

## Hippocampal Cell Distributions in Temporal Lobe Epilepsy: A Comparison Between Patients With and Without an Early Risk Factor

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**Summary:** Neuronal cell distributions were measured for anterior and posterior locations in the hippocampi of epilepsy patients who were seizure-free after temporal lobectomy. Patients were divided into two groups, those with an early risk factor, defined as a neurologic insult occurring in the first 4 years of life, and those with no early risk factor. Early-risk patients had lower hilar cell densities, lower granule cell densities, and fewer granule cells per millimeter, a measure related to total granule cell number, than no early risk patients. Moreover, each risk group had different anteroposterior density gradients for granule cells and hilar cells. These differences in cell distribution may arise from different patterns of cell loss or cell migration in the dentate gyrus during development. In the early-risk group, there was also a distinction between patients

with a history of febrile convulsions without CNS infection and patients with a history of meningitis or encephalitis. These two subgroups had similar numbers of granule cells. However, the meningitis/encephalitis subgroup exhibited a wider granule cell layer, suggesting that the granule cell layer was more dispersed. Our results support the hypothesis of a predominantly anterior hippocampal insult in temporal lobe epilepsy (TLE). In nonepileptic hippocampus, the ratio of putatively excitatory granule neurons to putatively inhibitory hilar neurons is highest in the anterior hippocampus. This ratio may explain in part why the anterior hippocampus is more prone to cell loss and seizures. **Key Words:** Cell loss—Epilepsy—Fascia dentata—Hilus—Hippocampus—Neuropathology.

Patients with intractable temporal lobe epilepsy (TLE) with an early risk factor, defined as a neurologic insult sustained in the first 4 years of life, are more likely than patients with no early risk factor to have a seizure-free outcome after temporal lobectomy (1-4). These early neurologic insults, which include prolonged febrile convulsions and convulsions associated with meningitis or encephalitis, are believed to produce particularly severe focal hippocampal lesions because they occur while development of the hippocampal formation is ongoing (5,6).

Conversely, epileptogenic tissue in patients with no early risk factor may be distributed more diffusely, i.e., beyond the posterior line of hippocampal resection or to unresected neocortical temporal lobe. Therefore, it is suspected that better seizure control is obtained in these early-risk patients be-

cause a focal hippocampal lesion is more easily excised than a diffuse or multifocal lesion (1-3,7-9).

Two measures of hippocampal atrophy have indicated that early-risk patients do indeed have a more severe hippocampal lesion than patients with no early risk factor. First, magnetic resonance imaging (MRI) volumetric measurements of the gross hippocampal formation have shown correlations between unilaterally reduced hippocampal volumes and either early neurologic insults or successful clinical outcome (10,11). Second, determinations of cell densities for specific hippocampal subfields have shown a correlation between an early risk factor and mesial temporal sclerosis (12-14).

The extent of cell loss in the epileptic hippocampus appears to display regional differences; e.g., decreased hippocampal cell densities, especially in the anterior hippocampal formation, have been described in a study comparing hippocampal cell populations in epileptic and nonepileptic autopsy groups (15). Furthermore, we demonstrated previously that TLE patients have fewer granule cells and lower

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TABLE 1. Patient information

| Risk factor               | No early risk<br>(n = 11) | Early risk<br>(n = 17) | Febrile convulsion<br>(n = 11) | Meningitis encephalitis<br>(n = 6) |
|---------------------------|---------------------------|------------------------|--------------------------------|------------------------------------|
| Sex                       | 6 M/5 F                   | 9 M/8 F                | 4 M/7 F                        | 5 M/1 F                            |
| Side of operation         | 8 R/3 L                   | 9 R/8 L                | 6 R/5 L                        | 3 R/3 L                            |
| Age at seizure onset      | 21.2 ± 3.2                | 8.9 ± 2.0 <sup>a</sup> | 10.0 ± 2.9 <sup>a</sup>        | 6.8 ± 2.7 <sup>a</sup>             |
| Age at time of operation  | 37.6 ± 3.5                | 30.7 ± 8.8             | 32.5 ± 2.6                     | 27.5 ± 4.1                         |
| Duration of epilepsy (yr) | 16.5 ± 2.9                | 22.1 ± 2.7             | 23.0 ± 3.2                     | 20.5 ± 6.1                         |
| No. of seizures           | 2,942 ± 1,147             | 1,221 ± 242            | 1,429 ± 365                    | 842 ± 185 <sup>a</sup>             |
| Auras                     | 8 Yes/1 No                | 14 Yes/2 No            | 9 Yes/1 No                     | 5 Yes/1 No                         |
| Tonic clonic seizures     | 5 Yes/6 No                | 11 Yes/6 No            | 7 Yes/4 No                     | 4 Yes/2 No                         |
| Full-Scale I.Q.           | 100.4 ± 14.3              | 90.5 ± 3.7             | 93.4 ± 5.3                     | 85.7 ± 5.6                         |

Patients were divided into two groups, early risk and no early risk. The early-risk group was further divided into two subsets: patients with a history of febrile convulsions without CNS infection and patients with a history of meningitis or encephalitis. Duration of epilepsy includes all ages and duration in years; total number of seizures is estimated.

Numerical values are mean ± SEM.

<sup>a</sup>  $p < 0.05$  statistically significant difference from the no-early-risk group (Student's *t* test).

hilar cell densities anteriorly than posteriorly in pairs of epileptic hippocampal biopsy specimens (16). These studies suggest that further investigations of hippocampal cell densities should include a comparative analysis at several locations in the hippocampus.

In addition, a distinction has been drawn between low granule cell density, which is believed to reflect cell loss, and granule cell dispersion, which may reflect an altered pattern of granule cell migration in the dentate gyrus during development (6). In Houser's study, granule cell dispersion was characterized histologically by a wider granule cell layer whose borders with the hilus and molecular layer were more irregular than those in nonepileptic tissue. Frequently, the granule cells had bipolar shapes and were arranged in columns. This pattern of granule cell dispersion was suggested to be associated with a history of severe febrile seizures or seizures associated with meningitis or encephalitis in the first 4 years of life (6).

Thus, clinical and pathological evidence suggests that early-risk and no-early-risk patients may indeed constitute two distinct patient populations. The purpose of the present study was to identify any antero-posterior differences in neuronal cell distribution in the dentate gyrus and hilus between the early-risk and no-early-risk groups and to determine whether low cell density or cell dispersion was more closely associated with either group. We examined both cell density and cell number measures to make a more complete assessment of neuronal distribution. Our results suggest that differences may exist in the patterns of granule and hilar cell loss between the early-risk and no-early-risk groups and, furthermore, between two subsets in the early-risk group: patients with a history of febrile convulsions without evidence of CNS infection and patients with a history of meningitis or encephalitis in the first 4 years of life.

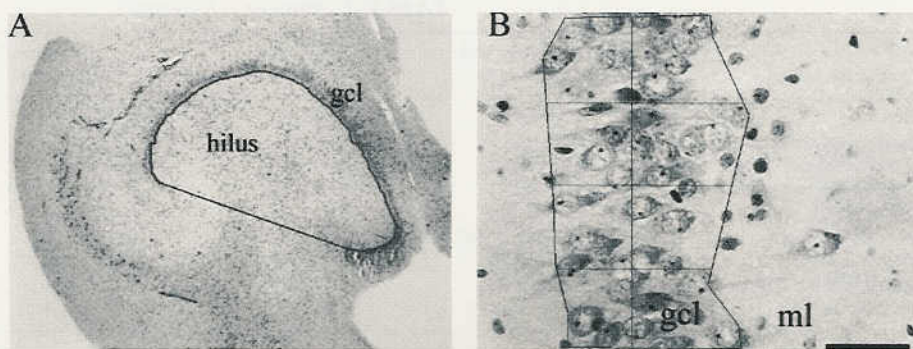
## MATERIALS AND METHODS

### Patients

All patients underwent therapeutic temporal lobectomy for control of medically intractable seizures according to the protocol of the Graduate Hospital Comprehensive Epilepsy Center (17). Table 1 summarizes some of the clinical characteristics of the patient population. There were 17 early-risk and 11 no-early-risk patients. All early-risk patients had a history of either febrile convulsions ( $n = 11$ ) or a history of meningitis ( $n = 2$ ) or encephalitis ( $n = 4$ ) in the first 4 years of life. Two patients from the meningitis/encephalitis group had seizures at the time of CNS infection: 1 patient with meningitis had a 30-min seizure, and 1 patient with encephalitis had a 2-h febrile convulsion. None of the patients in the no-early-risk group had a history of neurologic insult before age 8 years. Patients with tumors, as determined by computed tomography (CT) or MRI and subsequent pathological examination of resected tissue, were excluded from the study. The age of recurrent seizure onset was significantly lower for early-risk patients ( $p < 0.05$ , Student's *t* test). There was no statistically significant difference between risk groups in the mean duration of epilepsy, which was defined as the length of time between seizure onset and surgical treatment. However, on the average, the early-risk group had a lower estimate than the no-early-risk group for total number of seizures. This calculation of number of seizures is only a best estimate derived from recollections of patients or their family members and does not account for the possibility of subclinical seizures before seizure onset. One patient in this study, an early-risk patient who had experienced one prolonged febrile seizure at age 6 months, had one episode of status epilepticus.

All patients were seizure-free for a mean follow-up  $> 2$  years. A pure group of seizure-free patients

**FIG. 1.** Digital photomicrographs of cresyl violet-stained hippocampal sections showing the method of defining and measuring the area of the hilus and granule cell layer. **A:** Hilus area (hilus) is enclosed by the line following the inner border of the granule cell layer (gcl) and connecting the upper and lower blades of the gcl. **B:** gcl is enclosed by the line drawn along the borders of the gcl and across the top and bottom of the monitor screen. Granule cells that lay within the molecular layer (ml)



were not included in cell counts or area measurement. Width of the gcl was determined by drawing five horizontal lines across the gcl in each microscopic section and calculating the average for all widths in all sections. Calibration in A 1 mm; calibration in B 100  $\mu\text{m}$ .

was specifically selected to ensure that the tissue initiating or mediating the seizures had been removed.

#### Histopathological examination

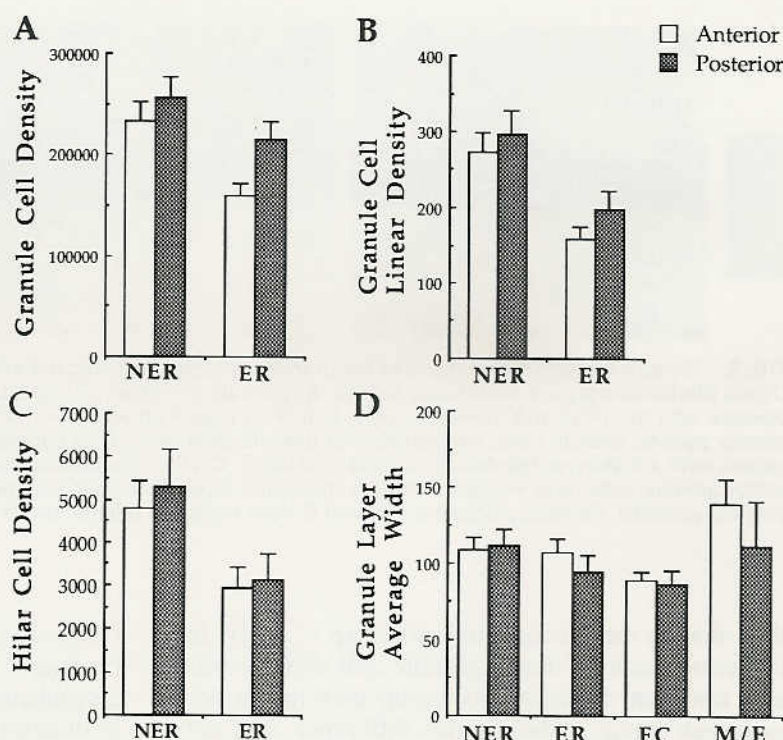
Hippocampal specimens were received in the operating room and immediately placed in ice-cold, low  $\text{Ca}^{2+}$ , high  $\text{Mg}^{2+}$ , artificial cerebrospinal fluid (concentrations (in mM): NaCl 124.0, KCl 5.0,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  1.25,  $\text{NaHCO}_3$  26.0,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.2, and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  5.0). Two coronal sections from each hippocampal specimen were fixed in 10% formalin in phosphate buffer for at least 24 h, embedded in paraffin, cut on a microtome into 10- $\mu\text{m}$  sections, and then mounted and stained in cresyl violet solution according to standard histological procedure. Sections which we have termed "anterior" were taken from within the anterior 15 mm of the pes hippocampus; "posterior" sections were taken from between 15 and 30 mm from the pes hippocampus. The mean distance between counted sections was  $12.2 \pm 1.2$  mm (mean  $\pm$  SEM) for the early-risk group and  $12.3 \pm 2.4$  mm (mean  $\pm$  SEM) for the no-early-risk group. All temporal lobectomies were performed by the same surgeon (M.J.O.). The location of the pes hippocampus was determined by the surgeon, and the posterior extent of hippocampal resection was measured relative to this marker.

Hippocampal sections were measured and counted with the aid of an image analysis system (Java, Jandel Scientific, Corte Madera, CA, U.S.A.) that produced a digitized microscopic image on a video screen and had a moving cursor that allowed measurement of area and length. Digital images in Figs. 1 and 3 were acquired by the Java image analysis system and were reproduced with image software (Adobe Photoshop, Adobe Systems, Mountain View, CA, U.S.A.). As shown in Fig. 1A, the area

of the hilus was measured at  $\times 5$  by tracing with the cursor along the border between the granule cell layer and the hilus and then connecting the upper and lower blades of the granule cell layer with a straight line from the tip of the upper blade to the tip of the lower blade. Hilus neurons were counted at  $\times 100$  by moving an ocular grid systematically over the entire hilus. No attempt was made to differentiate the various types of hilar neurons (18–20). CA3 neurons, when present in the surgical specimen, were not included in either the hilar cell counts or in the measurement of hilar area.

The granule cell layer was measured and counted at  $\times 100$  over at least 12 fields of a fixed length of 190  $\mu\text{m}$ . For each section, the number of counted fields approximated one half of all fields comprising the granule cell layer. Figure 1B is a typical view of the granule cell layer and illustrates our method of defining the borders of the granule cell layer. For these measurements, the granule cell layer was arranged with its length running vertically down the monitor screen. First, the area of the granule cell layer in the field was measured by tracing with a cursor along the inner and outer edges of the dentate granule cell layer and along the top and bottom edges of the monitor screen. In defining the edges of the granule cell layer, we excluded isolated granule cells which lay far outside (50–100  $\mu\text{m}$ ) the general outer border of the granule cell layer and invaded the molecular layer. Second, the granule cell layer width was measured at five regularly spaced intervals. We counted all granule cells that had a nucleolus surrounded by a clearly defined cell membrane and that fell within the measured area. In visualizing granule cells for counting, there was one plane of focus in which nucleolated granule cells were in focus; outside this plane, there were no cells in focus. Therefore, there was little chance that observer bias would

**FIG. 2.** Histograms of mean values for all morphological measurements for the no-early-risk group (NER) and early-risk group (ER) in anterior (open columns) and posterior (shaded columns) locations. **A:** Granule cell density (cells/mm<sup>3</sup>). In anterior regions, the mean value in the ER group was significantly lower than that in the NER group ( $p < 0.01$ ). In the ER group, mean granule cell density was significantly lower anteriorly than posteriorly ( $p < 0.05$ ). **B:** Granule cells per millimeter. In both anterior and posterior regions, the mean value in the ER group was significantly lower than that in the NER group ( $p < 0.01$  anteriorly;  $p < 0.02$  posteriorly). **C:** Hilar cell density (cells/mm<sup>3</sup>). For both anterior and posterior regions, mean values in the ER group were significantly lower than respective values in the NER group ( $p < 0.05$  for each region). **D:** Granule cell layer average widths. In anterior regions, the mean value in the febrile convulsion (FC) group was significantly lower than that in the meningitis/encephalitis (M/E) group ( $p < 0.01$ ). Average widths are expressed in microns. Histograms represent the mean  $\pm$  SE.



result in the selection of a maximum or minimum number of cells. All raw cell counts were corrected for the thickness of the tissue section by Abercrombie's formula (21). All cell counts and measurements were performed by 1 person who was blinded to clinical history. All morphological measurements were performed as a matter of routine, and this text is a retrospective analysis of existing data.

Neuronal cell distributions were analyzed by two different methods: first, granule and hilar cell densities (cells/mm<sup>3</sup>) were evaluated; second, granule cell density was separated into two components, granule cell layer average width ( $\mu\text{m}$ ) and granule cells per millimeter. Granule layer average width represents the average of at least 60 individual measurements of granule cell layer width for each section of tissue (five measurements per microscopic field). For a coronal tissue section with a thickness of 10  $\mu\text{m}$ , granule cells per millimeter provides a measure of the total number of granule neurons along a unit length of the granule cell layer, regardless of its width. Each value of granule cells per millimeter was determined by dividing the total number of counted granule neurons in all microscopic fields (corrected for section thickness by Abercrombie's formula) by the sum of the lengths of those fields as they were measured on the monitor screen. This measurement was described previously (16). These two separate measurements of the granule cell layer enable one

to distinguish between sections with similar numbers of cells but different cell distributions and thus represent a distinction between cell loss and cell dispersion. In turn, these two methods of comparison—by density and by dispersion—permit a more complete quantitative analysis of the complex anatomic variations of the granule cell layer.

#### Statistical analysis

Regression analysis was accomplished with a graphing software package (Cricket Software, Malvern, PA, U.S.A.). Differences in means between various groups, as described in the Results section, were analyzed by Student's *t* test for the following variables: granule cell density, granule cells per millimeter, granule cell layer average width, and hilar cell density, with  $p < 0.05$  as the level of statistical significance.

## RESULTS

#### Comparison of means for anterior and posterior locations

Mean values for all morphological measurements in both risk groups are shown in Fig. 2. All means were evaluated both across groups and within groups. Comparison of anterior granule cell densities across risk groups (Fig. 2A) showed the mean value in the early-risk group to be significantly lower



**FIG. 3.** Cresyl violet-stained sections of the granule cell layer showing the extremes of granule cell dispersion and depletion. Digital photomicrographs were taken at  $\times 40$ . **A:** Granule cell layer (gcl) exhibiting densely packed granule cells and distinct borders with the hilus and molecular layer (ml). This specimen was taken from the no-early-risk group. **B:** Narrow gcl with loosely packed granule cells, suggesting that granule cells have been depleted. This specimen was taken from an early-risk patient with a history of febrile convulsions in infancy. **C:** Hippocampal specimen exhibiting a dispersed granule cell layer in which granule cells have migrated into the molecular layer. Specimen was taken from an early-risk patient with a history of viral encephalitis. Photomicrographs in B and C have the same orientation as that in A. Calibration  $100 \mu\text{m}$ .

than that in the no-early-risk group ( $p < 0.01$ ). In posterior sections, mean granule cell density was also lower in the early-risk group than in the no-early-risk group, although this difference was not statistically significant ( $0.1 < p < 0.2$ ). Comparison of anterior granule cells per millimeter across risk groups (Fig. 2B) showed the mean value in the early-risk group to be significantly lower than that in the no-early-risk group ( $p < 0.01$ ). Posteriorly, granule cells per millimeter were also significantly lower in the early-risk group than in the no-early-risk group ( $p < 0.02$ ).

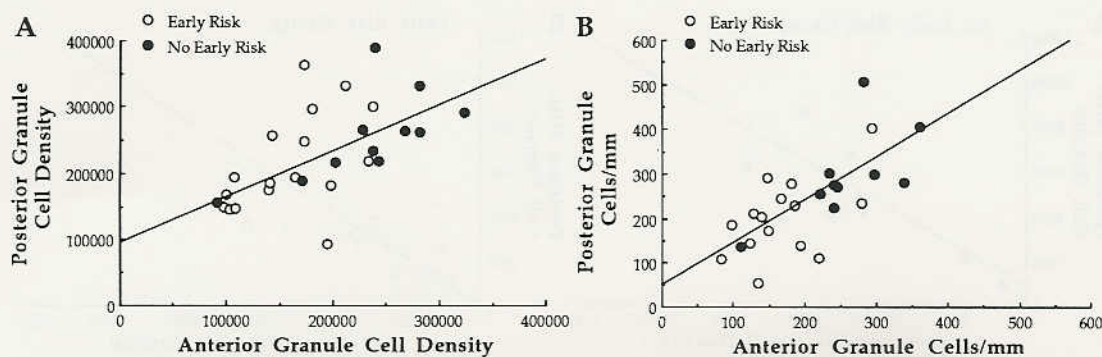
Mean hilar cell densities in anterior and posterior sections for both groups are compared in Fig. 2C. In both locations, hilar cell densities were significantly lower in the early risk group than in the no-early-risk group ( $p < 0.05$  for each location). These results indicate that the early-risk group incurred significantly greater cell loss than the no-early-risk group in anterior and posterior sections of the granule cell layer and hilus.

In comparing anterior and posterior cell population measurements within each risk group, we noted one statistically significant difference. For the early-risk group, granule cell density was 26% lower anteriorly than posteriorly ( $p < 0.01$ ) (Fig. 2A). Although no other comparison of cell population showed a statistically significant difference between anterior and posterior sections, mean cell populations were lower anteriorly for both risk groups in granule cell density, granule cells per millimeter, and hilar cell density (Figs. 2A–C).

The marked reduction in granule cell density in the early-risk group, especially in anterior sections, may be the result of cell loss or cell dispersion; e.g., an altered pattern of granule cell migration that

results in a wider, dispersed granule cell layer could produce a decrease in granule cell density without a significant change in the number of granule cells, as demonstrated by Houser (6). Figure 3 shows the extremes of granule cell depletion and dispersion present in this patient population. In Fig. 3A, the granule cell layer has distinct borders and densely packed granule cells; this specimen was taken from a no-early-risk patient. In Fig. 3B, the granule cell layer is extremely narrow and has very few granule cells, suggesting that the granule cell layer has been depleted. This section was taken from an early-risk patient who had a febrile convulsion at age 1 year. The granule cell layer in Fig. 3C shows evidence of granule cell dispersion: the border between the molecular layer and the granule cell layer is less distinct because the granule cells appear to have migrated into the molecular layer. This specimen is shown at lower magnification in Fig. 1A.

We measured the average width of the granule cell layer and granule cells per millimeter to identify evidence of dispersion. The granule cell layer mean average widths in anterior and posterior sections were not significantly different in either risk group, whether compared within groups or across groups (Fig. 2D). Mean widths were  $102 \pm 10 \mu\text{m}$  in anterior sections and  $90 \pm 12 \mu\text{m}$  in posterior sections for the early-risk patients and  $108 \pm 8 \mu\text{m}$  in anterior sections and  $111 \pm 11 \mu\text{m}$  in posterior sections for the no-early-risk patients. We further divided the early-risk group into two subsets: patients with histories of febrile convulsions only and patients with a history of meningitis or encephalitis. In the meningitis/encephalitis subgroup, granule cell layer mean widths in anterior sections were significantly wider than those in the febrile convulsion subgroup ( $p <$



**FIG. 4.** Relation between anterior and posterior granule cell measures are shown for both risk groups. **A:** Granule cell densities for anterior and posterior regions were significantly correlated. The best-fit line for the no-early-risk group was described by the equation  $y = 0.69x + 94,984$  ( $r = 0.67$ ,  $p < 0.05$ ). The regression line for the early-risk group was  $y = 0.79x + 88,725$  ( $r = 0.50$ ,  $p < 0.05$ ; data not shown). **B:** Values of granule cells per millimeter for anterior and posterior regions were also significantly correlated. The best-fit line for the no-early-risk group was described by the equation,  $y = 0.96x + 49$  ( $r = 0.67$ ,  $p < 0.05$ ). The regression line for the early-risk group was described by the equation  $y = 0.78x + 70$  ( $r = 0.54$ ,  $p < 0.05$ ; data not shown).

0.01) (Fig. 2D). Mean widths in the meningitis/encephalitis subgroup were  $139 \pm 17$  and  $111 \pm 30 \mu\text{m}$  in anterior and posterior sections, respectively. In the febrile convulsion subgroup, mean widths were  $89 \pm 5$  and  $86 \pm 9 \mu\text{m}$  in anterior and posterior sections, respectively. Although values of granule cells per millimeter tended to be higher in the meningitis/encephalitis subgroup (data not shown), there were no other significant differences between the two subgroups. Therefore, although all early-risk patients showed granule cell loss, the meningitis/encephalitis subgroup also showed evidence of granule cell dispersion, especially in anterior sections.

#### Relation between anterior and posterior cell densities

Mouritzen Dam showed that neuronal cell loss in the hilus and granule cell layer of epileptic tissue is accelerated with the additive effects of both epilepsy and age, a finding that lends support to the hypothesis of neuronal cell loss as an ongoing process in epileptic patients (15). This process of cell loss may reveal itself as a broad range of cell densities in epileptic patients. Indeed, in the present study, each risk group exhibited a range of cell densities for each tissue location. To identify the relative vulnerability of each hippocampal location to cell loss in patients with a range of pathology from mild to severe, we plotted anterior cell counts against posterior cell counts for each cell population measurement and for both risk groups (Figs. 4–6). For the no-early-risk group, combining these graphs may suggest a relation between granule and hilar cell loss.

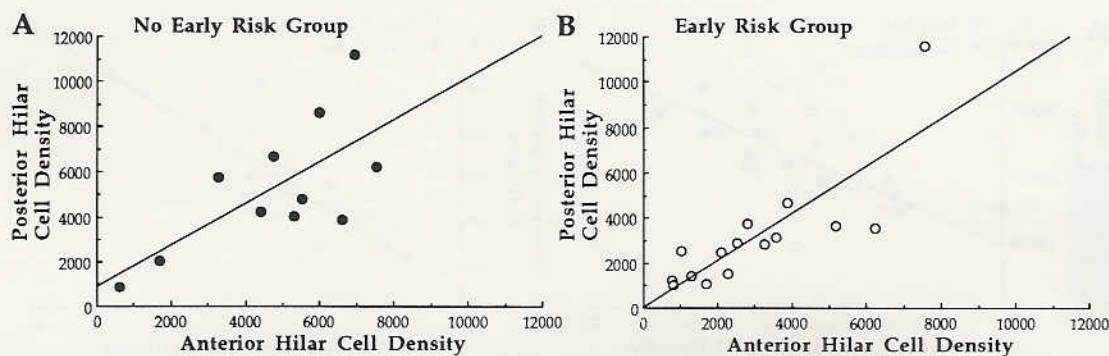
For granule cell density, anterior versus posterior cell counts were significantly correlated in each risk group ( $p < 0.05$ ) (Fig. 4A). Indeed, the data points

for both groups lay along essentially the same regression line. However, the early-risk data points appeared to occupy the lower end of the anterior granule cell density scale. This grouping was consistent with the particularly low mean anterior granule cell density in the early-risk group (Fig. 2A). In addition, the regression line for the no-early-risk group in Fig. 4A suggests that, as anterior granule cell density decreases to 0,  $\sim 100,000$  granule cells/mm<sup>3</sup> will survive posteriorly. Therefore, as compared with anterior granule cells, posterior granule cells may be relatively spared.

Values of granule cells per millimeter for anterior versus posterior sections from both risk groups are shown in Fig. 4B. In both groups, anterior and posterior values of granule cells per millimeter showed a significant correlation ( $p < 0.05$ ). Again, the no-early-risk data points were distributed over a wide range, whereas the early-risk data points largely occupied the low ends of the cell population axes. In the no-early-risk group, moreover, posterior granule cells appeared to be preserved relative to anterior granule cells.

Hilar cell densities for anterior versus posterior sections are compared for both risk groups in Fig. 5. In both groups, anterior and posterior cell densities were significantly correlated ( $p < 0.01$ ) (Fig. 5A and B). The regression line for the no-early-risk group (Fig. 5A) suggested slight preservation of posterior hilar cell density, as compared with that in anterior sections. However, in the early-risk group (Fig. 5B) anterior and posterior hilar cell densities appeared to decrease at nearly equal rates.

In the no-early-risk group, granule and hilar cell densities in anterior sections showed a linear correlation with a slope of 25.0 and a y-intercept of 114,120



**FIG. 5.** Anterior and posterior hilar cell densities were significantly correlated for the no-early-risk group (**A**) and the early-risk group (**B**): A: The regression line for the no-early-risk group was described by the equation  $y = 0.93x + 878$  ( $r = 0.70$ ,  $p < 0.01$ ). B: The regression line for the early-risk group was described by the equation  $y = 1.05x + 14$  ( $r = 0.82$ ,  $p < 0.01$ ).

cells/mm<sup>3</sup> ( $p < 0.01$ ) (Fig. 6A). Granule and hilar cell densities in posterior sections were also linearly correlated with a slope of 11.3 and a y-intercept of 197,020 cells/mm<sup>3</sup> ( $p < 0.05$ ) (Fig. 6B). These two correlations suggest that if all hilar neurons in both tissue locations were to disappear granule cell density would be  $>0$ . Therefore, the hilus may be more vulnerable to cell loss than the granule cell layer. In addition, the plot of hilar cell densities in Fig. 5A suggests that the hilus may be more prone to cell loss anteriorly than posteriorly. Therefore, the anterior hilus may be more vulnerable to cell loss than any other region of the hilus or dentate granule cell layer.

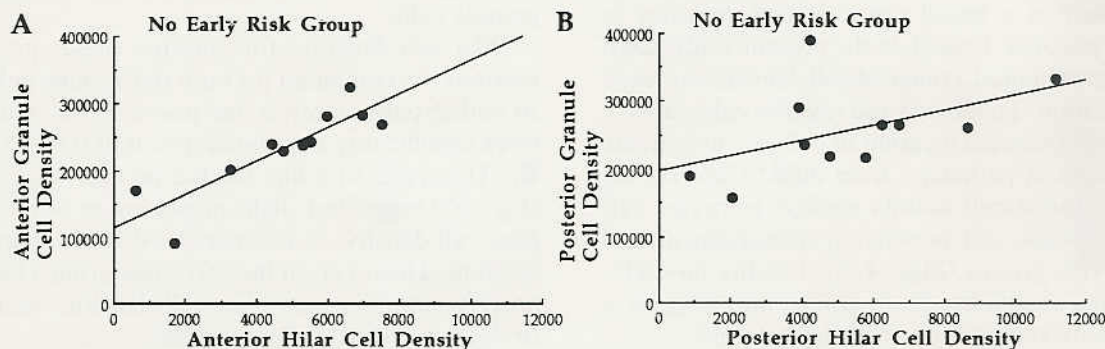
Moreover, comparison of respective y-intercepts and slopes in Figs. 6A and B suggests two additional conclusions. First, relative to hilar cell density, granule cell density is more preserved posteriorly than anteriorly. Second, relative to hilar cell density, the anterior granule cell density decreases about twice as fast as in posterior sections. Early-risk patients showed no statistically significant correlation between granule cell and hilar cell densities in anterior or posterior sections (data not shown), possibly be-

cause hilar and granule cell densities have been so depleted.

## DISCUSSION

In this study, the early-risk patients had lower mean granule cell densities, granule cells per millimeter, and hilar cell densities than did no-early-risk patients (Figs. 3A–C). These differences were statistically significant in anterior specimens with respect to granule cell density, granule cells per millimeter, and hilar cell density. In posterior specimens, differences between groups were statistically significant for granule cells per millimeter, and hilar cell density and emerged as a trend for granule cell density. These differences in hippocampal neuronal cell distribution suggest that the early-risk and no-early-risk patients may comprise two distinct patient populations.

To evaluate more completely the changes that occur in the epileptic hippocampus, it would be ideal to analyze by similar methods the hippocampal anatomy of control specimens with no known neurologic



**FIG. 6.** Granule and hilar cell densities in the no-early-risk group were significantly correlated in anterior (**A**) and posterior regions (**B**). A: The regression line for anterior regions was described by the equation  $y = 25.0x + 114,120$  ( $r = 0.86$ ,  $p < 0.01$ ). B: The regression line for posterior regions was described by the equation  $y = 11.3x + 197,020$  ( $r = 0.5$ ,  $p < 0.05$ ).

injury and no history of epileptic seizures. Mouritzen Dam studied such a nonepileptic patient group in comparison with a group of 20 epileptic patients who resembled our no-early-risk group with respect to history of neurologic risk factors. Only 1 patient had an early risk factor (15,22). Because the relation between cell densities obtained by different investigators using different methods cannot be precisely defined, our comparisons with these studies are limited to the percent differences between anterior and posterior cell densities. Moreover, comparisons between our study and Mouritzen Dam's findings are based on hippocampal sections from two of the five locations reported in her study: an "anterior" section lying 5–10 mm from the pes hippocampus and a "posterior" section lying 25–30 mm from the pes hippocampus.

Mouritzen Dam noted that granule cell density was 13% higher anteriorly than posteriorly in nonepileptic, postmortem specimens. In comparison with this nonepileptic tissue, epileptic specimens had almost the same granule cell densities in anterior and posterior sections, indicating proportionately greater reductions in anterior granule cell density relative to posterior granule cell density (15,22). In our early-risk patients, the mean granule cell density was 26% lower anteriorly than posteriorly, suggesting a 34% reduction in the anterior granule cell density relative to posterior sections as compared with nonepileptic specimens. In our no-early-risk group, there was no significant difference between anterior and posterior granule cell densities, suggesting a 12% reduction in anterior granule cell density relative to posterior sections as compared with nonepileptic tissue (22). From these relative differences in granule cell density, one can infer that the granule cell layer in both risk groups incurs a primarily anterior insult, but with particular severity in the early-risk group.

Mouritzen Dam's study of nonepileptic tissue showed that hilar cell density was 14% lower anteriorly than posteriorly. In comparison, epileptic hilar cell densities were lower than those in nonepileptic tissue, but with almost equal reductions in both anterior and posterior sections (15,22). In our early-risk group, anterior and posterior hilar cell densities were not significantly different ( $p > 0.5$ ; Fig. 2C). In our no-early-risk group, hilar cell densities were 10% lower anteriorly than posteriorly, although this difference was not statistically significant ( $p > 0.3$ ) (Fig. 2C).

These comparisons suggest that our no-early-risk group may parallel Mouritzen Dam's epileptic group, not only in terms of risk factor, but also with respect to the anteroposterior gradients of granule

and hilar cell density. Specifically, hilar cell densities in the no-early-risk group may sustain equal reductions in anterior and posterior regions whereas hilar cell densities in the early risk group may be reduced to uniformly and severely low levels in anterior and posterior sections.

The granule cell layer average widths in our early-risk and no-early-risk groups (Fig. 2D) were comparable to those of nonepileptic patients ( $99.5 \pm 7.8 \mu\text{m}$ ) and far below the widths of epileptic patients ( $182 \pm 14.9 \mu\text{m}$ ) reported in Houser's study (6). In light of Houser's description of granule cell dispersion and its association with early neurologic insults, this lack of difference in granule cell layer average widths between the two risk groups appeared to present a significant inconsistency. However, division of the early-risk group into two subsets—patients with a history of febrile convulsions without CNS infection and patients with histories of meningitis or encephalitis—suggested that although both subgroups had marked cell loss resulting in similar numbers of granule cells, there was a difference in the widths of their granule cell layers. The febrile convulsion subgroup exhibited a narrow granule cell layer which may arise from simple, severe cell loss. In contrast, the meningitis/encephalitis subgroup had a wider granule cell layer, which may result from the dispersion of these cells. This dispersion in the meningitis/encephalitis subgroup supports the hypothesis of an altered pattern of granule cell migration in the granule cell layer of patients with a history of meningitis or encephalitis occurring in the first 4 years of life and is consistent with Houser's observation of cell dispersion with early neurologic insults (6).

We have presented plots of anterior versus posterior cell counts to identify the relative vulnerability of each hippocampal location to cell loss in patients with a range of pathology from mild to severe. For the no-early-risk group, the combining of these granule, hilar, and granule-to-hilar correlations in Figs. 4–6 may suggest that granule cell loss and hilar cell loss occur at different rates in anterior and posterior regions. Specifically, the anterior hilus appears to be more vulnerable to cell loss than any other region of the hilus or dentate granule cell layer. Moreover, the decrease in granule cell density that accompanies a given decrease in hilar cell density is twice as great anteriorly as posteriorly.

Mouritzen Dam has shown that the anterior hippocampus of nonepileptic specimens displays a high ratio of granule neurons to hilar neurons (22). This ratio is consistent with a relatively lower inhibitory feedback from hilar neurons to granule neurons in anterior hippocampal regions, which may render the



anterior granule cell layer more excitable and anterior hilar neurons more susceptible to excitotoxic insult (23) than those in posterior regions. This regional difference in the ratio of granule to hilar cell density may explain in part why hippocampal damage is more severe and may begin anteriorly in most cases of TLE (16).

In the early-risk group, the correlation of anterior versus posterior hilar cell density (Fig. 5B) suggests that the hilus may undergo marked cell loss along the entire length of the resected hippocampus. This pattern of uniformly severe hilar cell loss is supported by our analysis of mean hilar cell density, which showed that early risk hilar cell densities were almost equal in anterior and posterior sections and that hilar cell densities in the early-risk group were almost 40% lower than in the no-early-risk group. This pattern of hilar cell loss is consistent with the classic description of "endfolium sclerosis" (24).

These differences in anteroposterior granule and hilar cell distribution suggest that cell loss in the early-risk group may be initiated by an event different than in the no-early-risk group; e.g., a sudden ischemic or convulsive insult (5) in the early-risk group has been suggested to initiate this pattern of severe cell loss. This hypothesis is consistent with numerous studies linking gross hippocampal atrophy or decreased hippocampal cell densities to febrile convulsions (7,10-14,25) or convulsions associated with meningitis or encephalitis in the first 4 years of life (6). Indeed, the disparity in cell populations between the early-risk and no-early-risk groups becomes more striking when we consider that the early-risk patients do not have a significantly longer duration of epilepsy and may experience a lower number of seizures. These calculations of duration of epilepsy and total estimated seizures do not account for the possibility in the early-risk group of subclinical seizures before the time of seizure onset. In contrast, the no-early-risk patients may incur a lesser, hyperexcitable insult that begins outside the hippocampus and results in more gradual loss of hilar and granule neurons, beginning with the anterior hilus and spreading posteriorly. Further investigation of the biochemical and physiological characteristics of the hippocampus along its anteroposterior extent may provide additional insight into the causes underlying these different forms of TLE.

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## REFERENCES

1. Falconer MA, Serafetinides EA. A follow-up study of surgery in temporal lobe epilepsy. *J Neurosurg Psychiatry* 1963;26:154-65.
2. Falconer MA, Serafetinides EA, Corsellis JAN. Etiology and pathogenesis of temporal lobe epilepsy. *Arch Neurol* 1964;10:233-48.
3. Falconer MA. Mesial temporal (Ammon's horn) sclerosis as a common cause of epilepsy: aetiology, treatment and prevention. *Lancet* 1974;2:767-70.
4. Abou-Khalil B, Andermann E, Andermann F, Olivier A, Quesney LF. Temporal lobe epilepsy after prolonged febrile convulsions: excellent outcome after surgical treatment. *Epilepsia* 1993;34:878-83.
5. Taylor DC, Ounsted C. Biological mechanisms influencing the outcome of seizures in response to fever. *Epilepsia* 1971;12:33-45.
6. Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res* 1990;535:195-204.
7. Falconer MA, Hill D, Meyer A, Mitchell W, Pond DA. Treatment of temporal-lobe epilepsy by temporal lobectomy, a survey of findings and results. *Lancet* 1955;1:827-35.
8. Babb TL, Brown WJ. Pathological findings in epilepsy. In: Engel J, Jr., ed. *Surgical treatment of the epilepsies*. New York: Raven Press, 1987:511-40.
9. Spencer DD, Spencer SS. Hippocampal resections and the use of human tissue in defining temporal lobe epilepsy syndromes. *Hippocampus* 1994;4:243-9.
10. Jack CR, Sharbrough FW, Cascino GD, Hirschorn KA, O'Brien PC, Marsh WR. Magnetic resonance image-based hippocampal volumetry: correlation with outcome after temporal lobectomy. *Ann Neurol* 1992;31:138-46.
11. Cendes F, Andermann F, Dubeau F, et al. Early childhood prolonged febrile convulsions, atrophy and sclerosis of mesial structures, and temporal lobe epilepsy: an MRI volumetric study. *Neurology* 1993;43:1083-7.
12. Ounsted C, Glaser GH, Lindsay J, Richards P. Focal epilepsy with mesial temporal sclerosis after acute meningitis. *Arch Neurol* 1985;42:1058-60.
13. Sagar HJ, Oxbury JM. Hippocampal neuron loss in temporal lobe epilepsy: correlation with early childhood convulsions. *Ann Neurol* 1987;22:334-40.
14. Marks DA, Kim J, Spencer DD, Spencer SS. Characteristics of intractable seizures following meningitis and encephalitis. *Neurology* 1992;42:1513-8.
15. Mouritzen Dam A. Epilepsy and neuron loss in the hippocampus. *Epilepsia* 1980;21:617-29.
16. Masukawa LM, O'Connor WM, Lynott J, et al. Longitudinal variation in cell density and mossy fiber reorganization in the dentate gyrus from temporal lobe epileptic patients. *Brain Res* 1995;678:65-75.
17. Sperling MR, O'Connor MJ, Saykin AJ, et al. A non-invasive protocol for anterior temporal lobectomy. *Neurology* 1992;42:416-22.
18. Lorente de No R. Studies on the structure of the cerebral cortex. II. *J Psychol Neurol* 1934;46:113-17.
19. Amaral DG. A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol* 1978;182:851-914.
20. Ribak CE, Seress L. Five types of basket cells in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. *J Neurocytol* 1983;12:577-97.
21. Abercrombie M. Estimation of nuclear population from microtome sections. *Anat Rec* 1946;94:239-47.
22. Mouritzen Dam A. The density of neurons in the human

- hippocampus. *Neuropathol Appl Neurobiol* 1979;5: 249-64.
23. Olney JW, Fuller T, de Gubareff T. Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res* 1979;76:91-100.
24. Margerison JH, Corsellis JAN. Epilepsy and the temporal lobes. *Brain* 1966;89:499-530.
25. Wyllie E, Chee M, Granström ML, et al. Temporal lobe epilepsy in early childhood. *Epilepsia* 1993;34: 859-68.