Macromolecular Structure of Axonal Membrane during Acute Experimental Allergic Encephalomyelitis in Rat and Guinea Pig Spinal Cord

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Abstract. Axonal membrane structure during acute experimental allergic encephalomyelitis (EAE) was examined with freeze-fracture electron microscopy. Axons without myelin sheaths were prevalent within EAE spinal cords. Often these axons were associated with astrocytic processes, though membrane specializations were not observed at these sites. The demyelinated axons exhibited a highly asymmetrical partitioning of intramembranous particles (IMP), with approximately 2,000 particles/μm² on P-faces and approximately 150/μm² on E-faces. This distribution and density of IMP is similar to myelinated internodal membrane. The IMP were generally randomly distributed along the axons. However, in some regions, E-faces of demyelinated axons without paranodal-like membrane specialization in the vicinity displayed a greater than normal (approximately 500/μm²) particle density. Many of the IMP in these regions of increased density were of a large (> 10 nm) diameter. Axonal membrane bounded by a single set of paranodal oligodendroglial loops ('heminodal') was also observed, and the axolemma adjacent to the terminal glial loop exhibited a gradient of morphologies. The E-faces of presumed heminodal membrane most often displayed a moderately low density of IMP. However, in several instances, heminodal membrane exhibited a moderately high IMP density (approximately 1,100/μm²), similar to that observed within normal nodal membrane. In all cases, a high percentage of the E-face IMP within heminodal membrane were large. The results demonstrate that acute demyelination is associated with a maintenance of the integrity of certain components of the axolemma and an apparent dedifferentiation in other constituents.

Key Words: Axolemma; Demyelinating diseases; Encephalomyelitis, experimental allergic; Membrane ultrastructure; Spinal cord.

INTRODUCTION

Along the length of myelinated axons, nodal, paranodal and internodal regions of the axolemma display unique macromolecular structure that is correlated with the association of adjacent glial cells (1, 2). Moreover, myelinated axons exhibit highly specific and regionally specialized cell–cell interactions with ensheathing oligodendrocytes, and with differentiated regions of astrocytic processes (3–5). The developmental processes governing axonal membrane differentiation during myelination have been suggested to be, at least in part, independent of glial cell association (3, 6, 7). However, it is likely that full maturation of myelinated axolemma is dependent upon specific interactions with oligodendrocytes and astrocytes (8, 9). In this regard, the effect that these glial cells exert on the stability of the structurally heterogeneous...
myelinated axonal membrane, once axolemmal specializations are established, is not entirely clear.

In experimental allergic encephalomyelitis (EAE), the normal pattern of axo-glial association in myelinated fibers is severely disrupted (10, 11). Within the central nervous system (CNS), there are focal regions of infiltration by hematogenous cells, and, at these sites, the demyelination of axons is present (12–15). While the most visible effect of EAE is a stripping of myelin from myelinated fibers, the direct effect of EAE on oligodendrocytes is not fully known (14). In explants of fetal mouse spinal cord, EAE serum immunoglobulin binds to oligodendrocytic processes and myelin, but not always to oligodendrocytic cell bodies (16). Moreover, in response to EAE serum, cultured oligodendrocytes produce a profusion of processes (17). Astrocyte morphology and biochemistry are also altered during the course of acute EAE (18), with reactive astrocytes being prevalent throughout the affected tissue.

Though the profound effects of EAE on glial cells, and axo-glial association are well-documented, it is not clear what effect this disruption of normal axo-glial relations has on the highly specialized structure of previously myelinated axonal membrane. In chronically demyelinated axons, resulting from an injection of the glial toxin ethidium bromide in the dorsal columns of the spinal cord, axolemmal ultrastructure has been reported to be dependent upon the location of the axon within the lesion and association, or lack of it, with adjacent glial elements (19). However, the effect of cellular-mediated demyelination and acute axo-glial dissociation on axonal membrane ultrastructure is unknown. Moreover, it is not clear whether acutely demyelinated axolemma exhibits a membrane ultrastructure similar to chronically demyelinated axonal membrane.

To examine the macromolecular structure of the axonal membrane in acutely demyelinated axons, spinal cords from EAE rats and guinea pig were examined with freeze-fracture electron microscopy. The present results demonstrate that the axonal membrane displays a gradient of ultrastructural appearances during acute EAE, with an apparent dedifferentiation of certain components of axolemma and a maintenance of structural integrity in other constituents.

MATERIALS AND METHODS

Acute experimental allergic encephalomyelitis (EAE) was induced in experimental animals according to a previously described protocol (20). Adult Lewis rats were injected with 0.25 ml of Freund's incomplete adjuvant containing 1 mg lyophilized guinea pig central nervous system (CNS) myelin and 1.0 mg Mycobacterium tuberculosis H37Ra, divided between the pads of the two hind feet. Crude myelin was prepared as previously described (20). In addition to rats, EAE was also induced in a guinea pig. Upon reaching a weight of 200 g, the guinea pig was injected with 0.5 ml of Freund's incomplete adjuvant containing 0.25 g homologous spinal cord and 5 mg Mycobacterium tuberculosis H37Ra in multiple sites in the nuchal region. At the time animals were killed for ultrastructural examination, all animals displayed clinical signs of acute EAE (i.e. quadriplegia, sialorrhea, incontinence and weight loss). This occurred approximately 12–14 days following injection of rats, and one month after guinea pig injection.

The animals were anesthetized with chloral hydrate and perfused through the heart with an isotonic saline solution at room temperature. Upon replacement of most of the blood, perfusion was continued with a solution containing 2% paraformaldehyde and 2% glutaraldehyde in 0.14 M phosphate buffer (pH 7.4). Following in situ fixation for one hour (h), lumbosacral spinal cords were excised, sectioned into approximately 2 mm slabs, and placed in fresh fixative.

Tissue for thin section examination was fixed overnight at 4°C, rinsed several times in phosphate buffer, and post-fixed in 2% OsO₄ in buffer for one h at 4°C. Following dehydration
in graded alcohols, the tissue was embedded in Epon-Araldite. Thin sections were placed on Formvar-coated slot grids and stained with uranyl acetate and lead citrate.

Tissue for freeze-etch fracture investigation was fixed for two to three h at 4°C, rinsed several times in phosphate buffer, and then cryoprotected, first for one to two h in 10% glycerol in phosphate buffer and then overnight in 30% glycerol in buffer. The tissue was placed on specimen supports and frozen in a slush of Freon 22. Samples were fractured in a Balzers 301 freeze-etch unit at a temperature of −115°C and a vacuum below 2 × 10⁻⁶ Torr. Platinum (approximately 2 nm) was evaporated over the exposed surface at an angle of 45°, and the replica was stabilized with a layer of carbon. Replicas were cleaned, first in bleach and then in dichromic acid, rinsed in double-distilled water, and placed on Formvar-coated 200 mesh grids. Thin sections and freeze-fracture replicas were examined with a JEOL 100 CX electron microscope. Methods for quantification of intramembranous particles have been described previously (21).

RESULTS

Previous light and electron microscopic descriptions (12–14, 22–24) of acute experimental allergic encephalomyelitis (EAE) have shown that affected regions are dispersed throughout spinal cord tissue, with the greatest inflammatory response and demyelination occurring in perivascular regions. Demyelination has been reported to be more prevalent within spinal cords from EAE-affected rats than from guinea pigs (13, 25). Similar observations were made in this study. However, since the axolemma of demyelinated fibers, or of axons displaying disrupted axogial association, exhibited similar patterns of ultrastructure in rat and guinea pig spinal cord, this report focuses on the macromolecular structure of demyelinated axolemma rather than on possible species differences of the onset and course of the disease.

In longitudinal sections of spinal cord, several morphological patterns of demyelination were observed. In some fibers, myelin sheaths were generally intact, but had become dissociated from the underlying axon for a portion of the length of the fiber (Fig. 1). In most of these instances, no cellular processes were observed intervening in the space between the axon and dissociated myelin sheath. Other axons displayed greater effects of EAE, with myelin sheaths being completely stripped from these axons (Fig. 1). These demyelinated axons exhibited regions that were completely bare, as well as segments that were associated with macrophages or astrocytic processes. Macrophages in the vicinity of demyelinated axons often contained large amounts of myelin debris and lipid inclusions, while astrocytic processes contained abundant glial filaments.

In demyelinated regions, occasionally only a single set of terminal oligodendroglial loops was observed associated with an axon (Fig. 1). At these 'heminodal' sites, the remaining set of terminal glial loops often displayed altered morphology (Fig. 1, inset). Some paranodal loops were detached from the underlying axolemma, in some cases extending outwards from the fiber, while adjacent glial loops maintained a normal relationship with the axon. In some instances, cellular processes were observed intervening between the axonal membrane and terminal paranodal loops (Fig. 2). At lower magnification (not shown), these processes originated from macrophages containing abundant myelin debris and lipid inclusions. Moreover, these processes did not contain glial filaments. In heminodal regions, it is difficult to unequivocally determine the location of previously nodal membrane due to the possible retraction and phagocytosis of the most terminal oligodendroglial loops. However, axonal membrane adjacent to heminodal terminal loops usually lacked electron-dense subaxolemmal undercoating (Fig. 3). While several patches of apparent subaxolemmal densification were observed, it was not possible to unequivocally identify these
Fig. 1. Longitudinal section of demyelinated axons from EAE spinal cord. The myelin sheath of an axon (A₁) is separated from the axon for part of its length (asterisk) and remains attached in other regions. A heminodal region with one set of terminal paranodal loops (arrowheads) is apparent. Distant from the terminal loops, the axolemma is not ensheathed by myelin (arrows). Two adjacent axons (A₂, A₃) are completely without myelin sheaths. Macrophages (M) containing lipid inclusions and myelin figures are in close association with
regions as possessing similar undercoating to nodal membrane. These areas of node-like membrane specialization were relatively punctate and never as extensive as that observed at nodes. Cytoplasm within the terminal loops at heminodal regions was invariably electron dense (Figs. 2, 3), in contrast to the terminal loops in unaffected regions of the spinal cord.

In freeze-fracture replicas, focal regions of the spinal cord affected by EAE were readily discerned. These areas were most often located near blood vessels, and within the surrounding space macrophages and reactive astrocytes were abundant. Demyelinated axons were prevalent within the focal areas affected by EAE. However, adjacent to demyelinated axons, normal-appearing myelinated fibers were also observed. In fibers unaffected by EAE, myelinated internodal axolemma exhibits a highly asymmetrical partitioning of intramembranous particles (IMP) between the P- and E-fracture faces (Fig. 4a, b). P-faces have an IMP density of approximately disrupted myelin sheaths. $\times 6,500$. Scale bar, 2 $\mu$m. Inset. The disrupted paranodal loops of the heminodal region are shown at increased magnification. Some of the loops are not in contact with the axolemma but turn outwards (arrowheads). $\times 35,000$. 

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2,000/μm², while E-faces have relatively few particles (approximately 150/μm²). These IMP densities are similar to those in normal rat spinal cord (3). At sites distant from paranodal specializations, particles on both fracture faces appear to be randomly distributed, and a relatively low percentage of large (>10 nm) diameter IMP are scattered among the smaller particles.

In EAE spinal cord, axons were judged to have been previously myelinated based on their diameter. Since virtually all unmyelinated axons in the CNS have axonal diameters considerably smaller than <1 μm (26, 27), fibers with diameters >1 μm were considered to be demyelinated axons. Demyelinated axolemma consistently exhibited a highly asymmetrical partitioning of IMP (Figs. 5, 6). P-faces of these demyelinated fibers displayed a high (approximately 2,000/μm²) density of IMP, and, in most regions, E-faces of demyelinated axons had relatively low densities of IMP. On both fracture faces, the particles appeared to be randomly distributed, and discrete aggregations of IMP were not observed. Astrocytic processes often appeared to envelope the demyelinated axons, though axolemmal specializations were not observed at these sites (Figs. 5, 6). Astrocytic processes were identified on the basis of orthogonal arrays of P-face particles ('assemblies') (28) on their membrane.

There were some regions of the E-face axolemma of demyelinated fibers where
the particle density was elevated compared to the generally low density of IMP observed on E-faces of these axons (Fig. 7). These areas were not punctate, but displayed a generalized increase in particle density for up to approximately 10 μm along the length of the axon. The density of IMP in these regions was greatest (approximately 500/μm²) in the central portion of these areas, with a gradient of decreasing particle density extending from the central region until background values were reached. Many of the IMP in these areas of increased E-face particle density were of a large (>10 nm) size, similar to the size distribution of IMP at nodes of Ranvier. The regions of increased particle density did not appear to be related in a specific manner to astrocytic processes or other cell types.

In freeze-fracture replicas of EAE spinal cords, axolemma displaying characteristics consistent with those observed in longitudinal thin sections of heminodal regions was encountered (i.e. bounded on only one side by terminal oligodendroglial loops). Examples of both P- and E-face heminodal axolemma were observed. However, since the E-fracture face of myelinated axonal membrane exhibits a high density of IMP only in nodal regions (Fig. 8), axolemma with nodal characteristics (i.e. high density of IMP, high percentage of large IMP) is more clearly apparent when examining the E-faces of demyelinated axons, in contrast to the more uniformly particulate P-faces. In most sites of presumptive heminodal membrane, axolemma adjacent to terminal glial loops had a much lower density of E-face IMP than normal.
Fig. 5. Freeze-fracture replica of demyelinated axon from EAE spinal cord. The myelin sheath of this large diameter (>1 μm) axon is no longer present, and an astrocytic process (asterisks) is closely associated with the desheathed axon. At increased magnification (not shown), orthogonal arrays of P-face IMP were observed on the astrocytic membrane. The P-face (PF) of the demyelinated axon has a high density of IMP, whereas the E-face (EF) has a low density of particles. ×40,000. Scale bar, 0.5 μm.

Nodal membrane (Figs. 9, 10). The density of particles in these regions appears intermediate between E-face densities observed for nodal and internodal membrane (Figs. 4b, 8). Many of the IMP in the juxta-heminoal regions are of a large (>10 nm) diameter, similar to those occurring within nodal membrane. In Figure 9, a small portion of the axolemma opposite the remaining set of presumed terminal loops is indented by an underlying process, and, at this site, paranodal-type specialization of the axolemma is observed. Faint linear striations (similar to those in paranodal axolemma) are observed on the axolemma where the underlying process indents the axonal membrane. It is not possible to identify the underlying process responsible for the indentation of the axolemma. However, it is most likely that the process is oligodendrocytic in origin, as such axolemmal ultrastructure has only been associated in the CNS with this type of glial contact. Consistent with thin section observations, astrocytic processes are present in proximity to the heminoal membrane. On the P-faces of these astrocytic processes, there are a large number of assemblies similar to those reported on the membrane of reactive astrocyte processes (29, 30).

Several examples of heminoal membrane in which E-face axolemma displayed a high density of IMP were observed (Fig. 11). Terminal paranodal loops are apparent on one side of the region, and the unensheathed axolemma adjacent to the loops has a high density of particles extending for at least 1.5 μm away from the last glial loop. The distribution of IMP is not uniform in the heminoal region, but a portion of the heminoal membrane is particle-poor. Many of the particles in the heminoal region are of a large size (Fig. 11, inset), similar to those reported in normal nodal membrane.
Fig. 6. Replica of demyelinated axon from EAE spinal cord. a. The demyelinated axon appears surrounded by astrocytic processes (asterisk). P-(PF) and E- (EF) fracture faces of the demyelinated axon are exposed. ×45,000. Scale bar, 0.5 μm. At increased magnification, the high density of IMP on the P-face (b) is apparent, whereas few particles are present on the E-face (c). Some of the particles on each fracture face are of large (>10 nm) diameter (arrows). Orthogonal arrays of particles (arrowheads) are present on the P-faces of astrocytic processes. b and c, ×125,000. Scale bar, 0.1 μm.
Fig. 7. Replica of demyelinated axon from EAE spinal cord. a. The E-face (EF) of a demyelinated axon is exposed, and this fracture face exhibits a differential IMP density along its length. An astrocytic process (which displays P-face assemblies at increased magnification; not shown) (asterisk) is adjacent to the demyelinated axon. ×16,500. Scale bar, 1 μm. The areas in brackets are shown at increased magnification in (b) and (c). Note the increased density of particles in b compared to c. In both regions, some of the particles are of large size (arrowheads). ×125,000. Scale bar, 0.1 μm.

DISCUSSION

A complex set of interactions between myelinated axons and ensheathing oligodendrocytes and astrocytic processes appears to influence the macromolecular structure of the axolemma (1, 3, 31, 32). The results presented here demonstrate that the
spatially heterogeneous structure of myelinated axonal membrane is not static, but that demyelination induces alterations in some components of the axolemma, whereas other constituents of the membrane are more stable.

Thin section observations of rat and guinea pig EAE spinal cord reported here are consistent with previous descriptions of the acute form of this disease (12–15, 22, 23). Demyelination occurred primarily in perivascular regions, and an abundance of hematogenous cells were present in the lesion sites. Heminodal regions were encountered with some regularity, suggesting that demyelination does not affect the paranodes on both sides of the nodal region to an equal extent. Oligodendrocytes are known to myelinate multiple axons (33–35), though it is not known if a single oligodendrocyte myelinates sequential segments of a single axon. Thus, it may be that oligodendrocytes exhibit differential sensitivity to EAE, with segmental demyelination the result of individual oligodendrocytic susceptibility. In this regard, it has been reported that some oligodendrocytes on the surface of fetal mouse spinal cord explants bind EAE serum immunoglobulin, whereas those oligodendrocytes within the explant do not (16). Furthermore, EAE serum immunoglobulin is always bound to oligodendrocytic processes and myelin. These data suggest that oligodendrocytes exhibit regional specialization of their plasmalemma, and perhaps indicate a basis for differential susceptibility to EAE.

In regions distant from paranodal-type axolemmal specialization (i.e. from hemi-
Fig. 9. Replica of probable haminodal axon from EAE spinal cord. a. The fracture plane exposes the E-faces of axonal membrane (EF), the most terminal glial loop (asterisk) and adjacent glial loop (eG). In the axonal membrane adjacent to the terminal glial loop, the density of E-face IMP is much lower than that observed at normal nodes (cf. Fig. 8). An astrocytic process (arrowheads) is in close proximity to the axolemma; at increased magnification (not shown) the astrocytic membrane contained assemblies. ×50,000. Scale bar, 0.5 μm. The area in brackets is shown at increased magnification in (b) and (c). b. Adjacent to the glial loops, the axonal membrane has a relatively low density of IMP. Several of the particles are of large diameter (arrowheads). c. In the region indented by an underlying process,
nodal areas), the macromolecular structure of demyelinated axonal membrane is similar to that of myelinated internodal membrane. Demyelinated and internodal axolemma exhibit similar densities of P-face particles, with approximately 2,000 IMP/μm² for each. These results suggest that the P-face ultrastructure of axolemma is not dependent, at least in terms of IMP density, upon myelin ensheathment for its integrity. In this regard, it has been reported that optic nerve axons display a marked increase in P-face particle density at about the time of oligodendroglial ensheathment (7). This observation might be interpreted as suggesting that oligodendrocytic association induces ultrastructural changes in axonal membrane. Such an inductive action of one cell type on the membrane structure of an adjacent cell

the axonal membrane exhibits faint linear striations (arrows) oriented parallel to the long axis of the axon. The appearance of this membrane is similar to E-face axolemmal specialization at paranodes. b and c, ×125,000. Scale bar, 0.1 μm.
Fig. 11. Replica of heminode from EAE spinal cord. The P-face (pPN) and a small portion of E-face (asterisk) of paranodal axolemma adjacent to the E-face (EF) of heminodal membrane is apparent. Arrowheads indicate the boundary between paranodal and heminodal membrane. The heminodal membrane exhibits a region with a high density of IMP. Note a portion of the E-face of adaxonal glial membrane with characteristic linear striations is apparent (arrow). × 50,000. Scale bar, 0.5 μm. Inset. The heminodal region is shown at increased magnification. Many of the IMP are of large size. Some of the heminodal membrane has a low density of particles. ×125,000. Scale bar, 0.1 μm.

may be similar to the clustering of acetylcholine receptors in muscle membrane following innervation (36, 37). However, recent studies indicate that the change in P-face membrane structure is governed by inherent qualities of the axon, and not by association with oligodendrocytes. In glial cell deficient environments, resulting

from irradiation or treatment with mitotic inhibitors during early gliogenesis, axons that would normally be myelinated, save for the paucity of myelinating cells, display P-face particle densities similar to those of age-matched, normal internodal membrane (3, 38). Thus, it appears that the factors governing the macromolecular structure of the P-face of axonal membrane, in regions destined to become myelinated, are independent of glial ensheathment, with a similar ultrastructure being observed for internodal membrane, axolemma deprived of glial association during development, or demyelinated axonal membrane.

While the ultrastructure of internodal membrane does not appear to be altered following demyelination, the stability of nodal membrane may be more sensitive to association with glial elements. At normal nodes, there is a moderately high density of IMP on both E- and P-fracture faces (approximately 1,100–1,400/μm²) (7, 8, 39). Moreover, a dense subaxolemmal undercoating is observed beneath nodal membrane (40, 41). In contrast, axolemma adjacent to the terminal glial loop in heminodal regions of EAE-affected spinal cords exhibited a gradient of E-face IMP densities, ranging from a moderately high to a moderately low density. This diversity of heminodal membrane ultrastructure is similar to that observed in heminodal regions of mature frog optic nerve (42). The relatively low E-face IMP density observed adjacent to heminodal axolemma of EAE tissue suggests that, following removal of one set of terminal oligodendroglial loops, certain components of nodal membrane diffuse away from the region. This hypothesis is consistent with the general lack of dense subaxolemmal undercoating in these regions. The observation that some heminodal regions appear to have a node-like E-face IMP density in the juxtaheminodal region suggests that diffusion of the particles away from nodal regions does not occur immediately after loss of paranodal specialization. In this context, in freeze-fracture studies of anti-galactocerebrosidase-induced demyelination, nodal specialization was reported to persist shortly after demyelination, though the nodal specialization was lost at later times (43).

In EAE-induced demyelination, all fibers are not affected in a synchronous manner, since a gradient of morphological appearances was observed, ranging from completely demyelinated axons to slightly disrupted axo-glial association. Thus, heminodal regions with a high density of E-face IMP may represent the most recently demyelinated segments of the axon. The area of increased E-face IMP density observed in demyelinated axollemma without apparent paranodal-type axonal membrane specialization (Fig. 7) may be the result of demyelination on both sides of a nodal region, with a slow diffusion of the E-face particles away from the previously nodal or paranodal sites. Alternatively, it must be considered that the axollemma adjacent to the heminodal terminal loops was not previously nodal membrane. Given the degree of disruption of axo-glial association in EAE tissue, it is conceivable that retraction and phagocytosis of the most terminal paranodal loops occurs. Thus, membrane adjacent to the existing terminal loops was previously paranodal, and, as such, it would not be expected to possess a high density of E-face IMP. However, aggregations of E-face particles with node-like densities were not observed within demyelinated axollemma, as would be expected if nodal membrane remained intact following removal of both sets of limiting paranodal loops.

The apparent loss of E-face particles from demyelinated nodal regions may be the result of the removal of a physical barrier (i.e. paranodal loops), or the result of some secondary effect, such as an alteration of the subaxolemmal cytoskeletal elements that may anchor particles within the nodal region (44). In this context, at neuromuscular junctions, it has been observed that there is a decrease in the density

of acetylcholine receptors at the synaptic gutters following denervation (45–47). Recently, it has been proposed that postsynaptic cytoskeletal elements play an important role in the clustering of ACh receptors (48). However, the molecular mechanisms governing ACh receptor clustering, and the involvement of cytoskeletal elements in this event, is far from resolved. Likewise, the factors influencing nodal membrane integrity are not fully understood. However, discrete aggregations of E-face IMP with node-like densities are not observed within demyelinated axolemma in EAE, suggesting that cytoskeletal elements do not anchor E-face IMP at specific sites in these pathological axons. Alternatively, except for one report on dysmyelinated spinal roots (49), axolemmal E-face IMP particle aggregations with node-like properties (i.e. high IMP density and high percentage of large particles) have not been reported except in association with glial elements (9, 50–52). Thus, these observations strongly suggest that nodal membrane development and maintenance represents a complex interaction between axon and glial cells.

The present ultrastructural reports on acutely demyelinated axons in EAE do not reveal generalized increased E-face IMP densities, as are seen in glial cell-deprived axons of the irradiated spinal cord (3). If some or most of the E-face axonal membrane IMP are related to voltage-sensitive sodium channels (8, 39), such increased IMP densities may provide a morphological correlate for the continuous conduction (53) observed in demyelinated fibers. Our results also provide no evidence for the development of “phi-nodes,” or clusters of sodium channels that are thought to underlie non-uniform conduction in other demyelinated fibers (54). In this respect, the ultrastructural results in acutely demyelinated axons in EAE are consistent with the clinical picture with quadriplegia. It is likely that conduction block occurs in acutely demyelinated axons in EAE. It is not clear, from the present results, whether reorganization of sodium channels, as described in chronically demyelinated axons in other model systems (55, 56), occurs in long-term demyelinated axons in EAE.

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