

## Blood-Brain Barrier, Aging, Brain Blood Flow, and Sleep

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### BLOOD-BRAIN BARRIER

The subject of the blood-brain barrier (BBB) has been referred to frequently in earlier chapters. The BBB is particularly important in view of its regulatory role in maintenance of an optimal neuronal microenvironment without which normal neurological function cannot occur. The BBB—whether at the level of the cerebral capillary endothelium, the choroid plexus epithelium, or arachnoid epithelium—constitutes a crucial interface between blood and brain. Its structural and/or functional integrity may be compromised by cerebral hypoxia consequent to diminished cerebral blood flow. Furthermore, recent studies show that structural alterations occur with increasing age in non-human primates (3,4). The condition of the BBB cannot readily be evaluated as a part of brain blood flow studies in the human; nevertheless, its status is extremely important to the final outcome of the entire diagnostic and therapeutic program. Comparable studies in animal models can provide some insight into alterations occurring in the BBB in man with increasing age as well as in response to chronic or acute hypoxia associated with diminished cerebral blood flow.

### EFFECTS OF AGING ON NEUROLOGICAL FUNCTION

Several theories regarding decline in cerebral functioning during senescence have been based on observed or hypothetical changes in the cerebrovascular system (2,16). Neurological changes peculiar to senescence may be related to diminished cerebral capillary perfusion, altered BBB integrity, a combination of these factors, or still some other cause. Little attention has been given to the BBB as a possible cause of declining neurological function during aging, as evidenced by a paucity of related experimental studies.

Schwink and Wetzstein (15) reported capillary endothelial attenuation with increasing age in rats. All of the rats included in their study, except two 18-month-olds, were 1 year of age or younger; thus their findings do not reflect the extent of changes occurring during senescence. Bar (1) in a recent light microscopic study in rats from birth through 30 months of age, found that the number of brain endothelial cells declined with increasing age, whereas the length of these cells increased with increasing age. Further, he found a significant decrease in the mean diameter of microvessels in 30-month-old

rats as compared with younger animals. A similar light microscopic study of the architecture of cerebral capillaries in aged normotensive and hypertensive human subjects by Hunziker et al. (7) indicated that changes in stereological parameters in these subjects were mainly age-related and only partially due to hypertension. In general, their findings relative to capillary diameter, volume, specific surface area, and minimal intercapillary distance are in agreement with those of Bar (1) with one notable exception, i.e., a significantly increased capillary diameter was found in both the normotensive and hypertensive groups, whereas Bar reported a significantly decreased capillary diameter with increasing age in rats. Observations by Ravens (13) at the light microscope level in 100 senile human brains, ranging in age from 64 to 118 years, revealed that capillary beds in both gray and white atrophic areas were "dense and sparsely disrupted" and that these changes increased progressively with advancing age. He concluded that changes at the capillary level directly involve transport mechanisms leading to disturbances of neuronal function, electrolyte imbalance, and synaptic activity. He concurs with Pickworth (9), who held that mental and emotional stages have histopathological representations in cerebral capillary patterns which are causally related to altered mental activity, abnormal behavior, and personality disintegration in mental illness.

It therefore seemed plausible to us that altered BBB morphophysiology may be reflected in altered sensory, motor, and cognitive functioning. For this reason we investigated cerebral capillary ultrastructure in aging Macaque monkeys.

## METHODS

Eighteen *Macaca nemestrina* were included in this study: three 4-year-old, ten 10-year-old, and five 20-year-old animals. Full-depth sections of cerebral cortex, 1 to 3 mm thick, were

<sup>1</sup>Random tissue sections of negligible thickness are used for volumetric analysis of tissue components by planimetry, lineal integration, or differential point counting.

removed from the frontal and occipital poles of the cerebrum within 3 min after cessation of respiration and immediately immersed in McDowell's fixative (8). Thin sections from the lateral surfaces of samples were post-fixed in osmium tetroxide, stained *en bloc* with uranyl acetate (5), dehydrated in a graded series of ethyl alcohol and propylene oxide, and embedded in Epon. Ultrathin sections of silver to gray interference colors were made perpendicularly to the cortical pial surface and stained with uranyl acetate and Reynold's lead. Electron micrographs were made using a Phillips 300 electron microscope.

Evaluation of age-related changes was based on the following measurements made from approximately 800 capillary profiles: cross-sectional area of the entire capillary as indicated in Fig. 1.1; cross-sectional area of the capillary lumen, Fig. 1.2; thickness of the basal lamina surrounding the entire capillary (two-point measurements were made perpendicularly across the basal lamina at 3 cm intervals along the entire perimeter of this structure), Fig. 1.3; cross-sectional area of pericyte basal lamina, Fig. 1.4; and cross-sectional area of the pericyte, Fig. 1.5. Further, endothelial mitochondria per capillary bed were counted.

All measurements were made either with an Optomax Modular image analyzer (courtesy of Optomax, Inc., Hollis, New Hampshire) or with a Grafacon planimeter (model #1010A, Bolt, Beranek and Newman, Inc., courtesy of Dr. Rudolph Vracko, General Medical Research Program, Veterans Administration Hospital, Seattle, Washington).

## RESULTS

A summary of measurements/counts is included in Table 1. A regression analysis revealed a significant decrease with age in the cross-sectional area of the capillary wall as indicated in Table 1.A and in Fig. 2. The cross-sectional area of the capillary lumen increased between 4 and 10 years; however, it decreased between 10 and 20 years, as shown in Table 1. B and Fig. 2. These changes were not statis-

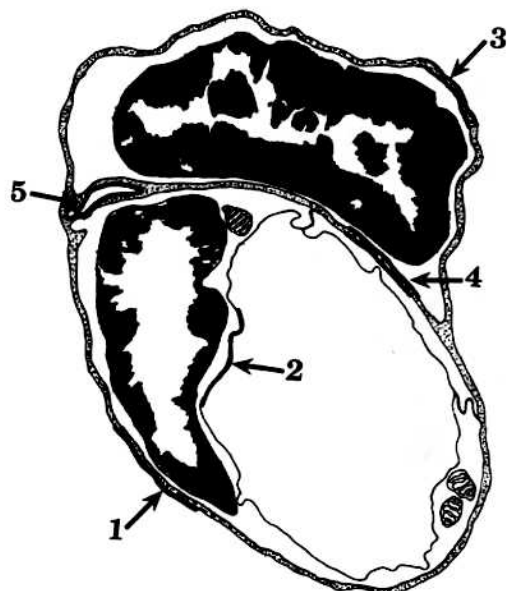


FIG. 1. Cerebral capillary cross section (diagrammatic). 1: External boundary of capillary profile delineated by the outer edge of the basal lamina ( $BL_o$ ) surrounding the capillary. 2: Luminal endothelial membrane. 3: Inner and outer edges of  $BL_o$ . 4: Portion of basal lamina ( $BL_i$ ) between endothelium and pericyte. 5: Small portion of a pericyte, cross-sectional view.

tically significant. A nonsignificant decrease in the cross-sectional area of pericytes occurred between 4 and 10 years with no further change after that time, as shown in Table 1.C and Fig. 2. The increase in thickness of the outer basal lamina with age was not statistically significant, as illustrated in Table 1.D and Fig. 2. Mean values for the number of endothelial mitochondria per capillary profile are shown in Table 1.E and Fig. 3. A regression analysis revealed a significant decline with age in endothelial mitochondria per capillary profile.

## DISCUSSION

The attenuation of the cross-sectional area of cerebral capillary walls with increasing age in Macaque monkeys is in agreement with that previously reported for the rat (15). The decrease in the cross-sectional area of the capillary lumen noted by us, although not statistically significant, is similar in magnitude to that reported for the rat by Bär (1); however, is not in agreement with that reported by Hunziker and co-workers (7) in normotensive and hypertensive humans. It should be remembered that

TABLE 1. Morphometric analysis of microvascular characteristics in frontal cortex of three age groups of macaca nemestrina

Characteristics	$\eta$	Age groups			Significance
		4 Years ( $N = 3$ )	10 Years ( $N = 10$ )	20 Years ( $N = 5$ )	
A Cross-sectional area of capillary wall in $\mu\text{m}^2$	10-18	$24.4 \pm 5.5^c$	$19.2 \pm 4.2$	$15.9 \pm 3.2$	$p = 0.019$
B Cross-sectional area of capillary lumen in $\mu\text{m}^2$	10-18	$7.3 \pm 2.7$	$10.1 \pm 6.1$	$6.7 \pm 3.3$	ns
C Cross-sectional area of pericytes in $\text{nm}^2$	12-34	$1,500 \pm 200$	$900 \pm 700$	$900 \pm 200$	ns
D Thickness of outer basal lamina in nm	159-354 <sup>b</sup>	$130 \pm 20$	$190 \pm 50$	$200 \pm 70$	ns
E Endothelial mitochondria per capillary profile	10-18	$3.5 \pm 1.0$	$3.3 \pm 0.9$	$2.3 \pm 0.5$	$p = 0.035$

<sup>a</sup> $\eta$  = Range of numbers of capillary profiles sampled per animal.

<sup>b</sup>Number of measurements sampled at 3 cm intervals. Significance was based on linear regression analysis of measurement vs. age groups.

<sup>c</sup>Means  $\pm$  SD.

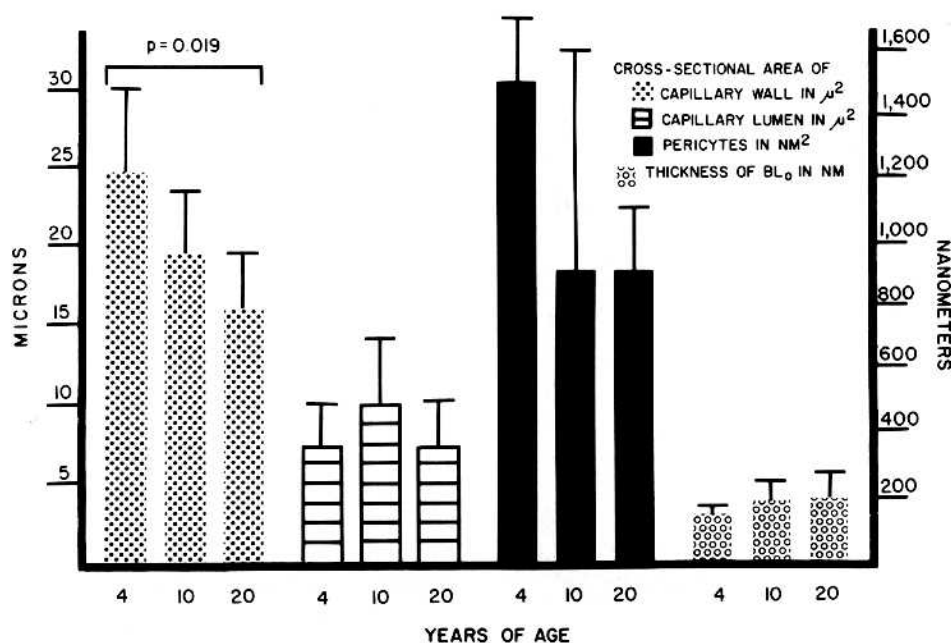


FIG. 2. Analysis of microvascular morphology. Morphometric analysis of microvascular characteristics in three age groups of the *Macaca nemestrina*. Values represent mean  $\pm$  SD.

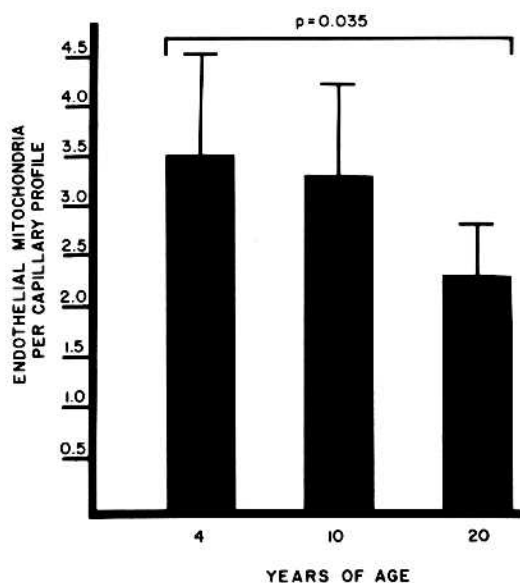


FIG. 3. Number of endothelial mitochondria (per capillary profile) in three age groups of *Macaca nemestrina*. Values represent mean  $\pm$  SD.

in the human study samples were taken 4 to 24 hr after death, whereas in the monkey, samples were obtained within 3 min after cessation of respiration. Also, the condition of the human prior to death might further contribute to the presence of artifacts. Autoregulation in man normally protects cerebral capillaries from changes in systemic blood pressure between 60 and 150 mm Hg. However, in defective autoregulation, increased systemic pressure results in increased cerebral blood flow, dilated cerebral capillaries, and opening of the BBB (12). In our study, no significant change was found in either pericyte cross-sectional area or thickness of the outer basal lamina, although the latter did increase in thickness with aging.

The observed attenuation of the capillary wall with aging is due primarily to a decrease in cross-sectional area of its endothelial component. Bär (1) reported endothelial cell loss from rat cerebral capillaries, and suggested that this loss might be compensated in part by elon-

gation of remaining cells. This seems a likely mechanism underlying changes in capillary walls in the Macaque monkey. Thinning of capillary walls, luminal narrowing, and thickening of the outer basal lamina may affect the capacity of the cerebral capillary bed to sustain an adequate cerebral blood flow, particularly during stress or when an increased blood flow might normally occur.

#### SLEEP, BRAIN METABOLISM, AND BLOOD FLOW

An important related question arises as to whether sleep variables may correlate with brain blood flow, which is inseparably linked physiologically with cerebral metabolism. Sleep pattern is a sensitive index of neurological function. Aging affects sleep pattern. Prinz and Raskind (11) have shown that delta sleep and rapid eye movement (REM) sleep are markedly attenuated and stage 4 sleep is either greatly reduced or absent in the elderly. Prinz and Raskind (10,11) noted that some elderly individuals had greater amounts of REM sleep than others and that the former also maintained cerebral blood flow levels closer to the norm for young adults.

More than a decade ago, Reivich et al., (14) reported a marked generalized increase in cerebral blood flow in REM sleep in cats. It is not known whether or not this phenomenon occurs in the human. Gusatinsky et al. (6) found protein synthesis and cell mass increased in large pyramidal neurons of the cat parietal cortex during REM sleep. They hypothesized that REM sleep plays a significant role in the restoration of neuronal proteins. If a generalized increase in metabolic rate occurs in nerve cells during REM sleep, an accompanying increase in cerebral blood flow should occur. Since brain metabolism regulates brain blood flow, only if the brain blood flow increases can an elevated metabolic rate be supported.

Ultrastructural changes observed in the Macaque monkeys might compromise brain blood flow by an effective decrease in the volume or capacity of the cerebral capillary bed, by an

increased resistance to blood flow through a more narrow capillary lumen, with the additional possibility of an increased rigidity of capillary walls due to the increased thickness of the outer basal lamina. It is not known whether ultrastructural changes affect passive permeability or carrier-mediated transport across the capillary wall. However, the decline in endothelial mitochondria per cerebral capillary profile suggests a decreased work capability of the BBB in aged nonhuman primates. This may alter BBB active transport mechanisms, thus adversely affecting the delivery of substrates needed by the brain for energy metabolism and day-to-day neuronal restorative processes. Thus it is possible that altered cerebral capillary morphophysiology may be reflected in an altered sleep pattern. Further, an altered sleep pattern may correlate with altered mental activity, abnormal behavior, and personality disintegration associated with senility.

#### SUMMARY

We have shown that significant ultrastructural changes occur with increasing age in the BBB in the nonhuman primate. It is probable that similar changes occur in aging humans. Clearly, morphophysiological changes, i.e., structural changes in cerebral capillaries, may alter the BBB mechanism as well as capillary perfusion which, in turn, may affect cerebral energy metabolism and neuronal function. Thus neurological function may be affected and sensitive indices of function such as sleep patterns altered.

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