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Commentary

In Aging, Is It Gray or White?

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The article by Marner et al. in this issue of the *Journal of Comparative Neurology* reports that there is an extensive loss of myelinated fibers from the white matter of the human cerebral hemispheres during normal aging. It raises the possibility that changes in gray matter, and especially any loss of its component neurons, might not be the principal reason for the mild cognitive decline that accompanies normal aging. To put this in perspective, we will first briefly examine some of the evidence for neuronal loss from human and nonhuman primate cerebral cortex in normal aging and then consider the changes that occur in white matter.

IS THERE A LOSS OF CORTICAL NEURONS DURING NORMAL AGING?

There is certainly a loss of cortical neurons in cases of Alzheimer's disease, and in what was probably the first study of the effects of normal aging on human cerebral cortex, Brody (1955) concluded that there is also a significant and progressive loss of cortical neurons during normal aging. The loss reported by Brody amounted to as much as 50%. This report received a lot of attention and was probably the seed for the common saying that "she/he is losing her/his neurons", to explain why an aging person is a little forgetful. Brody's studies were followed by those of others, who reached similar conclusions (see Peters et al., 1998, for references), and it was not until the 1980s that this concept was seriously challenged and some investigators presented evidence for the opposite conclusion, that there is a preservation of neurons. For example, Haug (1985) carried out morphometric analyses on over 120 human brains in an extensive study lasting several years and came to the conclusion that any loss of cortical neurons during normal aging is minimal. Haug showed that the previously reported losses could be attributed to the fact that, during preparation of tissue for microscopic examination, the brains of younger individuals shrink more than those of older ones. The consequence is that, when sections of cortical tissue are compared, those from younger brains show higher neuronal densities than sections from older brains.

In another study, Terry et al. (1987) examined 51 brains from individuals who had been carefully screened to ascertain that they were clinically normal: they ranged in age from 24 to 100 years. After examining microscopic sections from several areas of the cortex, Terry et al. concluded that, although neuronal packing density is not changed with age, there was a slight thinning of the cerebral cortex, which might indicate some loss of neurons. But like Haug, Terry et al. concluded that any loss was nowhere near as extensive as had been previously thought and suggested that some the earlier reports of extensive loss of cortical neurons from the normally aging human brain were probably due to the inclusion of brains of some individuals with Alzheimer's disease among the older samples.

NEW STEREOLOGIC METHODS AND THE OUTCOME FROM THEIR USE

Subsequent studies have been much more careful to ensure that brains of Alzheimer's patients are not included among groups of normal old brains and much more attention has been paid to fixation and potential shrinkage of tissue. Methods for counting neurons have also been scrutinized and much more attention paid to them. Attention to counting methods was catalyzed by the publication by Gundersen (Sterio, 1984) of the new, so-called, unbiased method, the disector, with its physical and optical versions. When used in conjunction with systematic random sampling procedures and estimates of the volumes of brain structures under examination, assessments of the total numbers of neurons, rather than densities of neurons, can be obtained (e.g., Coggeshall and Lekan, 1996). Pakkenberg and Gundersen (1997) used this methodology to examine a total of 94 normal human brains, ranging in age from 20 to 90 years, and concluded that there is an overall loss of approximately 9.5% of neurons with age.

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They found no change in packing density of neurons, but reached the conclusion that there is a loss of neurons because, as did Terry et al. (1987), they found some reduction in the overall volume of gray matter with increasing age.

Since then, several other investigators (e.g., see Morrison and Hof, 1997, and Peters et al., 1998, for references) have examined various portions of the neocortex and hippocampus in monkeys and humans, and essentially they all agree that, even if there is some loss of neurons from the cerebral cortex during normal aging, it is nowhere as extensive as earlier studies had depicted. It is also agreed that the loss is probably not of the magnitude that would account for all of the cognitive decline associated with normal aging. Of interest, the first studies of monkey cerebral cortex, which relied upon packing density measures also reported extensive neuronal losses with age (Brizzee, 1973), but again, more recent studies (see Peters et al., 1998) have shown this not to be the case.

The conclusion is that it is doubtful that cortical neuronal loss itself is the basis of the mild cognitive decline that characterizes normal aging. This is not to suggest that neuronal functioning is not compromised, because cortical neurons in primates have been shown to lose portions of their dendritic trees and dendritic spines (e.g., Page et al., 2002) and to show reductions in the levels of some neurotransmitters and their receptors (e.g., Beal et al., 199l; Rosene and Nicholson, 1999). But another explanation for cognitive decline might be the changes that occur with age in white matter, as reported by Marner et al.

MYELINATED NERVE FIBERS AND AGING

Among the earliest clues that myelinated nerve fibers alter with age is the report by Lintl and Braak (1983) that the intensity of myelin staining in the line of Gennari in the human visual cortex decreases with age and the observation of Kemper (1994) that the staining of white matter in old human brains is less intense than in young brains. However, these investigators were not able to determine whether the decrease in staining intensity was produced by a loss of nerve fibers or by alterations in myelin. And as recent evidence suggests, both of these factors might be contributory.

Changes in the structure of myelin with age

In electron photomicroscopic studies of the effects of aging in the cerebral cortex (Peters et al., 2000; Peters and Sethares, 2002), corpus callosum (Peters and Sethares, 2002), and optic nerve (Sandell and Peters, 2001) of monkeys, myelin sheaths have been found to show obvious age-related changes. Two types of alterations, the accumulation of dense cytoplasm in splits between lamellae of the sheaths and the formation of fluid-filled balloons, are considered to be degenerative alterations, and they increase in frequency with age (see Fig. 1). On the other hand, the formation of sheaths with redundant myelin, in which the sheath is too large for the enclosed axon, and the formation of thick sheaths with circumferential splits appear to be due to the continued formation of myelin with age (Peters et al., 2001). But in cerebral cortex, the frequency of these alterations in myelin with age also correlates significantly with the cognitive decline exhibited by monkeys.

Unpublished data from our group (Luebke and Rosene) on conduction across the corpus callosum of normally aging monkeys indicates that, with age, there is a significant reduction in suprathreshold amplitude and an increase in the duration of the compound action potential, even though latency to onset is relatively unaffected. This result could be explained if the myelin dystrophy observed in the corpus callosum (see Peters and Sethares, 2002) disrupts conduction in a fraction of the nerve fibers and suggests that alterations in myelin integrity and consequent disruption of conduction may alter the signal strength and timing that is critical for neuronal circuits to operate properly.

Loss of white matter as shown by stereology

An earlier study by Pakkenberg and Gundersen (1997), by using stereology to examine age changes in human brains, reported that, although the volume of the neocortex decreases by 12%, there is a much larger decline of 28% in the volume of white matter. This study was followed by a preliminary report by Tang et al. (1997), using five young and five old female subjects, which focused on myelinated nerve fibers in the white matter of human cerebral hemispheres. On the basis of these small numbers, they also reported a loss of white matter volume and, by using a newly developed stereologic method, they were able to ascertain that the decrease in volume is associated with a 27% overall loss in the total length of nerve fibers in the white matter. The present article by Marner et al. in this issue of the *Journal of Comparative Neurology* expands that study to include 36 subjects and concludes that the loss of white matter with age amounts to almost 23% and that the overall loss of myelinated nerve fibers length from age 20 to age 80 is 45%. This conclusion is even more striking than the one reached by Tang et al. (1997). Thus, Marner et al. find that males have a total myelinated nerve fiber length of 176,000 km at age 20 and 97,200 km at age 80, whereas in females, which generally have smaller brain volumes, the fiber length is 149,000 km at age 20 and 82,000 km at age 80. This amounts to a loss of fiber length of 10% per decade from age 20 to 80, and Marner et al. suggest that this loss of fiber length could be the basis of some of the cognitive decline in normal aging.

Unfortunately, there are few other studies on humans in which loss of nerve fibers with age have been assessed. However, Meier-Ruge et al. (1992) examined autopsied brains from cognitively intact humans by using semithick sections in which nerve fibers were stained and concluded that there is a 10.5% nerve fiber loss with age from corpus callosum and a 16% loss from the white matter of precentral gyrus.

By using the rhesus monkey as a model of normal aging, we have used design-based stereology to examine nerve fibers loss with age from the optic nerves (Sandell and Peters, 2001). We have found that the average number of nerve fibers in the optic nerves of young, 4- to 10-year-old, sexually mature rhesus monkeys is 1.6×10^6, whereas in old monkeys that are 27–33 years of age, the average number decreases to 9.1×10^5. This finding amounts to an average loss of 45% of the nerve fibers in elderly monkeys, but the optic nerve of one old monkey had as few as 4×10^5 fibers. More recently (Sandell and Peters, unpublished observations), we have been examining the anterior commissure of rhesus monkeys and our preliminary results indicate that approximately 40% of nerve fibers can be lost

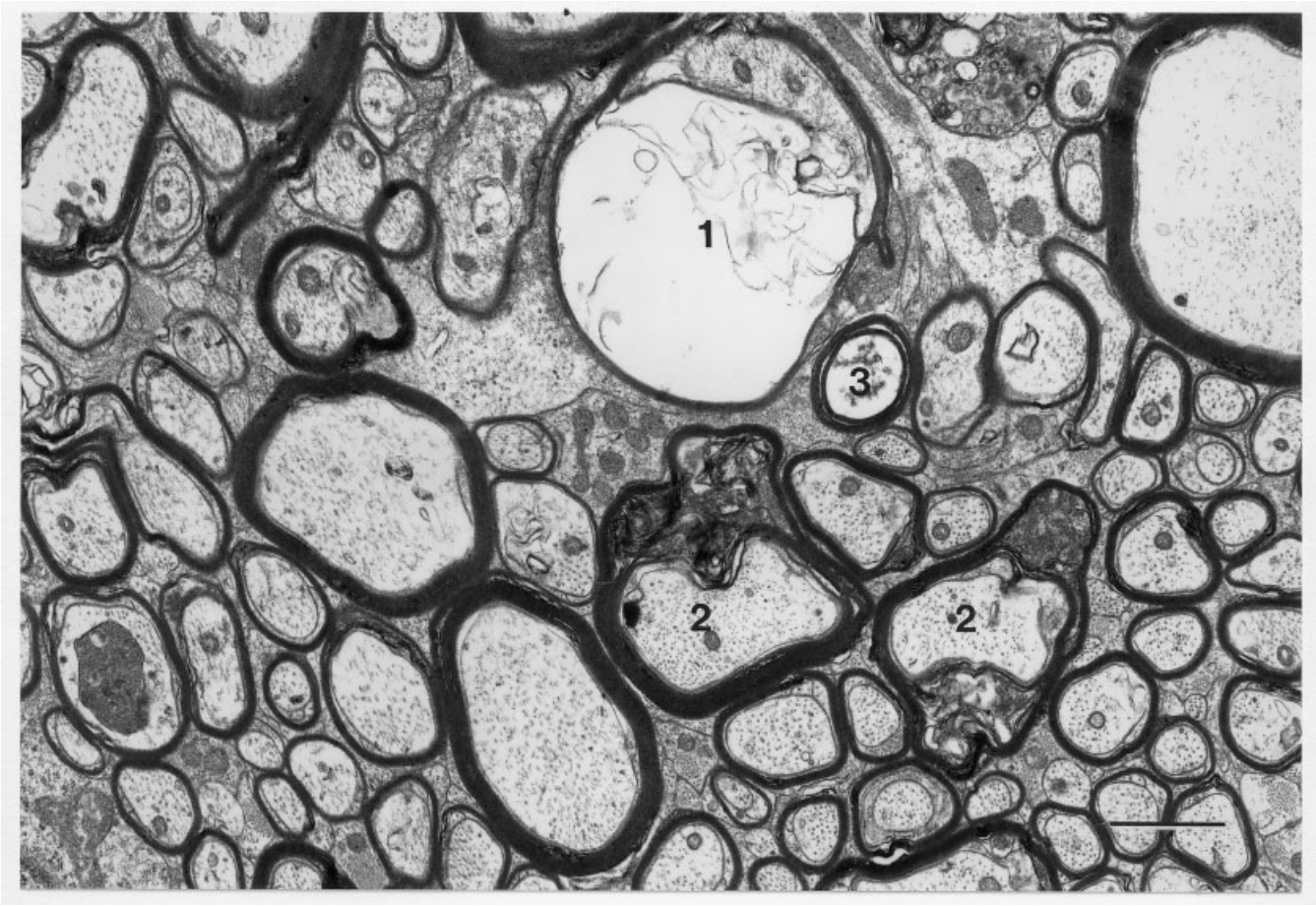

Fig. 1. An electron photomicrograph of nerve fibers from the anterior commissure of a 25-year-old rhesus monkey. Some of the myelinated nerve fibers show age-related changes, such as ballooning of sheaths (1) and sheaths that contain dense cytoplasm between their lamellae (2). In another case (3), the axon has been lost, leaving an empty sheath. Scale bar = 1 μm.

from that bundle with age. In each of these studies, some degenerating myelinated axons or empty myelin sheaths were encountered (Fig. 1).

Consequently, the available stereologic evidence indicates that there is a substantial loss of myelinated nerve fibers from white matter with age. A part of the puzzle that has not been solved is the origin of the myelinated nerve fibers lost from the white matter of the cerebral hemispheres. If there is no substantial loss of neurons from cerebral cortex with age, then it can only be presumed that some of the projection neurons are losing either all or a portion of their axonal plexuses that extend into white matter, while retaining local collaterals that may be sufficient to maintain functional and trophic support of the parent neurons. But there seems no straightforward way to make an assessment of whether this is true.

Loss of white matter as shown by magnetic resonance imaging

The results of magnetic resonance imaging (MRI) studies investigating the question of whether there is a loss of white or gray matter with normal aging are equivocal. There are some studies, such as those by Albert (1993) and Guttmann et al. (1998) on humans, and by Lai et al. (1995) on monkeys, which report a significant loss of white matter volume from primate cerebral hemispheres with age. But there are other studies, such as the one by Andersen et al. (1999) on monkeys, and by Pfefferbaum et al. (1994) on humans, which conclude that gray matter loss exceeds white matter loss. Why such conflicting results have arisen is not clear, but it might be due to a number of factors. First, there is always the difficulty in establishing criteria for where to place the boundary between white and gray matter, because, as is well known, in lower layer 6 of the cortex, there is an intermixing of nerve fibers and neuronal cell bodies, resulting in the tissue being intermediate between white and gray matter. How this boundary appears in any given scanning protocol and what criteria are used for placing the boundary are likely to differ between scans made by different groups. In addition, there is the well known “partial volume” effect, in which a voxel containing a mixture of gray and white matter may image as either white or gray matter, depending upon the scanning protocol used and the exact proportion of white matter in the voxel.

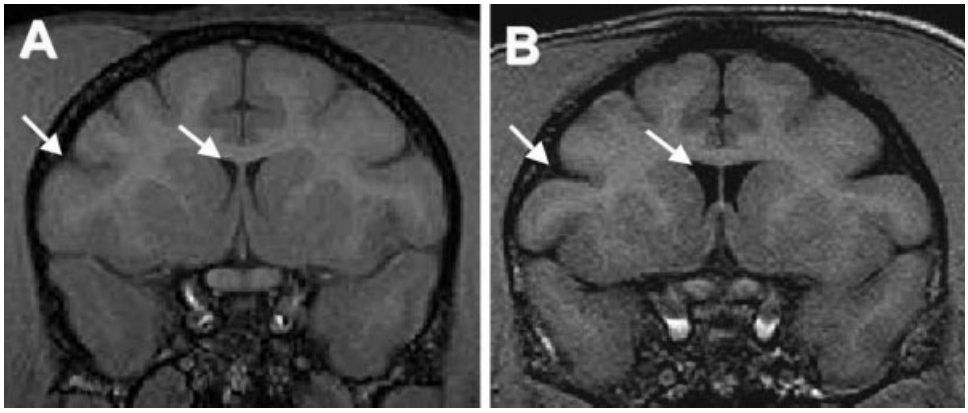

Fig. 2. Representative T2-weighted scans from two rhesus monkeys taken at the level of the temporal pole. **A:** A young monkey (7 years old). **B:** An elderly monkey (30 years old). Note the enlargement of the ventricles and the sulci in B (arrows). Quantitative analysis of segmented images from young and old monkeys indicates that this "gyral" atrophy results from a decrease in white matter volume with a compensatory increase in ventricular volume.

The criteria for setting the boundary can be addressed by examining tissue sections from the same brains that have been scanned, and the partial volume effect can be reduced by decreasing the voxel size. We have attempted to do this by using magnetic resonance imaging (MRI) and morphometric methods to obtain measures of myelin volume in normally aging rhesus monkeys (Wisco, Killiany, and Rosene, unpublished observations). We have scanned young and old monkeys by using a T2-weighted protocol with a voxel size of 0.7 mm and subsequently prepared Nissl-stained histologic sections from these same brains. By comparing the histologic sections with our T2 scans, it has been ascertained that the gray/white border in our MRI slices corresponds to the border identified in Nissl-stained sections of the same brain. As shown in Figure 2, by comparing MRI slices from similar levels of the brains of young and old monkeys, there is an obvious "gyral" atrophy and ventricular hypertrophy in the brains of the aged monkeys. By using our criteria, we segmented the entire forebrain (rostral to the superior colliculus) into gray matter, white matter, and ventricles. Preliminary results indicate that there is no evidence of a loss of gray matter but that there is a significant loss of white matter, with an accompanying increase in ventricular size. These observations lend further support to the notion that there is a little or no loss of neurons with age but that there is likely to be a global loss of myelinated nerve fibers.

White matter alterations with age can also be examined by using other MR methods such as diffusion tensor MRI, which uses diffusional anisotropy as a marker of white matter integrity. By using this technique, O'Sullivan et al. (2001) have shown that white matter disruption occurs with age. It is maximal in the frontal lobes, and O'Sullivan et al. (2001) suggest that the disruption could be due to loss of nerve fibers. Other MRI studies have examined the frequency of small white matter lesions with age. For example, De Groot et al. (2000) report that small lesions of periventricular white matter are quite common, and they conclude that such lesions could play a dominant role in bringing about cognitive decline, because in this locus, they would be likely to interrupt long corticocortical fiber tracts. In both cases, these changes in white matter could result in a disconnection syndrome and contribute to the cognitive decline in normal aging.

CONCLUSION

The article by Marner et al. in this issue of *Journal of Comparative Neurology* serves to emphasize the loss of myelinated nerve fibers from white matter as a potential contributor to the cognitive decline associated with normal aging. But not only is there a loss of nerve fibers, there are alterations in their myelin sheaths with age. This has been shown by changes in the staining properties of myelin and by electron microscopic studies that show the integrity of myelin sheaths is disrupted with age. Although MRI analyses do not all agree about whether there is a reduction of white matter volume in the forebrain with age, alterations in white matter are evident from diffusion tensor MRI imaging, and other MRI imaging indicates that, in humans, small lesions in white matter become more frequent with increasing age. The changes in myelin would result in alterations in conduction along affected nerve fibers, as we have seen and as has been reported in several systems (e.g., Aston-Jones et al., 1985; Morales et al., 1987; Xi et al., 1999). Overall, breakdown of myelin sheaths would cause disruption of conduction along nerve fibers and loss of nerve fibers would result in a reduction in the connectivity between parts of the brain. The data provided by Marner et al. adds further support to the increasing evidence that changes in myelinated nerve fibers are a major feature of normal aging and could contribute significantly to the cognitive decline normally experienced in the elderly. Clearly more attention needs to be paid to the mechanisms contributing to these age-related changes in myelinated nerve fibers. If alterations in myelin and myelinated nerve fibers can be alleviated, some of the cognitive decline associated with normal aging might be retarded.

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