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An Observation About Myelination*

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Summary. An analysis of the direction of myelination of fibers in the optic tract of a kitten shows that the direction of wrapping of neighboring fibers is not random. Adjacent fibers in contact with the same glial process tend to be wrapped in the same direction. A model for myelination is proposed to account for this observation.

Key words: Myelin – Myelination – Optic tract – Model of myelination

Introduction

Some years ago, Peters (1964) noted that there was an above chance tendency for the inner and outer tongues of the myelin sheath to lie in the same quadrant of their common optic nerve fiber of the rat or mouse. This observation was subsequently confirmed by Fraher (1972) and Sturrock (1975). Although the reason for this oddity was not clear, it did suggest that myelin growth is a controlled, phasic process. In some unknown manner, the myelin tongues must complete one cycle of wrapping, and then pause before proceeding on to the next cycle, thereby increasing the frequency of "snapshots" with the two tongues in the same quadrant. In the optic tract of the kitten, this cyclic wrapping process appears in the second postnatal week, when there is an explosive increase in the rate of myelination (Moore et al. 1976). The process is launched as if the spring of a clock has suddenly been released, for the axons become wrapped so quickly that their mean diameters tend to fall into one of two distributions. The first corresponds to the diameter of premyelinated axons, whereas the second is that for myelinated axons.

Such regularities in the myelin process suggest a well-organized machinery that may be exhibited in more than just the two ways cited above. Consequently, we reexamined our earlier sections of the optic tract of the kitten, paying particular attention to the direction of wrapping, and noted that very often neighboring axons tended to be wrapped in the same direction in any given enlargement. Because this tendency occurred more frequently than expected by chance, some non-random factor must influence the direction of wrapping. This report documents this observation and suggests a model for myelination that could cause this regularity.

Methods

The material examined in this study was identical to that used in an earlier report on the progress of myelination in the optic tract of the kitten (Moore et al. 1976), which describes the details of tissue preparation. For the present study, cross-sections of the optic tract were examined in photomicrographs at ×15,000 or ×28,000. The data reported here were collected on three and four week old kittens, which we found to be the most suitable for our purposes. At younger ages, the density of myelinated fibers was too low, whereas at older ages the myelin wrapping was so tight that the direction of myelination was hard to detect at our magnification.

Two different three week old kittens were used to obtain the statistical data reported here, plus one four week old normally reared and one four week old reared in the dark. A 0.2×0.2 mm^2 montage at ×10,000 was also prepared for the normal four week old in order to make comparisons within one large region of one optic tract. The optic nerves of one of the three week old kittens were also examined. Since no differences were observed in the measurements taken from the different animals, the results were pooled.

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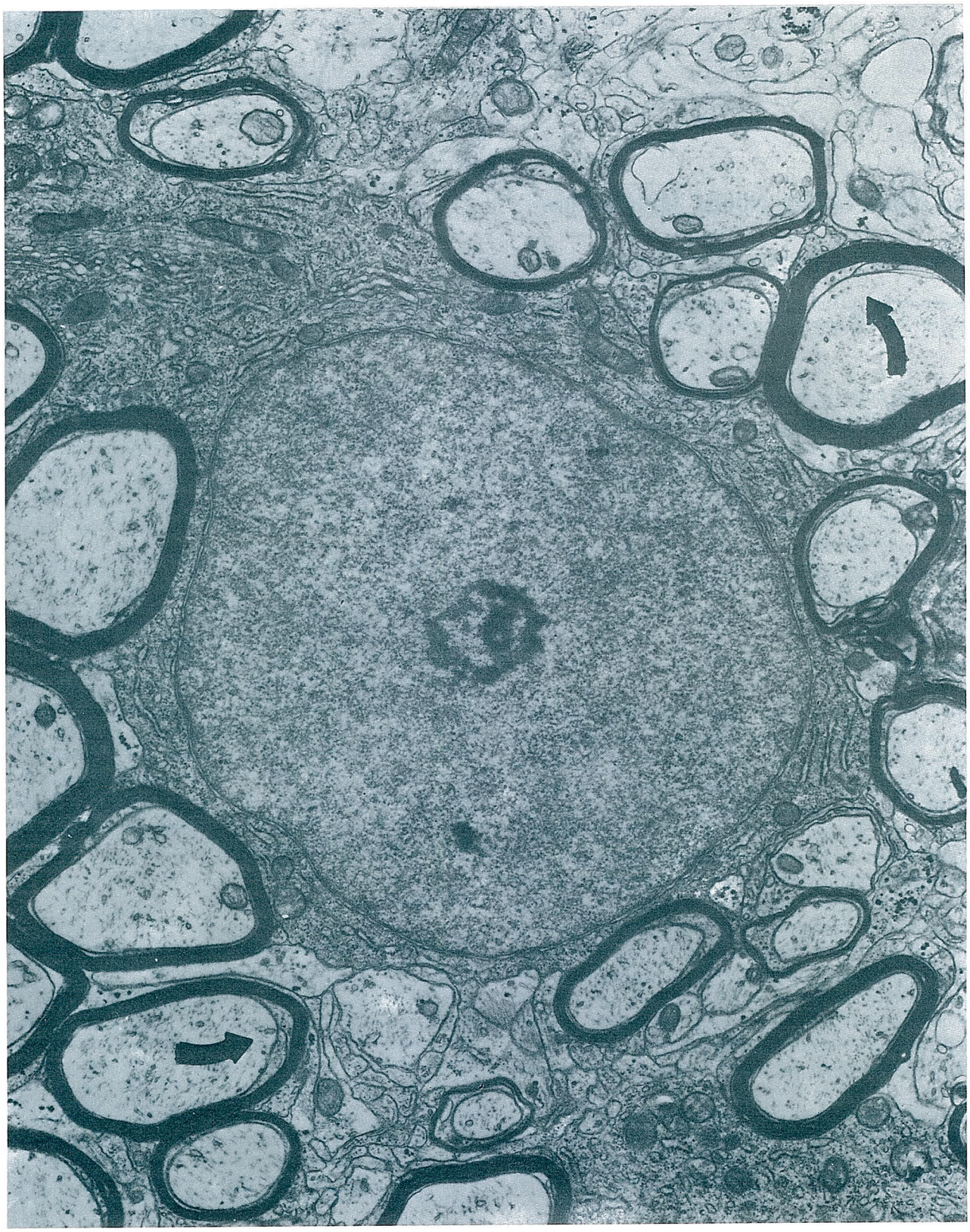

Fig. 1. Cross-section taken at ×28,000 from the optic tract of a three week old kitten. Note that all fibers in contact with the glial cell are wrapped in the same direction, as indicated by the direction that their internal mesaxon tongues are pointing. (See *arrow* in upper right quadrant for one very clear example)

Results

Two Illustrations

Figure 1 illustrates the major finding of this report. This cross-section of the tract of a normal three week old kitten at ×28,000 is centered on a glial cell. In direct contact with this cell are 11 myelinated fibers. In 10 of these fibers, the direction of myelination can be determined by the direction in which the internal tongue process is pointing, and is counterclockwise in each case. The probability of occurrence of this event by chance is 2^{10} or about 1/1000. Only one fiber in this figure clearly rotates in the opposite direction (lower left), and this fiber is well removed from the central glial process.

The montage of Fig. 2 is another illustration of the same phenomenon, but this time the ×15,000 cross section of the central figure is from a four week old kitten dark-reared from birth. Seventeen fibers are in direct contact with this glial process. The sequence is: A, B, D, E, H, J, K, M, with the internal tongues all counterclockwise; P, Q, R, clockwise; T, S, W, V, Y, Z; counterclockwise. (Fibers C, F, G, I, L, N, O, Θ, U and X are considered not to be in direct contact with the same glial process. Note, however, that their directions are consistent with the previous pattern.) Thus, 15 of the 18 fibers adjacent to the glial cell are all wrapped in the same direction, and these are all in sequence. The probability of this occurring by chance is extremely low.

Unfortunately, it is difficult to find many glial cells with a large number of fibers whose direction of wrapping can be determined. Instead, most of our samples contain only six or seven fibers that are legible. For further documentation of the non-random nature of the wrapping, a statistical sampling technique was used.

Statistical Sampling

To illustrate the statistical sampling technique used, consider the montage of Fig. 2 once again. Let the inspection of fibers proceed in a clockwise direction around the glial process. Starting with fiber A in Fig. 2, for example, continue inspecting the fibers and note when a reversal in direction of wrapping occurs (i.e. fiber P). Then continue until the wrapping direction changes once again (fiber T). Repeat this process, counting the total number of reversals until returning to the original fiber where the procedure began. In Fig. 2, therefore, the number of reversals is two. Clearly, this measure must always be an even number.

Figure 3 shows the result of such an analysis applied to all 132 glial cells seen in a 0.2×0.2 mm^2 montage prepared from a four-week-old kitten. The abscissa is the number of fibers that could be evaluated for any glia, and the ordinate is the number of reversals. The circles show the mean number of reversals actually found for glia that "captured" up to 22 different fibers. Only a few instances of more than 13 fibers per glia were found in the montage, as indicated by the filled circles; otherwise the mean reversal rate is based on at least eight or nine glial examples.

The heavy upper solid line rising monotonically to the right is the curve expected by chance, calculated as if the direction of wrapping of the fibers were determined by the flip of a coin. The lower curve that approximates the actual mean results falls well below the chance line beyond 10 fibers per glia. This lower curve through the means is the expected result if no more than four reversals in wrapping direction occurred for any given glial process. (Thus, this second theoretical curve must asymptote at four.) The observed direction of wrapping, therefore, cannot be random.

Discussion

Although the direction of myelin wrapping may appear random in a large sample, this is far from true for fibers in contact with the same glial process. These fibers have a probability of greater than 90% of being wrapped in clusters, with only four clusters per glia. In Fig. 3, which reflects this four cluster rule, there are only two exceptions. These are the two data points for glia with 14 and 15 fibers in contact, which are based upon only one or two examples out of our total of 132. Such data obtained from glia with a large number of adjacent fibers are the most subject to the two sources of error we encounter in evaluating the EM enlargements: first, the direction of myelination can easily be misread and confused with a parting of the inner sheath (such as in Fig. 2, fibers L or U); and second, it is often quite difficult to judge the extent of a glial process or protrusion (such as in the region between fibers P and T in Fig. 2, or also the lower left of Fig. 1). Therefore, it is reasonable to expect the observed reversal counts to exceed the conjectured maximum of four on occasion. The data of Fig. 3 thus support the four cluster rule to within observational error.

What is the significance of our observation that the direction of wrapping of neighboring fibers is not random? If no more than four reversals in wrapping direction occurs around any one glial process, as

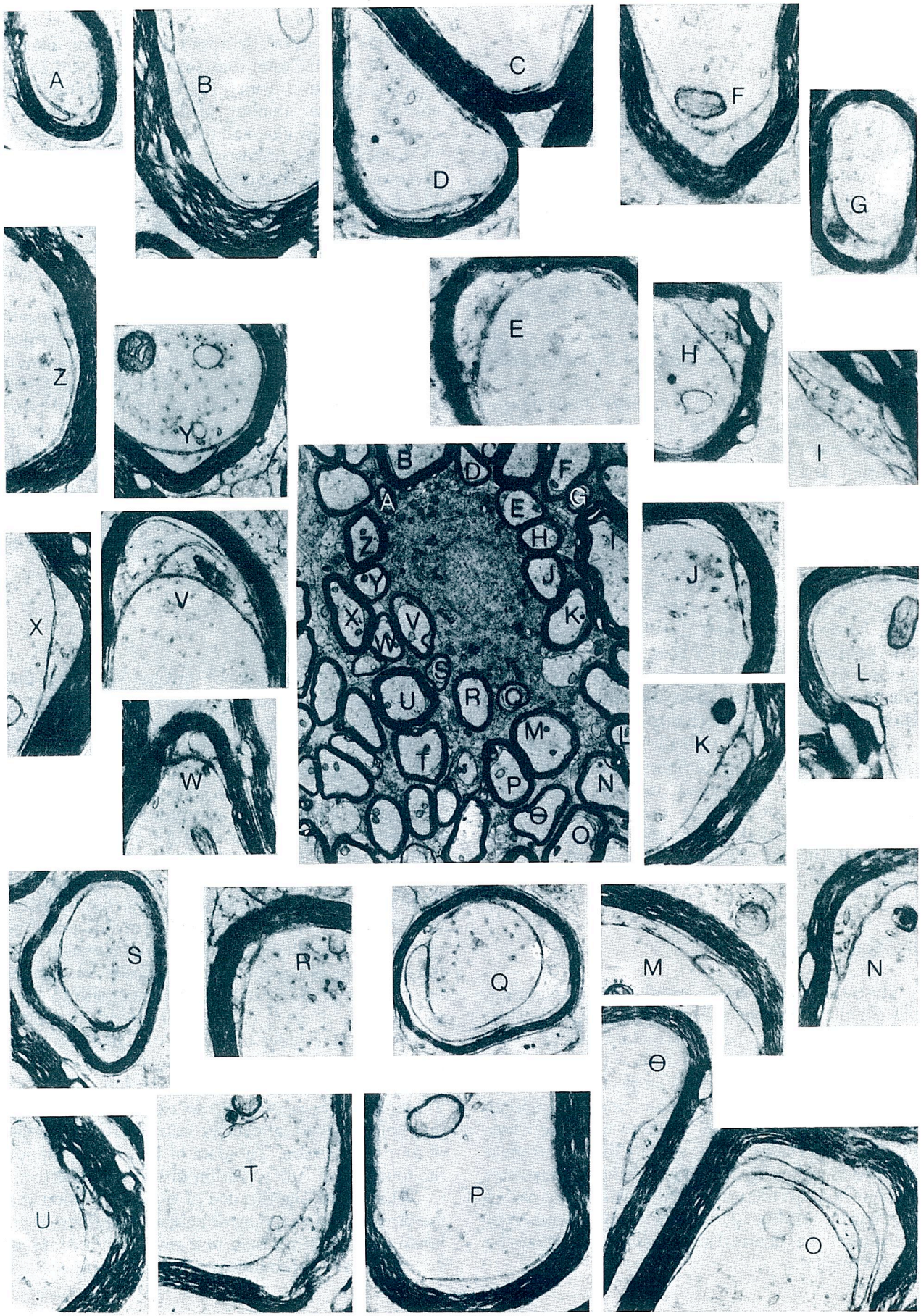

Fig. 2

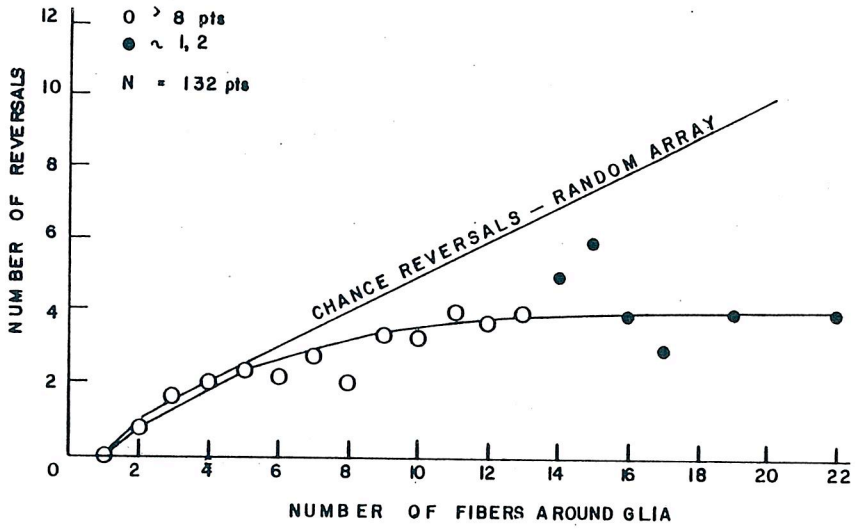

Fig. 3. Summary of the observed number of reversals in the direction of wrapping of myelinated fibers abutting glia. The abscissa includes how many fibers surrounded a particular glial cell (a total of 132 were studied). The ordinate shows the mean number of reversals in wrapping direction. If the fibers lying about an individual glial process were wrapping at random, then the expected number of reversals should increase along the upper solid line as the number of fibers surrounding the glia increases. Because the data asymptote near four reversals regardless of fiber count, the observed wrapping direction cannot be random. The open circles represent means obtained from at least eight examples of glia that captured the number of fibers indicated on the abscissa. Only a few examples were found in the montage of glia surrounded by 14 or more fibers. These data are indicated by the filled circles, which are based on only one or two measurements per point

Fig. 3 suggests, then the activity of the glia itself must be subdivided into quadrants, with each quadrant controlling the wrapping direction of its particular group of fibers. Such a grouping of wrapping directions seems incompatible with the idea that the glia rotate freely about the fibers in a single direction during myelination, as may occur in the peripheral nervous system (Peters et al. 1970; Morell and Norton 1980). In contrast, in the central nervous system, it must be possible for the same glial cell to move its processes in two directions at the same time. This excludes simple rotation and radially symmetric expansion and contraction of the glia. Instead, at the very least a bilaterally symmetric pattern of glial activity is required, where one process is expanding while the other part of the glia is contracting. (Evidence for pulsatile activity of oligodendrocites has been discussed by Murray 1965.) The wrapping process would then involve repeated elongation and contraction of the glial cell body about two orthogonal directions. This kind of activity would divide the direction of motion of the glial surface into four quadrants, as illustrated in Fig. 4. As one axis elongates, the other would contract, followed by a contraction of the elongated axis and an expansion of the previously shortened axis. Such a process would create similar gradients of motion of the glial process on opposite sides of the two axes of contraction and elongation. As shown in more detail in Appendixes I and II, a myelination process such as that proposed here would wrap fibers from the outside rather than from the inside.

Appendix I

A Speculative Proposal for Myelination

A model for myelination should account for at least three observations:

(i) The inner and outer mesaxon tongues tend to appear in the same clock position for their shared fiber (Peters 1964).

(ii) Neighboring fibers tend to be wrapped in the same direction (current report).

(iii) Fibers adjacent to one glial cell body tend to be divided into four quadrants, with the fibers in each quadrant having the same direction of myelination (current report).

Here we sketch a proposed scheme for myelination that incorporates the above observations. Appendix II then shows that such a speculative proposal, which requires that additional myelin be added to the outside of the sheath, rather than by growth of the inner tongue, is plausible by example.

Fig. 2. The central panel is a cross-section taken at ×15,000 of a region in the optic tract of a four week old kitten, dark-reared from birth. Surrounding the central panel are blow-ups showing the internal tongues for the fibers abutting the glial cell

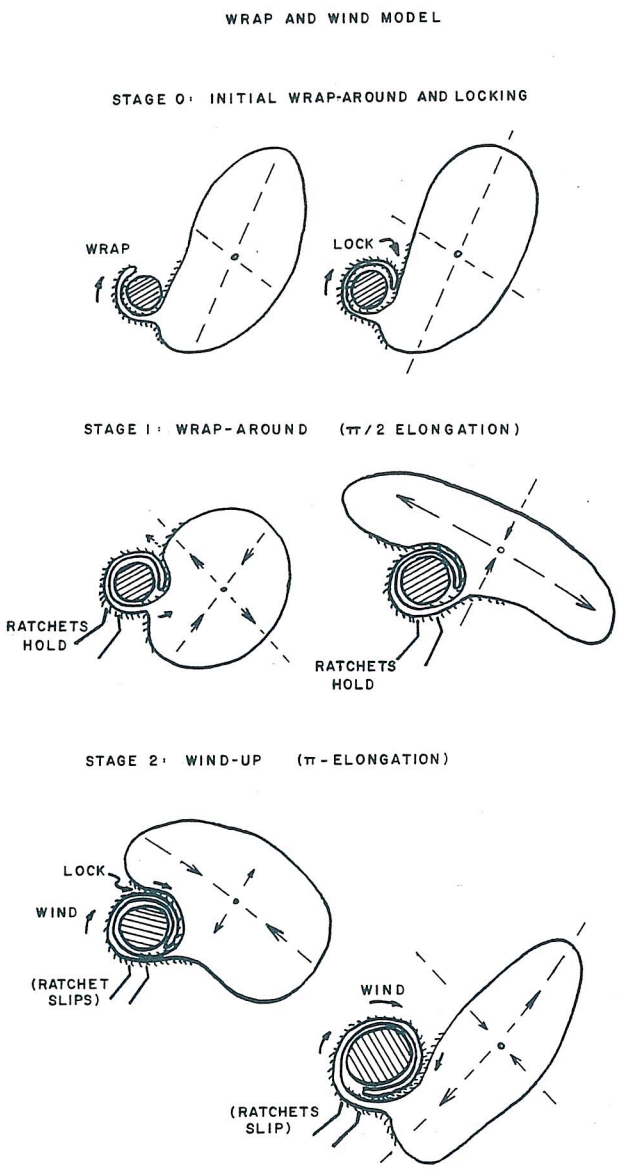

Fig. 4. Proposed stages of myelination in the CNS. After initially enveloping a fiber, glial pulsations would continue to wrap axons by repetition of the stages 1 and 2. See text and Appendix for details

Consider now a myelination process that proceeds as follows: in the pre- or pro-myelin stage, which is so evident in the one week old kitten, there are many small (0.6 μm diameter) fibers in contact with a glial cell. As the glial cell reaches maturity, it begins to "beat" as a relaxation oscillator, with its "beats" being in the form of an elongation of one axis and a contraction of the other [Pomerat et al. 1967, have reported glial pulsations in the peripheral nervous system and Murray (1965) has observed them in culture.] As the outer glial process streams past the premyelinated fibers, some of these fibers are caught by the glial surface and twist as the glial process streams by. During the relaxation phase of the glial beat, these netted fibers would counter-rotate in the local region near the glial cell. Then, just as if a bundle of threads is twisted in a pot of taffy, the fibers will either unwrap themselves or not, depending upon how far they have been twisted during the first pass. If the initial twisting is such that the glial process completely wraps the fiber, then the counter rotation by the fiber during the opposite phase of the glial beat will in fact cause more wrapping to occur. The additional wrapping will result from the glial surface sticking to itself as the central core of the fiber unwinds. But this can happen only if the initial wrapping approximates 360°. In this case, therefore, the inner and outer ends of the myelination processes (internal and external mesaxons) will be near to each other, as observed by Peters (1964) during the early stages of myelination.

Figure 4 illustrates the concept of the model in greater detail. In the initial wrapping stage 0, the glia either sends out a tongue that completely envelopes the fiber, or, alternatively, the pulsation of the glia is in itself sufficient to capture and wrap a fiber as its process streams past. Although Fig. 4 shows this first stage only for one fiber, it is important to note that several fibers must be "captured" before any routine wrapping can take place. Without counter-balancing on the opposite side of the glial cell, the glia will not be "anchored" and the streaming of its processes would simply result in a translation of the cell. [A similar anchoring with connective tissue may occur in the peripheral nervous system (Bunge and Bunge 1978)].

A further constraint on the initial wrap-around may be that fibers lying on opposite quadrants of the glia begin their wrapping in the same direction. If this does not occur, then the glia will be partitioned into more than four sectors and will have more than two axes of symmetry for elongation and contraction, resulting in a much less efficient oscillator. (Alternatively, the glial quadrants could be defined by the direction of a ratchet-like process proposed below.)

Once the initial wrap-around has taken place, then antiphase pulsations of the glial cell in two perpendicular directions could, in principle, continue to wrap many fibers at once. This is illustrated in the second and third panels of Fig. 4 for only one fiber. (A fiber in the opposite quadrant would be wrapped similarly, whereas fibers in the remaining two quadrants would be simultaneously wrapped in opposite directions.)

As the glial process elongates to the upper left, the membrane moves about the fiber approximately one-half of its diameter, adhering to the outer surface of its own membrane (Skoff et al. 1976). (This adherence may be viewed as molecular "ratchets" that will grip one another if pulled in one direction.) During the contraction phase along the same axis the glial membrane will now move in the opposite direction as shown in the lower panel (Stage 2), causing the fiber to rotate one-half turn as the contraction completes. (Further rotation of the fiber could occur as the glial process elongates on its opposite axis, continuing to wind the fiber up to a maximum of another one-half turn, thereby forcing more glial membrane into contact with the wrapped fiber.) If the original fiber is now held in place during the contraction of Stage 2, the first-stage of wrap-around can be repeated. Each complete cycle would add at least one-half turn of wrapping to the fiber (or a full turn if the process alternated sides). Appendix II describes a simple demonstration of the effectiveness of this wrapping process.

Appendix II

Simple Demonstration of Myelination Scheme

Materials

1. ½ inch masking tape, about 9 inches long.
2. Two pencils.

Procedure

1. Form a loop with the tape, about 3 inches in diameter, with the sticky surface outside.
2. Stick the middle of one pencil on one portion of the loop, with the pencil axis parallel to the loop axis.
3. Stick the second pencil in the same manner to the opposite side of the loop.
4. Grasp each pencil in separate hands, pulling the loop tight without unsticking it from the pencils.
5. Now rotate each pencil outward one turn (for example, by rotating the right pencil clockwise and the left pencil counterclockwise). The tape will now enclose each pencil, forming two back-to-back loops. The points where the tape contacts itself should be on the middle side of each pencil.
6. Hold the right pencil stationary and wrap the sticky tape *counterclockwise* one-half turn around the right pencil.
7. Repeat the process with the left pencil, but wrap the sticky tape one-half turn clockwise.
8. Repeat steps 6 and 7 as many times as necessary to wrap the tape completely around each pencil. Each step should wrap about one-half turn of the pencil. When finished, the pencils should be wrapped tightly together, but with the wrapping in opposite directions.

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