Scrapie Infection Diminishes Spines and Increases Varicosities of Dendrites in Hamsters: A Quantitative Golgi Analysis

R. Nick Hogan, Ph.D., J. Richard Baringer, M.D., and Stanley B. Prusiner, M.D.

Abstract. An altered morphology of neuronal dendrites has been shown to be associated with many degenerative diseases of the central nervous system (CNS). Scrapie is a CNS degenerative disorder caused by a novel infectious particle or prion. Golgi impregnation studies showed that neurons in the scrapie-infected brains of hamsters contained varicose swellings and diminished numbers of dendritic spines. In order to ascertain whether or not these differences were statistically significant, quantitative methods were applied to brain samples from scrapie-infected hamsters and compared to uninfected controls. Golgi impregnated layer III pyramidal neurons from both motor and visual cortex exhibited two types of changes in infected animals. First, loss of dendritic spines on the apical shaft of both motor and visual neurons were found from 50 to 200 μm from the cell body (p < 0.001). Second, spherical varicosities on dendritic stalks ranging from 7 to 25 μm in diameter were found. The average number of varicosities per cell was 18.1 in infected animals with varicosities on dendrites of controls numbering less than 3 per cell. Less than 2% of the control cells exhibited these varicosities, while greater than 80% of the scrapie dendrites exhibited varicosities. These changes in scrapie are similar to those reported in Creutzfeldt-Jakob and Alzheimer's disease in human patients.

Key Words: Dendrites; Golgi technique; Hamster; Scrapie; Slow viruses.

INTRODUCTION

Scrapie is a neurologic disorder of sheep and goats caused by an infectious pathogen or prion (1). The prion causing scrapie may be similar to that causing Creutzfeldt-Jakob disease (CJD) of humans (2, 3). The pathological changes in scrapie and CJD include intraneuronal vacuolation with neuronal degeneration and cell loss in the presence of a prominent astrocytic gliosis (4, 5). In both diseases, amyloid plaques can be found and recent studies suggest that these plaques may be composed of paracrystalline arrays of prions (6, 7).

Light microscopic analysis of neuronal dendrites in CJD is of interest in part because of the suggestion by Lampert et al (8) and Malamud (9) that ultrastructural vacuolar changes occur primarily within postsynaptic structures. While dendritic alterations have long been noted as a major site for pathologic change in many...
diseases characterized by retardation and dementia (10–14), alterations of dendritic architecture were only recently found in human CJD cases (15, 16). These authors noted dendritic “enlargements” and fewer dendritic spines on cortical pyramidal cells in CJD biopsies when compared with controls. Quantitative methods, however, were not employed in these studies and hence the extent of spine loss was not determined.

The present investigation was designed to assess quantitatively the dendritic change in scrapie using a controlled experimental environment. Hamsters were inoculated with the scrapie agent and killed 11 weeks later. At that time, the brains were processed for light microscopic analysis using Golgi and standard histological methods.

**MATERIALS AND METHODS**

**Source of Scrapie Prions and Inoculation of Hamsters**

Randomly bred female Golden Syrian hamsters (strain LVG/LAK, Charles River Laboratories) were inoculated intracerebrally as weanlings with 50 μl of a hamster-adapted preparation of the scrapie agent (17, 18). The inoculum contained ~10⁷ ID₅₀ units of prions as determined by end-point titration in hamsters. Scrapie-infected animals were paired with age-and sex-matched uninfected control animals.

**Histological Procedures**

The hamsters were anesthetized with ether 11 weeks after inoculation and killed by decapitation. The brain was quickly removed and hemisected along the midline. The left hemisphere was immersed in 10% formalin for analysis using rapid Golgi and hematoxylin and eosin techniques. The right hemisphere was immersed in Golgi-Cox fixative and processed according to the method of Van der Loos (19). Brains were embedded in low viscosity nitrocellulose and serially sectioned on a sliding microtome at 125 μm. All processing procedures were performed on the same day for both groups.

After one day in 10% formalin, cubes of cortex (5 mm³) from the left motor and visual areas (20) were washed in 0.01 M phosphate buffer and stained using the rapid Golgi method (21). Sections were triple impregnated by successive passages through osmium-dichromate and silver nitrate solutions. Sections were cut at 100 μm with a Sorvall tissue chopper and mounted with glycerol for quantitation of dendritic spines.

**Quantitation of Dendritic Spines**

The rapid Golgi method is considered unsurpassed for quantitative evaluation of dendritic spines (21). Coded slides of rapid-Golgi processed control and scrapie tissue sections were used for counting. Using sequential cell scanning methods and a random number generator (13), a total of 12 fully impregnated cells with apical dendrites of at least 200 μm in length were quantified in each of three animals from infected and uninfected groups. Spines were counted in 50 μm segment blocks along the apical dendrite progressing from the cell body to the terminal arborization, but not beyond 200 μm. Separate statistical analyses of spine numbers from motor and visual cortex were performed using Student’s t- and Mann-Whitney U-tests.

**Quantitation of Dendritic Varicosities**

The Golgi-Cox method is considered unsurpassed for quantitative evaluation of dendrite morphology (22). Layer III pyramidal cells with fully impregnated dendrites were chosen at random from Golgi-Cox stained sections of the right motor cortex in scrapie-infected animals. Varicosities larger than twice the diameter of the attendant dendrite were counted along each branch of the apical, oblique and basilar dendritic fields. Varicosities were counted on all branches for each of 10 cells per animal.
RESULTS

The titer of scrapie agent in hamsters killed 11 weeks after inoculation was $10^9$ ID$_{50}$ units/g of brain (23). These animals showed profound ataxia, tremor, bradykinesia and difficulty righting from a supine position. Control animals were neurologically intact.

Histopathology

Scrapie-infected hamsters possessed the neuropathologic changes commonly found in rodents with this disease (Fig. 1). Spongiform changes with vacuolation were extreme throughout the gray matter and reactive astrocytosis was severe in both cortex and hippocampus. Neuronal loss was evident in area CA$_2$ of the hippocampus and in some areas of layers I–V of the cortex. The population of granule cells, but not Purkinje cells, was decreased in the cerebellum.

Analysis of Golgi Stained Sections

Penetration of the Golgi solution was adequate in both scrapie and control material since very few dendrites trailed off as a series of dots and blood vessels were occasionally impregnated in deep as well as superficial brain regions. Blood vessels are usually the last structures to be impregnated by this method (22). Despite this fact, fewer cortical neurons were impregnated in Golgi-Cox preparations from scrapie animals than in controls (Fig. 2). However, the numbers of neurons which were impregnated in the hippocampus of scrapie animals approached that of controls. It is not clear whether this finding relates to the properties of the scrapie-infected material or to the capriciousness of the Golgi method. Those cortical neurons in scrapie-infected animals which were fully impregnated appeared to have thinner dendrites and were more irregular in their centrifugal course than those in control animals. Apical dendrites were attenuated and basilar dendritic trees often appeared to be nearly as thin as the axons.

Golgi impregnation of astrocytes was much more evident in scrapie cortex than in controls, but no difference was seen in impregnated astrocytes of subcortical white matter. Occasionally, dense clumps of astrocytes were found attached to processes of degenerating pyramidal cells in scrapie sections.

Dendritic Spines

Dendritic spines were examined in rapid Golgi sections of motor and visual cortex. Qualitatively, spine loss appeared to be more extreme on the apical and oblique dendrite systems than on the basal fields. Quantitative evaluation of spines was performed on apical dendrites of layer III pyramidal cells.

Figure 3A shows the number of spines counted in 50 μm segments along the apical dendrite of fully impregnated cells in motor cortex. Consistent with spine counts in other species (24, 25) as well as in hamsters (26), control animals exhibited a linear increase in spine numbers with increased distance from the cell soma. In contrast to human layer III cells and to hamster layer V cells (24, 25), spine density did not decline significantly as terminal arboris were approached. Spine numbers in scrapie animals increased progressively with distance from the cell body. This increase was not as great and the numbers were significantly lower at each measurement point as well as over the entire dendrite length ($p < 0.001$) compared with controls (Table 1).

Table 2 and Figure 3B show similar findings for pyramidal cells from visual cortex. While spine loss may have been related to the degree of vacuolation of the sur-
Fig. 2. Golgi-Cox impregnated sections of motor cortex of hamsters. A. Uninfected control. B. Scrapie-infected. Fewer neurons were impregnated in scrapie-infected cortex and dendritic stalks were relatively thin. An increase in astrocytes is evident in B. Note blood vessels in both sections indicating adequacy of impregnation. ×175.

Surrounding neuropil, this possibility was not analyzed directly in this study. Vacuolation of all areas of the cortex was severe with no apparent gradation in degree of spongy change within the cortical areas analyzed. Hence correlation between spine loss and vacuolation within a cortical area was not possible. Analysis of the longer apical dendrites of layer V pyramidal cells in animals less severely affected would better serve to answer this question.

Dendrite Varicosities

In addition to spine loss, varicose swellings of dendrites were found in Golgi preparations from several brain areas (Fig. 4). Detailed description of dendritic varicosities will be confined to cortical and hippocampal neurons.

Varicoses swellings were found on almost all neuron types within the cortex and hippocampus (Fig. 5A). Varicosities were not observed on isolated astrocytic processes. Those seen near astrocytes could be ascribed to background neuronal processes. The varicosities were spherical or ovoid with the long axis parallel to the dendrite cylinder, but occasionally appeared as bubble-like protrusions eccentric to the shaft.
Varicosities on terminal arborizations of pyramidal cells were usually multiple and resided on especially thin dendritic stalks. There did not appear to be a correlation between number of spines on a given dendrite and the presence of varicosities. Quantitative data on frequency of these dendritic varicosities is given in Table 3.

The size of dendritic varicosities ranged from 7 to 25 $\mu$m with the average swelling about 12 $\mu$m in diameter. There was no clear relationship between size of the varicosity and position on a dendrite branch although smaller swellings tended to occur more distally.

Infrequently, swellings similar to those found on dendrites were also seen in axons of scrapie-infected pyramidal cells (Fig. 5B). When present, they measured about 10 $\mu$m in diameter and were never found near the axon initial segment.

While quantitative methods were not employed in analysis of dendrite varicosities on hippocampal neurons, it was clear that most varicosities occurred on the basilar dendrites of the pyramidal cells in areas CA2 and CA3. Varicosities were found on terminal arborizations of dentate granule cells and on intrinsic neurons of the dentate gyrus. Axonal swellings were not seen in any region of the hippocampus.

Using a new method for counterstaining Golgi-Cox impregnated sections with hematoxylin and eosin (R. N. Hogan and S. B. Prusiner, unpublished data), it was found that regions of the hippocampus with extreme vacuolation did not contain an increase in the number of varicosities compared with those regions with less severe spongy change.

Dendritic varicosities could also be found in a small number of cells from control animals. Control cells which possessed varicosities never had more than 3 per cell. In addition, the number of cells with dendritic varicosities in controls were far fewer than for scrapie animals. Only rarely did layer III pyramidal cells have dendritic varicosities in control animals as compared with the majority of cells in scrapie
**TABLE 1**

Comparison of Dendritic Spines in Control and Scrapie-Infected Sections of Motor Cortex*

<table>
<thead>
<tr>
<th>Distance from cell body (µm)</th>
<th>Number of spines/50 µm segment ± SE</th>
<th>Control</th>
<th>Scrapie</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>9.6 ± 0.7</td>
<td>5.9 ± 0.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>24.3 ± 1.2</td>
<td>15.0 ± 1.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>39.3 ± 1.4</td>
<td>27.4 ± 0.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>47.1 ± 1.6</td>
<td>34.3 ± 1.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Quantitative analysis of spine numbers on apical dendrites of rapid Golgi stained layer III pyramidal cells in motor cortex. Twelve cells from each of three animals per group were analyzed for each point (N = 36 per group).
† p values determined using the Student's t-test with a two-tailed analysis for each distance point along the dendrite. Identical p values were obtained using the Mann-Whitney U-test with a two-tailed analysis over the entire distance.

animals. While the number of cells impregnated by the Golgi fixative was greater in control animals, it is doubtful that this factor alone was responsible for the decreased frequency of cells with varicosities in normal animals. On the contrary, this would have tended to increase the chance of finding cells with varicosities if present. Because of the rarity of control cells with swellings, they were not analyzed quantitatively.

**DISCUSSION**

This report is a quantitative investigation of dendritic spine loss in cortical neurons during scrapie infection. Our results are consistent with the qualitative impressions of other investigators who reported that dendritic spines are diminished in scrapie (16) and in cortical biopsies of CJD patients (15, 16). Beck et al (27) reported that the number of spines on Purkinje cell dendrites in monkeys with experimental kuru, as counted under the electron microscope, increased slightly over control numbers at early stages of the disease and then declined as the disease progressed.

Reduced spine density has been reported for a variety of CNS diseases. Spines are

**TABLE 2**

Comparison of Dendritic Spines in Control and Scrapie-Infected Sections of Visual Cortex*

<table>
<thead>
<tr>
<th>Distance from cell body (µm)</th>
<th>Number spines/50 µm segment ± SE</th>
<th>Control</th>
<th>Scrapie</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>10.4 ± 0.6</td>
<td>7.3 ± 0.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>27.0 ± 1.0</td>
<td>19.3 ± 0.8</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>40.9 ± 1.2</td>
<td>30.2 ± 1.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>49.3 ± 1.5</td>
<td>38.7 ± 1.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Quantitative analysis of spine numbers on apical dendrites of rapid Golgi stained layer III pyramidal cells in visual cortex. Twelve cells from each of three animals per group were analyzed for each point (N = 36 per group).
† p values determined using the Student's t-test with a two-tailed analysis for each distance point along the dendrite. Identical p values were obtained using the Mann-Whitney U-test with a two-tailed analysis over the entire distance.

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Fig. 4. Varicose swelling of dendrites in Golgi-Cox impregnated neurons in cortex of scrapie-infected hamster. × 315.

less frequent in pyramidal cells of patients with chromosomal abnormalities (28, 29) and in idiopathic mental retardation (10, 11). Spine numbers are decreased in Alzheimer’s disease and senile dementia (12, 30) and when afferent inputs to affected cells are removed (31, 32). Paula-Barbosa et al (33) found fewer spines on pyramidal cell dendrites in biopsies from patients with subacute sclerosing panencephalitis, an encephalopathy caused by measles virus.

While the degree of Golgi impregnation appeared adequate in both control and scrapie-infected brains consistently, fewer cortical cells were impregnated in the infected group. It is not clear if this finding is related simply to the fact that these brains were abnormal. A number of studies have shown that the Golgi methods predictably and randomly impregnated about 1% of all neurons (34–36). Schapiro and Vukovich (37) found that sensory stimulation increased the number of cells impregnated by the rapid Golgi method. However, mechanical injury (22) or alteration of neurotransmitter metabolism (14) did not affect cell impregnability. The only variable known to alter Golgi impregnation predictably is latency between the time of death and tissue fixation (38, 39). For our experiments, fixation was identical for both groups and took place immediately after decapitation. An apparent decrease in the number of neurons was detected in hematoxylin and eosin sections of scrapie
brains; thus, it could be argued that fewer neurons were available for impregnation. Alternatively, since it still is not known why only certain cells are impregnated by Golgi techniques (22, 38), some neurons in the extremely vacuolated scrapie-infected brains could simply have been too metabolically abnormal to allow adequate uptake of stain. It has been suggested that the metabolic state of the cortical cell prior to fixation might be an important determinant in the quality of Golgi impregnation (37, 39). Although the metabolic state of vacuolated neurons in scrapie-infected brains is unknown, 2-deoxyglucose studies have shown a widespread diminution of cerebral energy metabolism during scrapie infection (40). It remains to be determined, however, whether the finding of fewer Golgi impregnated cells in scrapie-infected hamsters is related only to the capriciousness of the Golgi stain.

The periodic varicosities of dendrites observed in scrapie-infected hamsters were numerous in all fields of the dendritic tree. While more varicosities were counted in basal fields, the relatively greater number of branches in that area suggest more swellings per unit length were present in oblique fields. The reasons for this difference remain unclear. The circuitry of the cortex suggests that specific afferent fibers synapse primarily with the basilar fields as well as with terminal and proximal portions of the apical dendrite (41, 42). Oblique dendrites and middle portions of the apical shaft obtain their input more frequently from smaller local neurons of various types. If dendritic swellings are related to deafferentation (see below), then a decrease in the input from small, local cells might be implicated rather than reductions in input from larger cells at more distant cortical and subcortical sites. Beck and Daniel (43) have suggested that smaller neurons may be more vulnerable to attack by the scrapie agent.

Dendritic varicosities observed in biopsy material obtained from CJD patients as reported by Landis et al (16) and Ferrer et al (15) differed in some respects from
TABLE 3

<table>
<thead>
<tr>
<th>Dendritic field</th>
<th>Mean number of primary dendrites</th>
<th>Mean number of swellings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per primary dendrite</td>
</tr>
<tr>
<td>Apical</td>
<td>1.00</td>
<td>1.40</td>
</tr>
<tr>
<td>Oblique</td>
<td>4.03</td>
<td>1.76</td>
</tr>
<tr>
<td>Basal</td>
<td>6.13</td>
<td>1.57</td>
</tr>
<tr>
<td>Per cell</td>
<td>11.16</td>
<td>1.62</td>
</tr>
</tbody>
</table>

* Quantitative analysis of dendritic varicosities in Golgi-Cox impregnated layer III pyramidal cells from motor cortex of scrapie-infected hamsters. Ten cells from each of three animals were analyzed.

those observed here. Varicosities in scrapie-infected animals were usually not as large as those in human CJD, but they were much more numerous and closely spaced. While it was clear that the number of varicosities in control cells were much fewer than those found in scrapie-infected cells, the fact that they were present at all suggests their origin is not specific to action by the scrapie agent. Spherical enlargements and swellings of dendrites have been seen in many pathological states: Alzheimer's disease (44), viral-induced brain tumors (45), kuru (4), CJD (8), phenylketonuria (14), infantile spongy cortical dystrophy (46), epilepsy (47), isoniazid toxicity (48), sodium chloride intoxication (49), respiratory acidosis (50), mannosidosis (51), and alcohol intoxication (52). Bernstein et al (53) have shown that experimental hemisection of spinal cord results in the progressive appearance of dendritic varicosities. They suggested that this occurs after a loss of synaptic input to the dendrite. Other workers have suggested that metabolic alterations of cells may also contribute to appearance of these neuronal swellings (50). In fact, puromycin given to animals after hemisection prevents, in large part, the appearance of dendritic varicosities, thus suggesting that active protein metabolism is required for their appearance (53).

Most dendritic swellings seen in Golgi impregnations of scrapie and CJD brain sections are presumed to be analogous to the vacuoles seen at the light microscopic level (15, 16). The dendritic swellings may be due either to effects caused by the prion or some secondary process such as loss of input to dendrites due to the death of afferent cells. The pathogenetic mechanisms responsible for vacuole formation in scrapie and CJD are unknown. Vacuolation of neurons is not coupled to scrapie agent replication since maximal titers are found in brain and retina before the appearance of pathologic changes (54, 55). Masters and Richardson (56), in their analysis of CJD cases, found that spongiform change was most apparent in disease of short duration. In cases of longer duration, neuronal loss and gliosis was more severe and seemed to mask vacuolar changes. In natural scrapie of sheep, spongiform change within the neuropil is rare, while vacuolation of individual neurons has been well documented (57). Thus, the formation of large vacuoles within the neuropil is not an obligatory part of prion infections. We have seen a patient with CJD whose brain transmitted the disease to monkeys and goats, but who had virtually no spongiform change (1). The analysis of dendritic varicosities at earlier stages in scrapie infection and in "non-spongiform" types of transmissible encephalopathy may provide some insight about basic alterations in neural membranes and the production of vacuolar changes.
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