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MORPHOMETRIC INVESTIGATIONS OF THE GLIAL CELL NESTS IN THE RHINENCEPHALIC ALLOCORTEX OF THE DOG

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ABSTRACT

The glial cell nests in the rhinencephalic allocortex in dogs were investigated in order to elucidate their involvement in glioma formation and to assess age-dependent changes. The glial cell nests are made up of cells with medium-sized dark nuclei and of cells with large pale nuclei. The volume of the whole brain, the allocortex and the glial cell nests were estimated following Cavalieri´s principle. Unbiased estimates of the numerical densities and total numbers of the two prevailing cell populations within these nests were obtained using the optical disector.
With increasing age, the volume fraction of the glial cell nests of the allocortex was significantly decreased as was the numerical density and the total number of cells with medium-sized dark nuclei. In contrast, the numerical density and the total number of cells with large pale nuclei was significantly increased. There was no significant difference in any of the measured parameters between dolichocephalic and brachycephalic dogs, the latter showing a predisposition for glioma formation.
The combination of Cavalieri´s principle and optical disector proved to be a very efficient morphometric tool to estimate volumes, numerical densities, and total cell number. The significance of the age-dependent changes remains just as obscure as does the function of the glial cell nests. The glioma disposition of the brachycephalic dog cannot be explained with the data of our study.

Key words: aging, canine rhinencephalic allocortex, Cavalieri´s principle, glial cell nests, glioma, optical disector.

INTRODUCTION

The rhinencephalic allocortex of dogs and other species usually contains multifocal accumulations of glial cells. Moreover, it is one of the most common sites of glioma formation in brachycephalic dogs, especially the boxer dog. The glial cell nests (GCN) are morphologically similar to the well known glial cells in the subependymal layer. It has experimentally been shown that the latter are involved in glioma genesis. The rhinencephalic

glial cell nests are composed of three cell populations: (1) cells with medium-sized dark nuclei (CMD), (2) cells with large pale nuclei (CLP), and (3) very few cells with very small dark nuclei. In the present investigation, the following two questions were addressed: (1) are the glial cell nests involved in glioma formation, i.e. do they differ between brachycephalic and dolichocephalic dogs?, (2) do glial cell nests undergo age-dependent changes?

MATERIAL AND METHODS

The brains of 9 brachycephalic boxer dogs and of 16 dolichocephalic dogs grouped into puppies (age group 1: 1 day to 9 weeks; 6 dogs), juveniles (age group 2: 1 to 2 years; 8 dogs), and old dogs (age group 3: 9 to 13 years; 11 dogs) were investigated. Using the method of water displacement (Scherle, 1970), the volume of the brain was determined immediately after removal as well as after fixation for three-and-a-half months in a 7% formalin solution. The formalin-fixed brains were cut into 2 mm thick coronal slices. In order to fulfill the requirements in design-based stereology (DBS), the first cut was chosen at random.

The slices were routinely processed for paraffin embedding. From the most rostral slice down to that slice containing the caudal end of the piriform lobe, three 20 μm thick paraffin sections, several 100 μm apart, were cut from each brain slice. From the slices posterior to the piriform lobe only one section of 20 μm thickness was cut. The sections were stained routinely with cresyl violet.

Volume estimation was carried out according to Cavalieri's principle (see Gundersen and Jensen, 1987; Weis et al., 1991): the volume (V) of any object may be estimated from randomized and parallel sections separated by a known distance (t) (i. e. section thickness) by summing up the areas of all cross-sections (A) of the object, and multiplying this sum by t. For the volume estimation of the brain one section of every second slice, for the volume estimation of the allocortex one section of each slice, and for the volume estimation of the glial cell nests up to three sections per slice were used.

Employing a semiautomatic image-analyser system the profile areas of the brain, allocortex, and glial cell nests were delineated with the cursor and registered by the computer. The difference in the volume between the unfixed brain and the embedded brain represented the shrinkage, which was calculated as the percentage of the volume of the unfixed brain. Every parameter measured on paraffin embedded slices was corrected for shrinkage occurring in each individual. In order to assess, whether the volumes of the brain, allocortex, and glial cell nests were precisely estimated, the coefficient of error (CE) was calculated for each structure using the formula of Matheron (1971) for nonindependent observations (for details see Gundersen and Jensen, 1987).

The numerical density of the glial cells within the glial cell nests was estimated using a modified optical disector (West and Gundersen, 1990; West et al., 1991) in combination with the unbiased counting frame, modified for three-dimensional microscopy by Howard et al. (1985). From each slice containing nests, three sections were evaluated. In each brain 20 optical disectors with a height of 10 μm were sampled in a random systematic way. The area of the counting frame was 1744 μm^2, resulting in a disector volume of 17440 μm^3. The cells within the optical disectors were counted using an 100x oil immersion objective. The cells within the optical disectors were counted at the moment they came into focus, if their nuclei were within the frame and did not touch the two forbidden lines or their extensions (West et al., 1991). The numerical density, i. e. the number of cells per unit volume, was then calculated by dividing the total number of cells in the total disector volume of each brain by the total disector volume.

The total number of cells within the glial cell nests of one brain was calculated by multiplying the numerical density with the total volume of the glial cell nests corrected for shrinkage.

Statistical analyses were carried out on a PC 80486 (Highscreen) using the statistical package SPSS/PC (Statistical Package for the Social Sciences). All parameters were analysed using the one-way analysis of variance (ANOVA) and the non-parametric Mann-Whitney-U-test.

RESULTS

Significant age-dependent changes were seen in dolichocephalic dogs: the volume of the unfixed, formalin-fixed and paraffin-embedded brain as well as the volume of the allocortex was larger in juvenile (D2) and old dogs (D3) as compared to puppies (D1). When relating the volume of the allocortex to that of the whole brain, no significant age-dependent changes were detected. The volume and number of the glial cell nests did not differ significantly between the three age groups. However, the volume fraction of the glial cell nests of the allocortex diminished remarkably with increasing age.

The numerical density as well as the total number of all cells and of the cells with medium sized dark nuclei decreased with increasing age. In contrast, the numerical density and total number of the cells with large pale nuclei increased (Fig. 1).

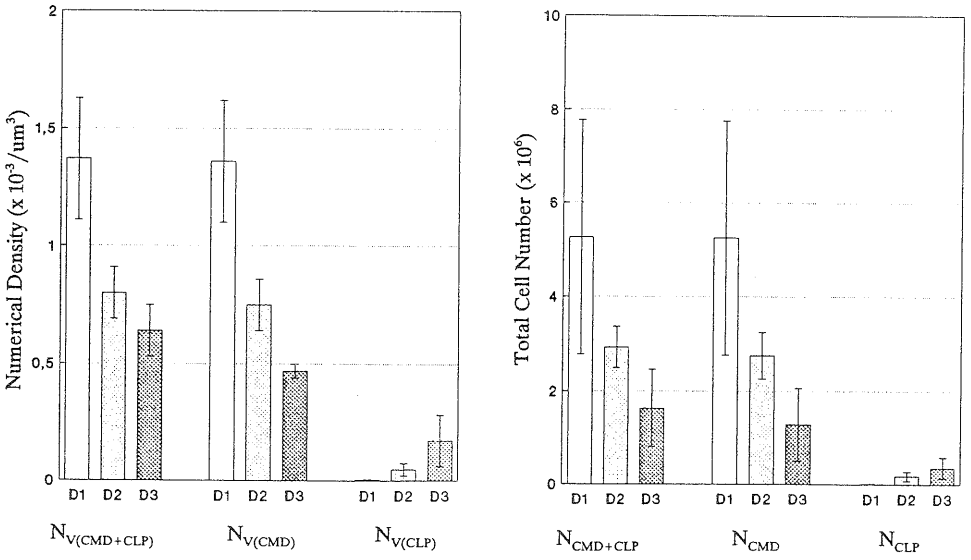

Fig. 1. Histograms showing the numerical density of all cells ($N_{V(CMD+CLP)}$), of cells with medium-sized dark nuclei ($N_{V(CMD)}$), and of cells with large pale nuclei ($N_{V(CLP)}$) and showing the total number of all cells ($N_{CMD+CLP}$), of cells with medium-sized dark nuclei (N_{CMD}), and of cells with large pale nuclei (N_{CLP}). Significant changes were found for: $N_{V(CMD+CLP)}$ between D1/D2 and D1/D3; $N_{V(CMD)}$ between D1/D2, D2/D3, and D1/D3; $N_{V(CLP)}$ between D1/D2 and D1/D3; $N_{CMD+CLP}$ and N_{CMD} between D1/D2, D2/D3, and D1/D3; N_{CLP} between D1/D2 and D1/D3.

Comparing the brachycephalic boxers to the dolichocephalic dogs, no significant changes were found for any of the measured parameters in the three age groups (Tbl. 1).

Table 1. Volume (V) of brain, allocortex, and glial cell nests (GCN), number (N) of GCN, numerical density (NV) and total number of cells within GCN as determined in old dogs. Data are given as mean values ± standard deviation.

Parameter	Brachycephalic dogs (n = 6)	Dolichocephalic dogs (n = 5)	p
V_{brain} (cm^3)	106 ± 12	84 ± 23	0.08
$V_{allocortex}$ (mm^3)	1162 ± 184	987 ± 223	0.27
V_{GCN} (mm^3)	2.49 ± 1.19	2.71 ± 1.64	1.00
N_{GCN}	61 ± 16	65 ± 22	0.58
$N_{V(CMD+CLP)}$ ($\cdot 10^{-3}/\mu m^3$)	0.75 ± 0.13	0.64 ± 0.11	0.20
$N_{V(CMD)}$ ($\cdot 10^{-3}/\mu m^3$)	0.60 ± 0.16	0.47 ± 0.03	0.07
$N_{V(CLP)}$ ($\cdot 10^{-3}/\mu m^3$)	0.15 ± 0.09	0.17 ± 0.11	0.72
$N_{CMD+CLP}$ ($\cdot 10^6$)	1.82 ± 0.79	1.63 ± 0.82	0.86
N_{CMD} ($\cdot 10^6$)	1.44 ± 0.59	1.28 ± 0.78	0.72
N_{CLP} ($\cdot 10^6$)	0.38 ± 0.32	0.35 ± 0.22	0.72

DISCUSSION

The peculiar glial cell nests within the rhinencephalic allocortex have hardly ever been described (Rose, 1927). The cells within these nests show a striking resemblance with the cells of the subependymal layer of the lateral ventricles described in dog and rat (Fischer, 1967; Lantos, 1977). The latter are known as pluripotent primitive cells (Nishio et al., 1990) playing an important role in the pathogenesis of periventricular brain tumors which has been shown experimentally (Vick et al., 1977). Spontaneous gliomas frequently appear in the rhinencephalic allocortex. Among the domestic animals they are most common in dogs, especially the boxer dog (Cordy, 1990). The reason for this striking breed predisposition is not clear. The aim of this morphometric study was to clarify possible breed differences between brachycephalic and dolichocephalic dogs in volume and composition of the glial cell nests allowing to make assumptions about the predisposition of the boxer dog to develop spontaneous gliomas.

The combination of Cavalieris principle and optical disector was the method of choice allowing the estimation of volumes, numerical densities, and total cell numbers in the same brain. As shown by Haug (1980) the embedding shrinkage is age-dependent and varies considerably between individuals. Therefore, the morphometric data obtained on paraffin material were corrected for the individual shrinkage.

Our morphometric results did not show any significant differences between brachycephalic and dolichocephalic dogs. However, age-dependent changes could be detected in dolichocephalic dogs. The volumes of the brain and allocortex of juvenile and old dogs exceeded those of the puppies. This difference was due to the still growing brain of puppies. There were no differences between juvenile and old dogs. Thus, an age-dependent brain atrophy as described in man (Boyd, 1861) could not be seen. However, it might occur in dogs older than those in our study. To our knowledge, there exists no systematic study on brain weight or volume changes with aging in dog.

The ratio of the volume of the allocortex to the volume of the whole brain did not change between the age groups; this means that the growth of the allocortex is proportional to that of the whole brain. The mean number of the glial cell nests was also nearly the same for the three groups, though the interindividual variation within one age group was quite high. The volume fraction of the glial cell nests of the allocortex decreased with increasing age. The cause of the decreasing volume fraction is not clear. It might be based upon the lack of production of new cells, since mitoses were not found in any of the dogs. On the other hand, degenerative processes may result in necrosis of some of the glial cells (Blakemore, 1969). However, we also did not find any single cell necrosis.

With increasing age, the composition of the glial cell nests shifted towards a higher number of cells with large pale nuclei. However, in no case did the number of these cells exceed 42% of the whole population. A similar shift has been observed in the subependymal layer of the mouse. In old mice, the CLP represented 90% of the whole cell population (Angevine et al., 1970). The shifting of cell populations observed in this study might be explained as follows: the glial cell nests of puppies consist of a homogeneous population of cells with medium-sized dark nuclei. With increasing age, these cells degenerate to cells with large pale nuclei. The latter undergo cell death, resulting in a low numerical density and a decreased total number of all cells.

Concluding, the combination of Cavalieri's principle and the optical disector proved to be a very efficient morphometric tool to estimate volumes, numerical densities, and total cell numbers. These two stereological methods could easily be applied for analysing the canine brain. Our results do not indicate an involvement of the glial cell nests in the development of gliomas nor do they explain the predisposition of the boxer dog for gliomas. Significant differences between dolichocephalic and brachycephalic dogs do not exist either in the volumes of brain, allocortex, and glial cell nests or in the numerical density or in the number of the two prevailing glial cell populations. The age-dependent changes of the canine brain volume and of the glial cell nests have been investigated by unbiased stereological methods for the first time. However, the significance of the age-dependent changes remains just as obscure as does the function of the glial cell nests within the rhinencephalic allocortex.

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