Epileptogenesis Is Associated With Enhanced Glutamatergic Transmission in the Perforant Path

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Scimemi, Annalisa, Stephanie Schorge, Dimitri M. Kullmann, and Matthew C. Walker. Epileptogenesis is associated with enhanced glutamatergic transmission in the perforant path. J Neurophysiol 95: 1213–1220, 2006. First published November 9, 2005; doi:10.1152/jn.00680.2005. The perforant path provides the main excitatory input into the hippocampus and has been proposed to play a critical role in the generation of temporal lobe seizures. It has been hypothesized that changes in glutamatergic transmission in this pathway promote the epileptogenic process and seizure generation. We therefore asked whether epileptogenesis is associated with enhanced glutamatergic transmission from the perforant path to dentate granule cells. We used a rat model of temporal lobe epilepsy in which spontaneous seizures occur after an episode of pilocarpine-induced status epilepticus. Whole cell patch-clamp recordings were obtained from dentate granule cells in hippocampal slices from control and epileptic animals 3 wk after pilocarpine-induced status epilepticus. The paired pulse ratio of perforant path–evoked AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) was reduced in tissue obtained from epileptic rats. This is consistent with an increase in release probability. N-methyl-D-aspartate (NMDA) receptor–mediated EPSCs were also prolonged. This prolongation could not be accounted for by decreased activity of glutamate transporters or by a change in NMDA receptor subunit composition in dentate granule cells, implying a change in NMDA receptor kinetics. This change in NMDA receptor kinetics was associated with the emergence of significant synaptic cross-talk, detected as a use-dependent block of receptors activated by medial perforant path synapses after lateral perforant path stimulation in MK-801. Enhanced glutamatergic transmission and the emergence of cross-talk among perforant path–dentate granule cell synapses may contribute to lowering seizure threshold.

INTRODUCTION

The hippocampus and parahippocampal gyri play an integral part in the generation of seizures in mesial temporal lobe epilepsy, and it is thus crucial to understand the propagation of excitation through these structures. The entorhinal cortex provides the major excitatory input to the hippocampus through the perforant path, which targets neurons in the fascia dentata and in the CA1-3 regions. The axonal tracts that form the perforant path split into two anatomically and functionally distinct pathways, the medial (MPP) and the lateral (LPP) perforant path, which travel along the middle and the outer third of stratum lacunosum-moleculare, respectively, and target different sections of the granule cell dendritic tree (Hjorth-Simonsen and Jeune 1972; Steward 1976; Witter 1993). Furthermore, the MPP and LPP have different targets within the hippocampus proper (Sewards and Sewards 2003). In addition to these distinctive anatomical properties, there are marked neurophysiological and pharmacological differences between the MPP and LPP (Bough et al. 2004; Colino and Malenka 1993; Dahl et al. 1990; Do et al. 2002; McNaughton 1980; Min et al. 1998; Pelletier et al. 1994; Rush et al. 2001). These two pathways may have distinct functional roles, relaying different information; the MPP provides feedback information into the hippocampus, whereas the LPP provides the main external input (Sewards and Sewards 2003).

Dentate granule cells have been proposed to act as a brake against seizure propagation through the limbic circuitry and changes in dentate granule cell excitability have been implicated in epileptogenesis (Behr et al. 1998; Collins et al. 1983; Heinemann et al. 1992; Lothman and Bertram 1993). During the kindling process, there is a transient enhancement of NMDA receptor–mediated transmission at the LPP–dentate granule cell synapse (Behr et al. 2001; Sayin et al. 1999). In addition, more permanent changes in NMDA receptor opening times have been described in granule cells both from kindled animals and from tissue resected from patients with temporal lobe epilepsy (Kohr et al. 1993; Lieberman and Mody 1999).

Here we apply several complementary methods to study glutamatergic transmission at the perforant path–granule cell synapse in a post-status epilepticus model of temporal lobe epilepsy. The goals of this study are to determine whether transmission is indeed enhanced at the perforant path to dentate granule cell synapse and whether this is accompanied by increased cross-talk within the perforant path that may disrupt normal physiological function. We found that epilepsy is associated with a decrease in the paired pulse ratio (PPR) of AMPA and N-methyl-D-aspartate (NMDA) receptor–mediated excitatory postsynaptic currents (EPSCs) in the LPP, consistent with an increase in release probability (Pr). In addition, stimulating the LPP elicits NMDA receptor–mediated EPSCs with a more prolonged time-course in granule cells from epileptic than from control tissue; this cannot be explained by decreased activity of glutamate transporters or by a change in NMDA receptor subunit composition. Furthermore, repetitive stimulation of the LPP results in detectable cross-talk from the LPP to the MPP in epileptic animals. These results reveal several mechanisms that point to an enhancement of glutamatergic transmission that may promote seizure generation and spread.

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Epilepsy model

Limbic status epileptics (SE) was induced in adult male Sprague-Dawley rats (8 wk old, ~250 g) by intraperitoneal pilocarpine injection (320 mg/kg) (Turski et al. 1989). To lessen peripheral cholinergic effects, scopolamine methyl nitrate (1 mg/kg, ip) was administered 30 min before and 30 min after pilocarpine (Chandler et al. 2003). The onset of SE was defined as the appearance of stage 3 seizures (Racine 1972), followed by continuous clinically detectable seizure activity. Clinically overt SE was terminated 90 min after its onset by injection of diazepam (10 mg/kg, ip). The animals were monitored daily for the appearance of spontaneous recurrent seizures. All rats with SE were observed to have spontaneous seizures by 2 wk (maximal severity 3–4 of the Racine scale). Rats were killed 3 wk after the pilocarpine injection.

All animal procedures followed the Animal (Scientific Procedures) Act 1986.

Tissue preparation and RT-PCR

Control (n = 5) and post-SE rats (n = 5) were killed with an overdose of pentobarbital sodium (500 mg/kg, ip). We dissected out both hippocampi and kept one of them for the electrophysiological recordings and the other for the RNA processing. The dentate gyrus was separated from the hippocampus proper under a light microscope, and the samples were rapidly frozen and stored at −80°C until RNA extraction. We homogenized each tissue sample and extracted the total RNA with Trizol according to the directions of the manufacturer (Invitrogen, Paisley, UK). One to 3 μg of each RNA sample was reverse-transcribed using random hexamers and Superscript II (Invitrogen, Paisley, UK). Each RT reaction was amplified using degenerate primers designed to amplify all four rat NR2 subunits, essentially as described by Hynd et al. (2003). The degenerate primer sequences were NR2up TRGCNGCCTTCATGATCCA; NR2down CAGCCTKGGTCRTCTACG. The resulting PCR product was digested with MboI, phenol:chloroform extracted, and separated on a 3% agarose gel stained with ethidium bromide. The bands were quantified using nonsaturating exposures on GeneGenius (Syngene, Cambridge, UK).

Electrophysiology and data analysis

Adult control rats (8–11 wk old, ~250 g) and rats 3 wk after pilocarpine-induced SE were deeply anesthetized with pentobarbital sodium (500 mg/kg, ip) and decapitated, and their brains were submerged in ice-cold carbogenated (95% O2-5% CO2) sucrose solution containing (in mM) 70 sucrose, 80 NaCl, 2.5 KCl, 1.3 MgSO4, 0.5 CaCl2, 25 NaHCO3, 1.25 NaH2PO4, and 22 glucose. The whole hippocampi were dissected out from surrounding brain tissue and placed in agar blocks before slicing. Transverse hippocampal slices (300 μm thick) from all hippocampal levels were obtained using a Leica VT1000S vibrating blade microtome. After cutting slices were transferred to an interface chamber containing EBSs medium (Invitrogen) supplemented with 1 mM CaCl2 and 2 mM MgCl2. Slices were stored in this solution for >1 h and transferred to a submersion-type recording chamber superfused with carbogenated artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO4, 2.5 CaCl2, 26.2 NaHCO3, 1 NaHPO4, and 22 glucose (21–23°C). Stimuli (100 μs square pulses; 0.1 Hz frequency) were delivered through bipolar stainless steel electrodes positioned in stratum moleculare, ~100 μm away from the recorded cell. Field potential recordings were made with glass electrodes filled with ACSF (tip resistance, ~1 MOhm). Whole cell recordings were made from dentate gyrus granule cells using patch pipettes filled with an intracellular solution containing (in mM) 97.5 Cs gluconate, 17.5 CsCl, 10 HEPES, 10 BAPTA, 8 NaCl, 2 MgATP, 0.3 GTP, and 5 QX314 Br (pH 7.2, osmolality 290 mOsm). The series resistance was monitored throughout the experiments using a −5 mV voltage step command, was in the range of 15–30 MΩ, and was not compensated. Cells in which the series resistance varied >20% were discarded from the analysis. All the experiments were performed in the presence of the GABA<sub>A</sub> receptor antagonist picrotoxin (PTX; 100 μM). In some experiments, the following cocktail of drugs was added to avoid interference from heterosynaptic signaling by GABA<sub>B</sub>, group II and III metabotropic glutamate, opioid, A<sub>1</sub>, and muscarinic receptors (in μM): 5 CGP52432, 100 (RS)-α-methylserine-O-phosphate (MSOP), 1 LY341495, 10 naloxone, 0.2 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), and 1 atropine. Evoked AMPA EPSCs were recorded by holding cells at V<sub>H</sub> = −70 mV, whereas NMDA EPSCs were recorded at V<sub>H</sub> = +40 mV, in the presence of 25 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt (NBQX). Currents were acquired with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) using a 2-kHz low-pass filter, digitized at 5 kHz, and stored on a personal computer for off-line analysis. All the experiments were performed in the presence of the NMDA antagonist MK-801, we analyzed only the first five trials after restarting stimulation (Scimemi et al. 2004). Wherever two inputs were compared, the stimulus intensity was adjusted to obtain EPSCs of similar amplitudes in both pathways.

Data are expressed as means ± SE and were considered significant at P < 0.05, as determined by Student’s paired or unpaired t-test (when measures were normally distributed) or Wilcoxon matched pair or Mann-Whitney U test (when distributions were not normal).

Drugs were purchased from Tocris Cookson (Bristol, UK) and Sigma (St. Louis, MO), except for QX314 Br (Alomone Laboratories, Jerusalem, Israel).

RESULTS

Paired pulse responses in the MPP and LPP

MPP and LPP EPSCs were evoked in granule cells through stimulating electrodes positioned in the middle and outer thirds of the dentate molecular layer, respectively (Abraham and McNaughton 1984) (Fig. 1A). In control animals, two consecutive stimuli delivered with a 50 ms interpulse interval with GABA<sub>A</sub> receptors blocked yielded paired pulse depression (PPD) of AMPA EPSCs in the MPP and paired pulse facilitation (PPF) in the LPP (Fig. 1, B and C). This is consistent with the different PPRs in these two pathways observed by others (Colino and Malenka 1993; McNaughton 1980; Min et al. 1998). In contrast, when the same protocol was applied to the epileptic hippocampus, although we still detected a clear PPD in the MPP, PPF in the LPP was no longer present (Fig. 1, B and C). The difference in the PPR in the LPP of control and epileptic dentate granule cells was significant at P = 0.02. Using field potential recordings in the outer third of the molecular layer of the dentate gyrus at 34°C, we confirmed that this change in PPR was not temperature dependent (PPR of LPP in control 1.30 ± 0.10, n = 4 and in epileptic 1.08 ± 0.02, n = 6, P = 0.02 for difference).
ACh receptors, A1 adenosine receptors, and opioid receptors in block GABAB receptors, group II and III mGluRs, muscarinic
concentrations that we and others have previously shown to measurement of the PPR in the presence of a cocktail of drugs at 2004; Klapstein et al. 1999). We therefore repeated the mea-
ded, epileptogenesis may affect the modulation of perforant 
modulation of the LPP synapses by endogenous factors; in-
acute hippocampal slices (see METHODS for details) (Bramham 
interaction, thus indicating the independence of the 2 pathways. **P
then LPP at 50-ms intervals (ML); this showed no significant pathway
the same stimulation parameters, single pulses were applied to the MPP and 
were placed in the middle and outer third of stratum lacunosum-moleculare to activate medial perforant path (MPP) and LPP fibers, respectively. B: delivering 2 pulses to the MPP at 20 Hz revealed paired pulse depression (PPD), whereas LPP stimulation resulted in PPF. Applying the same stimulation protocol to the epileptic hippocampus resulted in PPD in the MPP, but PPF in the LPP was no longer observed. All traces represent the average of 20 consecutive trials normalized by peak amplitude of the 1st excitatory postsynaptic current (EPSC), recorded in the presence of picrotoxin (PTX; 100 μM) to block GABA<sub>A</sub> receptors. C: summary of the paired pulse ratio (PPR) measured in control and epileptic granule cells following MPP (MM) and LPP (LL) stimulation. The PPR is significantly decreased in epileptic granule cells when delivering pulses to the LPP. D: summary histogram of the PPR measured after stimulation of MPP and LPP in the presence of a cocktail of PTX and drugs to block metabotropic receptors. Data were collected from control (left) and epileptic granule cells (right). Even in the presence of the cocktail, PPR in LPP was still significantly decreased during epilepsy. Using the same stimulation parameters, single pulses were applied to the MMP and then LPP at 50-ms intervals (ML); this showed no significant pathway interaction, thus indicating the independence of the 2 pathways. *P < 0.05, **P < 0.01, ***P < 0.001.

Such a change in PPR could be caused by a change in the modulation of the LPP synapses by endogenous factors; in-
deed, epileptogenesis may affect the modulation of perforant 
transmission by metabotropic glutamate receptors (Bough et al. 2004; Klapstein et al. 1999). We therefore repeated the measurement of the PPR in the presence of a cocktail of drugs at concentrations that we and others have previously shown to block GABA<sub>A</sub> receptors, group II and III mGlurRs, muscarinic ACh receptors, A<sub>1</sub> adenosine receptors, and opioid receptors in acute hippocampal slices (see METHODS for details) (Bramham and Harvey 1996; Doherty et al. 2004; Foster and Deadwyler 1992; Manzoni et al. 1994; Wagner et al. 1992). In the presence of these drugs, the PPRs in the MPP and LPP from control tissue were not significantly different from those ob-
served in the presence of PTX alone (P = 0.96 for MPP; P = 0.51 for LPP). This implies that these G protein–coupled receptors (GPCRs) do not contribute to PPR in either pathway. We repeated the measurement of PPR in epileptic tissue in the presence of the cocktail of antagonists (Fig. 1D). The reduction in PPR in LPP in epileptic tissue persisted when the GPCRs were blocked, further arguing against a role for these receptors in PPR. This reduction of PPR in the LPP with epilepsy is consistent with a higher baseline Pr in the epileptic hippocampus.

An alternative explanation for the reduced PPR in LPP in epileptic tissue is that the stimulating electrode positioned in the outer third of the molecular layer activated some MPP axons, possibly as a result of axonal rearrangements. We tested for such contamination by stimulating the MPP and LPP with a 50 ms interval and looking for evidence of cross-inhibition. The LPP was not significantly changed by prior stimulation of the MPP in either control or epileptic tissue (Fig. 1D), arguing against a major contamination from MPP fibers when stimulating the LPP.

Change in PPR with epilepsy is present at high and low Pr synapses

Since there is a nonuniform distribution of Pr at cortical synapses (Hessler et al. 1993; Rosenmund et al. 1993), changes in PPR at LPP synapses could be caused by a change in Pr across all synapses or a selective loss of low Pr synapses. To distinguish between these two possibilities, we took advantage of the use-dependent block of NMDA receptors by MK-801. MK-801 initially preferentially blocks NMDA EPSCs at high Pr synapses (Manabe and Nicoll 1994), and this is reflected in a gradual increase in PPR during MK-801 application (Manabe and Nicoll 1994). We therefore recorded paired NMDA EPSCs at V<sub>H</sub> = 40 mV in the cocktail of metabotropic receptor blockers (as used in Fig. 1D), supplemented with NBQX (25 μM) to block AMPA/kainate receptors. After establishing a stable baseline, we tested the effect of MK-801 (40 μM). Consistent with an increased Pr, there was a trend for the reduction in the amplitude of NMDA EPSCs in the presence of MK-801 to be faster in epileptic granule cells (data not shown). Similar to the PPR for AMPA EPSCs and in agreement with a higher baseline Pr at synapses in the epileptic tissue, the PPR for NMDA EPSCs calculated across a set of 20 traces before applying MK-801 was significantly greater in control than in epileptic granule cells (P < 0.01; Fig. 2, left). The PPR progressively increased throughout the MK-801 application, reaching a steady state ~20 min after the beginning of the application (Fig. 2). The increase in the PPR normalized by the baseline PPR followed a similar time-course and reached the same steady state in control and epileptic tissue (Fig. 2, right). This result suggests that the Pr at the LPP dentate granule cell synapses is increased across all synapses (high and low Pr) during epileptogenesis. It also argues further against the hypothesis that the LPP in epileptic tissue was contaminated by MPP fibers to a greater extent than in control tissue.

Time-course of NMDA EPSCs is prolonged in epileptic tissue

The results presented so far are consistent with an increase in Pr at LPP terminals during epileptogenesis. A transient
enancement of NMDA receptor-mediated transmission at the LPP–dentate granule cell synapse has been previously reported during kindling (Behr et al. 2001; Sayin et al. 1999), and has been suggested to be involved in the epileptogenic process. We therefore asked whether a similar phenomenon occurs in the post-status epilepticus model of epileptogenesis. We evoked NMDA EPSCs of similar amplitudes in the epileptic and post-status epilepticus model of epileptogensis. We evoked single pulses to the LPP to evoke similar amplitude NMDA EPSCs in control and epileptic granule cells. We measured the effect of the NR2B antagonist ifenprodil (5 μM) on the amplitude and on the decay of the EPSCs (Fig. 4A). Ifenprodil reduced the peak amplitude of NMDA EPSCs by 49 ± 3% in control (n = 10) and by 41 ± 3%, in epileptic tissue (n = 12); there was no significant difference between the reduction in control and epileptic tissue (P = 0.06), arguing against a major change in the contribution of the NR2B subunit. The effect of ifenprodil on the EPSC time-course was similar in control and epileptic cells (Fig. 4A, right), reducing it to 90 ± 4% in control (n = 10) and to 83 ± 3% in epileptic tissue (n = 12, P = 0.17 for comparison).

As a further test of the possibility that the change in decaying phase of EPSCs resulted from a shift in NMDA receptor subunit composition, we estimated the relative abundance of NR2A and NR2B transcripts in control and epileptic tissues using RT-PCR (Fig. 4B). There was no significant change in the ratio of NR2B/NR2A in RNA from control and

Effects of glutamate uptake on NMDA receptor responses

We first tested whether a reduction of glutamate uptake could explain the slower time-course of NMDA EPSCs in epileptic tissue. To block both neuronal and glial transporters, we perfused slices with the glutamate transporter inhibitor D-threo-beta-benzyloxyaspartate (TBOA; 50 μM; Fig. 3B). This evoked a large outward current that did not differ significantly between epileptic and control tissue (Fig. 3B, left). It also produced a significant increase in the time-course of EPSCs (control: 204 ± 14% of the baseline time-course, n = 10, P < 0.01; epileptic: 165 ± 20% of the baseline time-course, n = 10, P < 0.01; P = 0.12 for comparison between control and epileptic; Fig. 3B, right). Although these effects of TBOA on the holding current and on the time-course of NMDA EPSCs tended to be smaller in epileptic tissue, this trend did not reach significance, arguing against a major difference in the activity of glutamate transporters.

Role and expression of different NMDA receptor subunits

NMDA receptors containing NR2B subunits have slower kinetics than those that contain NR2A subunits (Erreger et al. 2005). We asked whether a different ratio of NR2B/NR2A receptors underlies the different time-course of EPSCs after LPP stimulation in the epileptic hippocampus. We delivered single pulses to the LPP to evoke similar amplitude NMDA EPSCs in control and epileptic granule cells. We measured the effect of the NR2B antagonist ifenprodil (5 μM) on the amplitude and on the decay of the EPSCs (Fig. 4A). Ifenprodil reduced the peak amplitude of NMDA EPSCs by 49 ± 3% in control (n = 10) and by 41 ± 3%, in epileptic tissue (n = 12); there was no significant difference between the reduction in control and epileptic tissue (P = 0.06), arguing against a major change in the contribution of the NR2B subunit. The effect of ifenprodil on the EPSC time-course was similar in control and epileptic cells (Fig. 4A, right), reducing it to 90 ± 4% in control (n = 10) and to 83 ± 3% in epileptic tissue (n = 12, P = 0.17 for comparison).
Thus neither the electrophysiological data nor the RT-PCR experiments lend any support to the hypothesis that a significant change in the NR2B/NR2A distribution occurs in the dentate gyrus during epilepsy.

Cross-talk from the LPP to the MPP can be detected in epileptic tissue

The results thus far suggest that there are no major changes in local glutamate uptake or NMDA receptor subunit composition. However, the change in decay time is consistent with an increased affinity of the NMDA receptor for glutamate, resulting in a prolonged opening time. An increase in receptor affinity should enhance the ability of the receptors to detect spill-over of glutamate, and this alone or in combination with increased Pr at the LPP could increase synaptic cross-talk. The MPP and LPP are distinct, spatially separated, pathways. We therefore asked if epileptogenesis could enhance cross-talk from LPP to MPP. To assay long-range cross-talk, we applied a highly sensitive approach that relies on the use-dependent blocker MK-801 (Carter and Regehr 2000; Scimemi et al. 2004). We added the cocktail of drugs used for the experiments described in Fig. 1D to the perfusing solution and verified that stimulating the MPP and LPP evoked AMPA receptor–mediated responses that were independent as confirmed by testing cross-PPR as previously described. We recorded baseline NMDA EPSCs at $V_H = +40$ mV, with AMPA-kainate receptors blocked, elicited by stimulating the MPP and LPP alternately every 10 s. MK-801 (4 μM) was subsequently washed in for 5 min, and the LPP was stimulated alone (5 pulses at 20 Hz, repeated every 10 s), until the LPP EPSC amplitude decreased to $\sim 15\%$ of baseline. Stimulation of the MPP was resumed 15–20 min after washout of MK-801.

When the first five MPP responses after MK-801 washout were compared with the baseline EPSCs, they showed a significant reduction in amplitude in epileptic tissue (Fig. 5A). To control for the effect of background activation of NMDA receptors by spontaneous glutamate release, we repeated the experiment without stimulating the LPP during the MK-801 application. The reduction of MPP EPSCs was significantly ($P = 0.01$) greater after LPP stimulation than in the no-stimuli control experiments (as described in Fig. 1D). The baseline drift from control rats, the reduction in the EPSC amplitude in the tissue. Specifically, it implies that the same NMDA receptors can be activated by glutamate released by either pathway. Thus neither the electrophysiological data nor the RT-PCR experiments lend any support to the hypothesis that a significant change in the NR2B/NR2A distribution occurs in the dentate gyrus during epilepsy.

**Discussion**

We have shown that an experimental model of temporal lobe epilepsy is associated with enhanced glutamatergic transmission at the perforant path to dentate granule cell synapse. This enhancement in glutamatergic transmission is manifest as an increased Pr and prolonged NMDA receptor–mediated EPSCs; these result in the emergence of detectable cross-talk from the lateral to the medial perforant path.
One of the features that distinguishes the LPP from the MPP is a difference in PPR of the EPSC; this has been proposed to be due to a difference in release probabilities in these two pathways (Colino and Malenka 1993; McNaughton 1980; Min et al. 1998). We found that the PPR was decreased at the LPP to dentate granule cell synapse during epileptogenesis. This decrease persisted despite blocking presynaptic adenosine, metabotropic glutamate, GABA<sub>B</sub>, and opioid receptors, arguing against a change in tonic presynaptic modulation of transmission, but consistent with an increase in baseline Pr. A potential pitfall is that the increase in Pr might be explained by contamination of the LPP by MPP axons, because this pathway exhibits PPD in control animals. This is, however, unlikely for the following reasons. First, we standardized the stimulating electrode positions to recruit the two pathways selectively (Abraham and McNaughton 1984). Second, we confirmed that there was no significant cross-interaction of the pathways by stimulating each pathway in turn. Finally, if the change in PPR was caused by a greater contamination of the LPP by MPP axons, this would lead to the prediction that progressively blocking NMDA receptors with MK-801 would lead to a change in PPR with a different evolution in control and epileptic tissue. Instead, the normalized PPR increased in a similar way, arguing for a uniform increase in Pr.

There have been variable observations of changes in PPF at the LPP to dentate granule cell synapse during epileptogenesis (Bough et al. 2004; Klapstein et al. 1999). To some extent, this may be caused by selection bias; LPP synapses are identified by the presence of PPF (Bough et al. 2004). Our result is, however, consistent with a study of kindled animals in which a long-lasting decrease in PPF was observed at the LPP (Klapstein et al. 1999), although the authors could not exclude contamination by MPP.

Changes in NMDA receptor kinetics

As further evidence of an enhancement in glutamatergic transmission from entorhinal cortex to dentate granule cells, we observed a prolongation of the NMDA receptor EPSC. This could be explained by a change in glutamate uptake (Arnth-Jensen et al. 2002; Diamond 2001), a change in glutamate diffusion (Savtchenko and Rusakov 2005), a change in NMDA receptor subtype (Chen et al. 1999; Lozovaya et al. 2004; Prybylowski et al. 2002), or a change in NMDA receptor kinetics (Jahr 1992; Rosenmund et al. 1993).

Blocking glutamate uptake had a similar effect on the NMDA EPSC decay in both epileptic and control granule cells, suggesting that glutamate transporter function in the granule cell molecular layer does not change significantly with epileptogenesis. This is in keeping with the finding that during epileptogenesis changes in glutamate transporter expression in the dentate molecular layer seem to be restricted to the inner molecular layer (Gorter et al. 2002). Thus a change in glutamate uptake cannot explain the prolongation of the NMDA EPSC in epilepsy.

Could the prolonged response be caused by changes in NMDA receptor subtype expression? Changes in NMDA receptor subtype have been found in experimentally induced cortical malformations and human cortical dysplasia (Andre et al. 2004; Calcagnotto and Baraban 2005; Hagemann et al. 2003; Ying et al. 2004) and would be expected to change NMDA receptor current kinetics. However, we observed no change in either the proportion of the NMDA EPSC mediated by NR2B or the mRNA ratios for NMDA receptor subtypes in the dentate gyrus (although this result does not specifically address the NMDA receptors expressed at the lateral perforant path synapse).

The most likely explanation of the prolonged response is thus a change in NMDA receptor open probability. Such a phenomenon is supported by evidence from dissociated granule...
ule cells from kindled rats and human tissue from patients with epilepsy (Kohr et al. 1993; Lieberman and Mody 1999). In these studies, the increase in opening time was explained not by a change in receptor subtype, but by a change in the phosphorylation state of the NMDA receptor.

Changes in synaptic cross-talk in epileptogenesis

Enhanced glutamate release in the LPP and an increase in NMDA receptor open probability should increase synaptic cross-talk in epileptic animals. There may be additional untested factors such as synaptic rearrangements and changes in diffusivity (Rusakov and Kullmann 1998; Savtchenko and Rusakov 2005). We therefore asked if we could detect glutamate spillover from the LPP to the MPP in epileptic tissue. In vivo, the pyramidal cells in the entorhinal cortex fire at 10 Hz (Frank et al. 2001). However, the entorhinal cortex from epileptic rats and humans exhibits high-frequency oscillations that may contribute to the excitatory input to dentate granule cells (Bragin et al. 2002, 2004). We therefore asked whether there was increased cross-talk between the pathways when there was a burst of action potentials in the lateral perforant path. In this circumstance, we detected a significant interaction between the pathways in the epileptic but not in the control animals. Although it is not possible to predict the consequences of this for entorhinal-hippocampal function in vivo because of differences in diffusion, temperature, glutamate release, and pattern of firing, this result does indicate that there is a greater propensity for cross-talk between the lateral and medial perforant path in epilepsy.

Implications of these changes

The dentate granule cells have been proposed to act as a brake on activity transmitted from the entorhinal cortex to the hippocampus that dysfunctions during epileptogenesis (Behr et al. 1998; Collins et al. 1983; Heinemann et al. 1992; Lothman and Bertram 1993). Indeed, there is increasing evidence that the entorhinal cortex plays a critical role in mesial temporal lobe epilepsy (Jones et al. 1992; Spencer and Spencer 1994). An increase in Pr will lead to a greater activation of glutamatergic synapses and thus enhanced neurotransmission. The prolonged decay of NMDA receptor responses will also result in enhanced transmission, but importantly has further implications for dentate function. During kindling, a transient increase in NMDA receptor-mediated transmission has been found that may promote the kindling process (Behr et al. 2001; Sayin et al. 1999). Inhibition of NMDA receptors retards the kindling process (McNamara et al. 1988), and also can inhibit epileptogenesis in post-status epilepticus models (Prasad et al. 2002; Rice and DeLorenzo 1998). The finding of increased NMDA receptor-mediated transmission at the perforant path to dentate granule cell may thus represent a common mechanism that enhances the epileptogenic process. We also found enhanced cross-talk between the pathways with high-frequency stimulation. This increased spillover may promote hyperexcitability. In addition, a decrease in perforant pathway specificity with epileptogenesis may adversely affect hippocampal function. Thus enhanced glutamatergic transmission at the perforant path to granule cell synapse may promote seizure generation and epileptogenesis while disrupting hippocampal function.

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