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## Cerebrovascular Hypoperfusion

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# Cerebrovascular Hypoperfusion: A Risk Factor for Alzheimer's Disease?

## Animal Model and Postmortem Human Studies

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**ABSTRACT:** Although cognitive impairment during aging is usually associated with neuronal alterations, the cerebrovascular system undergoes prominent alterations in aging as well. Using electron microscopy we previously showed a progressive deterioration of the capillary wall in the cerebral cortex of aged rats. In aged rats the capillary basement membrane (BM) is thickened, massive bundles of collagen fibrils are deposited within the BM, and pericytes are degenerating. A compromised cerebral circulation (e.g., in rats with chronic hypertension) is characterized by an increased number of capillary alterations. In autopsy material (gray matter, gyrus cinguli) of carefully diagnosed patient groups (controls, AD, Lewy body disease, MID and demented Lewy body disease patients) we observed significantly more morphological changes in the capillary bed of demented versus non-demented patients. In both animal and human material morphological evidence points to a relation between energy-dependent nutrient transport across the blood-brain barrier and the ultrastructural deviations. In the AD cases we did not find a correlation between the stage of the disease (Braak I-VI) and the incidence of capillary aberrations, which indicates that the capillary alterations are not a consequence of AD pathology. Simultaneously, we are conducting animal model studies to determine the effects of cerebral hypoperfusion in the rat. Permanent bilateral occlusion of the carotid arteries shifts the behavioral profile of the rats (Morris maze, open field) towards that of aged rats, while the sensitivity for muscarinic ligand agents is altered.

## INTRODUCTION

An adequately operating central nervous system (CNS) requires an optimal functioning and interplay of neuronal, glial and (micro)vascular systems. In aging, Alzheimer's disease (AD), and non-Alzheimer's dementia, the CNS is clearly af-

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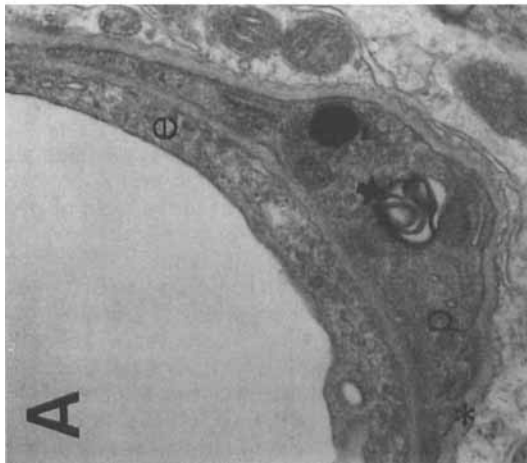
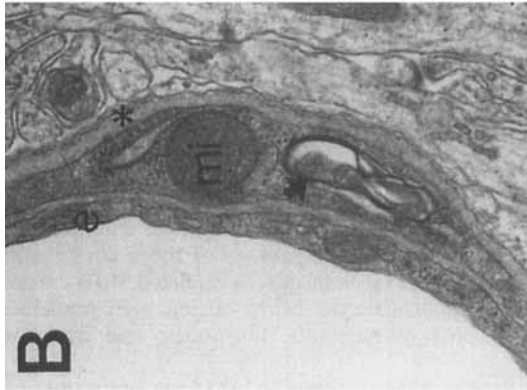
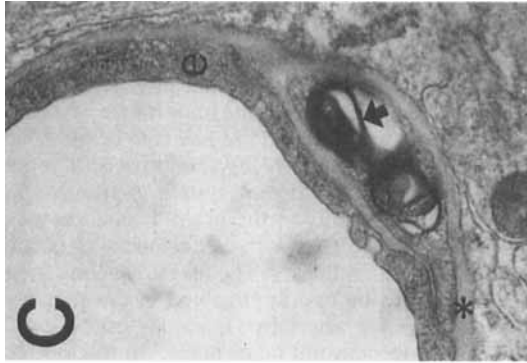
ected at all three levels, yielding prominent changes in brain structure and function. As a consequence, behavioral performance is considerably altered. The cognitive decline and its underlying mechanisms in aging and AD has been the subject of increasing extensive study throughout the last decades. Although most attention has been focused upon neuronal alterations, the importance of a disturbed cerebrovascular system in aging and dementia, and the key role of the endothelial cells in vascular and brain function, has become recognized in recent years.

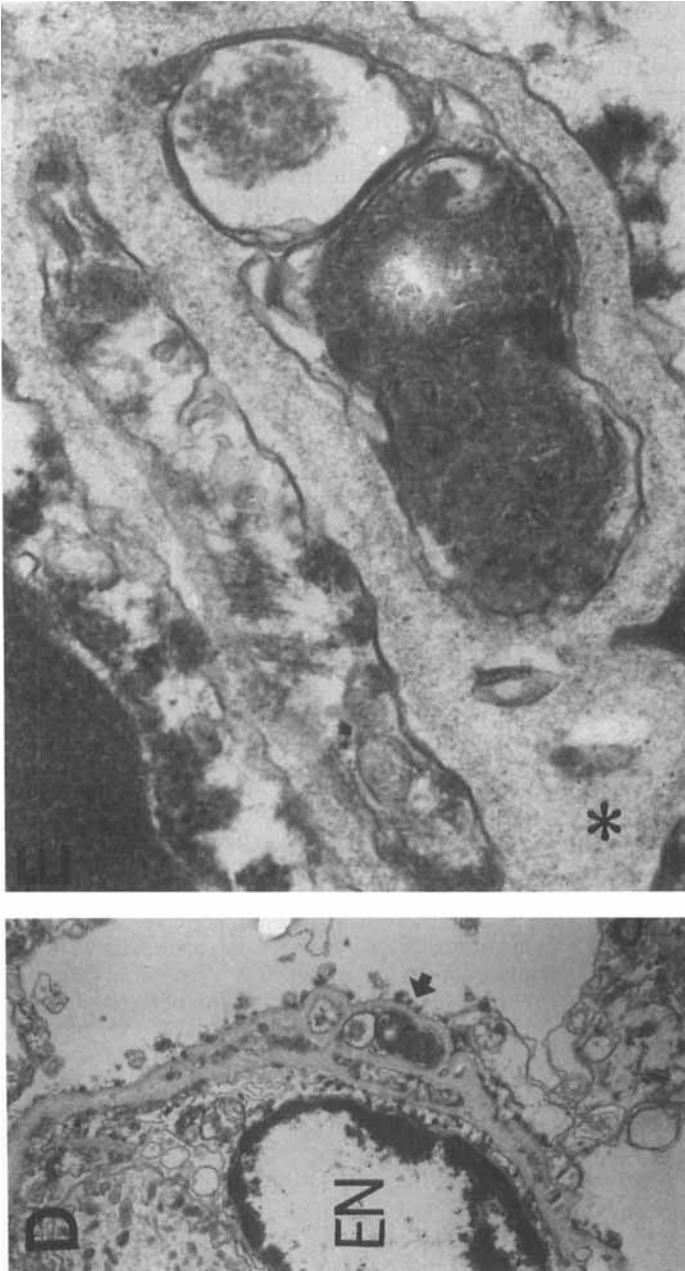
As in other organs, the cerebral vascular system guarantees an efficient and extremely well-controlled blood supply to the brain. Blood vessels providing flow to the brain can be classified into four types: the inflow tract arteries, arteries, intraparenchymal arterioles, and capillaries. The first three contain vascular smooth muscle cells and are thought to be mainly involved in the regulation of cerebral blood flow. Capillaries are the site where by far the largest bulk of active nutrient transport from blood-stream to neuropil takes place.<sup>1</sup> In this respect, cerebral microvessels possess unique properties comprising the blood-brain barrier (BBB,<sup>1</sup>). Electronmicroscopic techniques have shown that the major structural difference between cerebral vasculature and vessels in other organs can be recognized at the level of the endothelial cell. Endothelial cells within the CNS are closely interconnected by continuous tight junctions, lack fenestrations, and display very few pinocytotic vesicles.<sup>1</sup> These structural features of cerebral endothelial cells provide a rather selective barrier to prevent blood-borne substances from entering the neuropil. Yet, in order to meet the metabolic demands of the neuronal tissue, the transport of nutrients across the BBB becomes a prerequisite for brain function. Lipid-soluble substances can enter the brain by diffusion through the endothelial layer, although most nutrients are water-soluble. The passage of these components through the BBB requires active transport mechanisms. Specialized BBB carrier processes for glucose, amino acids, monocarboxylic acids, nucleic acid precursors and amines assure adequate transport of nutrients, hormones, and neurotransmitter precursors.<sup>2-4</sup>

Several studies in man have documented that the brain only oxidizes glucose to obtain energy in order to meet its functional and structural demands.<sup>5</sup> In the adult brain, the oxygen and glucose consumption ratio is well balanced to oxidative metabolism and energy production.<sup>5,6</sup> Metabolic requirements and cerebral blood flow are closely related, such that an increased energy demand is normally accompanied by an increased blood flow, while conversely, a reduced energy demand leads to an attenuated blood flow.<sup>7</sup> In both aged humans<sup>6</sup> and aged rats<sup>8,9</sup> a strong correlation is present between an impaired cerebrovascular system and a decreased neuronal activity. However, a sufficient cerebral blood flow is not the only vascular denominator for an adequate supply of nutrients to the brain to guarantee optimal neuronal functioning. Most important in this respect is the integrity of the BBB.

Aging and AD are associated with minimal, if any, alterations in BBB barrier function.<sup>3,10-15</sup> On the other hand, it has been demonstrated that transport of neutral amino acids,<sup>16</sup> small tyrosinated peptides,<sup>17</sup> choline,<sup>18</sup> glucose,<sup>19</sup> and tryptophan<sup>20</sup> across the BBB has been reduced in aged rats. Moreover, when the cerebral glucose transporter system in AD was compared with that of age-matched control brain samples, a specific reduction was encountered in the AD brain.<sup>21,22</sup> The latter may result in a reduced glucose availability in the brain, which in turn can have deleterious effects on neuronal metabolism and hence to normal neuronal functioning.

Altered integrity of BBB functioning is also reflected at the morphological level. Already in 1873, J. Batty Tuke<sup>23</sup> first described abnormalities in cerebral blood vessels of the elderly, which consisted of "straightness, tortuosity and kinking." In 1937, Pickworth reported that mental and emotional stages have histopathological





**FIGURE 1.** Degrating pericytes within the capillary basement membrane (\*) in the aged rat (A, B, C) and human (D, E) neocortex. Cytoplasmic degenerative inclusions can be observed in addition to normal pericytes.

representations in the cerebral capillary pattern and can be the fundamental cause of altered mental activity, abnormal behavior, and personality integration in mental illness.<sup>24</sup> Much later, detailed light- and electron microscopic examination of the cerebral blood vessels yielded a variety of morphometric and morphological alterations in various brain regions of aged rats,<sup>25-35</sup> aging humans,<sup>36,37</sup> and AD patients.<sup>38-41</sup> Growing evidence indicates that these morphological alterations directly hamper blood supply and nutrient transport across the BBB, leading to a disturbed electrolyte balance, synaptic activity, overall neuronal functioning, and concomitant impaired cognition in aging and AD. Whether the microvascular deterioration is a primary event, or a consequence of other neurodegenerative processes remains uncertain. The data presented in this paper, however, suggest a more than secondary role for an altered capillary bed in aging and dementia.

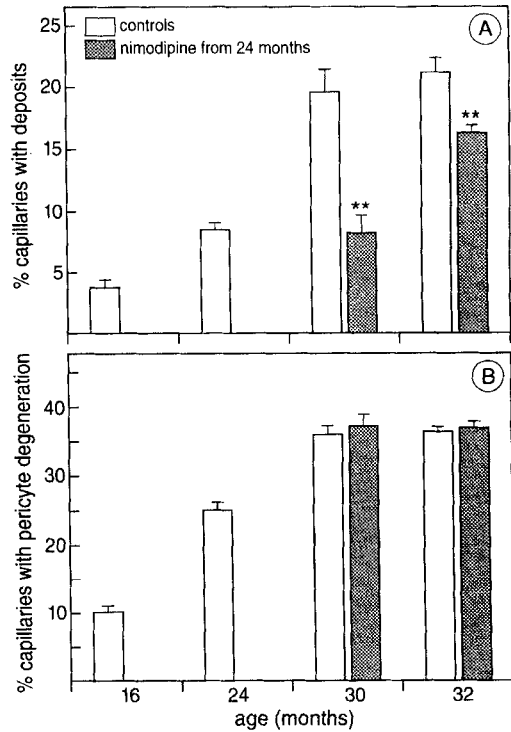
In our current studies, we use a bidirectional approach to gain insight into the relation between cerebral microvascular systems and AD neuropathology. At first, we performed a thorough analysis of the ultrastructure of the capillary bed of aging Wistar rats and rats with a compromised cerebral perfusion (i.e., spontaneously hypertensive rats). With an identical electron microscopic approach, we examined the capillary bed of human autopsy material from control, AD, and other neurodegenerative disease patients. In addition, we simulated a condition of chronic hypoperfusion, and possibly also chronic hypoglycemia, in the brain of Wistar rats by a permanent occlusion of bilateral carotid arteries. The impact of this hypoperfusion upon cognition, neuropathology and vulnerability to  $\beta$ -amyloid, for example, is under study.

## HISTOPATHOLOGICAL ALTERATIONS IN CORTICAL CAPILLARIES

### *Animal Studies: Cortical Capillary Breakdown and Aging*

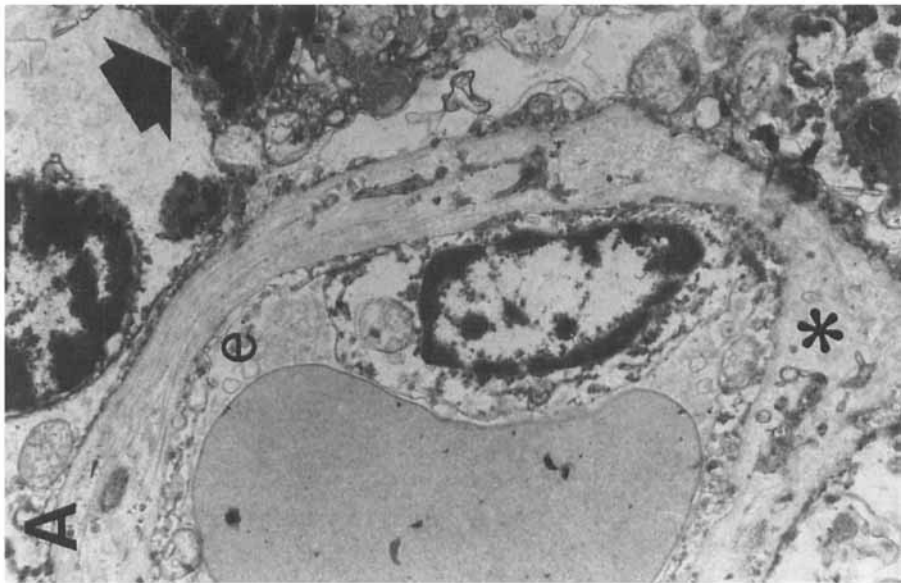
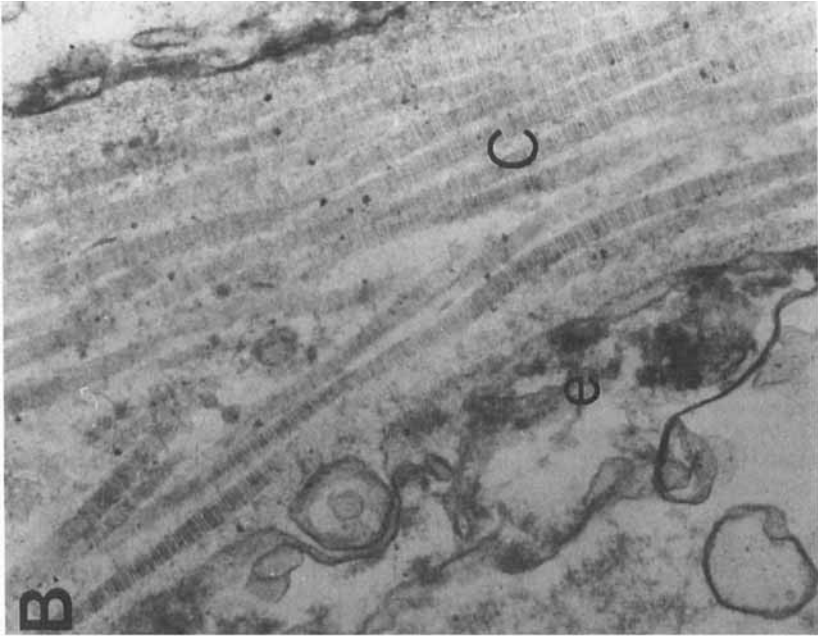
In our studies we specifically focused our interest on cortical capillaries because at this site the largest amount of nutrients are delivered to the surrounding neuropil. Moreover, Topple and colleagues<sup>42</sup> showed in aging rat hippocampus that capillaries are much more affected by the aging process than are larger vessels. A cerebral capillary typically consists of three cell types, the first of which is the specialized endothelial cell. The endothelial cell forms the actual blood-brain barrier (BBB) and is surrounded by a basement membrane. Furthermore, pericytes are embedded within the microvascular basement membrane, while the microvascular wall on the outside is covered by astrocytic endfeet. As reported in a series of papers, we extensively studied the occurrence of ultrastructural anomalies of the capillary wall throughout the second half of the lifespan of Wistar rats.<sup>33-35</sup> At the ultrastructural level we distinguished two basically different categories of age-related alterations: (1) membranous inclusions within the basement membrane, and (2) microvascular deposits consisting of collagen (fibrosis) or basement membrane components (thickening). The membranous inclusions (FIG. 1A-C) are enclosed and surrounded by the basement membrane and within the inclusions cytoplasmic elements such as mitochondria can be observed. Mainly because of the ultrastructural position in the microvascular wall, these membranous inclusions are considered to reflect degenerative stages of pericytes.<sup>32-35,40,43</sup> With advancing age, the incidence of capillaries with degenerative pericytes gradually increased and reached its maximum around 30 months of age, when 35% of the capillaries contained degenerative pericytes (FIG. 2B).

**FIGURE 2.** The percentage of capillaries with (A) deposits and (B) pericyte degeneration in the frontoparietal cortex in the second half of the lifespan of Wistar rats, and in animals treated with the calcium entry blocker, nimodipine, from 24–30 and from 24–32 months of age ( $\% \pm \text{SEM}$ ; \*\*  $p < 0.01$ ).

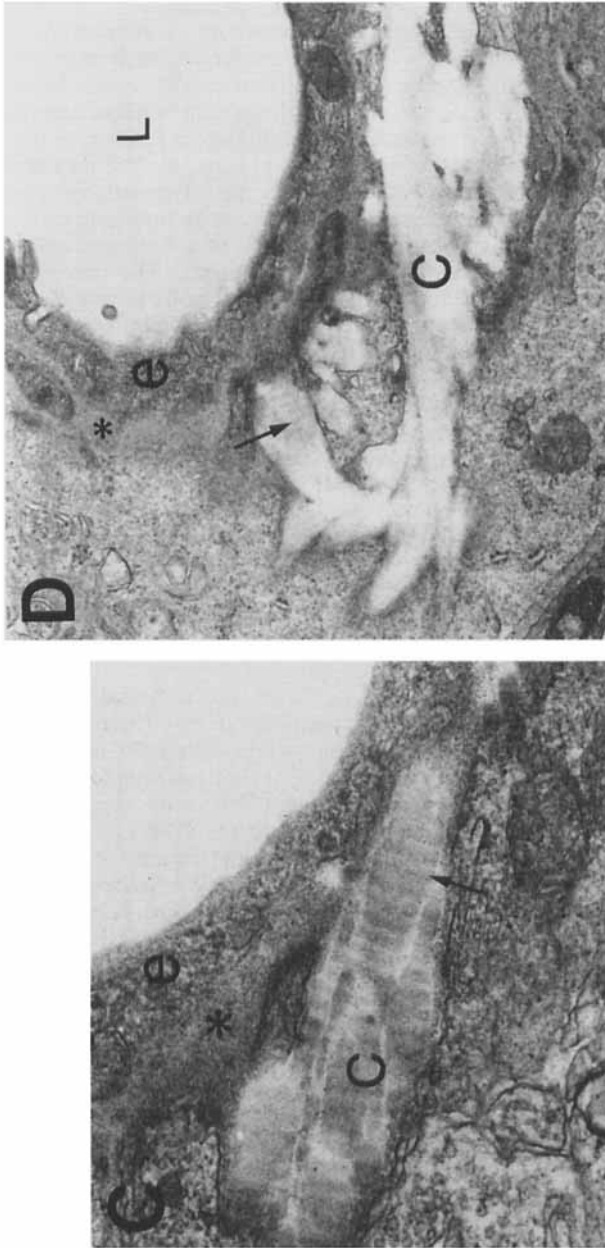


Deposition of collagen fibrils within the microvascular basement membrane has been reported before in the aged mammalian brain.<sup>11,29,32–35,40</sup> Such microvascular fibrosis (FIG. 3C, D) is characterized by banded fibrils deposited within the basement membrane. The periodicity of 64 nm of the fibrils lead to the identification of collagen as the main constituent of microvascular fibrosis.<sup>11,33–35</sup> In our own studies and those of others,<sup>11,29,33–35</sup> collagen deposits in the basement membrane were encountered between the endothelium and pericyte as well as in the outer basement membrane. Together with the identification of short collagen-like fibril fragments in the endothelial cytoplasm it may be concluded that the endothelial cell is involved in the formation and production of collagen fibrils within fibrotic plaques, and may be the source of perivascular fibrotic deposits. We also observed numerous thin basement membranes with a heavy investment of collagen fibrils extending into astrocytic endfeet (data not shown). This indicates also that astrocytes, possibly in concert with endothelial cells, may participate in the formation of fibrosis.

Many morphometric studies described an increased thickness of the capillary basement membrane in the brain of aged mammals.<sup>11,29,30,33–35,38,41,42</sup> We quantified local basement membrane thickenings (BMT) during aging and found a very low incidence in rat brain until the age of 30 months. At 32 months, however, BMT suddenly increased and was encountered in approximately 10% of all cortical microvessels. We observed thickening of the basement membrane between both endothelium and pericytes as well as in the outer basement membrane. This suggests that







**FIGURE 3.** Microvascular fibrosis in the capillary wall from human (A, B) and aged rat (C, D) neocortex. The deposited collagen fibrils (c) within the basement membrane (\*) are prominently present (e, endothelial cytoplasm, L, lumen).

in the aged rat brain endothelial cells, pericytes, and astrocytes all may potentially be involved in the formation of BMT.

Extensive ultrastructural examination justified a classification in which both fibrosis and BMT are combined into a single category of aberration: capillary deposits. The endothelial cytoplasm of most microvessels with deposits possessed a large number of pinocytotic vesicles, suggesting that microvascular deposits profoundly influence transport functions of the endothelial cell.<sup>35</sup> Others suggested that BMT formation may serve as a response to an altered barrier function of the BBB.<sup>42</sup>

In line with the "calcium hypothesis of aging and dementia"<sup>44,45</sup> we also investigated the influence of a long-term treatment with the L-type calcium antagonist nimodipine upon the occurrence of capillary aberrations in the aging rat brain.<sup>33-35</sup> This hypothesis is based on numerous observations of a deranged intracellular calcium [ $\text{Ca}^{2+}$ ], homeostasis during aging and in dementia. The outcome of this long-term drug treatment yielded some striking results. The frequency of capillaries with degenerative pericytes was not influenced by a chronic administration of nimodipine (FIG. 2A), while the formation of microvascular deposits was significantly delayed in the aging nimodipine treated rats (FIG. 2A).

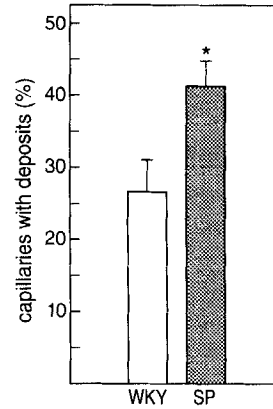
In conclusion, the aging process in the rat brain is accompanied by very specific and well-documented ultrastructural alterations of the capillaries, which might have its impact on the transport of nutrients over the BBB. The degeneration of pericytes is apparently not caused by an increased  $\text{Ca}^{2+}$  influx, whereas the occurrence of capillary deposits is related to an altered [ $\text{Ca}^{2+}$ ], homeostasis in either the capillary endothelial cells or in the circumventing astrocytes that involves voltage-dependent  $\text{Ca}^{2+}$  channels.

### ***Animal Studies: Cortical Capillary Breakdown and Hypertension***

Chronic hypertension affects cerebral perfusion and neuronal functioning in both rats<sup>46</sup> and humans<sup>47</sup> in such a way that cognitive and other behavioral functions can be impaired. The influence of a compromised cerebral perfusion on the incidence of capillary aberrations was studied by comparing neocortical samples from middle-aged (40 weeks) spontaneously hypertensive rats (SHR) with samples from age-matched normotensive controls from the Wistar Kyoto (WKY) strain. With an identical morphometric setup as previously used to determine the effects of age in normotensive animals, we demonstrated that a chronic hypertensive condition significantly accelerated the formation of capillary deposits (FIG. 4), whereas, again, the number of degenerative pericytes was not different between SHR and WKY animals.

### ***Human Postmortem Brain: Cortical Capillary Breakdown in Neurodegenerative Disorders***

Human autopsy samples of the gyrus cinguli were obtained from 15 patients, with a postmortem delay between 9–32 hours. Samples of approximately 20 mm<sup>3</sup> in size were immersed in a buffered fixation fluid containing 2% glutaraldehyde, 1% paraformaldehyde. The tissue blocs were cut into 50- $\mu\text{m}$ -thick sections with a vibratome, and were routinely embedded for electron microscopic examination. Of each sample the capillary ultrastructure was examined in the gray matter throughout the entire cortical depth. Irrespective of the postmortem delay before fixation, the ultrastructure of the autopsy material was preserved at such a high level that



**FIGURE 4.** The percentage of capillaries with deposits in the frontoparietal cortex of middle-aged normotensive (WKY) and hypertensive (SP) rats (%  $\pm$  SEM; \*  $p < 0.05$ ).

quantitative examination of the cerebral capillaries could be performed in exactly the same way as was previously done with the aged rat brain samples (see above). We preferred autopsy material over biopsy material because it enabled us to examine human control brain samples as well. Moreover, autopsy material is accompanied by an accurate neuropathological diagnosis.

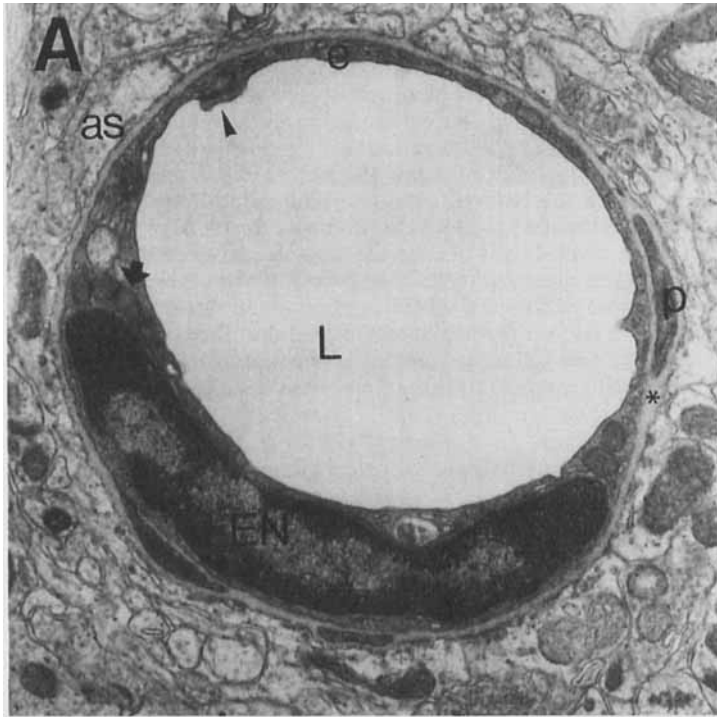
The patients were divided into five different groups, and each patient was carefully screened on the basis of extensive neurological and neuropathological diagnostic criteria. The first group was the *control subjects* ( $n = 5$ ). These patients did not display any clinical signs of cognitive disturbance or dementia, and showed no neuropathological abnormalities. The second group, those with *Alzheimer's disease* ( $n = 3$ ) scored  $<20$  on the MMSE, and were neuropathologically diagnosed according to both CERAD (probable or definite) and Braak and Braak (stage V or VI)<sup>48</sup> criteria. Patients suffering from *cerebrovascular disease* (CVD,  $n = 3$ ) were assigned to the third group; all three patients were demented (i.e., scored  $<20$  on the MMSE). The last two groups had *Lewy body disease* (LBD) with clinical signs of Parkinson's disease with ( $n = 3$ ) or without ( $n = 1$ ) dementia. The demented LBD patients showed early neuropathological (Braak stage III) signs of AD.

### *Pericyte Degeneration*

The ultrastructure of the autopsy material was well preserved (FIG. 5) and in the capillary bed we detected the same morphological categories of aberration as in the aging rat brain. Both degenerative pericytes (FIG. 1D,E) and capillary deposits (FIG. 3 A,B) were encountered. In all human samples, from either control or disease patients, approximately 35% of the capillaries were endowed with degenerative pericytes (data not shown), which was similar to the incidence found in the aged (30–32 month) rat brain. Previously, Claudio<sup>43</sup> described an equal frequency of capillaries with pericyte changes in biopsy material from five AD patients, and attributed this morphological aberration specifically to AD. We hereby demonstrate that capillary pericyte degeneration, a phenomenon that is not sensitive to calcium antagonist treatment in the rat, is not confined to AD patients, but can be observed with a similar frequency in human controls, and in patients with AD, LBD and CVD. This degeneration thus is unrelated to the occurrence of dementia.

*Microvascular Deposits*

Capillary deposits were commonly encountered in the human cortical material. Thickening of the basement membrane and deposition of collagen within the basement membrane were found to be morphologically identical to the appearance in the rat (FIG. 3A,B). Previous reports described the occurrence of fibrosis or BMT in the microvascular basement membrane of AD patients, and found an increased content of collagen type IV in cerebral microvessels of AD patients when compared to age-matched controls.<sup>49</sup> Using a quantitative approach, we observed a significantly higher percentage of capillaries with deposits not only in AD patients, but also in the LBD/AD group (FIG. 6A). CVD patients also displayed more aberrant capillaries, but this did not reach significance, possibly caused by the limited number of patients examined at present (FIG. 6B). When the non-demented patients are compared with all demented patients, a highly significant ( $p < 0.02$ ) difference in the occurrence of capillary deposits becomes apparent (FIG. 6B).



**FIGURE 5.** The ultrastructural features of a nonaberrant capillary in the neocortex of (A) rat and (B) human. (A) In the perfused rat brain capillary the lumen (L) is empty and the endothelial cytoplasm (e) with mitochondria (arrow) and nucleus (EN) can be identified, as can tight junction (arrowhead) interconnecting endothelial cells. Pericyte cytoplasm (p) is also enclosed by the capillary basement membrane (\*). (B) The human capillary is completely filled with an erythrocyte (ER). Within the endothelial cytoplasm (e) mitochondria can be observed (arrow) and the ultrastructural integrity of the delineating basement membrane can be studied in detail.

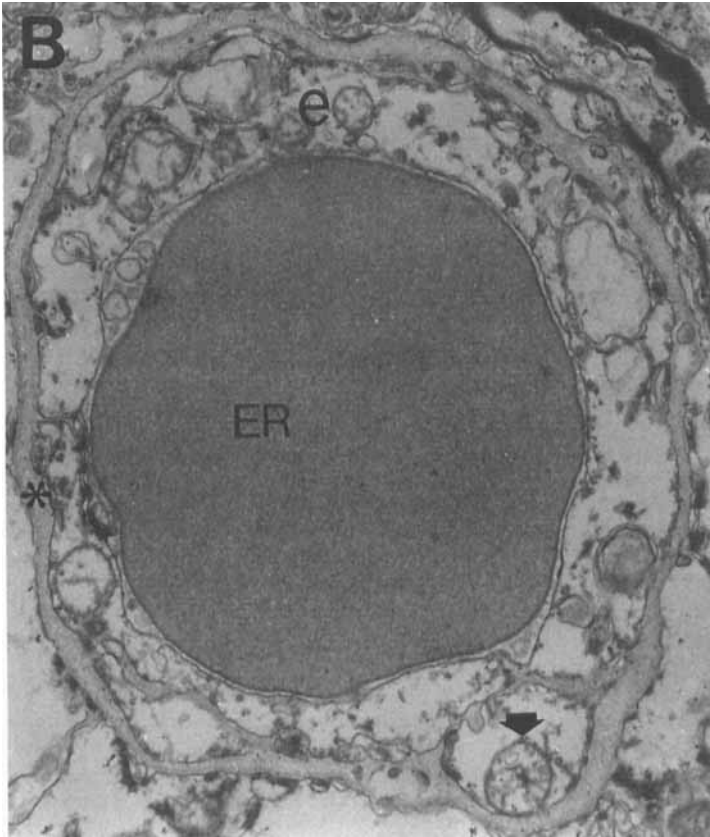
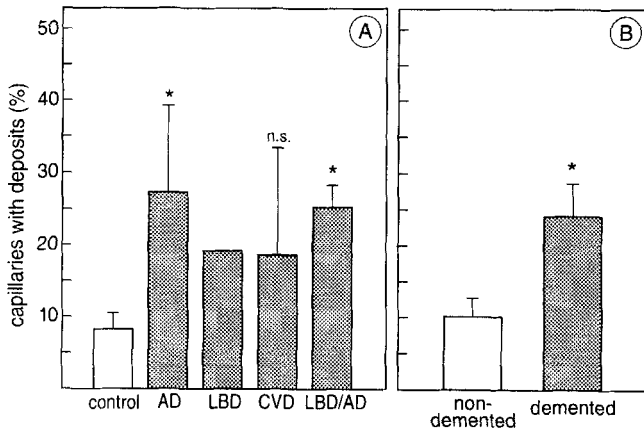


FIGURE 5 (Continued).

Using immunocytochemical markers, Kalaria and coworkers observed microvascular endothelial degeneration in the brains of AD, LBD/AD and Down syndrome patients, and suggested that this degeneration process was related to the deposition of  $\beta$ -amyloid in the neuropil.<sup>50</sup> With respect to the incidence of capillary deposits we were not able to differentiate between CVD, LBD (without severe amyloid deposits in the neuropil), and AD, LBD/AD patients. Therefore, it seems unlikely that capillary deposits are directly related to the deposition of  $\beta$ -amyloid in the neuropil. Moreover, we observed capillary amyloid angiopathy only in one patient and in two out of a total of 2,200 (!) capillaries studied. Vinters and colleagues concluded that cerebral amyloid angiopathy is related to vascular smooth muscle cell degeneration rather than endothelial destruction.<sup>51</sup> For this reason a distinction between capillaries (without smooth muscle cells) and other microvessels (arterioles) seems justified.

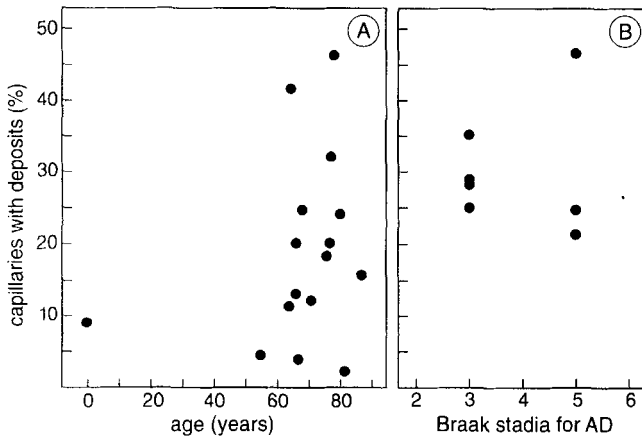
We hypothesize that the capillary deposits could lead to an impaired glucose/nutrient transport across the endothelial cell, inducing a chronic decreased glucose availability in the brain. The latter may be more closely related to cognitive distur-



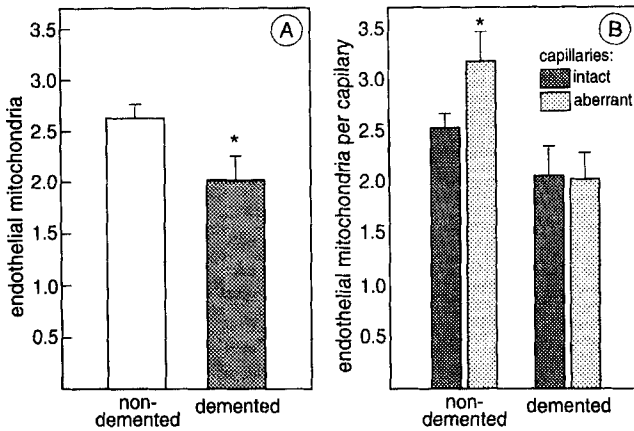
**FIGURE 6.** (A) the percentage of capillaries with deposits in the cingulate cortex of human controls, and patients with AD, CVD, and LBD without or with (LBD/AD) early AD characteristics. (B) the percentage of capillaries with deposits in the cingulate cortex of non-demented and demented patients (%  $\pm$  SEM; \*  $p < 0.05$ )

bances in general, rather than specifically to AD. The importance of a reduced glucose availability is reflected in the recent observation that induced hyperinsulinemia improves memory in AD patients.<sup>52</sup>

It has been questioned whether the capillary distortions observed with the electron microscope are a cause or a consequence of AD<sup>53</sup> or non-AD dementia.<sup>45</sup> In this respect we examined the relation between the degree of capillary deposits and neuropathological disease stage (according to Braak<sup>48</sup>) of the examined AD and LBD/AD patients (Fig. 7B). In spite of the limited number of data, we found



**FIGURE 7.** The percentage of capillaries with deposits in the cingulate cortex of the investigated human samples plotted against (A) age and (B) Braak stage of AD.



**FIGURE 8.** (A) the number of mitochondria in the endothelial cytoplasm per capillary encountered in non-demented and demented patients. (B) The number of mitochondria per capillary in intact or aberrant capillaries, classified for non-demented and demented patients (numbers  $\pm$  SEM;  $p < 0.05$ ).

no evidence that the integrity of the capillary wall is more compromised in the brains with more-advanced AD pathology. Also, the age factor was not a strong determinant for capillary aberrations in the gyrus cinguli (FIG. 7A).

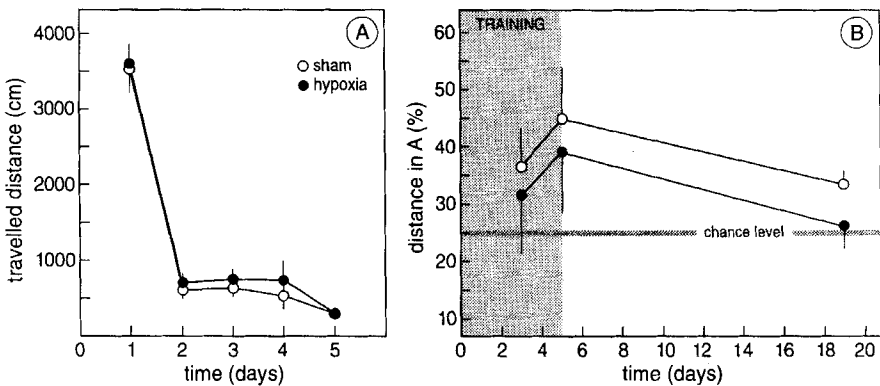
The deleterious consequences of the capillary deposits have been hypothesized to lead to a disturbed nutrient transport capacity of the affected endothelial cell.<sup>53</sup> In accordance with this view, the number of glucose transporters has been shown to be reduced in AD brains.<sup>21,22</sup> Morphological support for the disturbed nutrient transport theory came from observations in the aged rat brain where endothelial cells with abnormally high numbers of pinocytotic vesicles in combination with deposits were commonly encountered.<sup>35</sup> Also, a declining number or size of endothelial mitochondria was previously reported in the aging rat and human brain.<sup>26,43</sup> In the present study we found a negative correlation between the number of mitochondria encountered in one transversely sectioned capillary and the percentage of capillaries with deposits (data not shown), indicating a compromised energy status of endothelial cells of morphologically aberrant capillaries. We also observed significantly fewer mitochondria per capillary in demented patients when compared to non-demented controls (FIG. 8A,  $p < 0.03$ ). Even more interesting was the difference between intact capillaries and capillaries with deposits in non-demented and demented humans. FIGURE 8B shows that in non-demented humans, the number of mitochondria was higher in the aberrant capillaries with deposits than in intact capillaries. This suggests that the number of endothelial mitochondria is increased when morphological barriers such as thickening or collagen deposition compromise an appropriate energy-dependent nutrient transport across the endothelial cell. The capillaries of demented patients did not demonstrate such a compensation-like feature. In other words, they possessed fewer mitochondria in intact vessels, and missed the capacity to increase this number in aberrant capillary endothelial cells (FIG. 8B).

In summary, we observed an ultrastructural similarity between capillary anomalies in the aging rat and human brain. The age per se was not a prominent determi-

nant of the incidence of these anomalies in humans, and, also, different neurodegenerative disorders increased the incidence of capillary deposits, but not pericyte degeneration. The aberrations appeared more closely related to dementia in general rather than specifically to AD. In non-demented patients the number of endothelial mitochondria seemed to be compensationally increased in aberrant capillaries, a feature that is not encountered in the demented patients.

### ACUTE AND CHRONIC HYPOPERFUSION ANIMAL MODEL STUDIES

In order to obtain rats with a compromised cerebral microcirculation we used an acute and a chronic hypoperfusion model. In the acute model the rats were anaesthetized, followed by 5 minutes of unilateral carotid artery occlusion (mild reversible ischemia) combined with the inhalation of 10% O<sub>2</sub> and 90% NO<sub>2</sub> (hypoxia). In the hippocampus and neocortex of these rats, no silver degeneration was observed either 24 hours or 5 weeks after hypoxia. After a survival period of 5 weeks, the rats were trained to find the escape platform in the Morris water maze during five consecutive days with two trials per day. The hypoxia group learned to find the escape platform equally fast as the sham-operated control rats (FIG. 9A). During the training period (on day 3 and day 5, after the training sessions) and 14 days after the last training session the information retrieval was tested by placing the animals in the Morris water maze where the hidden platform was removed and the animals had 60 sec to search the maze. The maze is divided into 4 quadrants, and when the animal is searching for the hidden platform it will spend more than 25% of the search time in the correct quadrant. In this so-called probe trial, both groups stored the spatial information (that is, the % distance travelled increased throughout the training period). A remarkable difference between the hypoxic and sham rats was observed in the probe trial 14 days after training, where the hypoxic animals performed at chance level, whereas the sham-operated controls were still

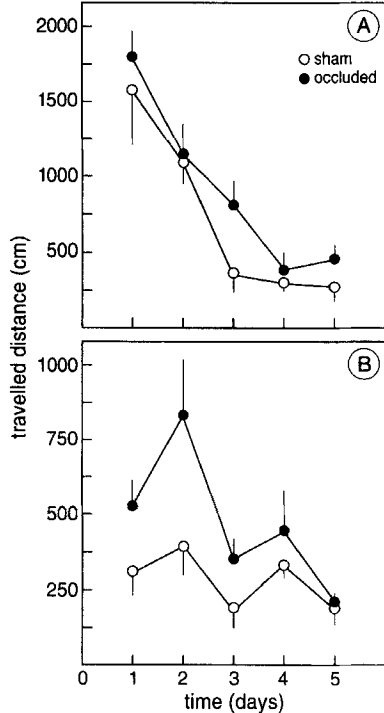


**FIGURE 9. (A)** The distance hypoxic and sham-operated groups swam in the Morris water maze before reaching the escape platform during 5 consecutive days, in which each day gives the average of two trials. **(B)** Swimming distance for both groups in the probe trials given after learning trials on days 3, 5, and 19. The animals were trained during days 1-5; thereafter the animals were not disturbed until day 19 (mean  $\pm$  SEM).

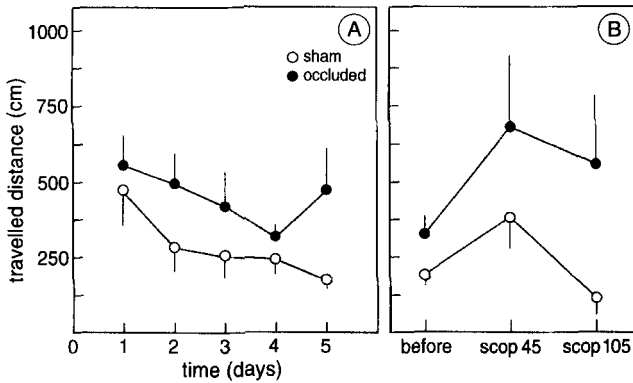


searching for the platform in the correct quadrant (FIG. 9B; the overall performance of the groups was significantly different:  $F(2,16) = 7.1; p < 0.006$ ). The same animals were re-tested in the Morris water maze after survival periods of 10 weeks and 1 year, yielding no significant differences between hypoxia and sham animals (data not shown). Thus, a very mild and short-lasting period of cerebral hypoperfusion yields a very subtle and transient effect on the retrieval of spatial information.

As a chronic model of cerebrovascular hypoperfusion, we used the permanent two-vessel occlusion (2-VO) model previously described by de la Torre *et al.*<sup>53</sup> and Ni *et al.*<sup>54</sup> The internal carotid arteries of young adult (3 months of age) Wistar rats were permanently occluded and the rats were followed for the extensive period of 1 year. After a relatively short survival period of 5 weeks, the rats were trained in the Morris water maze twice a day for 5 consecutive days. The 2-VO rats learned to find the escape platform in the Morris water maze one day later than did the sham animals (FIG. 10A; group difference:  $F(1,19) = 3.92, p < 0.06$ ). The same animals were re-tested 10 weeks after occlusion, where a significantly reduced cognitive performance was observed on the first training day (FIG. 10B). On the second training day, the location of the platform was altered, a situation to which the sham animals adjusted significantly quicker than did the 2-VO rats (FIG. 10 B; group difference:  $F(1,19) = 6.1, p < 0.02$ ). The same rats were again tested after a survival period of 1 year, where the 2-VO rats displayed a significantly impaired spatial memory (FIG. 11A; group difference:  $F(1,19) = 5.6, p < 0.03$ ) when compared to sham animals. Shortly after, we investigated whether these sham-operated



**FIGURE 10.** Distance travelled in the Morris swimming maze in 2-VO and sham-operated rats during training for five consecutive days **(A)** 5 weeks and **(B)** 10 weeks after surgery (mean  $\pm$  SEM).



**FIGURE 11.** Distance travelled in 2-VO and sham-operated rats **(A)** 1 year after surgery and **(B)** 1 year after surgery, 1 day before, and 45 and 105 minutes after an i.p. injection with 0.3 mg/kg scopolamine (mean  $\pm$  SEM).

and 2-VO rats reacted differently to a bolus injection of the muscarinic antagonist scopolamine. The classical response to cholinergic blockade in sham animals (that is, a transient impairment of cognitive function) was significantly attenuated in 2-VO rats (FIG. 11B; group differences:  $F(1,19) = 6.0, p < 0.02$ ), indicating that chronic hypoperfusion can induce specific neurochemical alterations as well.

The present and earlier data<sup>53,54</sup> strengthen the hypothesis that cerebral hypoperfusion leads to cognitive impairment. Whether this is sufficient to induce a pathologic picture that bears a resemblance to some of the specific AD features remains an unanswered question at this moment. The effects of chronic hypoperfusion on capillary ultrastructure and other neuropathological changes is currently under study using the animals that survived the chronic 2-VO for 1 year.

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