Interneurons in Area CA1 Stratum Radiatum and Stratum Oriens Remain Functionally Connected to Excitatory Synaptic Input in Chronically Epileptic Animals

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Rempe, D. A., E. H. Bertram, J. M. Williamson, and E. W. Lothman. Interneurons in area CA1 stratum radiatum and stratum oriens remain functionally connected to excitatory synaptic input in chronically epileptic animals. J. Neurophysiol. 78: 1504–1515, 1997. Past work has demonstrated a reduction of stimulus-evoked inhibitory input to hippocampus CA1 pyramidal cells in chronic models of temporal lobe epilepsy (TLE). It has been postulated that this reduction in inhibition results from impaired excitation of inhibitory interneurons. In this report, we evaluate the connectivity of area CA1 interneurons to their excitatory afferents in hippocampal-parahippocampal slices obtained from a rat model of chronic TLE. Rats were made chronically epileptic by a period of continuous electrical stimulation of the hippocampus, which establishes an acute condition of self-sustained limbic status epilepticus (SSLSE). This period of SSLSE is followed by a development of chronic recurrent spontaneous limbic seizures that are associated with chronic neuropathological changes reminiscent of those encountered in human TLE. Under visual control, whole cell patch-clamp recordings of interneurons and pyramidal cells were obtained in area CA1 of slices taken from adult, chronically epileptic post-SSLSE rats. Neurons were activated by means of electrodes positioned in stratum radiatum. Intrinsic membrane properties, including resting membrane potential, action potential (AP) threshold, AP half-height width, and membrane impedance, were unchanged in interneurons from chronically epileptic (post-SSLSE) tissue compared with control tissue. Single stimuli delivered to stratum radiatum evoked depolarizing excitatory postsynaptic potentials and APs in interneurons, whereas paired-pulse stimulation evoked facilitation of the postsynaptic current (PSC) in both control and post-SSLSE tissue. No differences between interneurons in control versus post-SSLSE tissue could be found with respect to the mean stimulus intensity or mean stimulus duration needed to evoke an AP. A multiple linear regression analysis over a range of stimulus intensities demonstrated that a greater number of APs could be evoked in interneurons in post-SSLSE tissue compared with control tissue. Spontaneous PSCs were observed in area CA1 interneurons in both control and post-SSLSE tissue and were markedly attenuated by glutamatergic antagonists. In conclusion, our data suggest that stimulus-evoked and spontaneous excitatory synaptic input to area CA1 interneurons remains functional in an animal model of chronic temporal lobe epilepsy. These findings suggest, therefore, that the apparent decrease of polysynaptic inhibitory PSPs in CA1 pyramidal cells in epileptic tissue is not due to a deficit in excitatory transmission from Schaffer collaterals to interneurons in stratum radiatum and stratum oriens.

INTRODUCTION

Disturbances in inhibitory neurotransmission have been postulated to contribute to seizure generation in the condition of human temporal lobe epilepsy (TLE) (for reviews, see Schwartzkroin 1994; Tasker and Dudek 1991). To evaluate changes in inhibitory neurotransmission, several models of chronic TLE, sharing multiple characteristics of the condition as it occurs in humans, have been developed (Cavalheiro et al. 1982, 1991; Cronin et al. 1992). Our laboratory has developed a model of TLE that uses electrical stimulation of the rat hippocampus to produce a self-limited period of nonconvulsive, self-sustaining limbic status epilepticus (SSLSE). Several weeks after SSLSE, rats develop a condition of chronic epilepsy with recurrent spontaneous seizures persisting for at least 12 mo after the induction of SSLSE (Bertram and Cornett 1994). The seizures are a mixture of nonconvulsive (Racine class 1 and 2) and limbic motor seizures (Racine class 3–5) with the majority of seizures occurring 3 mo after SSLSE being limbic motor seizures (Bertram and Cornett 1994). Histological examination demonstrates considerable neuronal loss in the dentate hilus, CA3, and CA1 with relative sparing of dentate granule cells and CA2 in post-SSLSE tissue, similar to the histopathological features of Ammon’s horn sclerosis described in human TLE (Lothman et al. 1990). The pathophysiological alterations observed in this model mirror several observations made in surgical specimens obtained from patients with epilepsy in that: abnormalities are chronic (lasting at least 12 mo post-SSLSE), epileptiform paroxysms are evoked readily with synchronized vigorous excitatory drive, and spontaneous paroxysms in vitro are rare (Rempe et al. 1995). Thus the post-SSLSE model faithfully replicates many aspects of human TLE (Wieser et al. 1993), and it provides a substrate to study enduring pathological changes within a model of TLE.

Using animal models of TLE, several studies have focused on inhibitory neurotransmission in area CA1 of the hippocampus. Previous work by other groups demonstrated that inhibitory postsynaptic potentials (IPSPs), evoked in CA1 pyramidal cells by stimulation of Schaffer-collaterals, were diminished markedly in slices taken from animals previously treated with intrahippocampal kainic acid (Ashwood et al. 1986; Franck et al. 1988; Meier et al. 1992; Nakajima et al. 1991; Williams et al. 1993). Similarly, in the post-SSLSE model, stimulus-evoked IPSPs in CA1 pyramidal cells were attenuated markedly when stimulating stratum radiatum, stratum lacunosum moleculare (L/M), and stratum oriens/alveus (Bekenstein and Lothman 1993; Mangan et al.
Preparation and maintenance of hippocampal slices

In experiments designed to determine the site of cellular dysfunction underlying the deficit in GABAergic inhibition, Nakajima and coworkers (1991) performed paired intracellular recordings between inhibitory interneurons in stratum radiatum (identified as basket cells) and pyramidal cells. A decrease in functional interactions between interneurons and pyramidal cells led to the conclusion that a decreased efficacy of inhibitory synapses exists in epileptic tissue. This conclusion since has been disputed in studies using both the kainic acid (Williams et al. 1993) and post-SSLSE (Bekenstein and Lothman 1993; Mangan et al. 1995) models of TLE. In these experiments, as well as others (Empson and Jefferys 1993; Sloviter 1987, 1991), the concept has emerged that the activation of inhibitory interneurons by their excitatory afferents may be compromised in epileptic tissue. The terms ‘dormancy’ and ‘functional disconnection of interneurons’ have been applied to this concept.

To evaluate the functional status of excitatory input to area CA1 interneurons, whole cell recordings of area CA1 interneurons were obtained in hippocampal slices taken from control and chronically epileptic (post-SSLSE) adult rats. Our results suggest that basic membrane properties of interneurons within stratum oriens, stratum radiatum, and stratum lacunosum/moleculare are not altered and that excitatory input to these interneurons remains functional in chronically epileptic tissue. We conclude, therefore, that the apparent loss of IPSPs in CA1 pyramidal cells in epileptic tissue occurs somewhere other than the Schaffer-collateral to interneuron synapse.

METHODS

Preparation of animals

All studies were carried out on adult (225–350 g) Sprague-Dawley rats. Three groups of animals were used: a naive control group, an electrode control group, and a chronically epileptic (post-SSLSE) group. The naive control group was not implanted with electrodes or stimulated before slice preparation, whereas the electrode control group was implanted with electrodes but not stimulated. Animals were 200–225 g at the time of electrode implantation. An electrode, consisting of two twisted Teflon-coated stainless steel wires, was implanted into the ventral CA3 subregion (AP −3.6, ML −4.9, DV −5.0 to dura, incisor bar +5.0) on one side of the brain. One week after surgery, the third group of animals underwent ‘continuous’ hippocampal stimulation for the purposes of inducing SSLSE and, stimulus trains (50 Hz of 1 ms 400 μA peak-to-peak biphasic square waves for 10 s) were delivered every 15 s for up to 90 min (see Lothman et al. 1989). During the period of status epilepticus, the animals behavior was predominantly nonconvulsive, but occasional limbic motor seizures (Racine 3–5) were seen in many of the animals especially during the first several hours of SSLSE (Lothman et al. 1989). Animals with continuous seizure activity for ≥2 h after cessation of stimulus trains were assigned to the post-SSLSE group. Using the hippocampus from the side of the brain opposite of the stimulation electrode, in vitro studies were performed for ≥30 days after the stimulation of these animals, a period in which the animals were having spontaneous limbic intermittent seizures.

Preparation and maintenance of hippocampal slices

Before preparing hippocampal-parahippocampal slices, rats were anesthetized deeply with halothane. After decapitation, brains were removed from the skull and blocked by dissecting away the cerebellum and coronal sectioning at the level of the midthalamus while retaining the hippocampus and adjacent parahippocampal region. The brain block was sectioned (300–400 μm) horizontally on a vibratome in chilled (4°C) and oxygenated bubbled with 95% O₂-5% CO₂ artificial cerebrospinal fluid (ACSF) containing (in mM) 153 Na⁺, 3.1 K⁺, 130.5 Cl −, 26 HCO₃⁻, 1.5 Ca²⁺, 1.5 Mg²⁺, 1.5 SO₄²⁻, 1.1 PO₄³⁻, and 10 dextrose with a pH of 7.4. After a minimum of 60 min of recovery (25°C), slices were placed into either a chamber for recording from submersed slices or a chamber for recording from interface slices. Submerged slices were maintained at between 28 and 30°C in oxygenated ACSF, whereas interface slices were maintained at between 35 and 37°C in oxygenated ACSF.

Criteria for selection of post-SSLSE slices

Previous reports demonstrated that the overwhelming majority of slices obtained from chronically epileptic post-SSLSE rats have markedly decreased stimulus-evoked polysynaptic IPSPs in area CA1 pyramidal cells (Mangan et al. 1995; Rempe et al. 1995). Because our aim was to study if a functional disconnection of interneurons is the mechanism of decreased CA1 pyramidal cell IPSPs, ‘sister’ slices taken from the same animal had to demonstrate an eradication of stimulus-evoked polysynaptic IPSPs in CA1 pyramidal cells using sharp intracellular recordings in an interface chamber.

Electrophysiological recording and stimulation

Extracellular and patch glass microelectrodes were made with capillary filled glass (1.5 mm OD; boro-silicate; World Precision Instruments) prepared on a vertical puller (model P-30; Sutter Inc.) and had resistances between 2 and 5 MΩ. In experiments designed to determine the site of inducing SSLSE and, stimulus trains (50 Hz of 1 ms 400 μA peak-to-peak biphasic square wave pulses for 10 s) were delivered every 15 s for up to 90 min (see Lothman et al. 1989). During the period of status epilepticus, the animals behavior was predominantly nonconvulsive, but occasional limbic motor seizures (Racine 3–5) were seen in many of the animals especially during the first several hours of SSLSE (Lothman et al. 1989). Animals with continuous seizure activity for ≥2 h after cessation of stimulus trains were assigned to the post-SSLSE group. Using the hippocampus from the side of the brain opposite of the stimulation electrode, in vitro studies were performed for ≥30 days after the stimulation of these animals, a period in which the animals were having spontaneous limbic intermittent seizures.

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FIG. 1. Intrinsic membrane properties of area CA1 neurons and methods of data analysis. A: intrinsic membrane properties of pyramidal cells. In most pyramidal cells, current steps evoked action potentials (APs) with a depolarization immediately after the AP (A2), whereas in other pyramidal cells, this depolarization was not seen (A1). Current steps and their corresponding voltage deflections (A, inset) demonstrate an impedance that was smaller than that found in interneurons. B and C: intrinsic membrane properties of a stratum oriens (B) and a stratum radiatum (C) interneuron. Fast afterhyperpolarizations (r) followed the APs evoked in these interneurons. Neurobiotin stains of these same interneurons is shown in Fig. 3. D: schematic diagram illustrating the location of stratum oriens and stratum radiatum interneuron whose intrinsic membrane properties are seen in B and C. E: depolarization width of the stimulus-evoked depolarization profiles were evaluated as shown. (Calibration bars located next to current and voltage steps in A also apply to insets in B and C. Similarly, calibration bars located next to AP traces in A also apply to AP traces in B and C. *, APs in all figures.)

distinguishing pyramidal cells from nonpyramidal cells. In addition to direct visualization, intrinsic properties (primarily the shape of the AP and membrane impedance) distinguished interneurons from pyramidal cells (Fig. 1). Because our internal solution contained a Ca\(^{2+}\) chelator, which has been shown to inhibit spike frequency adaptation (Staley et al. 1992), spike frequency adaptation was not used as a criterion for identification of interneurons.

Whole cell patch recordings were obtained in the current clamp mode so that the resting membrane potential (RMP) and impedance of the neurons could be assessed immediately. Only those neurons whose RMP was more negative than \(-50\) mV, having overshooting APs, and a membrane impedance \(>100\) MΩ \((>50\) MΩ for pyramidal cells\) were accepted into this study. Typically, the access resistance of the patch electrodes was between 10 and 25 MΩ, although sometimes as large as 40 MΩ. Spontaneous currents and paired-pulse stimulation experiments were recorded in interneurons when voltage clamped at \(-75\) mV without correcting for the error introduced by the series resistance.

In some cases, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) \((20\) μM, Cambridge Research Biochemicals\), \(d\)-(−)-2-amino-5-phosphonovaleric acid (d-APV; 50 μM, Cambridge Research Biochemicals\), were added to the perfusate to block non-N-methyl-

Histological procedures

Neurobiotin-filled cells were visualized by light microscopy using an established protocol (Buckmaster and Schwartzkroin 1995) to confirm morphology of cells. In addition to this protocol, the slices were Nissl stained with cresyl violet to allow visualization of cell body layers.

Data analysis

Analyses of data from extracellular and whole cell recordings were done with computer-assistance using Axograph software (Axon Instruments). AP overshoot was measured as the height of the action potential \(>0\) mV when the cell fired an AP to current injection. By using current steps, the membrane potential at which APs first were evoked (the AP threshold) was determined.

To avoid the confounding effects of APs on excitatory postsynaptic potentials (EPSP), the time to peak, amplitude, and half-height width of the stimulus-evoked EPSP was measured only when an EPSP clearly was evoked without an AP present. The maximal amplitude EPSP evoked without an AP present was used for analysis. Widths of the stimulus-evoked depolarization profiles were measured as demonstrated in Fig. 1E. The average membrane potential at which the different stimulus-evoked parameters (maximum number of APs, time to peak of EPSPs, width of depolarization profiles) were measured did differ between control \((\sim61.7\pm0.93\) mV, st. radiatum-L/M; \(-60.9\pm0.86\) mV, st. oriens\) and post-SSLSE \(\sim61.9\pm1.1\) mV, st. radiatum-L/M; \(-62.3\pm1.1\) mV, st. oriens\) tissue \((P<0.05\), t-tests\).

For paired-pulse recordings, postsynaptic currents (PSCs) were evoked in interneurons at different inter-pulse intervals (ranging from 20 to 500 ms). For each cell, five pairs of PSCs were evoked and averaged for measurement at each interpulse interval. Spontaneous events were measured using appropriate software (kindly provided by Dr. Steve Traynelis). The frequency and amplitude of spontaneous events were determined by measuring only those events that were significantly different from the preceding baseline. At least 100 events, and an average of 177±9 events, were measured per cell.

Statistical tests are indicated below in the text and include independent (grouped) t-tests, Fisher’s exact test, and multiple linear regression. Data are presented below as means \(\pm SE\). A value of \(P<0.05\) was selected for statistical significance.
### RESULTS

**Identification and location of area CA1 interneurons in the study**

Whole cell patch-clamp recordings were obtained from a total of 126 neurons from 83 animals. Because of reported differences in activity and function among interneurons based on location (Kawaguchi and Hama 1987, 1988; Lacaille and Schwartzkroin 1988b; Samulack and Lacaille 1993; Samulack et al. 1993; Segal 1990), interneurons were separated into two groups for the purpose of analysis: a first group from stratum oriens, and a second group from stratum radiatum and stratum L/M. The grouping of the recordings is detailed in Table 1. In both control and post-SSLSE tissue, interneurons throughout area CA1 were sampled as illustrated in Fig. 2.

We attempted to visualize neurons after filling with ne-robiotin to confirm morphology (Fig. 3). Our success was limited because of membrane disruption on exit from the cell. Therefore, in several cases, dendrites were visualized without their accompanying somata. Of those neurons in which the soma was visualized [31 cells/101 attempts (30%)], the classification of the neuron during electrophysiologic studies faithfully predicted the neuronal type. Pyramidal cells in area CA1 had a pyramidal shaped soma, a large apical dendrite, and basal dendrites, whereas both stratum oriens and stratum radiatum-L/M interneurons characteristically had ovoid-shaped somas and bilateral radially extending processes (Fig. 3). Although the soma and processes clearly were stained, axons were not consistently distinguished precluding classification of interneurons into different subtypes based on axon morphology.

**Intrinsic properties of area CA1 neurons**

Previous studies demonstrated no differences between CA1 pyramidal cell intrinsic properties or synaptic responses...
between electrode control and naive tissue (Bekenstein and Lothman 1993; Rempe et al. 1995). Because preliminary experiments in this study similarly demonstrated no difference in either intrinsic membrane properties or synaptic responses in neurons between electrode control and naive tissue, the data from these two groups were pooled into a single control group.

Intrinsic membrane properties of interneurons in epileptic tissue were generally unchanged from those in control tissue (Table 2). There were no significant differences with respect to the mean RMP, membrane impedance, AP half-height width, or AP threshold for either stratum radiatum-L/M or stratum oriens interneurons in control tissue versus those in post-SSLSE tissue (P > 0.05, t-tests; Table 2). Stratum radiatum-L/M interneurons in post-SSLSE tissue had a significantly greater (P < 0.05, t-test) AP overshoot compared with control tissue (Table 2). There were no statistical differences in the percentages of interneurons that exhibited spontaneous APs between control and post-SSLSE tissue for those located either in stratum radiatum-L/M or stratum oriens (P > 0.05, Fisher’s exact test; Table 2).

The intrinsic membrane properties of CA1 pyramidal cells differed from interneurons in four respects [all pyramidal cells (n = 32) are compared with all interneurons (n = 94)]: the mean membrane impedance of pyramidal cells was significantly lower, the mean AP overshoot of pyramidal cells was significantly greater, the mean RMP of pyramidal cells was significantly hyperpolarized, and unlike interneurons, pyramidal cells did not exhibit spontaneous APs (Table 2). No statistical differences were found between interneurons and CA1 pyramidal cells with respect to the AP half-height width (P > 0.05, t-tests).

### Interneuron and pyramidal cell stimulus-evoked responses

Responses in stratum radiatum-L/M and stratum oriens interneurons evoked by single shock stimulation ranged from only EPSPs at low stimulus intensities, to EPSPs, APs and late hyperpolarizations at high stimulus intensities (Fig. 4). Early hyperpolarizations were not observed unless glutamatergic neurotransmission was blocked (see below). In those interneurons exhibiting multiple APs, the APs typically progressed in a graded fashion from a single to multiple APs with increasing stimulus intensity. In post-SSLSE, but not control tissue, some interneurons (1/25 in stratum radiatum-L/M and 3/11 in stratum oriens), displayed an “all-or-none” burst of APs at the AP threshold stimulus intensity (Fig. 4). The amplitude and morphology of the EPSPs, evoked at a stimulus intensity just below AP threshold, did vary in their time course and amplitudes among cells (Fig. 4). However, the mean amplitude, half-height width, and time to peak of the EPSPs were not significantly different between post-SSLSE and control tissue for both stratum radiatum-L/M and stratum oriens interneurons (P > 0.05, t-tests; Table 3).

If interneurons are “functionally disconnected” from their excitatory input in epileptic tissue, then it may be expected that greater stimulus intensities would be required to elicit APs, a smaller numbers of APs would be elicited with comparable stimulation, and/or smaller depolarization widths would be elicited in interneurons of epileptic tissue. There was, however, no significant difference in the mean stimulus intensity or mean stimulus duration required to evoke an AP in interneurons in control versus post-SSLSE tissue for interneurons in either stratum radiatum-L/M or stratum oriens (Table 3; P > 0.05, t-tests). The maximal number of APs that could be elicited in interneurons varied among interneurons in both the control and post-SSLSE groups (Fig. 4). In addition, there was a clear trend toward an increased number of stimulus-evoked APs in post-SSLSE interneurons (Fig. 5). Using a multiple linear regression test, the number of stimulus-evoked APs was found to be significantly dependent on the group (control vs. post-SSLSE) for both stratum oriens and stratum radiatum-L/M interneurons (P < 0.01 in both cases). There was no significant difference in the mean width of the stimulus-evoked depolarization profile in control versus post-SSLSE stratum radiatum interneurons (P > 0.05, t-test). However, the mean width of the stimulus-evoked depolarization profile (P < 0.01, t-test) was significantly greater in post-SSLSE stratum oriens interneurons compared with control (Table 3).

To determine if the stimulus-evoked PSPs seen in interneurons were mediated by glutamatergic neurotransmission, non-NMDA (CNQX) and NMDA (d-APV) receptor channel blockers were added to the perfusate. Stimulus-evoked PSPs and APs in both stratum radiatum-L/M interneurons and stratum oriens interneurons were attenuated markedly by the glutamatergic antagonists in both control (n = 6, stratum radiatum; n = 3, stratum oriens) and post-SSLSE (n = 8, stratum radiatum; n = 2, stratum oriens) tissue (Fig. 4). In some cases, the first AP was not eliminated in CNQX/d-APV, suggesting antidromic activation of the interneuron.

### Table 2. Intrinsic membrane properties of CA1 area neurons

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>Group</th>
<th>RMP, mV</th>
<th>AP Overshoot, mV</th>
<th>AP Threshold, mV</th>
<th>Membrane Impedance, MΩ</th>
<th>AP Half-Height Width, ms</th>
<th>Spontaneous APs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum radiatum-L/M interneurons</td>
<td>Control (35)</td>
<td>−60 ± 0.9</td>
<td>20 ± 1.4*</td>
<td>−45 ± 0.7</td>
<td>279 ± 15</td>
<td>1.2 ± 0.1</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>post-SSLSE (27)</td>
<td>−39 ± 1</td>
<td>24 ± 1*</td>
<td>−46 ± 0.6</td>
<td>274 ± 19</td>
<td>1.2 ± 0.04</td>
<td>38%</td>
</tr>
<tr>
<td>Stratum oriens interneurons</td>
<td>Control (19)</td>
<td>−57 ± 1</td>
<td>24 ± 2</td>
<td>−47 ± 0.8</td>
<td>272 ± 25</td>
<td>1.1 ± 0.01</td>
<td>61%</td>
</tr>
<tr>
<td></td>
<td>post-SSLSE (12)</td>
<td>−59 ± 2</td>
<td>25 ± 3</td>
<td>−48 ± 1</td>
<td>394 ± 71</td>
<td>1.2 ± 0.1</td>
<td>50%</td>
</tr>
<tr>
<td>Pyramidal cells</td>
<td>All (32)</td>
<td>−63 ± 1**</td>
<td>42 ± 2**</td>
<td>Not Measured</td>
<td>70.6 ± 4**</td>
<td>1.3 ± 0.04</td>
<td>0%</td>
</tr>
</tbody>
</table>

Parentheses contain number of neurons. Values are means ± SE. The last column is the percentage of cells with spontaneous APs at their RMP. RMP, resting membrane potential; AP, action potential. *P < 0.05 (t-test) comparing interneurons (same location) in control versus post-SSLSE tissue; **P < 0.001 (t-tests) comparing all pyramidal cells to all interneurons.
The percentage of interneurons displaying antidromic activation in CNQX/D-APV was not statistically different between control (44%) versus post-SSLSE (60%) tissue ($P > 0.05$, Fisher’s exact test). Stimulus-evoked monosynaptic IPSPs were retained in some interneurons in the presence of CNQX/D-APV and had reversal potentials of (-60.0 mV, $n = 6$) and (-79.8 mV, $n = 4$) for the early and late components, respectively.

In some cases, interneurons and pyramidal cells were sampled consecutively in the same slice to compare and contrast responses of the two cell types. The stimulus-evoked response characteristics of pyramidal cells in post-SSLSE tissue consisted of multiple APs, broad depolarizing PSPs, and markedly diminished γ-aminobutyric acid-B (GABA_B)-mediated IPSPs in agreement with previous reports (Mangan et al. 1991; Rempe et al. 1995) (Fig. 6). As expected, pyramidal cell responses in control tissue consisted of a brief EPSP, single AP, and a late hyperpolarization. Although typically seen in sharp intracellular recordings of CA1 pyramidal cells, early GABA_A-mediated IPSPs were not apparent at RMP in these whole cell recordings because the Cl⁻ reversal potential was near the RMP. Using a multiple linear regression test, the number of stimulus-evoked APs was found to be significantly dependent on the group (control vs. post-SSLSE) for pyramidal cells ($P < 0.001$). It is important to note that in these same post-SSLSE slices, which contained decreased stimulus-evoked IPSPs, prolonged stimulus-evoked EPSPs, and multiple APs in CA1 pyramidal cells, robust EPSPs and APs were elicited in area CA1 interneurons (Fig. 6).

### Paired stimulation of interneurons

Several studies have employed paired-pulse protocols to evaluate the level of inhibitory activity in brain tissue (Bekenstein et al. 1993; Cornish and Wheal 1989; Sloviter 1991). Therefore paired stimulation of stratum radiatum-L/M and stratum oriens interneurons were measured to ascertain if interneurons responded differently to paired stimuli in post-SSLSE versus control tissue. In both control and epileptic tissue, the test PSC (second PSC in the pair) was larger than the conditioning PSC (first PSC in the pair) at interpulse-intervals of 20–100 ms, indicating a facilitation of the response. No facilitation was observed at interpulse-

### Table 3. Stimulus responses of interneurons

<table>
<thead>
<tr>
<th>Interneuron Location</th>
<th>Group</th>
<th>AP Threshold Stimulus Intensity, V</th>
<th>AP Threshold Stimulus Duration, ms</th>
<th>Width of Depolarization Profile, ms</th>
<th>EPSP Half-Height Width, ms</th>
<th>EPSP Amplitude, mV</th>
<th>EPSP Time to Peak, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. radiatum-L/M</td>
<td>Control</td>
<td>32.8 ± 3.8 (18)</td>
<td>0.27 ± 0.03 (18)</td>
<td>226 ± 16 (25)</td>
<td>39.5 ± 5 (17)</td>
<td>11.0 ± 0.7 (17)</td>
<td>11 ± 1.3 (17)</td>
</tr>
<tr>
<td></td>
<td>post-SSLSE</td>
<td>30.5 ± 5.9 (20)</td>
<td>0.24 ± 0.03 (20)</td>
<td>260 ± 18 (24)</td>
<td>35.2 ± 7 (17)</td>
<td>9.7 ± 0.9 (17)</td>
<td>12 ± 2.3 (17)</td>
</tr>
<tr>
<td>St. oriens</td>
<td>Control</td>
<td>32.5 ± 5.9 (11)</td>
<td>0.34 ± 0.03 (11)</td>
<td>173 ± 13* (12)</td>
<td>39.4 ± 5 (10)</td>
<td>6.9 ± 0.8 (10)</td>
<td>17 ± 2.7 (10)</td>
</tr>
<tr>
<td></td>
<td>post-SSLSE</td>
<td>30.6 ± 4.7 (11)</td>
<td>0.27 ± 0.03 (11)</td>
<td>249 ± 21* (11)</td>
<td>40.5 ± 11 (5)</td>
<td>7.9 ± 1 (5)</td>
<td>23 ± 7.8 (5)</td>
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</tbody>
</table>

Numbers in parentheses indicate number of cells. Values are means ± SE. * $P < 0.05$ (t-test) comparing interneurons (same location) in control versus post-SSLSE tissue. EPSP, excitatory postsynaptic potential.
DISCUSSION

This study, which examined the connectivity of area CA1 interneurons to their excitatory input in an animal model of temporal lobe epilepsy, contains six major findings. First, there was no difference in the stimulus intensity or stimulus duration required to elicit APs in interneurons in control versus post-SSLSE tissue. Second, the EPSPs and APs elicited with stimulation were sensitive to glutamatergic antagonists. Third, an increased number of APs were evoked with stimulation in interneurons in post-SSLSE compared with control tissue. Fourth, paired-pulse stimulation produced an equal amount of PSC facilitation in control and post-SSLSE interneurons. Fifth, spontaneous, glutamatergic excitatory currents were present in both control and post-SSLSE interneurons. Finally, except for AP overshoot in one subset of interneurons, the intrinsic membrane properties of the interneurons were unchanged between post-SSLSE and control tissue. Overall these findings indicate that stimulus-evoked and spontaneous excitatory synaptic input to area CA1 interneurons remains functional in an animal model of chronic temporal lobe epilepsy.

Functional connectivity of interneurons in epileptic tissue to excitatory afferents

Prior studies demonstrated a decrease in paired-pulse inhibition in vivo and a marked decrease in stimulus-evoked CA1 pyramidal cell IPSPs in vitro in animal models of TLE (Bekenstein and Lothman 1993; Bekenstein et al. 1993; Cornish and Wheal 1989; Mangan et al. 1995; Sloviter 1991; Williams et al. 1993). Based on these findings, it was proposed that area CA1 inhibitory interneurons were functionally disconnected from their excitatory input or “dormant” in epileptic tissue. Yet, in the current report, single stimuli delivered to stratum radiatum evoked EPSPs and APs in area CA1 interneurons of slices taken from animals with chronic TLE. These same chronically epileptic animals lacked stimulus-evoked polysynaptic IPSPs in CA1 pyramidal cells. Furthermore, paired-pulse stimulation produced equal amounts of facilitation of the PSC in interneurons from slices derived from control and epileptic animals.

Not only were EPSPs and APs evoked in interneurons in epileptic tissue, but they also were hyperresponsive in that a significantly greater number of APs were evoked in interneurons of post-SSLSE tissue compared with control. It is unlikely that a more direct or intense stimulation of interneurons in post-SSLSE tissue compared with control could account for this difference for several reasons. First, a similar percentage of interneurons displayed antidromically activated APs in control and post-SSLSE tissue (Table 4). The distribution of the amplitudes of the events for each group is shown in Fig. 8. The addition of CNQX and D-APV, to block non-NMDA and NMDA receptor dependent spontaneous activity, markedly diminished the amplitude and frequency of spontaneous events, in the overwhelming majority of interneurons tested from both control (100%, 7/7 stratum radiatum-L/M; 100%, 4/4 stratum oriens) and post-SSLSE (83%, 5/6 stratum radiatum-L/M; 100%, 2/2 stratum oriens) tissue (Fig. 9).

Spontaneous activity in interneurons

Spontaneous events were recorded in interneurons to determine if spontaneous excitatory input was present in both post-SSLSE and control tissue. The mean amplitude and frequency of the spontaneous events were not significantly different (P > 0.05, t-tests) in control versus post-SSLSE stratum oriens interneurons (Table 4). No significant differences in mean amplitude or frequency were found between control and post-SSLSE stratum radiatum-L/M interneurons (P > 0.05, t-tests; Table 4). The distribution of the amplitudes of the events for each group is shown in Fig. 8. The addition of CNQX and D-APV, to block non-NMDA and NMDA receptor dependent spontaneous activity, markedly diminished the amplitude and frequency of spontaneous events, in the overwhelming majority of interneurons tested from both control (100%, 7/7 stratum radiatum-L/M; 100%, 4/4 stratum oriens) and post-SSLSE (83%, 5/6 stratum radiatum-L/M; 100%, 2/2 stratum oriens) tissue (Fig. 9).
of stimulus intensities and durations were not significantly different between post-SSLSE and control tissue. Other evidence also suggests that interneurons are hyperresponsive in chronically epileptic tissue. Stratum oriens interneurons had a significantly longer stimulus-evoked depolarization profile compared with naïve, similar to hyperresponsive CA1 pyramidal cells in post-SSLSE tissue (Lothman et al. 1995) and, unlike interneurons in control tissue that only showed graded responses, some interneurons in post-SSLSE tissue demonstrated bursts of APs with stimulation at their AP threshold intensity. Several mechanisms may account for the interneuron hyperresponsiveness including: an increase in monosynaptic excitatory input to the interneurons from Schaffer-collaterals, a decrease in inhibitory input to the interneurons, or an increase in feedback excitation to interneurons from the pyramidal cells which have prolonged discharges in epileptic tissue. Currently, it is unresolved as to which, if any, of these mechanisms may account for the hyperresponsive interneurons. Nonetheless, these data suggest that interneurons functionally are connected to their Schaffer-collateral excitatory input in an animal model of TLE that lacks stimulus-evoked CA pyramidal cell IPSPs.

If interneurons are not functionally disconnected from their excitatory input in epileptic tissue, then what mechanism does account for the absent stimulus-evoked IPSPs in CA1 pyramidal cells of epileptic tissue? Prior studies suggest at least two different mechanisms may account for the lack of IPSPs in area CA1 pyramidal cells: a dysfunction of inhibitory receptors on the pyramidal cells and/or a “masking” of IPSPs by overwhelming excitatory input to the pyramidal cells. Recent work by our laboratory demonstrated that monosynaptic GABA<sub>A</sub> IPSPs, elicited in epileptic tissue with glutamatergic antagonists present, are shorter in duration compared with control (Mangan and Bertram 1997). The mechanism for this change remains to be elucidated. However, it is possible that this effect is the result of postsynaptic GABA<sub>A</sub> receptor dysfunction. Furthermore, it recently has been demonstrated that both presynaptic and postsynaptic GABA<sub>B</sub> receptors are markedly dysfunctional in post-chronically epileptic tissue. Stratum oriens interneurons had a significantly longer stimulus-evoked depolarization profile compared with naïve, similar to hyperresponsive CA1 pyramidal cells in post-SSLSE tissue (Mangan and Lothman 1996). In contrast, alterations in GABA mediated IPSPs on CA1 pyramidal cells are not seen in the kainic acid model of TLE when interneurons are stimulated directly in the presence of glutamatergic antagonists (Williams et al. 1993). The contribution of GABAergic receptor dysfunction to the epileptic state therefore remains to be elucidated fully. However, given the efficacy of GABAergic receptor modulators and agonists on treating human TLE, a relatively small alteration in GABA receptors in human TLE could have a large impact on epileptogenicity and thus deserves further study.

A second mechanism by which CA1 pyramidal cell IPSPs may appear to be absent in epileptic tissue is by masking of the IPSP by exuberant excitatory input onto the pyramidal cells. In an earlier report, monosynaptic GABA<sub>A</sub> IPSPs, evoked by directly stimulating interneurons in either stratum L/M or stratum pyramidale, only could be appreciated in CA1 pyramidal cells of post-SSLSE tissue if glutamatergic excitatory synaptic input was blocked (Lothman et al. 1995). In other words, when directly stimulating interneurons, a stimulus protocol that bypasses the excitatory input onto inhibitory interneurons, IPSPs were absent in CA1 pyramidal cells of epileptic tissue unless excitatory neurotransmission was blocked. This finding demonstrates that the stimulus-evoked monosynaptic GABA<sub>A</sub> IPSPs are present on pyramidal cells in epileptic tissue, and suggests that the IPSPs cannot be appreciated, or are masked, in the presence of overwhelming excitatory input in epileptic tissue. Given that monosynaptic IPSPs on pyramidal cells are masked by exuberant excitation, then it is reasonable to propose that polysynaptic IPSPs on pyramidal cells, such as those evoked...
with far-site stimulation in stratum radiatum, also can be
masked by excitatory input. In this way, the excitatory input
to inhibitory interneurons may not be dysfunctional, but the
effect of the excitation of the interneurons (postsynaptic
IPSPs on pyramidal cells) cannot be appreciated.

Sloviter has proposed that “dormant basket cells” are responsible for decreased paired-pulse inhibition in area CA1 and in the dentate gyrus in an animal model of TLE (Sloviter 1987, 1991). Basket cells were defined as dormant if they responded to one set of excitatory afferents but not to another. Because basket cells were not sampled in this report, our data neither disputes nor supports the dormant basket cell hypothesis. However, this study suggests that other CA1 interneuron subtypes in chronically epileptic tissue do not appear to be dormant at least to Schaffer-collateral input. Therefore, if dormancy of basket cells is responsible for the attenuated CA1 pyramidal cell IPSPs in epileptic tissue, our data suggests that this subtype of interneuron is unique in this property. Direct recordings from basket cells in vivo need to be obtained to resolve this issue definitively.

Interneurons sampled in this study were not categorized beyond the location of their cell body either in stratum radiatum-L/M or stratum oriens. Because several types of interneurons have been described in area CA1 (Buhl et al. 1994a,b; Kawaguchi and Hama 1988; Lacaille and Schwartzkroin 1988a,b; Ribak et al. 1990; Samulack and Lacaille 1993; Sik et al. 1995), presumably several types of interneurons in stratum radiatum and stratum oriens were sampled in this report. Therefore one could argue that different subsets of interneurons were sampled in control versus post-SSLSE tissue or that one subset of the interneurons was disconnected functionally from their excitatory input but underrepresented in the study. Neither of these possibilities is likely for several reasons. First, a similar distribution of interneurons throughout area CA1 with similar morphologies were sampled in control and post-SSLSE tissue (Fig. 2). Second, the interneurons in control and post-SSLSE tissue did not differ significantly in their membrane properties, suggesting a similar sampling. Third, there was no significant difference in the time to peak of the EPSPs between control and post-SSLSE interneurons. Finally, all interneurons sampled responded to stimulation making it unlikely that one subtype was disconnected functionally from its excitatory input. However, as discussed above, it should be noted that for technical reasons, interneurons within stratum pyramidale were not sampled as part of this study. Therefore, the basket cell subtype of interneuron is not represented in this study. Because of this limitation, the dormant basket cell hypothesis was not directly examined in this study.

It could be argued that although we sampled interneurons as part of this study, we did not sample “inhibitory” interneurons. Although interneurons were not specifically identified as inhibitory interneurons in this study, there is evidence that suggests that a large proportion of the sampled interneurons were inhibitory. First, inhibitory interneurons have been described throughout area CA1 (Buhl et al. 1994a,b; Kawaguchi and Hama 1988; Lacaille and Schwartzkroin 1988a,b; Ribak et al. 1978; Samulack and Lacaille 1993; Woodson et al. 1989). In fact, 80–95% of area CA1 interneurons are reported to contain the inhibitory neurotransmitter GABA in naive tissue (Woodson et al. 1989). Second, there is abundant evidence indicating the survival of area CA1 inhibitory interneurons in animal models of TLE (see Houser 1991 for review). In the post-SSLSE model, biochemical studies, in situ hybridization studies, and electrophysiological studies all indicate a preservation of inhibitory interneurons in the subfields of area CA1 (Bekenstein and Lothman 1993; Lothman et al. 1996; Mangan et al. 1995; C. R. Houser and E. W. Lothman, unpublished observations).

<table>
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<tr>
<th>Table 4. Spontaneous activity recorded in interneurons</th>
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<tr>
<td>Location</td>
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<tr>
<td>St. radiatum-L/M</td>
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<td>St. oriens</td>
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Numbers in parentheses indicate number of cells. Values are means ± SE.
area CA1 inhibitory interneurons survive in post-SSLSE tissue and a large percentage of the interneurons are inhibitory, it can be assumed that the majority of the interneurons sampled in this study were inhibitory interneurons.

**Spontaneous excitatory input is preserved in interneurons in epileptic tissue**

Because the intense and synchronized nature of the stimulus paradigms reported above may have “saturated” the system obviating more subtle changes in excitatory coupling, it was important to determine if spontaneous excitatory events were present in post-SSLSE tissue. In agreement with prior studies in stratum oriens interneurons in young naive tissue (McBain et al. 1994), spontaneous PSCs were observed in stratum oriens interneurons in this study in both control and post-SSLSE adult tissue. Furthermore, in both control and epileptic tissue, spontaneous events were observed in stratum radiatum-L/M interneurons. These events were attenuated by glutamatergic antagonists. Neither the mean amplitude nor frequency of the spontaneous events were significantly different between control and post-SSLSE tissue. These data demonstrate that glutamatergic excitatory events are retained on interneurons in post-SSLSE tissue and provide further evidence that interneurons are functionally connected to their excitatory input. Because the spontaneous events were recorded in ACSF, presumably both excitatory and inhibitory events were present. Therefore no definitive conclusion regarding whether spontaneous excitatory or inhibitory activity is altered in interneurons in epileptic tissue can be reached. This important topic is beyond the scope of this report and will require further study.

**Intrinsic properties of area CA1 interneurons**

Using direct visualization, laminar location, and differences in intrinsic properties, interneurons were discriminated from CA1 pyramidal cells. In agreement with published reports (Knowles and Schwartzkroin 1981; Lacaille and Williams 1990; Schwartzkroin and Mathers 1978), the shape of the interneurons’ APs were distinct, consisting of a large fast afterhyperpolarization after the AP. In those cells visualized histologically after filling with neurobiotin, electrophysiological characterization predicted accurately the neuronal type. Overall intrinsic membrane properties of area CA1 interneurons did not change in epileptic tissue, and the percentage of cells exhibiting APs also was not altered in control versus post-SSLSE tissue. These findings are consistent with other studies that found no change in intrinsic membrane properties of basket cells in area CA1 in the kainic acid model of TLE (Nakajima et al. 1991). These data suggest that alterations in basic cellular properties of area CA1 interneurons are not requisite for the expression of the epileptic state seen in models of TLE.
the methodology used in the study. We also thank L. Edwards for technical
Disorders and Stroke Grant NS-25605. L OTHMAN, E. W., BERTRAM, E. H., KAPUR, J., AND STRINGER, J. Recurrent
A1 radiatum-L/M (A1 and B1) and stratum oriens (A2 and B2) interneurons. Effect reversed after rinse (a = ACSF; b = CNQX/D-APV; c = after rinse in ACSF).

Summary

By using whole cell recordings of interneurons in adult brain slices in vitro, this report examined the functional connectivity of interneurons to their excitatory synaptic input in a chronic model of TLE. Excitatory synaptic input remained functional to interneurons with both single and paired stimulation and spontaneous excitatory input to interneurons remains intact in the chronically epileptic tissue. We therefore conclude that the apparent loss of IPSPs in CA1 pyramidal cells in epileptic tissue occurs somewhere other than the Schaffer-collateral to interneuron synapse.

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