The Formation Patterns of Central Myelin Sheaths in the Myelin Deficient Mutant Shiverer Mouse

By

YOSHIRO INOUE, REIKO NAKAMURA, KATSUHIKO MIKOSHIBA*,
and YASUZO TSUKADA*

Department of Anatomy, Hokkaido University School of Medicine,
Kita 15-jo, Nishi 7-chome, Kita-ku, Sapporo 060
*Department of Physiology, School of Medicine, Keio University
Shinano-machi, Shinjuku-ku, Tokyo 160, Japan

—Received for Publication, November 20, 1981—

Key words: Shiverer, Myelin formation, Central nervous system, Mutant mouse.

Summary: The myelin formation pattern of Shiverer mutant mice was investigated in detail by electron and light microscopy, especially with the Golgi silver-impregnation method. The myelin lamellae of Shiverer mice were mostly formed between stratified cytoplasmic sheets of oligodendroglia and their lamellae were only sporadically arranged in a spiral. We believe that some cytoplasmic sheets in these myelin sheaths might, even though in part, originate from plural processes either of the same or different oligodendroglia, and in addition, some cytoplasmic sheets might split to be multiplied in the number of stacked layers. Between these stratified cytoplasmic sheets incomplete compaction occurred to form a tri-lamellar membranous structure as we had described previously (Inoue et al. 1981), in which the major dense lines were mostly absent.

We believe that the internodal segments of myelin sheaths might be much shorter than those of normal ones either by Golgi impregnated images or by electron microscopy, and might be more numerous in number than those of the control, although myelinated nerve fibers were remarkably less. Oligodendroglia proliferated predominantly in the Shiverer mouse spinal cord. Most of them were of the mature type with an electron-dense appearance.


1. The total volume of myelin sheaths is markedly decreased.

2. In myelin lamellae, layers corresponding to intraperiod lines which are formed by the fusion of adjacent outer leaflets of the plasma membranes of oligodendrogial cytoplasmic sheets are commonly found, whereas the major dense lines are only sporadically observed. The cytoplasm of each sheet surrounding
an axon remains in varying amounts.

3. The myelin lamellae are frequently found to be formed between stacked cytoplasmic sheets, not arranged as spirals which are seen in the normal CNS.

4. These atypical myelin sheaths, on the other hand, does not completely lose the property of the central myelin, since electron-dense, intermittent dots between the axolemma and the paranodal cytoplasmic pockets at the nodes of Ranvier are present. In addition, radial densities in the myelin lamellae are precisely observed.

In normal central myelin sheaths, on the other hand, it is generally established that a single process of oligodendroglia is applied to one axon to wrap it spirally and its spirally extended cytoplasmic sheet gives rise to compaction between adjacent turns of plasma membranes to form an internodal segment of typical myelin lamellae which are composed of major dense and intraperiod lines (Bunge 1968, Peters et al. 1976, Inoue et al. 1973b, Sternberger et al. 1978). Thus, atypical myelin lamellae of the Shiverer mouse CNS give rise to the problem as to how stacked cytoplasmic sheets of oligodendroglia are formed or where they originate, since lamellae lacking major dense lines are formed by incomplete compaction between these sheets. We previously reported on the possibility that some cytoplasmic sheets multiplied by splitting of a process which adhered to an axon (Inoue et al. 1981).

In this present study we intend to investigate the myelin formation patterns in detail using electron and light microscopy, especially with the Golgi silver-impregnation method.

**Materials and methods**

Shiverer mutant mice at 18 and 30 days of age, 8, 20, 21 and 22 weeks of age were used for Golgi silver-impregnation, and at 2, 4, 8 and 18 weeks of age for electron microscopy and for routine staining of paraffin sections.

As a control, heterozygously affected or non-affected mice at 2, 4, 13 and 18 weeks of age were used for Golgi silver-impregnation and for paraffin sections and at 2, 4, 8 and 22 weeks of age for electron microscopy. Balb/C strain mice were also used for Golgi silver-impregnation.

For light microscopy, the Golgi method, hematoxylin-eosin and Klüver-Barrera stainings for paraffin sections, and toluidine blue staining for epoxy-resin sections, were used. For Golgi silver-impregnation, the modified methods of Stensaas and Stensaas (1968) and Colonnier (1961) were employed. Details of these methods have already been reported (Inoue et al. 1970). For paraffin sections materials were fixed by direct immersion fixation with Bouin's solution, although some materials were pre-fixed by perfusion of the same mixed aldehyde solution as that used for electron microscopy described below. The thickness of the paraffin sections was estimated at 10 μm. Epoxy-resin sections were sectioned in 1 μm thickness and stained with heated 1% toluidine blue in 1% borax aqueous solution.

For electron microscopy, the method employed in this study was the same as that reported previously in detail (Inoue et al. 1981). Briefly, a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in Millonig solution was perfused for pre-fixation. After the spinal cord was transversely cut into sections about 0.8 mm thick, tissue blocks were post-fixed with 2% osmic acid in Millonig buffered solution followed by alcohol-dehydration and embedding in Epon 812 or Epok 812. Ultrathin sections were double-stained with 2% aqueous uranyl acetate solution and Sato's mixed lead
solution.

Observations and discussion

The posterior funiculus of the spinal cord was chosen to be observed for description.

1. Light microscopy of Shiverer mouse spinal cord.

In the posterior funiculus of the Shiverer mouse spinal cord beyond 4 weeks of age glial cells were markedly increased in number as compared to those in control animals (Figs. 1 and 2), as has previously been reported (Mikoshiba et al. 1980, Inoue et al. 1981). The type of increased glial cells could be clearly identified as oligodendroglia by epoxyresin semithin-sections stained with toluidine blue and compared by electron microscopy of the same materials. Inoue et al. (1981) have already reported that oligodendroglia were typically grouped into two types by electron microscopy: light, immature and dark, mature types. At 18 weeks of age glial proliferation was predominant and most of the oligodendroglia were of the dark type (Fig. 3).

At 18 days of age, in the posterior funiculus of the Shiverer mouse spinal cord stained by the Golgi method, cells which extended numerous, fine processes from irregularly oval soma of about 10 μm in diameter were commonly observed (Fig. 5). The processes were markedly branched and some of them were found to be branched more than five times. They tended to extend along the entire length of some 50 to 100 μm of the nerve fibers in the white matter. They revealed a smooth surface and possessed small, nodular enlargements in the course of extension, and sometimes, at the ends of some of the processes, short, reticulate-tubular structures were present. This type cell was identical to the "multi-process-cell", i.e. the immature type of oligodendroglia in young chick and rat white matter, as has been demonstrated by Inoue et al. (1973a, b). In the spinal cord of Balb/C strain mouse of 13 days of age the same type of cell was often observed (Fig. 4). After 4 weeks of age oligodendroglia of the adult Shiverer mouse were observed to be somewhat similar to those found in controls (Figs. 6, 7, 8 and 9), or in other animal species, since they possessed a round or irregularly round soma with a smooth surface, from which 10 or so processes were extended. The processes became thinner and had several nodular enlargements either along their course or at bifurcations. However they branched more often at their peripheral portions and were more tortuous than those of control animals. In addition, they were densely interwoven with other oligodendroglial processes to form complicated networks (Fig. 8). At the ends or in the course of the processes several reticulate-tubular structures were attached, from which, further, fine processes often arose (Figs. 7, 8 and 9). These tubular structures of Shiverer mouse oligodendroglia were, however, much shorter than those of young chick or rat oligodendroglia as previously reported (Inoue et al. 1973a, b). In the previous report, it was shown that using the Golgi method, silver-particles were precipitated within whole cytoplasm of both perikaryon and processes of oligodendroglia, even within outer or inner cytoplasmic sheets of myelin sheaths, when compaction of their lamellae was still insufficient (Inoue et al. 1973b). Since, in myelin sheaths of Shiverer mouse the compaction of the lamellae was incomplete and contained cytoplasm of varying amounts in each layer (Rosenbluth 1980, Inoue et al. 1981), the short reticulate-tubular structures were considered to reveal the sites of cytoplasmic layers of internodal segments of myelin sheaths. Thus, internodal seg-
ments of Shiverer mouse myelin sheaths might be much shorter than those of normal animal species. When paying attention to one tubular structure, it was demonstrated that at least two processes originated either from a single oligodendroglia (Fig. 9) or that two different ones (Fig. 8) could take part in its formation. However, it was impossible to tell whether more than two processes might be associated with one tubule or not, since the processes of oligodendroglia formed complicated networks with each other. This fact suggests that in Shiverer mouse myelin sheaths some internodal segments, even though not all of them, might be formed from plural processes of oligodendroglia which simultaneously applied to an axon, showing a considerable difference from those in the normal CNS.

2. Electron microscopy of Shiverer mouse nerve fibers.

Using electron microscopy, as demonstrated in a previous report (Inoue et al. 1981), lamellae of Shiverer mouse myelin sheaths were mostly formed between stacked cytoplasmic sheets of oligodendroglia, not between spiral turns of a single cytoplasmic sheet. However, it was impossible to demonstrate the direct continuity of stacked cytoplasmic sheets to the processes which took part in myelin formation, since the processes were too tortuous to be demonstrated within a single ultrathin section. Thus, the formation pattern of these cytoplasmic layers which would, at least in part, originate from processes, as might be presumed from the Golgi images, could not be visualized by electron microscopy.

In the oligodendroglia cytoplasmic layers which adhered to axons, one of the layers was incidentally found to be split into a few layers to form atypical myelin lamellae between them (Fig. 10). In addition, in the cytoplasm of Shiverer mouse myelin sheaths or in the cytoplasm which adhered to axon without the myelin lamellae, the membranous structures composed of trilamellar structures characteristic of Shiverer mouse myelin lamellae were sometimes observed to be isolated, in places (Fig. 11). Such isolation of the lamellae probably represented a profile of the initial portion of cytoplasmic splitting (Inoue et al. 1981). Thus, these facts from Golgi images and electron microscopy possibly suggest that in some cases, even though not all, plural oligodendroglial processes might adhere to an axon and form a single internodal segment of myelin sheath and, in addition, some of them might split and be increased in number of stacked cytoplasmic layers.

At the nodes of Ranvier of Shiverer mouse myelin sheaths, even though they were not arranged spirally, paranodal cytoplasmic pockets were frequently found to line up along the length of the nerve fibers (Fig. 12), and electron-dense intermittent dots, which were formed by thickening of the outer leaflet of the axolemma between the axolemma and paranodal pockets, lay in a row at center-to-center intervals of 30 nm. These paranodal structures were somewhat more frequently found than those in control spinal cords. This fact shows that there are more numerous internodal segments of myelin sheaths present in Shiverer mouse spinal cord than in the normal spinal cord, even though myelinated nerve fibers were less in the former, and it agrees with the findings of the Golgi impregnation images that the internodal segments of Shiverer mouse myelinated nerve fibers might be much shorter than those of normal nerve fibers. In addition, Rosenbluth (1980) reported that single oligodendroglial processes often formed axon-glial junction with intermittent dots at extraparanodal sites. These processes,
The Formation Patterns of Shiverer Central Myelin Sheaths

However, were never found to adhere to astroglial processes to form such glial junctions or myelin sheaths.

Thus, from Golgi impregnation images and electron microscopy in Shiverer mouse CNS, it was considered that oligodendroglial processes might possess the ability of recognizing and distinguishing a target axon, whereas they might lack such inhibitory activity that they avoided the nerve fibers, which were already surrounded by the other processes. And furthermore, they might possess such an affinity for the oligodendroglial plasma membrane itself that some single internodal segments were certainly associated with plural cytoplasmic processes or aberrant myelin sheaths were formed around oligodendroglial perikaryon (Inoue et al. 1981). In addition, the spiral movement of the oligodendroglial processes in myelin formation was considered to be disturbed, since spiral turns of myelin lamellae were few, even though spiral myelin lamellae were sometimes found, or single oligodendroglial processes were sometimes found, or single oligodendroglial processes were often contacted, but not spirally, with axons to form axoglial junctions at extraparanodal sites (Rosenbluth 1980). Application of plural processes to single internodal segments to single internodal segments in myelin formation might further disturb a complete spiral movement of each cytoplasmic sheet and elongation of internodal segments.

At the posterior funiculus of the Shiverer mouse spinal cord oligodendroglia proliferated, even though myelin formation was insufficient. At the posterior funiculus of the Quaking mouse, which was a myelin deficient mutant, hyperplasia of oligodendroglia has been reported (Wisniewsky et al. 1971, Friedrich 1975). During the formation of normal central myelin sheaths, active and young oligodendroglia should adhere to 10 or so target axons for myelination by a somewhat cell-to-cell interaction (Inoue et al. 1973b, Sternberger et al. 1978). After these oligodendroglia begin to form myelin sheaths, the ability to adhere to axons might be lost, and the maturation of the oligodendroglia might advance, changing their external shape from “multi-process-cell” type to typical oligodendroglial type, as observed in Golgi-stained materials. Although this procedure of changing external configuration in accordance to maturation revealed the same pattern in principle in Shiverer mouse CNS as in the normal CNS, shorter internodal segments of myelin sheaths, the presence of internodal segments formed by plural processes, and furthermore, frequent extraparanodal attachments of single oligodendroglial processes to axons to form axon-glial junctions, might result in an easy loss of affinity of oligodendroglial processes to axons in order to advance maturation. The remaining non-myelinated axons, which would have been targets for myelination, might possibly activate the proliferation of oligodendroglia.

Aknowledgement

We are particularly grateful to Dr. Lachapell, Dr. J.-L. Guenet and Dr. Baumann for their generous supply of the Shiverer mutant mice. This study was supported by the Prof. Kato Memorial Research Fund for Physiology and Medicine.

References


PLATES
Explanation of Figures

Plate I

Fig. 1. The posterior funiculus of Shiverer mouse spinal cord of 18 weeks of age. Paraffin section stained with hematoxylin-eosin. Dark-stained nuclei were mostly of oligodendroglia.

Fig. 2. The posterior funiculus of the heterozygously affected mouse (a control mouse) spinal cord of 22 weeks of age. Paraffin section stained with hematoxylin-eosin.

Fig. 3. The posterior funiculus of Shiverer mouse spinal cord of 18 weeks of age. Low magnification electron microscopy. All cells in this figure are dark, mature type of oligodendroglia.

Fig. 4. The spinal cord of Balb/C mouse of 13 days of age. An immature type of oligodendroglia, "multi-process-cell" stained with Golgi method.

Fig. 5. The spinal cord of Shiverer mouse of 14 day of age. An immature type of oligodendroglia, "multi-process-cell", stained with Golgi method.

Fig. 6. The spinal cord of non-affected control mouse of 18 weeks of age. A typical oligodendroglia, stained with Golgi method. The long arrow shows silver precipitation around an axon, probably revealing the cytoplasmic sheet of myelin sheath. The short arrow shows the contact point between a process and myelin sheath.
Plate II

Fig. 7. The posterior funiculus of Shiverer spinal cord of 22 weeks of age. Oligodendroglia stained with Golgi method in a transverse section. Arrows reveal transverse profiles of reticulate-tubular structures at the tips or in the course of processes.

Fig. 8. The posterior funiculus of Shiverer spinal cord of 22 weeks of age. Two oligodendroglia (a and b) stained with Golgi method in a longitudinal section. Processes from two oligodendroglia form a complicate network (small arrows) and a reticulate-tubular structure (arrow-heads).

Fig. 9. The posterior funiculus of Shiverer spinal cord of 22 weeks of age. An illustration of oligodendroglia by camera-lucida, stained with Golgi method. Figures 9a and 9b are light microscopic photographs of this cell in different focus levels. The arrows a and b in Fig. 9a show short reticulate-tubular structures which are formed from plural processes originating from a single oligodendroglia, as shown in the illustration Fig. 9. Two thin processes (x and y) are also associated with a reticulatetubular structure (an asterisk).
Plate III

Fig. 10. The posterior funiculus of Shiverer mouse spinal cord of 4 weeks of age. Electron microscopy of oligodendroglia associated with an axon (Ax). The arrow reveals cytoplasmic splittings forming atypical myelin lamellae (My).

Fig. 11. The posterior funiculus of Shiverer mouse spinal cord of 4 weeks of age. Electron microscopy of the oligodendroglial cytoplasm applying to an axon (Ax). The arrow shows an isolated tri-lamellar structure, of which high magnification is shown in the lower right inset.

Fig. 12. The posterior funiculus of Shiverer mouse spinal cord of 4 weeks of age. Electron microscopy of the paranodal site of the node of Ranvier. Arrows show electron-dense intermittent dots between the paranodal cytoplasmic pocket and axolemma.