# Capillary changes in hippocampal CA1 and CA3 areas of the aging rhesus monkey

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# Capillary changes in hippocampal CA1 and CA3 areas of the aging rhesus monkey

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**Abstract** The rhesus monkey is considered a useful animal model for studying human aging, because non-human primates show many of the neurobiological alterations that have been reported in aging humans. Cognitive impairment that accompanies normal aging may, at least partially, originate from capillary changes in the hippocampus, known to be involved in learning and memory. Age-related effects on the cerebral capillaries in the non-human primate hippocampus have not yet been studied. Therefore, we investigated age-related microvascular changes in the hippocampus of the aged non-human primate. We examined by electron microscopy the microvascular ultrastructure in the CA1 and CA3 areas of 14 male rhesus monkeys *(Macaca mulatta)*, ranging from 1 to 31 years of age. The percentages of capillaries showing basement membrane thickening and deposits of collagen in the basement membrane were determined semiquantitatively in 4 young (1–6 years), 6 middle-aged (17–24 years), and 4 aged (29–31 years) monkeys. Aberrations in the basement membrane are few in young subjects (28 ± 6% of capillaries), and occur with increasing frequency during the aging process in rhesus monkeys (aged animals: 71 ± 5% of capillaries). This could be ascribed to an aging-associated increasing number of capillaries showing depositions of collagen fibrils, rather than local thickenings of the basement membrane. The observed changes in microvascular integrity are very similar to those seen in humans, supporting the view of rhesus monkeys as a model for human aging. The slow but steady progression of these changes could be detrimental for an efficient nutrient supply of the neuropil, and might therefore contribute to decreased cognitive functioning during normal aging.

**Key words** Aging · Microcirculation · Electron microscopy · Hippocampus · Primate

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## Introduction

Aging is associated with a deterioration of cognitive function, loss of memory, or decreased ability to process and store new information [5, 7, 23, 26, 27, 36]. In rats and humans, the pattern of memory deficits seen following hippocampalectomy or damage to the hippocampal formation is similar to that seen during normal aging [19]. This suggests that the hippocampus plays a critical role in memory processes and that the hippocampal integrity is affected during normal aging. Aged non-human primates performed poorly compared to young monkeys on a number of tasks that are sensitive to deficits in memory (e.g., delayed response and delayed non-match to sample) [5, 21, 23, 26, 27, 36, 38]. Deficits on those tasks, which are sensitive to aging in monkeys, are produced by damage to the prefrontal cortex and the medial temporal lobe, including the hippocampus [5, 21, 27].

Processes that may underlie cognitive decline during aging are numerous, but a very important role is to be attributed to the cerebrovascular system and blood supply to the central nervous system. Particularly the brain microvasculature plays a crucial role in maintaining local brain perfusion to meet the dynamic metabolic needs for normal cerebral function [15, 34]. The cerebral capillary endothelium is the anatomical basis of the blood-brain barrier (BBB) and isolates the neuropil from the systemic circulation. The capillary endothelium is readily permeable by diffusion or through transport to oxygen, water and other vital nutrients. Because of its specific transport systems, the endothelium only selectively allows certain compounds to pass the BBB, and in this way exerts a neuroprotective function to neuronal tissue besides maintaining a stable internal milieu. During aging, several active

endothelial mechanisms such as choline transport over the BBB and glucose influx into the brain become affected [22], indicating impairment of the BBB function. This functional decline of the barrier is accompanied with age-related structural alterations in the BBB. Thinning of the endothelium has been reported in rats [2, 8], primates [3] and humans [22, 31], and could be caused by a general shrinkage of the cytoplasm or a loss of endothelial cells [2, 31]. Yet the most consistent age-related change recorded in mammalian cerebral capillaries is thickening of the basement membrane (BM) or basal lamina [3, 4, 6, 8, 9, 13, 15, 17, 18, 32, 33]. In contrast to these studies in animals and to reports suggesting increased thickness of the BM in arteries and arterioles in animals and humans [15], it is not entirely clear whether the capillary BM is affected by the aging process in humans. Significant age-related increases in the BM thickness were reported for muscular [37] and conjunctival capillaries [29], whereas no such aging effects were observed in the capillary BM from the human neocortex [31]. On the other hand, an increased thickness was reported of the BM in cerebral microvessels of aged non-human primates [3, 4, 9]. Besides thickening of microvascular BM, deposition of fibrous material and collagen in the capillary wall, termed fibrosis, has been reported in aging rats [10–12, 17, 18] and humans [13, 15, 16]. Microvascular deposits have been considered to be associated with disturbed transport mechanisms through the endothelium [22], resulting in a derangement of neuronal functioning [28].

The rat has been the species utilized in a majority of studies on the anatomy, physiology, and behavioral functions of the hippocampal formation. However, the ultimate focus of our interest in the hippocampal formation is its general function in mammals, including man. The hippocampal formation has undergone progressive development in primate phylogeny, which is reflected in many levels of its morphological organization, and which also suggests a functional progression [30]. Among experimental animals, the rhesus monkey is the species closest to the human in which it is practical to conduct a full range of neurobiological studies [30]. Few of the above-mentioned studies on aging-associated microvascular aberrations were conducted in the rat hippocampal formation [8, 10, 32, 33]. Moreover, the microvascular integrity in the human hippocampal formation during the aging process has not yet been studied extensively. BM thickening (BMT) was described in the hippocampus of aged new world monkeys [9]; however, little is known about the course of microvascular anomalies during the aging process in primates. Rhesus monkeys are considered to serve as a useful model for normal human aging [1, 5, 23, 25, 26, 35,36]. Aged non-human primates show many of the age-related neurobiological alterations that have been reported in aging humans [35]. A very important role in human aging and, for example, in Alzheimer's disease has been ascribed to impaired integrity of the microvasculature [13, 14, 34]. However, to validate the rhesus monkey as a convincing model for human aging, it should be confirmed that the cerebral capillaries in non-human primates display similar age-associated capillary alterations as in humans. To our knowledge such structural aberrations in hippocampal capillaries have never been reported in the old world rhesus monkey. Therefore, the aim of the present study was to examine the effects of aging on BMT and microvascular fibrosis in the hippocampal formation of rhesus monkeys, ranging from 1 to 31 years of age.

In the present study, the hippocampal CA1 and CA3 subfields were investigated with respect to their function and connectivity in the hippocampal formation. The CA1 and CA3 are subfields in the so-called trisynaptic circuit, which consists of the perforant pathway projection from entorhinal cortex to dentate gyrus, the mossy fiber pathway from dentate gyrus to CA3, and the Schaffer collateral projection from CA3 to CA1. The mossy fibers terminate in the CA3 field, whereas the CA1 field receives its input from the Schaffer collaterals [30]. Apart from these intrinsic inputs, the CA1 and CA3 are also innervated from the entorhinal cortex directly [24, 30]. Additionally, extrinsic afferent projections to the CA1 subfield originate from the amygdala, basal forebrain, brain stem and medial septum. In contrast to the rat hippocampal formation, the rhesus monkey CA1 field also receives extrinsic input from the neocortex [30].

## Materials and methods

Brain tissue blocks, including the hippocampus, were obtained from autopsies of 14 male rhesus monkeys *(Macaca mulatta)* from the Wisconsin Regional Primate Research Center (Madison, USA), and stored in buffered formalin. None of the monkeys suffered from diabetes. The brain of one aged monkey was perfused with 4% paraformaldehyde (PFA) in phosphate buffer (PB) pH 7.4. In the present study, we used hippocampi of 4 young (1–6 years), 6 middle-aged (17–24 years), and 4 old (29–31 years) animals. Postmortem delay varied between 30 min and 15 h.

### Experimental procedure

A slab of approximately 2 mm from the main body of the hippocampus was cut perpendicular to the fimbria. Under a dissecting microscope, small blocks of the CA1 and CA3 areas (including strata pyramidale and radiatum) were selected and dissected from the slabs for further processing. Embedding of these tissue blocks was done in a Lynx EM tissue processor using a standard epoxy resin embedding protocol. In brief, tissue blocks were washed in 0.1 M phosphate buffer pH 7.4, treated with 1% osmium tetroxide (in 0.1 M PB), and rinsed again in 0.1 M PB. After dehydration in ethanol solutions of increasing concentrations and isopropanol, the tissue blocks were treated with propylene oxide. Prior to embedding in epoxy resin (Serva) the blocks were treated with mixtures (2:1, 1:1, and 1:2) of propylene oxide and epoxy resin. The blocks were flat-embedded in gelatin capsules containing epoxy resin. Polymerization occurred in an oven at 60 °C for 24 h. The gelatin capsules were removed by soaking in water of 60 °C.

The resin blocks were trimmed and semithin (1 μm) sections were cut and floated on a solution of Azur II-Methylene Blue at 60 °C. After rinsing in distilled water, they were placed on a drop of distilled water on a microscopic slide, and dried on a warming plate at 60 °C. After selecting the area of interest, the blocks were trimmed again for ultrathin sectioning, to an approximate size of 0.25–0.50 $\mu m^2$. Ultrathin sections (60–90 mm) were then mounted on one-hole grids coated with a Formvar film, and contrasted with uranyl acetate and lead citrate. Semi- and ultrathin sectioning was done with a Reichert ultramicrotome.

Quantification of microvascular deposits

Examination and quantification was done in a Zeiss EM 10 electron microscope. Prior to quantification, animal numbers were coded, and the code was only revealed after completion of the quantification. In the CA1 area 30–41 capillaries in one to three (non-consecutive) sections and in the CA3 area 30–59 capillaries in one or two (non-consecutive) sections were investigated per animal. Only completely visible microvessels were recorded.

Capillaries containing aberrations in the BM were counted according to three classifications, which have been described previously for rats [10–12]. First, capillaries displaying local BMT. The BM was considered locally thickened when it had an unevenly thickened appearance, and when its width was at least doubled. Another important criterion to distinguish BMT was that the BM at the thickened site had the same electron density as the neighboring BM. Second, capillaries showing microvascular fibrosis, characterized by collagen fibrils observed in various planes of cutting and deposited within the BM. Third, microvessels with both BMT and fibrosis in the same transverse section.

Thickening of the BM and deposition of collagen fibrils are collectively designated as microvascular deposits. So, after counting the vascular anomalies according to the above-mentioned categories, the percentage of microvessels with microvascular deposits could be calculated per animal for the CA1 and CA3 areas, as well as the percentage of all capillaries showing BMT or fibrosis. Statistical comparisons of the percentages were carried out by Welch test with a post hoc Games-Howell test. These tests are especially appropriate for comparing small groups of different sizes.

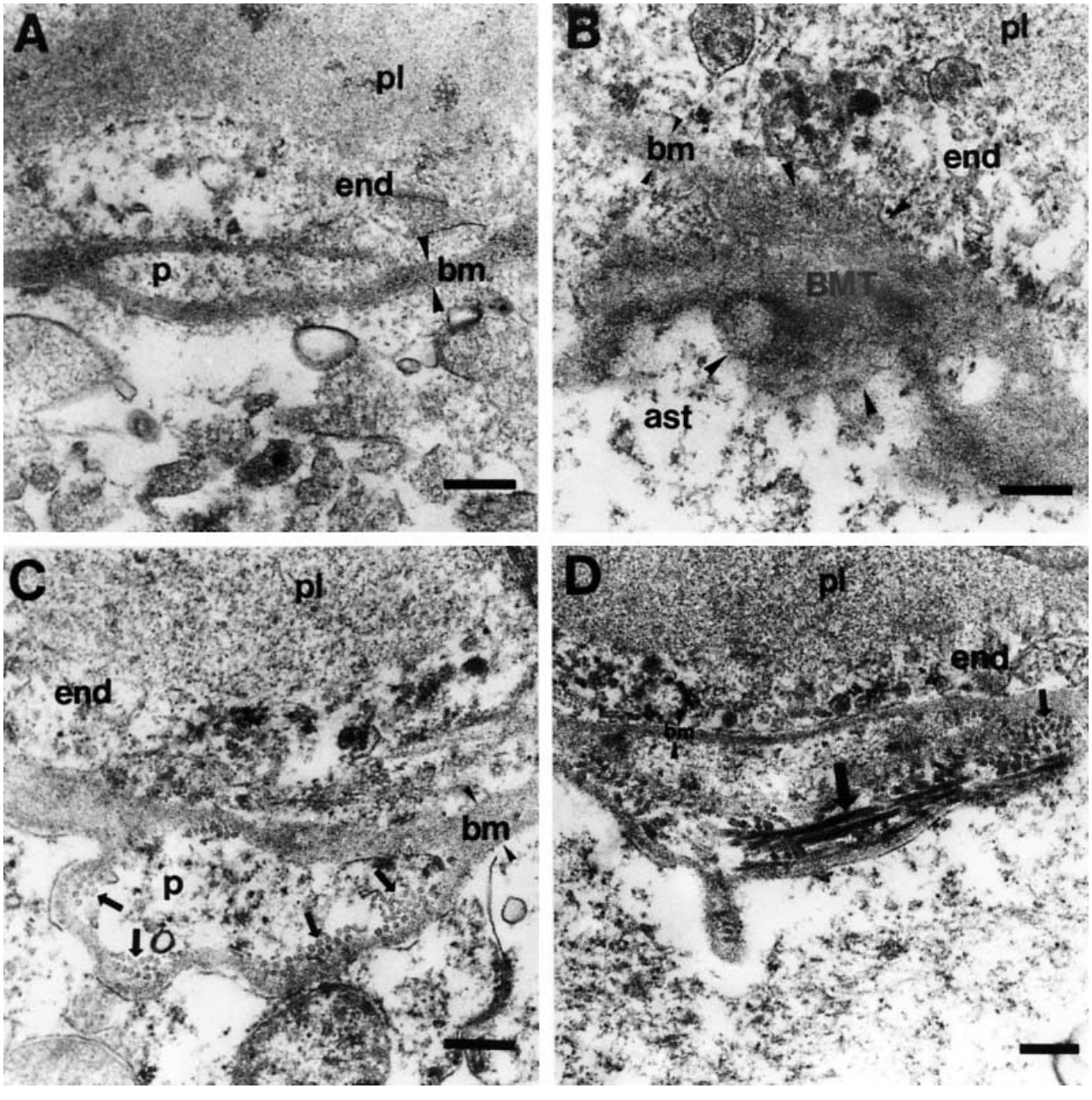


**Fig. 1 A** Normal appearance of a hippocampal capillary. The endothelial cell *(end)* is surrounding the lumen, which is filled with plasma *(pl)*. The BM *(bm, arrowheads)* circumvents the endothelial cell, and encloses an adjacent pericyte *(p)*. **B** The BM is locally thickened *(BMT)*, when compared to the normal BM (*ast* astrocyte). **C** Microvascular fibrosis is distinguished by deposition of collagen fibrils within the BM. Collagen fibrils can be encountered in a transverse way *(arrows)* and **D** in a longitudinal way *(large arrow)* (*BM* basement membrane). *Bars* **A–D** 0.25 μm

Additionally, correlations of the percentage of capillaries with deposits, BMT and fibrosis with age were assessed by linear regression. All statistical analyses were employed two-tailed, and the significance level was defined at $\alpha = 0.05$.

## Results

### Hippocampal microvascular integrity

The ultrastructure of the hippocampal tissue of the rhesus monkeys used in this study was typical for immersion-fixed tissue. Because of fixation delay, edema was regularly seen around blood vessels, including microvessels. This could lead to the appearance of empty space between the BM and the surrounding brain tissue. In addition, edema sometimes caused swelling of endothelial cells and astrocytes, and disruption of astrocytes. However, microvascular basement membranes were always intact and never expanded to yield a more electron-lucent appearance.

The ultrastructure of a normal capillary of a rhesus monkey is shown in Fig. 1 A. The microvascular lumen is filled with blood plasma. The endothelial cell is slightly swollen, but the BM forms a regular thin layer around the endothelial cell, and surrounds a pericyte. We could clearly detect local thickenings of the BM, as previously described [10–12]. Figure 1 B shows a locally and irregularly thickened BM, which is continuous with the normal and non-affected BM. The thickened BM displays a similar electron density as the neighboring BM. In Fig. 1 C, D capillaries displaying fibrosis are shown, which are characterized by the presence of collagen fibrils within the BM. The fibrils appeared in a transverse or longitudinal plane (Fig. 1 C, D, respectively), and had about the same electron density as the BM itself. The amount of collagen fibrils varied from one single fibril to large clusters of up to more than 20 fibrils. BMT and deposition of collagen fibrils could be detected everywhere in the BM, either directly adjacent to the endothelial cell, situated between the endothelial cell and pericyte, or between the pericyte and astrocyte. The described microvascular aberrations were encountered both in the CA1 and CA3 area of the hippocampus of rhesus monkeys, and no region specific differences were present.

BMT and microvascular fibrosis in the hippocampal CA1 and CA3 areas in a similar form and frequency occurred in an aged (24 years) rhesus monkey brain, which was perfused with 4% paraformaldehyde and 0.05% glutaraldehyde. The ultrastructure of a hippocampal capillary from this particular rhesus monkey is shown in detail in Fig. 2. The endothelial cells formed a thin, regular sheet on the inner side of the BM. Astrocytic endfeet continuously framed the outer side of the BM. Both cell types had clearly distinguishable mitochondria and tight junctions. The cell membranes could be clearly defined nearly continuously by the bilipid layers. Where these features partly had disappeared in immersion-fixed material, the overall BM was still found to be continuously regular and thin. This indicates that the anomalies seen in our material were not the result of the state of the tissue due to immersion fixation.

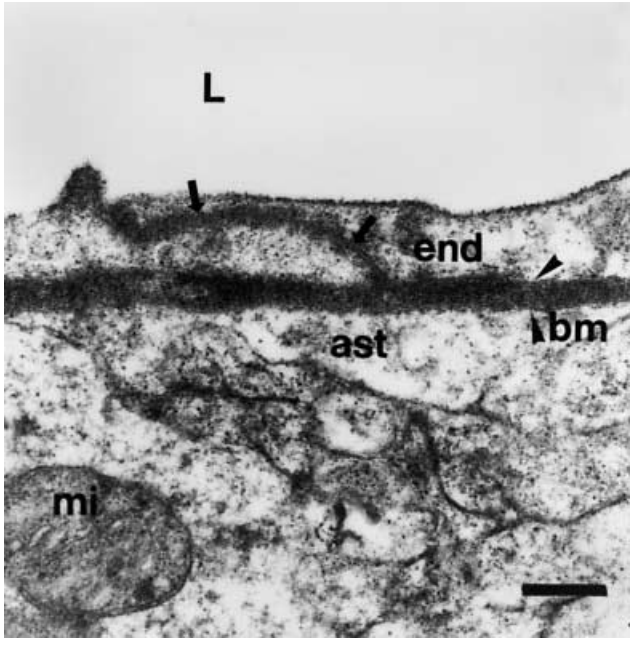


**Fig. 2** A hippocampal capillary of a perfused rhesus monkey. The capillary lumen *(L)* is, in contrast to the photographs in Fig. 1, free from blood cells and blood plasma. Two endothelial cells are connected with a tight junction *(arrows)*. Bilipid layers *(arrowheads)* of the endothelial cells *(end)* and astrocytes *(ast)* surround the BM *(bm)* (*mi* mitochondrium). Scale bar = 0.25 μm

### Quantitative analysis of aging-related microvascular changes

The mean percentages of capillaries with microvascular deposits in the CA1 and CA3 regions for the three age groups are shown in Fig. 3. In the CA3 area, a significant age-related increase in the percentage of capillaries displaying deposits in their BM was demonstrated. The middle-aged and aged rhesus monkeys displayed significantly more deposits in the CA3 area than young rhesus monkeys ($P < 0.005$ and $P < 0.025$, respectively). In the CA1 area, the same aging-related tendency was observed, but significance was not reached, probably due to greater inter-individual variability. The linear regression analysis showed a highly significant positive correlation between the percentage of CA3 capillaries with deposits and age (Table 1).

The frequency of occurrence of the two compounds of microvascular deposits, BMT and microvascular fibrosis, are shown in Fig. 4 A, B, respectively. No significant age-related increase in BMT could be detected in either the CA1 or CA3 area. Nevertheless, in the middle-aged and aged monkeys slightly more capillaries underwent local thickening of the BM. A moderate, but significant, correlation between BMT and age was detected for the CA3,

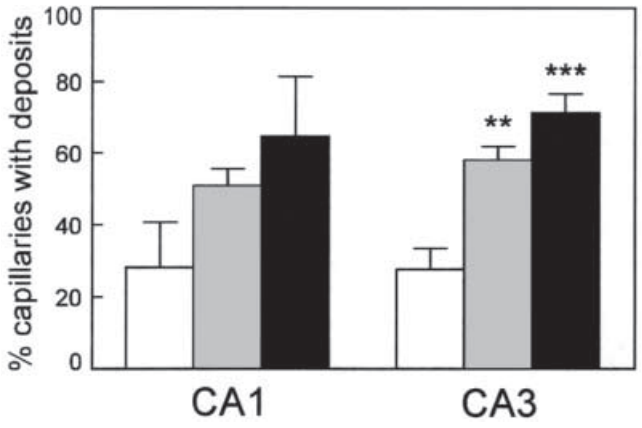


**Fig. 3** The percentage of capillaries with deposits in the CA1 and CA3 area for young *(white)*, middle-aged *(gray)* and aged *(black)* rhesus monkeys. Data are given as mean ± SEM (** $P < 0.025$, *** $P < 0.005$; middle-aged and aged, respectively, compared to young)

**Table 1** Linear regression analyses of the percentages of capillaries in CA1 and CA3 displaying deposits, BMT and fibrosis as a function of age (*BMT* basement membrane thickening, *r* correlation coefficient)

| | | Linear regression | | |
|---|---|---|---|---|
| | | r | *P* value | |
| CA1 | deposits | 0.505 | 0.066 | |
| | BMT | 0.330 | 0.249 | |
| | fibrosis | 0.548 | 0.042 | * |
| CA3 | deposits | 0.841 | 0.0002 | † |
| | BMT | 0.556 | 0.0391 | * |
| | fibrosis | 0.818 | 0.0003 | † |

* $P < 0.05$, † $P < 0.0005$

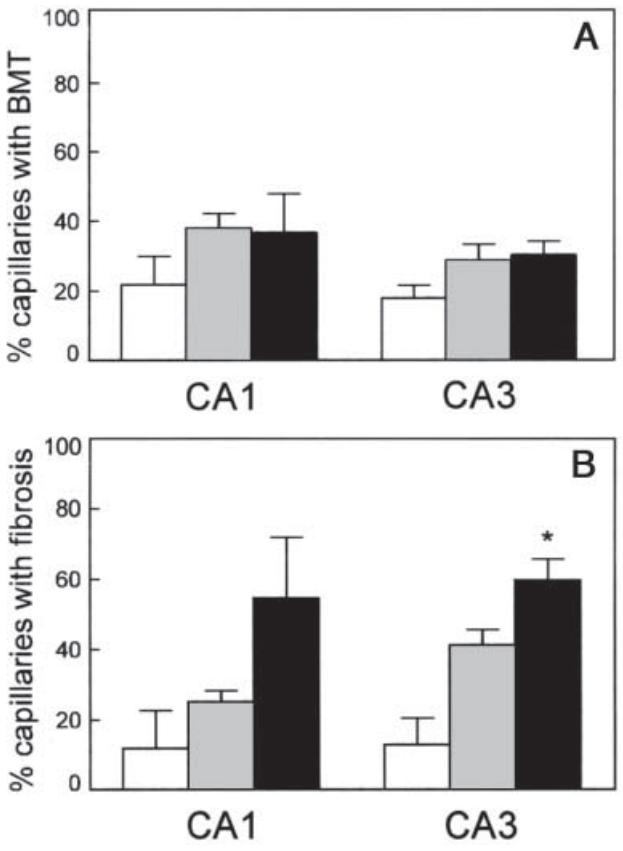


**Fig. 4** The percentages of capillaries with **A** BMT, and **B** microvascular fibrosis in the CA1 and CA3 area for young *(white)*, middle-aged *(gray)* and aged *(black)* rhesus monkeys. Data are given as mean ± SEM (* $P < 0.05$; aged compared to young) (*BMT* thickening)

but not the CA1 area (Table 1). For both CA1 and CA3 areas, an aging-associated tendency seems to exist for the occurrence of fibrosis. Microvessels in the CA3 area of aged monkeys showed significantly more fibrosis compared to young subjects ($P < 0.05$). For the CA1 area, similar trends could be observed. The linear regression analyses showed a positive age-associated correlation in the percentage of fibrotic capillaries in the CA1 and CA3 areas ($P < 0.0005$).

## Discussion

BMT of cerebral microvessels has previously been described as a common feature that accompanies aging [13, 15], but it is also frequently associated with diabetes mellitus [15, 20] and hypertension [13, 15]. This indicates that thickening of the BM is not an exclusively age-related phenomenon, but a relatively nonspecific change. In rats, the thickness of the microvascular BM has been shown to increase with advancing age in the cerebellum [6] and the frontal cortex [4, 8], as well as in the hippocampal dentate gyrus [32] and CA1 area [8, 33]. Besides increases in total thickness, several studies report local thickenings of the microvascular BM in brains of humans [13], new world monkeys [9], and rats [12, 13]. The latter two studies demonstrated that in the frontal cortex of aging rats capillaries displayed an increase of local thickenings of the BM. In the present study, we also quantified local thickenings of the BM, in the old world rhesus monkey. We found that the percentage of capillaries with local BMT in the hippocampal CA1 and CA3 areas slightly increased from young to middle-aged rhesus monkeys, but did not undergo further changes from middle-aged to aged individuals. Similar findings were observed in the frontal and occipital cortex from aging pigtailed macaques *(Macaca nemestrina)* [3]. Here, the total thickness of the BM was found to be increased between 4 and 10 years of age, but remained unchanged between 10 and 20 years of age. In samples from the human neocortex, although derived from diverse cortical regions, the BM thickness was found unchanged between 15 and 77 years of age [31]. These observations and those in non-human primates, including our own, might indicate that aging in primates in general is not associated with significant thickening of the cerebral capillary BM.

Microvascular fibrosis has not been investigated as extensively as BMT; however, its appearance has been de-

scribed not only in aging [13, 15], but also in Alzheimer's disease [13, 16] and hypertension [13, 17, 18]. From these findings it can be concluded that, like thickening of the BM, deposition of collagen fibrils is not a phenomenon that is specifically related to advancing age. Quantitative data on microvascular fibrosis during aging are scarce. However, microvascular fibrosis in the frontoparietal motor cortex of aging Wistar rats was found increased from 16 months up to 30 months, but did not further increase from 30 to 32 months [12]. The present study showed a significant age-related increase in the percentage of microvessels with fibrosis in the hippocampal CA3 area of rhesus monkeys. In addition, a similar trend was detected in the CA1 area; however, probably due to larger inter-individual variabilities in the occurrence of fibrosis especially in the young and aged groups, statistical significance was not reached.

In the present study, we demonstrate that the percentage of microvessels with deposits (whether BMT, fibrosis, or both) increases in the non-human primate hippocampus with age. This was significant for the CA3 area; however, a similar tendency was observed in the CA1 area. This was confirmed for CA3 by statistical analysis of group means and for CA1 and CA3 by linear regression analyses. Microvascular deposits were detected in the BM close to endothelial cells and astrocytes, as well as adjacent to pericytes. This suggests that endothelial cells, pericytes, and astrocytes all may be involved in the deposition of collagen fibrils and BM material in the capillary wall [13].

Whether deposits in the BM cause alterations in BBB function, or reversely whether attenuated functioning of the BBB induces microvascular deposits is not entirely clear. It was suggested that microvessels that are compromised in their BBB functioning by morphological barriers might be prone to compensatory mechanisms [13]. Endothelial cells of aging rat cerebral microvessels with deposits contained a large number of pinocytotic vesicles, which might be indicative for the detrimental effects of capillary depositions in the BM on transport functions of the BBB [12, 13]. Adequate functioning of the BBB requires mitochondrial energy for active transport of compounds through the capillary wall. Endothelial cells of morphologically aberrant microvessels are thought to be compromised in their energy status, as concluded from the negative correlation between the number of mitochondria in microvessels and the percentage of capillaries with deposits in the cortex of aged humans [13]. The same study showed that, in normal aged humans, the number of mitochondria was higher in the aberrant microvessels than in intact microvessels. In the rat hippocampal dentate gyrus and CA1, it was demonstrated that advancing age is associated with an increased thickness of the capillary BM and with an increased fraction of endothelial area occupied by mitochondria [32, 33]. The proposed compensation of the endothelial mitochondria suggests that microvessels in the hippocampus may suffer from an altered BBB functioning due to morphological barriers from microvascular deposits. However, thinning of the endothelium has been reported in various species, and has been considered to increase the vulnerability of cerebral capillaries to mechanical damage [2–4, 8, 22, 31] and to cause weakening of the endothelial barrier systems [33]. Therefore, it is possible that microvascular deposits serve as a compensatory response to an altered function of the BBB [15, 16, 32, 33].

In support of this view, a recent study on chronic hypoperfusion in rats showed local BMT and microvascular fibrosis in capillaries of the hippocampal dentate gyrus and CA1 area [14], as had been demonstrated in aging rat cerebral microvessels and in the present study. This study [14] showed a strong correlation between the percentage of intact microvessels in the CA1 area and cognitive performance. Moreover, a negative correlation was shown between the percentage of microvessels with deposits in the CA1 area and cognitive performance. From these observations, it can be concluded that the age-associated microvascular deposits in the hippocampal CA1 area are related to cognitive impairment. Furthermore, the condition of hippocampal microvessels is important and possibly crucial for normal brain functioning, which is probably true for the vascular supply to the brain in general.

In summary, the present study described the occurrence of aberrations in the BM of hippocampal capillaries, such as BMT and fibrosis, in the aged non-human primate, the rhesus monkey, which has been demonstrated previously in rats and humans [13, 15]. These microvascular deposits might compromise an appropriate energy-dependent nutrient transport across the BBB, and might consequently impair cognitive functioning. The semi-quantitative results suggest that aging of the rhesus monkey is associated with an increase in microvascular aberrations in the hippocampus. Although an increasing number of cerebral capillaries in aging rats [12] and non-human primates (present study) show such alterations, the development of the microvascular changes in the lifespan of these species seems to differ. From rat studies it was hypothesized that the percentage of capillaries with fibrosis increased during aging, without an increase in BMT. Then, in old age, degradation of collagen fibrils lead to thickening of the BM [12]. In contrast, we showed in the rhesus monkey hippocampus that the percentage of capillaries with BMT hardly increased during life, whereas a growing number of capillaries become affected by fibrosis, even into old age. These cerebral capillary deposits have been described in humans [13]; however, like most human studies, more emphasis was put on neuropathology rather than normal aging. Moreover, the development of such anomalies during the human aging process has not been studied so far. The present results, therefore, give evidence that microvascular fibrosis in non-human primates is a continuous and age-associated process, which might take place in humans in a similar form. The rhesus monkey may serve as a suitable model for investigation of how and why these capillary anomalies develop in the primate brain, and lead to a better understanding of human aging.

Several reports demonstrate that the degree of cognitive impairment increases with age. However, deteriorated

performance in learning and memory tasks is not a simple function of age [25]. Differential individual performances within age groups suggest that the varying cognitive abilities may reflect differentially impaired hippocampal function, and may result from individual differences [1, 5, 23, 25, 26], e.g., in hippocampal microvascular integrity. This phenomenon of differential individual cognitive aging is referred to as functional aging, in contrast to chronological aging. Functional aging in monkeys, also encountered in human aging, supports the view of rhesus monkeys as a model for human aging.

Although the presented data were not obtained using random, volume-weighted sampling of tissue blocks and sections, these results may be regarded as semiquantitative data, mirroring the relative density of aberrations in the BM along the longitudinal surface of a capillary. The larger inter-individual variability we observed in the integrity of CA1 capillaries might be the result from a lack of systematic sampling. However, using this semiquantitative approach, we did not observe such large inter-individual differences in the microvascular integrity of the CA3 area. Furthermore, it should be considered that, although studying the substrates of aging in the primate brain is a very interesting field of neurobiological research, it is difficult to obtain brain tissue from aged primates or humans in such a way that it is suitable for performing design-based studies. Instead, the present results merely give an impression that capillaries become affected probably over a larger longitudinal surface area during aging, which has deleterious implications for the surrounding neuropil.

The importance of intact hippocampal capillaries becomes clear when the cerebral hypoperfusion hypothesis in Alzheimer's disease [34] is considered. This hypothesis postulates that, preceding the neuronal changes that lead to the typical hallmarks of this disease, capillary changes in e.g. the BM causes disturbances in cerebral blood flow, which in turn compromises glucose transport into the neuropil. These slow but steady changes occur during normal aging and ultimately have widespread effects on neuronal mitochondrial functioning [34].

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