QUANTITATIVE IMAGE ANALYSIS OF BRAIN CAPILLARIES AND NEURONS IN HEALTH AND DEMENTIA*

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As the human life span lengthens, the importance of comparative morphological studies of the brain increases. Data on the architectonics of the capillary bed and changes in the size and shape of neurons taking place during human life are few in number and contradictory in nature. An age-linked shortening of dendrites [4], loss of dendritic branches [5], and a change in the soma-dendritic complex [10] may be either the cause or the result of changes in size and shape of the perikaryon [7, 13].

This paper describes an attempt to discover how the capillary network and the size, shape, and volume of neurons in the brain and putamen change during normal aging and in senile dementia of the Alzheimer's disease type (SDAT).

EXPERIMENTAL METHOD

The brain of 16 persons, made up of the control group aged 19-44 years (six persons), the control group aged 85-95 years (seven persons), and patients with demetia, with an Alzheimer's syndrome, aged 85-95 years (three persons), was studied. In the control groups, no neurological or serious mental disturbances were found in subjects of either sex. The group of patients with SDAT consisted entirely of women. The time interval between removal of the brain and freezing of the tissue blocks was 4-24 h. The brain tissue for investigation was supplied by the Department of Pathology of Basel University, and all morphometric investigations were conducted by the Department of Preclinical Research of the firm of "Sandoz" (Basel, Switzerland). Technical assistance was provided by Samir Abdel-Al.

The cortex of the frontal lobe and part of the brain stem (putamen) were investigated. Ten serial sections (14μ) were cut on a cryostat from each region: five odd sections for measurement of the capillaries and five even sections to study the neurons. Capillaries were revealed with the aid of alkaline phosphatase, and their diameter (in μ), volume (in %), ratio of surface to volume (in μ^{-1}), minimal intercapillary distance (in μ), length of capillaries in unit volume of brain (in cm/mm^3), and the number of capillary fragments in the measured region were determined on the "Leitz-Classimat" apparatus. Over-all mean values obtained on different series for separate regions of the brain and for groups were compared by Student's t test. Neurons were stained with cresyl violet. Stereologic parameters of the perikaryon were determined on the "Leits-TAC" system: the area of the neuron (in μ^2), its perimeter (in μ), and its shape factor [11, 13]. The scanning stage of the TAC system was programed so that scanning began with the pia mater, after which the gray matter was scanned along three horizontals 100μ long and the white matter along nine verticals. In this way a layer of cortex 3000 μ thick, divided into 10 measuring layers, was covered. In the putamen 10 measuring fields were chosen arbitrarily [12]. On the basis of the concept of isotropic "discovery" [14], 18 classes (hexagonal discoveries) were measured for each neuron, so that the size and shape of the perikaryon could be determined. The results were averaged for layers measured and for cases. To obtain comparable data on the size and shape of the perikaryon [11] mean values of area of the perimeter for the layers and cases measured, just as the corresponding classes of discoveries, were determined by multivariant statistical analysis.

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Fig. 1 Fig. 2

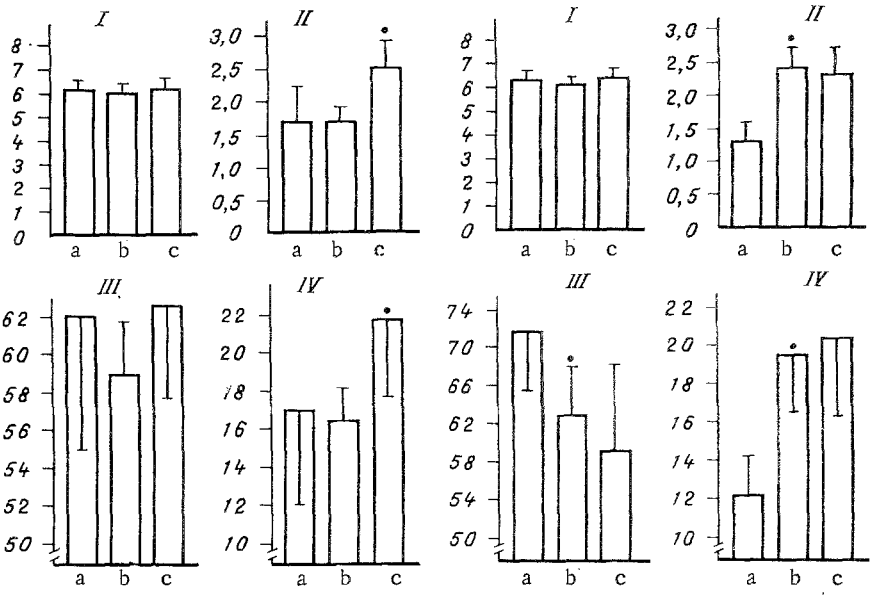

Fig. 1. Stereologic parameters of state of capillaries in frontal lobe. a) Subjects of control group aged 19-45 years, b) subjects of control group aged 85-95 years, c) patients with SDAT aged 85-95 years. I) Diameter of capillaries (in μ), II) volume of capillaries (in %), III) minimal intercapillary distance (in μ), IV) length of capillaries per unit volume (in cm/mm^3). *P < 0.005.

Fig. 2. Stereologic parameters of state of capillaries in putamen. *P < 0.01-0.0005. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

Compared with persons in the control group of the same age, patients with SDAT had a considerable increase in volume of the capillaries (+42.7%), and of their total length per unit volume of cortex (+32.6%), accompanied by a decrease in surface area of the capillaries (-5.9%) (Fig. 1). The increase in diameter of the capillaries under these cicumstances was very small. No changes in the architectonics of the capillaries or thickness of the cortex associated with age could be detected. Compared with the control group of old subjects, no significant changes could be observed in the capillaries of the putamen in patients with SDAT (Fig. 2).

The morphometric data and the results of comparison of the perikaryon of the frontal lobe confirmed a statistically significant decrease in size of the neurons in cortical layers II-IV in the group of patients with SDAT (Fig. 3a). Changes in shape of the perikaryon were observed sporadically.

Just as in the frontal lobe, the surface area of the neurons was reduced in the putamen of the patients with SDAT (Fig. 3c). A considerable change in the shape factor t_1 (acquiring values corresponding to a triangular shape) indicated a process of "shrinking" of the neurons.

In the precentral part of the cortex a considerable decrease in size of the neurons was observed in the old subjects compared with the young (Fig. 3b). In the putamen a considerable decrease in size of the nerve cells was found only in very old subjects.

In patients with presenile and senile demetia a considerable reduction of the regional cerebral blood flow (RCBF) was observed compared with that in subjects of the same age in the control group [8], and it occured uniformly throughout the brain. A definite reduction of RCBF could also be observed during normal aging. Since RCBF correlates with metabolism, the authors cited consider that the reduction in cerebral blood flow in patients with demetia reflects pathological changes in the CNS, leading to lowering of the intellectual level and to disturbances in the emotional sphere. At first glance, these data do not agree with those of the present investigation, which indicate a considerable increase in density of the capillary network in subjects with dementia (Fig. 1). On the other hand, these results reflect extensive atrophy of the frontal lobe in SDAT, and on the other hand it can be postulated that

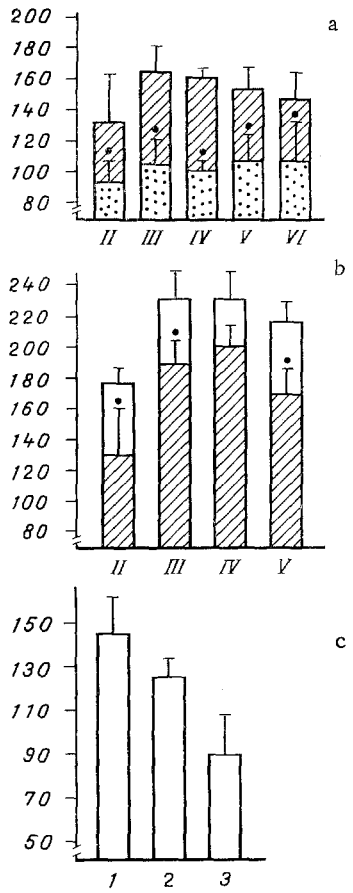

Fig. 3. Normal and pathological age changes in mean area of neurons (in μ^2) in different layers of cortex and putamen. Abscissa (a, b) — layers of cortex. a) Frontal gyrus: obliquely shaded area denoted healthy old people, dotted area — patients with SDAT; b) precentral gyrus: unshaded area represents healthy young subjects, obliquely shaded area — healthy old subjects: c) putamen: 1) in healthy young subjects, 2) in healthy old subjects, 3) in patients with SDAT. In a, c, *P < 0.0005; in b, *P < 0.005.

on account of the increased density of the capillary network, RCBF increases and, at the same time, the oxygen supply to the tissues is improved. That this conclusion is wrong is proved by electron-microscopic investigations of the cortical capillaries in SDAT [9], which showed a considerable increase in thickness of the capillaries and an increase in surface of the basement membrane in patients with SDAT.

Meanwhile the external diameter of the capillaries remained unchanged. During aging thickening of the basement membrane may be observed, but by a much lesser degree than in diseases accompanied by extensive atrophy of the brain. Thus thickening of the basement membrane of the capillaries is not necessarily accompanied by deposition of amyloid in the walls. This is evidently not specific for Alzheimer's disease but is the result of marked atrophy of the cerebral cortex. Unlike the cortex, a considerable increase in volume and absolute length of the capillaries per unit volume of tissue was observed in the putamen during aging, accompanied by a marked decrease in intercapillary distances [12]. Increased density of the capillary network did not arise in the putamen of patients with SDAT (Fig. 2).

The results of the morphometric investigations of the capillaries described above indicate that the cortex and putamen respond differently during aging and in Alzheimer's disease.

Morphometric investigations of neurons in the frontal cortex revealed considerable atrophy

(27-36%) of the perikaryon in all layers of the cortex in patients with SDAT compared with subjects of the control group of the same age (Fig. 3a). In subjects of this control group (aged 85-95 years) no significant change was observed in the neurons compared with the young subjects (Fig. 3b). Haug [6] found a considerable decrease in size of cells in different regions of the cortex during normal aging, but there was no corresponding change in their density. The reduction in size of the neurons during aging was accompanied by a slight change in their shape. In neurons of patiens with SDAT we found significant changes of shape in cortical layers III, V, and VI compared with subjects of the control group of the same age. As a result of dominance of atrophy these neurons become triangular in shape.

In this connection it must be pointed out that Scheibel and Scheibel [10] found a considerable decrease in dendritic branching in the cortex. Buell and Coleman [3] reported similar changes in the parahippocampal gyrus. Thus the length of the dendrites, like the number of branches, was reduced. These results are in good agreement with our morphometric data, which indicate considerable atrophy of the perikaryon of neurons of the frontal lobe and putamen, which in turn may reflect a diminution of metabolism and (or) in the number of certain intercellular organelles, on the one hand, and loss of synapses leading to inactivation of the nerve cells and to their death, on the other hand. Our results also agree with those obtained by Bowen [2] who, in a study of biopsy material from patients with SDAT, demonstrated the presence of cerebral atrophy (18%), decrease in the number of cells (33%), and a reduction in acetyl-transferase activity (by 64%). Other investigations [1] revealed a marked decrease in the noradrenalin concentration in the frontal cortex and putamen.

Morphometric studies can provide important quantitative data on the clinical-physiological state of the brain during aging and in various types of age-related pathology. Systems of quantitative image analysis of the Leitz-TAC type make the obtaining of such data very much easier; this is extremely important when the correct pharmacologic approach to the treatment of the cerebral insufficiency syndrome is being eleborated.

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