White Matter Neuronal Heterotopia in Temporal Lobe Epilepsy: A Morphometric and Immunohistochemical Study

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Abstract. A frequent abnormality in temporal lobes (TL) resected for pharmacoresistant epilepsy is the presence of heterotopic neurons within white matter (WM). We compared heterotopic neuron density in 22 TLs surgically resected from epilepsy patients with TLs from 22 non-neurologic cases obtained at autopsies. Neuronal density was assessed on LFB/PAS-stained and parallel sections immunostained for microtubule-associated-protein-2 (MAP-2). The white matter area was outlined by an image analysis system. Neurons, identified by morphologic features, were counted within the marked area. Results are expressed as mean ± SD neurons/mm². LFB/PAS sections: Epilepsy cases 4.11 ± 1.86, Autopsy (normal) 2.35 ± 0.96; MAP-2 sections: Epilepsy cases 4.08 ± 1.22, autopsy (normal) 1.68 ± 0.92 (significant at 0.05 level by Wilcoxon's Rank Sums test). The lower number of MAP-2-immunopositive neurons in the control group as compared with the histologically identified group is likely the result of antigen degradation resulting from an increased postmortem interval. These results indicate that normal TLWM contains a heterotropic population of neurons, and that this neuronal density is significantly higher in epilepsy patients. It is felt that this increased neuronal density is an epiphemomenon rather than the cause of seizures, and may be the result of decreased white matter either secondary to disruption of myelination, or loss of neurons as part of mesial temporal sclerosis.

Key Words: Epilepsy; Heterotopic neurons; MAP-2; Morphometry; Temporal lobe white matter.

INTRODUCTION

Epilepsy is the second most common neurologic disorder and is a major cause of morbidity worldwide (1). Various forms of epilepsy have been described in the medical literature; however, the association of epilepsy with localized temporal lobe (TL) pathology has been long recognized. Approximately half of all epileptics have seizure foci localized to the TL and up to 50% of these patients have pharmacoresistant seizures (1). Chronic complex partial seizure activity that is refractory to pharmacologic therapy is often treated surgically via temporal lobectomy, microdissection of the amygdala and hippocampus, or a neocortical resection (2). Histopathologic examination of these surgical specimens has yielded a significant body of information. In a review of four large studies evaluating 990 specimens, Ammon’s horn sclerosis, neoplasia, and vascular or glioneuronal malformations were the most common lesions identified (1). No pathologic abnormalities were identified in 1.8% (3) to 26.2% of the cases (4).

An infrequent occurrence described in TL specimens is the presence of heterotopic neurons in white matter. This finding is often included in the term microdysgenesis, a term used to describe microscopic malformations, and may variably constitute minor or significant components of the pathology (2–9). However, controversy exists over their pathologic significance (1, 10–12). In order to assess the significance of these heterotopic neurons and their possible role in seizure pathology, numerical limits for “normal” controls needed to be clearly defined. With this data, evaluation and relevance of neuronal heterotopia can be reliably correlated to clinical neuropathology of surgically resected TL specimens from patients with epilepsy.

In an earlier study we have demonstrated that subcortical white matter neuronal heterotopia was present to a very limited extent in normal occipital and frontal lobes (OL and FL). On the other hand, neuronal density in normal TL white matter was significantly higher (TLWM 2.3 neurons/mm² vs 0.3 neurons/mm² for OL and FL white matter) (13). Thus having established baseline values in normal brains from patients with no clinical or neuropathologic evidence of seizures, we have now applied similar methodology to the study of epilepsy specimens. Additionally, in an attempt to further improve the accuracy with which neurons could readily be identified, MAP-2 immunohistochemistry was also applied to sections. Thus, two distinct and independent measurements were obtained and compared.

MATERIALS AND METHODS

TL were examined in 2 populations: (a) in autopsy brains of 22 neurologically normal individuals ranging in age from 10 to 67 years (mean age 47.8 years), autopsied at either Shands Hospital, Veterans Administration Medical Center, or obtained through the Medical Examiner’s office, and (b) in surgery specimens of 22 patients with intractable epilepsy ranging in age.
from 6 to 51 years (mean age 34.2 years) from the Comprehensive Epilepsy Program at Shands Hospital at the University of Florida.

Four blocks (2 from each side) were obtained from TL of neurologically normal autopsied brains, taken from coronal slices at the level of the mammillary bodies. These brains had been routinely fixed in buffered formalin and specimens were embedded in paraffin. Hematoxylin-eosin–stained sections were also examined as part of the neuropathological examination to exclude other pathology.

Similarly, 4 blocks were selected from the surgically resected, right or left TL of patients with epilepsy. These specimens, obtained as part of the surgical resection, were examined in the neuropathology laboratory, and with the exception of mesial temporal sclerosis, had been determined to be without structural abnormality, “alien tissue” or neoplasm such as gangliogliomas, pleomorphic xanthoastrocytomas, or other gliomas. All 22 specimens consisted of a segment of anterior temporal cortex with underlying white matter, and in 19 cases a portion of anatomically identifiable hippocampus with or without amygdala was received. Surgical resections followed standard protocol yielding comparable TL specimens (2).

In both epilepsy and the control cases, 8 slides were prepared from each case, 4 were stained with LFB/PAS, and 4 adjacent slides were immunoreacted with MAP2 (Zymed Laboratories, Inc., South San Francisco, California). Slides were prepared for quantitation by outlining the white matter, at least 2–3 mm deep to the identifiable cortex, with permanent ink. This ensured the demarcation of white matter without incorporating neurons at the gray-white junction into the areas to be examined. This demarcated white matter area was then measured on a “Microcomputer Imaging Device” (MCID) system (Imaging Research Inc., St. Catherine’s, Ontario, Canada).

Heterotopic neurons within these areas were counted and neuronal density expressed as neurons/mm². Neurons were defined by major and minor criteria as described previously (13). These were as follows: major criteria: nucleus > 12 um, prominent nucleolus, open chromatin pattern and visible cytoplasm; minor criteria included: round nucleus, satellite glia, cytoplasmic lipofuscin and perineuronal space. Neuronal counts were independently determined either by a neuropathologist or by a resident. Random slides were independently counted by at least 2 investigators to ensure concordance between observers.

MAP2 immunohistochemistry was applied to parallel sections from all cases. Tissue sections cut at 5 microns were heat-fixed at 65°C for 60 minutes, deparaffinized, and hydrated. After rinsing in phosphate-buffered saline at pH 7.4 containing 0.3% Triton X-100, endogenous peroxidase was quenched with 0.3% hydrogen peroxide in methanol. Antigen retrieval involved microwaving tissue sections in 0.01 M citrate buffer at pH 6.0. The tissues were incubated in monoclonal mouse anti-MAP2 antibody (Zymed Laboratories, Inc., South San Francisco, Calif; dilution 1:100). This was followed by a biotinylated secondary antibody (Vector Laboratories, Inc., Burlingame, Calif), and streptavidin–complex reagent containing horseradish peroxidase (Zymed Laboratories, Inc., South San Francisco, Calif). The antibody complex was visualized using 3′, 3′ diaminobenzidine as the chromogen followed by a light hematoxylin counterstain. Slides were dehydrated through graded alcohols to xylene and coverslipped with a permanent mounting.
medium. The MAP-2 antibody is a monoclonal mouse anti-
microtubule-associated protein 2 antibody (14) that reacts with a
280 kDa cytoskeletal protein. It has been shown to clearly iden-
tify neurons by their prominent cytoplasmic immunoreactivity.
Although the antibody is directed against the somatodendritic
MAP of neurons, it also reacts to a limited degree with scattered
glial elements, and hence, additional criteria other than immu-
nopositivity were necessary. Only large (>15 micron diameter)
MAP-2-positive cells with nuclei greater than 10 microns and
usually prominent nucleoli were included. MAP-2 immunopos-
itivity was most marked in the neuronal soma, but could also
be seen to extend into neuritic processes, providing additional
morphologic confirmation of the cell type. White matter was
outlined with ink and measured by MCID as described above.
Large MAP-2 immunoreactive neurons were counted and re-
sults expressed as neurons/mm².

Additionally, random parallel sections from multiple cases
and sites were also examined immunohistochemically using the
avidin-biotin complex method. Primary antibodies included
Synaptophysin (Zymed, CA, polyclonal, 1:60), nonphosphory-
lated neurofilament (Zymed, CA, monoclonal, 1:10), and GFAP
(DAKO, CA, polyclonal, 1:5000). These immunoreactions
were performed in order to confirm the neuronal nature of the cells
being examined, and sections were not morphometrically
assessed.

Statistical analysis of data was performed using Statistical
Analysis System (SAS) v6.04 on a 486DX-based microcom-
puter system. The results were analyzed by Wilcoxon's Rank
Sums test, significant at a 0.05 level.

RESULTS

An MCID image of a surgically resected TL stained
with LFB-PAS, with the white matter outlined, is illus-
trated in Figure 1. The distinction between gray and white
matter is well defined and the outlined area specifically
excludes the immediate subcortical area, thereby mini-
mizing any inaccuracy of counts. The identification of
neurons was readily achieved on these sections using the
above defined criteria and a representative example is
shown in Figure 2a. MAP-2 immunopositive neurons
were identified in subcortical white matter by their promi-
nent cytoplasmic reaction with a well-defined nonreactive
nucleus (Fig. 2b). In addition to the larger size of
these cells, the shape and presence neuritic processes also
facilitated the identification of the cells as neurons (Fig.
2c). Results of the 2 independent morphometric assess-
ments are detailed below, expressed as mean ± SD neu-
rons/mm². Autopsy sections (nonseizure, normal con-
trols) stained by LFB/PAS had a neuronal density of 2.35
± 0.96, while epilepsy cases stained by the same method
had a significantly higher number, i.e. 4.11 ± 1.86. A
scatter plot of the mean value of the 22 normal cases,
when compared with the mean values of the epilepsy
cases by LFB-PAS method, clearly illustrates the notable
presence of heterotopic neurons in TL white matter in
both groups. Although there is some degree of overlap
between the two groups, there is a well-defined and sta-
tistically significant difference between these groups (Fig.
3). Results from MAP-2 immunoreacted sections from
epilepsy cases were similar to the LFB-PAS results with
recorded density of 4.08 ± 1.22 neurons/mm². Immu-
noreacted autopsy section counts, however, differed from
LFB-PAS counts, displaying a neuronal density of 1.68
± 0.92 neurons/mm² (Fig. 4). The difference between the
autopsy (normal) and epilepsy cases was significant by
both methods at 0.05 level when statistically analyzed by the
Wilcoxon's Rank Sums test.

The age distribution of the two groups was also com-
pared. The mean age of normal controls in our series was
45.7 years (range 10–67 years), while the mean age of
the epilepsy group was 36.5 years (6–51 years). With the
limited access to CNS tissue appropriate for the current
study, age-matched controls were only possible in a lim-
ited number of cases; thus, while the age range was com-
parable, the mean age of the normal group was higher
than the epilepsy group. As in the previous study (13),
possible age-related changes within each group were ar-
bitrarily assessed by dividing the cohort into 2 subgroups,
the youngest 11 patients in one and the oldest 11 patients
in the second, thus maximizing the age difference. Each
subgroup was then compared to its counterpart and with
the opposite groups. There was no statistical difference
between neuronal counts in the youngest 11 patients com-
pared to the older age group. Similarly, sections de-
derived from the younger control group did not differ from
those in the older age group. Comparisons of neuronal
counts were also made between controls derived from the
VA Medical Center and those derived from the Medical
Examiners office. Again, no significant differences were
identified.

DISCUSSION

The presence of heterotopic neurons in subcortical
white matter has been reported in many studies describing
the pathology of epilepsy surgery specimens (1, 2, 7–12,

Fig. 2. a. Examples of heterotopic neurons within TL white matter, with typical features of large nuclei with an open chromatin
pattern and prominent nucleolus (Arrows). Temporal lobe, autopsy (normal) specimen, LFB-PAS, original magnification 400X.
b. Examples of MAP-2-immunoreactive heterotopic neurons within temporal subcortical white matter. Note the intense cyto-
plasmic immunoreactivity in multiple large neurons within in a relatively negative background. Morphologic features typical of
large neurons as illustrated in Figure 2a are also evident in these cells. c. Additionally, the presence of neuritic processes as seen
here also facilitated the identification of these cells as neurons. Temporal lobe, surgical resection specimen from epilepsy patient,
MAP-2 immunoreaction, original magnification b: 100X; c: 400X.
15–18). The establishment of baseline levels of heterotopic neurons in subcortical white matter within different regions of normal (nonseizure) brains has only recently been documented (13). This normative data identified differences between TLWM and both OL and FLWM, revealing a higher neuronal density within TLWM.

The results presented here define a clear and statistically significant difference between neuronal density in subcortical white matter within normal TL and TL derived from epilepsy patients. The present study also adds an additional element of confirmation of the previous TL data. With regard to neuronal heterotopia in epilepsy patients, our results, while unique to the TL, have certainly been documented in other regions. Menck (17) investigated heterotopic neurons in WM of the inferior frontal gyrus from epilepsy patients and described an increase in the numbers compared with normal controls. Our study also bears similarity to the Hardmann et al study (9). The limitations of the latter study included inadequate distinction of the gray white interface and only a single criterion for the identification of neurons, i.e., cells with a lesser nuclear diameter of 10 microns. In addition to more rigorous criteria, we have also added a second method of identifying neurons, i.e., MAP-2 immunoreactivity.

The antibody used to identify neurons in this study is raised against microtubule-associated protein MAP-2 (14). Microtubules are one of the major components of the neuronal cytoskeleton, and while a variety of MAPs have been identified, MAP-2 is by far most abundant in the brain. Within the neuron, MAP-2 has been shown to be present in dendrites and cell bodies, and MAP-2 mRNA has been reported at these sites (19). There is strong evidence to suggest that MAP-2 is required both for the cessation of division and for neurite extension (20). Thus, identification of MAP-2 immunoreactivity of heterotopic neurons supports the morphologically mature appearance of these cells. The antibody very accurately identifies neurons with minimal background staining of the white matter, although immunopositive neurites are also seen. This greatly facilitated the counting process. MAP-2, however, also occasionally stained some glial elements, and it was therefore necessary to set additional criteria to ensure that only neurons were counted. While the size criteria used in this study may exclude some small neurons from being included in the results, it is extremely difficult to confidently separate the smaller elements. The utilization of LFB-PAS counts, however, greatly enhanced the reliability of results by providing a second independent morphometric measurement.

The results for MAP-2 immunoreacted and LFB-PAS–stained epilepsy surgery specimens were very similar in terms of heterotopic neuron density. However, the LFB-PAS–stained autopsy (normal) TL sections displayed a higher mean neuronal density as compared with parallel, MAP-2–immunoreacted sections. This is best explained by the fact that the surgical specimens were placed in fixative soon after resection, resulting in good antigen preservation and, hence, comparable results with both methods. The autopsy specimens had postmortem intervals ranging from 12 to 48 hours. Additionally, these specimens remained in fixative for varying intervals ranging from 8 to 14 days. Under these circumstances, there was most likely some degree of antigen degradation, which resulted in a false lower result. Neuronal staining and identification on LFB-PAS–stained sections would not be affected by the above circumstances and this method was therefore deemed to be more accurate.
In his description of neurons in the subcortical white matter of newborn mice, Cajal (21) used the term “interstitial neurons.” Investigative reports have confirmed that these neurons represent remnants of the embryonic subplate (22, 23). These neurons have also been shown to express a wide variety of neuropeptides and neurotransmitters, thus implying a significant functional role for these cells (23–25). It has been suggested that heterotopic neurons in white matter are remnants of subplate neurons and have failed to undergo programmed cell death (26). Alternatively, arrested migration of neuroblasts along radial glia, possibly secondary to injury to the latter, may be a contributing factor in the topographic distribution of these neurons (27).

The mechanisms that lead to the development of such neuronal heterotopia remain uncertain. Failed programmed cell death or aberrant migration, neither of which is mutually exclusive, may be viable explanations for these heterotopia in normal individuals. An examination for a further increase in heterotopic neurons in epilepsy patients may be derived from recent neuroimaging studies of TL of patients with epilepsy. Smaller, bilateral TL gray matter volumes have been described (28, 29) and white matter volume deficits as assessed by quantitative MRI studies have been reported in the hemisphere ipsilateral to the seizure focus (28–30). Similarly, in patients with a history of alcohol withdrawal seizures, smaller TL white matter volumes were measured (31). Thus, the decreased TL white matter volume may be a consequence of degenerating fibers in the context of neuronal loss as part of the spectrum of pathologic changes labeled mesial temporal sclerosis. In our study, 19 of the 22 cases did indeed have a neuropathologic diagnosis of mesial temporal sclerosis. In 2 of the 3 remaining cases, anatomically identifiable hippocampus was not available for examination, and in a single case, hippocampal pathology was not seen. Alternatively, if seizure activity is a very early phenomenon, the increased neuronal density may represent a measurable end point of disrupted myelination following or as a consequence of this seizure activity. In either circumstance, the higher heterotopic neuron counts in epilepsy cases in our study most likely represent increased density of “normal” heterotopic neurons within a smaller white matter volume (although absolute white matter volumes were not assessed). This explanation would therefore suggest that the increased heterotopic neuronal density is an “effect” rather than the “cause” of seizure activity and is hence better designated an epiphenomenon than a true pathologic entity.

In summary, the present morphometric analysis clearly shows that heterotopic neurons are normally present in hemispheric white matter and that there is a significant increase in neuronal density within temporal lobectomy specimens from epilepsy patients. It further supports previously reported normative data regarding TL white matter heterotopia. In the present study, we have made no attempt to correlate these findings with features such as clinical outcomes of surgery, duration, or number or severity of seizures. In the context of reported quantitative MRI studies of TL gray and white matter volumes, the likelihood of this increased neuronal density being an effect rather than a cause is also briefly addressed.

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