A Decrease in the Number of GABAergic Somata is Associated with the Preferential Loss of GABAergic Terminals at Epileptic Foci

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Previous studies have indicated that a loss of GABAergic terminals occurs at epileptic foci. The present study was undertaken to investigate if this loss is associated with a loss of GABAergic neuronal somata. Seven juvenile monkeys (M mulatta) received alumina gel injections to the pre-central gyrus of the left cerebral hemisphere to produce epileptic foci. Four of these monkeys were chosen for further quantitative study. One was sacrificed prior to seizure onset (‘pre-seizure’), one had seizures for 3 days (‘acute’), and two had a seizure record of one month (‘chronic’). Sections of tissue from the epileptic cortex and from the contralateral, non-epileptic cortex were processed for glutamate decarboxylase (GAD) immunocytochemistry at the light microscopic level. Quantitative analysis revealed that a loss of GAD-positive neuronal somata ranging from 24 to 52% occurred at epileptic foci for all monkeys. This decrease was significant (P < 0.01) for the two chronic monkeys. There was also a slight decrease in GAD-positive neurons 1 cm distal to the focus (‘parafocus’) in the chronic monkeys, but not in the acute or pre-seizure animals. In addition, small GAD-positive somata (50–150 μm²) were more severely decreased in number at epileptic foci than larger ones (200–250 μm²). As an experimental control, an additional monkey was given a surgical lesion in area 4 of one cerebral hemisphere. It did not display seizure activity prior to sacrifice and did not show a loss of GAD-positive neurons proximal to the control lesion. The results of this study indicate that a loss of GABAergic neuronal somata is associated with a loss of GABAergic terminals at epileptic foci, and that this loss may be more specific for the small GABAergic neurons.

INTRODUCTION

Numerous studies from our laboratory have indicated that a loss of GABAergic terminals occurs at epileptic foci. This loss was shown to be preferential for GABAergic, symmetric synapses with quantitative electron microscopic methods. The results of biochemical studies support these findings that indices for other neurotransmitters were not reduced as severely as those for GABA. In fact, these data obtained from experimental animal models of focal epilepsy have been predictive for the pathophysiology of human epilepsy because biochemical studies of human epileptic foci also indicate a GABA deficit. Therefore, a loss of GABAergic terminals is a hallmark of focal epilepsy.

The present study was undertaken to investigate if this loss of GABAergic terminals was associated with a loss of GABAergic somata. Since the terminals of both cortical basket and chandelier cells are dramatically reduced by over 80% at the epileptic focus, it was predicted that this loss was due to a neuronal loss and not simply a pruning back of the axonal plexus of these GABAergic neuronal types. In addition, the results of an analysis of Nissl-stained preparations of alumina gel epileptic foci have demonstrated a small neuronal loss. Therefore, it would be important to know if GABAergic somata were decreased in number at epileptic foci. To explore this point, we utilized an anti-glutamate decarboxylase (GAD) serum that detects somal GAD in many types of neurons without the use of colchicine. This antisera would facilitate a better comparison of epileptic and non-epileptic tissue because other antisera that require colchicine provide non-uniform laminar staining of GABAergic neuronal somata that is depend-

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MATERIALS AND METHODS

Eight juvenile monkeys (Macaca mulatta), weighing 2.5–4 kg, were used for this study. Each monkey displayed a normal scalp electroencephalogram (EEG) prior to surgery. Under general anesthesia a left frontal craniotomy was performed. The precentral gyrus (Brodmann's area 4) was identified and location of the hand/face area was confirmed by cortical stimulation. Seven monkeys received alumina gel applications using the Ward modification of the Kopeloff technique. No anticonvulsants were administered postoperatively. The eighth monkey was given a surgical control lesion which consisted of a subpial resection of approximately 5 x 5 mm down to the white matter (an area equivalent to that of a mature granuloma observed in this model). Serial scalp EEGs were subsequently performed on all monkeys on a weekly basis. Chronic seizure monitoring was similar to that previously reported. Of the 7 experimental monkeys, 4 were used for extensive quantitative analysis. The other 3 did not display a high enough quality of immunocytochemical staining for a meaningful interpretation of quantitative data. However, they were used for the qualitative description of the GAD-positive neuronal somata.

Of the 4 monkeys used for the quantitative analysis, one ('pre-seizure') was sacrificed prior to the development of clinical seizures (RB-161). An electrocorticogram (ECoG) demonstrated sharp waves adjacent to the developing granuloma. One animal ('acute') was sacrificed 6 weeks following the injection of alumina gel (RB-160). This animal had 4 days of clinically evident seizure activity with a seizure frequency of 2–3 per day. Another animal ('chronic') was sacrificed two months following the alumina injection (RB-163). This animal had 3 weeks of clinically evident seizure activity with a frequency of approximately one seizure every other day. The fourth monkey ('chronic') was sacrificed 2.5 months following the injection of alumina (RB-165). This animal had seizures for 6 weeks, demonstrating a seizure frequency of approximately one seizure per day. Each of the epileptic animals demonstrated an ECoG consistent with spike and wave discharges in the area immediately adjacent to the developing granuloma. The control monkey was sacrificed 2.5 months following the surgical control lesion (RB-166). This animal demonstrated no clinical seizure activity and the ECoG was normal.

Following the ECoG, each animal was deeply anesthetized with sodium phenobarbital and perfused transcardially with 0.2% paraformaldehyde in balanced phosphate buffer (pH 7.3). A low paraformaldehyde concentration was used in the fixative so that tissue from the same monkey could be analyzed for receptor binding. This primary fixative is not optimal for immunocytochemistry and may partially explain the variability in the number of immunoreactive somata between monkeys (Table I). The skull and dura were rapidly removed after the perfusion. The tissue block from the left precentral gyrus was immediately removed and included the area adjacent to the alumina injection ('proximal' tissue) and tissue 1–2 cm superior to the injection site ('distal' tissue). A block from the right precentral gyrus was cut for control tissue from the homotopic cortical area because the results of Harris and Lockard show that the contralateral homotopic cortex in monkeys with alumina gel-induced seizures does not show independent seizure activity. Both blocks were immediately placed in 10% formalin for further fixation and placed in a cryoprotectant solution of 30% sucrose for 24 h prior to sectioning. Sections 40 μm thick were cut on a freezing microtome and placed in 0.1 M phosphate-buffered saline for light microscopic immunocytochemistry.

Briefly, free-floating tissue sections were processed for the immunocytochemical localization of GAD using the GAD antibody characterized by Oertel et al. Sections from the experimental and contralateral hemispheres were incubated simultaneously. A modification of Oertel's protocol was used, employing the avidin–biotin–horseradish peroxidase complex (Vector Labs) in a double-bridged technique. Following immunocytochemical processing, the sections were mounted on subbed glass slides,
**TABLE I**

**Numbers of GAD-positive neuronal somata in 4 regions of cortex for 5 monkeys**

In each section of tissue processed for GAD immunocytochemistry (3–11 sections/animal), the number of GAD-positive somata was counted in an area of tissue covered by 3 adjacent, 240 μm-wide radial traverses throughout the entire depth of the cortex. For the first 4 (experimental) monkeys, counts were made at the focus, parafocus, and two contralateral sites. In the fifth (control) monkey, counts were made proximal and distal to the control lesion, and at two comparable contralateral sites.

| Monkey  | Number of GAD-positive somata counted | Tissue area (mm²) | Monkey  | Number of GAD-positive somata counted | Tissue area (mm²) |
|---------|---------------------------------------|------------------|---------|---------------------------------------|------------------|
| **RB-161** |                                       |                  | **Contralateral** |                                       |                  |
| Focus   | 40                                    | 2.8              | Focus   | 190                                   | 4.6              |
|         | 34                                    | 2.8              |         | 314                                   | 4.7              |
|         | 35                                    | 2.6              |         | 324                                   | 4.5              |
|         | 34                                    | 2.8              |         | 336                                   | 4.7              |
|         | 35                                    | 2.6              |         | 410                                   | 5.2              |
|         | 34                                    | 2.8              |         | 364                                   | 4.8              |
|         | 35                                    | 2.6              |         | 379                                   | 5.0              |
|         | 34                                    | 2.8              |         | 235                                   | 3.3              |
|         | 35                                    | 2.6              |         | 357                                   | 5.0              |
| Total   | 139                                   | 10.8             | Total   | 2909                                  | 41.8             |
| Average | 208                                   | 11.5             | Average | 69.6 somata/mm²                       |                  |
|         | 12.9 somata/mm²                       |                  |         |                                       |                  |
| Parafocus | 55                                    | 2.8              | Parafocus | 224                                   | 2.6              |
|         | 59                                    | 2.8              |         | 232                                   | 2.6              |
|         | 56                                    | 2.8              |         | 234                                   | 2.6              |
|         | 38                                    | 3.1              |         | 255                                   | 2.6              |
|         |                                        |                  |         | 252                                   | 1.8              |
|         |                                        |                  |         | 202                                   | 2.6              |
|         |                                        |                  |         | 257                                   | 2.4              |
|         |                                        |                  |         | 238                                   | 2.4              |
|         |                                        |                  |         | 251                                   | 2.6              |
|         |                                        |                  |         | 2145                                  | 22.2             |
| Total   | 148                                   | 11.9             | Total   | 1390                                  | 12.8             |
| Average | 209                                   | 17.6 somata/mm²  | Average | 108.6 somata/mm²                      |                  |
|         | 12.4 somata/mm²                       |                  |         |                                       |                  |
| Contra- | 32                                    | 2.6              | Contra- | 497                                   | 4.3              |
| lateral | 33                                    | 2.3              | lateral | 501                                   | 4.3              |
|         | 33                                    | 2.3              |         | 410                                   | 4.3              |
|         | 32                                    | 2.6              |         | 478                                   | 4.4              |
|         |                                        |                  |         | 1886                                  | 17.3             |
| Total   | 167                                   | 10.6             | Total   | 1886                                  | 17.3             |
| Average | 15.8 somata/mm²                       |                  | Average | 109.0 somata/mm²                      |                  |
dried, and defatted. They were then placed in alternating solutions of 0.0005% OsO4 and 0.05% thio-carbohydrazide (TCH) in order to intensify the reaction product, dehydrated, and coverslipped. In addition, 40 μm thick tissue sections were stained with cresyl violet for comparison with the immunocytochemical preparations.

One section from each hemisphere of every monkey was processed as described above except that normal sheep serum was used instead of anti-GAD serum. Such sections displayed no staining above a diffuse background level when examined with a light microscope.

Sections from the experimental and contralateral hemispheres were examined under the light microscope with a ×40 objective, at both the 'proximal' and 'distal' edges of the experimental tissue, and at corresponding sites in the contralateral tissue. A grid reticule (240 μm wide) was used to count the number of GAD-positive neuronal somata. Three adjacent

| Monkey | Number of GAD-positive somata counted | Tissue area (mm²) | Monkey | Number of GAD-positive somata counted | Tissue area (mm²) |
|--------|--------------------------------------|------------------|--------|--------------------------------------|------------------|
| RB-165 |                                      |                  | RB-166 |                                      |                  |
| Focus  | 33                                   | 2.6              | Focus  | 187                                  | 2.9              |
|        | 68                                   | 2.3              |        | 161                                  | 3.1              |
|        | 59                                   | 2.4              |        | 80                                   | 2.1              |
|        | 39                                   | 2.6              |        | 136                                  | 2.8              |
|        | 32                                   | 2.3              |        | 209                                  | 2.9              |
|        | 46                                   | 2.9              |        | 143                                  | 2.5              |
|        | 158                                  | 2.7              |        | 195                                  | 3.0              |
|        | 66                                   | 2.6              |        | 118                                  | 2.9              |
|        | 117                                  | 2.4              |        | 202                                  | 2.8              |
|        | 76                                   | 2.5              |        | 216                                  | 3.1              |
|        | 74                                   | 2.3              | Total  | 1599                                 | 28.1             |
| Total  | 768                                  | 27.6             | Average| 56.9 somata/mm²                       |                  |
| Parafocus |                                           |                  |        | 1188                                 | 27.8             |
|         | 86                                   | 2.3              |        | 67 2 somata/mm²                       |                  |
|         | 93                                   | 2.6              |        |                                      |                  |
|         | 74                                   | 2.9              |        |                                      |                  |
|         | 67                                   | 2.4              |        |                                      |                  |
|         | 49                                   | 2.6              |        |                                      |                  |
|         | 78                                   | 2.4              |        |                                      |                  |
|         | 168                                  | 2.6              |        |                                      |                  |
|         | 114                                  | 2.6              |        |                                      |                  |
|         | 141                                  | 2.4              |        |                                      |                  |
|         | 141                                  | 2.4              |        |                                      |                  |
|         | 193                                  | 2.4              |        |                                      |                  |
|         | 125                                  | 2.6              |        |                                      |                  |
| Total  | 1188                                 | 27.8             | Average| 65.0 somata/mm²                       |                  |
| Contralateral |                                   |                  | Contralateral | 248                                  | 4.0              |
|         | 126                                  | 2.9              |        | 250                                  | 4.5              |
|         | 148                                  | 2.5              |        | 291                                  | 4.5              |
|         | 144                                  | 2.1              |        |                                      |                  |
|         | 99                                   | 2.8              |        |                                      |                  |
|         | 157                                  | 2.4              |        |                                      |                  |
|         | 144                                  | 2.7              |        |                                      |                  |
|         | 207                                  | 2.6              |        |                                      |                  |
|         | 72                                   | 2.6              |        | 200                                  | 3.1              |
|         | 188                                  | 2.8              |        | 180                                  | 2.9              |
|         | 237                                  | 2.7              |        | 155                                  | 3.1              |
| Total  | 1522                                 | 26.1             | Total  | 535                                  | 9.1              |
| Average| 58.3 somata/mm²                       |                  | Average| 58.8 somata/mm²                       |                  |
traverses were made through the entire thickness of the cortex for each of the 4 sites from 3–11 sections per monkey. Cells which contained a dense, brownish reaction product within the perikaryal cytoplasm were considered to be GAD-positive. The average numbers of GAD-positive somata per 1.0 mm² were compared between each of the 4 tissue sites examined. An analysis of variance and the Newman–Keuls multiple comparison procedure were used to determine if the numbers of somata at each site were significantly different for each monkey. Camera lucida drawings of about 100 GAD-positive cell bodies per site were made at a magnification of ×700 from a plane of focus that demonstrated the greatest extent of the soma. These data were entered into an Apple II Plus computer by tracing them onto a Houston Instrument HI-PAD digitizing tablet. A Bioquant II program for computerized morphometry was used to calculate somal areas of GAD-positive cells.

RESULTS

Description of GABAergic neurons in normal and epileptic monkey motor cortex

GAD-positive neurons were found in all layers of non-epileptic monkey motor cortex. They were distributed rather homogeneously throughout the layers (Fig. 1). However, layer I and the deeper half of layer VI appeared to have slightly fewer GABAergic neurons than the other layers.

Neurons which were stained with the GAD antiserum contained a dark brown reaction product in their perikaryal cytoplasm. Many of the somata of these GAD-positive neurons appeared to have eccentrically-placed nuclei. The nuclei and most dendritic processes were unstained. Since the reaction product did not extend into the dendrites and axons, neurons could not be classified on the basis of their dendritic and axonal morphology. The shapes of the neuronal somata, however, indicated that the GAD-positive cells were non-pyramidal. Most somata were round, oval, teardrop-shaped, or fusiform, while some were irregular in shape (Figs. 1 and 3A). A few neurons had triangular somata, though not of a size to suggest that they were pyramidal cells. With the exception of layer I, there was no difference in shape distribution throughout the layers of cortex. Layer I neurons all had round or slightly oval somata.

The sizes of GAD-positive somata also varied. Almost all of the GAD-positive neurons in layer I had very small somal areas. In contrast, somata in layers II–VI displayed a wide variety of sizes. Thus, small, GAD-positive neurons were found in all cortical layers but the average somal area tended to increase from the superficial to deep laminae, such that increasing numbers of larger neurons were found in the deeper layers. Large somata were only occasionally observed in more superficial layers.

GAD-positive neurons located at sites both proximal and distal to epileptic foci were similar in size and shape to those in normal monkey motor cortex (Fig 2). In addition, they were observed throughout all of the cortical layers, although in reduced numbers as compared to the contralateral cortex. No particular layers seemed more affected than the others. Another observation for this focal region was the dramatic loss of GAD-positive puncta (cf. Fig 3A, B) as reported in an earlier study.

Quantitative differences between epileptic and normal cortex

The number of GAD-positive neuronal somata at sites proximal to the alumina gel focus was less than that found at distal or contralateral sites in the brains of epileptic monkeys (Table I). For example, monkey RB-163, which had seizures for 3 weeks, showed 70 GAD-positive cells/mm² at the focus, 97 cells/mm² at the distal ipsilateral site, and 108 and 109 cells/mm² at two corresponding sites in the contralateral cortex. Monkey RB-165, which had a 6-week history of seizures, displayed a similar distribution of cells. Since the average numbers of GAD-positive somata in the two examined regions of the contralateral cortex of each monkey were very similar (Table I), they were averaged together for each of the 4 epileptic monkeys.

The average number of cells/mm² at sites proximal and distal to each epileptic focus was expressed as a percentage of the contralateral cortex for the 4 monkeys used in the quantitative analysis. This manipulation of the data allowed for a direct comparison between monkeys because the raw data displayed a wide variation due to the fixation protocol (see Methods). The data (Fig. 4) indicated that the loss of GAD-positive cells at the site proximal to the focus ranged from 35 to 52% for the two chronic epileptic
Fig. 1. Light photomicrographs of normal monkey motor cortex obtained from sections that were incubated in anti-GAD serum. A. Entire depth of the cortex at low magnification from the pial surface (P) to layer VI. The GAD-positive immunoreaction product appears within small punctate structures and somata ×110. B, C and D. Enlargements of regions from layers I and II, lower layer III and upper layer V, respectively (selected areas denoted by corresponding letters in A). Numerous GAD-positive somata (arrows) are found in all layers ×600.
Fig. 2 Light photomicrographs of epileptic monkey motor cortex that show the reduction in the number of GAD-positive somata proximal to a chronic epileptic focus. A, representative section obtained from cortex adjacent to an alumina gel granuloma. The pial surface (P) is located at the top and layer VI is shown at the bottom ×110. B, C, and D enlargements of areas from layers I, III and V, respectively (denoted by corresponding letters in A). GAD-positive somata (arrows) are present in reduced numbers in these layers. Occasional red blood cells (arrowhead) are also found and should not be interpreted as GAD-positive neuronal somata ×600.
Fig 3 Light photomicrographs of layer V, GABAergic neuronal somata (open arrowheads) and punctate structures (arrows) A distribution of these structures in the non-epileptic cortex where GABAergic puncta (interpreted as axon terminals) form a dense pericellular plexus with the GAD-negative soma of a pyramidal neuron B dramatic loss of GABAergic puncta in an epileptic region of monkey motor cortex Note that only one GAD-positive soma is present in B whereas A displays two such somata ×900
monkeys. In contrast, monkey RB-160, which had only a 4-day record of severe seizures, showed a 24% loss of GAD-positive somata at the epileptic focus. The monkey (RB-161) that had been injected with alumina gel but had not displayed seizure activity prior to sacrifice showed a similar loss of GAD-positive somata (28%) proximal to the focus. The distal ipsilateral site (parafocus) in these two latter animals did not display a loss of GAD-positive somata. Instead, the number of cells at the parafocus was slightly more than that of the contralateral, control cortex. In contrast, the two monkeys with chronic seizure records (RB-163 and RB-165) displayed a loss of GAD-positive neurons at the parafocus.

Statistical analyses were made to determine if the decrease in the number of GAD-positive somata proximal to epileptic foci was statistically significant. A two-factor analysis of variance showed that, for the 4 alumina gel-injected monkeys as a group, the numbers of GAD-positive somata at the focus, parafocus and two control sites were significantly different ($P < 0.01$). The Newman–Keuls multiple comparison procedure did not replicate this finding. To determine if the numbers of cells at each site were significantly different for each individual monkey, a one-factor analysis of variance and the Newman–Keuls procedure were used. Both tests showed that

Fig 5 Distribution of GAD-positive somata according to somal area at the focus and contralateral, non-epileptic cortex of the 4 alumina gel-injected monkeys (A–D), and at two comparable sites in one surgical control monkey (E). The percentage of the total number of cells in each size range is plotted.
TABLE II
Comparison of somal areas for GAD-positive neurons in 5 monkeys

For the first 4 (experimental) animals, the range and mean somal area for GAD-positive neurons at the focus, parafocus and two contralateral sites are given. In the fifth (control) monkey, these measurements were taken proximal and distal to the control lesion, and at two comparable contralateral sites.

| Monkey | Number of somata measured | Smallest area $\mu$m$^2$ | Largest area $\mu$m$^2$ | Mean area $\mu$m$^2$ | $\pm$ S.D. $\mu$m$^2$ |
|--------|----------------------------|--------------------------|-------------------------|----------------------|----------------------|
| RB-161 | Focus 86                   | 65.6                     | 425.9                   | 160.6                | $\pm$ 86.1           |
|        | Parafocus 104              | 42.9                     | 366.6                   | 132.6                | $\pm$ 59.2           |
|        | Contralateral 94           | 52.3                     | 386.5                   | 136.1                | $\pm$ 65.7           |
|        | Contralateral              | 39.0                     | 514.8                   | 128.3                | $\pm$ 74.0           |
| RB-160 | Focus 105                  | 44.6                     | 389.6                   | 161.6                | $\pm$ 61.6           |
|        | Parafocus 98               | 54.4                     | 465.1                   | 176.7                | $\pm$ 84.7           |
|        | Contralateral 99           | 73.7                     | 555.0                   | 166.4                | $\pm$ 78.6           |
|        | Contralateral 100          | 58.1                     | 530.1                   | 154.5                | $\pm$ 78.5           |
| RB-163 | Focus 104                  | 44.5                     | 322.7                   | 140.9                | $\pm$ 53.9           |
|        | Parafocus 102              | 51.0                     | 225.9                   | 116.6                | $\pm$ 39.1           |
|        | Contralateral 113          | 36.5                     | 290.8                   | 115.1                | $\pm$ 50.3           |
|        | Contralateral 151          | 44.2                     | 286.8                   | 111.6                | $\pm$ 41.4           |
| RB-165 | Focus 104                  | 33.7                     | 269.4                   | 121.9                | $\pm$ 47.3           |
|        | Parafocus 106              | 55.9                     | 318.2                   | 124.4                | $\pm$ 46.9           |
|        | Contralateral 112          | 41.3                     | 335.8                   | 113.7                | $\pm$ 50.6           |
|        | Contralateral 120          | 38.6                     | 320.5                   | 111.7                | $\pm$ 49.4           |
| RB-166 | Proximal to lesion         | 111                      | 58.9                    | 299.1                | 129.5                | $\pm$ 52.5           |
|        | Distal to lesion           | 103                      | 63.7                    | 245.7                | 119.7                | $\pm$ 40.0           |
|        | Contralateral 125          | 46.5                     | 316.8                   | 127.3                | $\pm$ 50.0           |
|        | Contralateral 104          | 69.9                     | 306.7                   | 134.8                | $\pm$ 45.2           |

for the two chronic monkeys (RB-163 and RB-165), the number of GAD-positive somata at the focus was significantly less than the number at the control sites ($P < 0.01$), but not significantly different than the number at the parafocus. No statistically significant difference between any of the values was found for the pre-seizure (RB-161), acute (RB-160), and control (RB-166) monkeys.

Since a decrease of GABAergic neurons occurred proximal to chronic epileptic foci, we were interested in knowing if a particular type of neuron was preferentially lost. To test this notion, we calculated somal areas in each of the 4 examined sites. Average somal area and size range at each site varied from monkey to monkey (Table II). The smallest GAD-positive neuron found was 33.7 $\mu$m$^2$; the largest was 555.0 $\mu$m$^2$. In the pre-seizure and acute epileptic monkeys, the average area of neurons was markedly larger than that of the two chronic and the control animals. Data on areal distributions were graphed (Fig. 5) and apparent trends were noticeable. Distribution of GAD-positive cell bodies according to area was similar for each site, but small cells in the range of 50–150 $\mu$m$^2$ displayed a decrease in frequency at epileptic foci, whereas larger cells (200–250 $\mu$m$^2$) appeared to be less affected. Statistically, the standard deviations of the distributions were so large that these differences were probably not significant.

As an experimental control, a fifth monkey was given a surgical lesion in area 4 of one cerebral hemisphere. This animal did not display any seizure activity prior to sacrifice. It also did not show extensive
glial scarring at the lesion site, as did the monkeys injected with alumina gel. The numbers of GAD-positive neurons at the 4 sites examined in the control monkey were very similar (Table I) and indicated that GAD-positive neurons were not decreased in number at the site of the surgical lesion. Areal distribution was approximately the same in each of the 4 sites examined for this control monkey (Table II).

DISCUSSION

The results of this study are consistent with many previous studies that have described the GABAergic neurons and terminals in the cerebral cortex. These results also confirm the previously reported decrease of GABAergic terminals at chronic epileptic foci. Most importantly, the present study demonstrates a loss of GABAergic neuronal somata at epileptic foci. These findings are discussed in the following sections.

GABAergic neurons in the cerebral cortex

The analysis of the shapes, sizes and distribution of GABAergic neurons in the cerebral cortex as demonstrated with immunocytochemical methods has been the subject of numerous papers in the past few years. We first demonstrated GABAergic axon terminals and a heterogeneous population of GABAergic somata in the rat visual cortex and determined on the basis of Golgi-electron microscopic data that these neurons were aspinous and sparsely-spinous stellate cells. Our subsequent analysis in the monkey sensorimotor cortex showed a similar finding. However, colchicine injections were not used in that analysis to reveal the GABAergic neuronal somata. The distribution of axon terminals and the fact that they formed symmetric synapses provided adequate assurance that these neurons were non-pyramidal cells. The location of many terminals adjacent to pyramidal cell bodies indicated that basket cells were a major GABAergic cell type that could provide a powerful inhibition of the projection neurons of the cerebral cortex.

Other studies have confirmed these initial findings for the rat parietal cortex, cat visual cortex, and monkey visual and sensorimotor cortex. The most thorough quantitative analysis previously made of the sizes of GABAergic neuronal somata in monkey motor cortex was by Houser et al. The mean areas and ranges for GABAergic cells in our study are remarkably similar to the ones published in that study. This similarity is significant because different antibodies were utilized for these two immunocytochemical studies. Houser et al. utilized an anti-GAD serum produced in rabbit against purified GAD from mouse synaptosomal preparations, whereas our study used an antiserum raised against GAD in sheep. One further difference between these two studies was the fact that the present study did not require colchicine injections to produce somal staining. This difference may represent an advantage because colchicine may cause alterations to the internal structure of neurons (see ref. 5 for a review).

GABAergic terminals at epileptic foci

The distribution of GAD-positive puncta in the normal cortex and the cortex contralateral to alumina gel applications was similar to that previously described. These puncta, which have been shown to be axon terminals in electron microscopic preparations, are concentrated along the somata, proximal dendrites and axon initial segments of pyramidal neurons. In addition, they are found adjacent to non-pyramidal somata and scattered in the neuropil. The distribution and number of GAD-positive puncta at the epileptic foci are dramatically changed. Although the present quantitative study of somata did not include an analysis of GAD-positive puncta, the high magnification light micrograph of an epileptic focus indicates a reduction of these puncta. These findings are consistent with our previous quantitative analysis.

GABAergic neuronal somata at epileptic foci

The results of this study show that monkeys with alumina gel injections had reductions in the number of GAD-positive neuronal somata at epileptic foci. Only two monkeys demonstrated statistically significant decreases and they were the chronic ones that
had seizures for a period of several weeks or more. These findings are consistent with the previously described neuronal loss at alumina gel epileptic foci\textsuperscript{6}. Alumina gel must play some role in damaging GABAergic neurons because the surgical control did not display any loss of GAD-positive cells. Sloper et al.\textsuperscript{25} have shown that terminals with features similar to GABAergic terminals are sensitive to ischemia, and it is possible that alumina gel affects the vascular supply of the cortex to cause an ischemic insult that is selective for certain GABAergic neurons\textsuperscript{21}. The more active GABAergic neurons would be most susceptible to ischemia because they would have a higher demand for oxygen and nutrients. Since terminals that form synapses with pyramidal somata and axon initial segments have more mitochondria per terminal than other terminals in the cortex\textsuperscript{20,21}, it is likely that basket and chandelier cells are selectively affected if we assume that the number of mitochondria in a terminal is related to its activity. Nevertheless, since the magnitude of the decrease in GABAergic neurons is 35–50% in the chronic monkeys, we can speculate that a critical number of functional GABAergic neurons (70–75% of total) is required for the prevention of seizures in motor cortex.

The data on the changes in the number of GABAergic neurons at the parafocus are more difficult to interpret. Only the chronic monkeys displayed a decrease in the number of GABAergic somata at this site. This decrease was greater in the monkey with a 6-week record of daily seizures (RB-165) than in the animal that had seizures every other day for 3 weeks (RB-163). These data suggest that GABAergic neuronal loss may spread from the focus as the length and severity of seizures increases. Such a notion would be consistent with previous data in human clinical studies which show that seizures beget more seizures\textsuperscript{7,30}. This finding indicates the importance of prescribing effective anti-epileptic drugs for patients following their initial seizures. Surgical removal of the focus may provide an excellent treatment for focal epilepsy that is not controlled with drugs because the surgery itself does not cause a seizure focus\textsuperscript{7}. In our study, the size of the control lesion that produced no reduction of GABAergic somata was as large as the alumina gel granuloma that caused a significant decrease of GABAergic neurons. Thus, a gross loss of whole brain is not epileptogenic but a preferential loss of GABAergic neurons in cerebral cortex due to ischemia or some other insult may be the basis for epileptic activity in focal epilepsy.

The analysis of the sizes of GABAergic neurons at the focus and the contralateral cortex which lacks epileptic activity indicates that the small cells are more severely lost at epileptic foci than the larger GABAergic neurons. These data do not imply that large GABAergic cells are not reduced in number. All sizes of GABAergic neurons are decreased at the epileptic foci. However, the data indicate that a greater proportion of smaller cells is lost. Since chandelier cells are small and basket cells are large, these data may indicate that both of these GABAergic cell types are decreased at epileptic foci. Such a prediction was made based on the analysis of the axon terminal loss at epileptic foci in this same model\textsuperscript{20,21}. Therefore, the data from the present study do not provide an indication of the GABAergic cell types that remain at the epileptic foci. An analysis with a combined Golgi and immunocytochemical method\textsuperscript{4,26} may help to determine such information.

In conclusion, the results of this study add further data to demonstrate a GABAergic deficit at sites of focal epilepsy in the cerebral cortex. Also, they demonstrate that a loss of GABAergic somata is associated with the preferential loss of GABAergic terminals at epileptic foci.

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