J_ID: Z3O Customer A_ID: 11-0252.R1 Cadmus Art: Z3O-22809 Date: 16-November-11 Stage: Page: 1

RESEARCH ARTICLE

Age-Related Changes in the Morphology of Cerebral Capillaries Do Not Correlate with Cognitive Decline

Alan Peters* and Claire Sethares

Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts 02118

ABSTRACT

The effects of age on cerebral capillaries have been examined in area 46 of the prefrontal cortices of sixteen rhesus monkeys, ranging in age from 5 to 35 years. Fourteen of the monkeys had been behaviorally tested prior to their brains being prepared for electron microscopic examination. It was found that whereas the thickness of the outer basal lamina adjacent to the glial limiting membrane increased with age and showed increasing numbers of splits, the inner basal lamina between endothelial cells and pericytes did not become thicker with age, and did not show splitting. There were also no age-related changes in the extent of the coverage of endothelial cells by pericytes and no change in the frequency of mitochondria in endothelial cells. The factors that did change with age, namely, the thickness of the outer basal lamina and the increased numbers of splits in this lamina showed no correlations with the cognitive status of the monkeys, suggesting that thickening of the outer basal lamina does not contribute to cognitive decline. J. Comp. Neurol. 000:000–000, 2011.

INDEXING TERMS: rhesus monkey; aging; cerebral cortex; cognitive decline; capillaries

The potential factors underlying cognitive decline with age are numerous and include loss of some nerve fibers, breakdown of myelin sheaths (Peters, 2009), and loss of synapses (Peters et al., 2008). An important role in cognitive decline has also been attributed to age changes in the capillaries of the microvasculature, because the microvasculature is the site of the blood–brain barrier, which controls the passage of metabolites and other substances from the blood to the neurons in the cerebral cortex and other brain structures (Shah and Mooradian, 1997; Farkas and Luiten, 2001). In the cerebrum, endothelial cells are apposed to each other by tight junctions (zonulae occludentes), which seal the spaces between adjacent endothelial cells, so that metabolites cannot pass between endothelial cells. Consequently metabolites have to be transported through the walls of capillaries, where the endothelial cells can control what substances pass through them, and this is the basis of the blood–brain barrier.

The endothelium readily allows water, oxygen, and nutrients to pass through its walls, but its barrier properties retard the passage of some other compounds that may be harmful to the neurons. However, having passed through the endothelium of the capillaries, the nutrients have then to diffuse through the basal lamina that surrounds the endothelial cells and separates them from the end feet of astrocytes. Presumably, nutrients also have to negotiate the pericytes that occupy splits of the basal lamina and can cover much of the outer surfaces of capillaries; in addtion, as has been demonstrated recently, the pericytes appear to have a role in regulating the blood–brain barrier (Armulik et al., 2010). It is the thickening of the basal lamina that is the most commonly reported age-related change in capillaries in rats (Knox and Oliviera, 1980; Heinsen and Heinsen, 1983; Hicks et al., 1983; Topple et al., 1990), non-human primates (Burns et al., 1979, 1981; Honavar and Lantos, 1987; Keuker et al., 2000), and humans (Kalaria, 1996).

The subjects in the present study are rhesus monkeys. They live for some 35 years (Tigges et al., 1988) and because they do not develop neurofibrillary tangles and have few senile plaques, they do not suffer from Alzheimer's disease (Peters et al., 1996). However, like humans, rhesus monkeys exhibit cognitive decline with age and because their cognitive status can be determined before the brain is examined, it is possible to ascertain which morphological factors do, or do not, correlate with

Grant sponsor: National Institutes of Health, National Institute on Aging; Grant number: P01-AG0000001.

*CORRESPONDENCE TO: Alan Peters, Ph.D., Department of Anatomy and Neurobiology, Boston University School of Medicine, 72 East Concord Street, Boston 02118. E-mail: valan@bu.edu

Received August 1, 2011; Revised October 19, 2011; Accepted October 25, 2011

DOI 10.1002/cne.22809

Published online Month 00, 2011 in Wiley Online Library (wileyonlinelibrary.com)

ID: guganp | Black Lining: [ON] | Time: 18:43 | Path: N:/3b2/CNE#/Vol00000/110250/APPFile/JW-CNE#110250

TABLE 1.

Thicknesses of Basal Laminae

			Outer basal lamina			Inner basal lamina		
Animal	Age	CII	Mean thickness (μm)	Count	SEM	Mean thickness (μm)	Count	SEM
AM16	5		0.067	10	0.004	0.032	9	0.001
AM77	6	2.27	0.060	10	0.002	0.031	10	0.002
AM76	6	0.08	0.075	10	0.003	0.032	9	0.003
AM47	9	−0.51	0.067	10	0.003	0.025	9	0.002
AM96	9	2.12	0.084	10	0.003	0.043	9	0.001
AM221	18	2.71	0.084	11	0.005	0.034	9	0.002
AM209	19	0.75	0.090	10	0.005	0.036	10	0.001
AM100	25	3.59	0.092	10	0.005	0.030	10	0.004
AM12	27	3.31	0.103	8	0.007	0.049	8	0.003
AM15	27	1.76	0.084	9	0.004	0.043	9	0.004
AM62	27	3.81	0.080	11	0.006	0.035	11	0.002
AM27	28	1.24	0.078	10	0.004	0.034	10	0.003
AM26	29	1.05	0.104	10	0.005	0.029	10	0.002
AM41	32	4.51	0.089	10	0.004	0.033	10	0.003
AM91	32		0.070	9	0.005	0.023	9	0.001
AM13	35		0.096	10	0.004	0.044	10	0.008

normal cognitive decline. For these reasons rhesus monkeys provide an excellent model in which to study the effects of normal aging.

The point of the present study is to determine how the morphology of capillaries in area 46 of prefrontal cortex changes with age, and to ascertain whether any of the age-related changes correlate with cognitive decline. The prefrontal cortex was chosen for this study in light of the popular theory that frontal lobe impairment is one basis for cognitive decline in aging (Peters et al., 1994; West, 1996; Pugh and Lipsitz, 2002; Luebke et al., 2010). To this end we have determined the extent to which the basal lamina thickens with age, whether the extent of the covering of capillaries by pericytes and the frequency of mitochondria in endothelial cells alters with age, and whether any of these age-related changes correlate with the cognitive impairment shown by the monkeys. We have also examined age-related changes in the morphology of endothelial cells and pericytes.

MATERIALS AND METHODS

Tissue specimens and preparation

Sixteen rhesus monkeys (*Macaca mulatta*) were used in this study, and 14 of them had been behaviorally tested (Table 1). Five of the monkeys were young (5–10 years old), two were middle-aged (10–20 years of age), and nine were old (over 25 years of age). None of the monkeys had hypertension or suffered from diabetes.

Details of how the brains were fixed for morphological examination are given in Peters et al. (1994). Briefly a monkey was preanesthetized with ketamine (0.5 mg/kg) after which sodium barbitol was administered i.v. (15 mg/kg to effect) until a state of areflexia was achieved. The monkey was then intubated and artificially respired with a mixture of 5% CO_2 and 95% O_2. The chest was opened, and the monkey was perfused intracardially with a warm solution of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 cacodylate or phosphate buffer at pH 7.4. After the brain had been removed, one hemisphere, which was to be used for the present study, was transferred to a cold solution of 2.5% glutaraldehyde and 2% paraformaldehyde in the same buffer as used for the perfusion. The perfusions were carried out in full accordance 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 number of monkeys used and their suffering.

Several pieces of cortex were taken from the lower bank of the inside of the principal sulcus of the forebrain at the level of the rostral end of the corpus callosum of each monkey. This portion of the cortex is part of area 46. The pieces of cortex were then osmicated, dehydrated, stained en bloc with uranyl acetate, and embedded in resin.

Preparation of sections

One or two plastic-embedded blocks of area 46 were selected at random from each monkey and first sectioned at right angles to the surface of the cortex, so that the long axes of the apical dendrites of the pyramidal cells were evident by light microscopy in 1-μm sections stained with toluidine blue. Layer 4 was identified, and the blocks of tissue were reoriented so that sections could be taken parallel to the surface of the cortex. Each block was then sectioned until the depth of layer 4 was reached. At this level and plane most of the capillaries are in cross section.

ID: guganp | Black Lining: [ON] | Time: 18:43 | Path: N:/3b2/CNE#/Vol00000/110250/APPFile/JW-CNE#110250

Thin sections were then taken, mounted on copper grids, stained with lead citrate, and examined by using a JEOL (Peabody, MA) 100S electron microscope.

At least 15 electron micrographs of cross-sectioned capillaries were taken from layer 4 of each monkey. The micrographs were taken at a magnification of ×6,000 with care being taken to ensure that the entire profile of the capillary was included in the frame. This was to ensure that the entire perimeter of each capillary could be subsequently examined. Consequently each of the capillaries photographed had a lumen with a diameter of about 10 μm. The negatives were then scanned into an Epson Perfection V700photoscanner by using Epson (Long Beach, CA) plug-in for Photoshop CS3 (Adobe Systems, San Jose, CA) and saved as 150-dpi tif files. Adjustments were made to the scanned images to enhance contrast and evenness. Photographic prints were made from the scanned images of the capillaries. The prints were made at a magnification of ×12,500 by using a Kodak (Rochester, NY) Professional 9810 Digital Photo Printer.

Analysis of images of capillaries

The scanned images of capillaries were opened in ImageJ and analyzed by using a version of the radial grid plug-in program (A. Baker, Radial Grid, http://rsb.info/nih.gov/ij/plugins/radial-grid.html) modified by Chad Farris. Because the micrographs of the capillaries had been taken at a magnification of ×6,000 and scanned at a resolution of 800 pixels per inch, the scale for measurements using ImageJ was set such that there were 188.97 pixels per micrometer. The cross-hair tool was then used to mark the center of each capillary, and a radial grid with 12 radii or spokes, evenly spaced 30 degrees apart and centered on the cross hairs, was superimposed on the image of the capillary. By using the line tool, the width (or thickness) of the basal lamina at the sites where each of the 12 spokes of the radial grid crossed the basal lamina was measured, and the value was stored.

For the purposes of description, it needs to be pointed out that there is a thick basal lamina around a capillary and it surrounds the outsides of endothelial cells and pericytes where they are opposed by astrocytic end feet. This thick basal lamina will be referred to as the *outer basal lamina* (O in Fig. 1). However, where pericytes are present they are embedded in the basal lamina, so that the outer basal lamina passes on the outside of the pericyte (P in Fig.1), whereas a thinner basal lamina, which will be referred to as the *inner basal lamina* (I in Fig. 1), extends between the pericyte and the endothelial cell. The thicknesses of the outer and inner basal laminae were only measured where the limits of the basal laminae were clearly defined and had not been obliquely sectioned, and where the outer basal lamina did not show splitting (asterisks in Fig. 2). Eight to ten scanned images of different capillaries were analyzed for each monkey in the study, and so this approach resulted in about 75–100 measurements of outer basal lamina thickness and 20–30 measurements of the inner basal lamina thickness from the capillaries of each monkey.

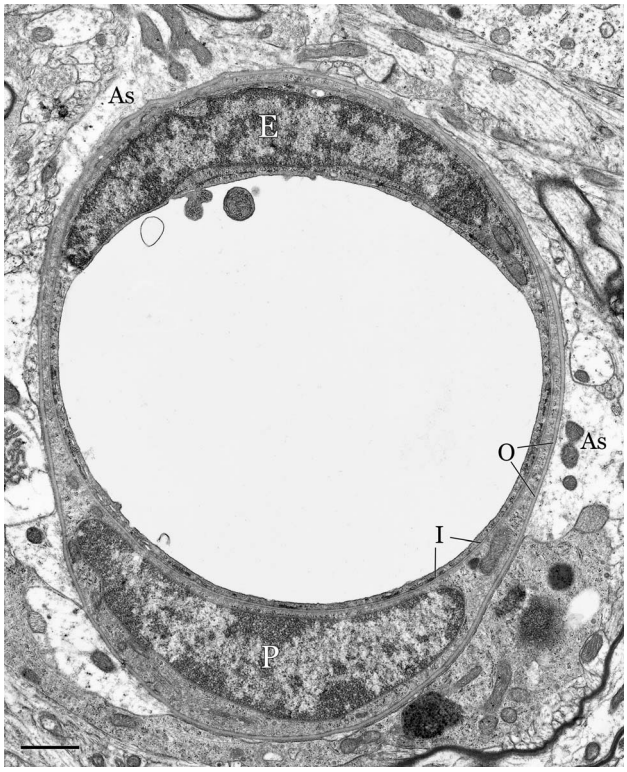

Figure 1. A capillary from a 6-year-old monkey. The capillary wall is formed by an endothelial cell (E) that is separated from astrocyte processes (As) by an outer basal lamina (O). This basal lamina splits to accommodate a pericyte (P), which is separated from the endothelial cell by a thin inner basal lamina (I). Scale bar = 1 μm.

After the thickness of the basal laminae was measured, the number of radii in the radial grid was doubled to 24, and an analysis was carried out to determine how many spokes in the grid intersected pericytes and what was the frequency of locations where the outer basal lamina was split (Figs. 2, 3). For each capillary profile, the number of spokes intersecting pericytes and splits was then divided by 24 to give the percentage of the capillary circumference occupied by these two entities.

The plots in Figures 5 and 6 show the means of these values and the standard errors of the means.

To determine whether there is a change in the frequency of endothelial mitochondria with age, the profiles of 10 capillaries each from five young (AM 16, AM 47, AM 76, AM 77, and AM 96) and five old (AM 12, AM 13, AM, 15, AM 26, and AM 27) monkeys were examined, and the

ID: guganp | Black Lining: [ON] | Time: 18:43 | Path: N:/3b2/CNE#/Vol00000/110250/APPFile/JW-CNE#110250

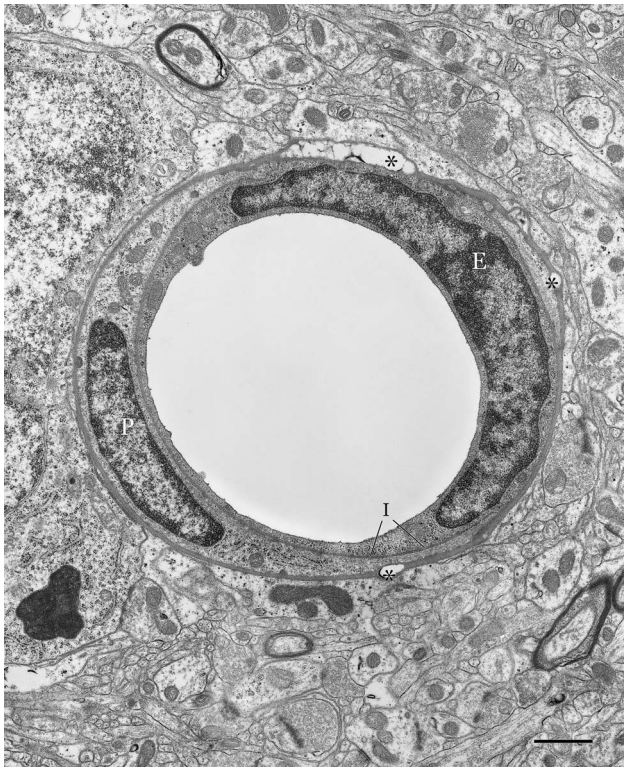

Figure 2. A capillary from a 27-year-old monkey. The capillary wall is formed by an endothelial cell (E) with a rather irregular nucleus. The endothelial cell is partially surrounded by a pericyte (P), and the two cells are separated from each other by a thin inner basal lamina (I). In this old monkey the outer basal lamina has a number of splits (asterisks), which are common in older monkeys. Scale bar = 1 μm.

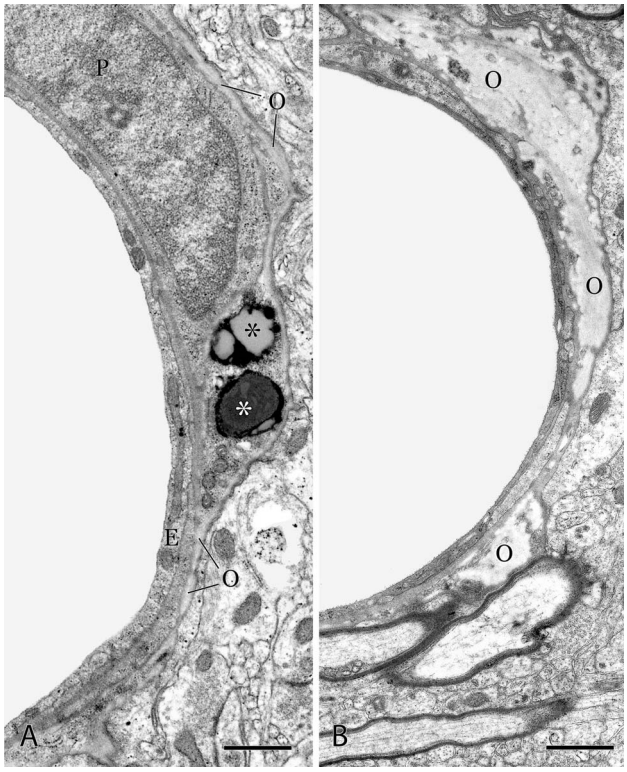

Figure 3. A: The wall of a capillary from a 27-year-old monkey. The outer basal lamina (O) that surrounds the endothelial cell (E) and the pericyte (P) is thick and contains some collagen fibers. Note the thin inner basal lamina (I) and the inclusions (asterisks) in the pericyte. B: The wall of a capillary from a 32- year-old monkey. The outer basal lamina (O) has become very thick and contains collagen fibers. Scale bar = 1 μm in A,B.

numbers of profiles of mitochondria within the endothelial cells were recorded. The values for the numbers of profiles of mitochondria in endothelial cells in the young and the old monkeys were pooled, and a mean value was obtained. This method does not give real numbers of mitochondria in endothelial cells, but it does provide an indication of whether there is a change in the frequency of endothelial mitochondria with age.

Behavioral testing

Of the 16 monkeys used in this study, 14 had been behaviorally tested. The behavioral tests used to assess the cognitive status of these monkeys have been described in earlier publications (Herndon et al., 1997; Killiany et al., 2000; Moss et al. 1999; Peters et al., 1996, 2000). Assessment of cognitive status is made on the basis of three visual recognition tasks; the delayed nonmatching to sample (DNMS) task, which is a test of rule learning; a DNMS task with a 2-minute delay, which is a test of short-term memory; and a delayed recognition memory span task (DRST), which is a test of working memory load in which a subject must identify the new object from an increasing number of familiar objects. From the combined scores achieved on these tasks, an overall measure of cognitive impairment, the Cognitive Impairment Index (CII) is derived (Peters et al., 2001). Essentially the higher the CII score, the more a monkey is cognitively impaired. In general, monkeys with scores lower than 1.5 are considered to be nonimpaired, monkeys with 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.

RESULTS

General description

The components of capillaries in the cerebral cortex are shown in Figures 1 and 2.

The walls of the capillaries are formed by endothelial cells, which are quite thin except where their nuclei occur, and to ensure a complete seal to the wall of the capillary, the processes of endothelial cells form tight junctions where they abut. These junctions are generally only discernible at high magnifications. In cross sections of capillaries the profiles of the endothelial cell nuclei are

crescent-shaped, and although they usually have smooth outlines, it is not uncommon for the nuclear envelope to have irregular contours. The heterochromatin of the nucleus is pronounced, and it forms a dense layer beneath the nuclear envelope as well as forming clumps throughout the nucleoplasm, giving the nucleus a mottled appearance. The cytoplasm of endothelial cells is also dark and contains ribosomes and a few mitochondria, but, unlike endothelial cells in other tissues, those in the cerebral cortex show few pinocytotic vesicles.

Surrounding the outer surfaces of endothelial cells is the thick outer basal lamina, which separates the endothelial cells and pericytes from the processes or end feet of astrocytes that completely surround the capillaries and form the glial limiting membrane. From a number of studies it is known that the thick outer basal lamina has three layers (Farkas and Luiten, 2001). There is a lamina rara externa, which is formed by the surrounding astrocytes, and a lamina rara interna, which is formed by the endothelial cells. Both of these laminae contain laminin, fibronectin, and heparan sulfate proteoglycan and between them is a lamina densa that contains intrinsic collagen type IV. Normally these three layers of the basal lamina are not evident as separate entities in micrographs of mature capillaries, but in both young and old monkeys the basal lamina can show partially splitting at the lamina densa. Sometimes the splits appear to be empty (Fig. 2), but in other cases the splits can be seen to contain collagen fibers (Fig. 3B). It should also be mentioned that sometimes the lamina rara externa of the outer basal lamina produces folds, and in sections these folds appear as finger-like processes that push into the surrounding astrocytic end feet (Fig. 3A).

Pericytes and their processes are enclosed within the basal lamina (Figs. 1, 2). To accommodate the pericytes the basal lamina splits, so that an outer layer (O in Fig. 1), which is similar in thickness to the outer basal lamina elsewhere, separates the pericytes from the astrocytic end feet, and an inner, thinner basal lamina (I in Fig. 1), separates the outer surface of the endothelial cells from the endothelial cells. As pointed out above, for the purposes of description the thicker basal lamina that separates the endothelial cells and pericytes from the astrocytic end feet will be referred to as the *outer basal lamina*, whereas the much thinner basal lamina that separates pericytes from the outer surfaces of the endothelial cells will be referred to as the *inner basal lamina*.

There appear to be two types of pericytes, granular and filamentous (Thomas, 1999). Both types have elongated nuclei with dark chromatin, so that their general appearance is very similar to that of the endothelial cells nuclei. The difference in the two types of pericytes is in the density of the cytoplasm. The granular pericyte has rather dense cytoplasm similar in appearance to the cytoplasm of the endothelial cells (P in Fig. 1), whereas the filamentous type has a paler cytoplasm (P in Figs. 2, 4). F4
Both types of pericytes can occur in monkeys of all ages, and so the difference between the two types of pericytes is not age related. One factor that is age related is the presence of lysosomes and inclusions within pericytes. The inclusions become more common with age, and their contents have a variety of appearances (Fig. 3A, asterisks). Some inclusions have a homogeneous electron density, and others have electron-lucent contents, but most of the inclusions have pale areas surrounded by electron-dense material. The origins of the inclusions appear not to be known, although it is generally assumed that they are phagocytic in origin.

With increasing age endothelial cells sometimes show vacuoles and small structural deficits in their walls, but they rarely show inclusions in their cytoplasm. However, as shown in Figure 4, occasionally in old monkeys the endothelial cells appear to be breaking down because the cytoplasm shows irregular vacuolation (Fig. 4, asterisks). However, it should be emphasized that such alterations in endothelial cells are not common.

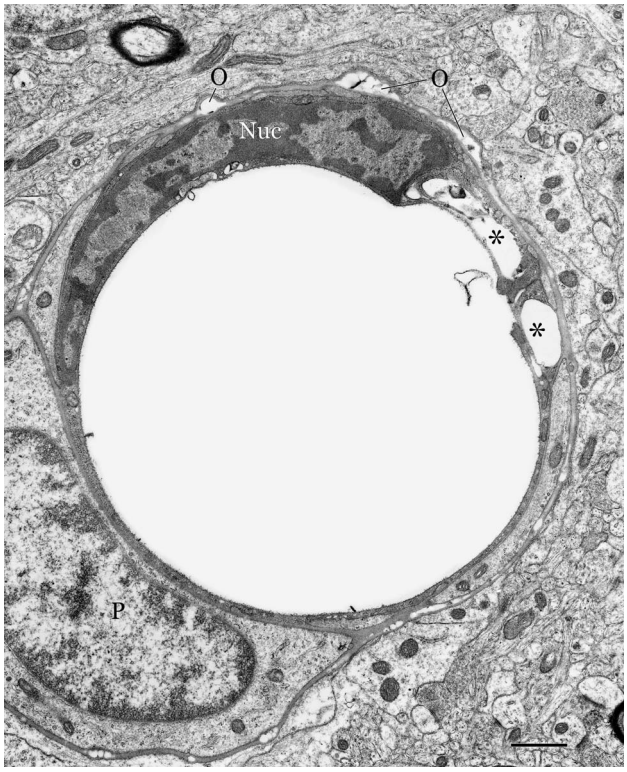

Figure 4. A capillary from a 32-year-old monkey. In this capillary the endothelial cell appear to be damaged, because the chromatin in the nucleus (Nuc) of the endothelial cell is clumped and the cytoplasm has become vacuolated (asterisks). Note the split outer basal lamina (O) and the pale pericyte (P). Scale bar = 1 μm.

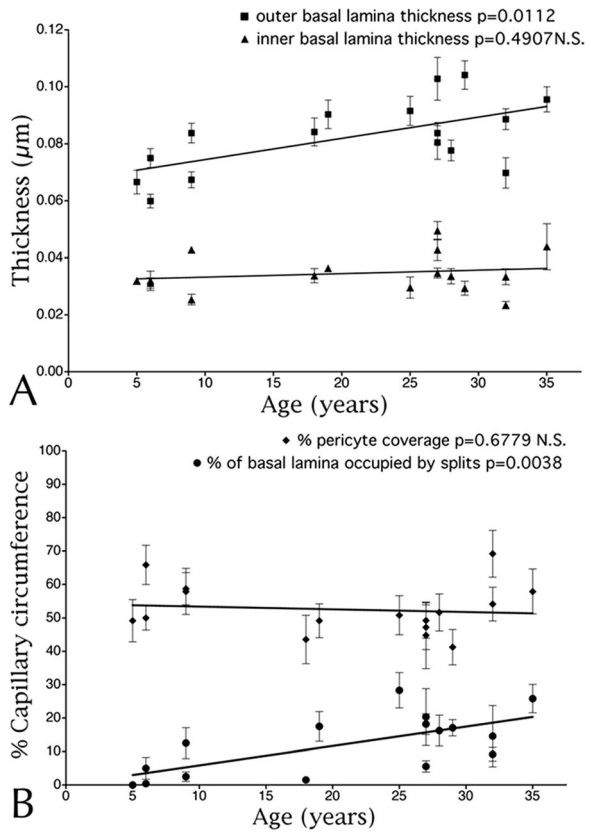

Figure 5. A: Plot of the thicknesses of the inner and outer basal laminae against age. B: Plot of age versus the percentage of the capillary surface occupied by pericytes and the percentage of the outer basal lamina occupied by splits.

Quantitative results

Outer basal lamina

Changes in the thickness of the intact outer basal lamina with age are shown in Table 1 and Figure 5. These values only apply to locations in which the outer basal lamina is intact and not split. In young monkeys, aged under 10 years of age, the mean thickness of the basal lamina is 0.071 μm, whereas in monkeys over 25 years of age the mean thickness increases by about 20% to a value of 0.087 μm, and as shown in Figure 5A the increase in the thickness of the outer basal lamina with age is significant ($P = 0.0112$). However, there is a great deal of variation in outer basal lamina thickness, so that in some of the older monkeys, such as AM 27 (28 years old) and AM 91 (32 years old), the intact outer basal lamina is no thicker than in young monkeys.

Inner basal lamina

The inner basal lamina between pericytes and endothelial cells is about half the thickness of the outer one (Table 1). It has a mean thickness of only 0.032 μm in young monkeys, and as shown in Figure 5A the thickness of the inner basal lamina does not change significantly with age ($P = 0.4907$). Moreover, the inner basal lamina shows no signs of splitting with age.

Splits in the outer basal lamina

As is evident in Table 2 and Figure 5B, although they can occur, splits in the outer basal lamina are not common in young monkeys, so that there is a large range in the frequency of splits, as indicated by the large SEM values. Splits begin to be common in middle age, and in old monkeys over 25 years of age splits are so frequent that almost every capillary profile shows some splitting of the outer basal lamina, and on average splits occupy between 15 and 20% of the outer basal lamina. As shown in Figure 5B, this increase in the frequency of splits is significantly correlated with increasing age ($P = 0.0038$).

Pericyte coverage

As shown in Table 2 and Figure 5B, the cell bodies and processes of pericytes cover some 50% of the outer surface of endothelial cells, and there is no significant change in this coverage with increasing age ($P = 0.6779$).

Frequency of endothelial cell mitochondria with age

The total number of profiles of mitochondria evident within endothelial cells in cross-sectioned capillaries was determined by examining images of 50 capillaries in five young and five old monkeys. In the capillaries of young monkeys, a mean of 3.3 $\pm$ 2.2 mitochondrial profiles is evident in the endothelial cells, and a mean of 3.8 $\pm$ 2.5 profiles in the endothelial cells from old monkeys, leading to the conclusion that there is not a significant increase in the frequency of endothelial cell mitochondria with age.

Correlations with cognitive impairment

The CIIs of 14 of the monkeys used in this study are given in Tables 1 and 2. A plot of CII against the thicknesses of the outer and inner basal laminae is shown in Figure 6A, and it is evident that the thicknesses of neither the outer nor the inner basal laminae correlate with cognitive decline. Furthermore, no correlations were found between the thicknesses of the basal laminae or any of the individual scores on the three tests that are used to generate the CII. Figure 6B shows that there is no correlation between the CII values and either the frequency of splits in the outer basal lamina or the extent of coverage of endothelial cells by pericytes, and again there are no correlations between these morphological changes and the scores on the three individual behavioral tasks used to generate the CII values.

TABLE 2.

Coverage by Pericytes and Splits

			Mean coverage by pericytes			Mean coverage by splits		
Animal	Age	CII	Mean	Count	SEM	Mean	Count	SEM
AM16	5		49.2%	10	6.3%	0.0%	10	0.00%
AM77	6	2.27	50.0%	10	3.6%	0.4%	10	0.42%
AM76	6	0.08	65.8%	10	5.9%	5.0%	10	3.27%
AM47	9	−0.51	57.9%	10	6.9%	2.5%	10	1.42%
AM96	9	2.12	58.8%	10	4.7%	12.5%	10	4.61%
AM221	18	2.71	43.6%	11	7.3%	1.5%	11	1.02%
AM209	19	0.75	49.2%	10	5.1%	17.5%	10	4.43%
AM100	25	3.59	50.8%	10	5.9%	28.3%	10	5.30%
AM12	27	3.31	44.8%	8	10.0%	20.3%	8	8.50%
AM15	27	1.76	47.2%	9	6.7%	5.6%	9	1.70%
AM62	27	3.81	49.2%	11	5.3%	18.2%	11	3.09%
Am27	28	1.24	51.7%	10	5.5%	16.3%	10	4.63%
AM26	29	1.05	41.3%	10	5.3%	17.1%	10	2.44%
AM41	32	4.51	54.2%	10	5.0%	9.2%	10	2.04%
AM91	32		69.2%	10	7.0%	14.6%	10	9.16%
AM13	35		57.9%	10	6.7%	25.8%	10	4.25%

DISCUSSION

In summary, this analysis of the effects of age on the capillaries in area 46 of the prefrontal cortex of the rhesus monkeys shows that whereas the thickness of the outer basal lamina, and the frequency of splits in this lamina, increase significantly with age, there is no change in the thickness of the inner basal lamina, or in the coverage of endothelial cells by pericytes. However, neither the thickness of the outer basal lamina nor the frequency of splits in this lamina correlates with cognitive decline shown by aging monkeys. This finding suggests that these normal age-related changes in the structure of cerebral capillaries do not contribute significantly to cognitive decline.

The thickening of the outer basal lamina with age, described here, agrees with observations of others on rats and non-human primates. In rats, in which most of the studies have been carried out, the basal lamina has been shown to increase in thickness during postnatal development and to continue to thicken into old age (Caley and Maxwell, 1970; Barr, 1978; Heinsen and Heinsen, 1983; Hicks et al., 1983; Topple et al., 1990; Alba et al., 2004). No previous studies on immature monkeys appear to have been carried out, but in agreement with the data shown here, others have recorded that in mature non-human primates the basal lamina thickens with age, not only in the neocortices, but also in the hippocampus (Burns et al., 1979; Honavar and Lantos, 1987; Keuker et al., 2000). However, in contrast to studies that show a progressive thickening of the outer basal lamina throughout life, Burns et al. (1981) found that the basal lamina in the frontal cortex of *Macaca nemestrina* thickened only between 4 and 10 years of age and then stabilized. In humans it has also been shown that the basal lamina becomes thicker with age (Perlmutter and Chui, 1990; Farkas et al., 2000).

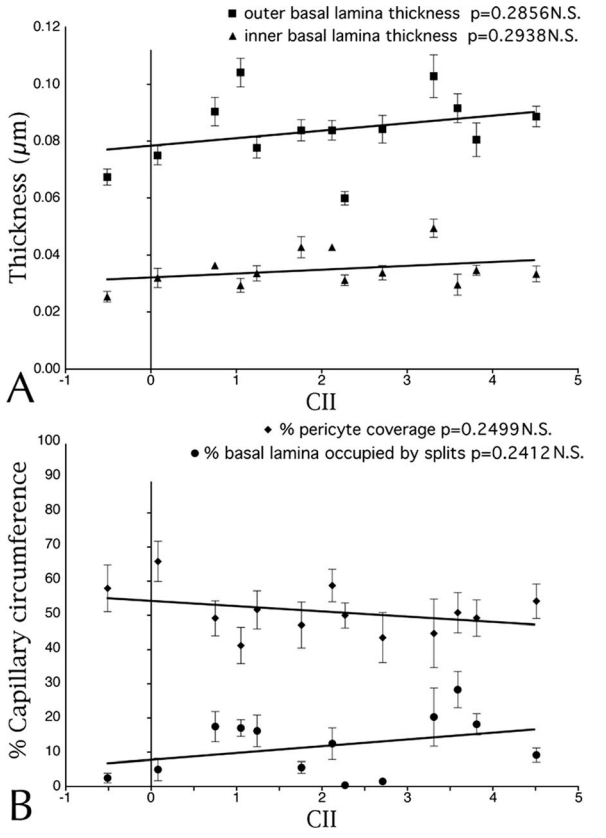

Figure 6. A: Plot of the thicknesses of the inner and outer basal laminae against the cognitive impairment index (CII) scores achieved by individual monkeys. B: Plot of cognitive impairment index (CII) versus the percentage coverage of capillary profiles by pericytes, and the percentage of the outer basal occupied by splits.

The present analysis shows no correlation between increasing outer basal lamina thickness and a decline in cognition, and this is consistent with the fact that the

barrier properties of capillaries appear to remain intact in older animals (Shah and Mooradian, 1997). However, it is interesting to note that the basal lamina becomes increasingly thickened in hypertension and in Alzheimer's disease, and in these cases it has been postulated that the thickening affects the blood–brain barrier and contributes to cognitive decline by perhaps interfering with nutrient and electrolyte transport (Farkas and Luiten, 2001).

As demonstrated here (Fig. 5) and in a number of previous studies, not only does the outer basal lamina become thicker with age, but it also shows an increased splitting of the lamina densa. The literature on this splitting has been reviewed by Farkas et al. (2001), who have shown that the splitting is accentuated by hypertension. The cause of the splitting appears not to be known, but Perlmutter and Chui (1990) have suggested that the formation of splits may be due to a breakdown of the basal lamina components, together with an increased production of some components, especially type IV collagen, which occupies much of the splits in the lamina densa. However, not only does the outer basal lamina split, the thickening of the lamina is also accompanied by some folding of the lamina rara externa adjacent to the astrocytic end feet, so that in thin sections finger-like profiles of the basal lamina are often seen pushing into the end feet.

As in the present study, Perlmutter and Chui (1990) made the observation that unlike the outer one, the inner basal lamina does not split during aging. Also, interestingly, as shown in the present study, not only does the inner basal lamina not split, it does not thicken with age. In their study of capillaries in the hippocampus of the rhesus monkey, Keuker et al. (2000) state that they found splits in the inner basal lamina with age. However, their tissue was fixed by immersion, and the rather poor preservation may have led to fixation artifacts.

Although no systematic studies appear to have been carried out on age changes in the capillaries of normally aging human brains, it is well known that abnormalities in brain vasculature, such as thickening of the basal lamina and increased frequency of inclusions in pericytes, are accentuated by diseases such as hypertension and type II diabetes (Schwartz et al., 2010; Dickstein et al., 2010). These diseases are risk factors for Alzheimer's disease, in which the brains show similar increases in the frequency and extent of vascular abnormalities (Buée et al., 1994; Farkas and Luiten, 2001).

In the present study the thickness of the endothelial cell walls and the diameters of the lumens of the capillaries have not been measured because these measures probably depend on whether the capillaries are distended during the perfusion with fixatives. This might be the reason why reports of capillary diameter have been inconsistent. Thus capillary diameter has been reported to be unchanged with age in monkey frontal and occipital cortices (Burns et al., 1979), decreased or increased in rat cortex (Barr, 1978; Hicks et al., 1983), and increased in human precentral gyrus (Hunziker et al., 1979). Also, a number a studies have made comments that the endothelial cell walls become thinner with age (Burns et al., 1979; Mooradian, 1988; Alba et al., 2004), and it has been considered that such a thinning might bring about a weakening of the endothelial barrier system (Topple et al., 1991). Also, as reported here, sometimes the endothelial cells show vacuolation with age (Fig. 4), which may also lead to a weakening of the blood–brain barrier.

Although no change in the frequency of capillary mitochondria has been found in the present study, Burns et al. (1979) have reported that in the frontal and occipital cortices of *Macaca nemestrina* there is a decline in the numbers of endothelial mitochondria with increasing age, and Burns et al. (1981) suggest that it is thinning of the endothelial cell walls together with a decrease in the numbers of mitochondria that interferes with various regulatory mechanisms such as the ability of the blood–brain barrier to maintain normal ionic differentials between blood and brain extracellular fluids. However, as in the present study, Hicks et al. (1983) found no age-related difference in the numbers of mitochondria in endothelial cells in the frontal cortex and hippocampus of the rat.

Pericytes can act as phagocytes (Peters et al., 1991; Thomas, 1999), and because they contain contractile proteins they may also be able to control blood flow in capillaries (Bandopadhyay et al., 2001; Peppiatt et al., 2006; Dalkara et al., 2011). Also, as recently reported, pericytes may play a role in regulating the blood–brain barrier (Armulik et al., 2010). From the present study it is concluded that the coverage of endothelial cells by pericytes does not change with age, the coverage remaining at an average value of about 50%. This is in agreement with the observations made by Burns et al. (1981), who also found that the pericyte coverage did not change with age in either the frontal cortex of *Macaca nemestrima* or in the frontal cortex of the aging rat. Similarly, Alba et al. (2004) found no significant change in pericytes in the lateral geniculate nucleus of the aging rat. Consequently, it appears that the major age change in pericytes is in the increased number of inclusions in their cytoplasm.

The main conclusion from the present study is that although there are some age-related alterations in the morphology of cerebral capillaries in rhesus monkeys, none of the alterations appear to be the underlying cause of cognitive decline.

ACKNOWLEDGMENT

The authors thank Chad Farris for his advice on how best to measure the thickness of the components of

capillary walls, and Dr. Tom Kemper for his comments on a earlier version of this manuscript.

LITERATURE CITED

Alba C, Vidal L, Diaz F, Villena A, Perez de Vargas I. 2004. Ultrastructural and quantitative age-related changes in capillaries of the dorsal lateral geniculate nucleus. Brain Res Bull 64:145–153.

Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betscholz C. 2010. Pericytes regulate the blood-brain barrier. Nature 468:557–561.

Bandopadhyay R, Orte C, Lawrenson JG, Reid AR, De Silva S, Allt G. 2001. Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. J Neurocytol 30:35–44.

Barr T. 1978. Morphological evolution of capillaries in different laminae of rat cerebral cortex by automatic image analysis. Changes during development and aging. Adv Neurol 20: 1–9.

Buée L, Hof PR, Buras C, Delacourte A, Perl DP, Morrison JH, Fillit HM. 1994. Pathological alterations of the cerebral microvasculature in Alzheimer's disease and related dementing disorders. Acta Neuropathol 87:469–480.

Burns EM, Kruckeberg TW, Comerford LE, Buschmann MT. 1979. Thinning of capillary walls and declining numbers of endothelial mitochondria in the cerebral cortex of the aging primate, *Macaca nemestrima*. J Gerontol 34:642–650.

Burns EM, Kruckeberg TW, Gaetano PK. 1981. Changes with age in cerebral capillary morphology. Neurobiol Aging 2: 285–291.

Caley DW, Maxwell DFS. 1970. Development of blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. J Comp Neurol 138:31–48.

Dalkara T, Gursoy-Ozdemir Y, Yemisci M. 2011. Brain microvascular pericytesin health and disease. Acta Neuropathol 122:1–9.

Dickstein DL, Walsh J, Brautigam H, Stckton SD, Gandy S, Hof PR. 2010. Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. Mt Sinai J Med 77: 82–102.

Farkas E, Luiten PGM. 2001. Cerebral microvascular pathology in aging and Alzheimer's disease. Prog Neurobiol 64: 575–611.

Farkas E, De Jong GI, de Vos RAI, Jansen Steur ENH, Luien PG. 2000. Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. Acta Neuropathol 100:395–402.

Heinsen H, Heinsen YL. 1983. Cerebellar capillaries. Qualitative and quantitative observations in young and senile rats. Anat Embryol (Berl.) 168:101–106.

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

Hicks P, Rolsten C, Brizzee D, Samorajski T. 1983. Age-related changes in rat brain capillaries. Neurobiol Aging 4: 69–75.

Honavar M, Lantos PL. 1987. Ultrastructural changes in the frontal cortex and hippocampus in the ageing marmoset. Mech Ageing Dev 41:161–175.

Hunziker O, Abel'Al S, Schulz U. 1979. The aging human cerebral cortex: a stereological characterization of changes in the capillary net. J Gerontol 34:345–350.

Kalaria RN. 1996. Cerebral vessels in ageing and Alzheimer's disease. Pharmacol Ther 72:193–214.

Keuker JIH, Luiten PGM, Fuchs E, 2000. Capillary changes in hippocampal CA1 and CA3 areas of the aging rhesus monkey. Acta Neuropathol 100:665–672.

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

Knox CA, Oliveira A. 1980. Brain aging in normotensive and hypertensive stains of rats. III. A quantitative study of cerebrovasculature. Acta Neuropathol (Berl) 52:17–25.

Kuhl DE, Metter EJ, Riege WH, Hawkins RA. 1984. The effects of normal aging on patterns of local cerebral glucose utilization. Ann Neurol 15(suppl):S133–S137.

Luebke J, Barbas H, Peters A. 2010. Effects of aging on prefrontal area 46 in the rhesus monkey. Brain Res Rev 62: 212–232.

Mooradian D. 1988. Effect of aging on the blood-brain barrier. Neurobiol Aging 9:31–39.

Moss MB, Killiany RJ, Herndon JG. 1999. Age-related cognitive decline in rhesus monkey. In: Peters A, Morrison JH. editors. Neurodegeneratve and age-related changes in structure and function of the cerebral cortex. Cerebral cortex, vol 14. New York: Kluwer Academic/Plenum Publishers. p 21–48.

Peppiatt CM, Howarth C, Mobbs P, Attwell D. 2006. Bidirectional control of CNS capillary diameter by pericytes. Nature 443:700–704.

Perlmutter LS, Chui HC. 1990. Microangiopathy, the vascular basement membrane and Alzheimer's disease. Brain Res Bull 24:677–686.

Peters A. 2009. The effects of normal aging on myelinated nerve fibers in monkey central nervous system. Front Neuroanat 3:11.

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

Peters A, Leahu D, Moss MB, McNally KJ. 1994. The effects of aging on area 46 of the frontal cortex of the rhesus monkey. Cereb Cortex 4:621–635.

Peters A, Rosene DL, Moss MB, Kemper TL, Abraham CR, Tigges J, Albert MS. 1996. Neurological bases of age-related cognitive decline in the rhesus monkey. J. Neuropathol Exp Neurol 55:862–874.

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

Peters A, Moss MB, Sethares C. 2001. The effects of aging on layer 1 of primary visual cortex in the rhesus monkey. Cereb Cortex 11:93–103.

Peters A, Sethares C, Luebke JI. 2008. Synapses are lost during aging in the primate prefrontal cortex. Neuroscience 152:970–981.

Pugh KG, Lipitz LA. 2002. The microvascular frontal-subcortical syndrome of aging. Neurobiol Aging 23:421–431.

Schwartz E, Wicinski B, Schmeidler J, Haroutunian, Hof PR. 2010 Cardiovascular risk factors affect hippocampal microvasculature in early AD. Transl Neurosci 1:292–299

Shah GN, Mooradian AD. 1997. Age-related changes in the blood-brain barrier. Exp Gerontol 32:501–519.

Thomas WE. 1999 Brain macrophages: on the role of pericytes and perivascular cells. Brain Res Rev 31:42–57.

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

Topple A, Fifkova E, Cullen-Dockstader K. 1990. Effect of age on blood vessels and neurovascular appositions in the rat dentate fascia. Neurobiol Aging 11:371–380.

Topple A, Fifkova E, Baumgardner D, Cullen-Dockstader K. 1991. Effect of age on blood vessels and neurovascular appositions in the CA1 region of the rat hippocampus. Neurobiol Aging 12:211–217.

West RL. 1996. An application of prefrontal cortex function theory to cognitive aging. Psychol Bull 120:272–292.