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Disrupted Myelin and Axon Loss in the Anterior Commissure of the Aged Rhesus Monkey

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ABSTRACT

This study assesses the effects of age on the composition of the anterior commissure of the rhesus monkey. The anterior commissures of nine young (5–10 years), five middle-aged (15–20 years), and eight old (25–35 years) monkeys were examined by light and electron microscopy. In all, 90–95% of the nerve fibers in the anterior commissure are myelinated. With age, the structure of the myelin sheaths of some nerve fibers is altered. Some of the axons also show signs of degeneration and this leads to a loss of nerve fibers. Thus, in young and the middle-aged monkeys the mean number of myelinated nerve fibers in the anterior commissure is 2.2×10^6, while in the old monkeys the mean is 1.2×10^6. Increasing age is correlated with a reduction in the number of myelinated nerve fibers in the anterior commissure, an increase in the frequency of structural alterations in myelin sheaths, and an increase in the frequency of occurrence of degenerating axons. However, the number of myelinated nerve fibers is the only variable that correlates with cognition: in monkeys 5–20 years of age the fewer the number of nerve fibers the poorer the cognitive performance, as measured by our Cognitive Impairment Index (CII). The most common neuroglial cells in the anterior commissure are oligodendrocytes. They account for 86% of all neuroglial cell profiles, while astrocytes account for 9%, and microglial cells for 5% of profiles. There is no apparent change with age in the total numbers of neuroglial cells, although as they age each of the neuroglial cell types acquires some inclusions in their cytoplasm. The data, together with those from previous studies, support the concept that in aging there is a ubiquitous loss of myelinated nerve fibers from the brain and that fiber loss is preceded by alterations in the structure of many of the myelin sheaths. J. Comp. Neurol. 466:14–30, 2003.

Indexing terms: aging; primate; astrocyte; oligodendrocyte; microglia; electron microscopy; axon; nerve fibers

One obvious structural difference between young and old primate brains occurs in the white matter. The brains of old humans and monkeys exhibit myelin pallor (Lintl and Braak, 1983; Kemper, 1994), reduced white matter volume, and structural alterations in myelin sheaths (e.g., Feldman and Peters, 1998; Peters et al., 2000). Presumably, these structural changes have functional consequences for the aging individual, which may range from reduced conduction velocity along myelinated tracts (Celesia and Daly, 1977; Xi et al., 1999) to the potential disruption of the connectivity that underlies cognition (Aston-Jones et al., 1985; Inzitari, 2000; O'Sullivan et al., 2001; Peters and Sethares, 2002).

Defects in myelin sheaths appear to be ubiquitous in the aging primate brain (Peters and Sethares, 2002) and in those locations where the incidence of myelin defects has been examined the frequency of these defects increases with age. These locations include the vertical bundles of myelinated nerve fibers in area 46 and area 17, the splenium of the corpus callosum (Peters et al., 2000; Peters

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and Sethares, 2002), and the optic nerve (Sandell and Peters, 2001). It has been harder to determine whether myelinated nerve fibers themselves are lost with age because it is difficult to assess entire populations of nerve fibers. However, Tang et al. (1997) used stereology to examine the myelinated nerve fibers in human cerebral hemispheres and estimated that with age there is a 27% reduction in the aggregate length of myelinated nerve fibers. We have also reported a substantial age-related loss of nerve fibers from the monkey optic nerve, in which degenerating axons are common (Sandell and Peters, 2001). But in contrast, no significant nerve fiber loss is detectable in the vertical bundles of myelinated axons in the layer 4Cβ of area 17 in aging monkeys, despite signs of the degeneration of myelin sheaths (Nielsen and Peters, 2000).

However, the results of studies of nerve fibers in the optic nerve and neocortex may not be generally representative of the effects of age on white matter. Thus, the optic nerve is an unusual white matter tract because the cells giving rise to it reside in a peripheral organ and the nerve contains a significant connective tissue component. These factors may contribute to the vulnerability of optic nerve fibers to aging. Conversely, the efferent nerve fibers in the vertical bundles that pass through the cortex are not in white matter and may be protected from the effects of age by the extensive local collateral axon branches of their parent neurons. Since Tang et al. (1997) found that nerve fibers are lost from white matter with age, it is possible that some of these nerve fibers passing through the cortex may only degenerate when they enter the white matter. To obtain more information about the effects of age on white matter, we have now turned our attention to the anterior commissure, which is a circumscribed tract whose cells of origin are mainly in the cerebral cortex.

In the rhesus monkey the anterior commissure provides an interhemispheric connection for the entire neocortex of the temporal lobe, as well as for parts of the orbitofrontal cortex, prepiriform cortex, and the amygdala (Jouandet and Gazzaniga, 1979; Demeter et al., 1990). The heaviest contribution to the anterior commissure arises in the rostral one-third of the temporal lobe, while the caudal two-thirds of the temporal neocortex primarily projects across the midline through the corpus callosum (Cipolloni and Pandya, 1985; Demeter et al., 1990). Numerous studies have demonstrated that the anterior commissure provides a functional pathway by which visual information can reach the opposite hemisphere to contribute to single unit activity or to mediate behavioral responses (Gross et al., 1977; Doty et al., 1994; Sobotka and Ringo, 1996).

Previous studies in young rhesus monkeys (1.5–5 years old) indicate that the mature anterior commissure contains about 3.15 million axons (LaMantia and Rakic, 1990). During development there is a significant overproduction of axons in the anterior commissure, so that the total peaks at over 10 million at the time of birth, but this is followed by a rapid elimination of axons in the first 3 months of life until the adult number of nerve fibers is reached (LaMantia and Rakic, 1994). Myelination begins just after birth and by 1 year of age over 90% of the axons are myelinated (LaMantia and Rakic, 1994). No information is available about the composition of the anterior commissure in monkeys older than 5 years of age. However, in the anterior commissure of the mouse the frequency of degenerating myelinated axons and of nerve fibers with redundant myelin increases between 5 and 31 months of age (Sturrock, 1980, 1987).

The present study examines the anterior commissure of the rhesus monkey (*Macaca mulatta*) to determine what structural changes occur between 5 and 35 years of age in its myelinated nerve fibers and neuroglial cells. The myelinated nerve fibers were counted in the midsagittal plane to determine whether some are lost with age, as in the optic nerve, or preserved, as in the vertical nerve fiber bundles in area 17. The frequency of myelin defects and of degenerating axons was determined by electron microscopy. The neuroglial cell populations were also examined to determine how they are affected by age and how their numbers might change with respect to the nerve fiber population. Finally, because many of the animals available to us have been behaviorally characterized by our collaborators (Moss et al., 1997; Killiany et al., 2000), we analyzed the number of nerve fibers and neuroglial cells and the frequency of myelin defects in relation to cognitive status in young and old animals. In previous studies we detected significant correlations between the frequency of myelin defects in the neocortex and diminished cognitive performance of old monkeys (Peters et al., 2000), but a similar correlation was not observed for the frequency of myelin defects in the splenium of the corpus callosum (Peters and Sethares, 2002).

MATERIALS AND METHODS

Tissue specimens and processing

The anterior commissures were obtained from nine young rhesus monkeys (5–10 years of age), five middle-aged monkeys (12–20 years old), and eight old monkeys (25–35 years old). The sexes and ages of the monkeys, rounded to the nearest whole year, are given in Table 1. The monkeys were part of a large population being used for studies of cognition and brain structure during normal aging. Consequently, most of the monkeys were behaviorally tested prior to the brains being perfused and fixed for anatomical examination.

Details of the protocol for fixing the brains are given in an earlier publication (Peters et al., 1994). 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 summary, the monkeys were pre-anesthetized with ketamine (6.5 mg/kg). Sodium pentobarbital was administered intravenously (~35–45 mg/kg) until the monkey was deeply anesthetized and a state of areflexia attained. Monkeys were then intubated and artificially respired using a mixture of CO_2 and O_2. The chest was opened and the monkeys perfused intraaortically with a warm solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate or cacodylate buffer at pH 7.4. Following perfusion the brain was removed and bisected in the midsagittal plane. The half of the brain to be used for electron microscopic examination was fixed further by immersion in a cold solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate or cacodylate buffer at pH 7.4.

Tissue preparation

The anterior commissure was cut out from the midsagittal surface of the brain to produce a block of tissue ~2

TABLE 1. Data Regarding Nerve Fibers in the Anterior Commissure

Number, sex	Age	Area, mm^2	Total myelinated fibers	% Myelinated axons	% Normal internodes	% Abnormal axons	% Paranodes	CII
R419, M	5	3.75	2628000	inadequate fixation	inadequate fixation	inadequate fixation	inadequate fixation	na
AM007, M	5	2.90	1951410	inadequate fixation	inadequate fixation	inadequate fixation	inadequate fixation	na
AM010, M	6	2.92	2406956	91	99.9	0	5.8	na
AM129, F	7	2.95	1741680	96	99.7	0	4	1.86
AM130, F	8	5.00	2448050	94	99.6	0.1	4.6	1.28
AM096, F	9	2.21	1411748	95	99.6	0	5.9	2.46
AM097, F	9	2.42	1681900	92	100	0	3.9	4.39
AM047, M	9	4.55	3096275	96	99	0	4.2	0.54
AM053, M	10	4.28	2425048	95	99.6	0	4.2	0.08
MEAN		**3.44**	**2199000**	**9.4**	**99.6**	**0.01**	**4.7**	
SD		**0.99**	**536550**	**1.95**	**0.32**	**0.04**	**0.84**	
AM140x, M	12	4.20	1923600	95	98.1	0.1	4.4	na
AM144, M	15	5.10	2825910	93	98.8	0.2	4.2	0.42
AM143, M	16	4.17	2176740	95	99.0	0	6	0.00
AM101, M	19	3.53	1487895	98	98.2	0.3	4.6	3.62
AM133, M	20	4.13	1913842	92	96.7	0.2	4.1	2.40
MEAN		**4.23**	**2065600**	**95**	**98.2**	**0.16**	**4.7**	
SD		**0.56**	**491770**	**2.30**	**0.90**	**0.11**	**0.73**	
AM100, F	25	2.34	1233414	90	94.6	0.5	7	4.12
AM062, M	27	1.71	734103	inadequate fixation	inadequate fixation	inadequate fixation	inadequate fixation	4.26
AM015, F	27	2.64	1355112	87	94.6	0.4	5.9	1.56
AM026, F	29	2.61	1347021	86	95.7	0.5	6.1	0.94
AM041, F	32	2.9	1390550	92	93.8	0.7	6.3	4.81
AM091, M	32	3.58	1476392	93	94	0.3	8.1	0.17
AM018, M	33	2.78	1166210	inadequate fixation	inadequate fixation	inadequate fixation	inadequate fixation	na
AM013, M	35	1.88	784524	*	94.8	0.3	10.6	na
MEAN		**2.56**	**1185900**	**90**	**94.6**	**0.45**	**7.3**	
SD		**0.59**	**279980**	**3.05**	**0.67**	**0.15**	**1.79**	

*Unable to determine because of the large number of astrocytic processes.

mm thick. The block of tissue was then osmicated, dehydrated in an ascending series of ethanols, and embedded in Araldite. Transverse sections of the anterior commissure, 1 μm thick, were collected and stained with Toluidine blue for light microscopic examination. Thin sections were then 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 myelinated nerve fibers was made for each anterior commissure. The semithick sections stained with Toluidine blue were analyzed using the Bioquant BQ-TCX-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 was carried out by one observer (JHS). The computer was used to generate the counting boxes (10 × 10 μm, spaced 200 μm from center-to-center in a randomly placed grid), move the stage, project the image of a counting box onto the video screen, mark the profiles that were counted, tally the counts, and calculate the cross-sectional area of the anterior commissure. In the counting box, all myelinated nerve fiber profiles were counted if they did not intersect the two forbidden margins of the box. The counting parameters were determined empirically to yield coefficients of error (CE = SEM/mean) for individual commissures that were well within the 10% recommended by West and colleagues for stereological analyses (West et al., 1996; Howard and Reed, 1998). The total number of myelinated nerve fiber profiles in an anterior commissure was calculated by multiplying the mean numbers of profiles per mm^2 by the cross-sectional area of the commissure being examined.

Neuroglial cell counts

The neuroglial cells were also counted using the semithick sections, the Bioquant system, and a ×100 oil immersion objective. In this case a 50 × 50 μm counting box was used, the counting boxes once again being spaced 200 μm apart. Profiles of oligodendrocytes, astrocytes, and microglial cells which displayed their nuclei were the objects counted. The counts were made by both authors working together. The criteria for distinguishing between these three types of neuroglial cells are given in the Results section. To calculate the total number of nuclear profiles of the three types of neuroglial cells, we applied a formula similar to that used to determine the total number of myelinated nerve fiber profiles. It was assumed that a sufficient number of counts had been completed when the coefficients of error for the numbers of profiles of oligodendrocytes and astrocytes counted was below 10%. It should be pointed out that microglial cells were so sparse that we did not attempt to attain sufficient numbers of counts of these cells to reach a 10% coefficient of error.

Sizes of neuroglial cell profiles

The areas of the profiles of neuroglial cell nuclei were determined to ascertain whether number estimates based on profile counts were likely to be affected by changes in the size of the nucleus. Profiles of neuroglial cell nuclei in Toluidine blue stained semithick sections were traced with the aid of a camera lucida using a ×100 oil immersion objective. At least 100 consecutive nuclei for each of the three neuroglial cell types were traced for each animal. The drawings were digitized and the areas of the nuclear profiles calculated using NIH Image 6.1 software. The mean nuclear profile area was calculated for each of the neuroglial cell types for each animal and the group means for each cell type were compared using two-tailed

t-tests. No significant differences in the neuroglial nuclear profile areas were found between young animals (5–10 years, n = 9) and old animals (25–35 years, n = 8): astrocytes (young mean = 30.3 μm^2, old = 30.5 μm^2, P = 0.8744), oligodendrocytes (young mean = 21.6 μm^2, old = 22.5 μm^2, P = 0.2886), and microglial cells (young mean = 13.9 μm^2, old = 13.8 μm^2, P = 0.8433).

Electron microscopic analyses

Each anterior commissure was cross-sectioned and examined by electron microscopy to determine that the state of preservation of the tissue was good enough to carry out useful analyses. All of the anterior commissures were considered to be well preserved, with the exception of those from four monkeys, R419, AM 007, AM 018, and AM 062. Consequently, the anterior commissures from these four monkeys were not analyzed by electron microscopy. For the remaining monkeys electron micrographs were taken of representative fields containing myelinated nerve fibers and of examples of the various types of neuroglial cells.

To ascertain the effects of age on the integrity of myelin sheaths, a random series of electron microscopic images was taken of fields of myelinated nerve fibers. The fields were photographed at a primary magnification of ×5,000, and the negatives printed to a final magnification of ×12,500. For each anterior commissure a sample of at least 800 internodal profiles of myelinated nerve fibers was examined to ascertain the percentage of these profiles that showed age-related changes in the sheaths and axons of myelinated nerve fibers.

From the total population of profiles of nerve fibers in the micrographs, a second analysis was carried out to determine the percentage of profiles that belonged to internodal lengths of myelin, paranodes, nodes of Ranvier, and unmyelinated nerve fibers. The features used to distinguish between profiles of these entities are given in the Results section, where the frequency of paranodes is described.

Behavioral testing

The behavioral tests used to determine the cognitive status of individual monkeys are described in earlier publications (e.g., Herndon et al., 1997; Moss et al., 1999; Peters et al., 1996, 2000; Killiany et al., 2000). The battery of tests included three visual recognition tasks. They are the delayed nonmatching to sample (DNMS) task, a DNMS task with a 2-minute delay, and the delayed recognition memory span task (DRS). From the scores achieved on these tasks an overall measure of cognitive impairment, the Cognitive Impairment Index (CII), was derived (Peters et al., 1998b). Specifically, the individual scores on the three tasks were transformed to scores normalized to a population of 53 adult rhesus monkeys, as described by Herndon et al. (1997), and a composite score, the Cognitive Performance Index (CPI) was derived. The CPI has been shown to be a practical index of global ability, and its inverse, the CII, is a measure of cognitive impairment. Essentially, the higher the CII, the more a monkey is cognitively impaired. The CIIs, for those monkeys that were tested, are given in Table 1.

Photographic production

The electron micrographs used to illustrate this article were produced using standard photographic darkroom procedures. Figure 1 was obtained digitally using a Nikon Cool Pix 995 camera and the light microscope negative used for Figure 1B was scanned using Adobe PhotoShop 5.5 (San Jose, CA), converted to grayscale, and minimally processed to improve contrast.

RESULTS

The anterior commissure is a well-delineated, compact bundle of nerve fibers that has an oval profile when cut in the midsagittal plane (Fig. 1A). It lies just behind the descending limb of the third ventricle and has a small basal telencephalic commissure on its dorsal anterior surface. In young monkeys (ages 5–10) the cross-sectional area of the anterior commissure averages 3.4 mm^2, while in five middle-aged monkeys (ages 12–20) it had a mean cross-sectional area of 4.2 mm^2. However, with increasing age the anterior commissure becomes smaller, so that in old monkeys (ages 25–35) the cross-sectional area averages only 2.6 mm^2, some 30% less than in young monkeys.

In addition to myelinated and unmyelinated nerve fibers, the anterior commissure contains neuroglial cells, of which oligodendrocytes are most common type. An occasional neuronal cell body can also be encountered.

Internodal nerve fibers and fiber number

Over 90% of the axons in the anterior commissure are myelinated and there is a slight tendency for young monkeys and middle-aged monkeys to have a greater proportion of myelinated axons than old monkeys (Table 1). The nerve fibers are closely packed together and they vary in size (Fig. 1B). The majority of myelinated nerve fibers in the anterior commissure have axonal diameters between 0.5–1.2 μm. The smallest myelinated nerve fibers have axonal diameters of ~0.3 μm, consistent with calculations that myelination improves conduction velocity for axons greater than 0.2 μm in diameter (Waxman and Bennett, 1972). The largest nerve fibers in the anterior commissure have axonal diameters of about 4.0 μm.

In young monkeys the myelin of the sheaths is generally compact and the axons appear normal (Fig. 2). However, because white matter has a poor blood supply, fixation of myelin sheaths is often less than optimal, with the consequence that some sheaths display shearing (Fig. 2, arrows). At such sites, which are often localized, the myelin lamellae become separated and detached from each other so that empty spaces occur between adjacent lamellae. This type of shearing can produce a bulge that indents the surface of the axon as well as sticks out into the extracellular space. In addition to such shearing defects, which occur in both young and old monkeys, nerve fibers in old monkeys exhibit age-related alterations of their myelin sheaths (Figs. 3, 4). The most common of these age-associated alterations is a splitting of sheaths at the major dense line and electron-dense cytoplasm is found within the splits. Other sheaths show a local splitting of the intraperiod line between one or two lamellae and the lamellae open up to enclose a fluid-filled sac or bleb, which can vary in size and sometimes be so large that its cross-sectional area matches that of the parent nerve fiber (Feldman and Peters, 1998). Other less common alterations are sheaths that have redundant myelin, such that the myelin sheath is much too large for the enclosed axon, and thick sheaths that show circumferential splitting. Ex-

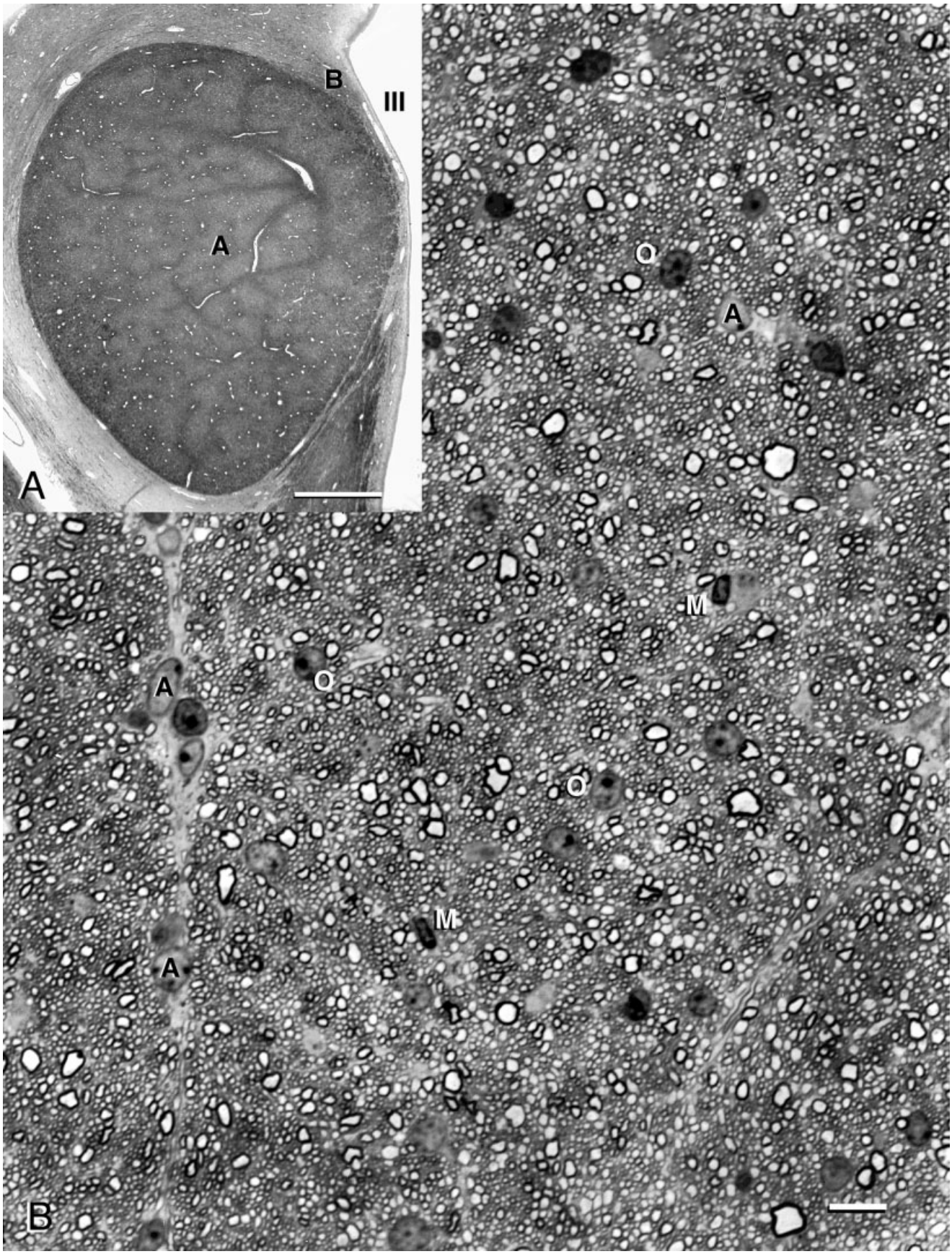

Fig. 1. Light micrographs of the anterior commissure of a 10-year-old monkey, AM 053, sectioned in the midsagittal plane. **A**: In this semithick section stained with Toluidine blue, the anterior commissure (A) has a cross-sectional area of 4.28 mm^2. The third ventricle (III) is to the right, and on the dorsal anterior aspect of the anterior commissure is the smaller basal telencephalic commissure (B). Scale bar = 0.5 mm. **B**: Light micrograph of part of a semithick section of the anterior commissure from an 8-year-old monkey, AM 130. The myelinated nerve fibers are closely packed and have a range of sizes. Oligodendrocytes (O) are the most common neuroglial cells. Astrocytes (A) are larger and have pale nuclei, while the few microglial cells (M) have small and dark nuclei. Scale bars = 0.5 in A; 10 μm in B.

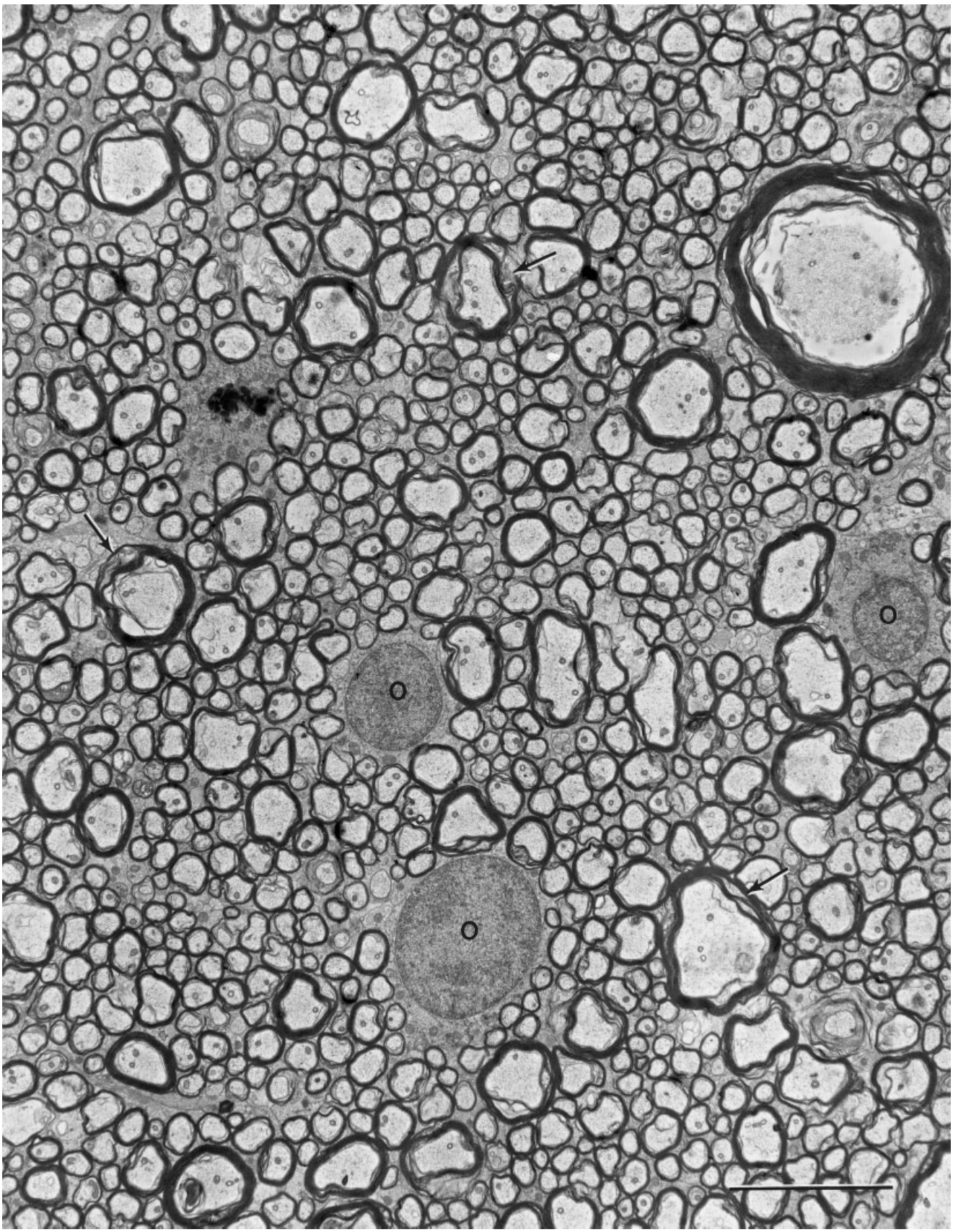

Fig. 2. Anterior commissure from a 12-year-old monkey, AM 140X. The commissure contains closely packed myelinated nerve fibers with a range of sizes. Myelin sheaths of some of the larger fibers show shearing defects (arrows). The most common neuroglial cells in the anterior commissure are oligodendrocytes (O). Scale bar = 5 μm.

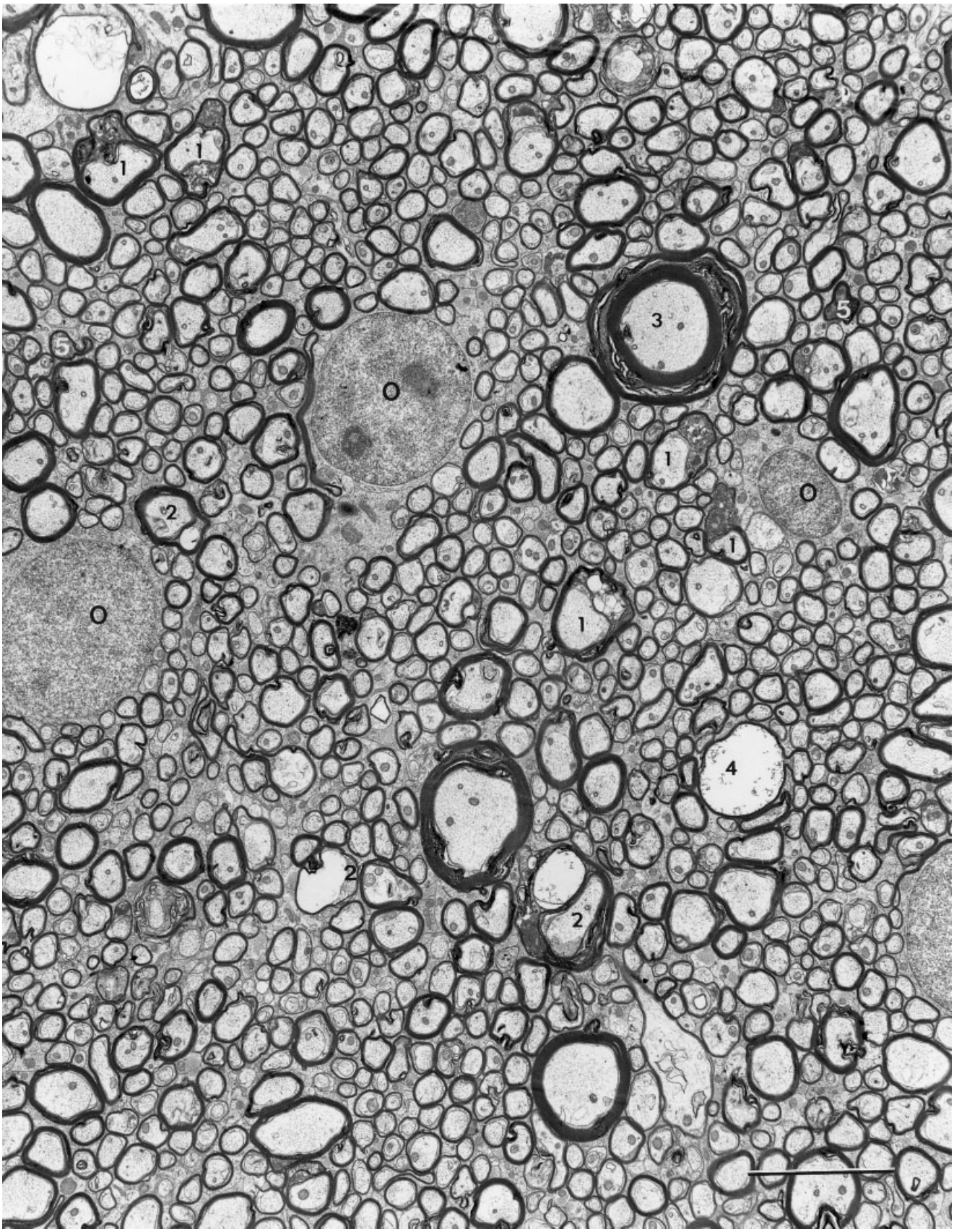

Fig. 3. Anterior commissure from a 25-year-old monkey, AM 100. In old monkeys the nerve fibers are less closely packed than in young monkeys and the myelin sheaths of some fibers show age-related changes, such as splits that contain dense cytoplasm (1), blebs (2), and circumferential splits (3). Some empty sheaths are also present (4) and some axons have dense cytoplasm (5). Note that the oligodendrocytes (O) are free of cytoplasmic inclusions. Scale bar = 5 μm.

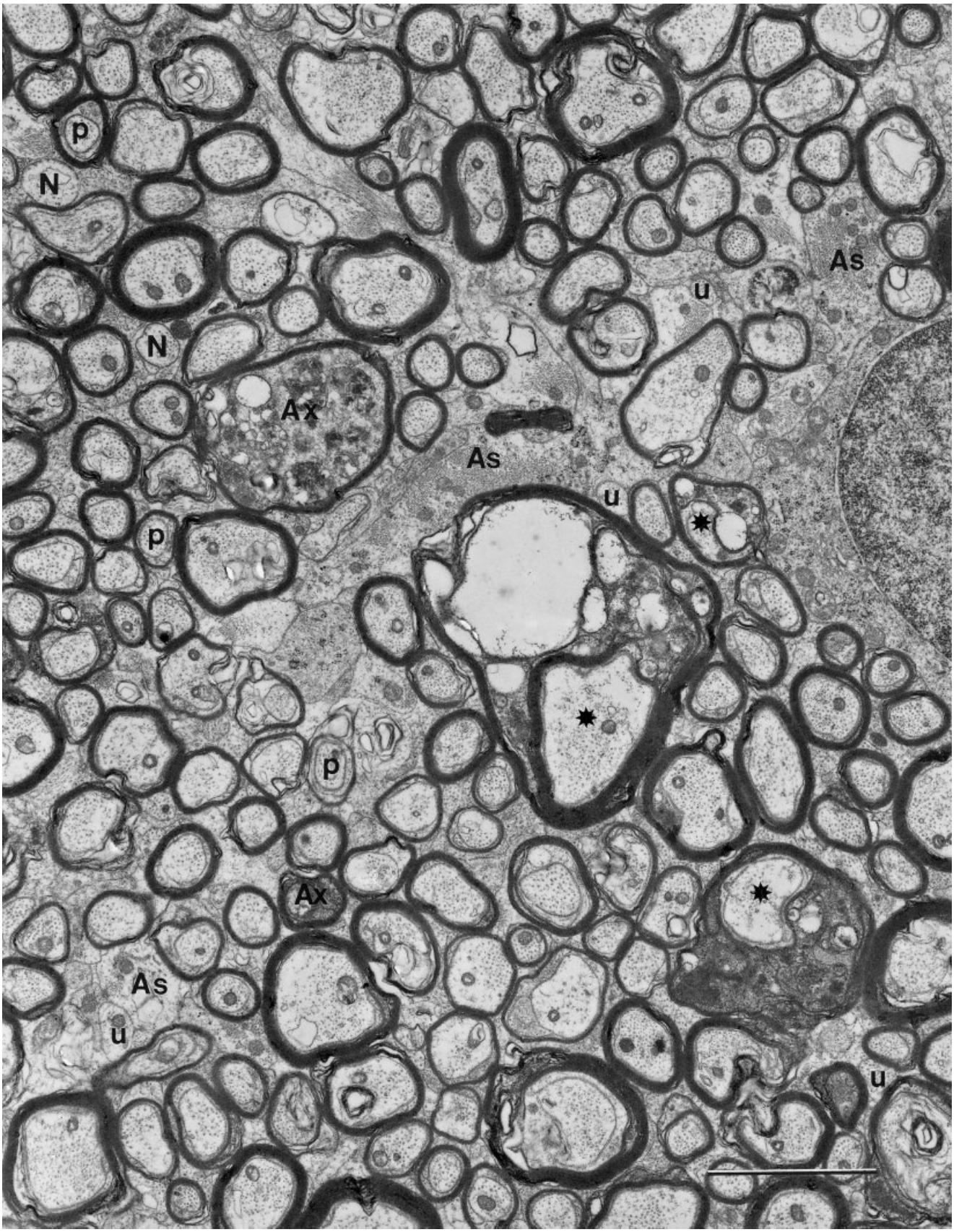

Fig. 4. Anterior commissure from a 32-year-old monkey, AM 091. In the field are three nerve fibers (asterisks) with split sheaths containing dense cytoplasm. Other fibers have degenerating axons (Ax). In old monkeys more astrocytic processes (As) are interposed between the nerve fibers. Only a few umyelinated axons (u) are present. Note the characteristic profiles of paranodes (p) and nodes (N). Scale bar = 2 μm.

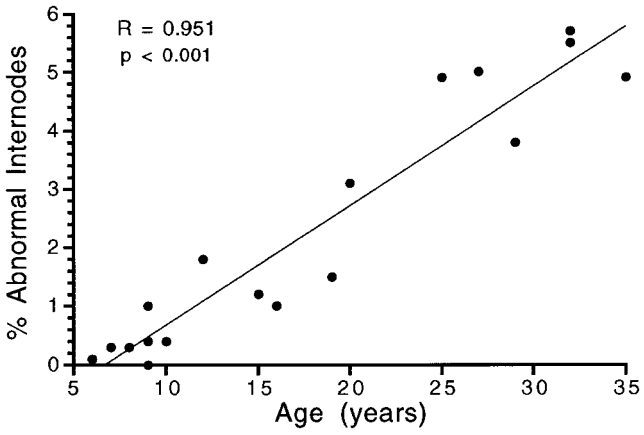

Fig. 5. Graph of the percentage of abnormal internodal profiles encountered in electron micrographs of the anterior commissure as a function of age.

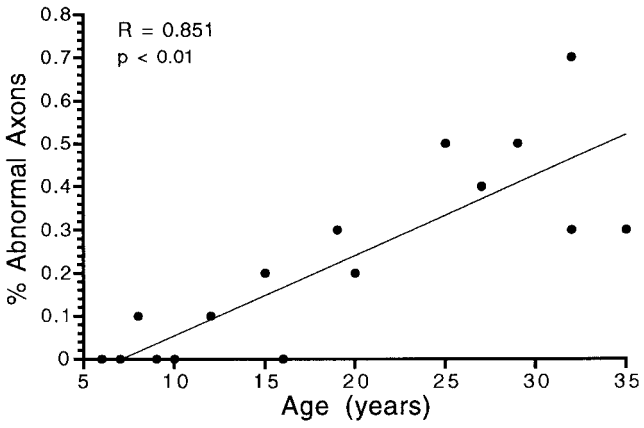

Fig. 6. Graph of the percentage of abnormal axonal profiles encountered in electron micrographs of the anterior commissure as a function of age.

amples of these alterations in the structure of myelin sheaths are already present at 12–15 years of age.

In general, the proportion of cross-sectioned profiles of myelin sheaths that show these age-related alterations is strongly correlated with increasing age ($r = 0.951$, $P < 0.001$; Fig. 5). Furthermore, analysis of variance reveals that an increase in the proportion of abnormal internodes is the only variable in which the middle-aged animals are significantly different from the young animals (one-way ANOVA $P < 0.0001$, Bonferroni-corrected post-hoc group comparison of young vs. middle-aged animals, $P < 0.01$). This indicates that myelin sheath alterations begin in middle age.

In addition to the alterations in their myelin, some of the axons in old monkeys appear to be degenerating. Some of them have granules and vacuoles in their cytoplasm, while others have electron-dense cytoplasm, which is generally regarded as a definite sign of axonal degeneration, and some empty sheaths are also encountered (Fig. 3). As with alterations in myelin, abnormal, degenerating axons make their appearance in middle age, when between 0.1

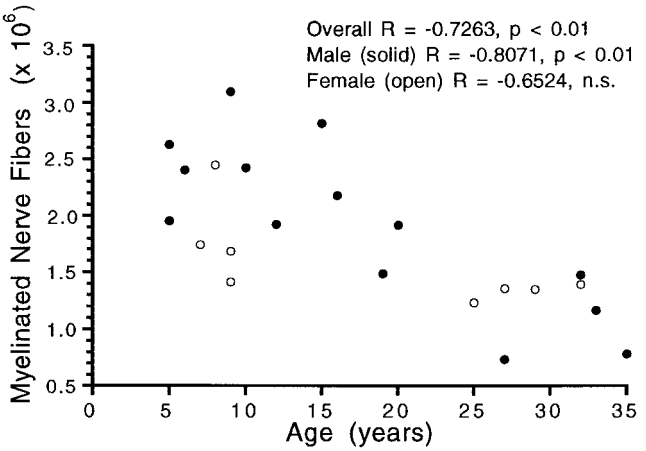

Fig. 7. Graph of the number of myelinated nerve fiber profiles counted in the anterior commissure as a function of age. These counts only include myelinated axons because these data were obtained using light microscopy. Counts from male monkeys are shown with solid symbols and counts from female monkeys are shown with open symbols.

and 0.3% of axons show cytoplasmic changes (see Table 1). By 25 years of age the percentage of abnormal axons increases to between 0.3 and 0.7%, and when all the data for the percentage of abnormal axons is examined there is an obvious correlation with increasing age ($r = 0.851$, $P < 0.001$, Fig. 6).

Since there are age-related alterations in both axons and their sheaths, and since the cross-sectional area of the anterior commissure decreases with age, it is not surprising that the total number of nerve fibers within the commissure decreases with age (Fig. 7, Table 1). In young monkeys the average number of myelinated nerve fibers in the anterior commissure is 2.2×10^6, but there is a wide range ($3.1–1.4 \times 10^6$). Likewise, there is a wide range of total fiber counts in the old animals, although the mean of 1.2×10^6 for old monkeys is well below that of young animals. In terms of total fiber counts, our small population of middle-aged animals resembles the group of young animals (mean = 2.1×10^6).

The overall correlation between age and the number of nerve fibers counted by light microscopy is significant ($r = -0.7263$, $P < 0.01$). Interestingly, in the sample of monkeys that we had available for analysis, most of this correlation appears to be attributable to age-related fiber loss in male monkeys (solid symbols in Fig. 7, $n = 14$). Although our sample of female monkeys (open symbols in Fig. 7) is relatively small ($n = 8$), they appear to have somewhat fewer fibers than males when they are young and to lose fewer nerve fibers as they age. Whether this is a genuine phenomenon or a consequence of the small female group has yet to be determined. Young females are particularly difficult to obtain for anatomical studies, since they are needed for captive breeding programs.

To our surprise, for the entire subset of monkeys for which data is available it was found that the total number of nerve fibers in the anterior commissure is significantly correlated with the overall cognitive performance, as measured by the cognitive impairment index ($r = 0.6862$, $P < 0.01$, Fig. 8A). Further analysis shows that the correlation

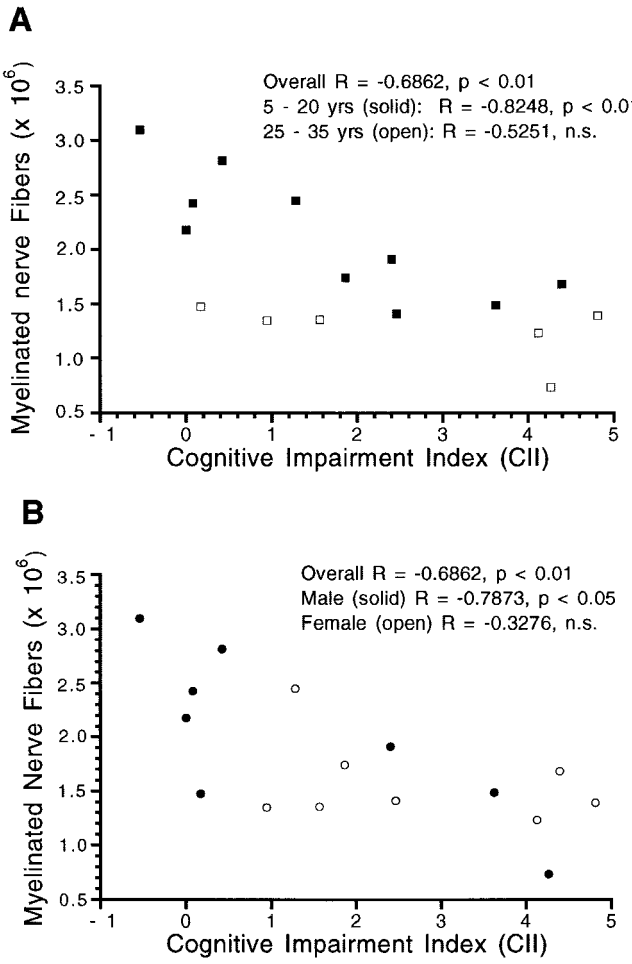

Fig. 8. Graphs of myelinated nerve fiber number in the anterior commissure as a function of cognitive impairment. **A**: Data from monkeys ≤20 years is graphed with solid squares. Data from old monkeys (25–35 years) is graphed with open squares. **B**: Data from male monkeys is graphed with solid circles. Data from female monkeys is graphed with open circles.

between CII and the number of nerve fiber in the anterior commissure is strong for monkeys that are between 5 and 20 years of age (solid symbols in Fig. 8A, $r = -0.8248$, $P < 0.01$), but it is not significant ($r = -0.5251$, $P > 0.05$) for monkeys 25–35 years of age (open symbols in Fig. 8A). These findings may be complicated, however, by the overrepresentation of female monkeys in the population of old animals for whom data on cognitive performance is available (see Table 1). When males and females are examined separately (Fig, 8B), a significant correlation is observed between fiber number and CII for male monkeys ($r = -0.7873$, $P < 0.05$), but not for female monkeys ($r = -0.3276$, $P > 0.05$).

Paranodes

In a study of the effects of age on the myelinated nerve fibers in cerebral cortex, we found that in transversely sectioned nerve fibers the frequency of paranodal profiles increased with age (Peters and Sethares, 2003), and so we examined the nerve fibers in the anterior commissure to ascertain if the same occurs in this structure.

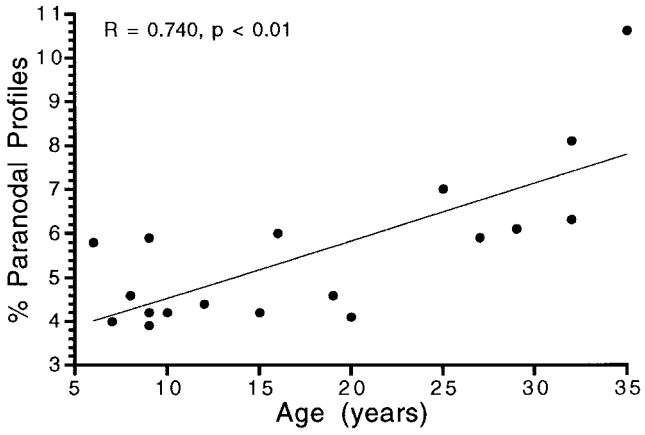

Fig. 9. Graph of the percentage of myelinated nerve fibers profiles belonging to paranodes that are encountered in electron micrographs of the anterior commissure at different ages.

Paranodes occur at the ends of internodal lengths of myelin and they are the locations where the myelin lamellae terminate (e.g., see Raine, 1984; Peters et al., 1991; Rosenbluth 1995). At a paranode the major dense line of the sheath opens up to accommodate cytoplasm, and because the lamellae of myelin gradually turn off in a spiral fashion, the cytoplasm is contained in a helical tunnel. The termination of the myelin leaves the axon exposed at the adjacent node of Ranvier (see Fig. 4; N). At a paranode a complex junction is formed between the axolemma and the plasma membrane on the inside of the sheath. In electron micrographs of transverse sections of paranodes the two trilaminar plasma membranes are seen to be closely apposed and to form a complex in which four dark layers are separated by three pale layers. The presence of this membrane complex, together with the presence of the cytoplasm in the helical tunnel, makes the paranode easy to recognize in transverse sections of nerve fibers (Fig. 4; p).

Transverse sections through nodes of Ranvier can be identified by the presence of a dense undercoating on the inside of the axolemma (Fig. 4; N), but unless the coating is obvious it is sometimes difficult to distinguish between profiles of transverse sections through nodes of Ranvier and those through the largest unmyelinated axons. However, for the most part profiles of unmyelinated axons have diameters of only 0.2–0.3 μm, and are smaller than the majority of profiles of nodes of Ranvier.

As seen in Table 1, in young monkeys the mean percentage of paranodal profiles is 4.7% and in middle-aged monkeys it is also 4.7%. This indicates that there is little or no change in the number of paranodes between 5 and 20 years of age. However, in the old monkeys the mean frequency of paranodal profiles increases to 7.3%. In other words, the mean number of paranodal profiles increases by about 60% and, as shown in Figure 9, there is a significant correlation between the increased frequency of paranodal profiles and increasing age ($r = 0.7407$, $P < 0.01$).

Such a result could be obtained if the mean lengths of internodes of myelin decrease with age. This could occur if there is a preferential loss of large nerve fibers with age, since large nerve fibers have longer internodes than small nerve fibers. In an attempt to ascertain if this occurs, we

compared the distribution of axon diameters in two young and two old anterior commissures, but did not detect a preferential loss of large diameter axons with age (data not shown).

Morphology of neuroglial cells

The three types of neuroglial cells encountered in the anterior commissure are oligodendrocytes, astrocytes, and microglial cells.

Oligodendrocytes have round to oval nuclei with a substantial amount of heterochromatin, so their nuclei are electron dense (see Figs. 2, 3, 10A) and stain darkly in semithick sections stained with Toluidine blue. The perikaryal cytoplasm is also dark and contains short cisternae of endoplasmic reticulum, as well as free ribosomes and microtubules. Although these neuroglial cells form the myelin sheaths of nerve fibers in their vicinity, processes are rarely seen to emanate from the cell bodies of oligodendrocytes. Most of the oligodendrocytes in the anterior commissure occur singly, although it is not uncommon to encounter a row of oligodendrocytes extending between two capillaries. In the anterior commissures of old monkeys the appearance of the oligodendrocytes is substantially similar to that in young monkeys, although in old monkeys dark inclusions are more common in the perikarya and processes of oligodendrocytes.

Microglial cells are the least common of the neuroglial cells. They have smaller and somewhat darker nuclei than oligodendrocytes and the profiles of the microglial cell nuclei tend to be oval or bean-shaped (Figs. 1A, 10A). Like oligodendrocytes, microglial cells have dark cytoplasm, but they have a more irregular shape and fit themselves to the contours of the nerve fibers surrounding them. The microglial cells often have rather thick processes extending from the cell body to pass between adjacent nerve fibers, and both the cell body and these processes may contain dense inclusions. With age, there is little change in the appearance of the microglial cells in the anterior commissure other than that they tend to contain a few more inclusions (Fig. 10A). However, microglial cells have not been seen to engulf degenerating nerve fibers or myelin.

Astrocytes have pale, oval, and sometimes indented nuclei that readily distinguish them from oligodendrocytes and microglial cells, both of which have much darker nuclei (Fig. 1A). In addition, the cytoplasm of astrocytes is much paler and it is common to see processes of astrocytes extending from the cell body to pass between nerve fibers. Both the cell bodies and the processes of astrocytes contain bundles of intermediate filaments and short cisternae of endoplasmic reticulum (Figs. 4, 10B). However, unlike the situation that we encountered in the optic nerves of these same monkeys (Sandell and Peters, 2002), the processes of astrocytes do not form septae that separate the nerve fibers of the anterior commissure into bundles. With age the astrocytes come to contain increasing numbers of intermediate filaments in their cytoplasm and as the nerve fibers become sparser in old monkeys the profiles of astrocytic processes between them become more common, as though they are filling spaces left vacant by degenerating nerve fibers (see Fig. 4). In addition, some of the astrocytes in old monkeys have inclusions in their cytoplasm.

Frequency of neuroglial cells

As seen in Table 2, the most common neuroglial cells in the anterior commissure are oligodendrocytes. On average they account for some 86% of the neuroglial nuclear profiles, while about 9% of the profiles belong to astrocytes and 5% to microglial cells. A midsagittal section of the anterior commissure contains an average of 4,837 profiles of neuroglial cells that show their nuclei (Table 2). There is no apparent change with age in the total number of neuroglial cells (one-way ANOVA $P = 0.773$) or in the frequency of the oligodendrocytes or astrocytes (one-way ANOVA $P = 0.895$, $P = 0.841$, respectively). Although we present data for microglial cells in Table 1 for the sake of completeness, these cells are so rare that we did not count sufficient profiles to reduce our error below 10%, so it is not possible to draw quantitative conclusions for these cells. As described in Materials and Methods, our analysis of the area of the oligodendroglial and astrocytic nuclear profiles did not reveal any significant change in nuclear size with age. This suggests that over the age range that we examined, neuroglial cell populations are relatively stable in the anterior commissure.

DISCUSSION

In this study we observed a strong association between advancing age and deterioration of the anterior commissure. The proportion of abnormal myelin sheaths and axons within this fiber bundle increases significantly between 5 and 35 years of age, but there is no correlation between the frequency of abnormal nerve fibers and the CII. There is a concomitant age-related reduction in the number of nerve fiber profiles in the commissure, which is more pronounced for male monkeys than for female monkeys in our sample. In our overall population, the number of myelinated nerve fibers in the anterior commissure was positively correlated with cognitive performance, although this relationship did not hold for six behaviorally tested animals 25–35 years of age. These older animals have fewer myelinated nerve fibers in the anterior commissure than the young animals, regardless of cognitive status.

Comparison with previous studies

The data in the present study complement those of LaMantia and Rakic (1990, 1994), who performed quantitative studies of the anterior commissure of rhesus monkeys from fetal life through 5 years of age. Calculated from their data, the mean number of myelinated axons in their adult group (1.5–5.0 years old) was 2.9×10^6, while on the basis of light microscopic counts the mean for our young animals (5–10 years old) was 2.2×10^6. Both studies observe variability in nerve fiber number from animal to animal, even when animals of similar age and sex are compared. The reason why the counts we obtained for young adults are somewhat lower than those of LaMantia and Rakic may be because the smallest myelinated axons are difficult to discern by light microscopy. Our electron microscopic analysis shows that in young monkeys 94% of the nerve fiber profiles belong to myelinated nerve fibers. This percentage drops to 90% in our group of old animals. However, a 4% reduction in the proportion of myelinated axons would not account for the entire age-related reduction in the number of myelinated nerve fibers, which

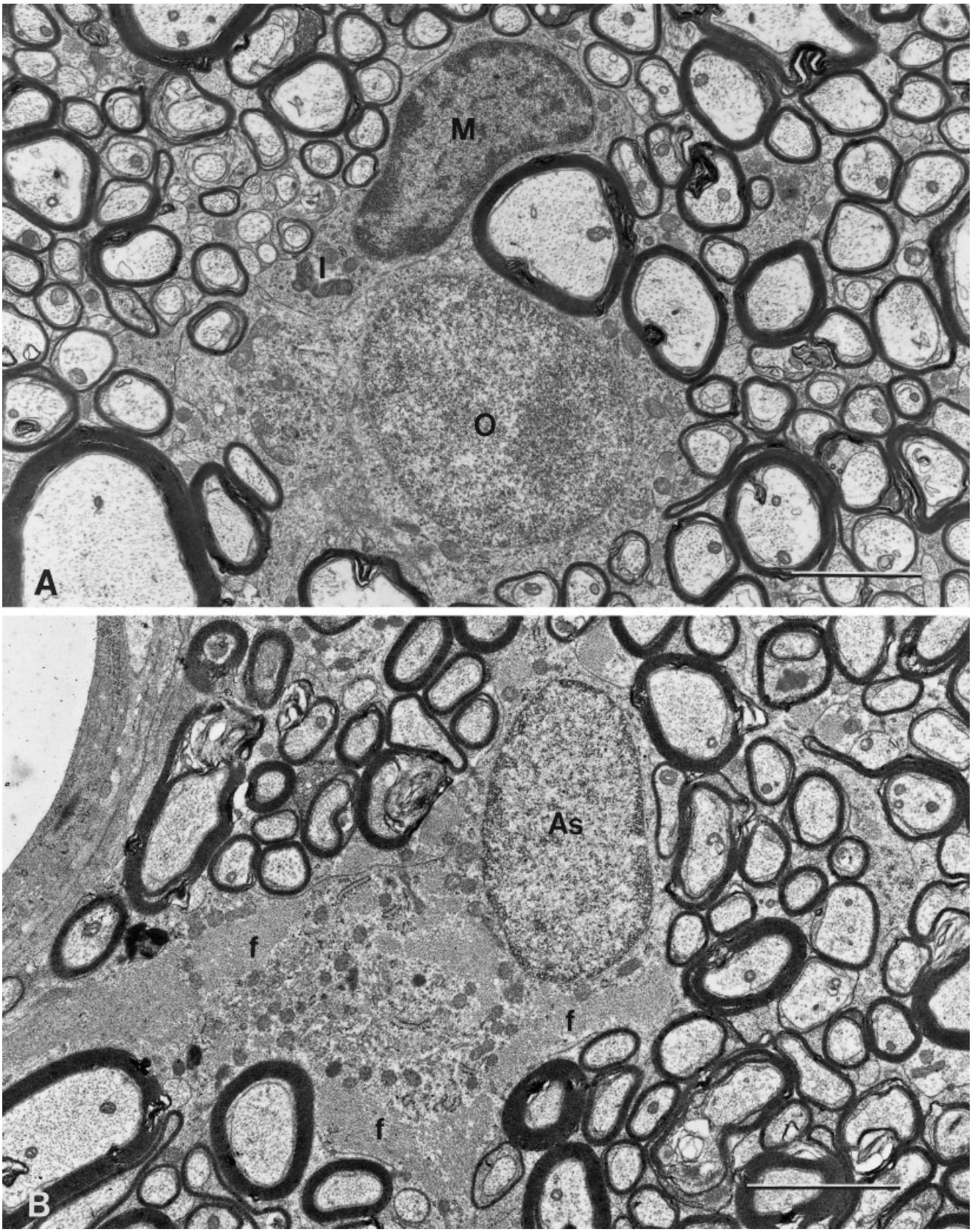

Fig. 10. Electron micrographs of neuroglial cells in the anterior commissure. **A**: Microglial cell (M) and an oligodendrocyte (O) from a 25-year-old monkey, AM 100. Both cells have dense cytoplasm, but the nucleus of the microglial cell is smaller, darker, and more elongate than that of the oligodendrocyte. The microglial cell has a small inclusion (I). **B**: An astrocyte (As) in the anterior commissure of a 32-year-old monkey, AM 41. In old monkeys the astrocytic cytoplasm becomes filled with filaments (f). Scale bars = 2 μm.

TABLE 2. Data Regarding Neuroglial Cells in the Anterior Commissure

Number, sex	Age	Total glia	% Astrocytes	# Astrocytes	% Oligodendrocytes	# Oligodendrocytes	% Microglia	# Microglia[1]
R419, M	5	4377	8	350	89	3896	3	131
AM007, M	5	4791	11	527	86	4120	3	144
AM010, M	6	5069	8	405	90	4562	2	101
AM129, F	7	4192	7	293	88	3689	5	210
AM130, F	8	6171	10	617	85	5245	5	309
AM096, F	9	3631	8	290	89	3232	3	109
AM097, F	9	3993	8	319	88	3514	4	160
AM047, M	9	4974	12	597	84	4178	4	199
AM053, M	10	4806	11	529	84	4037	5	240
MEAN		**4667**	**9.2**	**436**	**87.0**	**4052**	**3.8**	**178**
SD		**741**	**1.8**	**132**	**2.3**	**594**	**1.1**	**68**
AM140x, M	12	5217	8	417	86	4487	6	313
AM144, M	15	5871	11	646	83	4873	6	352
AM143, M	16	5359	10	536	85	4555	5	268
AM101, M	19	3926	8	314	86	3376	6	236
AM133, M	20	5013	10	501	83	4161	7	351
MEAN		**5077**	**9.4**	**482**	**84.6**	**4290**	**6.0**	**304**
SD		**717**	**1.3**	**125**	**1.5**	**570**	**0.7**	**51**
AM100, F	25	3773	9	340	86	3245	5	189
AM062, M	27	2473	9	223	83	2053	8	198
AM015, F	27	4115	12	494	83	3415	5	206
AM026, F	29	5552	7	389	88	4886	5	278
AM041, F	32	5806	10	581	85	4935	5	290
AM091, M	32	7073	8	566	88	6224	4	283
AM018, M	33	5490	9	494	87	4776	4	220
AM013, M	35	4777	13	621	81	3869	6	287
MEAN		**4882**	**9.6**	**463**	**85.1**	**4175**	**5.3**	**244**
SD		**1420**	**2**	**136**	**2.6**	**1292**	**1.3**	**44**

[1]Microglia were so scarce that they were not counted to an error criterion of <10%.

amounted to almost 50% when animals 5–10 years old are compared to the 25–35-year-old monkeys.

The magnitude of age-related fiber loss in the anterior commissure is similar to the loss we observed in the optic nerve (Sandell and Peters, 2001), although there is no significant correlation ($r = 0.587$) between the number of nerve fibers in the two structures in the eight animals that were common to the two studies. However, there are interesting differences in the variability of the fiber counts in the two structures. For the anterior commissure, the individual variation of the total fiber counts is about the same for both young and old animals (2.2-fold for young, 2.1-fold for old), even though the total number of fibers declines with age. For the optic nerve, however, the individual variation in total fiber number is much less for young animals (1.6-fold) than it is for old animals (3.8-fold). The greater variability in the old optic nerve suggests that two kinds of age changes may be taking place in this structure: an age-related increase in myelin abnormalities, which appears to be ubiquitous in the aging brain, and an age-related vulnerability of the retinal ganglion cells, which is unique to the eye.

Myelin abnormalities

The changes we observed in the myelin sheaths in the anterior commissure are commonly found elsewhere in the aging brain, including the corpus callosum and area 46 (Peters and Sethares, 2002), area 17 (Peters et al., 2000, 2001; Peters and Sethares, 2003), and in the optic nerve (Sandell and Peters, 2001). In all of these locations the lamellae of some myelin sheaths are split apart from one another along the intraperiod line to enclose fluid (Feldman and Peters, 1998), or, more commonly, along the major dense line to enclose abnormal dark cytoplasm that must belong to the parent oligodendrocyte. Other changes, such as the formation of abnormally thick myelin sheaths, or redundant sheaths (Peters et al., 2001), are less common in the aging anterior commissure than they are in the cerebral cortex or optic nerve.

Although the disruption of myelin sheaths appears to be a ubiquitous change in the aging brain, previously we have had no information on when this process begins. We now know that monkeys 12–20 years of age have a significantly greater frequency of myelin abnormalities than younger monkeys. Furthermore, this was the only variable that showed a significant difference between these two age groups. This suggests that some process that damages myelin begins quite early, before a decline in fiber number or a rise in the proportion of abnormal axons can be detected. As monkeys pass from middle to old age, the myelin abnormalities in the anterior commissure become much more frequent, and compared to both the young and middle-aged monkeys there are significantly more abnormal axons and fewer nerve fibers in the anterior commissure of the old animals.

Sloane et al. (2003) investigated the role of calpains in the metabolism of myelin proteins and found that in myelin fractions isolated from the white matter of rhesus monkeys there is an increase in the amount of floating, or degraded myelin, only in monkeys over 20 years of age. They also found that significant changes in the levels of CNPase, myelin/oligodendrocyte-specific protein (MOSP), and myelin-associated glycoprotein (MAG) only occur after 20 years of age, suggesting that changes in myelin only become pronounced in old monkeys.

Paranodes

An increase in the frequency of paranodal profiles in old monkeys has also been encountered in areas 17 and 46 of the cerebral cortex (Peters and Sethares, 2003). When young and old monkeys are compared, the increase in frequency in area 17 is 57% and in area 46 it is 90%, as compared to the 57% increase in the anterior commissure. For the cerebral cortex it was suggested that the increase

in frequency of paranodal profiles is due to an increase in the number of internodal lengths of myelin, as would occur if shorter lengths of myelin are produced by remyelination of demyelinated axons. And indeed, some abnormally short internodes are present in the old monkeys, as are abnormally thin myelin sheaths, both of which are considered the hallmarks of remyelination (e.g., Gledhill and McDonald, 1977; Ludwin, 1995; Kreutzberg et al., 1997). However, direct evidence that demyelination and remyelination are occurring in old monkeys is lacking and needs further study.

If there is an increase in the number of internodal lengths of myelin, it might be expected that there is a concomitant increase in the numbers of oligodendroglial cells that form myelin, but this is not detected in the anterior commissure.

Why are nerve fibers lost?

The extent of nerve fiber loss during aging must depend on a host of factors. One obvious possibility is the vulnerability of the cell bodies that give rise to the axons. This is the likely explanation of some of the fiber loss from the optic nerve, since retinal ganglion cells are subject to damage from ocular changes or systemic disease that occur more frequently in the elderly (Garner et al., 1994). In the cerebral cortex, age-related neuron loss has been shown to be 10% or less in those areas in which it has been examined with systematic counting measures (Pakkenberg and Gundersen, 1997; Morrison and Hof, 1997; Peters et al., 1998a; Hof et al., 2000; Merrill et al., 2000). The cell bodies that contribute axons to the anterior commissure are only a small fraction of the total number of cortical neurons. If these cells were selectively vulnerable, they might account for most of the modest amount of age-related cell loss. But this is unlikely, because studies like the one by Tang et al. (1997) suggest that myelinated nerve fibers are lost from the white matter in general.

To account for the relative preservation of myelinated nerve fiber number and morphology in the cortex (e.g., Nielsen and Peters, 2000), despite the presence of degenerating axons in the white matter, it might be suggested that only the part of the axonal plexus that extends into the white matter of the anterior commissure degenerates by a "dying back" process which begins distally and gradually extends towards the cell body, without the death of the parent neuron (see Raff et al., 2002), leaving the axonal plexus close to the cell body essentially intact. In this scenario the local axonal plexus would remain structurally intact, even though it may be affected by age-related changes in its myelin. The cortical neurons in layers II and III that send axons through the anterior commissure commonly have abundant collateral axonal arborizations (e.g., Feldman, 1984; Martin, 1988; Tamas et al., 1997). This scenario would also reconcile the absence of age-related neuron loss with the presence of an age-related reduction in subcortical nerve fiber length (Tang et al., 1997) and a reduction in white matter volume determined using MRI (Guttman et al., 1998).

Sex differences

Similar to the negative results reported by LaMantia and Rakic (1990), we found no systematic difference in the cross-sectional area of the monkey anterior commissure that could be attributed to the sex of the animals. However, a relatively small number of female monkeys have been examined (eight in our study, and two by LaMantia and Rakic), so individual variability may have masked an overall difference. The effect of sex on the size of the human anterior commissure is an open question. In one study the cross-sectional area of the anterior commissure was found to be larger in women than in men (Allen and Gorski, 1991); in another study the anterior commissure was reported to be larger in men than in women (Demeter et al., 1988), and in two studies no difference in size was observed between the sexes (Highley et al., 1999; Lasco et al., 2002).

The cross-sectional area of the commissures (particularly the area of the corpus callosum) has received attention because it is easy to measure, and because of the assumption that area is indicative of fiber number. In our study the cross-sectional area was indeed related to the number of fibers ($r = 0.8257$, $P < 0.0001$), although this correlation was not significant in the much smaller study by LaMantia and Rakic (1990). When we compared fiber number in male and female monkeys we did not find a significant difference between the sexes (two-tailed $P = 0.2232$), but we did find a sex difference in fiber number as a function of age. Male monkeys exhibited a significant negative correlation between fiber number and age, while female monkeys did not (Fig. 8B). Thus, in our population there was a tendency for young males to have more fibers than young females, but males lost more fibers with age, resulting in equivalence in terms of nerve fiber number in the old animals. One study of hemispheric volume in human brains found a greater age-related reduction in the volume of the temporal lobe in men than in women (Cowell et al., 1994), which may be significant because this lobe is the major source for the fibers of the anterior commissure. The only study we are aware of that examined fiber number in the human anterior commissure found more fibers in women than in men, although the effects of age were not investigated (Highley et al., 1999).

Relationship between cognitive performance and nerve fiber number

Overall, a modest but significant relationship ($P < 0.01$) is observed between the number of nerve fibers in the anterior commissure and the overall cognitive performance of the animals, as measured by the CII. However, additional information emerges when male vs. female animals and young vs. old animals are examined. Our population of females has a fairly uniform number of fibers, but a broad range of cognitive performance scores, resulting in no correlation between the two variables. In contrast, males with more fibers tend to have better cognitive performance than males with fewer fibers (Fig. 8B). It should be noted, however, that even within the males there is a wide range of cognitive performance, such that two animals with almost the same number of fibers can have very different levels of cognitive performance (compare AM 091 and AM 101, Table 1).

Of course, this study cannot address changes in fiber number and cognitive performance for individual animals over time. We do not know whether a 32-year-old animal such as AM 091, with 1.47×10^6 fibers and cognitive preservation, began life with more fibers or fewer fibers than AM101, which had 1.49×10^6 fibers and significant cognitive impairment by the age of 19 years. However, for animals 5–20 years of age there is a significant correlation between fiber number and cognitive performance that

does not hold for those 25 years and older (Fig. 8A). These results need further exploration with additional monkeys, since our old group only contained six behaviorally tested animals. Four of these were females, for whom we already suspect that the relationship between fiber number and cognitive performance is weak.

Neuroglial cells in the anterior commissure

In the anterior commissure the percentage of profiles of neuroglial cells identified as oligodendrocytes is considerably higher (86%) than the percentage of these cells in either the optic nerve (60%, Sandell and Peters, 2002) or the visual cortex (57%, Peters et al., 1991). Conversely, the percentage of astrocytes in the anterior commissure (9%) is correspondingly lower than the 35% observed in both the optic nerve and the visual cortex, while the percentage of microglial cells (5%) is approximately the same in all three structures (Peters et al., 1991; Sandell and Peters, 2002). The values obtained for the monkey anterior commissure are very similar to those reported for this structure in the mouse: 83% oligodendrocytes, 10% astrocytes, and 6% microglial cells (Sturrock, 1987).

The difference in the proportions of oligodendrocytes and astrocytes in the anterior commissure, compared to the visual cortex and optic nerve, may be explained by differences in the constituents of these structures. As white matter, the anterior commissure has proportionally more myelinated axons and fewer blood vessels than the gray matter of the visual cortex. Consequently, it is understandable that the anterior commissure has more oligodendrocytes and fewer astrocytes, since oligodendrocytes make myelin and an important function of astrocytes is to form the end feet of the glial limiting membrane around blood vessels. The optic nerve, on the other hand, contains only myelinated axons, but it contains many trabeculae of fibrous tissue that divide the nerve fibers into fascicles, each of which is surrounded by an astrocytic glial limiting membrane (Sandell and Peters, 2002). The presence of this extensive glial limiting membrane within the optic nerve might explain the relatively high proportion of astrocytes contained within it.

There is no change in the number of neuroglial cell nuclear profiles with age in the anterior commissure, which was somewhat surprising, given the age-related increase in glial number we observed in the optic nerve (Sandell and Peters, 2002). However, most aging optic nerves have more overt signs of nerve fiber degeneration than does the anterior commissure. In old optic nerves it is common to see activated microglia containing lamellar profiles of phagocytosed myelin. These are not seen in the anterior commissure. Perhaps nerve fiber loss occurs more gradually in the anterior commissure so that microglial cells are not activated, but we have no data to address this speculation.

Conclusions and functional implications

In every location examined thus far, myelin in the central nervous system is vulnerable to the aging process. Loss of the nerve fibers themselves has now been observed in two well-circumscribed bundles, the optic nerve and the anterior commissure. In this study we have the first indication that in white matter myelin damage precedes axon loss, as the proportion of abnormal myelin profiles increases significantly in middle-aged animals compared to young animals. Disruption of myelin sheaths in the optic nerve is known to impair the conduction velocity of action potentials (Gutierrez et al., 1995), and an age-related reduction in axonal conduction velocity has been reported in several other systems (Aston-Jones et al., 1985; Morales et al., 1987; Xi et al., 1999). Reduction in conduction velocity might explain why the prevalence of myelin abnormalities in monkey prefrontal and visual cortex relates to the magnitude of age-related cognitive impairment (Peters et al., 2000; Peters and Sethares, 2002), since the timing in neuronal circuits would thereby be altered with age. In humans, age-related white matter changes are visible by magnetic resonance imaging and cortical disconnection has been postulated as a cause of age-related cognitive impairment (de Groot et al., 2000; O'Sullivan et al., 2001).

In the present study it is fiber number, rather than the frequency of myelin abnormalities, that is associated with cognitive status, and the association is strongest for animals $\leq$20 years of age. It would be interesting to know whether greater interhemispheric connectivity is associated with superior cognitive performance, but to address that question all four interhemispheric commissures would need to be examined in a large sample of young behaviorally tested animals. Fiber number in the anterior commissure is certainly reduced in old animals, but no association between fiber number and cognitive status has been found within this age group. Several possible explanations can be suggested for this result. The first is that the number of behaviorally tested old animals is relatively small, compared to the population from age 5–20, so a larger sample size might reveal a relationship between fiber number and cognitive status among old animals. The second is that the tests used to compile a cognitive profile for our animals (tests of visual recognition and spatial memory) may not be particularly dependent on interhemispheric processing in the typical testing situation with intact animals. A related piece of information is that many of the temporal lobe regions that send axons through the anterior commissure also contribute to the splenium of the corpus callosum. A variety of experiments have shown that monkeys with a transected optic chiasm can perform well on visual tasks that require interhemispheric processing, provided that either of the two commissures is intact (Doty et al., 1988, 1994; Lewine et al., 1994). Perhaps fiber number in both the anterior commissure and the corpus callosum need to be examined in each animal in relation to cognitive status to reveal any association between fiber number in these structures and functional consequences in the aging monkey.

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