THE JOURNAL OF COMPARATIVE NEUROLOGY 419:364–376 (2000)

Effects of Aging on Myelinated Nerve Fibers in Monkey Primary Visual Cortex

ALAN PETERS,[1,2]* MARK B. MOSS,[1,2] AND CLAIRE SETHARES[1]
[1]Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts 02118
[2]Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322

ABSTRACT

In monkeys, myelin sheaths of the axons in the vertical bundles of nerve fibers passing through the deeper layers of primary visual cortex show age-related alterations in their structure. These alterations have been examined by comparing the myelin sheaths in young monkeys, 5–10 years old, with those in old monkeys, between 25 and 33 years of age. The age-related alterations are of four basic types. In some sheaths, there is local splitting of the major dense line to accommodate dense cytoplasm derived from the oligodendrocytes. Other sheaths balloon out, and in these locations, the intraperiod line in that part of the sheath opens up to surround a fluid-filled space. Other alterations are the formation of redundant myelin so that a sheath is too large for the enclosed axon and the formation of double sheaths in which one layer of compact myelin is surrounded by another one. These alterations in myelin increase in frequency with the ages of the monkeys, and there is a significant correlation between the breakdown of the myelin and the impairments in cognition exhibited by individual monkeys. This correlation also holds even when the old monkeys, 25 to 33 years of age, are considered as a group. It is suggested that the correlation between the breakdown of myelin in the old monkeys and their impairments in cognition has not to do specifically with visual function but to the role of myelin in axonal conduction throughout the brain. The breakdown of myelin could impair cognition by leading to a change in the conduction rates along axons, resulting in a loss of synchrony in cortical neuronal circuits. J. Comp. Neurol. 419:364–376, 2000.

Indexing terms: myelin; electron microscopy; cognitive decline; normal aging; senescence

To investigate the effects of normal aging on the primate nervous system we have used the rhesus monkey as a model, because when monkeys are subjected to a battery of behavioral tasks, similar to ones that can also be used to evaluate the effects of normal aging on cognition in humans, it is found that they display a similar decline in cognitive function (see Peters et al., 1996). The approach used has been to compare young monkeys with old monkeys. Consequently, after the monkeys have been behaviorally tested, their brains are examined microscopically to determine what kinds of morphologic alterations have taken place as a consequence of aging. An assessment is then made about whether any of the morphologic alterations correlate with the chronological age, the extent of the observed behavioral decline, or both, in the old monkeys.

In examining the neocortex, our efforts initially were focused on the effects of normal aging on its neurons, but we found no significant loss of neurons from either the visual (Vincent et al., 1989; Peters and Sethares, 1993; Peters et al., 1997), motor (Tigges et al., 1990), or prefrontal (Peters et al., 1994) cortices with age, and in these same monkeys, Rosene (1993) has encountered no loss of neurons from the hippocampus. Indeed, for both monkeys and humans, there is mounting evidence that there is no significant overall loss of neurons from the cortex during normal aging (Peters et al., 1998a). And, in monkeys, the majority of neocortical neurons show few changes with age beyond an accumulation of lipofuscin within some of them. The exception is layer 1. In area 46 of the prefrontal cortex, at least, layer 1 becomes much thinner with age,

Grant sponsor: National Institutes of Health, National Institute on Aging; Grant number: 2PO-AG 00001.

*Correspondence to: Alan Peters, Department of Anatomy and Neurobiology, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118.

Received 27 August 1999; Revised 2 December 1999; Accepted 9 December 1999

and in this cell-sparse layer some dying neurons have been encountered (Peters et al., 1998b).

However, despite the absence of significant changes in the population of neurons, the neuroglial cells display obvious age-related changes. Many of the astrocytes and the microglial cells accumulate inclusions, or debris, in their perikarya (Peters et al., 1991a). Presumably, this results from their phagocytic activity. Oligodendrocytes also acquire inclusions in both their perikarya and within bulbous enlargements that form along their processes (Peters, 1996), and this may correlate with the alterations that many of the myelin sheaths show throughout the central nervous system of the aging rhesus monkey. In the present study, we have chosen to examine the effects of age on the myelin sheaths of nerve fibers in the well-defined bundles of vertically oriented nerve fibers in primary visual cortex (see Peters and Sethares, 1996). The myelin in these nerve fibers is usually well preserved by perfusion fixation, making it feasible to carry out a fine structural analysis of the effects of aging on central nervous system myelin sheaths and to quantify the morphologic changes that occur in individual monkeys. As will be shown, the extent of the myelin changes correlates with both age and the behavioral status of the monkeys in which the analyses were performed.

MATERIALS AND METHODS

Blocks of primary visual cortex were removed from the opercular surface of the primary visual cortices of 14 rhesus monkeys (*Macaca mulatta*). Four of the monkeys, between 5 and 10 years of age, were used to assess the normal structure of nerve fibers in young mature monkeys, whereas the age-related changes were examined in the cortices of 10 old monkeys between 25 and 33 years of age. These ages were chosen because rhesus monkeys are sexually mature at approximately 5 years of age, and an analysis of the life-span of the rhesus monkey shows that only approximately 25% of them attain an age of 25 years, and only 6% live longer than 30 years (Tigges et al., 1988). The animals used were from an aging colony maintained at Yerkes Regional Primate Research Center at Emory University and at Boston University School of Medicine. The colony is maintained to determine the normal effects of aging on behavior and on the brain and is monitored to screen out individuals with known pathologic conditions or treatment that might adversely affect normal aging. The animals in the colony are cared for under professional veterinary supervision in accordance with the *Guide for the Use and Care of Laboratory Animals* (N.I.H. publication 86-23).

Tissue preparation

Tissue fixation was carried out by vascular perfusion under deep anesthesia as described by Peters et al. (1994) and in full accordance with approved Institutional Animal Care and Use Committee regulations. In brief, the monkeys were preanesthetized with ketamine, and a ketamine/Rompun mixture was administered intravenously to a state of areflexia. The anesthetized monkeys were artificially respired with a mixture of 95% oxygen and 5% carbon dioxide and transcardially perfused with a warm solution of 1% paraformaldehyde and 1.25% glutaraldehyde, in either 0.1 M cacodylate or 0.1 M phosphate buffer at pH 7.4. After cessation of the perfusion, the brains were removed and one hemisphere was placed for additional fixation in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in the same buffer. After several days, small blocks of primary visual cortex were removed, osmicated, dehydrated in an ascending ethanol series, and embedded in Araldite. Semithick sections were first taken through the depth of the cortex and stained with toluidine blue for light microscopic examination. The blocks were then turned, and a second set of semithick sections taken in the horizontal plane at the level of layer 4Cβ. In such sections, the nerve fibers in the vertical bundles that pass through the deeper layer of this cortex are seen in cross-section (see Peters and Sethares, 1996). Thin sections were then taken for electron microscopic examination and stained with uranyl acetate and lead citrate.

Quantitative assessment

After the thin sections had been examined to determine the kinds of structural alterations that affect myelinated nerve fibers as a consequence of aging, the frequency with which these alterations occur in individual monkeys was quantified. To achieve this, electron micrographs were taken at an initial magnification of ×4,000 or ×5,000 and printed to a final magnification of ×10,000 or ×12,500. For each monkey, at least 700 profiles of cross-sectioned nerve fibers were examined and a determination made of the percentages of nerve fiber profiles that were normal or showed age-related structural alterations. A separate determination was also made of the percentage of all nerve fibers in which the axons might be degenerating. The results of these analyses were then correlated with the age and with the behavioral data available for each monkey.

Behavioral testing

All monkeys were administered a battery of behavioral tasks to assess learning and memory function. The battery of tests included three visual recognition memory tasks: the delayed nonmatching to sample (DNMS) task, a DNMS task with a 2-minute delay, and the delayed recognition memory span (DRST) task. The normalized scores (Z scores) for each of these tasks, and the Cognitive Impairment Index (CII) for individual monkeys are listed in Table 1.

The DNMS is a behavioral task commonly used to assess visual recognition memory in the aged monkey (Presty et al., 1987; Rapp and Amaral, 1989; Arnsten and Goldman-Rakic, 1990; Bachevalier et al., 1991; Moss et al., 1999) and, in many respects, it resembles clinical tests that are used to assess memory function in patients with a variety of age-related neurologic disorders (Albert and Moss, 1996). As a group, aged monkeys evidence impairment on the acquisition and performance of this task, although several studies have reported normal to near-normal performance by individual aged monkeys (Presty et al., 1987; Rapp and Amaral, 1989; Bachevalier et al., 1991; Moss et al., 1999). Nevertheless, several studies spanning the past 10 years have collectively revealed a significant relationship between performance on the DNMS task and aging across the entire adult range in monkeys. The DNMS task relies upon a two-alternative, forced-choice paradigm in which the monkey is required to discriminate, after a delay, which one of two objects was previously presented. In one test, the delay was increased to 2 minutes.

TABLE 1. Nerve Fibers in Layer 4C of Area 17 and Behavioral Data

		Nerve fibers			Behavioral data[1]			
					DNMS		DRST	
Animal	Age (yr)	Total number counted	Normal myelin (%)	Altered axons (%)	Acquisition (errors)	2-Min delay (%correct)	spatial (mean span)	CII[2]
AM 16	5	994	99.2	0.10	−0.01	0.04	−0.03	0
AM 76	6	777	99.5	0.00	0.01	−0.45	0.72	0.09
AM 47	9	1,029	99.4	0.00	−1.28	−1.33	1.00	−0.54
AM 53	10	1,090	99.3	0.10	0.19	0.43	−0.38	0.08
AM 19	25	1,109	97.5	0.00	0.56	3.73	0.74	1.68
AM 12	26	1,089	94.5	0.30	7.92	2.63	1.61	4.05
AM 15	27	721	95.2	0.30	2.77	−0.01	1.91	1.56
AM 62	27	1,033	94.6	0.00	8.84	2.41	1.54	4.26
AM 27	28	1,223	93.7	0.20	1.57	1.09	1.35	1.34
AM 26	29	1,216	97.7	0.00	0.38	0.87	1.58	0.94
AM 91	32	1,741	95.7	0.00	−1.62	1.97	0.16	0.17
AM 17	29	919	91.7	0.00	2.73	2.41	1.79	3.24
AM 41	33	1,348	92.7	0.60	10.13	3.29	1.02	4.81
AM 65	33	1,112	94.6	0.00	5.62	2.41	1.79	3.73

[1]The individual behavioral Z scores shown in this table are based on a population of young adult and aged monkeys (see Herndon et al., 1997). DNMS, delayed nonmatching to sample; DRST, delayed recognition memory span.
[2]Cognitive Impairment Index (CII) expressed as number of standard deviation units from the mean performance of a cohort of young adult rhesus monkeys (Herndon et al. 1997).

In an effort to assess the memory capacity, or "load," we used an additional task of memory function, the delayed recognition span task (DRST) (Moss, 1983; Rehbein and Mahut, 1983). The DRST uses the same win-shift strategy as the DNMS and uses a nonmatching paradigm that requires the monkey to identify a novel stimulus among an increasing array of previously presented stimuli by using either spatial or nonspatial stimulus cues. The task has been used with monkeys to assess memory function after damage to the hippocampus (Rehbein and Mahut, 1983; Killiany and Mahut, 1991) as well as in normal aging (Moss et al., 1997; Herndon et al., 1997; Peters et al., 1998b). In humans, it has also been used in normal aging (Inouye et al., 1993) as well as to differentiate patterns of memory dysfunction in a variety of neurologic disorders (Moss et al., 1986; Salmon et al., 1989; Lange et al., 1992; Martin et al., 1995).

Although it is important to identify deficits in individual cognitive domains, we have found it useful to formulate a measure of overall cognitive impairment (Peters et al., 1998b), the Cognitive Impairment Index (CII). This index is derived from the normalized scores of each of the three behavioral outcome measures used in the battery. More specifically, the individual scores on the three behavioral measures (errors on DNMS acquisition, percent correct DNMS 2-minute delay, mean span length on spatial DRST) obtained by each of the monkeys in the present study were transformed to scores normalized to a population of 53 adult rhesus monkeys as described by Herndon et al. (1997). A composite based on these transformed scores (the Cognitive Performance Index) has been shown to be a practical index of global ability (Herndon et al., 1997). We have previously used the inverse of this score, the CII, as an overall behavioral measure that might be correlated with age-related changes in the frequency of synapses and in the thickness of layer 1 in area 46 (Peters et al., 1998b), and in the present study, we used the CII as a measure that might be related to the percent of normal myelin sheaths of nerve fibers in the vertical bundles in layer 4C of area 17 (see Table 1). The behavioral scores for the three cognitive tasks were also correlated with the percentage of normal sheaths in each of the 14 monkeys (Table 1). The Pearson product-moment correlation was used for all comparisons.

RESULTS

Nerve fibers in young monkeys

During development, processes of oligodendrocytes form a spiral wrapping around some of the axons (Peters, 1964). In that spiral wrapping, portions of the outer surface of the plasma membrane bounding the oligodendrocyte process come together to form a mesaxon, in which the outer leaflets of the apparent trilaminar plasma membrane become apposed to form what will become the intraperiod line of the mature sheath. In the initial phases on the formation of the myelin sheath, cytoplasm is retained within the turns of spiraling oligodendrocyte process. But eventually, this cytoplasm is lost. Consequently, the cytoplasmic faces of the spiraling process also come into apposition. This process results in the formation of compact myelin, in which the major dense line is darker and more prominent than the intraperiod line (Peters, 1960; Peters et al., 1991b).

In the primary visual cortex of young monkeys, profiles of almost all of the myelin sheaths in the vertical bundles display compact lamellae and surround normal axons. This is readily evident in horizontal sections taken at the level of layer 4Cβ, in which the nerve fibers are seen in cross-section (Fig. 1). But even in fiber bundles from young monkeys, it is common to find localized patches in the walls of sheaths where lamellae have sheared, splitting the lamellae slightly apart (Fig. 1, arrow). It is believed that such shearing defects are fixation artifacts, because they are most pronounced in poorly fixed material. At these sites, the lamellae simply separate. There is no cytoplasm in the splits and, indeed, in normal myelin sheaths it is not common to find cytoplasm between the turns of the sheath except at specific sites. These sites are the paranodes (Fig. 1, p), where the major dense line opens to accommodate a spiral of cytoplasm; within the outer tongue process, which is in direct continuity with the process of the oligodendrocyte forming that sheath; and within the inner tongue process on the inside of the sheath (see Peters et al., 1991b).

Myelin sheaths in old monkeys

In old monkeys, some of the sheaths show the same shearing defects that are encountered in the sheaths of

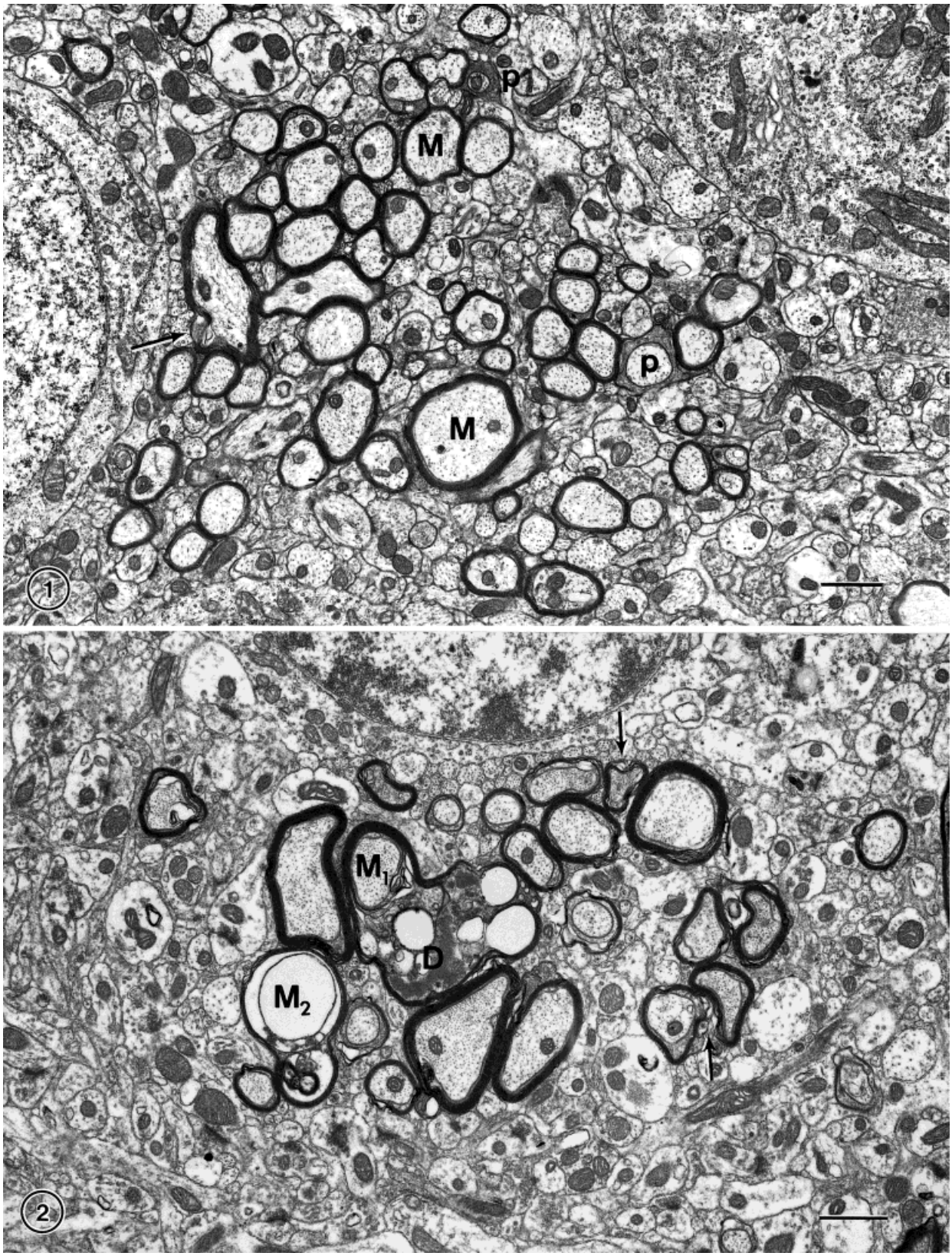


Fig. 1. Cross-sectioned bundle of nerve fibers at the level of layer 4C in primary visual cortex of a 6-year-old monkey. In the bundle, the myelinated nerve fibers (M), which are intermixed with unmyelinated axons and dendrites, all have compact sheaths. There is very little cytoplasm associated with the sheaths, except at paranodes (p). In one sheath, the myelin lamellae are sheared because of poor preservation (arrow). Scale bar = 1 μm.

Fig. 2. Cross-sectioned nerve bundle at the level of layer 4C from a 32-year-old monkey. Although some of the nerve fibers have intact sheaths, others have split lamellae (arrows). The sheath of one fiber (M_1) has an outpocketing that contains dense cytoplasm (D), whereas another sheath (M_2) has ballooned out and appears not to contain an axon. Scale bar = 1 μm.

some young monkeys (Figs. 2, 3; arrows), a finding that we consider to be fixation artifacts. However, many of the myelin sheaths in old monkeys show other alterations, which are age related, and generally the larger diameter nerve fibers are more affected than the smaller ones. The range of age-related alterations are illustrated in Figures 2 and 3, and basically the alterations are of four types.

(1) The most obvious and common alteration is that some sheaths develop bulges that are produced where there are pockets of electron-dense cytoplasm within the sheaths (Figs. 2–6; D, D_1, D_2). The cytoplasm in these pockets seems to be degenerating, because it is electron dense and usually contains dark amorphous bodies, which are not membrane bound, together with some membrane bound vesicles with clear contents. Sometimes the dark cytoplasm is contained within a single pocket, but in other examples dark cytoplasm is contained within two or three adjacent pockets, that are separated by myelin lamellae (see Figs. 4, 5). Examination of the profiles of such bulges show that the sheath accommodates the dark cytoplasm by splitting at the major dense line (Figs. 5, 6), indicating that the cytoplasm must belong to the oligodendrocyte forming the sheath.

(2) Other myelin sheaths in old monkeys are ballooned. Most commonly these balloons appear as large, rounded and smooth, isolated profiles that are formed by a thin myelin sheath of even thickness surrounding a space (Fig. 3). Structurally, the space usually appears to be empty, but it can contain membranous material (Figs. 3, M_3; Fig. 7), or dense granules, some of which adhere to the inside of the wall of the balloon. Because these myelin balloons generally have circular profiles, it is assumed that they are spherical in shape, and in fortuitous sections, it is apparent that at least some of the balloons are protrusions from the sides of otherwise intact myelin sheaths (Figs. 7, 8). In the electron micrograph shown in Figure 7, for example, there is a profile of a myelinated axon at the top of the balloon, and the cavity of the balloon is enclosed by a split on one side of the sheath of the axon. At the bases of the balloons, there may be some dark cytoplasm. An example of what might be an early stage in the formation of a balloon is shown in Figure 8. Here, there is a membrane-bound vesicle formed by a split of the innermost lamella of the sheath, and in this and other examples, it is apparent that the walls of balloons are produced by splits in the intraperiod line (see Fig. 8). This finding means that the space inside a balloon is in continuity with the extracellular space of the neuropil, and it is suggested that because balloons generally have smooth, round contours, they contain fluid that exerts some hydrostatic pressure, dilating the myelin bound structure (Feldman and Peters, 1998). The origin of the balloons is not known, but it is possible that they may be derived from swelling of the vesicles that can occur in embedded in the dark cytoplasm associated with some sheaths (e.g., Fig. 2D).

(3) Some axons are surrounded by what can be termed redundant myelin. Essentially, the sheath is much too large for the enclosed axon. Consequently, profiles of such sheaths show a normal axon at one end of a sheath that loops off to one side (Fig. 3; M_2). Such sheaths with redundant myelin can occur in young monkeys, but they are much more common in older ones.

(4) A few axons are surrounded by a double sheath, so that their profiles show an inner sheath of compact myelin that is separated by a space from a second and outer compact sheath (Fig. 9). This makes the sheath unusually thick.

Quantification of age changes in nerve fibers

The quantification of the effects of age on the structure of nerve fibers has been carried out by using horizontal sections taken at the level of 4Cβ. In such sections, the vertically oriented bundles of efferent nerve fibers are well defined (see Figs. 1–3). However, at this depth through the cortex, there are also some horizontally oriented nerve fibers that are sectioned longitudinally and others that are sectioned obliquely. Such profiles show that the cytoplasmic bulges and myelin balloons that alter the myelin sheaths of old monkeys can extend for some distance along the lengths of the nerve fibers and that several defects can occur successively along the length of an internode (see Fig. 10). Indeed, many of the profiles of longitudinally sectioned fibers from aged monkeys show some defect of their sheaths. Consequently, for the purposes of consistency in quantifying the extent of age-related changes in the myelin sheaths and their enclosed axons in individual monkeys, only transversely sectioned nerve fibers, in which the major axis of the profile was no more than twice that of the minor axis, were taken into account in ascertaining the proportion of nerve fiber profiles displaying defects.

Age changes in myelin sheaths

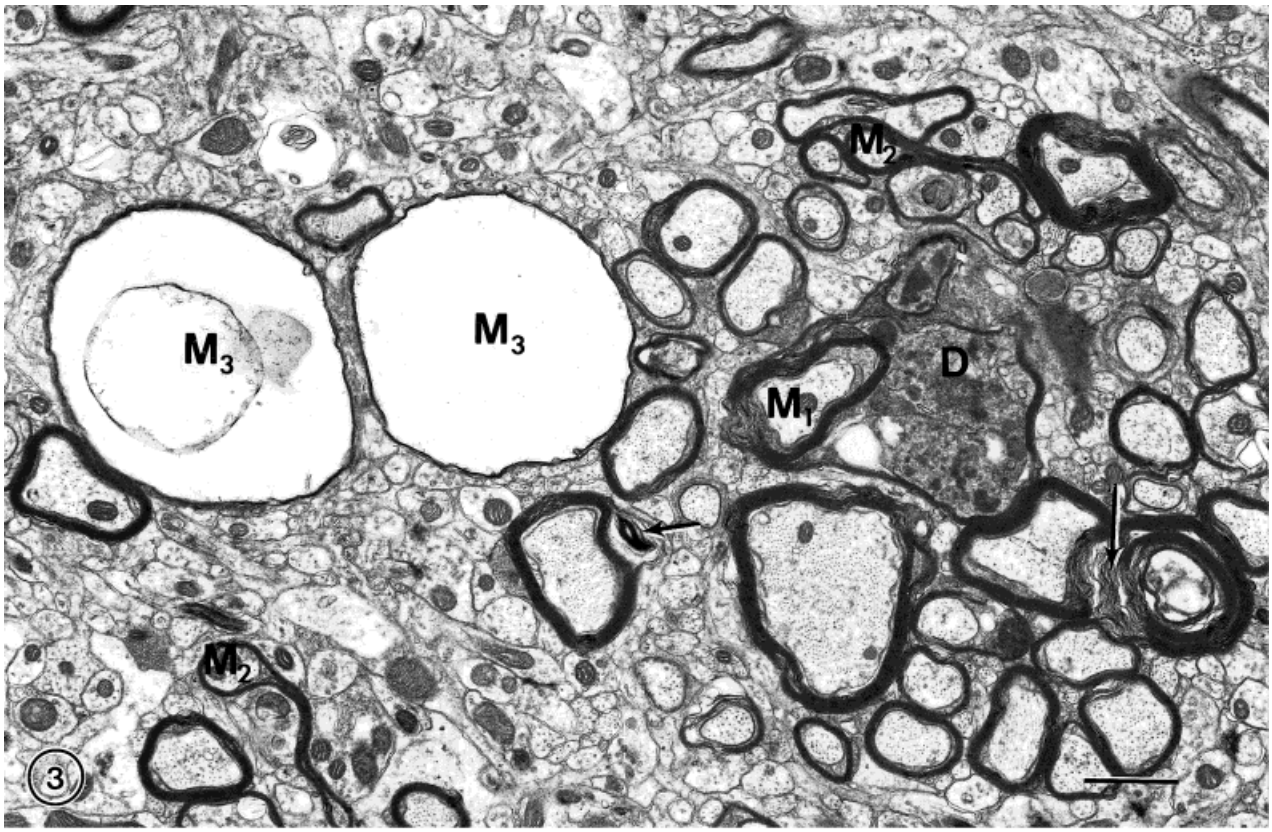
For each monkey, the myelin sheaths of at least 700 transversely sectioned nerve fibers were examined. A determination was made as to whether the profile of the sheath appeared normal or showed any of the four alterations described above. The results, given as the percentages of the profiles of sheaths with a normal appearance, are presented in Table 1.

Axons in old monkeys

Although many of the myelin sheaths in old monkeys display age changes, it is not common to find changes in the morphology of the enclosed axons. Normally, the cytoplasm of the axons contains neurofilaments, microtubules, mitochondria, and some small vesicles, and in the large majority of axons in aged monkeys, the distribution of these cytoplasmic components is similar to that in young monkeys. However, in old monkeys, occasional axons have lysosomes in their cytoplasm, some have large vacuoles, and the cytoplasm of a few others is dark (Fig. 9), suggesting that they are degenerating. In most cases, the surrounding sheath appear to be normal, whereas in other examples, the sheaths show alterations of the type described above. However, the frequency of occurrence of apparently altered axons is low (Table 1) and does not seem to have any correlation with age, because in some of the old monkeys, no profiles that could be construed as belonging to degenerating axons were encountered. Some profiles of empty sheaths were also encountered, but usually they were larger in diameter than the surrounding nerve fibers; therefore, they seemed to belong to myelin balloons.

Correlation of myelin sheath changes with age and behavior

As shown in Figure 11, when the percentages of profiles of nerve fibers with normal sheaths in the vertical bundles



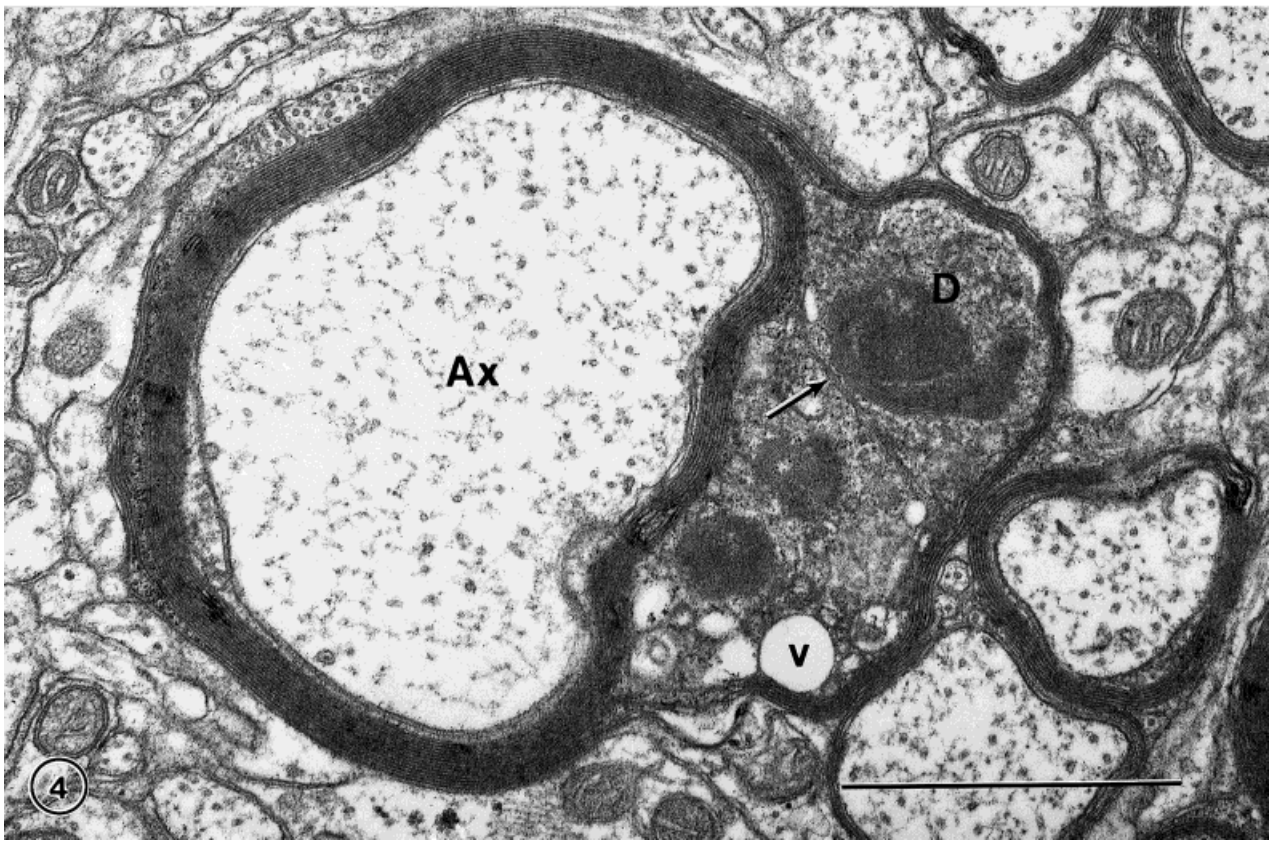
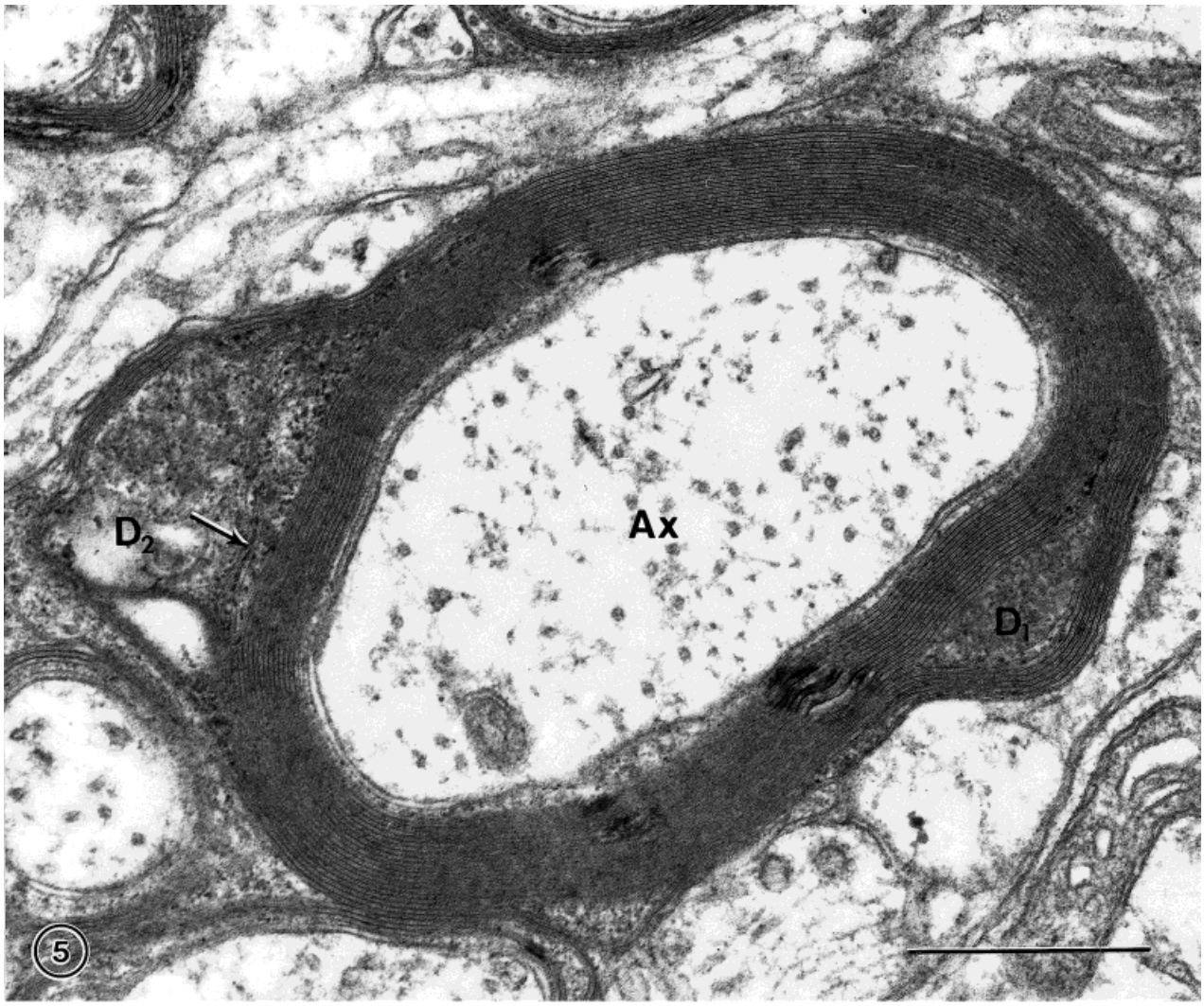


Fig. 3. Nerve fiber bundle from layer 4C of a 32-year-old monkey. Most of the myelin sheaths have some defects. Some have sheared lamellae (arrows) that we regard as fixation artifacts. These are significantly different from the age-related changes, such as that shown by one sheath (M_1) that has an extensive outpocketing that contains dense cytoplasm (D). Two other sheaths (M_2) have redundant myelin, so that the sheath is too large for the enclosed axon. The field also contains two myelin balloons (M_3) that appear to contain no axons. Scale bar = 1 μm.

Fig. 4. An axon (Ax) surrounded by a sheath that has split on one side to enclose dark cytoplasm (D) that contains some vesicles (V). The dark cytoplasm is partitioned by a lamella (arrow). The section is from a 35-year-old monkey. Scale bar = 1 μm.



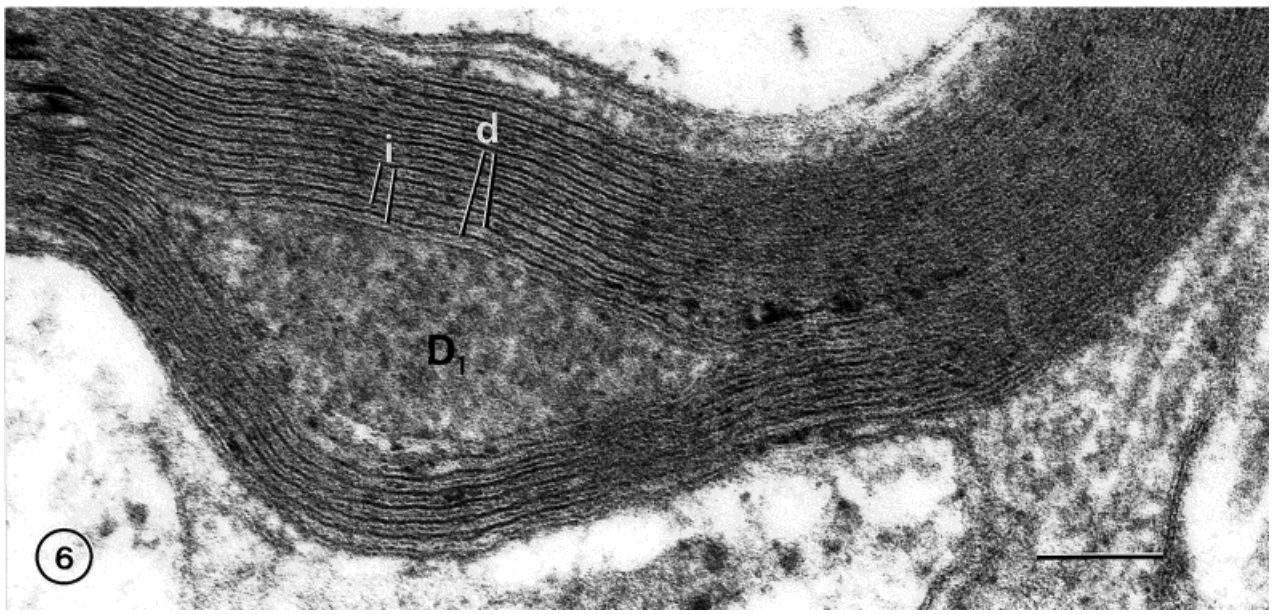
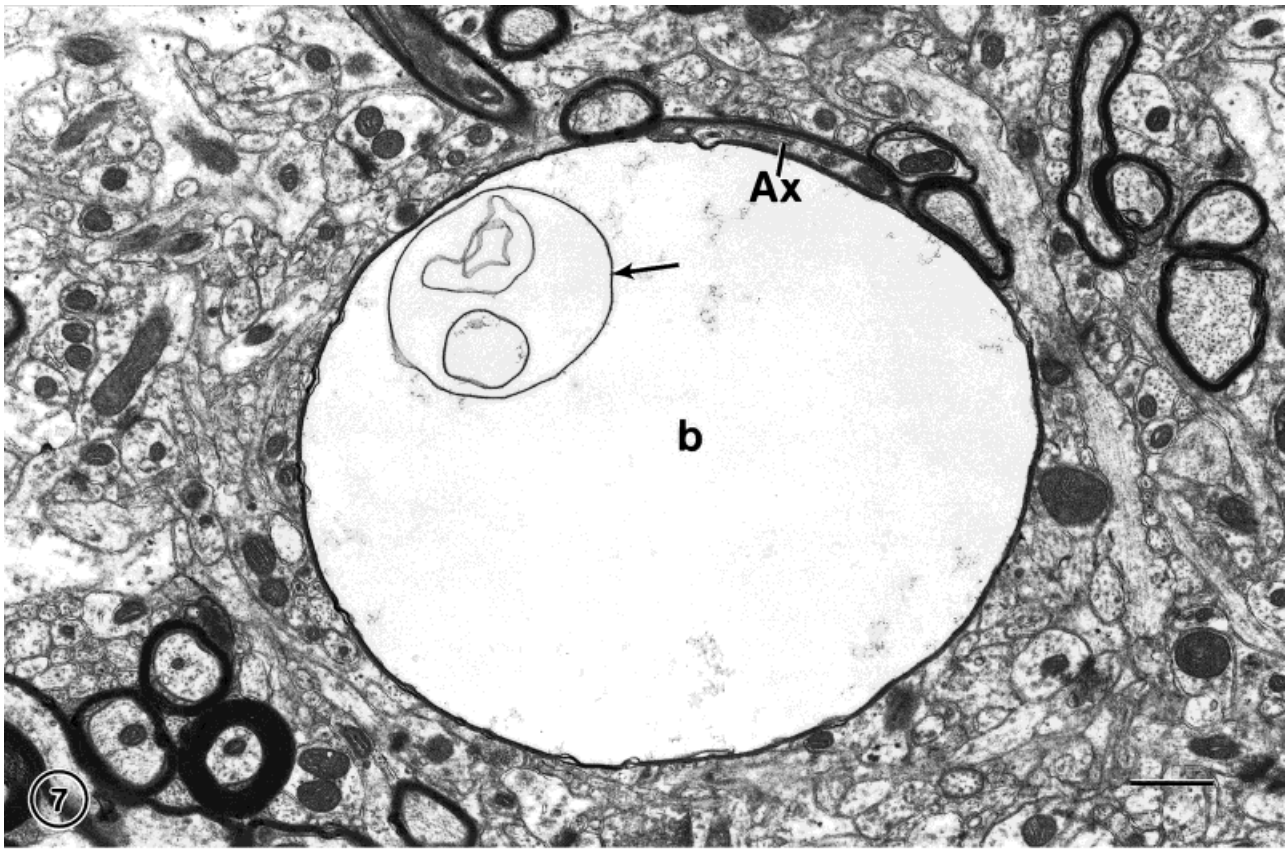


Fig. 5. An axon (Ax) surrounded by a sheath that has split in two places to enclose pockets of dense cytoplasm. One of the pockets of dense cytoplasm (D_1) is contained within a single split of the sheath, but the larger one (D_2) is partitioned by a lamella (arrow). The pocket of dense cytoplasm at D_1 is shown at higher magnification in Figure 6. The section is from a 35-year-old monkey. Scale bar = 0.5 μm.

Fig. 6. Higher-magnification photomicrograph of one pocket of dense cytoplasm (D_1) in the sheath in Figure 5. In this micrograph, the major dense (d) and intraperiod (i) lines of the myelin sheath are visible. It is apparent that the major dense line has split to accommodate the dense cytoplasm. The section is from a 35-year-old monkey. Scale bar = 0.1 μm.



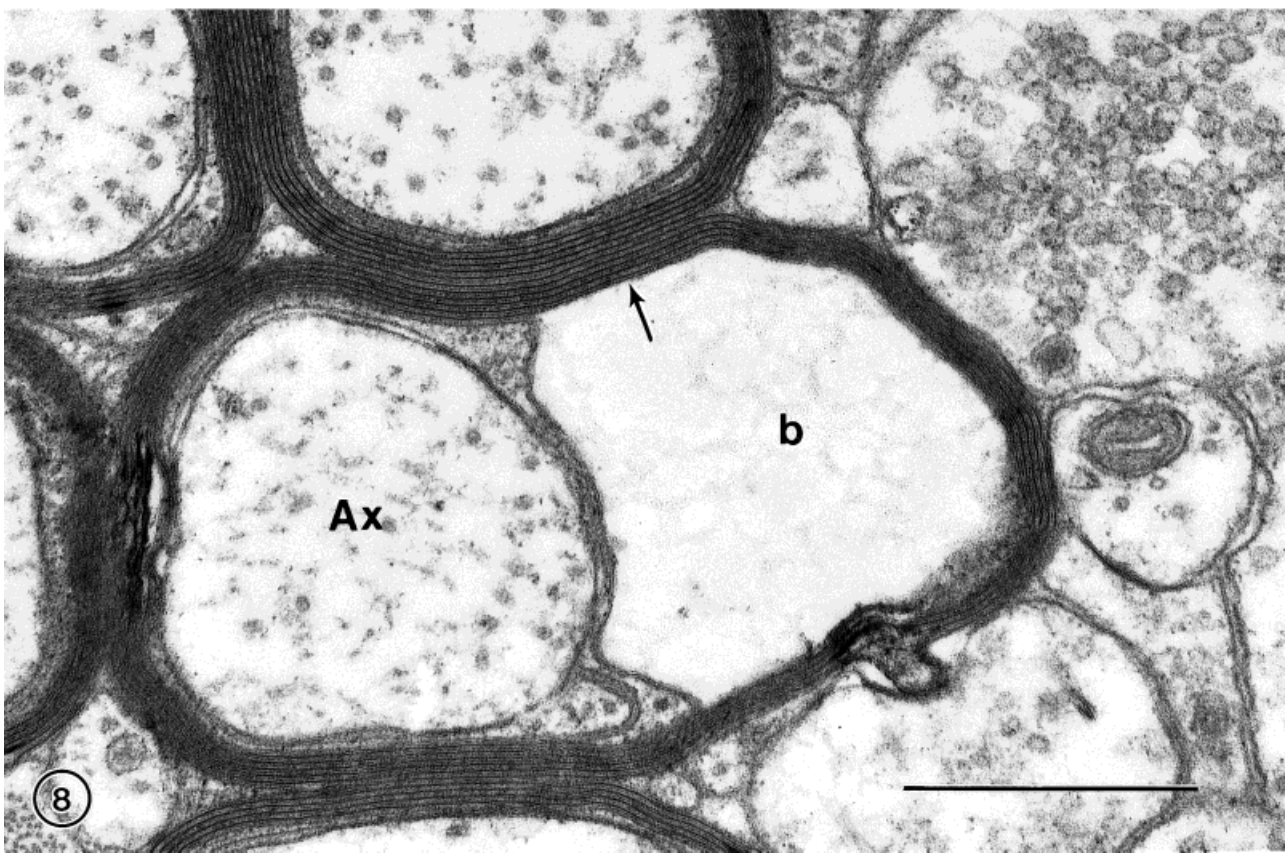


Fig. 7. A myelin balloon. The ensheathed axon (Ax) is flattened to one side of the sheath, which has herniated to produce a large balloon (b) approximately 7 μm in diameter. Apart from a membranous inclusion (arrow), the balloon appears to be empty. The section is from a 32-year-old monkey. Scale bar = 1 μm.

Fig. 8. An axon (Ax) surrounded by a myelin sheath that has split and ballooned out on one side. The contents of the balloon (b) appear to be largely fluid. To form such balloons, the sheath splits at the intraperiod line (arrow). The section is from a 29-year-old monkey. Scale bar = 0.5 μm.

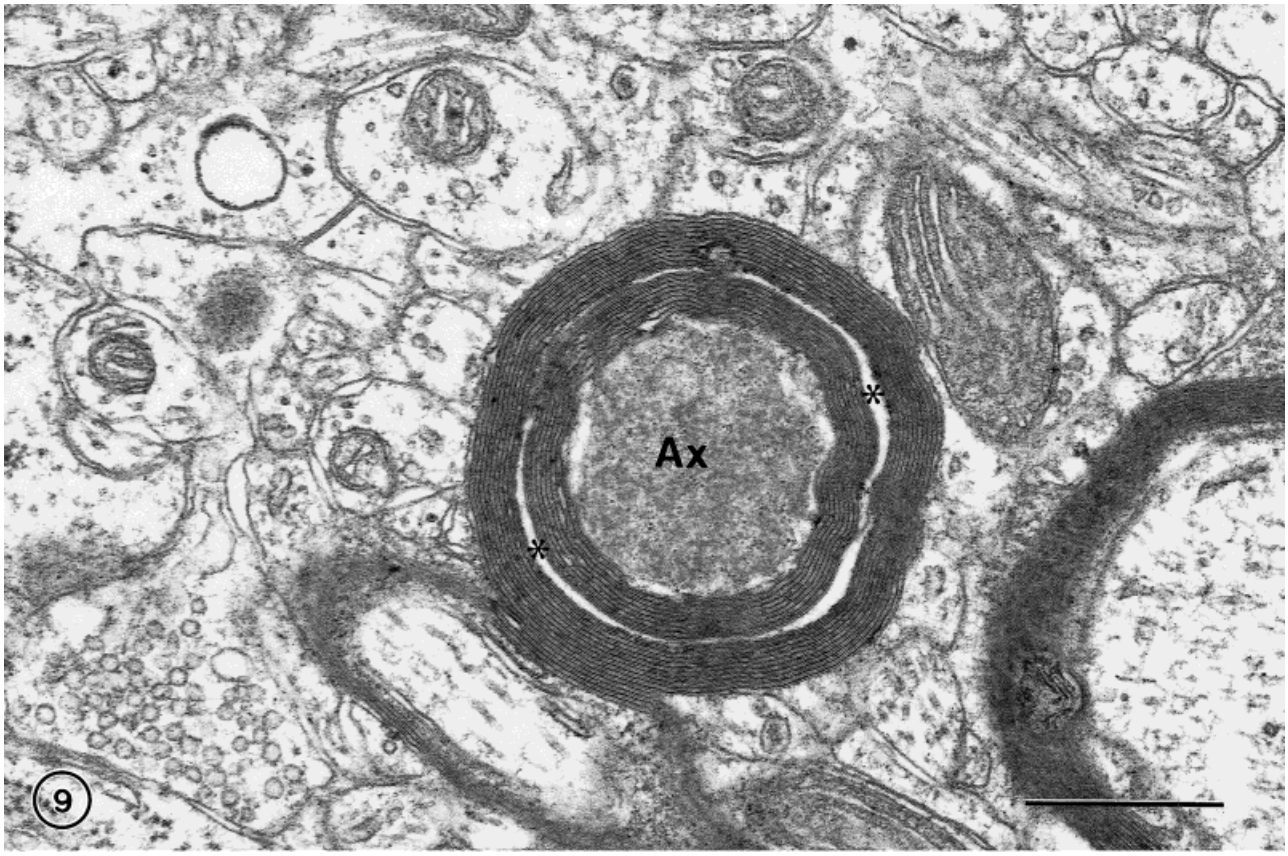


Fig. 9. A cross-section of a nerve fiber surrounded by two concentric sheaths of compact myelin separated by a space (asterisks). The axon (Ax) is dark and appears to be degenerating. The section is from a 32-year-old monkey. Scale bar = 0.5 μm.

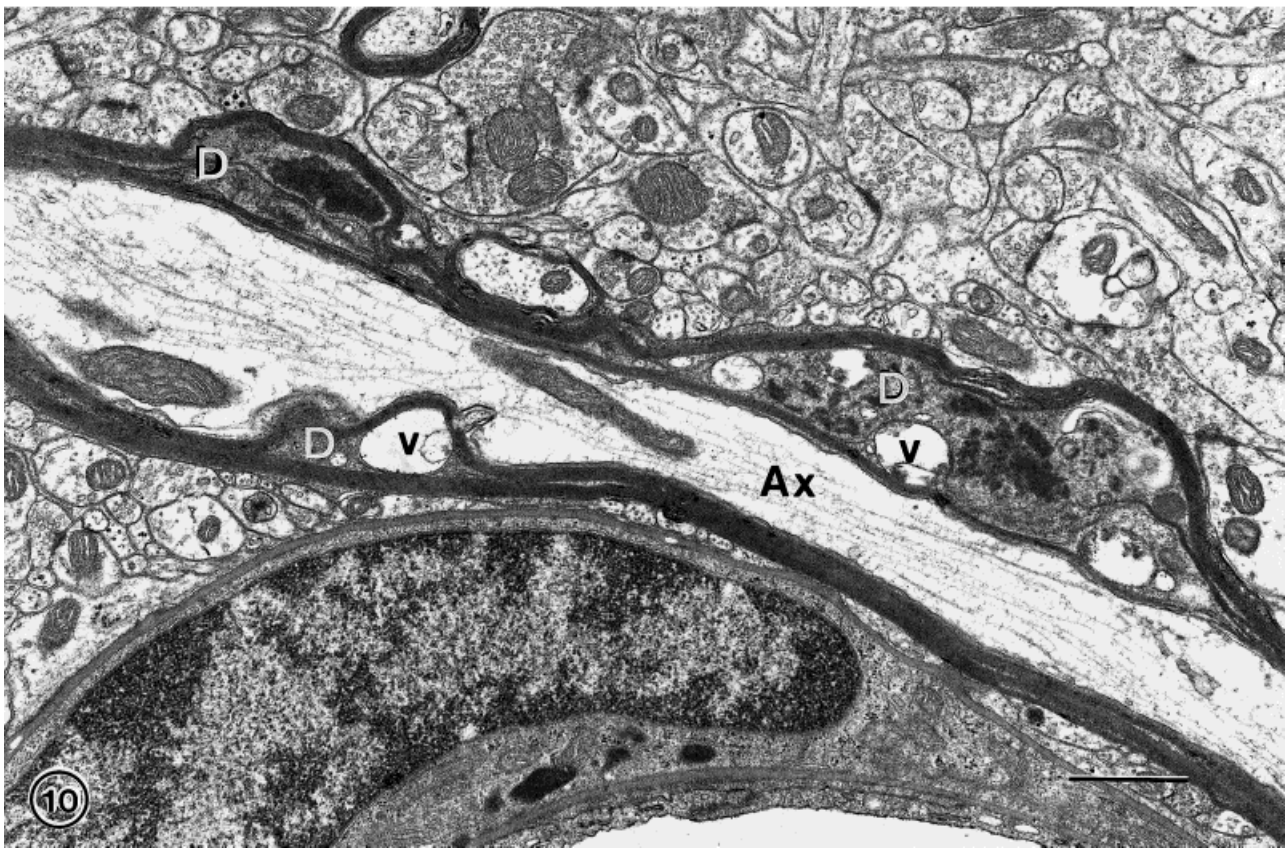


Fig. 10. Longitudinal section of an axon (Ax) enclosed by a myelin sheath, which in this micrograph shows three splits that contain dense cytoplasm (D). Some vesicles (v) are embedded within the dense cytoplasm. The section is from a 35-year-old monkey. Scale bar = 1 μm.

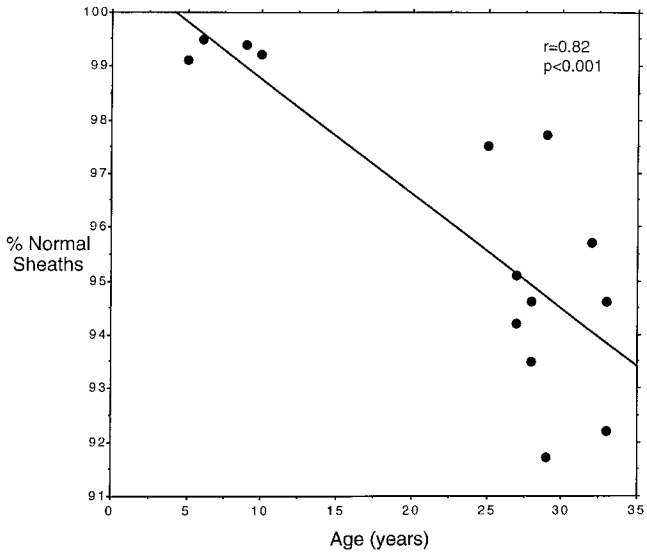


Fig. 11. A plot of the percentage of normal sheaths in the nerve fiber bundles in layer 4C vs. the ages of the monkeys.

in area 17 are plotted against the ages of the monkeys, there is a significant correlation between the ages of the monkeys and the increase in the percentage of nerve fibers with altered sheaths ($r = 0.82$, $P < 0.001$). Thus, the increase in the percentage of nerve fibers that have altered sheaths is a direct function of age.

In addition to the correlation with age, Figure 12 shows that there is also a significant correlation between the percentage of nerve fiber profiles with normal myelin sheaths and the overall efficiency of learning of the 14 monkeys as evaluated by the Cognitive Impairment Index (CII) ($r = 0.82$, $P < 0.001$). Furthermore, there is also a significant correlation between the percentage of normal myelin sheath profiles and each of the three individual

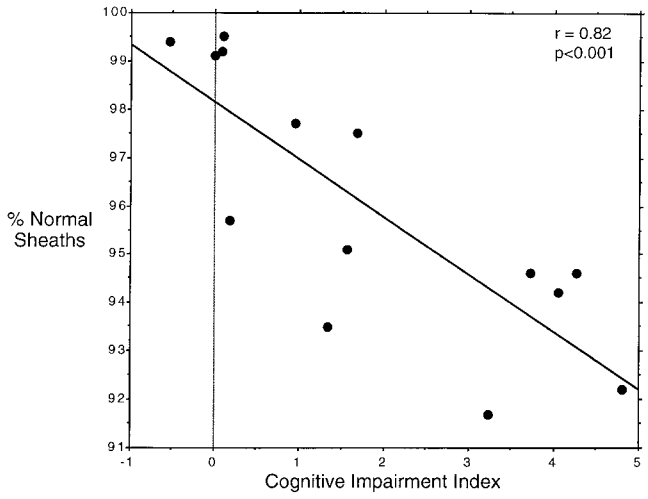


Fig. 12. A plot of the percentage of normal nerve fibers in the nerve fiber bundles vs. the cognitive impairment index determined for individual monkeys.

behavioral measures ($r = 0.66$, $P < 0.01$; $r = 0.66$, $P < 0.01$; and $r = 0.63$, $P < 0.01$, for DNMS acquisition, DNMS delays, and DRST spatial, respectively).

To assess the relationship between cognitive status and morphologic alterations excluding age as a factor, comparisons between performance and the percentage of normal sheath profiles were made among the 10 old animals alone. In this group of old monkeys between 25 and 33 years old, the analysis revealed a significant correlation between the percent of fibers with normal sheaths and the Cognitive Impairment Index ($r = 0.59$, $P < 0.05$).

DISCUSSION

In this study, it has been shown that with age the integrity of the myelin sheaths surrounding many of the nerve fibers in the primary visual cortex of the rhesus monkey is compromised. However, the structural changes observed in the myelin sheaths do not seem to be the consequence of a Wallerian type of degeneration, in which the primary degeneration is of the axons and the degeneration of myelin is secondary (e.g., Franson and Ronnevi, 1989). In experimental lesions, this type of degeneration results in the breakdown of the sheaths and the active uptake of their debris by reactive microglial cells (e.g., Ludwin, 1990). In the normally aging monkey cortex, very few degenerating axons have been encountered, even in axons surrounded by sheaths that are severely affected, and so it seems that the primary effects of age on the nerve fibers involve their myelin sheaths. This might account for the fact that Kemper (1994) has reported that in the normally aging cerebral hemispheres of primates, the white matter staining in old brains is paler than that in young brains and that Lintl and Braak (1983) found the intensity of staining of myelin in the stripe of Gennari in human visual cortex to be reduced with advancing age.

The most common alteration in the myelin sheaths from aged monkeys is the appearance of dark cytoplasm within pockets where the myelin lamellae are split at the major dense line. This finding indicates that the dark cytoplasm is derived from the myelin-forming oligodendrocytes (e.g., see Peters, 1964; Peters et al., 1991b). When this study was begun the question was raised about whether the cytoplasm in the pockets was derived from microglial cells, which also have a dark cytoplasm (Peters et al., 1991b), and whether they were phagocytizing the myelin. If this were the case, then the dark cytoplasm would be contained within a cell process outside the plasma membrane of the oligodendroglial cell forming the myelin, and be accommodated by a splitting of the intraperiod line. This type of invasion of the myelin sheath by thin processes of microglial cells occurs, for example, as degenerating myelin is split off from the sheath in JHM mouse hepatitis virus encephalomyelitis (Powell and Lampert, 1975).

In the cortices of young monkeys, cytoplasm is not normally present between lamellae in the internodes of myelin sheaths; therefore, it seems that during aging, the oligodendroglial cells are producing extraneous cytoplasm that becomes inserted into sheaths. A somewhat similar situation seems to occur in Cuprizone toxicity, in which Ludwin (l978, 1995) describes the cytoplasm of the inner tongue process in some degenerating sheaths as becoming dark and containing dense inclusions. Such dark cytoplasm is also present in mice with a myelin-associated glycoprotein deficiency (e.g., Lassmann et al., 1997). As in

the aging monkey, the density of the cytoplasm, and the presence of vacuoles and of amorphous dark bodies within it, suggests that the cytoplasm is undergoing degeneration.

The other common change that some myelin sheaths undergo with age, is the formation of balloons. As recently shown by Feldman and Peters (l998) such myelin balloons are common in the central nervous system of aged monkeys, as it is in aged animals of other species (see Faddis and McGinn, 1997). Typically, these balloons have round profiles with smooth contours and may be as large as 10 μm in diameter. Although the profiles of myelin balloons are usually seen in isolation, in fortuitous sections, it is obvious that they protrude from the sides of myelin sheaths and that they are accommodated by splits of the intraperiod line. Consequently, in effect, the interiors of the balloons are in communication with the extracellular space, suggesting that they are fluid-filled structures, the internal fluid exerting a positive pressure on the walls of the balloons to generate their spherical shapes (Feldman and Peters, 1998). However, the formation of myelin balloons is not restricted to aged animals. They occur, for example, in the early phases of Wallerian degeneration (Franson and Ronnevi, 1989); in experimental toxicity produced by triethyl tin (Hirano, 1969; Malamud and Hirano, 1973), by Cuprizone (Ludwin, 1978), and by isolecithin (Blakemore, 1978); in severe diabetes (Tamura and Parry, l994); in genetically engineered mice that have either an excess or a deficit of proteolipid protein (e.g., Monuki and Lemke, 1995; Anderson et al., 1998); and in mice that are lacking the galactolipid galactocerebroside (Coetzee et al., 1996, 1998).

It is also of interest that Waxman et al. (1993) have found that when isolated rat optic nerves are kept anoxic, in the presence of calcium in the perfusion bath, membrane-bound profiles can form in the cytoplasm on the inside of the sheath. The appearance of the cytoplasm in these cases is somewhat reminiscent of the vesicle containing dark cytoplasm that can occur in aging myelin sheaths, perhaps indicating that some of the abnormalities that occur during aging may be caused by an accumulation of axonic damage.

The other myelin defects common in old monkeys are the formation of sheaths of redundant myelin and the formation of one concentric sheath around another. These defects suggest that the oligodendrocytes in old monkeys are generating excess myelin with age. Sturrock (1976) also encountered an increased frequency of redundant myelin sheath when he compared myelinated fibers in the anterior commissures of 5-month-old mice with those of 18-month-old mice.

Myelin and oligodendrocytes

Obviously, because the myelin sheaths are affected by age, so must the oligodendrocytes that form them be affected, and a fundamental and unanswered question is whether the age-related alterations in the myelin are secondary to the inability of the parent oligodendrocytes to maintain their sheaths. The effect of age on oligodendrocytes was described earlier (Peters, 1996), when it was shown that in old monkeys the perikarya of oligodendrocytes come to contain dark inclusions resembling age pigment, and many of their processes develop swellings filled with similar inclusions. Perhaps some of the material in the inclusions is derived from the degenerating cytoplasm in the pockets of split sheaths, and the observations of LeVine and Torres (1992) on the twitcher mouse suggest that this might be the case. In the twitcher mouse, which is an animal model for globoid cell leukodystrophy, there is disintegration of myelin sheaths, and LeVine and Torres (1992) report that in these animals the processes of oligodendrocytes also have large swellings. They suggest that the material in the swellings comes from the sheaths and moves toward the cell bodies of the oligodendrocytes. However, this conclusion needs confirmation. It is also relevant that several studies have indicated that oligodendrocytes are susceptible to damage and that microglial cells produce several substances, such as cytokines (Hartung et al., 1992) and nitric oxide (Merrill et al., 1993; Mitrovic et al., 1994)) that are toxic to oligodendrocytes (see Ludwin, 1997, for a review). With time, some of these substances may accumulate sufficiently to bring about the age-related changes that we have observed in the myelin/oligodendrocyte complex.

Nerve fiber loss

Despite that few axons display possible signs of degeneration in the nerve bundles in primary visual cortex of the monkey, there are indications from axial MRI scans of both human (Albert, 1993) and rhesus monkey brains (Lai et al., 1995; Rosene et al., manuscript submitted for publication) that white matter is lost from the cerebral hemispheres during normal aging. Pakkenberg and Gundersen (1997) have also reported a significant decline in the total volume of white matter from the human brain with age, and Tang et al. (1997) concluded that this decline is largely caused by a loss of small diameter fibers. Obviously, this finding raises the question as to why we encountered few degenerating axons in our preparations. It may be that any axonal degeneration occurs over a short time span, and at such a fast rate that few such axons are present at any one point in time. This explanation needs further investigation.

Myelin breakdown and behavior

Of particular interest is the significant correlation between the percentage of nerve fiber profiles with altered myelin in layer 4 of area 17 and all four measures of behavioral performance among the 14 animals in the study. More importantly, when this analysis was performed stratifying for age, so that only the group of 10 old monkeys was assessed, a significant relationship was found between the percentage of nerve fibers with altered myelin sheaths in layer 4 and the degree of impairment on the CII, which is an overall measure of cognitive dysfunction. This suggests that, independent of age, cognitive performance among the old monkeys is related to the integrity of myelin sheaths in area 17.

Because the CII is based on tasks that place demands on learning and memory functions, it is unlikely that morphologic alterations within area 17 alone could account for the relationship with the behavioral findings, because this is a cortical area that subserves primary visual function. Nor is it likely that the relationship could be accounted for by a deficit in primary visual sensory function, particularly given that old monkeys are generally unimpaired in basic visual discrimination tasks (Moss et al., 1999). Rather, the findings suggest that altered myelin sheaths in area 17 may reflect a more ubiquitous morphologic change throughout the cerebral cortex, the effect of which

would be to impede or alter information processing, particularly for more complex or integrative cognitive tasks. Based on this supposition, one would predict that alterations paralleling those in visual cortex will be found in other cortical regions in these same aged monkeys. Furthermore, one would hypothesize that changes in nerve fiber sheaths in these other cortical regions would, as in area 17, correlate with performance on the CII.

A hypothesis

Presumably, the basis for the correlation between the breakdown of myelin in the old monkeys and their cognitive state rests on the role of myelin in axonal conduction. It seems likely that the age-related defects in the myelin sheaths could bring about changes in the rates of conduction along their axons. Evidence for this comes from the study of Xi et al. (1999), who compared the conduction velocity of axons in the pyramidal tracts of aged cats with those in young cats. They found that 51% of pyramidal tract neurons in young cats were fast conducting, compared with 26% in old cats, and that compared with young cats, the old cats showed an overall 43% decrease in median conduction velocity. Similarly, Morales et al. (1987) have shown that there is a decrease in the conduction velocities of lumbar neurons in old cats, and Aston-Jones et al. (1985) found that the conduction latencies from nucleus basalis to frontal cortex are some 31% longer in aged compared with normal adult rats. Decreases in conduction rates also occur in demyelinating disorders (see Waxman et al., 1995) and in proteolipid protein–deficient mice in which the myelin is not compact (Gutiérrez et al., 1995). It seems then, that myelin defects lead to a decrease in the conduction velocity along axons. This finding would be expected to result in changes in the timing of events in neuronal circuits, perhaps leading to extended excitation or inhibition of neurons, which might underlie some of the cognitive deficits that characterize aged primates.

LITERATURE CITED

Albert M. 1993. Neuropsychological and neurophysiological changes in healthy adult humans across the age range. Neurobiol Aging 14:623–625.

Albert M, Moss MB. 1996. Neuropsychology of aging: findings in humans and monkeys. In: Schneider EL, Rowe JW, Morris JH, editors. Handbook of the biology of aging, 4th ed. San Diego: Academic Press. p 217–233.

Anderson TJ, Schneider A, Barrie LA, Klugman M, McCulloch MC, Kirkman D, Kyriades E, Nave KA, Griffiths IR. 1998. Late onset neurodegeneration in mice with increased dosage of the proteolipid protein gene. J Comp Neurol 394:506–519.

Arnsten AFT, Goldman-Rakic PS. 1990. Analysis of alpha-2 adrenergic agonist effects on the delayed nonmatching to sample performance of aged rhesus monkeys. Neurobiol Aging 11:583–590.

Aston-Jones G, Rogers J, Shaver RD, Dinan TG, Moss DE. 1985. Age-impaired impulse flow from nucleus basalis to cortex. Nature 318:462–464.

Bachevalier J, Landis LS, Walker LC, Brickson M, Mishkin M, Price DL, Cork LC. 1991. Age monkeys exhibit behavioral deficits indicative of widespread cerebral dysfunction. Neurobiol Aging 12:99–111.

Blakemore WF. 1978. Observations on remyelination in the rabbit spinal cord following demyelination induced by lysolecithin. Neuropathol Appl Neurobiol 4:47–59.

Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Susuki K, Popko B. 1996. Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell 86:209–219.

Coetzee T, Susuki K, Popko B. 1998. New perspectives on the function of myelin galactolipids. Trends Neurosci 21:126–130.

Faddis BT, McGinn MD. 1997 Spongiform degeneration of the gerbil cochlear nucleus: an ultrastructural and immunohistochemical evaluation. J Neurocytol 26:625–635.

Feldman ML, Peters A. 1998. Ballooning of myelin sheaths in normally aged macaques. J Neurocytol 27:605–614.

Franson P, Ronnevi L-O. 1989. Myelin breakdown in the posterior funiculus of the kitten after dorsal rhizotomy: a qualitative and quantitative light and electron microscopic study. Anat Embryol 180:273–280.

Gutiérrez R, Bioson D, Heinemann U, Stoffel W. 1995. Decompaction of CNS myelin leads to a reduction of the conduction velocity of action potentials in optic nerve. Neurosci Lett 195:93–96.

Hartung HP, Jung SF, Stoll G, Zielasek J, Schmidt B, Archelos JJ, Toyka KV. 1992. Inflammatory mediators in demyelinating disorders of the CNS and PNS. J Neuroimmunol 40:197–210.

Herndon J, Moss MB, Killiany RJ, Rosene DL. 1997. Patterns of cognitive decline in early, advanced and oldest of the old aged rhesus monkeys. Behav Res 87:25–34.

Hirano A 1969 The fine structure of the brain in edema. In: Bourne GH, editor. The structure and function of nervous tissue, vol 2. New York: Academic Press. p 69–135.

Inouye SK, Albert MS, Mohs R, Sun K, Berkman LF. 1993. Cognitive performance in a high-functioning community-dwelling elderly population. J Gerontol 48:146–151.

Kemper TL. 1994. Neuroanatomical and neuropathological changes during aging and dementia. In: Albert ML, Knoefel JE, editors. Clinical neurology of aging. New York: Oxford University Press. p 3–67.

Killiany RJ, Mahut H. 1991. Ontogenetic development of recognition memory span in rhesus macaques. IBRO Abstract 165.

Lai ZC, Rosene DL, Killiany RJ, Pugliese D, Albert MS, Moss MB. 1995. Age-related changes in the brain of the rhesus monkey: MRI changes in white matter but not gray matter. Soc Neurosci Abstr 21:1564.

Lange KW, Robbins TW, Marsden CD, James M, Owen AM, Paul GM. 1992. L-dopa withdrawal in Parkinson's disease selectively impairs cognitive performance in tests sensitive to frontal lobe function. Psychopharmacology 107:394–400.

Lassmann H, Bartsch U, Montag D, Schachner M. 1997. Dying-back oligodendrogliopathy: a late sequel of myelin-associated glycoprotein deficiency. Glia 19:104–110.

LeVine SM, Torres MV. 1992. Morphological features of degenerating oligodendrocytes in twitcher mice. Brain Res 587:348–352.

Lintl P, Braak H. 1983. Loss of intracortical myelinated fibers: a distinctive alteration in the human striate cortex. Acta Neuropathol 61:178–182.

Ludwin SK. 1978. Central nervous system demyelination and remyelination in the mouse: an ultrastructural study of Cuprizone toxicity. Lab Invest 39:597–612.

Ludwin SK. 1990. Phagocytosis in the rat optic nerve following Wallerian degeneration. Acta Neuropathol 80:266–273.

Ludwin SK. 1995. Pathology of the myelin sheath. In: Waxman SG, Kocsis JD, Stys PK, editors. The axon: structure, function, and pathophysiology. New York: Oxford University Press. p 412–437.

Ludwin SK. 1997. The pathobiology of the oligodendrocyte. J Neuropathol Exp Neurol 56:111–124.

Malamud N, Hirano A. 1973. Atlas of neuropathology. Berkeley: University of California Press.

Martin EM, Pitrak DL, Pursell KJ, Mullane KM, Novak RM. 1995. Delayed recognition memory span in HIV-1 infection. J Int Neuropsychol Soc 1:575–580.

Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE. 1993. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. J Immunol 151:2132–2141.

Mitrovic B, Ignarro LJ, Montestruque S, Smoll A, Merrill JE. 1994. Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells in vitro. Neuroscience 61:575–585.

Monuki ES, Lemke G. 1995. Molecular biology of myelination. In: Waxman SG, Kocsis JD, Stys PK, editors. The axon: structure, function, and pathophysiology. New York: Oxford University Press. p 144–163.

Morales FR, Boxer PA, Fung SJ, Chase MH. 1987. Basic electrophysiological properties of spinal cord motoneurons during old age in the cat. J Neurophysiol 58:180–194.

Moss MB 1983 Assessment of memory in amnesic and dementia patients: adaptation of behavioral tests used with non-human primates. INS Bull 3:15–17.

Moss MB, Albert MS, Butters N, Payne M. 1986. Differential patterns of memory loss among patients with Alzheimer's disease, Huntington's disease, and alcoholic Korsakoff's syndrome. Arch Neurol 43:239–246.

Moss MB, Killiany RJ, Lai ZC, Rosene DL, Herndon JG. 1997. Recognition span in rhesus monkeys of advanced age. Neurobiol Aging 18:13–19.

Moss MB, Killiany RJ, Herndon JG. 1999. Age-related cognitive decline in rhesus monkey. In: Peters A, Morrison JH, editors. Neurodegenerative and age-related changes in structure and function of the cerebral cortex cerebral cortex, vol 14. New York: Kluwer Academic/Plenum Publishers. p 21–47.

Pakkenberg B, Gundersen HJG. 1997. Neocortical neuron number in humans: effect of sex and age. J Comp Neurol 384:312–320.

Peters A. 1960. The structure of myelin sheaths in the central nervous system of *Xenopus laevis* (Daudin). J Biophys Biochem Cytol 7:121–126.

Peters A. 1964. Further observations on the structure of myelin sheaths in the central nervous system. J Cell Biol 20:281–296.

Peters A. 1996. Age-related changes in oligodendrocytes in monkey cerebral cortex. J Comp Neurol 371:153–163.

Peters A, Sethares C. 1993. Aging and the Meynert cells in rhesus monkey primary visual cortex. Anat Rec 236:721–729.

Peters A, Sethares C, 1996 Myelinated axons and the pyramidal cell modules in monkey primary visual cortex. J Comp Neurol 365:232–255.

Peters A, Josephson K, Vincent SL. 1991a. Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex. Anat Rec 229:384–398.

Peters A, Palay SL, Webster DeFH. 1991b. The fine structure of the nervous system: neurons and their supporting cells. New York: Oxford University Press.

Peters A, Leahu D, Moss MB, McNally KJ. 1994. The effects of aging on area 46 of the frontal cortex of the rhesus monkey. Cereb Cortex 6:621–635.

Peters A, Rosene DL, Moss MB, Kemper TL, Abraham CR, Tigges J, Albert MS. 1996. Neurological bases of age-related cognitive decline in the rhesus monkey. J Neuropathol Exp Neurol 55:861–874.

Peters A, Nigro NJ, McNally KJ. 1997. A further evaluation of the effects of age on striate cortex of the rhesus monkey. Neurobiol Aging 18:29–36.

Peters A, Morrison JH, Rosene DL, Hyman BT. 1998a. Are neurons lost from the primate cerebral cortex during aging? Cereb Cortex 8:295–300.

Peters A, Sethares C, Moss MB. 1998b. The effects of aging on layer 1 in area 46 of prefrontal cortex in the rhesus monkey. Cereb Cortex 8:671–648.

Powell HC, Lampert PW. 1975. Oligodendrocytes and their myelin-plasma membrane connections in JHM mouse hepatitis virus encephalomyelitis. Lab Invest 33:440–445.

Presty SK, Bachevalier J, Walker LC, Struble RG, Price DL, Mishkin M, Cork LC. 1987. Age differences in recognition memory of the rhesus monkey (*Macaca mulatta*). Neurobiol Aging 8:435–440.

Rapp PR, Amaral DG. 1989. Evidence for a task-dependent memory dysfunction in the aged monkey. J Neurosci 9:3568–3576.

Rehbein L, Mahut H. 1983. Long-term deficits in associative and spatial recognition memory after early hippocampal damage in monkeys. Soc Neurosci Abstr 9:639.

Rosene DL. 1993. Comparing age-related changes in the basal forebrain and hippocampus of the rhesus monkey. Neurobiol Aging 14:669–670.

Salmon DP, Granholm E, McCullough D, Butters N, Grant I. 1989. Recognition memory loss in mild and moderately demented patients with Alzheimer's disease. J Clin Exp Neuropsychol 4:429–443.

Sturrock RR. 1976. Changes in neuroglia and myelination in the white matter of aging mice. J Gerontol 31:513–522.

Tamura E, Parry GJ. 1994. Severe radicular pathology in rats with long-standing diabetes. J Neurol Sci 127:29–35.

Tang Y, Nyengaard JR, Pakkenberg B, Gundersen HJG. 1997. Age-induced white matter changes in the human brain: a stereological investigation. Neurobiol Aging 18:609–615.

Tigges J, Gordon TP, McClure HM, Hall EC, Peters A. 1988. Survival rate and life span of rhesus monkeys at the Yerkes Regional Primate Research Center. Am J Primatol 15:263–272.

Tigges J, Herndon JG, Peters A. 1990. Neuronal population of area 4 during life span of the rhesus monkey. Neurobiol Aging 11:201–208.

Vincent SL, Peters A, Tigges J. 1989. Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex. Anat Rec 223:329–341.

Waxman SG, Black JA, Ransom BR, Stys PK. 1993. Protection of the axonal cytoskeleton in anoxic optic nerve by decreased extracellular calcium. Brain Res 614:137–145.

Waxman SG, Kocsis JD, Black JA. 1995. Pathophysiology of demyelinated axons. In: Waxman SG, Kocsis JD, Stys PK, editors. The axon: structure, function, and pathophysiology. New York: Oxford University Press. p 438–461.

Xi M-C, Liu R-H, Engelhardt KK, Morales FR, Chase MH. 1999 Changes in the axonal conduction velocity of pyramidal tract neurons in the aged cat. Neuroscience 92:219–225