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The Neuroglial Population in the Primary Visual Cortex of the Aging Rhesus Monkey

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KEY WORDS
neuroglial cells; aging; myelin alterations; rhesus monkey; visual cortex; cognitive decline

ABSTRACT
The effects of age on neuroglial cells have been examined in the primary visual cortices of rhesus monkeys that had been behaviorally tested. The assessment of changes in the neuroglial populations was made on the basis of the frequency of occurrence of profiles of neuroglial cells in semithick sections of osmicated tissue stained with toluidine blue. No changes were found in the numbers of astrocytes and microglial cells with age, but the numbers of oligodendrocytes increased by about 50%. The myelinated nerve bundles at the level of layer 4 were also examined by electron microscopy to assess the effects of age on the nerve fibers. The numbers of nerve fiber profiles showing age-related alterations in their myelin sheaths increase with age. There was also an age-related increase in the frequency of profiles of nerve fibers sectioned through paranodes, indicating that shorter lengths of myelin are being produced by remyelination. These changes in sheaths both correlate significantly with the frequency of oligodendrocyte profiles, suggesting that with age additional oligodendrocytes are required to remyelinate nerve fibers whose sheaths have broken down, probably by death of the original parent oligodendroglial cell. Also the most cognitively impaired monkeys had the greatest numbers of oligodendrocytes, but this is probably a secondary correlation, reflecting the fact that altered myelin slows down the rate of conduction along nerve fibers, which leads to cognitive decline.

INTRODUCTION

In the monkey, primary visual cortex occupies much of the occipital poles of the cerebral hemispheres. Morphologically this cortical area is characterized by having almost twice the density of neurons than other cortical areas (e.g., O'Kusky and Colonnier, 1982), and in layer 4B there is also a distinct band of horizontally oriented nerve fibers, which constitute the stripe of Gennari. In addition there are well-defined vertical bundles of myelinated nerve fibers that pass through the lower layers of the cortex and it has been proposed that each bundle of myelinated nerve fibers constitutes the efferent output from a single pyramidal cell cortical module (Peters and Sethares, 1991b, 1996).

With age there is no significant loss of neurons from the monkey primary visual cortex (e.g., Peters and Sethares, 1993; Vincent et al., 1989), although neurons in the aging primary visual cortex accumulate lipofuscin in their cell bodies. Inclusions also accumulate in the perikarya of the neuroglial cells (Peters et al., 1991a; Peters, 1996), and in addition electron microscope studies show that there are age-related alterations in the morphology of the myelin sheaths of nerve fibers (Peters et al., 2000).

Essentially there are four kinds of alterations in the myelin sheaths of aging monkeys (Feldman and Peters, 1998; Peters et al., 2000; Peters and Sethares, 2002b). Two kinds of alterations, the localized accumulation of electron dense cytoplasm between the lamellae of sheaths and the blebbing or ballooning of some sheaths, appear to be degenerative. The other two kinds of age-related alterations appear to be due to the continued production of myelin by oligodendrocytes. This results in the formation of sheaths with redundant myelin and in an increase in the mean numbers of lamellae in myelin sheaths (Peters et al., 2001b).

Another change that occurs during aging is an increase in the frequency of profiles of paranodes of myelinated nerve fibers (Peters and Sethares, 2003). Since the extent of this increase cannot be explained by an age-related lengthening of paranodes, it is concluded that some internodal lengths of myelin degenerate with age, to be subsequently replaced by a series of shorter internodes, as is known to occur in remyelination (Gledhill and McDonald, 1977; Hirano, 1989; Kreutzberg et al., 1997; Ludwin, 1978, 1995; Prineas and McDonald, 1997). The contention that some myelin sheaths degenerate with age is supported by the fact that some astrocytes in old monkeys contain electron dense inclusions with the characteristic lamination of myelin, while in other astrocytes some of the amorphous inclusions label with antibodies to myelin basic protein (Peters and Sethares, 2003). It has not been possible to obtain direct proof, but it seems likely that it is the sheaths with either dark cytoplasm or ballooning degenerate. And the suggestion that there is remyelination is supported by the fact that in the primary visual cortex of old monkeys

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TABLE 1. Numbers of Astrocytes per mm^2

			Layers 1–3		Layer 4		Layers 5–6		
Animal	Sex	Age (yrs)	Mean	SEM	Mean	SEM	Mean	SEM	CII
AM7	M	5	177.8	13.2	181.5	14.6	246.4	17.1	no data
AM76	F	6	232.2	12.6	159.6	11.4	178.8	12.8	0.08
AM77	F	6	124.8	10.8	70.8	6.9	160.2	13.5	2.27
AM130	F	8	129.6	9.1	86.4	7.5	140.6	11.9	1.28
AM 96	F	9	192.2	12.4	162.3	13.7	184.0	13.9	1.12
AM 47	M	9	180.4	11.9	143.8	11.4	170.7	11.2	0.51
			172.8	**11.7**	**134.1**	**10.9**	**180.1**	**13.4**	
AM42	M	12	144.0	12.1	147.0	11.0	211.7	14.8	0.95
AM140	M	12	109.7	10.8	70.9	6.0	120.7	11.9	no data
AM143	M	16	141.0	14.0	57.6	11.6	125.3	11.6	0.00
AM133	M	19	116.6	11.2	71.6	7.1	141.7	12.6	2.46
			127.8	**12.0**	**86.8**	**8.9**	**149.9**	**12.7**	
AM12	F	27	162.5	11.4	114.1	9.4	127.6	10.7	3.31
AM62	M	28	193.6	11.9	138.7	11.4	258.8	19.2	3.81
AM26	F	29	161.6	15.6	95.5	8.7	195.6	16.9	1.05
AM91	M	32	207.5	15.8	98.7	9.6	200.7	15.1	incomplete
AM65	F	33	187.4	13.3	144.0	10.0	190.8	15.1	3.24
AM13	M	35	215.6	14.2	140.1	11.4	183.8	14.0	no data
			188.0	**13.7**	**121.8**	**10.1**	**192.9**	**15.2**	

we have found very short internodal lengths of myelin, as well as sheaths that are inappropriately thin for the size of the enclosed axon (Peters and Sethares, 2003).

Oligodendrocytes are the myelin-forming neuroglial cells in the central nervous system and since the increased paranodal frequency shows that additional internodal lengths of myelin occur with increasing age, it is likely that the remyelination process requires additional oligodendrocytes. We have two indications that the numbers of oligodendrocytes in visual cortex increase with age. The results from an early study suggested that there might be an increase in the numbers of oligodendroglial cells with age, but at that time too few monkeys were available to produce acceptable statistical data (Peters et al., 1991a). Then, more recently, we have examined the effects of age on oligodendroglial cells in layer 4Cβ and found a 50% increase in the number of oligodendroglial cell profiles (Peters and Sethares, 2004).

The present study has been undertaken using design-based stereology. The aim is to assess the effects of age on the frequency of occurrence of neuroglial cells throughout the depth of monkey primary visual cortex, and to correlate the results with the cognitive statuses of the monkeys from which the cortical tissue was obtained, as well as the frequency of alterations in myelin sheaths and the frequency of occurrence of paranodes. To that end the frequencies of the three types of neuroglial cells, oligodendrocytes, astrocytes, and microglia, have been determined in the upper, middle, and deep layers of the monkey primary visual cortices of young, middle aged, and old rhesus monkeys. One micron thick sections of osmicated material embedded in plastic and stained with toluidine blue were used, since this is the only type of preparation in which the profiles of these cells can be distinguished from each other and from neurons by light microscopy. In addition, we have examined profiles of myelinated nerve fibers in the vertical bundles of myelinated fibers in the cortices of the same monkeys at the level of layer 4C by electron microscopy, to determine the frequency of occurrence of altered myelin sheaths and of paranodal profiles, so that it could be determined if these alterations in morphology correlate with changes in oligodendroglial frequency.

MATERIALS AND METHODS

Tissue Specimens and Processing

Sixteen rhesus monkeys (*Macaca mulatta*) were used in this study. Six of them were young (5–9 years of age), four were middle aged (12–19 years of age), and six were old (over 25 years of age). The ages of the monkeys, given to the nearest year, and their sexes are given in Tables 1–4. Details of the method used to fix the brains of these monkeys for preparation of semithick plastic sections for light microscopic examination are given in earlier publications (e.g. Peters et al., 1994). In brief, the monkeys were perfused intracardially with a warm solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in a 0.1 M cacodylate or phosphate buffer at pH 7.4. The brains were then removed and one hemisphere from each brain was stored in a cold solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M buffer of the same type used for the perfusion. The perfusions were carried out in full accord with the approved Institutional Animal Care and Use Committee Regulations, and in accordance with the NIH publication *Guide for the Care and Use of Laboratory Animals.* All efforts were made to minimize the numbers of animals used and their suffering.

Several blocks of tissue were taken from the primary visual cortex, area 17, of each monkey. The tissue blocks were taken from the opercular surface, about 3 mm caudal to the lunate sulcus, where the center of the visual field is represented. The tissue blocks were osmicated, dehydrated in an ascending series of alcohols and embedded in Araldite.

The tissue blocks were oriented in the vertical plane, normal to the pial surface, and sectioned at a thickness of

1 μm. These semithick sections were mounted on glass slides, stained with toluidine blue, and coverslipped. Other blocks of visual cortex were oriented so that the plane of sectioning was parallel to the pial surface, and thin sections cut at the level of layer 4C, so that the profiles of nerve fibers in the vertically oriented myelinated fiber bundles could be examined by electron microscopy.

Neuroglial Cells Counts

Each semithick section of area 17 to be counted was first examined by light microscopy so that its depth could be divided into upper, middle, and deep tiers. The upper tier extended from the pial surface to the boundary between layer 2/3 and layer 4A; the middle tier from the top of layer 4A to the bottom of layer 4Cβ; and the lower tier from the bottom of layer 4Cβ to the boundary between layer 6A and the white matter. Separate counts of the numbers of the profiles of neuroglial cells displaying their nuclei were made in each of the three tiers. These counts of profiles of neuroglial cells were made using the Bioquant BQ-TCX-95 system (R & M Biometrics, Nashvillle, TN) with a motorized stage, and a 60× oil immersion objective. A section was viewed with the 60× oil immersion lens and the image of the section projected onto a video monitor. The image of the section was then overlain with a two dimensional counting grid with the lines spaced at intervals of 100 μm, and at each intersection of the grid a 75 × 75 μm^2 counting box was applied. In each box the profiles of astrocytes, oligodendrocytes, and microglial cells displaying their nuclei were then counted. Sufficient numbers of profiles were examined to ensure that acceptable coefficients of error were obtained. For astrocytes and oligodendrocytes it was assumed that sufficient counts had been made when the coefficient of error (CE = SEM/mean) was 10% or less, as recommended by West et al. (1996) for design-based analyses. For the sparse microglial cells it was decided that an acceptable coefficient of error was 20% or below. To obtain these levels for the coefficient of error, at least two full sections generated from different blocks of cortex were counted for each monkey. Sometimes a third count had to be made to obtain acceptable levels of coefficients of error, especially for the counts of microglial cells, in which case a second section from one of the tissue blocks was used. This section was at least 10 μm away from the first section counted from that tissue block.

As an example of the number of counts that needed to be made to obtain satisfactory coefficients of error is the following. For monkey AM 96 we counted 560 boxes from three sections, which contained profiles of a total of 566 astrocytes, 682 oligodendrocytes, and 52 microglial cells.

Alterations in the Structure of Myelinated Nerve Fibers

In 14 of the 16 monkeys in which neuroglial cell counts were made, tangential thin sections through layer 4C were examined in the electron microscope to determine the percentages of profiles of cross sectioned myelinated axons in the vertical oriented bundles that showed any morphological alterations in their myelin sheaths. That is the percentages of sheath profiles that had either ballooned sheaths, dense cytoplasm between their lamellae, sheaths with redundant myelin, or thick sheaths with splits. These morphological alterations have been described previously (e.g., Peters et al., 2000; Peters and Sethares, 2003; Sandell and Peters, 2003) and outlined in the Introduction. The percentage of nerve fiber profiles belonging to paranodes was also determined, as a measure of the extent of remyelination (e.g., Peters and Sethares, 2003). It should be pointed out that analyses of the alterations in myelin sheaths and the frequency of occurrence of paranodal profiles in three of the young monkeys and five of the old monkeys have been presented previously in the article by Peters and Sethares (2003).

Behavioral Testing

Of the 16 monkeys used in this study 12 had been behaviorally tested. The behavioral tests used to assess the cognitive status of these monkeys have been described in previous publications (e.g. Herndon et al., 1997; Killiany et al., 2000; Moss et al., 1999; Peters et al., 1996, 2000). The assessment is made on the basis of the scores obtained on three visual recognition tasks. These are the delayed nonmatching to sample (DNMS) task, a DNMS task with a 2 min delay, and the delayed recognition memory span task (DRST). From the scores obtained on these tasks an overall measure of cognitive impairment, the Cognitive Impairment Index (CII), is derived (Peters et al., 2001a). Essentially the higher the CII score the more a monkey is cognitively impaired. In general, monkeys with CII scores lower than 1.5 are considered to be nonimpaired, ones with CII scores between 1.5 and 2.5 are considered to be mildly impaired, and those with scores over 2.5 are considered to be severely impaired. The most cognitively impaired monkeys that we have encountered in our program have CIIs of about 8, but the most impaired monkey used in this study, AM 62, had a CII of 3.81 (see Tables).

RESULTS

Morphology of Neuroglial Cells and Pericytes

As shown in Fig. 1, in semithick plastic sections stained with toluidine blue and examined by light microscopy, the profiles of oligodendrocytes (O) can be recognized because they have dark round or oval nuclei surrounded by a dark cytoplasm. The microglial cells (M) also have dark nuclei, but their nuclei are smaller than those of oligodendrocytes, and are either bean-shaped or elongate, while the cytoplasm of microglial cells is somewhat paler than that of oligodendrocytes.

Astrocytes (A) have pale, round or oval nuclei, with a thin, dark rim of chromatin beneath the nuclear enve-

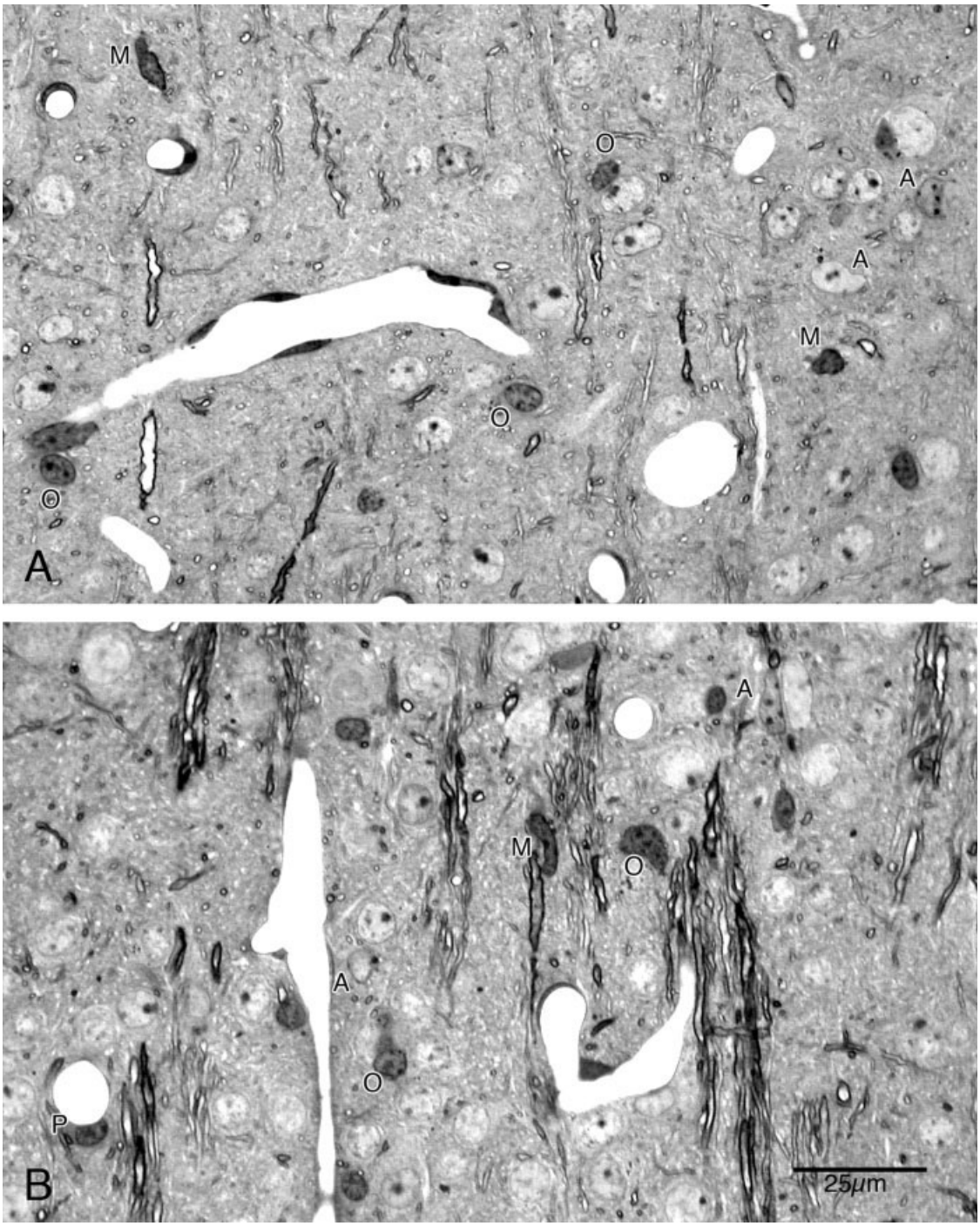

Fig. 1. Light microscopy. Vertical semithick sections of primary visual cortex stained with toluidine blue to show the different types of neuroglial cells. (**A**). Layer 2/3 from a 6-year-old monkey (AM 77). (**B**). Layer 4 from a 29-year-old monkey (AM 26). Oligodendrocytes (O) have dark nuclei that are round or oval in shape, and have dark cytoplasm. Microglial cells (M) have dark nuclei that are either bean-shaped or irregular; their cytoplasm is also dark, but usually more scant than that of oligodendrocytes. Astrocytes (A) have pale nuclei, with a thin, dark, layer of heterochromatin beneath the nuclear envelope. Their nuclei tend to stain more evenly than the round and mottled neuronal nuclei. The cell labeled P is a pericyte.

lope, and the nucleus is surrounded by pale cytoplasm. The profiles of astrocytes can be distinguished from those of neurons, because the nuclei of neurons are generally larger and their chromatin is less homogeneous. However, there is a cell type that is very difficult to distinguish from the astrocytes, and that is the fourth type of neuroglial cell in the central nervous system, which we have suggested should be called a β neuroglial cell (Peters, 2004). These cells, which label with antibodies to NG2 chondroitin sulphate proteoglycan, are often

referred to as oligodendroglial progenitors, (Levine et al., 2001; e.g. Nishiyama et al., 1999). They are so similar in appearance to astrocytes that usually they can only be reliably distinguished from astrocytes on the basis of ultrastructural characteristics. Consequently, in the present light microscopic study they are necessarily included in the astrocytic counts.

In the old monkeys a few hypertrophied astrocytes were encountered in layer 1, which becomes thinner with age and loses many branches of apical dendrites of pyramidal cells (Peters and Sethares, 2002a). However, even though the perikarya of these astrocytes become enlarged, there is no indication that the sizes of their nuclei change with age.

The Numbers of Neuroglial Cells

When the total neuroglial cell population in area 17 of young monkeys is analyzed it is evident that overall 40% of the profiles of neuroglial cells are astrocytes, 53% are oligodendrocytes, and 7% are microglial cells. In layers 1–3 astrocytes account for 57% of the total number of neuroglial cell profiles and oligodendrocytes only 36%, while in layer 4, where myelinated axons become more prominent, the proportion of oligodendrocyte profiles increases to about 62% and the frequency of astrocytes decreases to only 30% of the total number of neuroglial cell profiles. Then in layers 5–6 the profiles of oligodendrocytes account for about 55% of all neuroglial profiles and the astrocytes increase to 39%. As for the microglial cells, they account for only 7–10% of neuroglial cell profiles in all layers.

In old monkeys, the frequency of profiles of astrocytes and microglial cells in all layers are similar to those in young monkeys, but in old monkeys, the frequency of profiles of oligodendrocytes increases by about 50% in all layers. Since, as we showed in our earlier study (Peters and Sethares, 2004), the sizes of the nuclei of the neuroglial cells do not alter with age, the age-related increase in the frequency of profiles of oligodendrocytes is not due to an increase in the sizes of the nuclei.

Astrocytes

As shown in Table 1, there are similar numbers of astrocytic profiles per mm^2 in layers 1–3 and in layers 5–6, and slightly lower numbers in layer 4. When the numbers of profiles of astrocytes per mm^2 are plotted against age (see Fig. 2) it is apparent that with increasing age there are no significant changes in the frequency of astrocytes in any layers of the cortex.

It should be borne in mind that in this study the oligodendroglial progenitors have been included in the population of cells referred to as astrocytes, and while we do not know the frequency of these progenitor cells in monkey cerebral cortex, according to a review by Levine et al. (2001) oligodendroglial progenitors cells make up 5–8% of the total glial cell population. In the present study, the cells counted as astrocytes account for an average percentage of about 40% of the total neuroglial cell population, and using the values cited by Levine et al. (2001) the oligodendroglial cell progenitors probably account for between 12 and 20% of the cells counted as astrocytes.

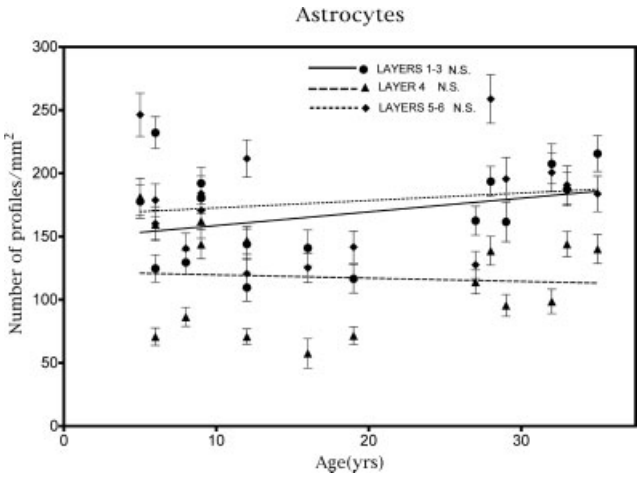

Fig. 2. A plot of the number of astrocytic profiles per mm^2 against the ages of the monkeys.

Oligodendrocytes

The frequency of occurrence of profiles of oligodendrocytes is given in Table 2, and the data are plotted in Fig. 3. Layers 1–3 have the lowest frequency of oligodendrocytes, and there are almost twice as many oligodendrocytes both in layer 4 and in layers 5–6. This parallels the numbers of myelinated nerve fibers present in these layers, because in layers 1–3 there are few myelinated nerve fibers, but in layer 4 there is an obvious increase, because layer 4B contains the stripe of Gennari, as well as the vertical bundles of myelinated axons that extend from the pyramidal cells in layer 2/3. In layer 5 most of these vertical nerve fibers are still present, and additionally there are the myelinated nerve fibers that belong to the inner band of Baillarger.

With age there is a significant increase in the frequency of oligodendrocytes throughout the cortex ($P < 0.002$ for layers 1–3; $P < 0.0001$ for layer 4; and $P < 0.012$ for layers 5–6), so that when young and old monkeys are compared the age-related increase in the frequency of oligodendroglial cell profiles amounts to about 50% (Table 2, Fig. 3).

Microglial cells

The frequency of occurrence of profiles of microglial cells is given in Table 3 and the data are plotted in Fig. 4. It will be seen that there are similar numbers of profiles of microglial cells per mm^2 in all layers of the

TABLE 2. Numbers of Oligodendrocyte Profiles per mm^2

			Layers 1–3		Layer 4		Layers 5–6		
Animal	Sex	Age (yrs)	Mean	SEM	Mean	SEM	Mean	SEM	CII
AM7	M	5	117.7	11.6	226.5	17.2	316.3	24.5	no data
AM76	F	6	104.2	9.6	309.3	19.6	233.4	19.6	0.08
AM77	F	6	95.8	9.4	258.5	14.8	292.6	19.7	2.27
AM130	F	8	99.6	7.5	272.5	14.8	198.0	19.7	1.28
AM 96	F	9	122.3	11.4	286.2	18.1	266.7	19.4	1.12
AM 47	M	9	115.4	9.8	267.4	16.2	203.9	15.5	0.51
			109.2	**9.9**	**270.1**	**16.8**	**251.8**	**19.7**	
AM42	M	12	112.2	10.3	277.0	17.8	229.3	17.2	0.95
AM140	M	12	190.0	14.6	333.9	15.5	254.2	20.6	no data
AM143	M	16	162.1	12.8	332.8	15.8	347.4	2.0	0.00
AM133	M	19	135.3	11.4	246.9	15.3	274.1	20.4	2.46
			149.9	**12.3**	**297.6**	**16.1**	**276.3**	**15.1**	
AM12	F	27	160.5	12.3	344.0	20.6	266.1	16.4	3.31
AM62	M	28	200.5	11.9	432.0	19.6	429.5	27.0	3.81
AM26	F	29	185.8	13.3	350.6	21.0	379.7	26.0	1.05
AM91	M	32	209.2	18.5	434.7	27.0	410.0	25.8	incomplete
AM65	F	33	189.0	13.2	385.8	19.0	396.8	26.7	3.24
AM 13	M	35	125.5	12.4	374.4	25.2	254.0	17.8	no data
			178.4	**13.6**	**386.9**	**22.1**	**356.0**	**23.3**	

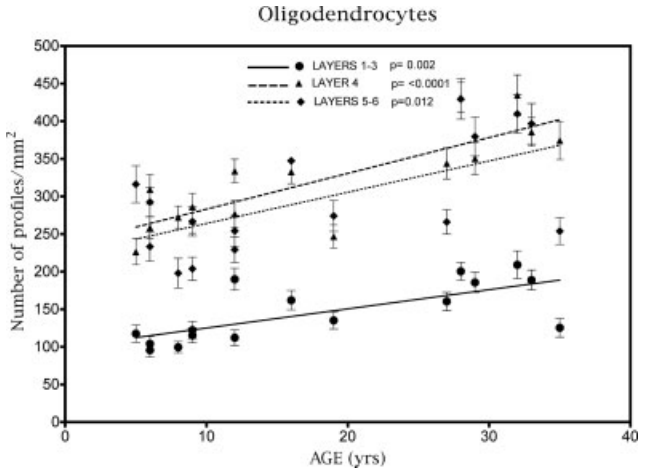

Fig. 3. A plot of the number of oligodendrocyte profiles per mm^2 against the ages of the monkeys.

cortex and that there is no change in their frequency with age.

Neuroglial Cells and Gender

As will be seen in Tables 1–4 there is a very uneven distribution of the sexes among the three age groups used in this study, and this is largely due to the fact that monkeys of both sexes for all ages are not readily available. This is the reason that all of the monkeys in the middle-aged group are males, although the young group of monkeys consists of two males and four males and the old group contains three males and three females, so that some comparisons can be made between males and females.

When the frequencies of occurrence of neuroglial cell types in male and female monkey are plotted separately, the results are essentially similar to those shown in Figs. 2–4. Neither the males nor the females show a significant increase with age in the frequencies of occurrence of astrocytes and microglial cells. In females there is a significant increase in the frequency of oligodendroglial cells with age, while for males there are significant increases in layers 4 and 5–6 with age, but for layer 2–3 which contains the fewest oligodendrocytes, the correlation between age and oligodendrocytes frequency is not significant.

When the numbers of profiles per mm^2 in the three cortical depths are compared by sex, it is found that there are significantly more astrocytes in males in layer 1–3 of old monkeys ($P = 0.03$), and more oligodendrocytes in males in layer 4 of old monkeys ($P = 0.036$). There are no sex differences for neuroglia in any other layers, in either old or young monkeys and so the significance of these two specific differences for old monkeys is not evident. Before any conclusions about sex differences can be drawn a larger population of monkeys needs to be examined.

Correlations with Behavioral Data

When the data on the numbers of oligodendrocyte profiles per mm^2 are plotted against the cognitive impairment indices (CIIs) of individual monkeys it is found (see Fig 5) that for the upper, middle, and lower layers of the cortex there is a weak correlation (P = about 0.1) between the two sets of data. There are no correlations between the behavioral data and the frequency of astrocytes and microglial cells.

The Effects of Age on Myelin Sheaths

As pointed out in the Introduction, there are alterations in myelin sheaths with age, and the most obvious alteration is the inclusion of dense cytoplasm between

TABLE 3. Numbers of Microglial Profiles per mm²

			Layers 1–3		Layer 4		Layers 5–6		
Animal	Sex	Age (yrs)	Mean	SEM	Mean	SEM	Mean	SEM	CII
AM7	M	5	22.0	4.3	26.8	5.3	27.0	5.3	no data
AM76	F	6	15.3	3.0	28.1	5.0	29.0	5.7	0.08
AM77	F	6	31.6	4.6	51.9	6.2	28.4	5.3	2.27
AM130	F	8	14.2	2.5	27.9	4.4	28.1	5.3	1.28
AM 96	F	9	18.5	3.7	31.8	5.3	32.2	5.7	1.12
AM 47	M	9	15.8	3.2	21.9	3.9	24.5	4.3	0.51
			19.6	**3.6**	**31.4**	**5.0**	**28.2**	**5.3**	
AM42	M	12	50.0	5.7	64.2	7.8	54.9	6.8	0.95
AM140	M	12	43.7	5.5	53.5	5.5	34.5	6.2	no data
AM143	M	16	57.1	7.3	43.6	5.2	33.6	5.0	0.00
AM133	M	19	50.1	6.6	46.4	6.8	45.3	6.6	2.46
			50.2	**6.3**	**51.9**	**6.3**	**42.1**	**6.1**	
AM12	F	27	23.8	4.1	25.6	5.2	21.0	4.1	3.31
AM62	M	28	24.5	3.9	29.2	5.2	32.7	6.6	3.81
AM26	F	29	28.3	5.2	41.6	5.7	34.3	6.2	1.05
AM91	M	32	28.6	5.5	41.2	6.8	31.5	6.0	incomplete
AM65	F	33	32.7	5.0	47.1	5.9	50.7	7.3	3.24
AM13	M	35	22.8	4.1	24.9	4.8	26.5	5.2	no data
			26.8	**4.6**	**34.9**	**5.6**	**32.8**	**5.9**	

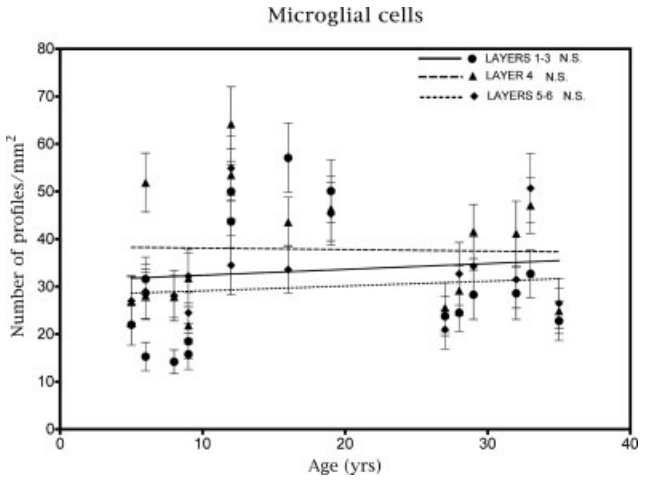

Fig. 4. A plot of the number of microglial cell profiles per mm² against the ages of the monkeys.

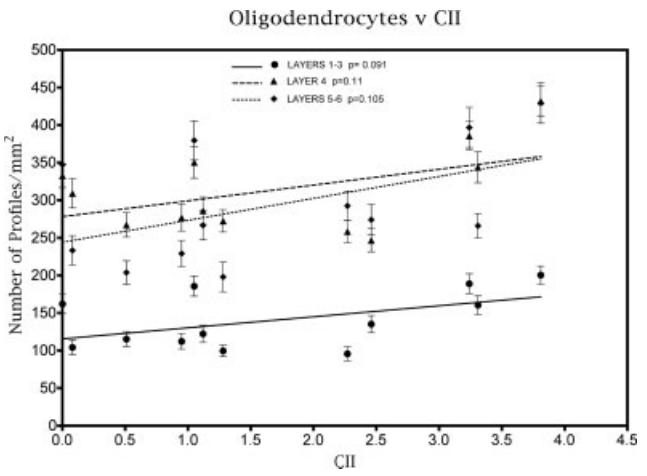

Fig. 5. The number of profiles of oligodendrocytes per mm² in the monkeys plotted against their cognitive impairment indices (CIIs).

the lamellae of some sheaths (see Fig. 6). Other alterations are the ballooning of some sheaths, the formation of redundant myelin, and the circumferential splitting of thick sheaths (also see Sturrock, 1987). The frequency of occurrence of these four types of age-related alterations, as a percentage of all profiles of myelinated nerve fibers, has been assessed by electron microscopy in tangential thin sections through layer 4C in 13 of the 16 monkeys in which neuroglial cells counts were made. In these same thin sections the percentage of nerve fiber profiles that belong to paranodes has also been determined (see Fig. 6). The results of this analysis are given in Table 4, which also repeats the data on the frequency of occurrence of oligodendrocyte profiles in layer 4 given in Table 2.

Plotting the percentage of altered myelin sheath profiles against the number of oligodendroglial profiles per mm² in layer 4 (see Fig. 7) shows that as the frequency of occurrence of altered sheaths increases, there is a parallel increase in the frequency of occurrence of profiles of oligodendrocytes, and there is a very significant correlation between the two sets of data ($P = 0.0005$). Similarly, there is a significant correlation ($P < 0.0001$) between the frequency of occurrence of profiles of paranodes and the frequency of oligodendrocyte profiles in layer 4 of individual monkeys (see Fig. 8).

DISCUSSION

Neuroglial Cells in Primary Visual Cortex

This more complete stereological analysis of the effects of age on the frequency of neuroglial cells confirms what had been suspected from our previous electron microscopic analysis of the effects of age on the populations of these cells in monkey primary visual cortex (Peters et al., 1991a). This earlier study was primarily aimed at assessing the effects of age on the morphology of neuroglial cells, and the effects of age on the numbers of the

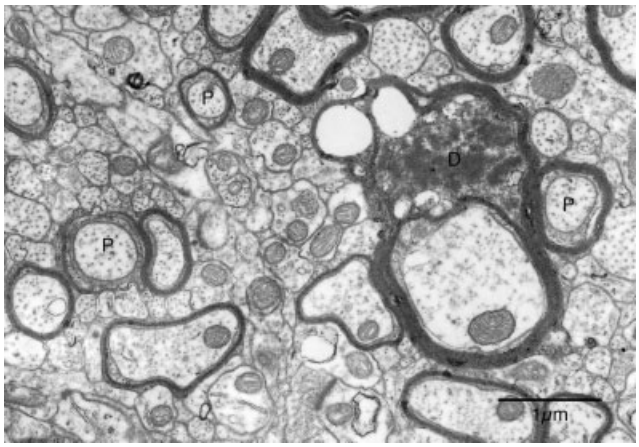

Fig. 6. Electron micrograph of a tangential section through area 17, taken at the level of layer 4C from a 35-year-old monkey, to show a large nerve fiber with a myelin sheath that has split to accommodate dense cytoplasm (D), even though the axon within the sheath appears to be normal. The micrograph also contains profiles of three paranodes (P), which are identified by the close apposition between the axolemma and the plasma membrane on the inside of the sheath.

neuroglial cells was ascertained by determining how frequently profiles of these cell types were encountered in the thin sections from only a few monkeys. More recently, we examined the effects of age on the populations of the three types of neuroglial cells in layer 4Cβ of area 17 of the rhesus monkey and we found that while there is no increase in the frequency of astrocytes and microglial cells with age, there is a 50% increase in the numbers of oligodendrocytes in layer 4Cβ (Peters and Sethares, 2004).

The present study shows that the increase in the frequency of oligodendrocytes occurs in all layers of this cortex, but there is no increase in the frequency of astrocytes and microglial cells in any layers. Not surprisingly, the frequency of oligodendrocytes somewhat parallels the prevalence of myelinated nerve fibers in the various layers, so that there are fewest oligodendrocytes in layers 1–3, and a doubling of their frequency in layers 4, 5, and 6, which contain many more myelinated nerve fibers. It should be added that in a study centered on the effects of age on layer 1 in primary visual cortex, no changes in the frequency of occurrence of any of the glial cells types was encountered, (Peters and Sethares, 2002a). The likely reason for this result is the scarcity of myelinated nerve fibers in layer 1.

The Origin of the Increased Number of Oligodendrocytes

As shown by Cerghet et al. (2006), during aging some cells are dying and others are being generated, and it is an imbalance between these two processes that leads to the increase in the numbers of oligodendroglial cells with age. The origin of the new oligodendrocytes formed in primary visual cortex is not known. It is possible that they are produced by division of existing oligodendrocytes, and in support of this view is the fact that with age, pairs, rows, and groups of mature oligodendrocytes become increasingly common (Peters, 1996; Peters and Sethares, 2003). However, there seems little evidence that mature oligodendrocytes divide (Keirstead and Blakemore, 1997; Norton, 1996; Ludwin, 1995). This is supported by in a recent study of the ultrastructure of BrdU labeled cells in adult monkey dentate gyrus (Ngwenya et al. 2008). We found that the only cell types labeled were immature neurons, immature astrocytes, and oligodendroglial progenitor cells. No labeled oligodendrocytes were encountered, which would suggest that the division of existing oligodendrocytes in cortex is probably not the source of the increased numbers of oligodendrocytes that occur with age.

It seems more likely that new oligodendrocytes are generated from oligodendroglial cell progenitors present throughout the central nervous system, although the role of these cells in the normal mature brain is not known (Chen et al., 2002; Levine et al., 2001; Norton, 1996; Watanabe et al., 2002). These progenitor cells are visualized by labeling with antibodies to NG2 chondroitin sulfate proteoglycan and the platelet derived growth factor α receptor (see Levine et al., 1993; Nishyama et al., 1997; Stallcup, 2002). In tissue examined by electron microscopy these NG2 positive cells are very similar in appearance to protoplasmic astrocytes, although they lack intermediate filaments and do not play a role in the formation of the glial limiting membrane (Peters and Sethares, 2004). However, because of their similarity to astrocytes, the progenitor cells cannot usually be distinguished from astrocytes in semithick sections examined by light microscopy. Consequently, in the present study they have necessarily been included in the counts of astrocytes, and as shown above it is likely that oligodendroglial progenitor cells account for between 12 and 20% of the cells counted as astrocytes. How to carry out differential counts of astrocytes and of progenitor cells concomitant with counts of the other neuroglial cell types, is not clear at present, although double labeling with specific antibodies would allow for a determination of the numerical densities of these two cells types.

Relations Between Oligodendroglial Increases and Myelin Sheath Alterations with Age

Interesting relationships to emerge from this study are those between age-related changes in the frequency of oligodendrocytes and the frequency of profiles of altered sheaths (see Fig. 7) and paranodes (see Fig. 8) in layer 4. The existing data suggest that with age some of the original myelin internodes degenerate, probably due to the degeneration of their parent oligodendrocytes, and such neuroglial cell death has been shown in rodent white matter (Cerghet et al., 2006). To remyelinate axons that have lost their myelin sheaths, new oligodendrocytes must be generated, and these new cells form internodes that are shorter and more numerous than

TABLE 4. Oligodendrocytes, Altered Sheaths, and Paranodes in Layer 4

Animal	Sex	Age (yrs)	Number of Oligo profiles per mm^2	% Altered sheaths	% Paranodal profiles
AM 7	M	5	226.5	no data	no data
AM 76	F	6	309.3	0.5%	9.6%
AM 77	F	6	258.5	0.9%	6.4%
AM 130	F	8	272.5	no data	no data
AM 96	F	9	286.2	1.5%	8.7%
AM 47	M	9	267.4	0.6%	10.9%
AM 42	M	12	277.0	1.5%	6.4%
AM 140	M	12	333.9	2.4%	9.6%
AM 143	M	16	332.8	2.4%	10.6%
AM 133	M	19	264.9	1.1%	6.8%
AM 12	F	27	344.0	5.6%	12.7%
AM 62	M	28	432.0	5.4%	13.5%
AM 26	F	29	350.6	2.3%	12.9%
AM 91	M	32	434.7	4.3%	13.9%
AM 65	F	33	385.8	no data	no data
AM 13	M	35	374.4	4.8%	16.5%

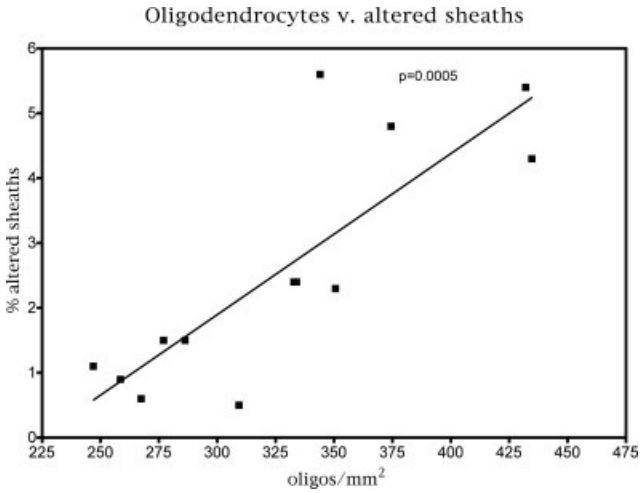

Fig. 7. The percentage of nerve fiber profiles that show altered sheaths in layer 4 plotted against the numbers of oligodendrocyte profiles per mm^2 in that layer for the monkeys examined.

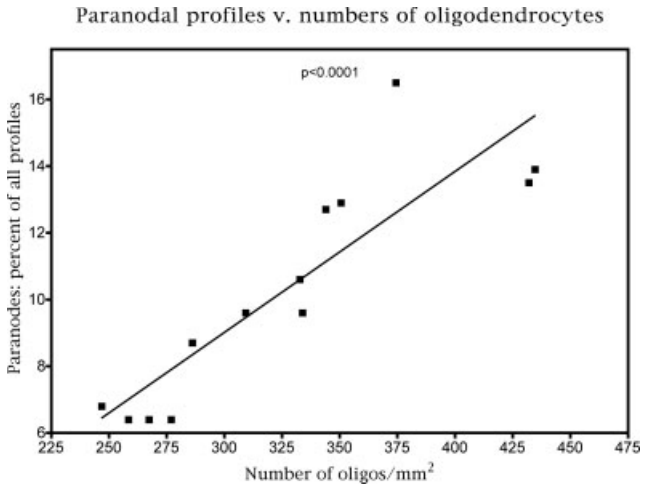

Fig. 8. The percentage of profiles of myelinated nerve fibers in layer 4 that belong to paranodes plotted against the numbers of oligodendrocyte profiles per mm^2 in that layer.

the original ones, so that increased numbers of oligodendrocytes are required.

The only other structure in which we have data on both the changes in frequency of neuroglial cells and of altered sheaths and paranodes is the anterior commissure (Sandell and Peters, 2003). But while the frequency of both altered sheaths and paranodes increase significantly with age in this fiber tract, there is no apparent parallel increase in the frequency of oligodendrocytes. However, unlike the primary visual cortex, in which there is no evidence for nerve fiber loss with age (Nielsen and Peters, 2000), there is a 50% loss of nerve fibers from the anterior commissure. Consequently, it is possible that the oligodendrocytes that were responsible for the sheaths of the lost nerve fibers become available to produce the new and shorter internodes along nerve fibers whose sheaths degenerate and are remyelinated. In such a scenario no new oligodendrocytes would be required. However, to determine if this is true, further data must be generated on the effects of age on nerve fibers and oligodendrocytes in gray and white matter in other parts of the central nervous system.

The Effects of Age on Neuroglial Cells in Other Structures

As pointed out above, there is no increase in the frequency of oligodendrocytes in the anterior commissure with increasing age and the same is true for astrocytes and microglial cells (Sandell and Peters, 2003). However, in the optic nerve, in which there can be a substantial loss of nerve fibers with age (Sandell and Peters, 2001), there is no change in the frequency of astrocytes with age, but there are increases in the frequency of oligodendrocytes and microglial cells (Sandell and Peters, 2002). In the young optic nerve are about 1.6 million nerve fibers, and our data suggest that when nerve fiber loss from old monkeys is moderate, the frequency of oligodendrocytes increases, but when the number of nerve fibers falls below 0.9 million the frequency of oligodendrocytes declines. It was suggested (Sandell and Peters, 2002) that with moderate nerve fiber loss from optic nerve no additional oligodendrocytes are necessary to produce new internodes, but that with extensive nerve fiber loss some oligodendrocytes degenerate because so

few fibers remain on which to form myelin. The additional microglial cells are required to phagocytose the nerve fibers and other components of the nerve that are degenerating.

A different picture emerges from our studies on the effect of age on layer 1 of the cortices in both area 17 (Peters et al., 2001a) and area 46 (Peters et al., 1998). Layer 1 shows significant thinning with age and this is accompanied by a loss of some dendrites and dendritic spines, and a decrease in the frequency of synapses. At the same time there is a very obvious thickening of the glial limiting membrane, which is due to hypertrophy of the astrocytes and not to an age-related increase in the numbers of any of the neuroglial cells (Peters and Sethares, 2002a). A similar hypertrophy of astrocytes occurs with age occur in optic nerve (Sandell and Peters, 2001), corpus callosum (Peters and Sethares, 2002b), and anterior commissure (Sandell and Peters, 2003). As nerve fibers are lost, the astrocytes hypertrophy to occupy the increase in inter-fiber space and at the same time they become more filamentous.

Apart from the study by Hansen et al. (1987), which showed no increase in the numbers of astrocytes with age in the human cerebral cortex, the only other studies of the effects of aging on neuroglial populations appear to have been done on rodents. In one of the earliest studies, Vaughan and Peters (1974) examined the auditory cortex of mature rats and found little change in the frequency of oligodendrocytes and astrocytes, but a 65% increase in microglial cells. Similarly Diamond et al. (1977) examined the occipital cortex of rats and found no change in the numbers of astrocytes and oligodendrocytes with age, but they did not examine microglial cells. However, Peinado et al. (1993, 1997) arrived at a different conclusion, because after an examination of the total number of neuroglial cells in rat frontal cortex (Peinado et al., 1993) and in rat parietal cortex (Peinado et al., 1997) they reported a 16–17% increase in the total number of all neuroglial cells. In the hippocampus on the other hand, Long et al. (1998) found no age related change in astrocytes and microglial cells in the aging mouse. Similarly, Bhatnagar et al. (1997) found no increase in the total number of astrocytes in the hippocampus of rats, while Pilegaard and Ladefoged (1996) report a slight increase in number of astrocytes with age. Although there are some differences in these reports, the likely conclusion to be drawn is that in the cortices of normally aging rodents there is little, if any, significant increase in numbers of neuroglial cells with age.

However, more recently Mouton et al. (2002) examined the numbers of astrocytes and microglial cells in the hippocampal formation of mice and concluded that there are some 20% more microglia and astrocytes in the brains of aged females compared with young mice. They also showed gender differences, since in the dentate gyrus and CA1 region of the hippocampus females at all ages had significantly more microglia and astrocytes than male mice. Our data suggest that except for the possibility that there are more oligodendrocytes in layer 4, and astrocytes in layer 1–3 of old male monkeys, there are no differences in the frequency of the three neuroglial cell types in monkey visual cortex. But before it can be assumed that these two sex differences are significant, the visual cortices of larger numbers of monkeys need to be analyzed and other cortical areas examined.

Changes in neuroglial cell populations have also been reported in white matter. Thus Cerghet et al. (2006) have shown that in the corpus callosum, fornix, and spinal cord of rodents, the density of oligodendrocytes is 20–40% greater in males than in females. Moreover, the generation of new glia and the apoptosis of glia is greater in the corpus callosum of females than in males, indicating that the lifespan of oligodendrocytes is shorter in females than in males, and in addition Cerghet et al. (2006) indicate that the turnover of myelin is greater in females than in males. Unfortunately, there is no information available about the turnover of oligodendrocytes and of myelin in primates.

Correlations with Behavior

How to interpret the weak correlation between oligodendrocyte frequency and cognitive impairment displayed by individual monkeys (see Fig. 5) is not readily apparent. This weak correlation may be a reflection of the strong correlation ($P < 0.0001$) between the frequency of altered sheaths and cognitive impairment in the aging visual cortex (Peters et al., 2001a). There is a similar strong correlation between the frequency of altered sheaths and CIIs in area 46 of prefrontal cortex (Peters and Sethares, 2002b), as well as in the corpus callosum and fornix (unpublished). We have proposed that the basis of the correlation between cognitive status and altered sheath frequency is that the breakdown of myelin sheaths interferes with, and slows down, the rate of conduction along nerve fibers with altered sheaths. This would affect the timing in neuronal circuits and lead to cognitive impairment.

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