THE JOURNAL OF COMPARATIVE NEUROLOGY 429:541–553 (2001)

Effects of Age on Nerve Fibers in the Rhesus Monkey Optic Nerve

JULIE H. SANDELL* AND ALAN PETERS
Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT

During normal aging there is a reduction in white matter volume in the cerebral hemispheres and structural abnormalities in myelin in some parts of the central nervous system, but whether nerve fibers are lost with age and whether the myelin changes are ubiquitous is not known. Studying the optic nerve, which is a circumscribed bundle of nerve fibers, offers an opportunity to gain further insight into the effects of normal aging on white matter. The present study examined the optic nerves from young (4–10 years) and old (27–33 years) rhesus monkeys using light and electron microscopy. These nerves had been perfused transcardially to obtain optimal preservation of the tissue. Varying degrees of degeneration were encountered in all the optic nerves from the old monkeys. The changes included myelin abnormalities, similar to those reported in other parts of the central nervous system; the presence of degenerating axons and their sheaths; changes in neuroglial cells; and thickening of the trabeculae of connective tissue in the nerve. The total number of nerve fibers was reduced from an average of 1.6×10^6 in the young optic nerves to as few as 4×10^5 in one old monkey, and with one exception in all of the old optic nerves the packing density of nerve fibers was less than in any of the young optic nerves. The degenerative changes were most marked in those optic nerves that contained the fewest nerve fibers. J. Comp. Neurol. 429: 541–553, 2001.

Indexing terms: aging; primate; myelin; axons; degeneration; electron microscopy

Previous studies of the effects of age on the optic nerve in monkey and human have reported a modest and variable loss of nerve fibers, perhaps masked by a large variation in the total number of nerve fibers within individual optic nerves (Mikelberg et al., 1989; Repka and Quigley, 1989; Morrison et al., 1990). However, these studies have all relied on immersion-fixed material, which is unsuitable for characterizing age-related changes in the nerve fibers themselves. The present study was designed to combine nerve fiber counts with structural analyses of the same nerves in young and old rhesus monkeys.

The impact of aging on the optic nerve is of general interest because it appears that aging has global effects on white matter, but relatively little effect on cortical gray matter (Peters, 1999). For example, magnetic resonance imaging (MRI) scans show a reduction in the volume occupied by white matter in the aging brain, whereas the volume of gray matter stays constant. This is true for both humans (Albert, 1993; Guttmann et al., 1998) and monkeys (Lai et al., 1995). At present it is not known what brings about the loss in volume of aging white matter, but it is probably due to a loss of nerve fibers, and Tang and colleagues have reported a 27% decrease in the total length of myelinated fibers present in the subcortical white matter of human cerebral hemispheres (Tang et al., 1997). Although it is unknown whether nerve fiber loss with age is ubiquitous in the central nervous system, degenerative age-related changes have been observed in myelin sheaths in the few locations in which nerve fibers have been examined in detail (reviewed by Feldman and Peters, 1998; Peters, 1999).

We have chosen to examine the optic nerve of the monkey as an example of a circumscribed tract of white matter to determine whether it loses nerve fibers with age and whether there are concomitant alterations in myelin sheaths in normal aging. The optic nerve has the practical advantage that a single section contains all the axon profiles, which can be readily counted. Furthermore, the optic

Grant sponsor: National Institutes of Health, National Institute on Aging; Grant number: 2PO1 AG00001.

*Correspondence to: Julie H. Sandell, Department of Anatomy and Neurobiology, 715 Albany Street, Boston, MA 02118.
E-mail: jsandell@cajal-1.bu.edu

Received 3 August 2000; Revised 2 October 2000; Accepted 2 October 2000

TABLE 1. Quantitative Analyses of Optic Nerves

Age (yr), sex	ID no, optic nerve	Total fibers	Area (μm^2)	Fibers/100 μm^2	% Area occupied by trabeculae
4, M	AM058, right	1692379	7126800	23.74	
4, M	AM058, left	1514860	5345143	28.34	
5, M	R419, left	1470878	5256300	27.98	7.08
5, M	R419, right	1507763	4898781	30.78	
6, F	AM076, left	1335603	6129383	21.79	6.91
6, F	AM076, right	1431711	5749000	24.90	
6, F	AM077, left	1581199	5154934	30.67	
6, F	AM077, right	1694823	5580480	30.37	6.38
9, F	AM097, left	2061735	6381900	32.31	
9, F	AM097, right	2066343	6289400	32.85	8.69
9, M	AM047, right	1697390	6595035	25.74	
10, M	AM053, left	1627496	4975000	32.71	7.33
10, M	AM053, right	1541652	4685205	32.90	
Mean, young		**1632600**	**5705200**	**28.86**	**7.28**
27, M	AM062, left	999820	6266828	15.95	
29, F	AM026, right	912342	5311018	17.18	
29, F	AM026, left	974749	5012941	19.44	9.14
32, M	AM091, right	712036	4927558	14.45	12.74
32, M	AM091, left	635159	4979621	12.76	
32, F	AM041, left	384044	3589836	10.70	10.83
32, F	AM041, right	410499	3425151	11.98	
32, F	AM023, right	679475	4342971	15.65	12.63
32, F	AM023, left	1306000	5604437	23.30	
33, F	AM065, left	1481563	6890098	21.50	
33, F	AM065, right	1485347	6150889	24.15	8.62
Mean, old		**907370**	**5136500**	**17.18**	**10.79**
Two-tailed *P* value, young vs. old		<0.0001	0.1426, n.s.	<0.0001	0.0059

nerves are available from monkeys whose brains have been fixed by transcardial perfusion, which ensures that the fixation of the tissue is optimal. As will be shown, there is loss of nerve fibers from the normally aging monkey optic nerve, and this loss is due to a degeneration of nerve fibers.

MATERIALS AND METHODS

Tissue specimens and processing

These studies used optic nerves from rhesus monkeys (*Macaca mulatta*). Optic nerves were obtained from seven young monkeys (4–10 years of age) and six old monkeys (27–33 years of age). In most cases both left and right nerves were available and were processed, although in one 9-year-old monkey (AM 047) and one 27-year-old monkey (AM 062), only a single nerve was available (Table 1). The monkeys were part of a large population that has been used for studies of cognition and brain structure during normal aging. The monkeys were maintained at the Yerkes Regional Primate Research Center at Emory University and at Boston University School of Medicine. Three of the old monkeys (AM 062, 065, and 091) had received ophthalmoscopic examinations performed by Dr. Alcides Fernandes while they were part of the Yerkes colony. All three had some degree of cataractous lens changes. Two of the three (AM 062 and AM 065) had normal intraocular pressure; the intraocular pressure was not measured in the third animal. One of these animals (AM 065) had some retinal pigment epithelium atrophy near the macula, but no other significant age-related changes were noted. No visual psychophysical tests were performed by the animals in this study, although all the monkeys were highly practiced and successful at visual discrimination tasks that are part of the behavioral battery used to assess cognition (e.g., Killiany et al., 2000).

The procedures used for tissue preparation have been described previously (Peters et al., 1994, 2000). All procedures regarding the care and euthanasia of these animals were approved by the Institutional Animal Care and Use Committee of Boston University School of Medicine and were in accordance with the NIH publication *Guide for the Care and Use of Laboratory Animals*. In brief, the animals were preanesthetized with ketamine (10 mg/kg) and further anesthetized to a state of areflexia with intravenous sodium pentobarbital (35 mg/kg). The anesthetized animals were given artificial respiration with 95% oxygen and 5% carbon dioxide and perfused transcardially with 4 L of warm fixative (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M PO_4 or 0.1 M cacodylate buffer at pH 7.4). The eyes were removed, together with a segment of the optic nerve that extended from the globe to near the chiasm. The nerves were cut into several segments, each approx. 2 mm in length, and were stored in the cold in a stronger fixative (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M PO_4 or 0.1 M cacodylate buffer, pH 7.4) until further processing. The segments of optic nerve were osmicated, dehydrated in ascending concentrations of ethanol, and embedded in Araldite. Sections 1 μm thick were collected and stained with toluidine blue for light microscopic studies. Thin sections were taken for electron microscopy, stained with uranyl acetate and lead citrate, and subsequently examined in a JEOL 100 electron microscope.

Nerve fiber counts

An estimate of the total number of nerve fiber profiles was made for each optic nerve. Sections stained with toluidine blue were analyzed using the Bioquant BQ-TCW-95 system (R & M Biometrics, Nashville, TN) and a motorized stage. A section was viewed with a 100× oil immersion objective (NA 1.40) and the image projected onto a video monitor. The counting of profiles was done by two human observers, not the computer, but the computer was used to generate the counting grid, move the stage, project the counting box onto the video image, mark the

profiles as they were counted, tally the counts, and calculate the cross-sectional area of the nerve. The fibers in several nerves were counted by both observers, and the interobserver variability averaged 6.9%. The counting boxes were 15 × 15 μm each, spaced 200 μm from center to center in a grid that was placed randomly on the section of the nerve. All myelinated nerve fiber profiles were counted in each counting box, provided that they did not intersect the forbidden margins of the counting box (left side and bottom). The counting parameters, such as counting box size and grid spacing, were determined empirically to yield coefficients of error (CE = SEM/mean) for individual nerves of 2.3–6.7%, well within the 10% recommended by West and colleagues for stereological analysis (West et al., 1996; Howard and Reed, 1998). The following formula was used to estimate the total number of nerve fiber profiles in each nerve:

$$\text{total profiles} = (\text{cross-sectional area of nerve} / \Sigma \text{ area of counting boxes}) \times \text{profiles counted}$$

It should be noted that the optic nerve contains fibrous trabeculae that contribute to the cross-sectional area but that do not contain nerve fibers. We chose to simply count all the nerve fiber profiles in each counting box, regardless of whether some portion of the counting box was occupied by fibrous trabeculae. These counts are then valid for estimating the total number of profiles when they are used in conjunction with a cross-sectional area that likewise includes the area occupied by the fibrous trabeculae. This strategy allowed us to use an unbiased sampling scheme to count profiles in a consistent counting grid that could be applied to all nerves, rather than manipulating the size or placement of the counting boxes to avoid trabeculae.

Area of fibrous trabeculae

To determine the magnitude of any age-related change in the area occupied by the fibrous trabeculae within the optic nerve, we determined the proportion of the cross-sectional area occupied by the trabeculae in individual nerves from five young animals and five old animals. Each section was drawn with the aid of a camera lucida, and after they had been drawn the trabeculae were inked in. The drawings were then scanned and digitized with Adobe Photoshop 4.0 and analyzed with NIH Image 6.1, to calculate the proportion of the total area of the cross-sectioned nerve occupied by the trabeculae.

Packing density of nerve fiber profiles

Two methods were used to determine whether the packing density of the nerve fiber profiles was affected by age. First the packing density for each nerve was determined by dividing the estimate of the total number of profiles by the total cross-sectional area of the nerve. All these packing densities are underestimates, however, because a small proportion of the cross-sectional area is occupied by fibrous trabeculae, and the greater the area occupied by trabeculae, the greater the potential underestimate of packing density. Consequently, for a subset of animals (five young and five old) the profile packing density was recalculated excluding the fibrous trabeculae, using only the area of the optic nerve sections occupied by nerve fibers (see above).

Electron microscopy

Thin sections from each nerve were examined by electron microscopy to ascertain the presence or absence of age-related abnormalities in the nerve fibers and neuroglial cells.

Statistical analysis

For statistical purposes the optic nerves were divided into two groups. "Young" designates nerves from animals that were ≤10 years old, and "Old" designates nerves from animals that were ≥27 years old. In most cases both nerves from each animal were analyzed, and these were treated as separate data points. Student's t-tests were used to determine the significance of differences between the group means for total fiber profile number, area of the nerve, fiber profile packing density, and proportion of the cross-sectional area occupied by fibrous trabeculae. All significance values were obtained from two-tailed tests, on the assumption that we could not predict a unidirectional effect of age on any variable. Although a linear regression analysis is very common in studies of this type to assess the relationship between age and other variables, we considered that such an analysis was not appropriate for this study, given the absence of data from animals between 10 and 27 years of age.

RESULTS

Light microscopy

The optic nerves of young monkeys are about 3 mm in diameter, and in cross sections the nerve fibers are seen to be closely packed and partially segregated into bundles by intervening trabeculae that contain the blood vessels supplying the nerve (Fig. 1). The nerve fibers in the optic nerve are between 0.5 and 3.0 μm in diameter, with the smaller nerve fibers predominating. Between the nerve fibers the pale staining cell bodies of astrocytes are visible (arrows), and some of them show processes that extend between the nerve fibers.

The optic nerves of some old monkeys are basically similar in appearance to those of young monkeys (Fig. 2), although the fibrous trabeculae are usually thicker than in the optic nerves of young monkeys, and the astrocytes (arrows) generally have larger cell bodies with prominent processes (arrowheads) extending between the nerve fibers. In the optic nerves of other old monkeys the effects of age are much more apparent. In these optic nerves, there is a more extensive thickening of the fibrous trabeculae, with the result that the nerve fibers are segregated into bundles (Fig. 3). In addition, the astrocytes (arrows) have hypertrophied so that they have large cell bodies, most of which are star-shaped with thick processes extending between the nerve fibers. In such optic nerves the darker cell bodies of oligodendrocytes and microglial cells can be discerned.

Electron microscopy

Even in optic nerves from young monkeys the fixation of the myelin is never optimal. As shown in Figure 4, a large proportion of the nerve fibers as viewed in cross sections have myelin sheaths showing shearing defects (arrows) in one part of their circumference. Such shearing defects result in a separation or splitting of the lamellae in one segment of the sheath profile. The other common fixation

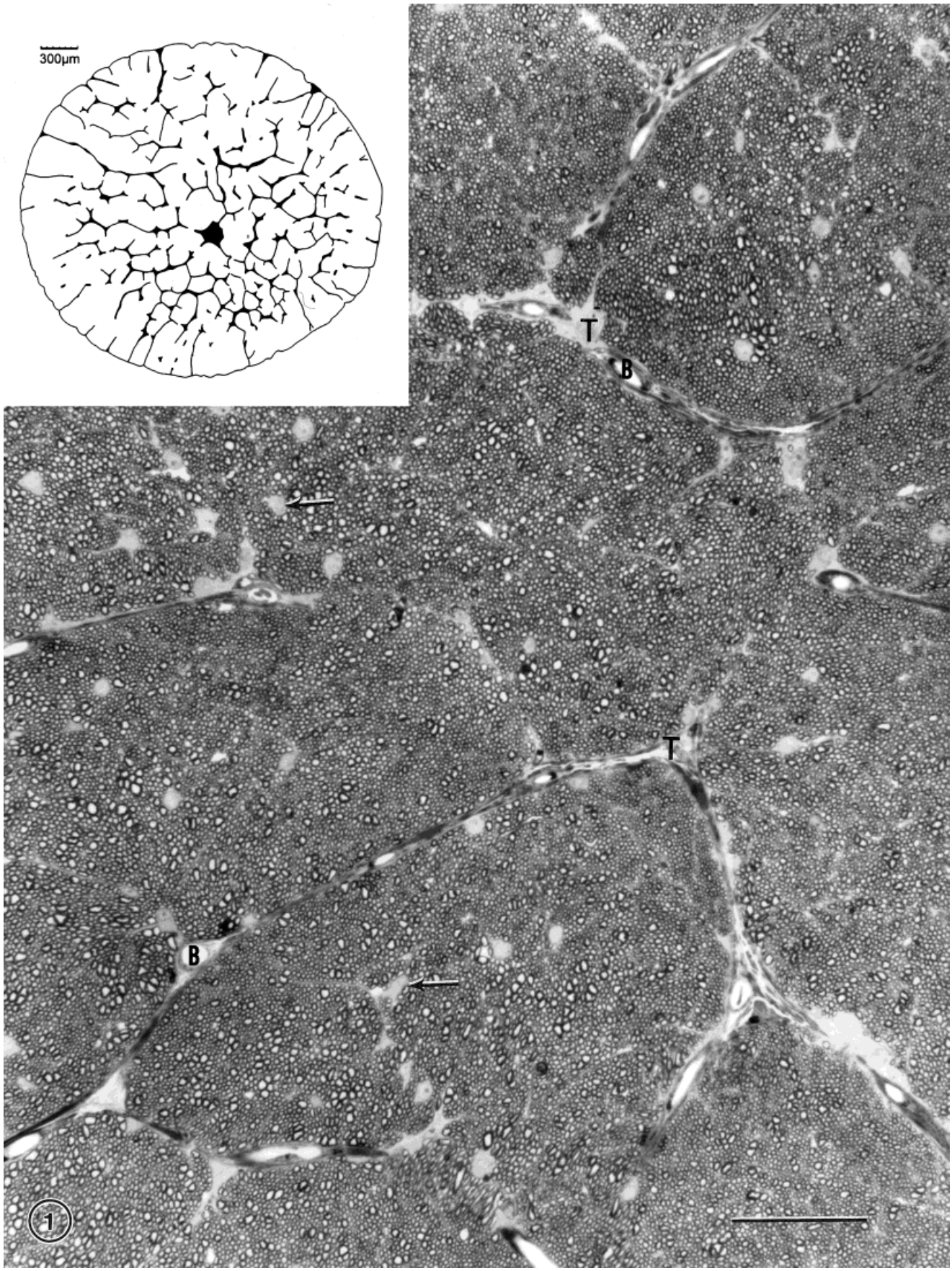


Fig. 1. Figures 1–3 are light microscope photographs of semithick sections of cross-sectioned optic nerves stained with toluidine blue. The insets are drawings of the entire cross sections of the optic nerves to show the disposition of the connective tissue trabeculae. Fig. 1. The optic nerve of a 6-year-old monkey, AM 076. The optic nerve is composed of closely packed myelinated nerve fibers that have a range of sizes. The nerve fibers are incompletely separated into bundles by the connective tissue trabeculae (T), which contain the blood vessels (B) supplying the nerve. Within the bundles of nerve fibers the cell bodies of astrocytes (arrows) are visible. Scale bar = 50 µm.

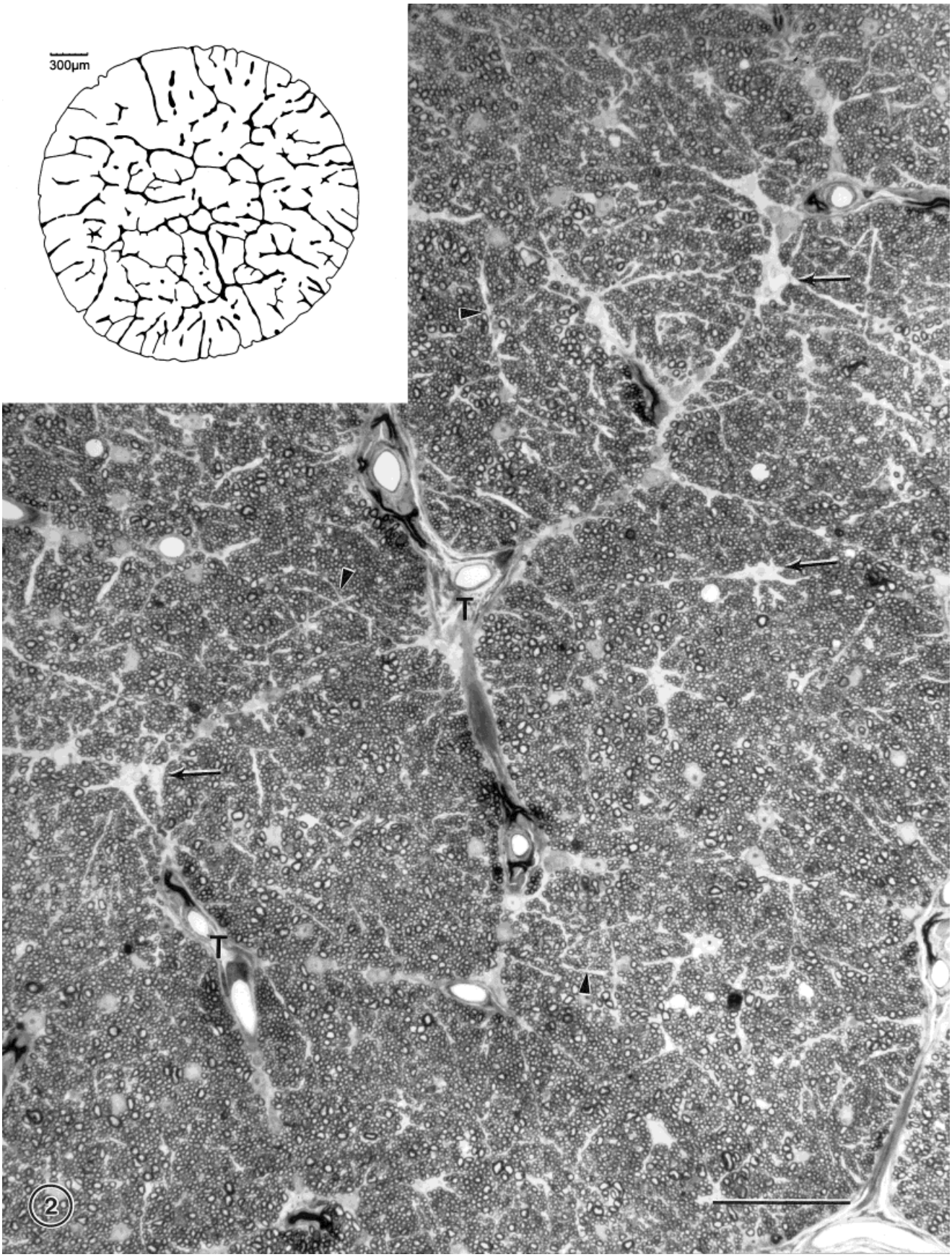


Fig. 2. See introductory comment, Figure 1. The optic nerve of a 29-year-old monkey, AM 026. The optic nerve of this particular old monkey shows few signs of degeneration. The nerve fibers are still closely packed, but the connective tissue trabeculae (T) are thicker than in young nerves, and the astrocytes (arrows) have prominent processes (arrowheads) extending between the nerve fibers. Scale bar = 50 μm.

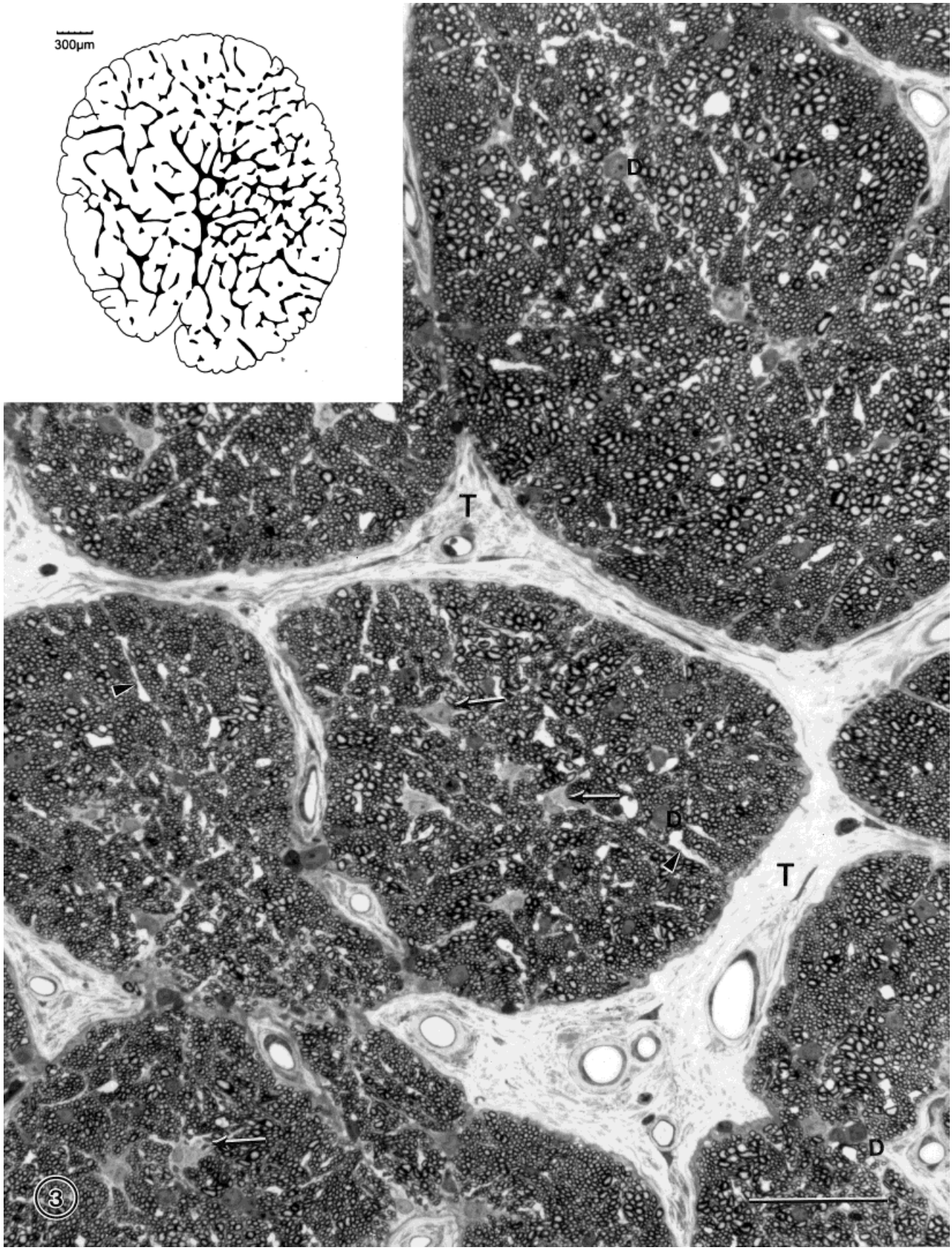


Fig. 3. See introductory comment, Figure 1. The optic nerve of a 32-year-old monkey, AM 091. In this optic nerve the fibrous trabeculae (T) are very thick and have essentially segregated the nerve fibers into bundles. Although the nerve fibers are still closely packed, the astrocytes (arrows) have hypertrophied to produce thick processes (arrowheads) that pass between the nerve fibers. In these optic nerves the darkly staining cell bodies (D) of microglia and oligodendrocytes are apparent. Scale bar = 50 μm.

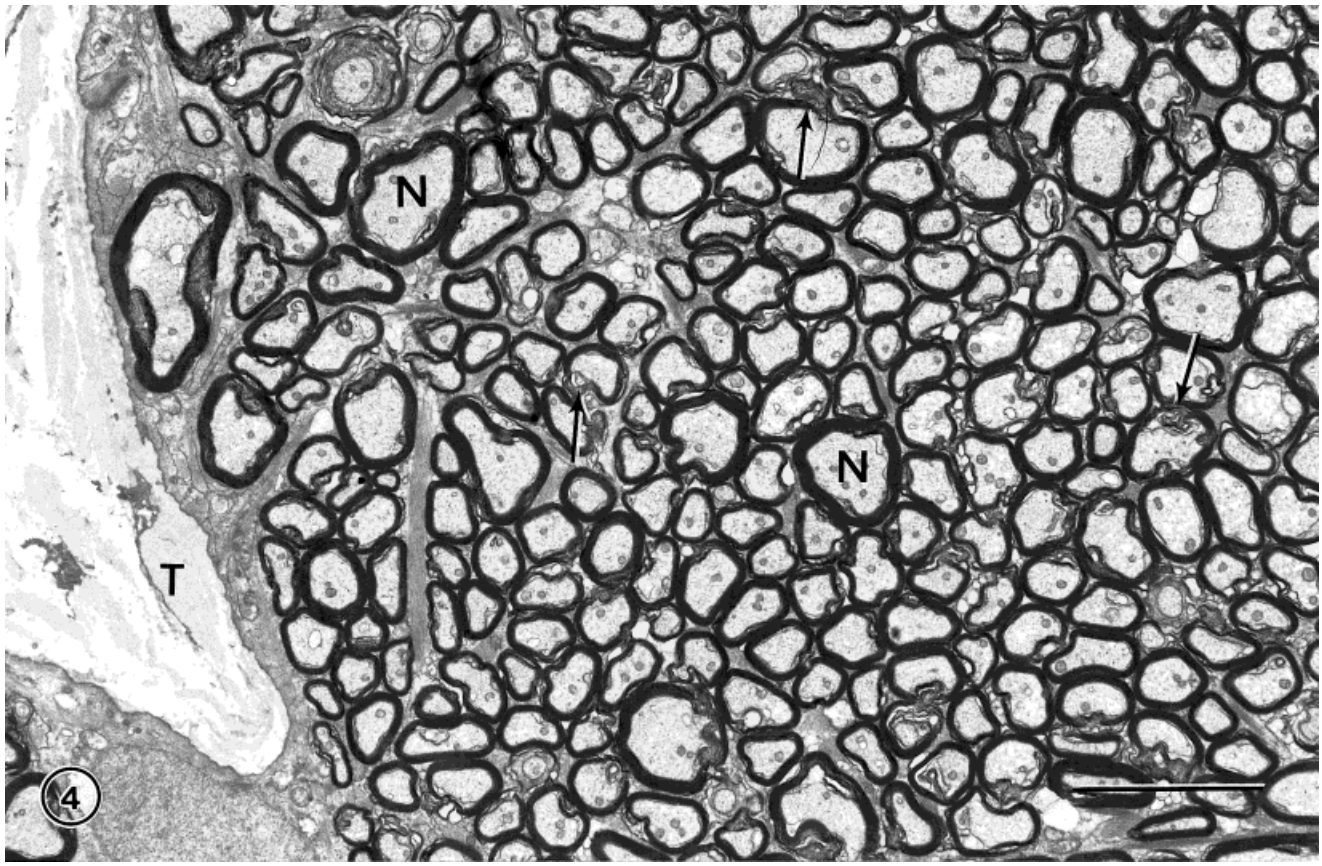


Fig. 4. Electron micrograph of part of the optic nerve of a 4-year-old monkey, AM 058. In young optic nerves the nerve fibers (N) are closely packed so that their myelin sheaths often abut each other. Frequently the myelin sheaths show shearing defects, such that over part of the circumference of the profile of a sheath the lamellae are separated (arrows). On the left of the micrograph, part of a trabeculum (T) contains collagen fibers. Scale bar = 5 μm.

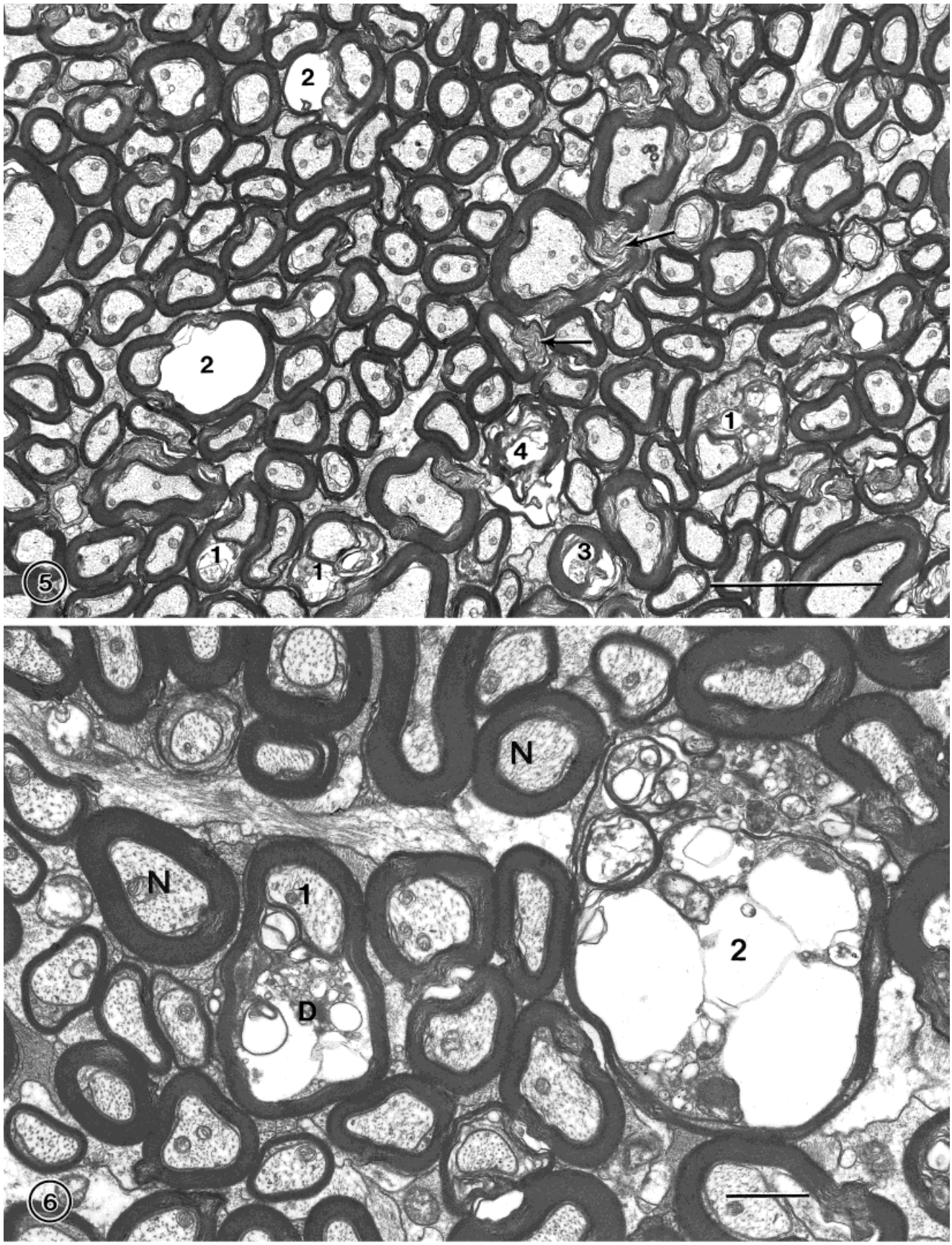
defect is a swelling of the processes of astrocytes that course between the nerve fibers in the form of sheets, although the astrocytic processes that form the glial limiting membrane are rarely swollen.

In the optic nerves of young monkeys, the nerve fibers are tightly packed, so that the outer surfaces of nerve fibers are often apposed to the outsides of their neighbors, and the large majority of the myelin sheaths show no defects beyond the shearing described above (Fig. 4). The cytoplasm of the axons is pale and contains mitochondria surrounded by a matrix of evenly spaced microtubules and neurofilaments. Between nerve fibers are the cell bodies of neuroglial cells, and there is some separation of the nerve fibers into bundles by the fibrous trabeculae that contain fibroblasts and bundles of collagen fibers. Embedded in these trabeculae are the blood vessels that supply the optic nerve.

Some of the optic nerves from old monkeys, like those of AM 065 (33 years old), have an appearance quite similar to those of young monkeys, in that the nerve fibers are closely packed and show little change other than fixation defects. However, in most optic nerves from old monkeys the nerve fibers show a decreased packing density, which varies in different portions of the nerve. Filling the spaces between the separate nerve fibers are an increased number of processes of astrocytes. All the optic nerves from old monkeys have at least a few myelin sheaths that display morphological alterations similar to those that have been encountered in the cerebral cortex (Feldman and Peters, 1998; Peters et al., 2000). Examples are shown in Figures

Fig. 5 (Overleaf). Electron micrograph of part of the optic nerve of a 27-year-old monkey, AM 062. Although the myelin sheaths of some of the nerve fibers show shearing defects (arrows), others show signs of degeneration. Some sheaths (1) shows a splitting of the myelin lamellae to accommodate electron-dense cytoplasm, whereas others (2) have sheaths that have ballooned out. In other cases the axons appear to have degenerated, so that the myelin sheath surrounds debris (3), or the axon has disappeared and the sheath has started to degenerate (4). Scale bar = 5 μm.

Fig. 6 (Overleaf). Electron micrograph of part of the optic nerve of a 27-year-old monkey, AM 062. In this higher magnification micrograph it is apparent that the myelin sheaths of most of the nerve fibers (N) are intact, but two nerve fibers show degeneration. In one case (1) the myelin sheath has split to accommodate dense cytoplasm (D) containing vacuoles. In another nerve fiber (2) the axon appears to have been lost, leaving a myelin sheath that has swollen and is beginning to degenerate. Scale bar = 1 μm.



Figures 5–6 (Overleaf)

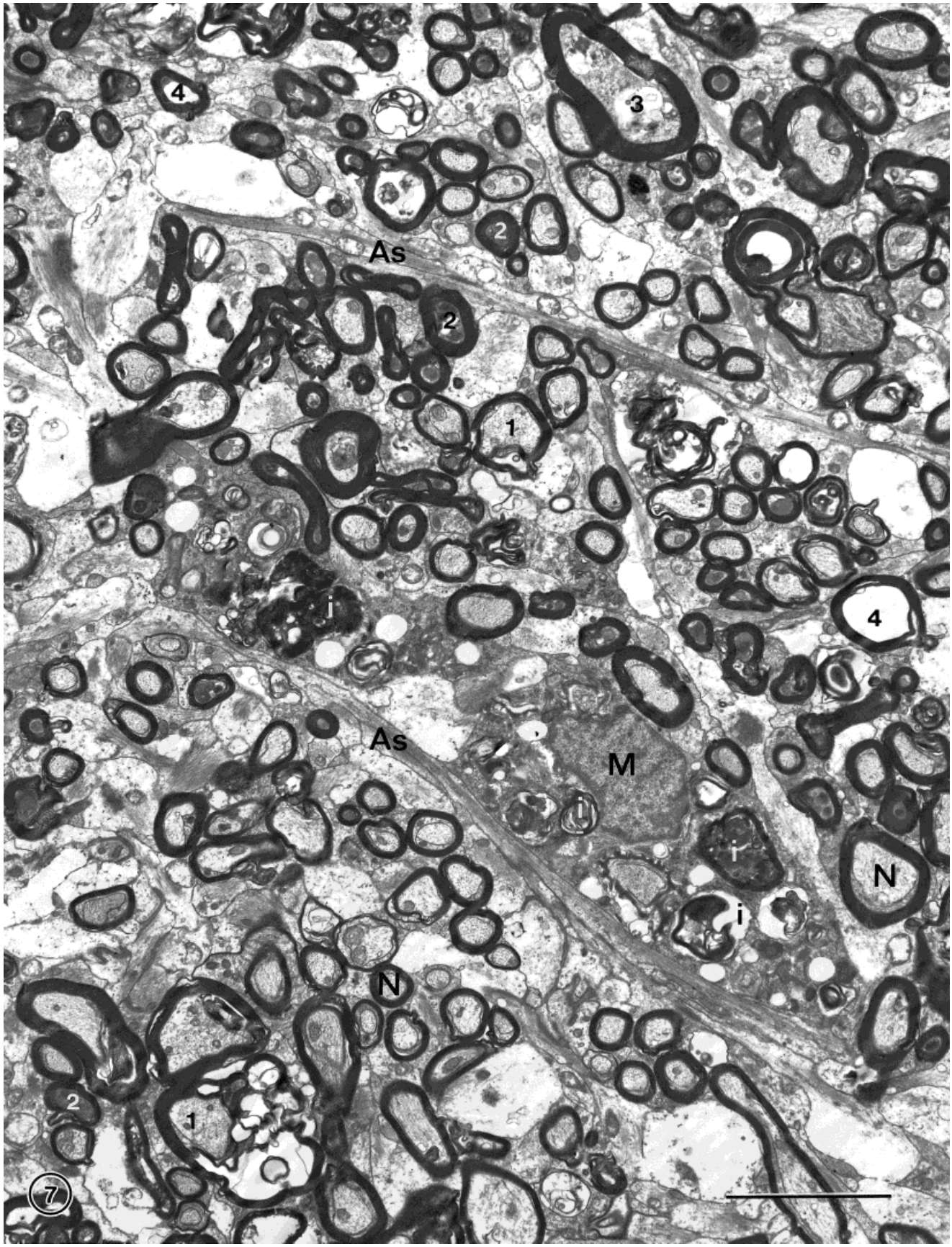


Fig. 7. Electron micrograph of part of the optic nerve of a 32-year-old monkey, AM 041. The optic nerve of this monkey shows extensive degeneration. Although some nerve fibers (N) appear to be intact, other nerve fibers have intact axons, but altered sheaths (1). In other nerve fibers it is the axon that is affected, so that some axons have electron-dense cytoplasm (2), and other axons have cytoplasm that is vacuolated and granular (3). In yet other cases the axon appears to have degenerated so that the myelin sheath is empty (4). In the middle of the field is a microglial cell (M). Its cytoplasm contains a large number of inclusions (i) that are obviously derived from degenerating nerve fibers, and passing between the nerve fibers are the processes of astrocytes (As). Scale bar = 5 μm.

5 and 6, which are from AM 062, a 27-year-old monkey. These alterations include the ballooning of some myelin sheaths through splits along the intraperiod line to form a fluid-filled vesicle (label 2 in Fig. 5), and the separation of other myelin sheaths at the major dense line to accommodate dark cytoplasm that frequently contains vesicles (label 1 in Figs. 5, 6). This splitting is different from the shearing brought about by imperfect fixation (arrows in Fig. 5) because the shearing defects are not associated with accumulations of cytoplasm and are generally confined to a small portion of the circumference of the sheath. However, in addition to these alterations in myelin sheaths, other nerve fibers exhibit degeneration of their axons (labels 3 and 4 in Fig. 5), and such degeneration is especially common in optic nerves that are most seriously affected by age. In such nerves (Fig. 7, AM 041, 32 years old) some of the axons have electron-dense axoplasm that may contain vacuoles (labels 2 and 3 in Fig. 7), whereas in other cases the axons appear to have degenerated, leaving myelin sheaths that surround debris, or sheaths that are empty (label 4 in Fig. 7) and breaking down (also see Fig. 6).

Age also affects the neuroglial cells. In the optic nerves from old monkeys neuroglial cells appear to be more frequent than in young monkeys, and the neuroglial cells frequently have inclusions in their cytoplasm. This increase in the frequency of neuroglial cells is most obvious in the optic nerves that show the most extensive degeneration of nerve fibers. In such optic nerves the microglial cells are very prominent and contain numerous inclusions that are obviously derived from the phagocytosis of myelin (Fig. 7).

These observations indicate that although nerve fibers in all optic nerves display some breakdown of myelin with age, similar to that which occurs in other parts of the central nervous system, in those nerves that are most severely affected by age there is also axonal degeneration. This axonal degeneration leads to the subsequent degeneration of the myelin sheaths and the phagocytosis of the degenerating myelin by microglial cells.

Total nerve fiber profile counts and profile packing density

As suggested by the microscopic examination of the optic nerves, the mean number of nerve fiber profiles in the optic nerves of young monkeys is significantly greater than the mean number present in the optic nerves of old monkeys (estimated mean ± SD for young = 1,632,600 ± 219,650 profiles; for old = 907,370 ± 389,960 profiles; two-tailed $P < 0.0001$). There is considerable variability among the optic nerves taken from old animals of similar age, as can be seen in Figure 8 and Table 1. However, in general, the light and electron microscopic appearance of the two nerves from an individual monkey, and the number of nerve fibers present, are similar to each other (Table 1). We did not observe any obvious effect of sex on the population of nerve fibers in either young or old animals, although our sample is too small to draw any firm conclusions on this point. The aged nerves with the most and the least fibers both came from females, although two-thirds of our specimens were obtained from female monkeys.

Despite the overall reduction in nerve fiber number with age, there is no significant difference in the cross-sectional areas of the nerves in young vs. old animals (mean area ± SD for young = 5,705,200 ± 745,990 μm^2; for old = 5,136,500 ± 108,010 μm^2). However, because there is a loss of nerve fibers with age, the packing density of the nerve fibers is significantly greater in the nerves from young animals, as seen in Figure 9 (mean ± SD for young = 28.85 ± 3.7/100 μm^2; for old = 17.18 ± 4.4/100 μm^2; two-tailed $P < 0.0001$). However, the age-related reduction in nerve fiber packing density is not uniform in all nerves. In the nerves from some old monkeys there are patches in which the packing density of the nerve fibers is reduced: sometimes the patches are at the periphery, and in other cases they are at the center of the nerve. In addition to loss of nerve fibers, it is clear that at least three other factors contribute to the reduced packing density. These are a thickening of the fibrous trabeculae, an increase in the abundance of the astrocytic cytoplasm that fills the space between adjacent nerve fibers, and an increase in the number of neuroglial cell bodies with age.

Changes in fibrous components of the nerve with age

Compared with the optic nerves of young monkeys, many, but not all, optic nerves from old animals exhibit a thickening of the trabeculae of fibrous connective tissue (Figs. 1–3). Consequently, these trabeculae occupy a significantly greater fraction of the cross-sectional area of the nerves in the old animals (mean ± SD for young is 7.28 ± 0.86%; mean for old is 10.79 ± 1.91%; two-tailed $P = 0.0059$). To determine whether the expansion of the fibrous trabeculae could account for all the change in the fiber profile packing density with age, we calculated what the packing density of nerve fibers would be if the space occupied by the fibrous trabeculae were omitted from the calculation of nerve fiber packing density. To accomplish this, sections of the optic nerves from five monkeys in each age group were drawn using a camera lucida, the drawing was digitized, and the area occupied by the fibrous trabeculae was determined.

As expected, when the nerve fiber profile packing densities are recalculated using cross-sectional areas adjusted to reflect the space occupied by trabeculae, the packing densities increase slightly (mean ± SD for young = 29.40 ± 5.0/100 μm^2; for old = 17.61 ± 5.3/100 μm^2). However, the difference between the packing densities for the young and old nerves remains significant (two-tailed $P = 0.0067$). Therefore the trabeculae do not simply thicken to encroach on space that was occupied by nerve fibers that have been lost with age. Indeed, as visualized by the electron microscopic analyses, in the optic nerves of old monkeys the remaining nerve fibers are spaced further apart within the nerve, and the space that was occupied by nerve fibers that have degenerated is replaced not only by fibrous trabeculae, but by the processes of hypertrophied astrocytes and other neuroglial cells (Fig. 7).

DISCUSSION

This study shows that compared with young monkeys, old monkeys as a group have fewer nerve fiber profiles in their optic nerves, and the packing density of the profiles is reduced. Although electron microscopy shows some degenerative changes in the myelin sheaths of nerve fibers in the optic nerve in *all* old monkeys, in the optic nerves most affected by age the dominant change is the degeneration of axons. This axonal degeneration ultimately leads

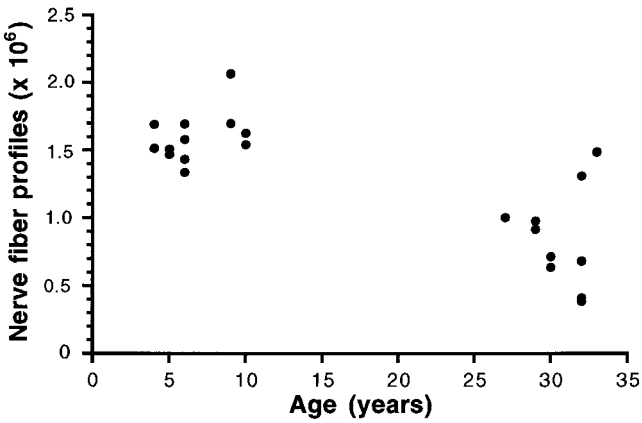


Fig. 8. Scatterplot of estimates of total nerve fiber profiles in the optic nerve as a function of age. Counts are shown for a total of 13 nerves from seven young monkeys and 11 nerves from six old monkeys. Some counts are almost identical in the two nerves from a single animal (Table 1) and so cannot be resolved as separate data points.

to a degeneration of myelin and its phagocytosis by neuroglial cells. At the same time the space in the optic nerve occupied by the fibrous trabeculae increases with age. These results are in agreement with previous studies in humans and monkeys, which have suggested that optic nerve fibers are vulnerable to age, although to our knowledge no previous studies have combined fiber counts with examination of the fine structure of the nerve.

Counting studies

Several previous studies of optic nerves from normal humans have documented a significant age-related loss of nerve fibers (Balazsi et al., 1984; Mikelberg et al., 1989; Jonas et al., 1992), although in one study the loss was not statistically significant (Repka and Quigley, 1989). The only previous study of optic nerve fiber numbers in the aging rhesus monkey (Morrison et al., 1990) reported a negative correlation between age and nerve fiber number, which did not reach statistical significance ($P = 0.069$). The authors attribute this to normal individual variability and note that as a group animals older than 10 years of age have significantly fewer fibers than animals less than 10 years of age. The packing density of fibers was also noted to be significantly greater in young animals (1.5–2 years of age) than in older animals (Morrison et al., 1990), which is in agreement with our results.

When old optic nerves are examined by electron microscopy, it is evident that all of them contain some degenerating axons, and this is particularly obvious in the nerves with the fewest total number of fibers. Indeed, axonal degeneration is the only explanation for the age-related fiber loss. Such degeneration was not reported in previous studies, perhaps because the degeneration is not particularly obvious when optic nerves are examined by light microscopy. Previous studies of aging monkey optic nerves did not include fine structural analyses, and adequate fixation is difficult to achieve with human optic nerves. A single study of human nerves reported axonal swelling (Munari et al., 1989), which was not common in our specimens. Even with optimized intracardiac perfusion procedures, it is still difficult to obtain adequate fixation of white matter for electron microscopy. Inadequate fixation caused us to discard optic nerves from a number of monkeys, and it may be that the very factors that make adequate fixation of the optic nerve so difficult, such as thick connective tissue surrounding blood vessels, also increase the likelihood of damage to the fibers in vivo.

Our findings raise several interesting questions about the relationship between retinal ganglion cell axons within the nerve and the parent cell bodies in the eye. Although most studies have assumed that nerve fiber number reflects ganglion cell number, nerve fibers and ganglion cells have not been counted in the same specimens. Retinal ganglion cells are notoriously difficult to count, in part because there is no marker that labels all retinal ganglion cells and only retinal ganglion cells. In addition, a significant fraction of the neurons in the retinal ganglion cell layer represents displaced amacrine cells (Hughes and Wieniawa-Narkiewicz, 1980; Perry and Walker, 1980; Koontz et al., 1993; Masland et al., 1993), making the use of nonspecific markers problematic. Despite these difficulties, two groups have used high-resolution Nomarski microscopy to examine retinal ganglion cell number in whole-mount retinae, with conflicting results. Curcio and Drucker (1993) concluded that compared with young controls there is a 25% decrease in the number of retinal ganglion cells representing the central 11° of the visual field in four 66–86-year-old humans. In contrast, a similar study in rhesus monkey failed to find a significant loss of retinal ganglion cells with age (Kim et al., 1996).

This raises the question of whether there is a concomitant age-related loss of nerve fibers in the optic nerve and of ganglion cells in the retina. Although it is unlikely that an axon can survive in the absence of its sustaining cell body, the converse may not be true. Perhaps retinal ganglion cells can survive damage to their axons, provided that the damage takes place gradually, as may occur over the lifespan of the monkey, rather than abruptly, as occurs in experimental axotomy. In any case, the relationship between numbers of nerve fibers in the optic nerve and numbers of ganglion cells in the retina will not be resolved until both are counted in the same aged individuals.

Fibrous trabeculae

Several studies of human optic nerve across the lifespan have noted thickening of the fibrous trabeculae with age (Dolman et al., 1980; Giarelli et al., 1989; Munari et al., 1989). At the periphery of the nerve these trabeculae are in continuity with the meninges, but in the semithick plastic sections that we have examined, most trabeculae appear as isolated branching islands of connective tissue. Usually the trabeculae contain blood vessels and fibroblasts, and one potential consequence of thickened trabeculae may be an age-related decrease in the availability of oxygen and nutrients to the axons and glial cells. The present study is the first to quantify this change in the connective tissue components of the monkey nerve with age. The overall contribution of the trabeculae to the cross-sectional area of the nerve is rather modest—the trabeculae occupy an average of 7.28% of the cross-sectional area in young nerves vs. 10.79% of the area in old nerves. Nonetheless, this is an increase of 48% in the volume of this tissue compartment with age.

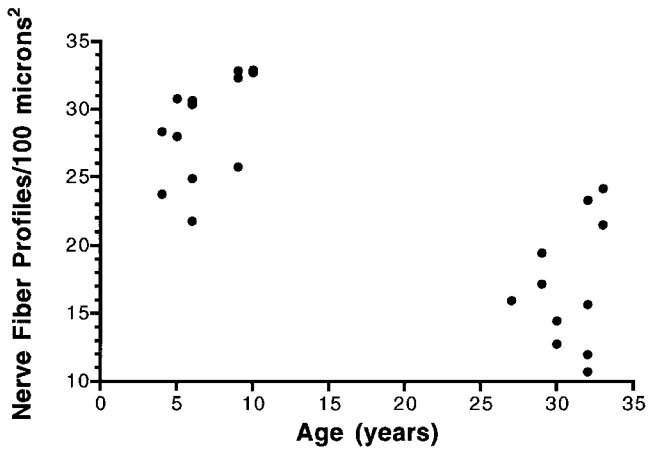


Fig. 9. Scatterplot of overall density of nerve fiber profiles as a function of age in the same nerves shown in Figure 8.

Changes in myelin sheaths

With the exception of the shearing defects that are brought about by fixation, the myelin sheaths in the optic nerves of young monkeys appear to be normal, but a proportion of the nerve fiber profiles in all of the old monkeys shows age-related changes in their myelin sheaths. However, the occurrence of such changes is greatest in those old optic nerves with the fewest nerve fibers. The most common myelin abnormalities in the aging optic nerve are splitting of the major dense line to accommodate dense cytoplasm and ballooning of a myelin sheath produced by a splitting of the intraperiod line. These age-related myelin changes are identical to those we have observed in other parts of the aging brain (Feldman and Peters, 1998; Peters et al., 2000).

The age-related loss of nerve fibers from the optic nerve is reminiscent of the age-related loss in the volume of white matter in the cerebral hemispheres. As stated earlier, this loss of white matter with age has been documented by MRI (Albert, 1993; Lai et al., 1995; Guttmann et al., 1998), and Tang and colleagues (1997) have shown an age-related reduction in total length of myelinated fibers in white matter of the cerebral hemispheres. Presumably this loss of myelinated nerve fiber length in the cerebral hemispheres is a result of degeneration of nerve fibers, but as far as we are aware, such nerve fiber degeneration has not yet been visualized. It is of interest, however, that the loss of white matter in the cerebral hemispheres seems to take place in the absence of significant age-related nerve cell loss for the cerebral cortex (Peters et al., 1998a; Hof et al., 2000; Merrill et al., 2000) and in the absence of a significant loss of efferent fibers from the cortex (Nielsen and Peters, 2000).

Functional implications

The aged optic nerves we examined were obtained from rhesus monkeys between 27 and 33 years of age. This range is equivalent to approximately 81–99 years of human age, using the 1:3 metric for development to compare the two species (Tigges et al., 1988). Elderly humans experience a variety of visual deficits with age, including changes in acuity, accommodation, dark adaptation, visual thresholds, and contrast sensitivity (reviewed by Spear, 1993). Some of these deficits arise from age-related optical factors, including smaller, less responsive pupils (senile miosis) and changes to the lens. Aging is also associated with an increased incidence of ocular diseases including macular degeneration and glaucoma, as well as systemic diseases with ocular complications, such as hypertension and diabetes (reviewed by Garner, 1994; Garner et al., 1994). However, with the exception of glaucoma, these conditions all have a far greater impact on the optical apparatus, or on the outer retina, than on the retinal ganglion cells whose axons comprise the optic nerve.

From a practical standpoint the survival of retinal ganglion cell axons is critical, since the axons form the obligatory link between the eye and the brain, but it is unclear precisely how the number of optic nerve fibers is related to visual function. One might expect that more fibers could support better visual acuity, if more retinal ganglion cells allowed a finer grained analysis of the retinal image. However, the variation in fiber number is large in young, presumably normal individuals, whereas acuity as measured by standard tests is fairly constant. Perhaps visual testing under conditions of low contrast or illumination might reveal functional differences related to the number of nerve fibers in the optic nerve.

The aging process

What can the relative vulnerability of optic nerve fibers tell us about the aging process? The optic nerve is unusual in several informative respects. *If* fiber loss from the optic nerve reflects retinal ganglion cell loss, then this raises the question of why these neurons die with age, whereas most neurons in other parts of the visual pathway such as the dorsal lateral geniculate body and the primary visual cortex do not die (Vincent et al., 1989; Ahmad and Spear, 1993; Peters and Sethares, 1993; Kim et al., 1997). Of course, retinal ganglion cells are unusual elements of the central nervous system because their cell bodies are located in a peripheral organ, the eye, where they have potential exposure to mechanical stresses and environmental factors, such as ultraviolet radiation, that do not affect the rest of the brain. Gradual damage to retinal ganglion cells over the lifespan due to such factors could be the primary cause of age-related decline in nerve fiber number in the optic nerve.

On the other hand, loss of nerve fibers could originate within the nerve itself. Unlike other bundles of nerve fibers within the central nervous system, the optic nerve is encased by a full set of meninges, and it is interesting that in the aging brain the cortex closest to the meninges, namely, layer 1, appears more vulnerable than the deeper cortical layers (Peters et al., 1998b). Although we do not yet understand the mechanism for this vulnerability, toxic substances in the cerebrospinal fluid may underlie the age-related damage that is common to both structures.

Finally, it should be noted that age-related visual deficits may be more subtle than simple changes in acuity that might result from the attrition of optic nerve fibers. For example, electrophysiological studies have demonstrated that orientation selectivity and direction bias are diminished in neurons in striate cortex in old monkeys (Schmolesky et al., 2000), even though neuron numbers are preserved in the striate cortex (Vincent et al., 1989; Peters and Sethares, 1993; Kim et al., 1997; Hof et al.,

2000) and the LGN (Ahmad and Spear, 1993). Neuron numbers, by themselves, are likely to be poor predictors of functional abilities in the aging brain, since numerous studies have now demonstrated that age-related cognitive decline is not associated with neuronal loss from the relevant regions of the cortex or hippocampus, although neuron loss in cortically projecting nuclei has been related to cognitive impairment (reviewed by Kemper, 1999). Ubiquitous changes in myelin, however, are likely to have deleterious effects, as system-wide neuronal function depends on the speed and fidelity of axonal transmission by ensembles of axons. The deterioration of myelin sheaths that we observed in the aged optic nerve, as in other parts of the brain, suggests that transmission of visual signals is likely to be impaired in old animals, regardless of the extent to which the neurons survive.

ACKNOWLEDGMENTS

The authors thank Ms. Claire Folger for expert technical and photographic assistance, Ms. Gillian Hamilton for some of the initial fiber counts, and Alcides Fernandes, M.D. for ophthalmological examination of some of the old monkeys.

LITERATURE CITED

Ahmad A, Spear PD. 1993. Effects of aging on the size, density, and number of rhesus monkey lateral geniculate neurons. J Comp Neurol 334:631–643.

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

Balazsi AG, Rootman J, Drance SM, Schulzer M, Douglas GR. 1984. The effect of age on the nerve fiber population of the human optic nerve. Am J Ophthalmol 97:760–766.

Curcio CA, Drucker DN. 1993. Retinal ganglion cells in Alzheimer's disease and aging. Ann Neurol 33:248–257.

Dolman CL, McCormick AQ, Drance SM. 1980. Aging of the optic nerve. Arch Ophthalmol 98:2053–2058.

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

Garner A. 1994. Vascular diseases. In: Garner A, Klintworth GK, editors. Pathobiology of ocular disease: a dynamic approach. New York: Marcel Dekker. p 1625–1710.

Garner A, Sarks S, Sarks JP. 1994. Degenerative and related disorders of the retina and choroid. In: Garner A, Klintworth GK, editors. Pathobiology of ocular disease: a dynamic approach. New York: Marcel Dekker. p 631–674.

Giarelli LG, Grandi G, Delendi M, Falconieri G. 1989. The pathology of optic nerve aging. Metab Pediatr Syst Ophthalmol 12:61–63.

Guttmann CRG, Jolesz FA, Kikinis R, Killiany RJ, Moss MB, Sander T, Albert MS. 1998. White matter changes with normal aging. Neurology 50:972–978.

Hof RP, Nimchinsky EA, Toung WG, Morrison JH. 2000. Numbers of Meynert and layer IVB cells in area V1: a stereologic analysis in young and aged macaque monkeys. J Comp Neurol 420:113–126.

Howard CV, Reed MG. 1998. Unbiased stereology: three dimensional measurement in microscopy. New York: Springer-Verlag.

Hughes A, Wieniawa-Narkiewicz E. 1980. A newly identified population of presumptive microneurones in the cat retinal ganglion cell layer. Nature 284:468–470.

Jonas JB, Schmidt AM, Muller-Bergh JA, Schlotzer-Schrehardt UM, Naumann GOH. 1992. Human optic nerve fiber count and optic disk size. Invest Ophthalmol Vis Sci 33:2012–2018.

Kemper TL. 1999. Age-related changes in subcortical nuclei that project to the cerebral cortex. In: Peters A, Morrison J, editors. Cerebral cortex, vol 14. New York: Plenum Press. p 365–397.

Killiany RJ, Moss MB, Rosene DL. 2000. Recognition memory function in early senescent rhesus monkeys. Psychobiology 28:45–56.

Kim CB, Tom BW, Spear PD. 1996. Effects of aging on the densities, numbers, and sizes of retinal ganglion cells in rhesus monkey. Neurobiol Aging 17:431–438.

Kim CB, Pier LP, Spear PD. 1997. Effects of aging on numbers and sizes of neurons in histochemically defined subregions of monkey striate cortex. Anat Rec 247:119–128.

Koontz MA, Hendrickson LE, Brace ST, Hendrickson AE. 1993. Immunocytochemical localization of GABA and glycine in amacrine and displaced amacrine cells of macaque monkey retina. Vision Res 33:2617–2628.

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 grey matter. Soc Neurosci Abstr 21:1564.

Masland RH, Rizzo JF Jr, Sandell JH. 1993. Developmental variation in the structure of the retina. J Neurosci 13:5194–5202.

Merrill DA, Roberts JA, Tuszynski MH. 2000. Conservation of neuron number and size in entorhinal cortex layers II, III, and V/VI of aged primates. J Comp Neurol 422:396–401.

Mikelberg FS, Drance SM, Schulzer M, Yidegiligne HM, Weis MM. 1989. The normal human optic nerve. Ophthalmology 96:1325–1328.

Morrison JC, Cork LC, Dunkelberger GR, Brown A, Quigley HA. 1990. Aging changes of the rhesus monkey optic nerve. Invest Ophthalmol Vis Sci 31:1623–1627.

Munari PFR, De Caro R, Colletti D. 1989. Anatomy of the optic nerve in elderly men. Metab Pediatr Syst Ophthalmol 12:13–16.

Nielsen K, Peters A. 2000. The effects of aging on the frequency of nerve fibers in rhesus monkey striate cortex. Neurobiol Aging 21:621–628.

Perry VH, Walker M. 1980. Amacrine cells, displaced amacrine cells and interplexiform cells in the retina of the rat. Proc R Soc Lond (Biol) 208:415–431.

Peters A. 1999. Normal aging in the cerebral cortex of primates. In: Peters A, Morrison J, editors. Cerbral cortex, vol 14, New York: Plenum Press. p 49–80.

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

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, Morrison JH, Rosene DL, Hyman BT. 1998a. Are neurons lost from the primate cerebral cortex during normal 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 8:671–684.

Peters A, Moss MB, Sethares C. 2000. Effects of aging on myelinated nerve fibers in monkey primary visual cortex. J Comp Neurol 419:364–376.

Repka MX, Quigley HA. 1989. The effect of age on normal human optic nerve fiber number and diameter. Ophthalmology 96:26–32.

Schmolesky MT, Wang Y, Pu M, Levental AG. 2000. Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. Nature Neurosci 3:384–390.

Spear PD. 1993. Neural bases of visual deficits during aging. Vis Res 33:2589–2609.

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 the rhesus monkey. Am J Primatol 15:263–273.

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

West MJ, Ostergaard K, Anreassen OA, Finsen B. 1996. Estimation of the number of somatostatin neurons in the striatum: an in situ hybridization study using the optical fractionator method. J Comp Neurol 370: 11–22.