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STEREOLOGICAL CHANGES IN THE CAPILLARY NETWORK AND NERVE CELLS OF THE AGING HUMAN BRAIN*

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SUMMARY

Stereologic parameters of the capillaries and nerve cells of the brain cortex and putamen were investigated. Thirty-eight brains from subjects aged between 19 and 94 years were examined. All cases were free of metabolic, neurologic and psychiatric diseases.

It is demonstrated that the capillary diameter remains unchanged during aging in both brain cortex and putamen. However, in the putamen the total capillary length per unit volume and the capillary volume fraction increase (~60%) progressively with age. Consequently the mean inter-capillary distances in the putamen decrease (~15%). These age-induced changes in the putamen indicate shrinking of subcortical brain structures.

In contrast to those of the putamen, the morphometric data of the capillaries in the cortex remain unchanged during the aging process.

Stereologic investigations of nerve cells in the brain cortex and putamen revealed that only in brains over 85 years of age can a significant decrease in nerve cell size be demonstrated.

A correlation of all the data by a correspondence analytical procedure showed that only the surface/volume ratio of the capillaries correlates with the nerve cell size. This observation suggests a functional interaction between the nerve cells and the capillaries.

From the data presented it becomes apparent that the shrinkage of the gyri in the aging brain is not a change in the volume of the cortex, but is a decrease in the volume of subcortical structures.

INTRODUCTION

Since the investigations by Kety and Schmidt, we have known that the microcirculation and glucose and oxygen uptake in the aging brain decrease [1–3]. The observation of a decrease in microcirculation led to the false assumption that "an aging brain is as old

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as its vessels", and to the therapeutic concept of vasoactive treatment to improve microcirculation in the brain. Vasoactive therapy, however, failed to improve disturbed cognitive functions of the aging brain [4–6]. Contrary to microcirculation which was decreased, total blood volume in the brain was found to be increased [6].

The correlation between decrease in microcirculation and oxygen uptake on the one hand, and the alteration of the capillary network and a loss of nerve cells on the other, is at present an open question. We were therefore interested in studying the questions:

(1) What changes does the capillary network undergo during the aging process?

(2) How are the capillary parameters correlated with changes in nerve cell parameters?

MATERIAL AND METHODS

The 38 human brains studied come from cases of sudden death (myocardial infarction, *etc.*). All subjects, who were aged between 19 and 94 years, were free of metabolic, neurologic and psychiatric diseases.

Putamen and brain cortex (frontal, occipital, pre- and post-central gyrus, superior and inferior temporal gyrus) were stereologically examined.

The time post-mortem up to withdrawal and deep-freezing the tissue blocks varied between 4 and 24 hours.

In all cortical regions the capillaries were specifically stained with an alkaline phosphatase reaction in 14-μm thick sections of fresh tissue [7].

The selectivity of capillary staining was studied by three-dimensional reconstruction of the capillary network [8]. The effect of post-mortem delay on morphometric parameters of the brain cortex capillaries was investigated experimentally by Hunziker and Schweizer [9]. The capillaries were measured with a Leitz-Classimat optical–electronic image-analysis system [10]. The data determined were statistically evaluated by computer to yield the overall average values of morphometric parameters.

Quantitative investigations of neuronal cells in the cortex and putamen were performed with a Leitz Texture Analyser. The stereological study of nerve cells with a great variety of shape and size is considerably facilitated by the two-dimensional logic system [11] used in quantitative image analysis in the texture analyser.

In defined areas from the putamen and pre-central gyrus of the human brain, ten serial frozen sections of 14 μm thickness were randomly selected. The scanning stage of the texture analyser was programmed to start at the pial surface and to reach the white matter in steps of 300 μm (magnification factor of the objective: 40 X). An electronically set measuring field (mask) of the texture analyser measured each neuron of which the nucleolus was visible in the section [12]. On the basis of the concept of an isotropic opening procedure [11], the texture analyser measured 18 size classes (hexagonal openings) from each neuron, thus allowing the determination of perikaryal size and shape (Schulz and Hunziker, *J. Gerontol.*, in press).

The morphometric results were evaluated according to the correspondence analysis theory of Benzecri [13, 14].

EXPERIMENTAL RESULTS

The *capillary parameters* investigated were: diameter, volume fraction, specific area, mean intercapillary distances, and total capillary length per unit brain volume (Figs. 1–3).

Fundamental differences in the capillary parameters in brain cortex and putamen occur (Fig. 2), which are independent of the aging process and have anatomical causes. The capillary diameter in cortex and putamen is identical (6.3 ± 0.3 μm; diameter of an erythrocyte 7.0–7.5 μm). The putamen has, however, significantly (≅80%) shorter capillaries, and consequently a lower capillary volume (Table I). The capillary distances in the putamen are greater than in the cortex. In other words, the putamen has a less-developed capillary network than the cortex.

Investigations of age-induced changes in the capillary parameters of the *putamen* demonstrated no changes of the capillary diameter (Fig. 1). With increasing age, however, there is a significant increase in the total capillary length (≅ +62%), and a decrease in the mean intercapillary distance (≅ −13%), which results in an increase in the capillary volume (≅ +54%). The surface/volume ratio decreases by about 10% (Table II).

Age-related changes in the capillary network of the *brain cortex* are slight (Fig. 3). The morphometric values of the 75–95-year-old subjects oscillate around the mean values of the 19–54-year age group (the age group of 64–75 years is disregarded).

A surprising observation in the aging brain cortex was the significant increase in the capillary diameter (from 6.38 μm ± 0.33 to 7.06 μm ± 0.24; ≅ +10%), which was found exclusively in the 64–75 years age group. In this group, an increase in capillary length (from 21.93 cm/mm^3 ± 3.57 to 25.7 cm/mm^3 ± 3.33) and capillary volume (from 2.32% ± 0.38 to 3.08% ± 0.56; ≅ +26%) (Fig. 3), and a decrease in the surface/volume ratio (≅ −8%; from 0.489 μm^{-1} ± 0.017 to 0.450 μm^{-1} ± 0.011) were also observed. These changes were found in all other cortical areas investigated.

Because of the difficulty of counting *nerve cells* in the brain cortex, in the following perikaryal area, perimeter and shape in randomly selected neurons from the different layers of the brain cortex were measured.

We observed that only in patients over 85 years do the nerve cells become significantly smaller in comparison with those of the group aged between 19 and 44 years (Fig. 4). The mean area of the cortical nerve cell layer II (cortical depth 330–530 μm) decreased from 176.1 μm^2 in the young brains (19–44 years, n = 6) to 130.2 μm^2 in the aged brains (85–94 years, n = 7). The values in the external pyramidal layer III show similar changes (19–44 years, 231 μm^2; 85–94 years, 189 μm^2). No significant nerve-cell alterations were seen in the group aged between 65 and 74 years.

Identical results were obtained in the putamen: in the young brain (19–44 years, n = 7) the mean area of the nerve cells was 145 μm^2 and significantly decreased in the old brain (85–94 years, n = 6) to 126 μm^2.

With the aid of correspondence analysis it was shown that only the surface/volume ratio of the capillary network is directly correlated with age-dependent size changes of the nerve cells (Fig. 5).

Stereological parameters of the human putamen

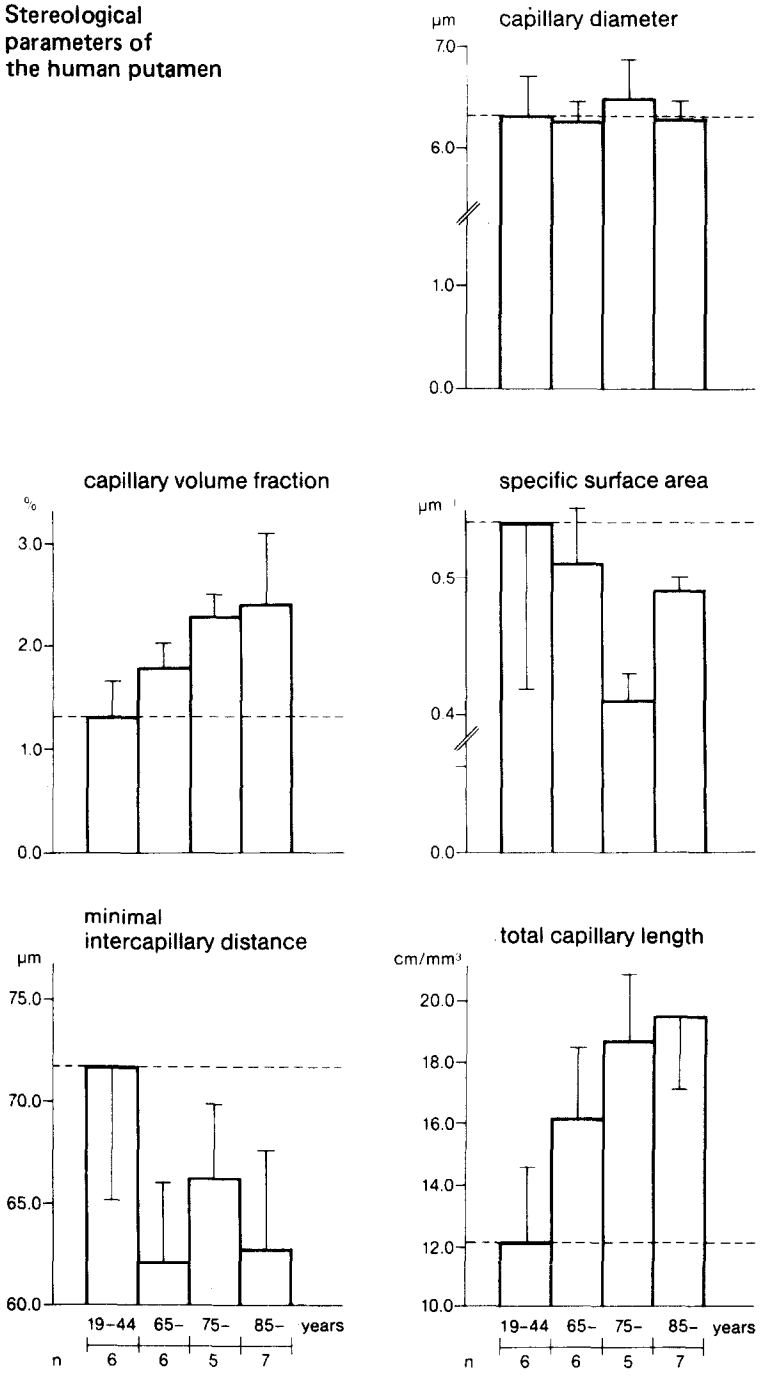

Fig. 1. Age-induced changes in the capillaries of the putamen. With increasing age a progressive increase in capillary length and capillary volume develops. The mean capillary distances decrease with age.

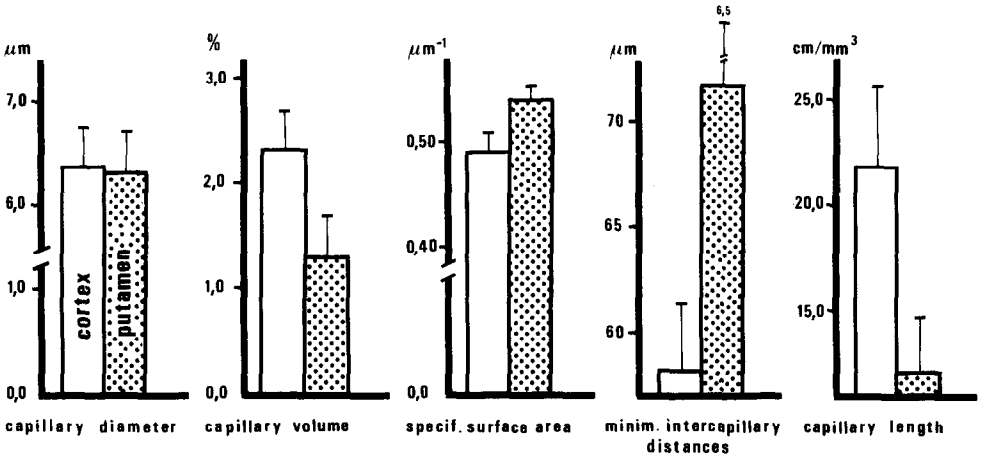

Fig. 2. Comparison of basic stereologic anatomic parameters of the capillary network in brain cortex (19–54 years, n = 10) and putamen (19–44 years, n = 6). It is shown that the capillary diameters in both structures are the same (6.3 µm). Capillary length and volume are greater in the cortex (≅ +80%) than in the putamen. Consequently the intercapillary distance in the cortex is smaller (≅ −20%).

DISCUSSION

In the brain cortex we have observed that the capillary parameters in patients older than 75 years were similar to those in subjects of 19–54 years of age.

Investigations of the brain cortex of subjects between 65 and 74 years of age revealed a significant increase in capillary diameter, capillary volume, and capillary length per unit cortex volume [15]. This surprising observation was not a consequence of visceral circulatory disturbances such as hypertension, heart failure or other circulatory diseases. According to our observations, this capillary alteration seems to be an isolated phenomenon in the brain cortex.

Two hypotheses might explain this unexpected result:

(1) The existence of two different genetic populations: one dying between the ages of 65 and 74 years, and another with a life expectancy of more than 80 years, as demonstrated by stereologic investigations of the capillary network.

(2) A disease of the vascular network in the brain cortex might be the cause of death in the population with the shorter life expectancy. Preliminary submicroscopic studies, however, do not indicate conclusively that morphologic changes of the capillaries would be the reason for the stereologic changes observed.

In contrast to the brain cortex, which does not show a progressive, age-dependent trend in capillary changes, we observed in the putamen an age-dependent decrease in intercapillary distances, and an increase in total capillary length and capillary volume.

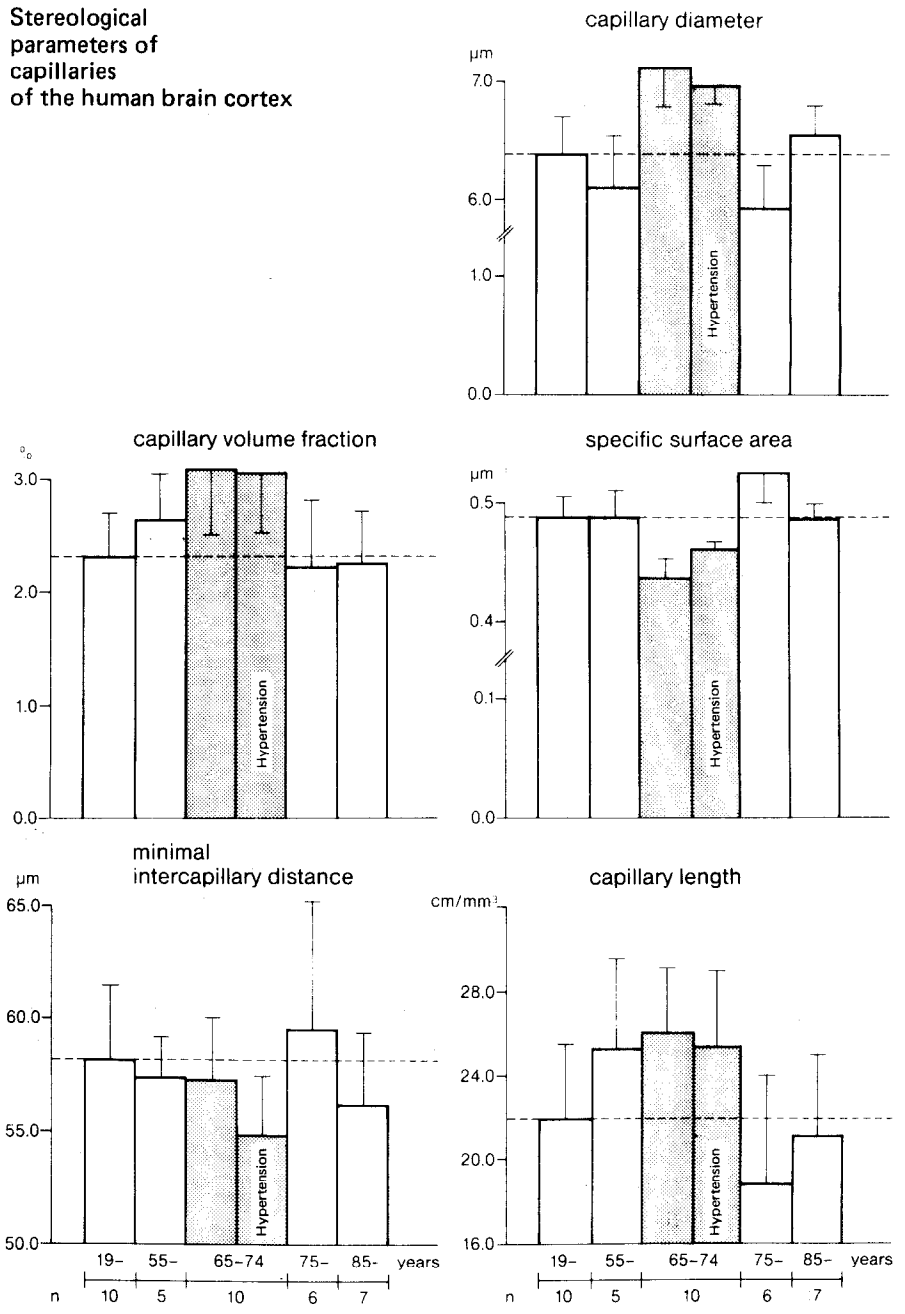

Fig. 3. Stereologic results of the cortical capillaries (precentral gyrus) in five age groups between 19 and 94 years (n = 38). The capillary parameters of the age groups 19–54 and 75–94 years are in the same range. Only the age group 65–74 years (n = 10) is characterized by pronounced capillary changes: capillary diameter (≅ +10%), capillary length (≅ +15%) and volume (≅ +25%) increase; surface/volume ratio decreases accordingly (≅ −8%).

TABLE I

ANATOMICAL DIFFERENCES IN THE CAPILLARY PARAMETERS OF BRAIN CORTEX AND PUTAMEN

Values are the means (±SD) of subjects between 19 and 44 years of age

	Capillary length *(cm/mm^3)*	*Capillary volume* *(%)*	*Mean intercapillary distance* *(μm)*
Brain cortex	21.93 ± 3.57	2.32 ± 0.38	58.18 ± 3.33
Putamen	12.20 ± 2.53	1.30 ± 0.35	71.73 ± 6.51

Consequently these results show that the narrowing of the gyri and the broadening of the sulci in old age are not the result of atrophy of the brain cortex [16], but the consequence of a considerable decrease in the volume of subcortical structures as shown in the putamen.

The capillaries undergo changes during the aging process, which are—except for the cortical changes in the age group 65–74 years—a secondary phenomenon resulting from changes in the surrounding tissue and result in a drawing together of the capillary network. Therefore it seems reasonable to interpret the 10–20% decrease in cerebral blood flow in old age as a phenomenon of adaptation to changed stereologic parameters of the brain. The increase in the capillary volume, on the other hand, explains the increase in the blood volume in the aged brain. The question regarding the correlation between the capillary parameters and changes of the nerve cells was investigated in identical brain structures.

Nerve-cell counting in the olivary nucleus revealed a progressive loss of nerve cells of about 20% by the age of 94 years [16]. Again because of the difficulty in counting nerve cells in the brain cortex, we have tried to evaluate age-induced changes in nerve cell area and perimeter which seemed indicative of nerve cell-related volume changes in the brain tissue. The fact that a significant decrease in nerve cell area and perimeter in putamen and cortex could be observed only in subjects over 85 years of age, permits the conclusion that nerve cell changes are not linked to shrinking processes in the brain. The decrease in brain volume may possibly be the result of a loss of extracellular space and a shrinkage of the myelin sheaths in the white matter. There are at present, however, only indirect results available to support this assumption.

The only link between the observed age-dependent changes in the stereologic parameters of the capillaries and the nerve cells is the correspondence-analytical demonstration that the nerve cell size is correlated with the surface/volume ratio of the capillary network. This finding is further evidence for the working hypothesis that nerve cell activity and microcirculation are functionally interdependent [17–20]. Recent findings, using non-invasive techniques, by Sylvia and Rosenthal [21] revealed that the cerebral microcirculation is directly regulated by functional and metabolic brain activity.

TABLE II

AGE-INDUCED CHANGES IN THE CAPILLARIES OF THE BRAIN CORTEX AND PUTAMEN

Cortex: 19–44 years, $n = 10$; >85 years, $n = 7$. Putamen: 19–44 years, $n = 6$; >85 years, $n = 7$. Values are expressed as mean ± SD.

	Capillary length (cm/mm^3)		*Capillary volume (%)*		*Surface/volume (μm^{-1})*		*Mean intercapillary distance (μm)*	
	19–44 years	*>85 years*	*19–44 years*	*>85 years*	*19–44 years*	*>85 years*	*19–44 years*	*>85 years*
Cortex	21.93 ± 3.57	21.10 ± 3.82	2.32 ± 0.38	2.26 ± 0.46	0.49 ± 0.02	0.49 ± 0.01	58.18 ± 3.34	56.20 ± 3.32
Putamen	12.20 ± 2.53	19.51 ± 3.34	1.30 ± 0.35	2.39 ± 0.70	0.54 ± 0.12	0.49 ± 0.01	71.73 ± 6.51	62.77 ± 4.87

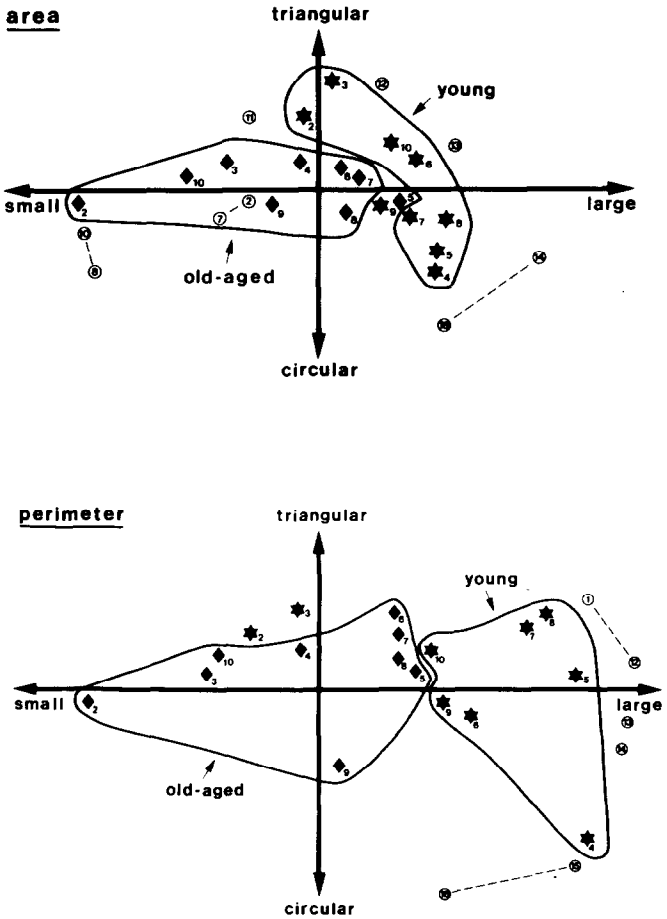

Fig. 4. Changes in cortical nerve-cell size in the precentral gyrus can not be stereologically identified before 85 years of age (young = 19–44 years (★); old = 85–94 years (♦); Arabic numerals correspond to the ten 300-μm-thick layers which were consecutively measured from the pial surface to the white matter). The most pronounced differences can be seen in the first (2–4) and the last (9–10) measuring fields; smaller changes occur in the great pyramidal cell layers (5–7).

CONCLUSIONS

The age-related changes in the brain capillaries suggest that a decrease in microcirculation is not a symptom of a disturbed cerebral blood supply, but rather a phenomenon of adaptation to changed stereologic parameters in the brain tissue of elderly patients. It is the result of a drawing together of the capillary network due to shrinkage of subcortical brain structures. These findings provide an explanation for an age-dependent decrease in brain volume and increase of brain ventricles. Accordingly, the narrowing of the gyri and the broadening of the sulci is not the result of a significant volume change of the brain cortex, but the consequence of a decrease in volume of subcortical brain

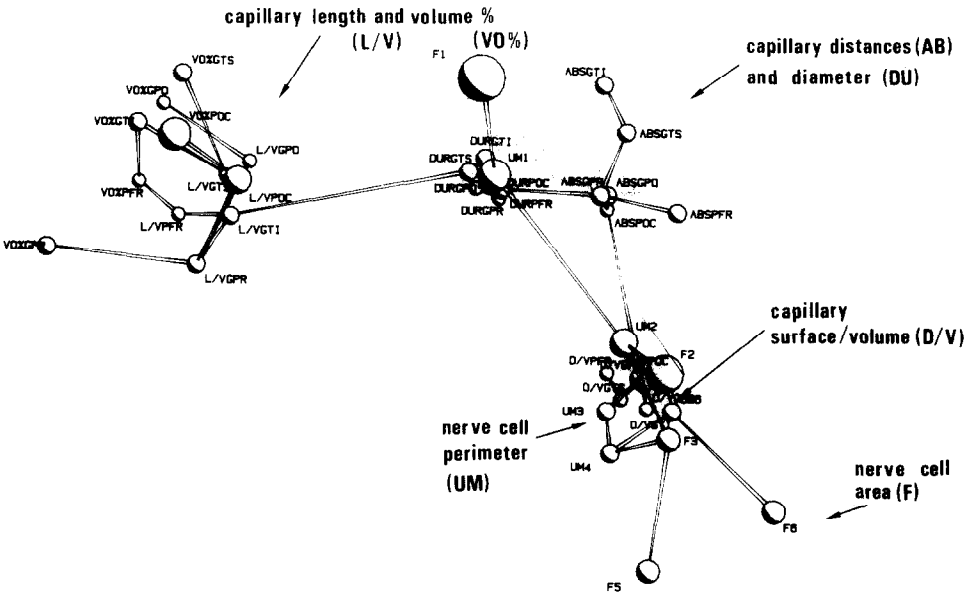

Fig. 5. Correspondence-analytical evaluation of the stereologically determined parameters of capillaries and nerve cells in the brain cortex. The only parameter of the capillary network which correlates with the nerve-cell size is the capillary surface/volume ratio. All the other capillary parameters are different from the nerve-cell parameters. Capillary distance and diameter, capillary length and volume, all correlate with each other. (F = nerve-cell area; UM = nerve-cell perimeter; DV = capillary diameter; VO% = percentage capillary volume; O/V = capillary surface/volume ratio; ABS = minimal capillary distance; L/V = capillary length/mm^3; PFR = polus frontalis; GPR = precentral gyrus; GPO = polus occipitalis; GTS = superior temporal gyrus; GTI = inferior temporal gyrus.)

structures. The increase in total capillary length and capillary volume could explain the observation of an increase in total blood volume in the aging brain.

The stereologic results of nerve cell measurements in the putamen and precentral gyrus demonstrate that nerve cell changes as a result of physiological aging are an extraordinarily moderate phenomenon, which is usually not apparent before the age of 85 years.

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