Excitatory Input Onto Hilar Somatostatin Interneurons Is Increased in a Chronic Model of Epilepsy

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Halabisky B, Parada I, Buckmaster PS, Prince DA. Excitatory input onto hilar somatostatin interneurons is increased in a chronic model of epilepsy. J Neurophysiol 104: 2214–2223, 2010. First published July 14, 2010; doi:10.1152/jn.00147.2010. The density of somatostatin (SOM)-containing GABAergic interneurons in the hilus of the dentate gyrus is significantly decreased in both human and experimental temporal lobe epilepsy. We used the pilocarpine model of status epilepticus and temporal lobe epilepsy in mice to study anatomical and electrophysiological properties of surviving somatostatin interneurons and determine whether compensatory functional changes occur that might offset loss of other inhibitory neurons. Using standard patch-clamp techniques and pipettes containing bicytin, whole cell recordings were obtained in hippocampal slices maintained in vitro. Hilar SOM cells containing enhanced green fluorescent protein (EGFP) were identified with fluorescent and infrared differential interference contrast video microscopy in epileptic and control GIN (EGFP-expressing Inhibitory Neurons) mice. Results showed that SOM cells from epileptic mice had $J$) significant increases in somatic area and dendritic length; $2$) changes in membrane properties, including a small but significant decrease in resting membrane potential, and increases in time constant and whole cell capacitance; $3$) increased frequency of slowly rising spontaneous excitatory post synaptic currents (sEPSCs) due primarily to increased mEPSC frequency, without changes in the probability of release; $4$) increased evoked EPSC amplitude; and $5$) increased spontaneous action potential generation in cell-associated recordings. Results suggest an increase in excitatory innervation, perhaps on distal dendrites, considering the slower rising EPSCs and increased output of hilar SOM cells in this model of epilepsy. In sum, these changes would be expected to increase the inhibitory output of surviving SOM interneurons and in part compensate for interneuronal loss in the epileptogenic hippocampus.

Introduction

Loss of hippocampal neurons, including some GABAergic interneurons, is a common neuropathological finding in human temporal lobe epilepsy (Margrison and Corsellis 1966). It has been proposed that some surviving interneurons lose excitatory synaptic input and become less active (Sloviter et al. 2003). Alternatively, surviving interneurons might undergo structural and functional changes that compensate for epileptogenic injuries. We sought to address this question by evaluating the excitatory synaptic input to hilar somatostatin-immuno-reactive interneurons (“SOM” interneurons in the following text) labeled with enhanced green fluorescent protein (EGFP) in GIN (EGFP-expressing Inhibitory Neurons) mice (Oliva Jr et al. 2000) that were epileptic following pilocarpine-induced standard error (SE). Somatostatin-containing cells are a major class of GABAergic interneurons in the dentate gyrus (Austin and Buckmaster 2004; Buckmaster and Jongen-Rêlo 1999; Esclapez and Houser 1995; Kosaka et al. 1988; Somogyi et al. 1984). Their axon collaterals primarily synapse with the dendrites of glutamatergic granule cells (Buckmaster et al. 2002; Katona et al. 1999; Leranth et al. 1990; Milner and Bacon 1989), so that their output would be an important factor in regulating excitability in the dentate synaptic network.

Several anatomical abnormalities occur in the dentate gyrus of both patients (de Lanerolle et al. 1985; Mathern et al. 1995; Robbins et al. 1991; Sundstrom et al. 2001) and animals with temporal lobe epilepsy (Freund et al. 1991; Houser and Esclapez 1996; Lowenstein et al. 1992; Schwarzer et al. 1995; Sloviter 1987; Sun et al. 2007). There is sprouting of granule cell (GC) axons (Sutula et al. 1989), so that an average individual GC in epileptic rats projects 75% more axon into the hilus compared with controls (Buckmaster and Dudek 1999). There is also a substantial loss of hilar SOM interneurons; however, somata of surviving SOM interneurons appear qualitatively larger than those in controls (Buckmaster and Dudek 1997). Associated changes in neuron dendritic length have not previously been evaluated in epileptic animals. However, if the apparent somatic enlargement were accompanied by enlargement and lengthening of dendritic processes, both alterations in intrinsic electrophysiological properties (Rall 1962) and increased excitatory synaptic input from the sprouting GC axons targeting these dendrites might be expected. One potential consequence of such structural alterations and accompanying increased excitatory input would be a compensatory increase in feedback inhibition from surviving SOM interneurons.

We used hippocampal slices from GIN mice (Oliva Jr et al. 2000) to obtain whole cell patch recordings from hilar SOM interneurons in control and epileptic mice to compare their intrinsic electrophysiological characteristics, somatic and dendritic morphology, excitatory synaptic inputs, and spontaneous activity. Results show that somatodendritic structure of surviving SOM interneurons is altered in epileptic mice and that SOM interneurons receive more excitatory synaptic input and are more active than in controls.

Methods

All experiments were carried out according to protocols approved by the Stanford Institutional Animal Care and Use Committee. GIN mice (Oliva Jr et al. 2000), strain FVB-TgN(GadGFP)45704Swn, were obtained from Jackson Laboratories.
Induction of temporal lobe epilepsy

To induce status epilepticus (SE), 34 ± 7-day-old (mean ± SE) mice (n = 40) of either sex were treated with atropine methyl bromide (5 mg/kg, administered intraperitoneally [ip]), followed 20 min later by pilocarpine hydrochloride (300–320 mg/kg, ip). After 2 h of SE, mice were treated with diazepam (10 mg/kg, ip; Hospira, Lake Forest, IL), which was repeated, as necessary, to suppress convulsions. They were used in an in vitro slice experiment 46 ± 3 days later. Beginning ±10 days after SE, mice were video-monitored for behavioral seizures of ≥grade 3 (Racine 1972). Of the 40 mice treated with pilocarpine, 14 developed SE, survived, and displayed at least two spontaneous seizures before being used in a slice experiment. Controls consisted of the remaining 26 mice that were treated with pilocarpine but did not develop SE or display spontaneous seizures. We previously found no differences in naïve control GIN mice and those that failed to develop SE and epilepsy after treatment with pilocarpine (and atropine and diazepam) (Zhang et al. 2009). Therefore to reduce otherwise specified.

that a small number of control mice actually belong in the epileptic is arguably the best control because it was exposed to the same drug consisted of pilocarpine-treated mice that failed to develop SE, all of (and atropine and diazepam) (Zhang et al. 2009). Therefore to reduce otherwise found no differences in naïve control GIN mice and those that

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All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise specified.

Immunohistochemistry

For morphological characterization of individual interneurons, patch pipettes were filled with 0.05% biocytin (Sigma). Slices containing labeled cells were fixed overnight in 4% paraformaldehyde in phosphate buffer, rinsed with PBS, permeabilized with 0.25% Triton X-100 plus 0.5% bovine serum albumin, and later incubated for 2 h in Texas Red Avidin D (1:200; Vector Laboratory, Burlingame, CA) at room temperature.

Cell morphology

Confocal z-stack images (Zeiss LSM 10; Carl Zeiss MicroImaging, Thornwood, NY) were obtained from somata and dendritic processes of biocytin-filled GFP-labeled hilar SOM cells (n = 10) from which electrophysiological data had been obtained. Filled cells were located >50 μm from the slice surface. Somata and dendrites were traced using the confocal module of the NeuronLucida system (MicroBrightField, Williston, VT), which allowed measurement of somatic areas. Dendritic lengths and branch patterns were measured using a modified Sholl analysis in which concentric circles were drawn at increasing distances from the center of the soma at 10-μm intervals and numbers of dendritic crossings counted. Numbers of dendritic endings were assessed in the same group of filled cells.

Acute slice preparation

Forty GIN mice (26 control, 14 epileptic), 80 ± 7 days old, were used for in vitro recordings. Techniques for preparing and maintaining brain slices in vitro were as previously described (Halabisky et al. 2006). Mice were deeply anesthetized with sodium pentobarbital (55 mg/kg, ip), decapitated, and the brains were rapidly removed and placed in cold (4°C) oxygenated cutting solution containing (in mM): 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4, 0.5 CaCl2, 26 NaHCO3, 11 glucose, and 234 sucrose. A block of brain containing temporal hippocampus (Franklin and Paxinos 1997) was fastened to the stage of a DSK vibratome (Dosaka, Kyoto, Japan) with cyanoacrylate (Krazy Glue) and 350-μm horizontal slices were cut in the cutting solution. Slices were then incubated for ≥30 min in standard artificial cerebrospinal fluid (ACSF; 30°C) that contained (in mM): 2.5 KCl, 126 NaCl, 10 glucose, 1.25 NaH2PO4, 1 MgSO4, 2 CaCl2, and 26 NaHCO3 (pH ~ 7.4 when gassed with a mixture of 95% O2-5% CO2), after which they were maintained at room temperature. Single slices were transferred to a submerged recording chamber where they were maintained at 30°C and perfused at a rate of 2 mL/min.

Recordings

Recordings were made from single EGFP-expressing SOM interneurons located within the hilus of the dentate gyrus, identified under fluorescent and infrared differential interference contrast (IR-DIC) video microscopy with a Zeiss Axioskop 2 FS plus microscope. Patch pipettes (4–6 MΩ) were pulled from borosilicate glass (WPI, Sarasota, FL) and filled with (in mM) 5 KCl, 135 K-glucuronate, 2 NaCl, 10 HEPES, 4 EGTA, 4 MgATP, and 0.3 Na3GTP (pH ~ 7.2; 285 mosmol) for current-clamp and voltage-clamp recordings. Liquid junction potentials were measured using the junction potential calculator in Clampex 9.0 software and corrected during data analysis. Electrophysiological data for analysis were obtained from interneurons that had a resting membrane potential (Vr) more negative than ~50 mV, action potentials (APs) overshooting beyond +10 mV, and membrane input resistance (Ron) >100 MΩ. Series resistance was monitored throughout recordings and, if >15 MΩ or varied >15%, recordings were discarded.

Using the same internal solution as before, cell-attached recordings were obtained from granule cells located within stratum granulosum, identified on the basis of their position and morphology under IR-DIC video microscopy. To evoke synaptic currents a monopolar glass stimulating electrode filled with ACSF was used. Stimulus pulses were delivered through a WPI 850 A/B stimulus isolation unit. Stimulating electrodes were placed in the granule cell layer at a point close to the recorded interneuron. Minimal stimulation intensity was defined for each cell as the minimal current amplitude (30–200 μA) for a 25-μs stimulus pulse that evoked a just detectable excitatory postsynaptic current (EPSC). A series of graded intensity stimuli was then applied by increasing the pulse duration to 50, 75, 125, 250, and 375 μs (2-, 3-, 5-, 10-, and 15-fold threshold).

Electrophysiological analysis

Current pulses were delivered from the cell’s Vm with no holding current applied. Membrane input resistance (Ron) was obtained from the linear portion of a current–voltage (I–V) plot, where Vm was determined as the maximal negative potential during the 500-ms hyperpolarizing pulse (see Fig. 3A). Membrane time constant (τm) was obtained by fitting voltage responses to hyperpolarizing current pulses with a single standard-exponential decay (y = yo + Arex), where y0 is the y offset or asymptote, A is amplitude, τm is decay time constant, and t is time. Series resistance (Rs) was calculated for all cells based on the amplitude of the capacitive current in response to a 10-mV step.

Currents were analyzed with event-detection software (wDetecta; JR Huguenard). For amplitude, rise time, and decay time measurements, currents uncontaminated by subsequent events were isolated. Rise times were measured as the time from 10 to 90% of peak amplitude and vice versa for decay times. Frequencies of spontaneous and miniature EPSCs (sEPSCs and mEPSCs, respectively) were determined from the average frequency over 3 min for each. The ratio of mEPSCs/sEPSC frequency was determined for each cell and then averaged across the population.
To determine whether the morphology of SOM-EGFP neurons is altered in epileptic mice, we obtained confocal images of biocytin-filled cells. Somatic area, taken from sections through the center of somata, was significantly larger in hilar SOM-EGFP neurons of epileptic (340.8 ± 5.5 μm², n = 7) versus control mice (258.2 ± 14 μm², n = 7; P < 0.01; Table 1). Total dendritic length was also greater in the cells from epileptic (1,375 ± 28 μm, n = 6) versus control mice (780 ± 15 μm, n = 4; P < 0.05; Fig. 1, A–C). Scholl analysis (Fig. 1D) confirmed the increased length of dendrites in SOM-EGFP neurons and showed a trend toward more dendritic branches in the filled cells from epileptic mice (103 ± 30 crossings for epileptic and 30 ± 16 for control; P = 0.14). Analysis of dendritic endings in the same groups of neurons studied in the Sholl analysis (6 epileptic compared with 4 control cells) also showed a trend toward more dendritic branches that did not reach significance. These results suggest that soma size and dendritic length of SOM-EGFP interneurons are increased in epileptic mice.

The induction of epilepsy in these mice could potentially have changed the expression profile of the GFP transgene. To confirm that GFP expression remains confined to somatostatin-positive cells a one-in-six series of coronal sections from cerebral hemispheres were processed for somatostatin-immunoreactivity in four epileptic pilocarpine-treated GIN mice. A total of 75 hilar GFP-positive cells were examined and all 75 were somatostatin-immunoreactive. Therefore it is likely that all of the GFP-positive hilar neurons evaluated were somatostatin cells.

### RESULTS

**Soma size and dendritic length of EGFP-expressing interneurons are increased in epileptic mice**

Statistical significance for group means was determined with unpaired two-tailed Student’s t-test (*P < 0.05, **P < 0.01). Results from modified Sholl analyses were analyzed by two-way ANOVAs. Data are presented as means ± SE. Origin and Microsoft Excel software were used to perform all statistical analyses.

### Statistical analysis

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### Table 1. Soma size of single hilar EGFP-expressing interneurons

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>Perimeter, μm</th>
<th>Area, μm²</th>
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<tr>
<td><strong>Control</strong></td>
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<tr>
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<td>59</td>
<td>234</td>
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<tr>
<td>7</td>
<td>57</td>
<td>215</td>
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<tr>
<td>Mean</td>
<td>63.2 ± 2.1 (7)**</td>
<td>258.2 ± 14 (7)**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>Perimeter, μm</th>
<th>Area, μm²</th>
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</thead>
<tbody>
<tr>
<td><strong>Epileptic</strong></td>
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<tr>
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</tr>
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<td>6</td>
<td>77</td>
<td>340</td>
</tr>
<tr>
<td>7</td>
<td>85</td>
<td>344</td>
</tr>
<tr>
<td>Mean</td>
<td>79.2 ± 1.7 (7)**</td>
<td>340.8 ± 5.5 (7)**</td>
</tr>
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</table>

Values are means ± SE (number of cells). **P < 0.01, difference between epilepsy and control groups.

### Figure 1

**FIG. 1.** Morphological features of somatostatin–enhanced green fluorescent protein (SOM-EGFP) hilar interneurons in control and epileptic mice. A and B: images of representative biocytin-filled interneurons in epileptic (A) and control (B) mice. Arrows in A point to elongated dendrites. Calibration in B: 25 μm for A and B. C: graph of dendritic lengths in 6 epileptic and 4 control cells measured with Neurolucida from confocal image stacks. D: plot of results of modified Sholl analysis for neurons in C showing dendritic crossings (intersections) made by concentric circles separated by 10 μm, centered on the soma. SOM-EGFP cells from epileptic mice (black squares): 103 ± 30 dendritic crossings; controls (open circles): 30 ± 16 crossings (P = 0.14). Results confirm increases in dendritic length shown in C and show a trend toward increased dendritic branching at increasing distances. Quantification of somatic areas shown in Table 1.
The morphological characteristics described earlier are likely to affect the intrinsic membrane properties of the SOM neurons, their responses to synaptic inputs, and their functional role in the dentate gyrus network. To assess whether the physiological properties of SOM neurons are altered in epileptic mice, we performed whole cell patch-clamp recordings from EGFP-containing neurons within the hilus of the dentate gyrus. Increased somatic area and dendritic length should be accompanied by an increase in cell membrane area and thus whole cell membrane capacitance. Whole cell membrane capacitance was measured by determining the charge transfer during the initial dynamic current response to a 5 mV depolarizing step while the cell was voltage clamped at −60 mV (Fig. 2A). Membrane capacitance recorded from SOM-EGFP neurons from epileptic mice was significantly larger (65.8 ± 4.7 pF) than that in nonepileptic mice (40.7 ± 1.9 pF; Fig. 2B, Table 2), a finding consistent with the increased somatic area and dendritic length of these cells. To further examine differences in intrinsic membrane properties we also performed current-clamp experiments. Compared with controls, SOM-EGFP neurons from epileptic mice had a more depolarized $V_m$ ($−54.9 ± 1.5$ vs. $−58.9 ± 0.5$ mV; $P < 0.05$), significant decreases in membrane input resistance (Table 2; cf. responses to hyperpolarizing current pulses in Fig. 3, A vs. B and graph of Fig. 3C), and slower membrane time constants (Fig. 3D, Table 2). Membrane capacitance and time constants had similar relative increases in SOM-EGFP neurons from epileptic versus control mice (Table 2; see DISCUSSION).

**Excitatory input onto EGFP-expressing interneurons is increased in epileptic mice**

In current-clamp recordings, we noted many more spontaneous excitatory postsynaptic potentials (sEPSPs) in SOM-EGFP neurons from epileptic mice than from controls (Fig. 3B, arrowheads). In models of temporal lobe epilepsy, there is extensive remodeling of granule cell axons within the hilus (Buckmaster and Dudek 1999). To test whether this remodeling results in increased excitation of SOM-EGFP neurons, we obtained whole cell voltage-clamp recordings of spontaneous excitatory postsynaptic currents (sEPSCs) from fluorescein-labeled hilar interneurons from control and epileptic mice at a holding potential ($V_h$) of −60 mV in control ACSF (Fig. 4A) in the presence of 10 μM gabazine to block inhibitory postsynaptic currents. Rise times of sEPSCs were slower in epileptic mice than those in controls, but decay times were similar (Table 3; see DISCUSSION). Although the frequency of sEPSCs in each group of neurons was variable (control range: 0.94–27.6 Hz; epileptic range: 2.2–35.8 Hz), analysis of a large sample showed that there was a significant (−196%) increase in sEPSC frequency in SOM neurons from epileptic mice (Fig. 4B). These findings suggest that granule cell axon reorganization in this model of epilepsy might result in increased synaptic excitation of SOM-EGFP neurons (see DISCUSSION).

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**TABLE 2.** Intrinsic membrane properties from SOM neurons in epileptic and control mice

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>Epileptic</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_m$ ms</td>
<td>22.5 ± 1.6</td>
<td>37.4 ± 3.5</td>
<td>165.8 ± 19.5</td>
</tr>
<tr>
<td>$C_m$ pF</td>
<td>40.7 ± 1.9</td>
<td>65.8 ± 4.7</td>
<td>161.6 ± 13.7</td>
</tr>
<tr>
<td>$R_m$ MΩ</td>
<td>321.4 ± 25.0</td>
<td>233.7 ± 15.7</td>
<td>72.7 ± 7.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. % Change: epileptic versus control mice.
Increased excitatory input is due to an increased number of excitatory synapses

The higher sEPSC frequencies seen in SOM-EGFP neurons from epileptic mice may reflect an increased number of excitatory synapses, an increased probability of release (Pr) from terminals, or an increased rate of AP discharge from excitatory presynaptic neurons within the dentate gyrus and hippocampus. To begin to dissect each of these possibilities and assess their relative contribution to the increased sEPSC frequency seen in epileptic mice, we first recorded miniature (m)EPSCs contain both AP-related events and mEPSCs and we found there was a significant increase in mEPSC frequency is likely related to an increase in the number of excitatory synapses or release sites, rather than an increase in Pr.

**Firing of excitatory neurons presynaptic to SOM-EGFP neurons is not increased**

The sEPSCs recorded in Fig. 4A contain both AP-related events and mEPSCs and we found there was a significant increase in the frequency of mEPSCs in SOM-EGFP neurons from epileptic versus control mice (Fig. 5B). To determine whether increased AP firing in excitatory presynaptic neurons might also contribute to increased sEPSC frequency in epileptic mice, we analyzed the mEPSC/sEPSC frequency ratio in the subpopulation of SOM-EGFP neurons of Fig. 4. In each neuron, we recorded EPSC frequency before and after application of TTX. The ratio was significantly increased in SOM-EGFP hilar neurons of control and epileptic slices (black). Number of cells indicated at bottom of bars. *P < 0.05; **P < 0.01, in 2-sample t-test. Number of mice: 17 control, 7 epileptic.

*Fig. 3. Comparison of intrinsic membrane properties of SOM hilar interneurons from control and epileptic mice. A and B: representative voltage responses to 500-ms hyper- and depolarizing current pulses (−100, −75, −50, −25, and 100 pA) in SOM-EGFP hilar neurons of control (A) and epileptic (B) mice. Resting membrane potential is indicated above beginning of traces. Note slower charging responses to hyperpolarizing current pulses in B vs. A. Dashed line in B response of cell in A to a −100-pA current pulse scaled to match amplitude of response of cell in B to same current pulse. Arrows in A and B denote faster responses of cell from control mouse. Arrowheads point to frequent spontaneous excitatory postsynaptic potentials (EPSPs) seen in cells from epileptic mice (see Fig. 4). C and D: graphs of membrane input resistance (C) and time constant (D) for hilar SOM-EGFP cells from control (white) and epileptic slices (black). Number of cells indicated at bottom of bars. *P < 0.05; **P < 0.01, in 2-sample t-test. Number of mice: 17 control, 7 epileptic.*
release was quite low, judging from changes in sEPSC frequency after TTX perfusion (Figs. 4B and 5B), making a sizable contribution from this effect unlikely.

**Evoked excitatory synaptic transmission onto EGFP-expressing SOM interneurons is increased in epileptic mice**

If there is an increase in the number of excitatory presynaptic contacts onto SOM-EGFP neurons in epileptic mice, an increase in the slope of the input–output (I–O) curve might be expected. To obtain I–O curves, a stimulating electrode was placed as for PPR recordings, a threshold stimulus strength was found that evoked a minimal response (1T), and then input was varied by increasing the duration of the stimulus (2T, 3T, 5T,...; see METHODS; Fig. 7A) during whole cell recordings at $V_h = -60$ mV. Peak amplitudes of responses at lower stimulus intensities (1T, 2T, and 3T) were similar in the two groups (1T values: $-20.7 \pm 7.3$ pA in control, $n = 4$, vs. $-15.6 \pm 5.5$ pA in epileptic, $n = 4$; $P > 0.5$; Fig. 7B). In contrast, the amplitude of EPSCs evoked by stimuli of $\pm 5T$ was significantly larger in SOM-EGFP neurons from epileptic versus control mice (Fig. 7B). The I–O slope, expressed as pA/normlized stimulus unit, was steeper for SOM-EGFP neurons from epileptic than that from control mice ($-5.15 \pm 0.60$ in control, $n = 4$, vs. $-14.9 \pm 0.8$ in epileptic, $n = 4$; $P < 0.01$; $\sim 289\%$ increase; Fig. 7C). This result is compatible with an increased excitatory innervation of SOM-EGFP neurons.

**Firing frequency of EGFP-expressing interneurons is increased in epileptic mice**

The increased frequency of EPSCs onto hilar SOM-EGFP neurons and the significant resting depolarization of $V_m$ in epileptic mice could give rise to an increase in their inhibitory output, although other factors, such as an increase in membrane conductance (Fig. 3B), could counteract the increased excitatory input. To further assess this issue, we evaluated spontaneous firing frequency by obtaining cell-attached patches from hilar SOM-EGFP neurons in control and epileptic mice in normal bathing medium without pharmacological blockade of synaptic transmission. SOM-EGFP neurons from epileptic mice had a firing rate that was significantly greater than that from control mice (Fig. 8; $-5$ vs. $-0.9$ Hz; $P < 0.01$), suggesting the increased excitatory input onto these neurons. However, we cannot rule out that changes to the intrinsic properties of these neurons resulted in an increase of AP firing rate. Either way, the increased AP firing would presumably increase inhibitory output.

| TABLE 3. Properties of spontaneous (s)EPSCs and miniature (m)EPSCs from SOM-EGFP neurons |
|---------------------------------|----------------|----------------|----------------|
|                                | Peak Amplitude, pA | Rise Time, ms | Decay Time, ms |
|                                | Control | Epileptic | Control | Epileptic | Control | Epileptic |
| sEPSCs                          | $-31.1 \pm 1.5$ | $-28.6 \pm 1.8$ | $1.43 \pm 0.07$ | $2.00 \pm 0.16$ | $4.78 \pm 0.27$ | $5.14 \pm 0.41$ |
| mEPSCs                          | $-27.1 \pm 1.5$ | $-30.0 \pm 2.6$ | $1.62 \pm 0.08$ | $1.91 \pm 0.12$ | $4.88 \pm 0.29$ | $5.18 \pm 0.99$ |

Values are means ± SE. Number of cells as shown in Figs. 4 and 5.
Griffey et al. 2006), as they are in the human disorder. It has been observed that interneuronal hypertrophy is present in a mouse model of cortical dysplasia (Spreafico et al. 1998). Also of interest is the observation that interneuronal hypertrophy is present in a cortical dysplasia (Spreafico et al. 1998). Also of interest is the observation that interneuronal hypertrophy is present in a mouse model of temporal lobe epilepsy, surviving dentate hilar SOM-containing interneurons (Prince et al. 2009).

Increased interneuronal size has been previously described in animal models of epileptogenesis

Glutamic acid decarboxylase (GAD)67-containing interneurons in dentate gyrus and CA3 subfield enlarge after intraventricular kainic acid injections in rats (Shetty and Turner 2001) and hypertrophy of calbindin-containing interneurons, including their dendrites, occurs in microgyri that develop in neocortex after epileptogenic neonatal freeze lesions (Kharazia et al. 2000). Interneuronal hypertrophy also occurs in calbindin-containing interneurons of dentate gyrus in human temporal lobe epilepsy (Magloczky et al. 2000), in interneurons of neocortex of children with epilepsy following perinatal brain damage (Marin-Padilla 1997), and possibly in some cases of human cortical dysplasia (Spreafico et al. 1998). Also of interest is the observation that interneuronal hypertrophy is present in a mouse model of ceroid lipofuscinosis (Batten’s disease; Cooper et al. 1999), in which seizures are present (Eliason et al. 2004; Griffey et al. 2006), as they are in the human disorder. It appears that different subtypes of interneurons can survive and enlarge in some epileptogenic pathophysiological processes, although in some models, interneuronal atrophy rather than hypertrophy has been found (Prince et al. 2009).

The mechanisms leading to increases in interneuronal size are not known, although a variety of experimental results suggest that brain-derived neurotrophic factor (BDNF) may be involved. BDNF is up-regulated by increased neuronal activity (Rocamora et al. 1996; Wetmore et al. 1994; Zafra et al. 1991; reviewed in Marty et al. 1997). Increases in BDNF mRNA and protein expression occur in dentate gyrus of rats following seizures (Binder et al. 1999; Danzer et al. 2004; Dugich-Djordjevic et al. 1992; Inoue et al. 1998; Isackson et al. 1991; Kokai et al. 1996; Rocamora et al. 1992) and we have found a similar increase in BDNF immunoreactivity in hilar neurons of epileptic versus control GIN mice (Supplemental Fig. S1).1 This agent has known trophic effects on interneuronal growth and development (Jin et al. 2003; reviewed in Marty et al. 1997), including hypertrophy of GABAergic cells (Aguado et al. 2003), and may also be involved in hypertrophy of dentate granule cells (Inoue et al. 1998) and regulation of both excitatory and inhibitory connectivity (Marty et al. 2000; Olofsson et al. 2000). BDNF TrkB receptor immunoreactivity is present on interneurons in the dentate hilus (Han et al. 1993).

**FIG. 5.** Increased frequency of miniature excitatory postsynaptic currents (mEPSCs) from SOM-EGFP hilar interneurons of epileptic mice. A: continuous recordings of mEPSCs from typical cells in a control mouse (left) and in an epileptic mouse (right). B: summary graph of mEPSC frequencies. C: summary graph showing the mean ratio of mEPSC to sEPSC frequency for cells from control and epileptic mice. Number of cells indicated at the bottom of bars. **P < 0.01, 2-sample t-test. mEPSC recordings obtained in artificial cerebrospinal fluid (ACSF) containing 1 μM tetrodotoxin (TTX). Number of mice: 14 control, 7 epileptic.

**DISCUSSION**

These results show that in a mouse model of temporal lobe epilepsy, surviving dentate hilar SOM-containing interneurons have both structural and functional alterations, including increases in somatic and dendritic size, altered intrinsic membrane properties, increased excitatory synaptic innervations, and increased frequency of action potential generation. Such changes would be expected to affect the functional role of SOM interneurons within the epileptogenic dentate gyrus.

Increased interneuronal size has been previously described in animal models of epileptogenesis

1 The online version of this article contains supplemental data.

**FIG. 6.** Paired-pulse ratios (PPRs) for evoked excitatory postsynaptic currents (eEPSCs) are not different between SOM-EGFP hilar interneurons from control and epileptic mice. A: representative traces from SOM-EGFP hilar interneurons in control and epileptic mice showing pairs of eEPSCs evoked with minimal (threshold) stimulation at a 50-ms interpulse interval. B: summary graph of PPRs for 20-, 50-, and 100-ms interpulse intervals. Number of cells indicated above (control, open symbols, dashed line) or below (epileptic, black, solid line) data points. No significant difference in PPR between control and epileptic neurons at any interpulse interval. PPR calculated as the peak amplitude of response 2/response 1. Number of mice: 3–4 control, 3–4 epileptic.
and on interneuronal axons and terminals (Drake et al. 1999), in addition to prominent expression in dentate granule cells. The anatomical changes in SOM interneurons shown here and in other recent experiments (Zhang et al. 2009) would have potential effects on both their intrinsic properties and their function in the dentate circuit. Increased cell (somatic and dendritic) size would increase the total membrane area and result in an increase in whole cell membrane capacitance (Limón et al. 2005; Sooksawate et al. 2005) and a decrease in input resistance. Differences in the relative changes in these parameters may be due to different contributions of somatic versus dendritic membrane areas and conductances to the measurements obtained from whole cell somatic recordings, as well as potential contributions to measurements of somatic area by membrane infoldings not detectable by our methods of analysis. We did not assess possible mechanisms underlying the depolarized resting membrane potential in SOM cells of the epileptic animals. Injury-induced alterations in Na+ K+ -ATPase activity (Ross and Sotjes 2000), I_h (Brauer et al. 2001), or a leak conductance might underlie this depolarization. The net effect of intrinsic changes and their effect on synaptic inputs, such as a decreased input resistance that will reduce the efficacy of synaptic inputs, is therefore hard to predict from the available data. However, the increase in excitatory synaptic input onto SOM cells is a significant consequence of the injury induced by the SE and possibly by subsequent seizures. There was an approximate doubling of sEPSC frequency (Fig. 4) that was predominantly a consequence of increased mEPSC frequency (Fig. 5C) rather than an increase in AP-dependent transmitter release. In the absence of a significant increase in Pr (Fig. 6), these results indicate a larger number of synapses most likely due to innervation by increased numbers of sprouting axonal

![Fig. 7](http://www.jn.org)  
**FIG. 7.** Amplitude of eEPSCs is increased in SOM-EGFP neurons from epileptic mice. **A:** representative superimposed traces showing EPSCs recorded from interneurons from both a control mouse and an epileptic mouse evoked by stimuli of increasing intensity delivered to the granule cell layer. Input–output (I–O) slopes for peak amplitudes of evoked EPSCs vs. stimulus intensity are steeper in cells from epileptic mice than from control mice. **B:** I–O plots of amplitudes of EPSCs evoked by 6 different stimulus intensities in 4 SOM-EGFP neurons from control (open symbols, dashed lines) and 4 from epileptic mice (black symbols, solid lines). Each line represents plot from a single cell. Error bars: SE from 3 repeated measures in each cell. **C:** mean amplitudes for eEPSCs in the groups of cells of **B** at the same stimulus increments. *P < 0.05; **P < 0.01. Number of mice: 4 control, 4 epileptic.

![Fig. 8](http://www.jn.org)  
**FIG. 8.** Increased firing frequency of hilar interneurons from epileptic mice. **A:** representative cell-attached recordings of action potentials of hilar SOM-EGFP neurons from a control mouse (left) and an epileptic mouse (right). **B:** summary graph of action potential frequency for 9 cells from 3 control mice (white circles) and 8 cells from 2 epileptic mice (black circles). Each point represents the average firing frequency for an individual cell. Horizontal bars: mean value for control (black) or epileptic cells (gray). **P < 0.01, 2-sample t-test.
terminals, although a contribution by boutons with multiple synapses to increased mEPSC frequency is possible (McNeill et al. 2003; White et al. 2004).

In addition, the increased dendritic lengths and branches in biocytin-filled SOM cells (Fig. 1, A, C, and D) would provide an increased dendritic territory for new synapse formation. The input–output results (Fig. 7) show no significant increase in peak amplitude of EPSCs evoked by stimuli close to threshold in control versus epileptic SOM cells, suggesting that sprouting axonal terminals from single presynaptic neurons do not hyperinnervate single SOM cells. Rather, sprouting presynaptic cells likely contact a greater number of postsynaptic SOM interneurons in epileptic mice. This result is similar to that reported in injured CA3 pyramidal cells of hippocampal slice cultures (McKinney et al. 2007) and in chronically epileptogenic neocortical slices (Jin et al. 2006). With increasing stimulus intensity, increased numbers of presynaptic elements are activated, generate APs, and release glutamate onto SOM cells. The increased slopes of the I–O relationships at higher stimulus intensities (Fig. 7, B and C) reflect recruitment of more presynaptic axons in slices from the epileptic mice.

One source of this hyperinnervation could be from sprouting mossy fibers of granule cells, although only a very small proportion (<5%) of synapses made by these axons innervate GABAergic dendrites in stratum molecular (Buckmaster et al. 2002). However, granule cell projections in the hilus are likely contributors to new excitatory synaptic input to hilar SOM neurons because granule cell axon length in the hilus is significantly greater than that in controls in epileptic kainate-treated rats (Buckmaster and Dudek 1999). A more distal localization of the new excitatory synapses onto SOM cells is suggested by the significantly slower rise time for both spontaneous and miniature EPSCs without an alteration in decay time (Table 3). Other sources of new excitatory innervation of SOM interneurons might be CA3 collateral backprojections, mossy cells, and perforant path from entorhinal cortex. One consequence of this increased excitatory innervation, together with the small but significant depolarization of resting membrane potential noted earlier, is an increase in spontaneous AP generation by SOM cells in the slices from epileptic mice. This enhanced output, together with the increased axonal lengths of hilar SOM cells and the significant increase in their innervation of granule cells (Zhang et al. 2009), would strengthen the feedback GABAergic inhibition in the dentate and could be regarded as a partial compensatory mechanism for loss of hilar SOM interneurons in the epileptogenic hippocampus.

**Disclosures**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**References**


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