Quantified patterns of mossy fiber sprouting and neuron densities in hippocampal and lesional seizures

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Quantified hippocampal mossy fiber synaptic reorganization and neuron losses were measured to determine the pathological features associated with epileptogenic fascia dentata. Twenty-five patients with temporal lobe epilepsy (TLE) were classified as having either mesial temporal sclerosis (MTS; 16 patients), with seizure genesis in the hippocampus, or temporal mass lesions (nine patients), with seizures that were probably extrahippocampal. Neo-Timm’s histochemistry identified mossy fiber sprouting, and aberrant fascia dentata puncta densities were objectively measured by light microscopic analysis on an image-analysis computer. Neuron densities determined cell losses and the two seizure groups were compared to control specimens obtained from autopsies. Results showed significantly greater fascia dentata mossy fiber puncta densities and neuron losses in TLE patients compared to autopsy specimens (p < 0.026). Furthermore, there were significant differences between the two seizure groups: 1) mossy fiber puncta densities in the inner molecular layer were significantly greater in MTS compared to lesions (p < 0.02), and 2) mossy fiber puncta densities were greater in the inner molecular layer than in the stratum granulosum in 14 of 16 MTS patients (88%) compared to four of nine patients with lesions (44%, p < 0.01). Neuron densities were significantly different comparing MTS, lesion and control groups for stratum granulosum (p = 0.0001) and Ammon’s horn (p = 0.0001), with each group significantly different (p < 0.05) compared to another. All patients were either seizure-free or significantly improved 1 year or more after en bloc temporal lobectomy. There were no significant correlations between fascia dentata mossy fiber puncta densities and counts of hilar neurons, CA4 pyramids, granule cells, or years of seizures. This indicates that inner molecular layer mossy fiber puncta densities and neuron losses are greater in patients with MTS than in those with lesions, and mossy fiber sprouting probably contributes to the pathophysiology of hippocampal seizures. Furthermore, these data show that some patients with extrahippocampal lesions have mossy fiber sprouting similar to MTS patients, suggesting that hippocampi in lesion patients may be capable of epileptogenesis from synaptic reorganization.

KEY WORDS • seizures • pathology • synaptic reorganization • intractable epilepsy • tumor • hippocampus

THE association of hippocampal damage with temporal lobe seizures has a well-established history in the literature. It was first reported by Bouchet and Cazauvielh and confirmed in postmortem studies of chronic epileptics by Sommer and others. Furthermore, in surgical studies of patients with temporal lobe epilepsy (TLE), the most frequent pathological finding has been severe hippocampal damage, termed mesial temporal sclerosis (MTS), and the next most common finding has been temporal mass lesions. Electrophysiological studies with depth macroelectrodes and microelectrodes have shown that in MTS the region of seizure onset is the damaged hippocampus, and patients who demonstrate MTS or temporal lesions in their pathological specimens have better seizure outcomes than those without either finding.

These pathological findings present a conceptual paradox. The hippocampus in MTS has been shown to be the region of severest neuronal losses and the zone of epileptogenesis. How a damaged hippocampus could generate synchronized neuronal hyperexcitability remained unclear until the relatively recent discovery of axon and synaptic reorganization. One axon system of special interest has been the excitatory mossy fibers of the granule cells. Animal studies had shown mossy fibers were capable of dense sprouting and reinnervation onto granule cells following hippocampal injury, and the same pattern of aberrant mossy fiber sprouting has been found in adult humans with epilepsy. Sprouted mossy fibers in animal studies have been associated with neuronal hyperexcitability and spontaneous chronic hippocampal seizures. This has led to the hypothesis that reorganized mossy fibers form a monosynaptic excitatory feedback circuit on granule cells that contributes to local circuit hyperexcitability and chronic seizures.

This hypothesis has been criticized by several investigators who have noted that damaged epileptic hippocampi show preservation and sprouting of inhibitory neurons and.
seizures. Hence, it is suggested that mossy fiber reactive hippocampi of two elderly individuals with no history of compared to those with temporal mass lesions, and some be variable from one case of MTS to the next. Also, it is thermore, critics note that mossy fiber sprouting seems to er reason for synchronized epileptic discharges. Fur- EEG telemetry (Phase II). After localization of the sei-

tution using a standardized protocol approved by the institution’s Human Subject Protection Committee. Initial evaluation (Phase I) included a detailed history and neurological examination, interictal and ictal scalp electroencephalography (EEG) using sphenoidal electrodes, structural imaging with magnetic resonance (MR) imaging, functional imaging with 18-fluoro-2-deoxyglucose positron emission tomography, an extensive neuropsychological test battery, and intracarotid sodium amobarbital injections (Wada test) for memory and speech representa-
tion. If this failed to lateralize and localize the epileptic region, patients underwent intracranial depth electrode EEG telemetry (Phase II). After localization of the sei-

urse focus to one anterior temporal lobe, a standard en bloc resection including 3 to 4 cm of the hippocampus was performed (Phase III). The lateral temporal neocortex was serially sectioned and microscopically examined by the neuropathologist. The hippocampus was evaluated as described below.

Each patient’s follow-up data was obtained using a standardized format abstracted from the medical record and research files. This information was collected independently of the clinical and pathological information. Outcome was classified based on the incidence of seizures for the most recent 12-month period. The number of years since surgery was also recorded.

Clinical Material and Methods

Patient Selection

Patients with intractable complex partial seizures of probable temporal lobe origin were evaluated at our institution using a standardized protocol approved by the institution’s Human Subject Protection Committee. Initial evaluation (Phase I) included a detailed history and neurological examination, interictal and ictal scalp electroencephalography (EEG) using sphenoidal electrodes, structural imaging with magnetic resonance (MR) imaging, functional imaging with 18-fluoro-2-deoxyglucose positron emission tomography, an extensive neuropsychological test battery, and intracarotid sodium amobarbital injections (Wada test) for memory and speech representation. If this failed to lateralize and localize the epileptic region, patients underwent intracranial depth electrode EEG telemetry (Phase II). After localization of the seizure focus to one anterior temporal lobe, a standard en bloc resection including 3 to 4 cm of the hippocampus was performed (Phase III). The lateral temporal neocortex was serially sectioned and microscopically examined by the neuropathologist. The hippocampus was evaluated as described below.

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Cell Counts

Sections adjacent to the neo-Timm’s processed slides were stained with cresylecht violet for histopathological review (30 μm thick) and cell counts (10 μm thick). Counts were at × 400 using grid morphometric techniques, and the hippocampal subfields counted were based on the classification of Lorente de Nó (Fig. 1A). The subfields were granule cells of the stratum granulo-
sum and hilar neurons of the fascia dentata, and CA4, CA3, CA2, and CA1 stratum pyramidal and prosubiculum

Hippocampal specimens consecutively collected from 1988 to 1992 and investigated with Timm’s histochem-

Study Design

Hippocampal specimens consecutively collected from 1988 to 1992 and investigated with Timm’s histochemistry and neuron density measurements of good quality were the basis of this study. As described by Wieser, et al., patients were grouped based on the clinical, pathological, and neuroimaging studies into those with MTS or extra-hippocampal mass lesions. The MTS group consist-
ed of patients without mass lesions, with a childhood history of seizures, and with the hippocampus as the probable source of seizures. Lesion patients had a temporal lobe macroscopic mass identified by neuroimaging and confirmed pathologically. The hippocampus may or may not have been epileptogenic. Lesions included low-grade astrocytoma, macroscopic cortical dysplasia, dysembry-onic neuroepithelial tumor, anaplastic astrocytoma, and epidermoid tumor. Microscopic heterotopias and hamar-tomas were not considered to be temporal neocortical epileptic lesions. Of the 96 temporal resection during this time, a total of 35 hippocampal specimens were studied with neo-Timm’s procedures. Six were excluded because of significant tissue damage from surgery or use of an old Timm’s method. Of the remaining cases, 25 met the criteria for inclusion into the MTS group (16 patients) or the lesion group (nine patients).

Surgical Collection and Processing

Hippocampal specimens were collected in the operating room and a 1-cm block was placed in neo-Timm’s fixative (0.1% sodium sulfide in Millonig’s buffered 4% gluta
taraldehyde). From this single block two sets of 30-μm sections (total of six adjacent sections spanning approximately 200 μm) were processed for Timm’s histochemistry, one set lightly and the other darkly developed, to confirm that the staining was specific for mossy fi-

bers, Development time for light sections was 50 to 60 minutes and for dark sections 70 to 80 minutes. The collection, processing, and development was regimented and uniform between surgical cases, similar to previous studies. The histochemical staining patterns for the six sections in each patient were essentially identical.

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the hilar neurons (see Fig. 1A). The counts were performed by one person (J.K.P.), who was blinded to other collected data.

Human control comparison tissue consisted of autopsy specimens from people of comparable ages without cerebral pathology, processed in the same manner as the surgical material and collected within a few hours after death prior to autolysis. Previous studies have found that autopsy controls as used in this study do not introduce artifacts that affect human neuron density measurements or neo-Timm’s staining, and normal hippocampal cell densities are stable from age 2 to age 55 years.

**Neo-Timm’s Quantification**

The Timm’s process consists of a zinc–sulfide–silver complex similar to a photographic print in the axons and terminals of mossy fibers. The major variable from one case to the next was the size of each silver deposit, which depends on the time of development. An image-analysis computer was programmed to estimate the number and density of puncta, controlling for the variation in size of individual puncta. This analysis was performed by a researcher blinded to the TLE type. The Timm’s sections were from a narrow region of the hippocampus, the staining was essentially identical between adjacent sections, and hence any slide represented a single sample of mossy fiber sprouting from each patient. One of the three darkly developed slides that had a representative histochemical reaction with the fewest artifacts was imaged using a black and white charge-coupled device camera attached to a microscope and interfaced with an image-analysis computer. For a single field of view, several focal planes were digitally integrated to image the depth of the 30-μm Timm’s sections. The microscopic regions imaged were the fascia dentata infragranular hilus, the stratum granulosum, and the inner molecular layer, with a single image covering approximately 10,000 μm², representing a magnification of ×1620 on the video monitor. The video image was sufficient to encompass the entire width of the stratum granulosum or inner molecular layer. For each specimen, seven to 10 isolated black Timm’s single puncta were visually identified by the computer operator, and their area measured and averaged. As a measure of the uniformity of the histochemical technique we compared the single silver puncta size for all specimens. It ranged between 0.177 and 0.265 μm² (mean 0.22 μm²). The computer considered puncta clusters two or more times larger than a single punctum to be multiples of the basic unit. The image-analysis computer divided these clusters to obtain the approximate number of overlapping puncta within clusters, and the density of puncta per unit area was determined. A total of 10 images per slide that sampled the majority of the upper and lower stratum granulosum blades was quantified and the results averaged. Each specimen was processed in a uniform manner but they were not batch-processed. To further control for variability from differences in Timm’s development, a calculated measure compared the ratio of puncta between two adjacent regions (inner molecular layer and stratum granulosum). This was a relative measure of the difference in mossy fiber puncta between these two regions of the fascia dentata.

**Data Analysis**

Data were entered into a database on a personal computer and analyzed using a statistical program. Differences between the seizure groups and autopsy specimens were statistically compared using an analysis of variance (ANOVA) and further compared between individual groups (at p < 0.05) using the Games–Howell test that controls for multiple comparisons of unequally sized samples and of unassumed variances. Other statistical

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*Microscope and IBAS image-analysis computer manufactured by Carl Zeiss, Thornwood, New York.

† Super ANOVA Version 1.1 obtained from Abacus Concepts, Inc., Berkeley, California.
tests included Student’s t-test and chi-square test. Results were plotted using a commercially available software program.

Results

Clinical Profiles

The 16 MTS patients had clinical histories of an initial precipitating injury (for example, meningitis, trauma, or multiple febrile seizures) prior to the onset of chronic TLE. The nine patients with lesions did not have initial precipitating injuries and chronic seizures began de novo. The clinical features of both groups are summarized in Table 1; there were no statistically significant differences between the two groups.

Aberrant Fascia Dentata Mossy Fiber Sprouting

Qualitatively and quantitatively, both sclerotic and lesioned hippocampi showed, by Timm’s histochemistry, aberrant mossy fiber staining and significant differences in puncta densities for the stratum granulosum or inner molecular layer that were not observed in autopsy specimens. Figure 1 shows at low magnification the typical patterns of Nissl and Timm’s staining in MTS. Figure 2 shows that qualitatively and quantitatively mossy fiber puncta densities varied between patients. A control section is shown in Fig. 2A. Some lesion patients had mossy fiber puncta limited to the stratum granulosum (Fig. 2B), other hippocampi showed nearly equal amounts in the stratum granulosum and inner molecular layer (Fig. 2C), and in MTS patients there were significantly greater mossy fiber puncta in the inner molecular layer compared to the stratum granulosum (Fig. 2D). The quantitative computer-generated mossy fiber puncta densities verified the qualitative light microscopic assessments in individual specimens.

Comparing sections from the seizure groups with autopsy specimens showed that mossy fiber puncta densities were significantly different in the stratum granulosum (ANOVA; $F = 4.1; p = 0.026$) and inner molecular layer ($F = 5.2; p = 0.012$). The stratum granulosum mossy fiber puncta counts were significantly greater ($p < 0.05$; Games–Howell) for MTS and lesion compared to autopsy specimens, and in the inner molecular layer puncta counts were greater for MTS compared to autopsy specimens. Comparing the two seizure groups, excluding autopsy cases, also showed significant differences (Table 2). The inner molecular layer mossy fiber puncta counts were greater in MTS compared to lesion groups ($p < 0.02$) without correction for any granule cell loss. Correcting for granule cell loss showed an even greater difference ($p < 0.01$). The inner molecular layer mossy fiber puncta appeared to be slightly but not significantly greater in lesion patients compared to MTS patients. Correction for granule cell loss showed no significant differences between MTS and lesion patients. The ratios of inner molecular layer to stratum granulosum puncta counts (IML/SG) were significantly different between MTS and lesion groups ($p < 0.01$; see Table 2). Hence, patients with MTS showed more inner molecular layer mossy fiber puncta compared to the stratum granulosum (ratio 2.77:1), whereas lesion patients had mossy fiber puncta in the stratum granulosum (ratio 0.24:1). The anteroposterior location of the hippocampal sections did not influence the mossy fiber puncta densities in the stratum granulosum ($F = 1.9; p = 0.19$), inner molecular layer ($F = 0.15; p = 0.86$), or the IML/SG ratios ($F = 0.04; p = 0.96$).

The inner molecular layer mossy fiber puncta densities and IML/SG ratios for the seizure groups are shown in Fig. 3. In MTS patients, 14 of 16 hippocampi (88%) showed more mossy fiber puncta in the inner molecular layer compared to the stratum granulosum (IML/SG > 1), whereas five of the nine lesion patients (56%) showed greater mossy fiber puncta in the stratum granulosum compared to the inner molecular layer (IML/SG < 1). Figure 3 also shows the biological variability within the two seizure groups. For example, four of the nine lesion patients showed inner molecular layer densities and IML/SG ratios similar to the MTS group. Averaged

### Table 1

<table>
<thead>
<tr>
<th>Seizure Etiology</th>
<th>Males:Females</th>
<th>Age at Surgery (yr)*</th>
<th>Time Since Onset of TLE (yr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mesial temporal sclerosis</td>
<td>8:8</td>
<td>30.7 ± 2.1</td>
<td>17.6 ± 2.1</td>
</tr>
<tr>
<td>lesion†</td>
<td>2:7</td>
<td>27.4 ± 2.9</td>
<td>15.1 ± 2.8</td>
</tr>
<tr>
<td>significance</td>
<td>p &lt; 0.3</td>
<td>p = 0.32</td>
<td>p = 0.72</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± standard error of the mean.
† Lesions: Low-grade astrocytoma in four patients; macroscopic cortical dysplasia in two; one dysplastic neuroepithelial tumor, one anaplastic astrocytoma, and one epidermoid tumor.

### Table 2

<table>
<thead>
<tr>
<th>Specimen Group*</th>
<th>No. of Puncta/10 μm²†</th>
<th>IML:SG Ratio</th>
</tr>
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<tbody>
<tr>
<td>autopsy (controls)</td>
<td>0.31 ± 0.1</td>
<td>0.70 ± 0.3</td>
</tr>
<tr>
<td>MTS</td>
<td>15.31 ± 2.6‡</td>
<td>3.82 ± 0.8‡</td>
</tr>
<tr>
<td>lesion</td>
<td>7.01 ± 2.9</td>
<td>6.73 ± 1.6</td>
</tr>
<tr>
<td>MTS:lesion ratio = 2.18</td>
<td>MTS:lesion ratio = 0.57</td>
<td></td>
</tr>
<tr>
<td>MTS</td>
<td>43.79 ± 9.4‡</td>
<td>10.5 ± 2.4‡</td>
</tr>
<tr>
<td>corrected lesion</td>
<td>7.56 ± 3.1</td>
<td>8.64 ± 2.3</td>
</tr>
</tbody>
</table>

* MTS = mesial temporal sclerosis; correction is for number of remaining granule cells as an estimate of available dendritic sites for mossy fibers; the value expressed is the density of mossy fiber puncta per granule cell (that is, puncta/10 sq μm/granule cell).
† Data are presented as mean ± standard error of the mean.
‡ p < 0.02 for MTS compared to lesion.
§ p < 0.01 for MTS compared to lesion.
∥ p = not significant.
Ammon’s horn neuron losses (CA4 to prosubiculum; see Fig. 4) in the four lesion patients with greater inner molecular layer mossy fiber sprouting were not different than the counts in the five patients without inner molecular layer mossy fiber sprouting (23.5% and 20.3%, respectively; p = 0.43). This would indicate that hippocampi from patients with lesion may show mossy fiber sprouting patterns similar to MTS patients whose hippocampi are capable of generating seizures.

Hippocampal Neuron Losses

The neuron densities for the seizure groups compared to autopsy control specimens showed significant differences in all subfields except subiculum (p < 0.02), as shown in Fig. 4. The averaged Ammon’s horn neuron losses for autopsy (19,632 ± 1254), lesion (14,755 ± 408), and MTS (7346 ± 691) specimens were significantly different (ANOVA; F = 47.5; p = 0.0001), with each group significantly different (p < 0.05; Games–Howell) compared to any other group. Compared to autopsy specimens, lesion patients averaged 19% to 26% neuron loss, and MTS patients averaged 46% to 79%. Neuron counts in MTS patients were significantly less (p = 0.05) than in autopsy specimens for all subfields except subiculum, and significantly less than in lesion patients in the stratum granulosum, CA3, CA2, CA1, and prosubiculum.

Denervation-Reinnervation Analysis

Correlation analyses compared the stratum granulosum and inner molecular layer mossy fiber puncta densities with the neuron counts of hilar neurons, CA4 pyramids (estimate of denervation of the fascia dentata) and/or amounts of granule cells (estimate of available dendrites and cell bodies for reinnervation). No significant correlations (r values between 0.001 and 0.143) were found. Similar analyses of comparisons within MTS or lesion groups found no significant correlations. Lastly, no correlation was found between the years of clinical seizures and fascia dentata mossy fiber puncta densities (r values between 0.002 and 0.279).
Fig. 3. Graph comparing the density of inner molecular layer (IML) mossy fiber puncta against the ratio of puncta in the IML and stratum granulosum (SG) for 25 patients with mesial temporal sclerosis (MTS; closed circles) or lesions (open triangles). Note that the IML/SG ratio scale is logarithmic, and IML/SG ratios less than 1 (below the dashed line) indicate more puncta in the SG than in the IML, whereas above the dashed line there are more puncta in the IML compared to the SG. Patients with MTS whose seizures began in the mesial temporal region showed greater amounts of IML mossy fiber puncta and higher IML/SG ratios than lesion patients. Within the lesion group, four of the nine patients had IML mossy fiber puncta and higher IML/SG ratios than lesion patients. The three patients who continued to have seizures without seizures.

Discussion

Hippocampi from TLE patients showed quantifiable and statistically significant differences in fascia dentata mossy fiber puncta densities and neuron counts compared to autopsy controls. There were also significant differences in the same measures between patients with MTS and lesions. Compared to lesion patients, MTS patients had significantly greater inner molecular layer mossy fiber puncta, larger ratios of mossy fiber puncta in the inner molecular layer compared to the stratum granulsum, and greater neuron losses in Ammon’s horn and stratum granulsum. Mesial temporal sclerosis and lesion patients had similar seizure outcomes, supporting the notion that the temporal lobe pathologies were the seizure generators. No significant correlations were found that would explain the densities of fascia dentata mossy fiber puncta when compared to the amount of hilar neuron or CA4 pyramid loss as an estimate of inner molecular layer denervation, granule cell densities as an estimate of dendritic and somatic synaptic sites available for reinnervation, or the number of years of chronic seizures. Hence, this study did not support the hypothesis that mossy fiber sprouting was a simple reactive consequence of hippocampal neuron damage with denervation-reinnervation of the fascia dentata. This study does support the hypothesis that inner molecular layer mossy fiber sprouting and the ratio of inner molecular layer to stratum granulsum puncta densities were significantly associated with MTS, and probably contribute to the pathophysiology of epilepsy in the sclerotic hippocampus. Furthermore, this study

Seizure Outcome

Information on postoperative seizure control was available in 21 of the 25 patients, as summarized in Table 3. One patient from each group was lost to follow-up review; one patient in the MTS group died 4 weeks postoperatively for unknown reasons (autopsy performed), and one patient from the lesion group was less than 1 year from surgery at last follow-up examination. Both MTS and lesion patients had excellent seizure outcomes in 86% of cases. The three patients who continued to have seizures showed no differences in mossy fiber fascia dentata puncta densities and neuron losses compared to the 18 patients without seizures.

Fig. 4. Bar graph illustrating the differences in neuron densities between patients with mesial temporal sclerosis (MTS) and lesions compared to control specimens obtained at autopsy. The mean (± standard error of the mean) stratum granulsum (SG) densities (neurons per cubic millimeter) for control (289,422 ± 16,072), lesion (192,900 ± 22,408), and MTS (116,700 ± 10,289) groups were significantly different (analysis of variance; F = 24.9; p = 0.0001), with each group significantly different compared to any other group (⁎⁎ p < 0.05; Games–Howell). The hilar counts for control (3582 ± 266), lesion (2246 ± 376), and MTS (1311 ± 388) groups were significantly different (F = 10.5; p = 0.0003), with MTS significantly less than control (⁎⁎ p < 0.05). The CA4 counts for control (11,938 ± 1528), lesion (9421 ± 773), and MTS (7207 ± 784) groups were significantly different (F = 4.5; p = 0.021), with MTS significantly less than control (⁎ p < 0.05). The CA3 counts for control (21,792 ± 947), lesion (16107 ± 568), and MTS (10,891 ± 1116) groups were significantly different (F = 12.8; p = 0.0001), with each group significantly different compared to any other group (⁎⁎ p < 0.05). The CA2 counts for control (21,224 ± 1198), lesion (18,192 ± 1637), and MTS (11,078 ± 1386) groups were significantly different (F = 9.5; p = 0.0014), with MTS significantly less compared to lesion and control groups (⁎⁎ p < 0.05). The CA1 counts for control (22,929 ± 1733), lesion (17,434 ± 1149), and MTS (4223 ± 516) groups were significantly different (F = 129; p = 0.0001), with MTS significantly less than lesion and control groups (⁎⁎ p < 0.05). The prosubiculum (PRO) counts for control (20,276 ± 1814), lesion (16,044 ± 1798), and MTS (5401 ± 1063) groups were significantly different (F = 27.6; p = 0.0001), with MTS significantly less than lesion and control groups (⁎⁎ p < 0.05). The subiculum (SUB) counts for control (13,833 ± 1528), lesion (14,023 ± 1215), and MTS (14,618 ± 890) groups were not significantly different (F = 1.8; p = 0.88).
showed that some lesion patients had patterns of mossy fiber sprouting similar to MTS patients and suggests that these hippocampi may be epileptogenic. It should be emphasized that a retrospective study can only infer a causal relationship between mossy fiber sprouting and epileptogenesis. Yet, our robust associations strongly favor our conclusions.

The validity of this study’s findings and conclusions were based on the quantified differences in mossy fiber puncta densities as assessed by light microscopy. It is important to understand the limitations of this method and not to confuse mossy fiber puncta densities with the number of mossy fiber synaptic terminals. Electron microscopy sampling five neo-Timm’s–stained human MTS hippocampi has previously shown that multiple zinc deposits were present in the vesicles and free intervesicular spaces of aberrant mossy fiber synaptic terminals. The average size of mossy fiber synaptic terminal “diameters” (not the individual silver granules) was between 1.3 and 2.3 μm, and several zinc–silver complexes were present in the vesicles and free intervesicular spaces of aberrant mossy fiber synaptic terminals. The average size of mossy fiber synaptic terminal “diameters” (not the individual silver granules) was between 1.3 and 2.3 μm, and several zinc–silver complexes were present in the vesicles and free intervesicular spaces of aberrant mossy fiber synaptic terminals. The average size of mossy fiber synaptic terminal “diameters” (not the individual silver granules) was between 1.3 and 2.3 μm, and several zinc–silver complexes were present in the vesicles and free intervesicular spaces of aberrant mossy fiber synaptic terminals.

The present study does support another important concept. Our data suggest that the mechanisms of aberrant mossy fiber sprouting and hippocampal epileptogenesis were probably different between the two TLE groups. One could still argue that fascia dentata mossy fiber puncta densities were only a reflection of hippocampal damage, because the data did show that as a group MTS patients showed the greatest amount of fascia dentata mossy fiber sprouting and neuron losses. However, if mossy fiber–reactive synaptogenesis was a simple compensatory mechanism to reorganize axons after neuron damage, then the amount of individual fascia dentata mossy fiber sprouting should correlate with neuron counts. It is possible that the developmental age and manner of hippocampal injury influence the amount of synaptic reorganization. In support of this idea, recent animal experiments have shown that if the hippocampus was selectively injured with a direct injection of kainate in either adult or developing animals, there were progressive increases of mossy fiber sprouting over several months. During a latent period, inner molecular layer mossy fiber sprouting gradually increased, neuron densities in regions receiving aberrant mossy fiber synapses gradually decreased, and no in vivo evidence of hippocampal seizures was recorded with depth EEG. Only after animals showed robust inner molecular layer sprouting were chronic spontaneous hippocampal seizures recorded. This animal model would be similar to patients with MTS. Kindled animals have shown less hippocampal mossy fiber sprouting, which is a model that may be more like the lesion patients in this study. Furthermore, surgical studies of children have shown hippocampal mossy fiber sprouting with intractable extrahippocampal seizures without profound neuron loss, suggesting that mossy fiber sprouting may be age dependent.

These data suggest that mossy fiber reactive synaptogenesis may not be a single compensation to reinnervate the damaged brain operating under a single principle. Instead, hippocampal mossy fiber sprouting may be regulated by several molecular mechanism that are influenced.
by the patient’s developmental age and dependent on the manner of injury. For example, studies have supported the importance of astrocytes in the initiation of reactive synaptogenesis. Further, proteins such as neurotrophic factors may play a role in the initiation and/or regulation of growth cones. Neurotrophic factors are expressed at high levels in the hippocampus, and there are differential expressions of different factors during development. Seizures and neuron injury alter the acute and chronic expression of neurotrophic factors. It is not known if neurotrophic factors control aberrant mossy fiber sprouting, and further studies will be necessary to understand the interplay and expression of astrocytes and molecular proteins such as neurotrophic factors in relation to aberrant mossy fiber sprouting.

Conclusions

This study has demonstrated that patients with TLE have significant increases in mossy fiber sprouting and neuron loss compared to control autopsy specimens. Furthermore, patients with MTS have different patterns of aberrant fascia dentata mossy fiber sprouting and hippocampal neuron loss than patients with temporal lobe lesions. This suggests that mossy fiber sprouting may be critical in the pathophysiology of seizures in MTS and may be important in the propagation of secondarily epileptogenic hippocampal seizures in some patients with temporal mass lesions.

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References

27. Feldblum S, Ackermann RF: Increased susceptibility to hippocampal and amygdala kindling following intrahippocampal kainic acid. Exp Neurol 97: 255–269, 1987

G. W. Mathern, J. K. Pretorius, and T. L. Babb
Pathology in hippocampal epilepsy


44. Mouritzen Dam A: Epilepsy and neuron loss in the hippocampus. Epilepsia 21:617–629, 1980


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