Topological Determinants of Epileptogenesis in Large-Scale Structural and Functional Models of the Dentate Gyrus Derived From Experimental Data

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Dyhrfjeld-Johnsen J, Santhakumar V, Morgan RJ, Huerta R, Tsimring L, Soltesz I. Topological determinants of epileptogenesis in large-scale structural and functional models of the dentate gyrus derived from experimental data. J Neurophysiol 97: 1566–1587, 2007. First published November 8, 2006; doi:10.1152/jn.00950.2006. In temporal lobe epilepsy, changes in synaptic and intrinsic properties occur on a background of altered network architecture resulting from cell loss and axonal sprouting. Although modeling studies using idealized networks indicated the general importance of network topology in epilepsy, it is unknown whether structural changes that actually take place during epileptogenesis result in hyperexcitability. To answer this question, we built a 1:1 scale structural model of the rat dentate gyrus from published in vivo and in vitro cell type–specific connectivity data. This virtual dentate gyrus in control condition displayed globally and locally well connected (“small world”) architecture. The average number of synapses between any two neurons in this network of over one million cells was less than three, similar to that measured for the orders of magnitude smaller C. elegans nervous system. To study how network architecture changes during epileptogenesis, long-distance projecting hilar cells were gradually removed in the structural model, causing massive reductions in the number of total connections. However, as long as even a few hilar cells survived, global connectivity in the network was effectively maintained and, as a result of the spatially restricted sprouting of granule cell axons, local connectivity increased. Simulations of activity in a functional dentate network model, consisting of over 50,000 multicompartmental single-cell models of major glutamatergic and GABAergic cell types, revealed that the survival of even a small fraction of hilar cells was enough to sustain networkwide hyperexcitability. These data indicate new roles for fractionally surviving long-distance projecting hilar cells observed in specimens from epilepsy patients.

**INTRODUCTION**

The dentate gyrus, containing some of the most vulnerable cells in the entire mammalian brain, offers some unique opportunities to investigate the importance of structural alterations during epileptogenesis. Many hilar cells are lost in both human and animal models after repeated seizures, ischemia, and head trauma (Buckmaster and Jongen-Re loft 1999; Ratzliff et al. 2002; Sutula et al. 2003), accompanied by mossy fiber (granule cell axon) sprouting. In temporal lobe epilepsy, loss of hilar neurons and mossy fiber sprouting are hallmarks of seizure-induced end-folium sclerosis (Margerison and Cordellis 1966; Mathern et al. 1996), indicating the emergence of a fundamentally transformed microcircuit. Because structural alterations in experimental models of epilepsy occur concurrently with multiple modifications of synaptic and intrinsic properties, it is difficult to unambiguously evaluate the functional consequences of purely structural changes using experimental techniques alone.

Computational modeling approaches may help to identify the importance of network architectural alterations. Indeed, prior modeling studies of idealized networks indicated the importance of altered network architecture in epileptogenesis (Buzsáki et al. 2004; Netoff et al. 2004; Percha et al. 2005). However, to test the role of structural changes actually taking place during epileptogenesis, the network models must be strongly data driven, i.e., incorporate key structural and functional properties of the biological network (Ascoli and Akeson 2005; Bernard et al. 1997; Traub et al. 2005a,b). Such models should also be based on as realistic cell numbers as possible, to minimize uncertainties resulting from the scaling-up of experimentally measured synaptic inputs to compensate for fewer cells in reduced networks.

Within the last decade, large amounts of high-quality experimental data have become available on the connectivity of the rat dentate gyrus both in controls and after seizures. From such data, we assembled a cell type–specific connectivity matrix for the dentate gyrus that, combined with in vivo single cell axonal projection data, allowed us to build a 1:1 scale structural model of the dentate gyrus in the computer. We characterized the architectural properties of this virtual dentate gyrus network using graph theoretical tools, following recent topological studies of biochemical and social networks, the electric grid, the Internet (Albert et al. 1999; Barabási et al. 2000; Eubank et al. 2004; Jeong et al. 2000; Watts and Strogatz 1998), the Caenorhabditis elegans nervous system (Watts and Strogatz 1998), and model neuronal circuits (Lin and Chen 2005; Masuda and Aihara 2004; Netoff et al. 2004; Roxin et al. 2004). To test the functional relevance of the alterations observed in our structural model, we enlarged, by two orders of magnitude, a recently published 500-cell network model of the dentate gyrus, incorporating multicompartmental models for granule cells, mossy cells, basket cells, and dendritically projecting interneurons reproducing a variety of experimentally determined electrophysiological cell-specific properties (Santhakumar and Barlow et al. 2005).

Taken together, the results obtained from these data-driven computational modeling approaches reveal the topological

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characteristics of the control dentate gyrus and demonstrate that hyperexcitability can emerge from purely structural changes in neuronal networks after loss of neurons and sprouting of new connections, in the absence of changes in synaptic or intrinsic cellular properties.

**METHODS**

A three-step strategy was implemented to investigate the functional role of the structural reorganizations that take place in the rat dentate gyrus during epileptogenesis: 1) construction of the database; 2) construction of the structural models (control and diseased versions); and 3) construction of the functional models (control and diseased versions). These three steps will be described first, followed by details of the implementation and assessment of the structural and functional models. Additional details can be found in appendixes A1–A3.

**Construction of the database and the models**

**CONSTRUCTION OF THE DATABASE.** The database for the normal and epileptic biological dentate networks was assembled from published data. This process itself entailed several distinct steps. As an initial step, eight types of dentate cells were identified as anatomically well described: granule cells, mossy cells, basket cells, axo-axonic cells, molecular layer cells with axonal projections to the perforant path (MOPP cells), hilar cells with axonal projections to the perforant path (HIPP cells), hilar cells with axonal projections to the commissural-associational pathway (HICAP cells), and interneuron-specific (IS) cells (Fig. 1A). Next, the numbers of cells for each of these eight neuronal types were estimated from the published data (see cell numbers in the left column of Table 1, with references). For a full description of how the cell numbers were estimated, see APPENDIX A1. As a third step in assembling the database, the connectivity matrix was filled in (Table 1). This matrix contains estimates of how many postsynaptic cells among each of the eight cell types a single presynaptic neuron of a given type innervates (for example, from the third row, second column in Table 1: a single basket cell innervates about 1,250 granule cells; mean and ranges are indicated, with references). For full justification of the estimates in the connectivity matrix, see APPENDIX A2. As a final step, spatial constraints in connectivity were considered. For each cell type, the extent of the axons of single cells was fitted with a single Gaussian (Fig. 2). For example, in the case of control mossy fibers, the averaged in vivo axonal distribution of 13 granule cells (Buckmaster and Dudek 1999) was fitted with a single Gaussian (Fig. 2). For a full description of the construction of the axonal distributions from the in vivo single-cell filling data and the single or double Gaussian fits, see APPENDIX A3.

**CONSTRUCTION OF THE STRUCTURAL MODEL—CONTROL CONDITION.** Once the database was assembled, a structural model of the dentate gyrus was created in the computer. This was a so-called graph network, consisting of “nodes” (corresponding to neurons) and “links” (corresponding to synaptic connections). Each node carried the identity and connectivity pattern of a particular cell type (in other words, there were “granule cell nodes” and “mossy cell nodes,” etc.). The links were directed (like synapses) but nonweighted (meaning that a link simply represented the existence of a connection from cell A to cell B, irrespective of the number of synapses between cells A and B or the functional strength of that connection; note that the functional model, described later, takes some of these factors into account). The structural model was full scale (1:1, meaning that the number of nodes in the graph equaled the total number of cells in the dentate gyrus) and captured the salient connectivity and axonal distribution of the various cell types. Overall, the resulting structural model of the dentate gyrus was similar to graph representations of other real-world systems (e.g., Watts and Strogatz 1998).

**CONSTRUCTION OF THE STRUCTURAL MODEL—PROGRESSIVE SCLEROSIS.** In terms of the structural reorganization of the neuronal networks during limbic epileptogenesis, the loss of hilar cells and the sprouting of mossy fibers are two key factors underlying the process of “end-folium” (meaning the dentate gyrus) sclerosis (Margerin and Corsellis 1966; Mathern et al. 1996) (in the rest of the paper, we will use the shorthand “sclerosis” for end-folium sclerosis; note that end-folium sclerosis is distinct from the broader term “hippocampal sclerosis”). Herein, we simulated the structural changes in sclerosis by removing hilar cells (mossy cells, HIPP cells, HICAP cells, and IS cells) and adding mossy fiber contacts. The biological process of sclerosis (original meaning: “hardening of the tissue”) encompasses more than the loss of cells and sprouting of axons (importantly, it also entails gliosis). However, from the perspective of neuronal network reorganization in the dentate gyrus, the loss of hilar cells and the sprouting of mossy fibers are clearly the two major factors.

There were three important features that needed to be considered during the implementation of sclerosis in the structural model. First, just as in the biological network, the loss of hilar cells entailed the loss of both the excitatory mossy cells and the inhibitory HIPP, HICAP, and IS interneurons in the hilus (Buckmaster and Jongen-Reilo 1999). Second, just as in the biological network, the spatial extent of sprouted mossy fibers from a single granule cell remained restricted to a single hippocampal lamella (about 600 μm) like the control mossy fibers (Buckmaster et al. 2002b). Third, the progression of sclerosis was implemented by considering full (100%) sclerosis the state of maximal hilar cell loss (when all hilar cells are removed) and the addition of a maximal number of previously nonexistent mossy fiber connections to other granule cells [the densest, anatomically quantified sprouting reported in the literature from an experimental epilepsy model was an average of 275 extra mossy fiber contacts per granule cell (Buckmaster et al. 2002b)—we considered this number 100% sprouting]. Therefore intermediate stages in the progression of sclerosis could be distributed between the control (0% sclerosis) and the maximally sclerotic (100% sclerosis) states. For example, at 50% sclerosis, 50% of mossy cells and 50% of hilar interneurons were lost, and 50% of the maximal sprouting of mossy fibers was implemented (Fig. 1B2). Sclerosis could also be studied in networks containing only the nodes representing the excitatory cells (“isolated excitatory graph”) or only the interneurons (“isolated inhibitory graph”). However, mossy fiber sprouting obviously could not be implemented in the isolated inhibitory graph. Similarly, sprouting could be studied without hilar cell loss (“sprouting-only networks”). However, the reverse was not necessarily true because mossy cell loss without mossy fiber sprouting in the isolated excitatory graph caused the graph to become disconnected as sclerosis progressed (because granule cells do not make synapses on each other in the control network). It should also be noted that in the isolated interneuronal graphs, axo-axonic cells were included only as synaptic targets for other interneurons, but not sampled for the L and C calculations, because they exclusively projected to excitatory neurons. In addition, the interneuronal graphs were characterized only ≤96.66% sclerosis because 100% sclerosis resulted in a disconnected graph.

**CONSTRUCTION OF THE STRUCTURAL MODEL—EQUIVALENT RANDOM GRAPHS.** Specific topological measures (the average path length and the clustering coefficient; see following text) were calculated for each structural model representing different stages in the progression of sclerosis, to quantify how network architecture changes during sclerosis. However, because the numbers of nodes and links change during sclerosis, these topological measures are meaningful only if they are contrasted with similar measures taken for equivalent random graphs at each stage of sclerosis. An
FIG. 1. Schematic of the basic circuitry of the dentate gyrus and the changes to the network during sclerosis. A: relational representation of the healthy dentate gyrus illustrating the network connections between the 8 major cell types: GC, granule cell; BC, basket cell; MC, mossy cell; AAC, axo-axonic cells; MOPP, molecular layer interneurons with axons in perforant-path termination zone; HIPP, hilar interneurons with axons in perforant-path termination zone; HICAP, hilar interneurons with axons in the commissural/associational pathway termination zone; and IS, interneuron selective cells. Schematic shows the characteristic location of the various cell types within the 3 layers of the dentate gyrus. Note, however, that this diagram does not indicate the topography of axonal connectivity (present in both the structural and functional dentate models) or the somatodendritic location of the synapses (incorporated in the functional network models). B1: schematic of the excitatory connectivity of the healthy dentate gyrus is illustrated (only cell types in the hilus and granule cells are shown). Note that the granule cell axons (the mossy fibers) do not contact other granule cells in the healthy network. B2: schematic of the dentate gyrus at 50% sclerosis shows the loss (indicated by the large x symbols) of half the population of all hilar cell types and the 50% sprouting of mossy fibers that results in abnormal connections between granule cells (note that, unlike in this simplified schematic, all granule cells formed sprouted contacts in the structural and functional models of sclerosis; thus progressive increase in sprouting was implemented by increasing the number of postsynaptic granule cells contacted by single sprouted mossy fibers; see METHODS). C: schematics of 3 basic network topologies: regular, small-world, and random. Nodes in a regular network are connected to their nearest neighbors, resulting in a high degree of local interconnectedness (high clustering coefficient C), but also requiring a large number of steps to reach other nodes in the network from a given starting point (high average path length L). Reconnection of even a few of the local connections in a regular network to distal nodes in a random manner results in the emergence of a small-world network, with a conserved high clustering coefficient (C) but a low average path length (L). In a random network, there is no spatial restriction on the connectivity of the individual nodes, resulting in a network with a low average path length L but also a low clustering coefficient C.
TABLE 1. Connectivity matrix for the neuronal network of the control dentate gyrus

<table>
<thead>
<tr>
<th>Cells</th>
<th>Granule Cells</th>
<th>Mossy Cells</th>
<th>Basket Cells</th>
<th>A xo-axonic Cells</th>
<th>MOPP Cells</th>
<th>HIPP Cells</th>
<th>HICAP Cells</th>
<th>IS Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granule cells</td>
<td>X</td>
<td>9.5</td>
<td>X</td>
<td>15</td>
<td>3</td>
<td>X</td>
<td>110</td>
<td>40</td>
</tr>
<tr>
<td>Mossy cells</td>
<td>32,500</td>
<td>350</td>
<td>7.5</td>
<td>5</td>
<td>600</td>
<td>200</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Basket cells</td>
<td>1,250</td>
<td>75</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A xo-axonic cells</td>
<td>3,000</td>
<td>150</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>MOPP cells</td>
<td>7,500</td>
<td>40</td>
<td>1.5</td>
<td>7.5</td>
<td>X</td>
<td>7.5</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>HIPP cells</td>
<td>1,550</td>
<td>35</td>
<td>450</td>
<td>30</td>
<td>15</td>
<td>X</td>
<td>15</td>
<td>X</td>
</tr>
<tr>
<td>HICAP cells</td>
<td>700</td>
<td>35</td>
<td>175</td>
<td>X</td>
<td>15</td>
<td>50</td>
<td>50</td>
<td>X</td>
</tr>
<tr>
<td>IS cells</td>
<td>X</td>
<td>X</td>
<td>7.5</td>
<td>X</td>
<td>7.5</td>
<td>7.5</td>
<td>450</td>
<td>-</td>
</tr>
</tbody>
</table>

Cell numbers and connectivity values were estimated from published data for granule cells, Mossy cells, basket cells, axo-axonic cells, molecular layer interneurons with axons in perforant-path termination zone (MOPP), hilar interneurons with axons in perforant-path termination zone (HIPP), hilar interneurons with axons in the commissural/associational pathway termination zone (HICAP), and interneuron-selective cells (IS). Connectivity is given as the number of connections to a postsynaptic population (column) from a single presynaptic neuron (row). The average number of connections used in the graph theoretical calculations is given in bold. Note, however, that the small-world structure was preserved even if only the extreme low or the extreme high estimates were used for the calculation of L and C (for further details, see APPENDIX B1(3). References given correspond to: *Gaarskjaer (1978);* *Buckmaster et al. (1996);* *Wenzel et al. (1997);* *Han et al. (1993);* *Gulyás et al. (1992);* *Boss et al. (1985);* *West (1990);* *Patton and McNaughton (1995);* *Freund and Buzsáki (1996);* *Buckmaster and Dudek (1999);* *Acsády et al. (1998);* *Geiger et al. (1997);* *Blasco-Ibáñez et al. (2000);* *Gulyás et al. (1992);* *Buckmaster and Jongen-Relo (1999);* *Buckmaster et al. (1996);* *Wenzel et al. (1997);* *Han et al. (1993);* *Gulyás et al. (1996);* *Babb et al. (1998);* *Woodson et al. (1989);* *Halasy and Somogyi (1993);* *Acsády et al. (2000);* *Sik et al. (1997);* *Bartos et al. (2001);* *Li et al. (1992);* *Ribak et al. (1985);* *Frotscher et al. (1991);* *Katona et al. (1999);* *Soriano et al. (1990);* *Claborne et al. (1990);* *Buckmaster et al. (2002a);* *Nomura et al. (1997a);* *Nomura et al. (1997b).
mossy fiber connections were included in sclerotic graphs. (Andre et al. 2001; Esclapez and Houser 1999), only sprouting of that because the neuronal origin of GABAergic sprouting is unknown.

Implementation and assessment of the models

IMPLEMENTATION OF THE STRUCTURAL MODEL. The dentate gyrus was represented as a 6-mm strip (corresponding to the approximate septotemporal extent of the rat dentate gyrus; West et al. 1978) subdivided into 60-μm bins. Cells of the eight distinct neuronal types were represented in the structural model as individual nodes and distributed evenly among the bins. The nodes were linked according to cell-type-specific connection probabilities derived from the average number of projections from the pre- to the postsynaptic neuronal class in the literature (i.e., according to the connectivity matrix shown in Table 1; appendixes A1 and A2). In general, in addition to the mere existence of connections between two particular cell types (codified in Table 1), the probability of connections from one particular cell A to a given cell B also depends on the extent of the axonal arbor of cell A and the relative distance between cells A and B. Therefore the cell-type-specific connection probability was further modified by a factor obtained by the normalized Gaussian fits to the experimentally determined axonal distributions of the presynaptic cells (Appendix A3 and Fig. 2) and the relative positions of the pre- and postsynaptic neurons in the graph. Within these cell-type-specific constraints, connections were made probabilistically on a neuron to neuron (or, more specifically, because we are talking about a graph, a “node to node”) basis with a uniform synapse (“outgoing link”) density along the axon [in agreement with the in vivo data in Sik et al. (1997)], treating multiple synapses between two cells as a single link and excluding autapses. Note that this implementation of the structural model did not take into account certain potential factors that may distort local connection probabilities (see Discussion). Also note that because the neuronal origin of GABAergic sprouting is unknown (Andre et al. 2001; Esclapez and Houser 1999), only sprouting of mossy fiber connections were included in sclerotic graphs.

ASSESSMENT OF THE STRUCTURAL MODEL: CALCULATION OF GRAPH CHARACTERISTICS. To quantify the topological characteristics of the structural model, the approach of Watts and Strogatz (1998), originally applied to the neuronal network of the worm C. elegans, was used. Two measures were used to assess the salient features of the structural models: the average path length L (average number of steps to reach any node in the network) reflecting global connectivity and the average clustering coefficient C (for a given node, the fraction of possible connections between its postsynaptic nodes that actually exist) as a measure of local connectivity. In human societies, for example, C describes the probability that friends of person X also know each other (i.e., it is a measure of local “cliquishness”), whereas L describes what is commonly known as “the six degrees of separation” between any two persons on the planet (i.e., it is a measure of large-distance or “global” connectivity). These two key topological measures for the structural model of the dentate gyrus were calculated using custom C code on a Tyan Thunder 2.0 GHz dual Opteron server (32 GB RAM). Graph calculation times were roughly 50 h per graph.

In general, there are three distinct major network topologies (for reviews, see Buzsáki et al. 2004; Soltesz 2006): 1) Regular (high L, high C); 2) Random (low L, low C); and 3) Small world (low L, high C) (Fig. 1C). The graph of a regular (or “ordered” or “lattice-like”) network is characterized by a high degree of local interconnectedness (because each node is linked to its nearest neighbors, resulting in a high C), but nodes at the two ends of the graph are separated by a large number of nodes (leading to a high L). In other words, a regular network has an abundance of local connections (thus the comparison to a “lattice” or a fishing net), but has no long-distance connections. Conversely, the graph of a random network is well connected globally (low L), but its local connectivity is low (low C) (this is because random connectivity does not typically form local clusters, but it results in numerous long-distance connections). A small-world structure can be best understood by considering that it can be derived from a regular network by disconnecting and randomly reconnecting a few of its connections (leading to at least a few long-distance connections, which, in turn, results in a low L while retaining the high C of the regular network) (Fig. 1C; note that the term “random reconnection” is used here for didactic purposes to describe a commonly used...
derivation of a small-world network from a regular network, without implying that long-distance connections in an actual biological small-world network are random). Therefore small-world networks are both locally (high C) and globally (low L) well connected (again, in the language of social networks, humans tend to have a strongly interconnected local cluster of friends, but also at least a few acquaintances with connections far outside of the local circle). The quantitative determination of the small-world topology of a given network is performed by comparison to an equivalent random graph: for a small-world network, $L \approx L_{\text{random}}$ and $C \gg C_{\text{random}}$.

Given the large size of the graph, L and C were determined from the weighted averages of randomly sampled nodes ("weighted" here refers to the fact that our sampling took into account the ratio of the nodes representing granule cells, mossy cells, and the six interneuronal classes; i.e., the sampling of the nodes in the structural model had to reflect the ratio of the constituent cell types). A minimum of 1/1,000 granule and mossy cells and 1/100 interneurons were sampled and control calculations were performed to verify the accuracy and stability of the sampling method, as described in Appendix B1.

IMPLEMENTATION OF THE FUNCTIONAL MODEL. The functional model network was implemented using the NEURON 5.6 simulation environment (Hines and Carnevale 1997). The required simulation times ranged from about 35 to 70 h per model network. The single-cell models were taken from Santhakumar et al. (2005), with morphologies, voltage-gated conductances, and intrinsic properties based on detailed experimental data. Briefly, the single-cell models had nine to 17 compartments including a somatic compartment and two to four dendritic segments. Minimally, each dendrite was modeled with a proximal, 17 compartments including a somatic compartment and two to four detailed experimental data. Briefly, the single-cell models had nine to 17 compartments including a somatic compartment and two to four dendrites. Minimally, each dendrite was modeled with a proximal, middle, and distal dendritic segment. The models contained nine classes of active conductance mechanisms such as sodium channels, three types of potassium channels (A-type and fast and slow delayed rectifier), three types of calcium channels (L-, N-, and T-type), two types of calcium-dependent potassium channels (SK and BK channels), $I_{Na}$, and an intracellular calcium clearance process. The intrinsic properties of the cell types were modeled to simulate the passive (membrane potential at rest, input resistance, and membrane time constant) and active (amplitude and threshold of action potential, fast afterhyperpolarization, spike frequency adaptation, and sag ratios) properties observed in experimental data (Lubke et al. 1998; Staley et al. 1992). For granule cells, the somatodendritic distribution of active conductances was adapted from Aradi and Holmes (1999). In all other cell types, the active conductances, with the exception of sodium and fast delayed rectifier potassium channels, were distributed uniformly in all compartments. Sodium and fast delayed rectifier potassium conductances were present only in the soma and proximal dendritic compartments. Additionally, correction for the membrane area contribution of spikes was implemented for the granule and mossy cell models. The multicompartmental single-cell models of 50,000 granule, 1,500 mossy, 500 basket, and 600 HIPP cells were evenly distributed in 100 bins along the septotemporal axis.

Connectivity in the functional model network was established using the procedure described for the structural model. All connection probabilities were increased fivefold compared with the structural model, to compensate for the fewer number of cells in the functional model and ensure that no cells in the model networks were disconnected (note that even with this increase in connection probability, each presynaptic cell still made fewer connections in the functional model network than in the full-scale structural model because the postsynaptic cell populations were reduced by a factor of 20; compare Tables 1 and 2). The synaptic conductances between cell types, based on unitary conductances from the literature, were taken from Santhakumar et al. (2005). Excitatory synaptic conductances were adjusted to avoid depolarization block in postsynaptic cells arising from the higher value of the clustering coefficient C in the functional model network (see Fig. 3B). Except when specifically stated (see Appendix B3), distance-dependent axonal conduction delays were not included. Perforant path stimulation was simulated as in Santhakumar et al. (2005), by a single synaptic input to 5,000 granule cells, 10 mossy cells (note that only a fraction of all mossy cells receive direct perforant path input; Buckmaster et al. 1992; Scharffman 1991), and 50 basket cells (situated in the middle lamella of the model network) at $t = 5$ ms after the start of the simulation. Additional details of the functional model network, including the convergence and divergence of the connections and the synaptic weights, are listed in Table 2. Note that the current functional model has three primary differences from the network model of Santhakumar et al. (2005). First, we have enlarged the network by two orders of magnitude, making it possible to study the small-world network characteristics of the dentate gyrus.

### Table 2. Parameters of functional network model

<table>
<thead>
<tr>
<th>From</th>
<th>To</th>
<th>GC</th>
<th>MC</th>
<th>BC</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granule cells*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(50,000)</td>
<td></td>
<td>68.03</td>
<td>78.05</td>
<td>370.95</td>
<td>2,266.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68.03</td>
<td>2.34</td>
<td>3.71</td>
<td>27.19</td>
</tr>
<tr>
<td></td>
<td>Synapse weight, nS</td>
<td>1.00</td>
<td>0.20</td>
<td>0.94</td>
<td>0.10</td>
</tr>
<tr>
<td>Mossy cells</td>
<td></td>
<td>243.62</td>
<td>87.23</td>
<td>5.59</td>
<td>375.53</td>
</tr>
<tr>
<td>(1,500)</td>
<td></td>
<td>8,120.82</td>
<td>87.23</td>
<td>1.86</td>
<td>150.21</td>
</tr>
<tr>
<td></td>
<td>Synapse weight, nS</td>
<td>0.30</td>
<td>0.50</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td>Basket cells</td>
<td></td>
<td>3.11</td>
<td>6.31</td>
<td>8.98</td>
<td>n/a</td>
</tr>
<tr>
<td>(500)</td>
<td></td>
<td>313.22</td>
<td>18.93</td>
<td>8.98</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>Synapse weight, nS</td>
<td>1.60</td>
<td>1.50</td>
<td>7.60</td>
<td>n/a</td>
</tr>
<tr>
<td>HIPP cells</td>
<td></td>
<td>4.82</td>
<td>3.76</td>
<td>140.13</td>
<td>n/a</td>
</tr>
<tr>
<td>(600)</td>
<td></td>
<td>401.86</td>
<td>9.39</td>
<td>116.77</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>Synapse weight, nS</td>
<td>0.50</td>
<td>1.00</td>
<td>0.50</td>
<td>n/a</td>
</tr>
<tr>
<td>Perforant path†</td>
<td></td>
<td>20.00</td>
<td>17.50</td>
<td>10.00</td>
<td>n/a</td>
</tr>
</tbody>
</table>

The cell numbers (column 1) and synaptic connectivity values and strengths in the functional model network are used for the activity calculations in Fig. 4 (quantified in Fig. 5). Note that this network is smaller (50,000+ cells) than the full-scale dentate gyrus (>1,000,000 cells); thus the connectivity had to be adjusted from what is shown in Table 1. Convergence is given as the number of connections converging onto a single postsynaptic neuron (row 1) from a presynaptic neuronal population (column 1). For example, 243 mossy cells converge on a single granule cell in this network. Divergence is given as the number of connections diverging to a postsynaptic population (row 1) from a single presynaptic neuron (column 1). For example, a single mossy cell makes synapses on 8,120 postsynaptic granule cells in this network. The strengths of the connections are given in nanosiemens (nS). For example, the strength of the excitatory synapse formed by a single mossy cell on a single granule cell is 0.3 nS. *Granule cell to granule cell connections represent values at 100% sprouting. †Perforant path input to 5,000 granule cells (two synapses each), 50 basket cells (two synapses each), and 10 mossy cells (one synapse each) in the central 10 bins of the network model.
Second, in contrast to the Santhakumar et al. (2005) study that focused on moderate (<50%) sclerosis, the current model examines the structural and functional effects of the progression of sclerosis from 0 to 100%. Third, the use of Gaussian fits to constrain axonal distributions instead of an uniform probability adopted in the earlier model considerably increased topological accuracy of this model. Moreover, the current study also tested the effects of hilar interneuronal loss and parallel increases in sprouting and hilar cell loss in contrast to the independent examination of sprouting and mossy cell loss performed in Santhakumar et al. (2005).

ASSESSMENT OF THE FUNCTIONAL MODEL: MEASURES OF HYPEREXCITABILITY. Excitability of the functional model was assessed by a number of measures, including: 1) total duration of the granule cell discharges in the network (defined as the time from the first spike fired by a granule cell in the network to the last spike fired by a granule cell in the network; note that the first and the last granule cell spikes may originate from different granule cells); 2) mean number of spikes per granule cell; 3) latency to spread of activity from the perforant path activation to the firing of the most distant granule cells in the network; and 4) synchrony of granule cell discharges. Because the latter measure is the most complicated, it will be described below separately.

To assess synchrony, the coherence of granule cell firing between 100 and 200 ms (i.e., sufficiently far in time from the initial stimulus, and during a period where networkwide activity could be observed at most degrees of sclerosis; see Fig. 4) was calculated, using a published coherence measure (Foldy et al. 2004; White et al. 1998). The local coherence was calculated by all-to-all comparison of the activity in granule cells #25000 to #25999. Pairwise comparison of the activity in granule cells #25000 to #25999 and #45000 to #45999 provided the long range coherence. To calculate coherence from the network simulations during the poststimulation analysis, trains of square pulses were generated for each firing cell in a pair with each pulse of unitary height centered on the spike peak and the width equal to 20% of the mean interspike interval of the faster spiking cell in the pair. Subsequently, the shared area of the unit height pulse trains was calculated (equivalent to the zero time lag cross-correlation). Coherence was defined as the sum of their shared areas normalized by the square root of the product of the total areas of the individual trains (Foldy et al. 2004; White et al. 1998).

Data analysis and plotting were done using Matlab 6.5.1 (The MathWorks, Natick MA) and Sigmaplot 8.0 (SPSS, Chicago IL).

Note that the structural and functional model networks are available for download from ModelDB (http://senselab.med.yale.edu/senselab/ModelDB).

RESULTS

Key features of the biological net captured by the structural model

The structural model of the healthy, nonsclerotic dentate gyrus contained over one million (1,064,000) nodes, with the majority (94%) representing granule cells. The million nodes in the control dentate graph were richly linked by over a billion links (1,287,363,500). As in the biological network, there was a large difference in the degree of interconnectedness between nodes representing different cell types. The nodes representing granule cells gave the fewest links (Table 1) and these links were also the most spatially restricted (corresponding to the restricted septotemporal extent of the in vivo filled granule cell axons shown; Figs. 1B and 2). In contrast, nodes representing mossy cells formed by far the highest number of links to other nodes (Table 1) and these links spanned almost the entire length of the dentate graph (corresponding to the large extent of single mossy cell axon arbors; Figs. 1B and 2).

Globally and locally well connected nature of the control dentate gyrus

We assessed the quantitative topological properties of the control structural model of the dentate gyrus by calculating L and C for the graph at 0% sclerosis and for the equivalent
The average path length for the control dentate graph was remarkably low (L/̅ = 2.68, marked E in Fig. 3A), considering the presence of over one million nodes in the network. The L/̅ = 2.68 value indicated that, on average, fewer than three synapses separated any two neurons in the dentate gyrus. Therefore the low L showed that the graph was well connected globally. To our knowledge, this is the first measurement of L for a mammalian microcircuit, where each neuron is represented by a unique node in the graph. It is interesting to note that the average path length for the control structural model was virtually identical to the L/̅ = 2.65 reported for the much smaller nervous system of the worm *C. elegans* with a connected graph of only 282 nodes (Watts and Strogatz 1998) (note, however, that the *C. elegans* simulations were done on a nondirected graph, whereas our graphs take into account the directionality of the connections).

The average path length calculated for the equivalent random graph was only slightly lower (L/̅_random = 2.25) than the L for the control structural model, resulting in a L/L/̅_random ratio close to one (1.19; indicated by the solid black line at 0% sclerosis in Fig. 3C). However, the control structural model was much more highly connected locally than the equivalent random graph, as shown by the high value of the relative clustering coefficient (C/̅_random = 0.026751 / 0.001135 = 24.7, indicated by the solid black line at 0% sclerosis in Fig. 3D; note that the control value for C is marked E in Fig. 3B). The relatively low average path length and high clustering coefficient of the control dentate graph fulfilled the dual requirements of L/̅_c ≈ L/̅_random and C_̅_c ≫ C/̅_random, demonstrating that the normal, healthy biological dentate gyrus is a small-world network (Watts and Strogatz 1998).

### Enhanced local and global connectivity with submaximal sclerosis and the transition to a more regular network structure at severe sclerosis

Next, we determined how the graph characteristics of the dentate gyrus change during the progression of sclerosis, characterized by the loss of hilar neurons and mossy fiber sprouting (Longo et al. 2003; Nadler 2003; Ratzliff et al. 2004). The fully sclerotic dentate graph exhibited only a small (4.5%) decrease in the total number of nodes (48,000 nodes representing hilar cells lost out of 1,064,000), but there was a dramatic (74%) reduction in the number of links (953,198,800 links removed out of the total 1,287,363,500), indicating that maximal sprouting did not replace the lost links resulting from the removal of all richly connected hilar cells (Table 1). To determine how small-world topology was affected by the removal of so many links, L and C values were calculated for dentate graphs...
constructed at various degrees of sclerosis (solid black lines in Fig. 3, A and B). Because the average path length and clustering coefficient of the equivalent random graphs also changed with deletion of nodes and addition of links, these were recalculated for each degree of sclerosis (dashed lines in Fig. 3, A and B).

The results revealed that the progression of sclerosis (increased loss of the predominantly long distance projecting mossy cells and hilar interneurons, with increasing degrees of spatially restricted mossy fiber sprouting) did not significantly elevate L, until about 90% of the hilar cell nodes were lost (solid black line in Fig. 3A). In fact, the relative average path length (L/L\text{random}) actually decreased below the control, 0% sclerotic level (illustrated by the horizontal dotted line in Fig. 3C). These data indicated that, despite the loss of long-distance projecting hilar cells and the resulting massive decrease in connections, there was a seemingly paradoxical enhancement of long-distance connectivity and conservation of the small-world topology during submaximal (<90%) sclerosis. However, the relative L did not stay below its control value because it showed a sudden increase during the last stages of sclerosis. Therefore the changes in relative L during sclerosis were strongly biphasic (i.e., the initial decrease in L was followed by a sudden increase). Importantly, as illustrated in Fig. 3C, it was only at 96.6% sclerosis (vertical dotted line) that the relative average path length (solid black line) started to increase above the control value (horizontal dotted line), indicating that global connectivity was preserved until the final stages of sclerosis.

The high C value (the second characteristic feature of small-world topology) of the control dentate graph was also preserved and actually enhanced during submaximal sclerosis. Although the initial increase in C values was followed by a subsequent decrease at midlevel sclerosis (around 40%) (solid black line in Fig. 3B), the relative clustering coefficient (C/C\text{random}, solid black line in Fig. 3D) increased above the control value (indicated by horizontal dotted line in Fig. 3D) up to about 90% sclerosis, showing a sclerosis-related enhancement of local connectivity. Similar to the biphasic changes in relative L, it was only shortly before the onset of full sclerosis that the relative C values decreased below the control level (dotted lines in Fig. 3D); note that, even though relative clustering coefficient decreased at 100% sclerosis in Fig. 3D, the absolute clustering coefficient in Fig. 3B remained more than tenfold higher than C\text{random} even at maximal sclerosis.

The decreasing relative average path length (solid black line in Fig. 3C) and increasing relative clustering coefficient (solid black line in Fig. 3D) during submaximal sclerosis together demonstrated an unexpected enhancement of the features characterizing a small-world topology. However, a transition to a more regular or lattice-like network structure (Watts and Strogatz 1998), characterized by high values of both L and C (i.e., poor global but rich local connectivity), occurred shortly before maximal (100%) sclerosis (note that the fully sclerotic network is not a true lattice structure with only nearest-neighbor connections because, e.g., the axonal arbors of basket cells span roughly 25% of the septotemporal extent of dentate gyrus, providing a large number of midrange connections in the network even at 100% sclerosis).

Analysis of the changing roles of topological factors using isolated excitatory and inhibitory structural models

To determine the mechanisms underlying the transient enhancement of small-world properties during sclerosis, graphs of the excitatory and inhibitory parts of the dentate network were considered separately. First, the isolated excitatory graph was examined. The sclerosis-induced changes in L and C in the isolated excitatory graph (mossy cells and granule cells alone; solid lines in Fig. 3, E and F) were generally similar to the alterations in the full dentate graph (solid black lines in Fig. 3, A and B), suggesting that it was the loss of long-range connections (in this case, from mossy cells) and mossy fiber sprouting that played key roles in alterations of graph structure. Sprouting without mossy cell loss did not significantly affect the average path length in the excitatory graph (dashed line in Fig. 3E), indicating that the added local connections from sprouted mossy fibers mattered little for L when the long-range connections of the mossy cells were retained. However, sprouting without mossy cell loss in the excitatory graph produced similar changes in the clustering coefficient (dashed line in Fig. 3F) as sprouting with mossy cell loss (solid line in Fig. 3F). (Note that the essentially unchanged L and the biphasic changes in C observed in the sprouting-only isolated excitatory network will play an important role in determining the role of L and C in network hyperexcitability during sclerosis; see following text.) The decrease in C at higher degrees of sclerosis (which is also observed in the structural model containing both excitatory and inhibitory neurons) was the result of each granule cell primarily contacting other granule cells after mossy fiber sprouting. Because the probability of sprouted connections between any two granule cells is low, the fraction of actually existing connections between pairs of postsynaptic granule cells is also low, resulting in a decreasing C (see METHODS, ASSESSMENT OF THE STRUCTURAL MODEL: CALCULATION OF GRAPH CHARACTERISTICS). In the structural model network containing both excitatory and inhibitory neurons, this dominant influence of granule-to-granule cell connections on the clustering coefficient was more gradual as a result of the larger number of nongranule cell postsynaptic targets of each granule cell.

In contrast to the isolated excitatory graph, the isolated interneuronal graph (i.e., without granule cells and mossy cells) showed a steady increase in average path length and decrease in clustering coefficient with sclerosis (dotted lines in Fig. 3, E and F) because the progressive loss of hilar interneurons resulted in an increasingly sparse graph (note that there were no granule cells and thus no sprouting of mossy fibers in the isolated interneuronal graph). Interestingly, the control interneuronal graph had an order of magnitude higher clustering coefficient (C = 0.0561) than the control excitatory graph (C = 0.0060), reflecting the significantly more interconnected nature of interneuronal circuits.

These results showed that, during submaximal sclerosis, it was primarily the sprouting of mossy fibers that played a key role in determining topology because the local shortcuts provided by sprouting not only increased C, but also maintained a low L: for granule cells (GC), the loss of mossy cells (MC) removed a number of two-step (GC → MC → GC) and three-step (GC → MC → MC → GC) paths that were partially compensated by the introduction of a large number of new connections.
three-step paths (GC → MC → GC → GC; GC → GC → MC → GC). This mechanism was similar for connections involving other long-range projecting hilar neurons. However, with severe sclerosis, the primary role shifted to the loss of hilar cells because their numbers became too small to maintain the low average path length (even with maximal sprouting). Therefore these calculations, carried out in the isolated excitatory and inhibitory networks, revealed that mossy fiber sprouting was primarily responsible for the transient increase in C at sub-maximal sclerosis, whereas the dramatic increase in L at maximal sclerosis was mostly explained by the loss of the last distantly projecting hilar neurons. In other words, the key topological determinant switches from sprouting to hilar cell loss during severe sclerosis.

**Biphasic alterations in network topology during sclerosis result in biphasic changes in network excitability**

Next, the functional consequences of the biphasic alterations in network topology observed in the structural model were explored in our large-scale, topographically, and biophysically realistic functional model of the dentate gyrus (for details on the model, see Methods). Before the simulations, we first verified that the changes in L and C for the graph of the functional model network with 50,000+ cells were similar to those observed for the graph of the biological network containing 1,000,000+ cells (blue lines in Fig. 3, A–D; compare with solid black lines in the same panels).

As with the biological dentate gyrus (Santhakumar et al. 2001), the functional model network of the normal, nonsclerotic dentate gyrus showed only limited firing in response to single simulated perforant path stimulation (Fig. 4A). Between 20 and 80% sclerosis (when the features characteristic of small-world topology were found to be enhanced in the structural model), the functional model network showed increasing hyperexcitability (Fig. 4, B–E), with activity spreading to the entire network by 40% sclerosis, reminiscent of the epileptiform activity recorded in vitro (Rafiq et al. 1995). However, beyond 80% sclerosis, corresponding to the change from a small-world network to a more regular network in the structural model, the activity in the functional model network decreased (Fig. 4F). In other words, changes in network activity during sclerosis, as assessed by a probing stimulus to the perforant path, appeared to be biphasic, similar to the biphasic nature of the topological alterations during sclerosis observed in the structural model. It is interesting to note that the structural changes also affected network dynamics, as indicated by the transition from a spatially relatively uniform pattern (40–60% sclerosis; Fig. 4, C and D) to a pattern with distinct waves of activity (80–100% sclerosis; Fig. 4, E and F), that could collide and mutually annihilate (Netoff et al. 2004; Roxin et al. 2004).

Next, we quantified the changes in activity seen in Fig. 4 in the functional model network during sclerosis, using a number of measures of hyperexcitability (see Methods). Both the maximal duration of granule cell activity and the mean number of spikes fired per granule cell (black lines in Fig. 5, A and B) increased and peaked at 80% sclerosis, followed by a decrease at 100% sclerosis. For degrees of sclerosis where the activity spread to the entire functional model network (40–100% sclerosis), the latency from perforant path activation to the firing of the most distant granule cells (black line in Fig. 5C) was the shortest (i.e., activity spreads the fastest) at 60% sclerosis (89.7 ms), followed by an increase in latency to the maximal value at 100% sclerosis (106.2 ms). The topological alterations also affected synchrony of firing in the network. Comparison of local and global synchrony in granule cell firing showed that local and long-range coherences initially were similar at 40 and 60% sclerosis (Fig. 5D, dashed black line: local coherence; solid black line: long-range coherence), but the long-range context.

![Image](http://jn.physiology.org/)

**FIG. 5.** Biphasic changes in granule cell firing in the functional model of the dentate gyrus during progressive sclerosis and the lack of biphasic changes when sprouting occurs without hilar neuron loss. Black lines: sclerosis; green lines: sprouting alone, without concurrent hilar cell loss. A: changes in the maximal duration of perforant path stimulation-evoked granule cell firing (for a description of this measure, see Methods) as a function of sclerosis or sprouting alone (note that granule cell firing persisted beyond 2,000 ms at 80 and 100% sprouting in the sprouting-only case). B: mean number of spikes fired per granule cell with progressive sclerosis or sprouting without cell loss. C: latency to firing of granule cells in all 100 bins, in networks with persistent activity in all granule cell bins. D: local (dashed line, all-to-all comparison of action potential firing in granule cells #25000 to #25999) and long range (solid line, pairwise comparison of the activity in granule cells #25000 to #25999 to granule cells #45000 to #45999) coherence of granule cell firing in the time interval of 100 to 200 ms for 40–100% sclerosis or sprouting without cell loss. Note that in C and D, results are shown only for 40–100% sclerosis or sprouting, where activity spread to the entire network and persisted beyond 100 ms. E and F: changes in average total peak conductance of excitatory (E) and inhibitory (F) synapses onto the model granule cell during sclerosis or sprouting in the absence of cell loss. Note the lack of biphasic changes in A–D when sprouting took place without hilar cell loss.
coherence subsequently decreased with increasing sclerosis, whereas the local coherence dramatically increased. This divergence of local and long-range synchrony with progressive sclerosis reflected the increasing dominance of short-range connections over long-range hilar cell axonal projections. Taken together, these measures all indicated that biphasic changes in the structural model resulted in biphasic alterations in hyperexcitability during sclerosis.

We also tested the potential caveat that changes in the activity of the functional model network simply reflected alterations in net excitatory and inhibitory conductances to granule cells, rather than transitions in network architecture. As shown in Fig. 5, E and F (black lines), there was a monotonic decrease in both net excitatory and inhibitory peak conductances received by individual granule cells during sclerosis. Clearly, these monotonic changes in synaptic innervation of the granule cells could not be directly responsible for the biphasic changes in network activity.

Network activity parallels changes in sprouting in the absence of cell loss

The described biphasic changes in network hyperexcitability during sclerosis (solid black lines in Fig. 5, A–D) occurred in conjunction with biphasic changes in both relative average path length and relative clustering coefficient (solid black and blue lines in Fig. 3, C and D). Which of these two topological measures primarily determine changes in network hyperexcitability? How do the changes in L and C correspond to cell loss and mossy fiber sprouting? To answer these questions, we took advantage of the fact that (as noted before), in graphs of the isolated excitatory network with sprouting but no cell loss (dashed lines in Fig. 3, E and F), the average path length did not change significantly, but the clustering coefficient still underwent a biphasic change with increasing degree of sprouting. Therefore we could use sprouting-only networks (showing unchanged L but biphasic changes in C) to test whether biphasic changes in the clustering coefficient alone would conserve the biphasic changes in network hyperexcitability. Accordingly, we constructed functional model networks where mossy fiber sprouting occurred in the absence of hilar neuron loss. Again, before the actual simulations were performed, we first verified that the graphs of these sprouting-only functional model networks indeed showed the expected biphasic changes in absolute and relative clustering coefficient (green lines in Fig. 3, B and D), without significant changes in absolute and relative average path length (green lines in Fig. 3, A and C). (Note that these calculations were crucial because the functional model network contained 20 times fewer neurons than the number of nodes in the structural model network. Therefore topological changes taking place in the structural model cannot be automatically assumed to occur in the graph of the functional model.)

In response to perforant path stimulation, the sprouting-only functional model networks showed increasing granule cell activity with progressive sprouting, which spread to the entire model network in a self-sustained manner at 40–100% sprouting (Fig. 6). Detailed analysis of the activity in the sprouting-only networks (green lines in Fig. 5, A–D) showed that both the maximal duration of activity and the mean number of spikes fired per granule cell increased monotonically with increasing degree of sprouting and exceeded those in the corresponding sclerotic networks (compare black and green lines in Fig. 5, A and B; note the difference in scale of the y-axes), whereas the latency of the spread of activity to the most distant granule cells steadily decreased (green line in Fig. 5C). In contrast to the sclerotic networks (black lines in Fig. 5D), the local and long-range coherence of the granule cell firing remained similar to each other for all degrees of sprouting in the sprouting-only networks (green lines in Fig. 5D). The monotonic increase in granule cell firing and decrease in latency of activity propagation in the sprouting-only networks reflected the monotonic increase in excitatory drive to granule cells with progressive sprouting (68 nS increase per granule cell at maximal sprouting; green line in Fig. 5E), whereas inhibition remained constant (green line in Fig. 5F).

These results with the sprouting-only networks demonstrate that biphasic changes in the absolute and relative clustering coefficient alone (i.e., without corresponding alterations in path length) do not result in biphasic changes of network hyperexcitability. Therefore these findings further support the conclusion that, in networks with concurrent sprouting and hilar neuron loss, the biphasic changes in relative average path length and the corresponding transition from a small world to a more regular network structure were primarily responsible for the biphasic change in network hyperexcitability.

Control simulations and tests of robustness

To examine the robustness of our results, we tested the effects of changes in structural and functional parameters on L and C values and on network activity in an extensive series of control simulations. These controls are described in appendixes B2 and B3 and illustrated in Figs. 7–9. The control simulations concerning structural parameters [APPENDIX B2, (1)–(5)] included tests of cell-type–specific changes in neuronal density along the septotemporal axis, inhomogeneity in connectivity along the transverse axis, axonal distributions at the septal and temporal poles of the model dentate gyrus, offset degrees of cell loss and sprouting, and the bilateral dentate gyrus model with both associational and commissural projections. The control simulations concerning functional parameters [APPENDIX B3, (1)–(3)] included increasing (doubling) the strength of inhibitory synaptic connections, inclusion of axonal conduc-
tion delays, and simulation of spontaneous instead of stimula-
tion-evoked activity.

All variations in structural parameters in these control calculations yielded L and C values that were similar to the L and C values of the base model used in the rest of the paper. Similarly, the simulations of activity with altered structural and functional parameters all displayed the characteristic decrease in network activity with the transition from 80 to 100% sclerosis, demonstrating the robustness of the major conclusions.

DISCUSSION

The following are major findings of this study. 1) The control dentate gyrus is a locally and globally well connected small-world network. 2) Structural alterations during epileptogenesis result in biphasic changes of small-world topology. Initially, and during the majority of the sclerotic process, the
features defining the small-world characteristics of the dentate gyrus are enhanced (the dentate gyrus becomes “more small world” than in its control state). This is shown by a decreased relative L and increased relative C, indicating enhanced global and local connectivity. However, just before maximal sclerosis is reached, the relative L sharply increases as a result of the loss of the last long-distance projecting hilar cells, while relative C declines, leading to an overall decrease in the small-world characteristics. Analyses of isolated excitatory and inhibitory graphs show that biphasic changes in small-world characteristics correspond to changing roles of topological factors: During submaximal sclerosis, the key factor is the predominantly local mossy fiber sprouting, which (as long as at least some long-distance projecting hilar cells survive) compensates for the loss of hilar cells (leading to a decrease in relative L, despite the loss of many long-distance connections). However, during the last stages of sclerosis, the loss of hilar neurons becomes the major topological determinant: as the last long-distance connections are removed, the network evolves into a more regular, lattice-like structure. Simulations in the functional network show that changes in network topology predict the development of hyperexcitability: during submaximal sclerosis, when small-world characteristics increase, network hyperexcitability increases; however, during the last stages of sclerosis, when small-world characteristics decrease, the network hyperexcitability declines. Sprouting-only networks, showing insignificant changes in L but biphasic changes in C, exhibited monotonic increases in hyperexcitability. These latter results further underline the importance of network topology by indicating that, in networks with concurrent sprouting and hilar cell loss, the biphasic changes in relative L (and the corresponding transition from a small-world to more regular network topology) are primarily responsible for the biphasic change in network hyperexcitability. Consequently, these results suggest that the survival of even a few hilar cells is critically important in maintaining networkwide hyperexcitability in the epileptic dentate gyrus.

Limitations of the models and robustness of the conclusions

Although our structural model was full scale and incorporated eight neuronal classes with cell-type–specific and topographic connections, the “virtual dentate gyrus” did not replicate the complete connectivity of the biological network. In most cases, specific components were not represented in the structural model because no precise data were available. For example, recent results indicate that local connection probability may be modified by intraclass correlations (Yoshimura and Callaway 2005; Yoshimura et al. 2005) and overrepresentation of small network motifs (Milo et al. 2002; Reigl et al. 2004; Song et al. 2005; Sporns and Kotter 2004). However, there is no evidence for such factors in the dentate gyrus. In the functional model, the neuronal populations were simulated with homogeneous cellular properties and synaptic connectiv-
ity strengths. However, a large degree of heterogeneity was still present, stemming from the use of four very different neuronal populations with different synaptic characteristics, variability in the number and distribution of connections, and a random, noisy conductance implemented in the mossy cell models to emulate the experimentally described spontaneous firing. Furthermore, simulations with continuous random synaptic activation of the network (see Fig. 9, C and D) did not alter the described behavior. The functional model deliberately did not incorporate a number of factors that reportedly change in epilepsy because our goal was to test the functional roles of purely structural changes, while keeping all functional parameters unaltered.

Despite model limitations, the conclusions are remarkably robust. Confidence in the results are strengthened by two factors: 1) the multicompartmental single-cell models were specifically developed to replicate a large number of electrophysiological properties, including resting membrane potential, input resistance, action potential amplitude, threshold, adaptation, afterhyperpolarization, maximal firing rate, and sag ratio (Santhakumar et al. 2005); and 2) the functional networks constructed from these model cells simulate biological responses, including action potential firing in only a small fraction of granule cells in response to a single-shock stimulation of perforant path fibers under control conditions (Santhakumar et al. 2005) (Fig. 4A), resulting from the low resting membrane potential of granule cells and strong feedforward inhibition from basket cells. Also, hyperexcitability already emerged in the functional model at mild (20–40%) levels of sclerosis, in agreement with experimental observations (Lowenstein et al. 1992; Santhakumar et al. 2001; Toth et al. 1997). In addition, an extensive series of control simulations (appendixes B2 and B3) tested the dependency on structural and functional parameters that may not have been well constrained by experimental data: e.g., the biphasic change in network hyperexcitability was unaltered despite doubling of all inhibitory conductances in the functional model to compensate for the excluded interneuronal subtypes. Remarkably, control simulations with the bilateral model showed that the conclusions persisted even when the network was doubled in size and interconnected with anatomically realistic commissural projections. The robustness of the conclusions was further supported by the strong predictive powers of the different models: e.g., although the structural model was based on nonweighted graphs, consistent functional effects were observed in the functional model with weighted synaptic connections.

Control dentate microcircuit as a small-world network

Our results demonstrated that neurons in the healthy dentate gyrus form a both locally and globally well connected small-world network microcircuit. Interestingly, any two neurons in the healthy dentate gyrus were separated by fewer than three synapses on average, just as in the C. elegans neuronal network (Watts and Strogatz 1998), despite the severalfold difference in network size. It is interesting to speculate that perhaps an...
evolutionary pressure exists to keep $L$ constant as network size increases. However, more networks must be analyzed to test this hypothesis. Currently, the only neuronal networks for which $L$ and $C$ values have been determined, with individual neurons considered as distinct nodes, are the networks of the *C. elegans* (Watts and Strogatz 1998), culture systems (Shefi et al. 2002), and the dentate gyrus (present study). In all other studies where small-world characteristics were determined from anatomical data, the nodes were entire brain areas (Achard et al. 2006; Sporns and Zwi 2004; Stephan et al. 2000), not single cells. Studies describing small-world topology of interarea brain connectivity (Sporns and Zwi 2004; Stephan et al. 2000)
predict that the low $L$ of the dentate gyrus is unlikely to increase considerably when larger parts of the limbic system are considered, in agreement with the presence of long-distance connections between distinct limbic areas (Buzsáki et al. 2004; Ceranik et al. 1997; Sik et al. 1994).

**Functional relevance of topological changes during epileptogenesis and correspondence to experimental data**

Based on the presence of both local and long-distance connections, it may be predicted that small-world topology should allow both fast local computations and efficient relay of signals to distant parts of the network. Indeed, previous modeling studies demonstrated that small-world networks display fast signal propagation and long-range synchronization (Barahona and Pecora 2002; Lago-Fernandez et al. 2000; Li and Chen 2003; Masuda and Aihara 2004). We found a marked enhancement of small-world characteristics of the submaximally sclerotic dentate that was accompanied by increasing degrees of hyperexcitability, as assessed by a number of different measures. The enhancement of small-world network characteristics during submaximal sclerosis was counterintuitive (it took place despite a massive loss of connections), occurring because mossy fiber sprouting compensated for the loss of long-range hilar neurons, leading to only slight increases in $L$. However, this compensation was a double-edged sword: because of the highly localized mossy fiber sprouting, submaximal sclerosis increased the clustering coefficient, leading to an overall enhancement of small-world network properties, and thus enhanced network hyperexcitability.

The importance of network topology was perhaps best demonstrated by the surprising decrease of hyperexcitability with maximal sclerosis. This decrease in hyperexcitability took place at the transition from small-world topology to a more regular network structure, resulting from the loss of the last hilar mossy cells that normally project several millimeters in the dentate gyrus, innervating tens of thousands of granule cells. This result not only supported the functional role of hilar mossy cells, forming contacts 15 basket cells and three axo-axonic cells, assuming no axonal arborizations in the commissural/associational pathway termination zone (HIPP cells), but also predicted that the low $L$ of the dentate gyrus is unlikely to increase considerably when larger parts of the limbic system are considered, in agreement with the presence of long-distance connections between distinct limbic areas (Buzsáki et al. 2004; Ceranik et al. 1997; Sik et al. 1994).

**APPENDIX A1: ESTIMATION OF CELL NUMBERS**

The number of granule cells (GCs) in the dentate gyrus of the rat was estimated to be 1,000,000 (Boss et al. 1985; Freund and Buzsáki 1996; Gaarskjaer 1978; Patton and Mcnaughton 1995; West 1990). Buckmaster and Jongen-Relo (1999) estimated the number of GAD-mRNA negative neurons in the dentate hilus [presumed mossy cells (MCs)] to be 30,000 (see also control calculations in APPENDIX B1). The maximal fraction of GABAergic cells in the granule cell layer of the dentate has been estimated to be 2% (Babb et al. 1988; Woodson et al. 1989), many of which are likely to be basket cells (BCs) (Patton and Mcnaughton 1995). Thus because the number of granule cells is 1,000,000, we set the number of basket cells to 10,000 (also in agreement with Patton and Mcnaughton 1995). In the CA1 region of the rat hippocampus, the ratio of pyramidal cells to axo-axonic cells (AACs) is estimated to be 200–600:1 (Li et al. 1992), whereas the ratio of granule cells to axo-axonic cells in the dentate is estimated to be in the higher end of this range (Patton and Mcnaughton 1995). Assuming a GC: AAC ratio of 500:1, we estimated the number of axo-axonic cells to be 2,000. Buckmaster and Jongen-Relo (1999) estimated the total number of GAD-mRNA positive neurons in the molecular layer of the dentate gyrus to be about 10,000. Assuming an even distribution between inner-, medial- and outer molecular layers, we estimated that there were 4,000 molecular layer interneurons with axonal arborization in the perforant path termination zone (MOPCs), with somata located in the inner molecular layer (Han et al. 1993). Note that molecular layer interneurons with a majority of their postsynaptic targets outside the dentate gyrus, like the outer molecular layer interneurons projecting to the subiculum (Ceranik et al. 1997), have not been included. Because the hilar interneurons with axonal arborization in the perforant path termination zone (HIPP cells) are thought to be identical to the somatostatin positive interneurons in the dentate hilus (Freund and Buzsáki 1996; Katona et al. 1999) and because Buckmaster and Jongen-Relo (1999) estimated that there were 12,000 somatostatin-positive neurons in the hilus, we included 12,000 HIPP cells in the dentate network. Hilar interneurons with axonal arborizations in the commissural/associational pathway termination zone (HICAP cells) are thought to be NOS-positive (Freund and Buzsáki 1996). Because roughly 50% of the nearly 7,000 NOS-positive cells in the hilus are single labeled (i.e., not somatostatin/neuropeptide-Y or calretinin positive) (Nomura et al. 1997a,b), we estimated the number of HICAP cells in the dentate gyrus to be 3,000. The hilus contains about 6,500 calretinin-positive cells (Nomura et al. 1997a,b), roughly 30% of which are somatostatin-positive (presumably spiny CR positive cells) and some of which overlap with the NOS-positive cells (Nomura et al. 1997a,b). Hilar interneuron-selective cells (IS cells) are asynp and calretinin-positive (Gulyás et al. 1996), and, assuming that maximally 50% of the calretinin-positive cells are asynp, we estimated the number of IS cells to be 3,000.

**APPENDIX A2: ESTIMATION OF CONNECTIVITY**

The connectivity for each cell type is summarized in Table 1 and described below in detail. In the estimates given below, we used a uniform bouton density along the axon of the presynaptic cell, in agreement with the in vivo data in Sik et al. (1997).

**Granule cells**

Mossy fibers (granule cell axons) in the healthy rat dentate gyrus are primarily restricted to the hilus (97%), with few collaterals (3%) in the granule cell layer (Buckmaster and Dudek 1999). In addition to mossy cells (Acsády et al. 1998), mossy fibers were also shown to contact basket cells (Buckmaster and Schwartzkroin 1994; Geiger et al. 1997) and parvalbumin-positive interneurons (Blasco-Ibanez et al. 2000). With a total of 400–500 synaptic contacts made by a single mossy fiber (Acsády et al. 1998), the 3% of each axon located in the granule cell layer (Buckmaster and Dudek 1999) was estimated to contact 15 basket cells and three axo-axonic cells, assuming no preferential targeting of either interneuron type. In the hilus, a single granule cell was shown to project to seven to 12 mossy cells, forming...
large complex mossy fiber boutons (Acsády et al. 1998), whereas an estimated 100–150 mossy fiber terminals target hilar interneurons with approximately one synapse per postsynaptic interneuron (Acsády et al. 1998). Gulyás et al. (1992) estimated that a single spiny CR-positive cell (presumed HIPP cell) is contacted by about 9,000 granule cells. With 12,000 HIPP cells and 1,000,000 GCs, each granule cell is estimated to contact about 110 HIPP cells. The remaining contacts were distributed among HICAP cells (40 contacts). Additionally, in agreement with the presence of mossy fiber terminals on aspiny calretinin-positive interneurons (Acsády et al. 1998), we included 15 mossy fiber synapses to IS cells. Because mossy fibers avoid the molecular layer (Buckmaster and Dudek 1999) in the healthy dentate gyrus, it is assumed that they do not contact MOPP cells. During sclerosis, sprouted mossy fibers were shown to contact ≤500 postsynaptic granule cells (Buckmaster et al. 2002b); thus we estimate an average of 50 to 500 connections from a single granule cell to other granule cells at maximal sclerosis.

**Mossy cells**

A single filled mossy cell axon was reported to make 35,000 synapses in the inner molecular layer (Buckmaster et al. 1996; Wenzel et al. 1997). Assuming a single synapse per postsynaptic cell, a single mossy cell is estimated to contact 30,000–35,000 granule cells. Of the 2,700 synapses made by a single mossy cell axon in the hilus, about 40% (about 1,000 synapses) target γ-aminobutyric acid (GABA)–negative neurons (Wenzel et al. 1997). Because each mossy cell is estimated to make one to five synaptic contacts on a postsynaptic mossy cell (Buckmaster et al. 1996), we estimate that each mossy cell contacts about 350 other mossy cells. The remaining 60% of the hilar mossy cell axons target GABA-positive cells (Buckmaster et al. 1996; Wenzel et al. 1997), with no reports supporting mossy cell targeting of IS cells. Assuming that there is no preferential target selectivity between HIPP and HICAP cells, and that each postsynaptic hilar interneuron receives two synaptic contacts from a single mossy cell axon (Buckmaster et al. 1996), we estimated that each mossy cell contacted 600 HIPP and 200 HICAP cells. With very low mossy cell to interneuron connectivity in the inner molecular layer (Wenzel et al. 1997), we estimated that each mossy cell contacts five to ten basket and axo-axonic cells and nearly five MOPP cells with somata in the inner molecular layer (Han et al. 1993).

**Basket cells**

In the CA3 region of the rat hippocampus, each principal cell is contacted by about 200 basket cells (Halasy and Somogyi 1993), whereas it is suggested a granule cell in the dentate is contacted by as few as 30 basket cells. Assuming that each of the 1,000,000 granule cells is contacted by 115 basket cells, each making one to 20 synaptic connections (Acsády et al. 2000; Halasy and Somogyi 1993), we estimated that each basket cell contacted about 1,250 granule cells. Mossy cells receive 10–15 basket cell synapses (Acsády et al. 2000), leading to an estimate of 75 mossy cells contacted by a single basket cell. Roughly 1% of the 11,000 synapses made by a single basket cell axon in the granule cell layer of the dentate are onto other basket cells (Sik et al. 1997) with three to seven synapses per postsynaptic cell (Bartos et al. 2001). Consequently, we estimated that each basket cell in the dentate gyrus contacted 35 other basket cells. Because hilar and molecular layer interneurons are not a major target of basket cells (Halasy and Somogyi 1993), we estimated that a basket cell contacted zero to one HIPP cells (i.e., every second basket cell contacted a HIPP cell). Similarly, the basket cell synapses onto axo-axonic cells, HI-CAP, and MOPP cells were assumed to be negligible. Because parvalbumin-positive (PV) cells preferentially contact other PV-positive cells in the hilus (Acsády et al. 2000), we assume that basket cells do not contact the calretinin-positive IS cells (Gulyás et al. 1992).

**Axo-axonic cells**

Most synapses made by axo-axonic cell axons are thought to target granule cell axon initial segments (Halasy and Somogyi 1993), although a small fraction of axon collaterals also descend into the superficial and deep hilus (Freund and Buzsáki 1996; Han et al. 1993). In neocortex, an axo-axonic cell makes four to ten synapses on the postsynaptic cells’ axon initial segment (Li et al. 1992). With 22,000,000 estimated axon initial segment synapses in the granule cell layer (Halasy and Somogyi 1993) and assuming four synapses per postsynaptic cell [based on the data from the neocortex from Li et al. (1992)], we estimated that each of the 2,000 axo-axonic cell targeted about 3,000 granule cells. Mossy cells receive axo-axonic cell inhibition (Ribak et al. 1985) and, with the comparatively small fraction of axons from axo-axonic cells in the hilus (Freund and Buzsáki 1996; Han et al. 1993), it was estimated that axo-axonic cells targeted a number of mossy cells equal to about 5% of their granule cell targets, corresponding to 150 mossy cells. Because axo-axonic cells primarily target the axon initial segment of non-GABAergic cells (Freund and Buzsáki 1996; Halasy and Somogyi 1993), we assumed that these cells did not project to interneurons.

**HIPP cells**

HIPP cells were previously estimated to contact about 1,600 granule and 450 basket cells with one to five synapses per postsynaptic cell (Sik et al. 1997). Mossy cells can have one dendrite in the molecular layer (Buckmaster et al. 1996), which can be targeted by HIPP cell axons, whereas granule cells have two primary dendrites (Claiborne et al. 1990; Lübbe et al. 1998). With the mossy cell population corresponding to 1/30 of the granule cell number, the mossy cell dendrites constitute a target for HIPP cells about 1/60 of that of granule cells. Increasing this fraction to about 1/45 to account for the presence of a few HIPP cell contacts on mossy cells in the hilus (Buckmaster et al. 2002a) allowed us to estimate that each HIPP cell contacts about 35 mossy cells. HIPP cell axonal divergence onto HICAP and MOPP cells in the molecular layer was assumed to be similar to that found for somatostatin-positive cells in CA1 (Katona et al. 1999) and set to 15 connections to each population. The HIPP cell axonal divergence to axo-axonic cells was estimated to be between the divergence to basket and HICAP cells and thus the HIPP cell axon was assumed to contact 30 axo-axonic cells.

**MOPP cells**

MOPP cells target an estimated 7,500 granule cells in the rat dentate gyrus. Although MOPP cells have a horizontal axonal extent similar to that of HIPP cells, they show considerably less branching (Han et al. 1993), leading us to estimate that they contact only half as many MOPP and HICAP cells as HIPP cells. Because MOPP cell axons are restricted to the molecular layer (Han et al. 1993) and do not target the basal dendrites of basket cells, they were assumed to contact <1/10 the number of basket cells targeted by HIPP cells. Likewise, because MOPP cells with axons restricted to the outer and middle molecular layers (Han et al. 1993) would not target the hilar dendrites of axo-axonic cells (Soriano et al. 1990) and the axo-axonic cells with somata and proximal dendrites in the hilus (Han et al. 1993), we estimate that MOPP cells contact only one to two axo-axonic cells. Because the MOPP cell axonal arbors in the molecular layer (Han et al. 1993) do not overlap with major parts of the dendritic arborizations of mossy cells (Frotscher et al. 1991), HIPP cells (Han et al. 1993; Katona et al. 1999; Sik et al. 1997), and IS cells (Gulyás et al. 1996), the connectivity to these cells was deemed negligible.

**HICAP cells**

Sik et al. (1997) estimated that the septotemporal extent and bouton density of HICAP cell axons was similar to those of HIPP cell axons,
whereas the estimated axonal length of HICAP cells was nearly half that of the HIPP cell axonal length. Thus we estimated that HICAP cells contacted about half the number of granule cells contacted by HIPP cells, although because the HICAP cells have an additional 3% of axon collaterals in the hilus (Sik et al. 1997), their number of postsynaptic mossy cells was assumed to be the same as that of the HIPP cells. HICAP cells were assumed to contact less than half the number of basket cells targeted by HIPP cells (about 175) and a negligible number of axo-axonic cells. With a total of 26,000 from a single HICAP cell axon (Sik et al. 1997), nearly 700 synapses should be present in the hilus. Assuming two to five synapses per postsynaptic cell, each HICAP cell could contact 100–300 hilar cells. We assumed that each HICAP cell targeted 50 HIPP and HICAP cells, which, along with 35 synapses on mossy cells, was in the estimated range. Although the total axonal length of HICAP cells is only about half that of HIPP cells, the number of MOPP cells targeted was assumed to be the same (about 10–20) because the HICAP cell axons primarily project to the inner molecular layer where both cell bodies and proximal dendrites of MOPP cells are located (Han et al. 1993).

**IS cells**

IS cells contact an estimated 100–800 other IS cells and five to ten (presumably CCK-positive) basket cells (Gulyás et al. 1996). Acsády et al. (2000) suggested that CCK cells would include both BC and HICAP morphologies and that, furthermore, IS cells project to somatostatin-positive presumed HIPP cells. We therefore estimate that IS cells also project to five to ten HICAP cells and HIPP cells.

**APPENDIX A3: FITS TO THE EXPERIMENTALLY DETERMINED AXONAL DISTRIBUTIONS**

The density of connections made by each neuron type as a function of distance from the soma was assumed to be proportional to the length of axonal branches (Sik et al. 1997). For each cell type, Gaussian distributions were fitted to normalized, binned distributions of data from in vivo fills of the respective cells (Fig. 2). The distribution of sprouted mossy fiber to granule cell connections during sclerosis followed the same distribution as that of the healthy mossy fibers (Buckmaster and Dudek 1999) (Fig. 2A). Because of the bimodal distribution of the mossy cell axonal branches (Fig. 2E), a single Gaussian was fitted to the average of the length of axonal branches on both sides of the soma and mirrored around the somatic coordinate to give the final fit. No experimental data are available on the axonal distributions of MOPP and IS cells. However, because the horizontal axonal extent of MOPP cells was previously reported to be about 2 mm (Han et al. 1993), they were assigned a distribution based on the average of the basket and HIPP cell axonal distributions. IS cells were assumed to have axonal distributions similar to those of MOPP cells. Note that these axonal distributions are all for ipsilateral projections; for axonal distributions concerning commissural projections, see APPENDIX B2(5) and Fig. 8A.

**APPENDIX B1: CONTROLS FOR CALCULATING L AND C**

To ascertain the robustness of our calculations of L and C in the full-scale structural model of the dentate gyrus, a number of control calculations were performed.

1. **Accuracy of sampling**

Because the structural model of dentate gyrus contained a total of 1,064,000 interconnected nodes, it was not computationally feasible to calculate the properties of all nodes. Therefore a minimum of 1/1,000 granule and mossy cells and 1/100 interneurons were sampled randomly, and the weighted averages of L and C were calculated (see METHODS). To ensure the accuracy and stability of this sampling method, control calculations were performed using different random seeds to establish connectivity and randomly sample different subpopulations of cells. Comparison of L and C of the network graphs generated using different random seeds to establish connectivity and/or sampling different cells did not reveal significant differences; in fact, the SD of L and C calculated for these control networks was <0.2%. Additionally, the clustering coefficient calculated for the equivalent random graph of the control dentate gyrus agreed with the theoretical value for a random graph C = 0.0011, where k is the number of connections per node and n is the number of nodes (Watts and Strogatz 1998).

2. **Variations in cell numbers**

The small-world structure of the structural model of the dentate gyrus was preserved even when large changes in the estimated cell numbers were introduced. For example, control calculations showed that reducing the number of mossy cells by 50% did not dramatically change the graph structure (L = 2.79, C = 0.0263).

3. **Variations in connectivity estimates**

The calculations of L and C presented in this paper were based on the means of connectivity ranges estimated from the literature (Table 1). The small-world structure was preserved even if only the extreme low or the extreme high connectivity estimates (Table 1) were used for the calculation of L and C (extreme low: L = 2.76; C = 0.0163; extreme high: L = 2.62; C = 0.0379), indicating the robustness of the basic findings.

**APPENDIX B2: CONTROLS FOR STRUCTURAL PARAMETERS**

In five distinct sets of control simulations, we tested the effects of changes in structural parameters on L and C values and network activity, comparing the obtained data to the L and C and activity measures calculated for the base model described in the main part of the paper.

1. **Inhomogeneous distributions of neurons along the septotemporal axis**

The density of neurons along the septotemporal axis was previously shown to vary in a cell-type-specific manner in the dentate gyrus. Specifically, the density of granule cells decreases dramatically at the temporal pole (Fricke and Cowan 1978), whereas the densities of mossy cells and hilar interneurons increase at the temporal pole (Buckmaster and Jongen-Relo 1999) (Fig. 7A). Calculations of L and C for structural models implementing these inhomogeneous cell distributions along the septotemporal axis did not show any significant differences from the baseline structural model described in the main text (Fig. 7, C_f and C_s) and the corresponding functional model network with inhomogeneous cell distributions also displayed the characteristic decrease in activity with the progression of sclerosis from 80 to 100% (Fig. 7C).

2. **Inhomogeneity in connectivity along the transverse axis**

Earlier autoradiographic studies showed that within a given lamella, the density of associational fibers from hilar cells to their molecular layer targets appears to double from the tip of the ventral blade to the tip of the dorsal blade (Fricke and Cowan 1978). This nonuniform projection density in the transverse plane (perpendicular to the septotemporal axis) was modeled by including a linear ventrodorsal scaling in the probability of connecting mossy cells and HIPP cells to their molecular layer targets (e.g., granule cell #10,000 in each bin had
twice the probability of receiving mossy or HICAP cell input compared with granule cell #1. The results obtained with the structural and functional models implementing inhomogeneous connectivity in the transverse plane for either L and C (Fig. 7, C1, and C2) or the granule cell activity (Fig. 7C3) did not show any significant differences from the base model described in the main text.

(3) Axonal distributions at the septal and temporal poles (anatomical boundary)

The cell-type–specific axonal distributions (Fig. 2) were based on in vivo fills obtained from cells situated some distance away from the septal and temporal poles of the dentate gyrus. However, the structural and functional model networks contained cells whose axons reached these boundaries. Because it is not known how the axonal distributions are skewed at the two poles of the biological dentate gyrus, control simulations with four different boundary conditions were implemented affecting all cell types, resulting in the conservation or reduction of the septotemporal span (i.e., the tail-to-tail width) of the Gaussians shown in Fig. 2, and the conservation or reduction of the total axonal length (i.e., the area under the Gaussians; note that the total axonal length corresponds to the total number of connections): 1) reduced septotemporal span, reduced total axonal length (i.e., the axons beyond the anatomical boundary were simply cut off and not preserved; this was the baseline method used for all simulations described in results); 2) reduced septotemporal span, conserved total axonal length (i.e., higher density of axons in the arbor that remained after the cutoff at the boundary); 3) conserved septotemporal span, conserved total axonal length; 4) conserved septotemporal span, reduced axonal length. These four boundary conditions are illustrated for the case of a granule cell situated at the septal pole of the dentate gyrus in Fig. 7A. The results of these control calculations showed that the L and C values obtained for the three additional boundary conditions were similar to the L and C values calculated for the baseline case (reduced septotemporal span, reduced total axonal length) used in the rest of the paper (Fig. 7, C1, and C2). Similarly, the networks with the distinct boundary conditions all showed the characteristic decrease in activity as sclerosis progressed from 80 to 100% (Fig. 7C3). Therefore these anatomical boundary conditions further support the robustness of our main results.

(4) Offset degrees of sprouting and hilar neuron loss

A strong correlation between the degree of hilar neuron loss and mossy fiber sprouting was previously reported in both specimens from patients with temporal lobe epilepsy (Gabriel et al. 2004) and animal models (Nadler and Jiao 2004). However, to test whether a potential offset in the degrees of sprouting and hilar cell loss would affect our main conclusions, control calculations with sprouting lagging 20% behind the hilar neuron loss were performed (70% sprouting with 90% cell loss, 80% sprouting with 100% cell loss). These calculations did not show any significant differences from the baseline model described in the main text (Fig. 7, C1–C3).

(5) Bilateral model of the dentate with commissural projections

The full-scale structural model consisted of only one side of the dentate gyrus (and, consequently, contained only ipsilateral, associative connections). To test whether the main findings of the paper (on L and C and the biphasic activity) also apply when both sides of the dentate gyrus are taken into account, bilateral structural and functional model networks were implemented. For both the structural and functional bilateral models, two individual unilateral models were implemented and anatomically realistic commissural connections were established between them (Table B1). Cell-type specificity of the commissural projections and their axonal distributions (Fig. 8A) were constrained based on data from studies using anterograde tracer techniques (reviewed in Deller 1998). Briefly, 1) because mossy fibers do not cross the commissure (Blackstad et al. 1970; Zappone and

### TABLE B1. Connectivity matrix for commissural projections of the control dentate gyrus

<table>
<thead>
<tr>
<th></th>
<th>% Commisural Projections</th>
<th>Granule Cells</th>
<th>Mossy Cells</th>
<th>Basket Cells</th>
<th>A xo-axonic Cells</th>
<th>MOPP Cells</th>
<th>HIPP Cells</th>
<th>HICAP Cells</th>
<th>IS Cells</th>
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<tbody>
<tr>
<td><strong>Granule cells</strong></td>
<td>0%</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><strong>Mossy cells</strong></td>
<td>100%</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<td>(30,000)</td>
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<td>2–10</td>
<td>X</td>
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<td><strong>Basket cells</strong></td>
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<td><strong>A xo-axonic cells</strong></td>
<td>38%</td>
<td>1000</td>
<td>X</td>
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<td><strong>MOPP cells</strong></td>
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<td><strong>HIPP cells</strong></td>
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<tr>
<td><strong>HICAP cells</strong></td>
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<tr>
<td><strong>IS cells</strong></td>
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</table>

Connectivity values were estimated from published data [see Appendix B2(5)] and are given as number of connections to the contralateral postsynaptic population (row) from a single presynaptic neuron (column). The fraction of a population projecting contralaterally and the average number of connections used in the graph theoretical calculations are given in bold. References given correspond to: 1Blackstad et al. (1970); 2Zappone and Sloviter (2001); 3Frotscher et al. (1991); 4Deller (1998); 5Deller et al. (1994); 6Fricke and Cowan (1978); 7Seress and Ribak (1984); 8Deller et al. (1995a).
granule cells were modeled without contralateral projections. 2) Mossy cells have extensive commissural projections (Frotscher et al. 1991) with almost all cells projecting bilaterally (Deller 1998). Similar to their ipsilateral side, the commissural projections of mossy cells terminate exclusively in the inner molecular layer and on both granule cells and parvalbumin-positive interneurons (Deller et al. 1994; Seress and Ribak 1984). In agreement with tracer studies demonstrating similar densities and longitudinal extent for both associational and commissural projections to the inner molecular layer (Fricke and Cowan 1978), the number and septotemporal span of the ipsilateral distribution of mossy cell axons were conserved on the contralateral side. However, commissural contacts of the mossy cell axons were restricted to the molecular layer because they are known to avoid synaptic contacts in the hilus (Deller 1998; Deller et al. 1995b). In contrast to the bimodal ipsilateral distribution, the contralateral distribution of mossy cell axons is unimodal with the maximal density in the homotopic contralateral lamella (Deller 1998). Therefore the commissural axonal distribution of mossy cells was described by a single Gaussian (Fig. 8A) with the same septotemporal width as the ipsilateral mossy fiber axons (Fig. 2), with the peak located in the contralateral lamella homotopic to the soma. 3) Previous studies showed that nearly 38% of parvalbumin cells, nearly 98% somatostatin cells, and nearly 33% of calretinin interneurons in the dentate project contralaterally (Zappone and Sloviter 2001). Additionally, with the exception of avoiding hilar targets, the cell-type specificity of the interneuronal axonal termination zone is preserved in contralateral projections (Deller et al. 1995b). Therefore we included contralateral projections from basket cells, axo-axonic cells, HIPP cells, and IS cells to their appropriate targets in the bilateral network (Table B1). However, in keeping with the more restricted septotemporal span of the interneuronal contralateral projections (Deller et al. 1995a,b) we reduced the number of contacts and longitudinal extent of commissural terminals (Fig. 8A) compared with the corresponding associational connections (Fig. 2).

To test whether the biphasic change in network hyperexcitability was present in a bilateral dentate gyrus, a bilateral functional model network was also implemented, by scaling down the connectivity of the bilateral structural model in exactly the same way as for the ipsilateral model, resulting in a bilateral functional model with 100,000+ cells (containing the various cell types in anatomically realistic proportions, as in the ipsilateral model). The commissural connection strengths and kinetics were assumed to be identical to those for the ipsilateral model (Table 2). Because of the additional inputs to granule cells from spontaneously active contralateral mossy cells, the bilateral functional model network showed greater granule cell discharge activity (Fig. 8B) than that of the unilateral model after perforant path stimulation at 0% sclerosis. However, in agreement with the low firing frequency of biological granule cells (Santhakumar et al. 2005), 87.3% of granule cells did not spike and the average firing frequency of granule cells remained low (0.27 Hz) at 0% sclerosis even in the bilateral model (as illustrated by the similar activity measures for the bilateral and base models at 0% sclerosis in Fig. 8C).

The results of these bilateral control simulations showed that the L and C characteristics of the bilateral network with >2 million nodes and possessing both commissural and associational links were virtually identical to the L and C values calculated for the ipsilateral structural network (Fig. 8, C1 and C2). Furthermore, the bilateral functional model network also displayed the characteristic drop in activity as sclerosis progressed from 80 to 100% (Fig. 8, B2, B3, and C3). Note that the hyperactivity in the bilateral model at 100% sclerosis did not spread to the contralateral side (Fig. 8B), arising from the complete loss of mossy cells, resulting in an even more pronounced decrease in the overall granule cell firing at 100% sclerosis in the bilateral network than in the ipsilateral base model (compare open and filled circles in Fig. 8C).

APPENDIX B3: CONTROLS FOR FUNCTIONAL PARAMETERS

In addition to simulations testing the effects of changes in the structural model (described above), three sets of control simulations were performed to determine the effects of varying specific parameters in the functional network model. In all cases, we tested how large parameter changes affect the biphasic nature of the changes in hyperexcitability during sclerosis.

(1) Double inhibition

Stemming from the lack of sufficiently detailed data on four interneuron types, only the two major somatically and dendritically projecting interneuronal classes (basket and HIPP cells) were included in the functional model network, effectively removing nearly 50% of the inhibitory connections (see Table 1). Therefore control simulations were carried out to determine if large increases in inhibition in the network alter the basic conclusions. As illustrated in Fig. 9, A2–A3 (and summarized in Fig. 9D), the biphasic changes in network activity in the functional model during sclerosis persisted even after doubling of the maximal conductances of all inhibitory synapses (base values listed in Table 2). Therefore these data indicate that the main conclusions do not depend on the strength of inhibition included in the functional model.

(2) Axonal conduction delay

In the baseline network model described in the main text, a fixed, connection-type–specific synaptic delay was used (Santhakumar et al. 2005). In additional control simulations, an axonal conduction velocity of 0.25 m/s (Bartos et al. 2002) was used to add distance-dependent delays to connections made outside the 60-μm bin of the presynaptic neuron. This resulted in an additional 0.24-ms delay for each bin separating the pre- and postsynaptic cells. As illustrated in Fig. 9, B2–B3 (and summarized in Fig. 9D), these simulations with distance-dependent delays displayed biphasic changes in network activity during sclerosis similar to those in networks with fixed synaptic delays. These data also support the robustness of our conclusions.

(3) Spontaneous instead of stimulation-evoked activity

In most simulations, a perforant path stimulus was used to investigate the functional consequences of network architectural changes. To show that the main conclusions were stimulus independent, spontaneous network activity, simulated by uncorrelated activation of each granule cell, each basket cell and 100 evenly distributed mossy cells was implemented by assigning individual perforant path inputs with Poisson-distributed interspike intervals. As illustrated in Fig. 9, C2–C3 (and summarized in Fig. 9D), these simulations revealed the characteristic biphasic changes in activity with sclerosis: the average granule cell activity showed a peak at 80% sclerosis, followed by a decrease at 100% sclerosis. Therefore the main conclusions were not dependent on the specific parameters of the stimulus.

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