontaneous and miniature EPSCs in dentate basket cells were only half control values. It is unclear whether excitatory input to other dentate interneurons also is reduced. Basket cell dendritic length does not decrease

Figure 6. High failure rate of unitary IPSCs in epileptic rats is independent of presynaptic mACh and GABA_B receptor activation. **A**, Unitary IPSCs in granule cells evoked by single action potentials in basket cells of epileptic rats in normal ACSF (top traces), 5 μ M atropine (left bottom traces), and 10 μ M CGP 55845 (right bottom traces). Red traces indicate failures. **B**, There were no significant changes in failure rates in the presence of atropine or CGP 55845. Black bars indicate average failure rates of six pairs tested with atropine and three pairs tested with CGP 55845. Gray symbols indicate values of individual pairs.

following status epilepticus, so target area for excitatory synaptic input is maintained. However, status epilepticus kills glutamatergic neurons, including granule cells (Obenaus et al., 1993), layer II entorhinal cortical neurons (Kumar and Buckmaster, 2006), and CA3 pyramidal cells (Nadler et al., 1978), all of which synapse with basket cells (Kneisler and Dingledine, 1995a,b; Geiger et al., 1997). Mossy cells also are vulnerable (Buckmaster and Jongen-Rêlo, 1999) and might synapse with basket cells (Buckmaster et al., 1996). Thus, loss of excitatory afferents is a possible mechanism for reduced excitatory input to basket cells.

It has been proposed that after status epilepticus, basket cells lose excitatory synaptic input especially from vulnerable mossy cells (Sloviter, 1994) and that subsequently excitatory drive to basket cells is restored by sprouted granule cell axons (Sloviter et al., 2003). If so, one might expect mEPSC frequency in basket cells to decrease initially but then increase later. Although mEPSC frequency was reduced in rats 3–7 d after status epilepticus, it did not recover in epileptic rats but instead remained low. Recordings were obtained from epileptic animals an average of 36 d and up to 111 d after status epilepticus, sufficient time for mossy fiber sprouting to develop. Densities of apposed synaptophysin-positive punctae were similar in control and epileptic rats. Synaptophysin is a presynaptic marker of both GABAergic and glutamatergic synapses (Jahn et al., 1985; Wiedenmann and Franke, 1985), but in hippocampus <8% of synaptophysin immunoreactivity coincides with GABA markers (Hiscock et al., 2000). These findings suggest if basket cells lose excitatory input after status epilepticus it might be restored to control levels in epileptic rats.

Another possible mechanism for reduced excitatory input is reduced probability of release at glutamatergic synapses with basket cells. Excitatory synaptic inputs from granule cells and CA3

pyramidal cells to interneurons at the hilar/granule cell layer border, including basket cells, display enhanced short-term depression in the week after pilocarpine-induced status epilepticus, suggesting functional disconnection of basket cells from their afferents (Doherty and Dingledine, 2001). Results of the present study are consistent with that conclusion.

Impaired transmission at basket cell-to-granule cell synapses

Inhibitory synaptic input to dentate granule cells is reduced in patients with temporal lobe epilepsy (Williamson et al., 1995, 1999). And in rat models of temporal lobe epilepsy, frequencies of mIPSCs in granule cells are only 35–70% of control values (Kobayashi and Buckmaster, 2003; Shao and Dudek, 2005; Sun et al., 2007) (but see Leroy et al., 2004). Loss of inhibitory interneurons might contribute to reduced mIPSC frequencies, but most GABAergic interneurons survive in dentate gyrus of patients (Babb et al., 1989) and models of temporal lobe epilepsy (Buckmaster and Jongen-Rêlo, 1999). Another mechanism that could contribute to less frequent mIPSCs in granule cells is reduced probability of GABA release. In support,

uIPSCs at basket cell-to-granule cell synapses were 2.3 times more likely to fail in epileptic rats compared with controls. It is unclear whether synaptic efficacy to granule cells from other dentate interneurons is reduced. Some CA1 basket cells are cholecystikinin (CCK)-immunoreactive and express cannabinoid receptors on their axon terminals (Katona et al., 1999; Tsou et al., 1999). At those synapses, GABAergic transmission is modulated by cannabinoid receptor activation (Glickfeld and Scanziani, 2006), and cannabinoid-dependent presynaptic inhibition of GABA release increases long-term after febrile seizures (Chen et al., 2003). In dentate gyrus, however, CCK-immunoreactive interneurons are HICAP cells not basket cells (Hefft and Jonas, 2005). In the present study, HICAP cells were excluded from analysis on the basis of axon projections, so cannabinoid receptor activation is unlikely to account for failure of dentate basket cell-to-granule cell synapses in epileptic tissue. Unitary IPSC failure does not appear to involve activation of presynaptic muscarinic acetylcholine or GABA_B receptors, the most likely candidates (Hefft et al., 2002), but we cannot exclude the possibility that other receptor systems may contribute.

Another potential mechanism of increased uIPSC failure is fewer vesicles in RRP at basket cell-to-granule cell synapses. We estimate an average of 6.4 vesicles/RRP in control rats, which is similar to numbers of functional release sites at basket cell-to-granule cell synapses (Kraushaar and Jonas, 2000) and synaptic contacts formed by hippocampal basket cells with individual principal neurons (Buhl et al., 1994; Miles et al., 1996). Together, these findings suggest basket cells normally may release one vesicle per release site, unlike mossy fiber-associated interneurons (Biró et al., 2006). Epileptic rats had only 4.3 vesicles/RRP at basket cell-to-granule cell synapses. Smaller RRP could contrib-

ute to increased failure rate of uIPSCs. It is unclear whether RRP size is smaller because of fewer synaptic contacts, reduced probability of release at individual contacts, or both. There were no obvious deficits in axon projections of basket cells. Probability of finding uIPSCs in granule cells postsynaptic to a basket cell was similar in control and epileptic rats, and granule cells from patients with temporal lobe epilepsy receive abundant structural synapses from perisomatic parvalbumin-positive axon terminals (Wittner et al., 2001).

Previous studies revealed plasticity in RRP size at hippocampal GABAergic synapses. Estrogen reduces inhibition of CA1 pyramidal cells and shifts synaptic vesicles away from release sites at GABAergic synapses with pyramidal cell somata (Ledoux and Woolley, 2005). Brain-derived neurotrophic factor increases release probability and RRP size at GABAergic synapses in hippocampal cell cultures (Baldelli et al., 2005). In models of temporal lobe epilepsy, mIPSCs are less frequent in CA1 pyramidal cells, and densities of synaptic vesicles are reduced in GABAergic synapses with pyramidal cell bodies, but that was attributed to reserve pools, not RRP (Hirsch et al., 1999). Visual deprivation causes neocortical fast-spiking GABAergic interneurons to reduce amplitudes of uIPSCs they generate in star pyramidal neurons, possibly by reducing release probability (Maffei et al., 2004). By analogy, when dentate basket cells receive less excitatory synaptic input in epileptic animals, they may reduce release probability by some homeostatic mechanism gone awry.

However, some aspects of basket cell-to-granule cell synaptic transmission appear normal or enhanced. During high-frequency trains of action potentials, vesicle resupply rates were similar in control and epileptic rats. These findings suggest once recruited basket cells can sustain inhibition of granule cells during periods of high activity. Furthermore, in epileptic rats, quantal size at basket cell-to-granule cell synapses was 1.4 times control levels. Similarly, amplitudes of mIPSCs, which likely arise from multiple types of interneurons, are 1.3–1.5 times control values in granule cells of rat models of temporal lobe epilepsy (Cohen et al., 2003; Kobayashi and Buckmaster, 2003; Leroy et al., 2004) (but see Shao and Dudek, 2005). In kindled rats, granule cells have larger amplitude mIPSCs and more GABA_A receptors per somatic synapse (Nusser et al., 1998). However, although quantal size increases at GABAergic synapses, uIPSCs fail more often, RRP size decreases, and uIPSC amplitude tends to decrease at basket cell-to-granule cell synapses. Therefore, despite some preserved and enhanced aspects, synaptic transmission at basket cell-to-granule cell synapses is impaired in epileptic animals.

Figure 7. High-frequency transmission and quantal size at basket cell-to-granule cell synapses reveal smaller RRP in epileptic rats. **A**, Trains (20 pulses at 50 Hz) of uIPSCs in a control (left) and epileptic rat (right). Averages of at least 10 traces. **B**, Corresponding plots of cumulative amplitude versus time. Regression lines were calculated from last 10 responses. Regression line y-intercepts indicate RRP cumulative amplitude, and slopes indicate steady-state vesicle resupply rate. **C**, Putative quantal uIPSCs (blue traces) recorded in bathing medium that included 0.5 mM Ca²⁺/3.5 mM Mg²⁺. Quantal averages shown in black, failures in red. **D**, Group averages of regression line y-intercepts (RRP cumulative amplitude) and slopes (vesicle recycling rate). No significant differences. **E**, In a subset of recordings, quantal amplitude was measured. In those cases, number of vesicles per RRP was calculated by dividing cumulative amplitude intercept by quantal size. Error bars indicate SEM. * $p = 0.04$, ** $p = 0.002$, t test.

Functional implications

Reduced excitatory input might delay basket cell recruitment and reduce feed-forward and feed-back inhibition of granule cells. However, alone, reduced excitatory input to basket cells appears insufficient to cause epilepsy, because spontaneous seizures were not observed in mice with reduced AMPA-mediated excitatory synaptic input to parvalbumin-positive basket cells (Fuchs et al., 2007). In epileptic animals, however, additional aspects of basket cell circuitry are dysfunctional. Normally, putative (Buzsáki and Eidelberg, 1981) and morphologically identified dentate basket cells (Buckmaster et al., 2002) are easily and quickly recruited by mild afferent activation *in vivo*. Similarly, fast-spiking basket cells in CA1 respond immediately to mild stimulation (Glickfeld and

Scanziani, 2006) and inhibit pyramidal cells most effectively at stimulus train onsets (Pouille and Scanziani, 2004). These findings and results of the present study suggest in epileptic animals granule cells would be less inhibited during mild activation and early phases of sustained input to dentate gyrus, because of smaller RRP size and increased uIPSC failure rate at basket cell-to-granule cell synapses. A speculative hypothesis is that reduced inhibitory control at these times might allow activity of subsets of granule cells to progressively build and recruit other granule cells, eventually developing into electrographic seizures (Bower and Buckmaster, 2008).

References

- Andrioli A, Alonso-Nanclares L, Arellano JJ, DeFelipe J (2007) Quantitative analysis of parvalbumin-immunoreactive cells in the human epileptic hippocampus. *Neuroscience* 149:131–143.
- Austin JE, Buckmaster PS (2004) Recurrent excitation of granule cells with basal dendrites and low interneuron density and inhibitory postsynaptic current frequency in the dentate gyrus of macaque monkeys. *J Comp Neurol* 476:205–218.
- Babb TL, Pretorius JK, Kupfer WR, Crandall PH (1989) Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. *J Neurosci* 9:2562–2674.
- Babb TL, Kupfer WR, Pretorius JK, Crandall PH, Levesque MF (1991) Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience* 42:351–353.
- Baldelli P, Hernandez-Guio JM, Carabelli V, Carbone E (2005) Brain-derived neurotrophic factor enhances GABA release probability and non-uniform distribution of N- and P/Q-type channels on release sites of hippocampal inhibitory synapses. *J Neurosci* 25:3358–3368.
- Bartos M, Vida I, Frotscher M, Geiger JR, Jonas P (2001) Rapid signaling at inhibitory synapses in a dentate gyrus interneuron network. *J Neurosci* 21:2687–2698.
- Bernard C, Esclapez M, Hirsch JC, Ben-Ari Y (1998) Interneurons are not so dormant in temporal lobe epilepsy: a critical reappraisal of the dormant basket cell hypothesis. *Epilepsy Res* 32:93–103.
- Biró AA, Holderith NB, Nusser Z (2006) Release probability-dependent scaling of the postsynaptic responses at single hippocampal GABAergic synapses. *J Neurosci* 26:12487–12496.
- Bower MR, Buckmaster PS (2008) Changes in granule cell firing rate precede locally recorded spontaneous seizures by minutes in an animal model of temporal lobe epilepsy. *J Neurophysiol* 99:2431–2442.
- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA (1998) Selective changes in single cell GABA_A receptor subunit expression and function in temporal lobe epilepsy. *Nat Med* 4:1166–1172.
- Buckmaster PS (2004) Laboratory animal models of temporal lobe epilepsy. *Comp Med* 54:473–485.
- Buckmaster PS, Dudek FE (1997) Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol* 385:385–404.
- Buckmaster PS, Jongen-Rêlo AL (1999) Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. *J Neurosci* 19:9519–9529.
- Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA (1996) Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *J Comp Neurol* 366:271–292.
- Buckmaster PS, Yamawaki R, Zhang GF (2002) Axon arbors and synaptic connections of a vulnerable population of interneurons in the dentate gyrus in vivo. *J Comp Neurol* 445:360–373.
- Buhl EH, Halasy K, Somogyi P (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* 368:823–828.
- Buzsáki G, Eidelberg E (1981) Commissural projection to the dentate gyrus of the rat: evidence for feed-forward inhibition. *Brain Res* 230:346–350.
- Chen K, Ratzliff A, Hilgenberg L, Gulyás A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I (2003) Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. *Neuron* 39:599–611.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378:75–78.
- Cohen AS, Lin DD, Quirk GL, Coulter DA (2003) Dentate granule cell GABA_A receptors in epileptic hippocampus: enhanced synaptic efficacy and altered pharmacology. *Eur J Neurosci* 17:1607–1616.
- Doherty J, Dingledine R (2001) Reduced excitatory drive onto interneurons in the dentate gyrus after status epilepticus. *J Neurosci* 21:2048–2057.
- Engel Jr J, Williamson PD, Wieser HG (1997) Mesial temporal lobe epilepsy. In: *Epilepsy: a comprehensive textbook* (Engel Jr J, Pedley TA, eds), pp 2417–2426. Philadelphia: Lippincott-Raven.
- Freund TF, Katona I (2007) Perisomatic inhibition. *Neuron* 56:33–42.
- Fuchs EC, Zivkovic AR, Cunningham MO, Middleton S, Lebeau FE, Bannerman DM, Rozov A, Whittington MA, Traub RD, Rawlins JN, Monyer H (2007) Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron* 53:591–604.
- Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ER, Eilers A, Kivi A, Zeller M, Meencke HJ, Cavalheiro EA, Heinemann U, Lehmann TN (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. *J Neurosci* 24:10416–10430.
- Galarreta M, Hestrin S (1998) Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex. *Nat Neurosci* 1:587–594.
- Geiger JR, Lübke J, Roth A, Frotscher M, Jonas P (1997) Submillisecond AMPA receptor-mediated signaling at a principal neuron-interneuron synapse. *Neuron* 18:1009–1023.
- Glickfeld LL, Scanziani M (2006) Distinct timing in the activity of cannabinoid-sensitive and cannabinoid-insensitive basket cells. *Nat Neurosci* 9:807–815.
- Han ZS, Buhl EH, Lörinczi Z, Somogyi P (1993) A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus. *Eur J Neurosci* 5:395–410.
- Harney SC, Jones MV (2002) Pre- and postsynaptic properties of somatic and dendritic inhibition in dentate gyrus. *Neuropharmacology* 43:584–594.
- Hefft S, Jonas P (2005) Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. *Nat Neurosci* 8:1319–1328.
- Hefft S, Kraushaar U, Geiger JR, Jonas P (2002) Presynaptic short-term depression is maintained during regulation of transmitter release at a GABAergic synapse in rat hippocampus. *J Physiol* 539:201–208.
- Hirsch JC, Agassandian C, Merchán-Pérez A, Ben-Ari Y, DeFelipe J, Esclapez M, Bernard C (1999) Deficit of quantal release of GABA in experimental models of temporal lobe epilepsy. *Nat Neurosci* 2:499–500.
- Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R (2007) How common are the “common” neurologic disorders? *Neurology* 68:326–337.
- Hiscock JJ, Murphy S, Willoughby JO (2000) Confocal microscopic estimation of GABAergic nerve terminals in the central nervous system. *J Neurosci Methods* 95:1–11.
- Holtzman DM, Lowenstein DH (1995) Selective inhibition of axon outgrowth by antibodies to NGF in a model of temporal lobe epilepsy. *J Neurosci* 15:7062–7070.
- Jahn R, Schiebler W, Ouimet C, Greengard P (1985) A 38,000-dalton membrane protein (p38) present in synaptic vesicles. *Proc Natl Acad Sci U S A* 82:4137–4141.
- Katona I, Sperlág B, Sik A, Kálfalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 19:4544–4558.
- Kneisler TB, Dingledine R (1995a) Synaptic input from CA3 pyramidal cells to dentate basket cells in rat hippocampus. *J Physiol* 487:125–146.
- Kneisler TB, Dingledine R (1995b) Spontaneous and synaptic input from granule cells and the perforant path to dentate basket cells in the rat hippocampus. *Hippocampus* 5:151–164.
- Kobayashi M, Buckmaster PS (2003) Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *J Neurosci* 23:2440–2452.
- Kraushaar U, Jonas P (2000) Efficacy and stability of quantal GABA release at a hippocampal interneuron-principal neuron synapse. *J Neurosci* 20:5594–5607.
- Kumar SS, Buckmaster PS (2006) Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci* 26:4613–4623.

- Ledoux VA, Woolley CS (2005) Evidence that disinhibition is associated with a decrease in number of vesicles available for release at inhibitory synapses. *J Neurosci* 25:971–976.
- Leranth C, Malcolm AJ, Frotscher M (1990) Afferent and efferent synaptic connections of somatostatin-immunoreactive neurons in the rat fascia dentata. *J Comp Neurol* 295:111–122.
- Leroy C, Poisbeau P, Keller AF, Nehlig A (2004) Pharmacological plasticity of GABA_A receptors at dentate gyrus synapses in a rat model of temporal lobe epilepsy. *J Physiol* 557:473–487.
- Lübke J, Frotscher M, Spruston N (1998) Specialized electrophysiological properties of anatomically identified neurons in the hilar region of the rat fascia dentata. *J Neurophysiol* 79:1518–1534.
- Maffei A, Nelson SB, Turrigiano GG (2004) Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nat Neurosci* 7:1353–1359.
- Margerison JH, Corsellis JAN (1966) Epilepsy and the temporal lobes. *Brain* 89:499–530.
- Mello LE, 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, Tóth K, Gulyás AI, Hájós N, Freund TF (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16:815–823.
- Morozov YM, Freund TF (2003) Post-natal development of type I cannabinoid receptor immunoreactivity in the rat hippocampus. *Eur J Neurosci* 18:1213–1222.
- Mott DD, Xie CW, Wilson WA, Swartzwelder HS, Lewis DV (1993) GABA_B autoreceptors mediate activity-dependent disinhibition and enhance signal transmission in the dentate gyrus. *J Neurophysiol* 69:674–691.
- Mott DD, Turner DA, Okazaki MM, Lewis DV (1997) Interneurons of the dentate-hilus border of the rat dentate gyrus: morphological and electrophysiological heterogeneity. *J Neurosci* 17:3990–4005.
- Moulder KL, Mennerick S (2005) Reluctant vesicles contribute to the total readily releasable pool in glutamatergic hippocampal neurons. *J Neurosci* 25:3842–3850.
- Nadler JV, Perry BW, Cotman CW (1978) Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. *Nature* 271:676–677.
- Nusser Z, Hájós 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.
- Oliva AA Jr, Lam TT, Swann JW (2002) Distally directed dendrotoxicity induced by kainic acid in hippocampal interneurons of green fluorescent protein-expressing mice. *J Neurosci* 22:8052–8062.
- Pouille F, Scanziani M (2004) Routing of spike series by dynamic circuits in the hippocampus. *Nature* 429:717–723.
- Racine RJ (1972) Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281–294.
- Ribak CE, Nitsch R, Seress L (1990) Proportion of parvalbumin-positive basket cells in the GABAergic innervation of pyramidal and granule cells of the rat hippocampal formation. *J Comp Neurol* 300:449–461.
- Sayin U, Osting S, Hagen J, Rutecki P, Sutula T (2003) Spontaneous seizures and loss of axo-axonic and axo-somatic inhibition by repeated brief seizures in kindled rats. *J Neurosci* 23:2759–2768.
- Scharfman HE (1995) Electrophysiological diversity of pyramidal-shaped neurons at the granule cell layer/hilus border of the rat dentate gyrus recorded in vitro. *Hippocampus* 5:287–305.
- Schikorski T, Stevens CF (1997) Quantitative ultrastructural analysis of hippocampal excitatory synapses. *J Neurosci* 17:5858–5867.
- Schneggenburger R, Meyer AC, Neher E (1999) Released fraction and total size of a pool of immediately available transmitter quanta at a calyx synapse. *Neuron* 23:399–409.
- Shao LR, Dudek FE (2005) Changes in mIPSCs and sIPSCs after kainate treatment: evidence for loss of inhibitory input to dentate granule cells and possible compensatory responses. *J Neurophysiol* 94:952–960.
- Sloviter RS (1994) The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol* 35:640–654.
- Sloviter RS, Sollas AL, Barbaro NM, Laxer KD (1991) Calcium-binding protein (calbindin-D28K) and parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. *J Comp Neurol* 308:381–396.
- Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M (2003) “Dormant basket cell” hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. *J Comp Neurol* 459:44–76.
- Somogyi P, Klausberger T (2005) Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J Physiol* 562:9–26.
- Soriano E, Frotscher M (1993) GABAergic innervation of the rat fascia dentata: a novel type of interneuron in the granule cell layer with extensive axonal arborization in the molecular layer. *J Comp Neurol* 334:385–396.
- Soriano E, Nitsch R, Frotscher M (1990) Axo-axonic chandelier cells in the rat fascia dentata: Golgi-electron microscopy and immunocytochemical studies. *J Comp Neurol* 293:1–25.
- Straessle A, Loup F, Arabadzisz D, Ohning GV, Fritschy JM (2003) Rapid and long-term alterations of hippocampal GABA_B receptors in a mouse model of temporal lobe epilepsy. *Eur J Neurosci* 18:2213–2226.
- Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J (2007) Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based model of temporal lobe epilepsy. *J Comp Neurol* 500:876–893.
- Tsou K, Mackie K, Sañudo-Peña MC, Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* 93:969–975.
- Varela JA, Song S, Turrigiano GG, Nelson SB (1999) Differential depression at excitatory and inhibitory synapses in visual cortex. *J Neurosci* 19:4293–4304.
- Wiedenmann B, Franke WW (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of M_r 38,000 characteristic of presynaptic vesicles. *Cell* 41:1017–1028.
- Williamson A, Spencer SS, Spencer DD (1995) Depth electrode studies and intracellular dentate granule cell recordings in temporal lobe epilepsy. *Ann Neurol* 38:778–787.
- Williamson A, Patrylo PR, Spencer DD (1999) Decrease in inhibition in dentate granule cells from patients with temporal lobe epilepsy. *Ann Neurol* 45:92–99.
- Wittner L, Maglóczy Z, Borhegyi Z, Halász P, Tóth S, Eross L, Szabó Z, Freund TF (2001) Preservation of perisomatic inhibitory input of granule cells in the epileptic human dentate gyrus. *Neuroscience* 108:587–600.
- Zhu ZQ, Armstrong DL, Hamilton WJ, Grossman RG (1997) Disproportionate loss of CA4 parvalbumin-immunoreactive interneurons in patients with Ammon’s horn sclerosis. *J Neuropathol Exp Neurol* 56:988–998.