ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LI, 21 SECTIO D 1996

Katedra i Zakład Histologii i Embriologii z Pracownią Cytologii Doświadczalnej
Akademii Medycznej w Lublinie
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Morphometrical and Histological Alterations in the Hippocampus Cells Affected by Anoxia

Morfometryczne i histologiczne zmiany komórek hipokampa pozostających pod wpływem niedotlenienia

Clinical reports suggest that the hippocampus is a structure closely related to the functioning of memory and it plays a significant role in the learning process (15).

It is also known that anoxia frequently induces degenerating alterations in the CNS whose characteristic features are lesion and cell necrosis. The alterations of this type appear in various fields and also in the hippocampus [2, 8, 9]. Sommer [13] was the first to report that this CNS area is very sensitive to damages during anoxia or epilepsy.

Since there are different relations between the Willis' arterial circle and the system of vertebral and carotid arteries in different animals [4, 5], the effects of any vessel occlusion will vary.

The aim of the present study was to examine the degree of damage in the rat's hippocampus after a twenty-minute complete ischemia.

MATERIALS AND METHODS

Male Wistar rats (3 animals) were anesthetized with pentobarbital in the dose of 60 mg/kg and their vertebral arteries were clamped due to coagulation. Twenty-four hours after coagulation both cervical arteries were closed for 20 min. After further 48 hrs the animals were killed after a brain perfusion with 4% formalin (*pH* 7.2) and their brains were preserved in such a solution for 24 hrs.

10μ thick paraffin scraps were stained with H+E and assessed by the light microscopy. Furthermore, in the field CA3 the circumference and surface of the section through cell nuclei were measured using a computer analyser of histological pictures (Lobophot 2, Nicon).

Alterations of these parameters were determined statistically by 1-factor analysis of variance and multiple Tukey's confidence intervals. Mean values of the studied features, standard deviation and the differences between the means were calculated.

RESULTS

The hippocampus cortex of the experimental animals showed distinct histological differences in comparison to control animals. Both within the molecular layer and stratum oriens blood vessels were slightly shrunken, whereas the zona glomerulosa was markedly enlarged (Figs 1, 2). The most distinctively marked degeneration changes were observed in the CA1 field (Figs 3, 4). The pyramidal cells showed far-reaching changes displayed by severely shrunken and strongly basophilus cytoplasm. In most cells, apparently dead, the nucleus was invisible, in others — hardly visible. In stratum oriens basket cells also showed the signs of prominent damages. Numerous neurones were surrounded by an "empty" space (Figs 3, 4).

Similar destructive alterations were observed in the field CA2 in the pyramidal layer. This layer is narrower in the CA2 field in comparison with the CA1 field, though the pyramidal cells are arranged here much more densely (Figs 5, 6).

The changes in the fields CA3 and CA4 varied in particular animals. In two cases mild changes were observed in the form of the slightly shrunken cytoplasm and condensed tigroid or its lack (Figs 7, 8). However, in one animal a serious damage was detected in the form of the prononucedly staining shrunken cytoplasm and even more strongly staining shrunken nucleus (Figs 9, 10, 11, 12, 13).

In all animals statistically significant changes of the section field of the cellular nuclei and the changes of the circumference of the section in the CA3 field were marked in contrast with the controls. A particular decreasing of these parameters was observed in rat I (Tables 1, 2)

Tab. 1. The surfaces of neuron's nucleus in field CA 3 of the hippocampus [μm²]

Animal	Average	Standard deviation	Minimum	Maximum	Difference with control
Control	308.6[c]	65.3	171.4	446.7	—
Animal I	146.1[a]	42.7	64.1	250.8	162.4
Animal II	199.8[b]	55.0	82.2	365.7	108.8
Animal III	192.9[b]	41.8	96.5	268.9	115.6

Tab. 2. The perimeters of neuron's nucleus in field CA 3 of the hippocampus [μm]

Animal	Average	Standard deviation	Minimum	Maximum	Difference with control
Control	37.4[C]	4.4	27.3	52.7	—
Animal I	25.9[A]	4.3	16.8	36.5	11.5
Animal II	29.2[B]	4.2	12.7	40.8	8.2
Animal III	30.6[B]	4.1	19.5	40.8	6.8

REMARK: The same letter near averages denotes lack of statistically significant difference between them (significance level $p=0.01$).

so that in comparison with rats II and III the difference was also statistically significant. However, between rat II and rat III no such statistically significant changes were found.

DISCUSSION

In rats blood flows to the CNS from vertebral and carotid arteries, so in order to obtain complete ischemia the coagulation of vertebral arteries and a 20-minute occlusion of the common carotids were applied. This had to be done because the reduction of the blood flow caused by clamping just the carotids would not bring about any damage in rats [3]. Yet an 8% reduction of oxygen following the occlusion of the carotids induces a pronounced lesion of the pyramidal cells in the hippocampus and cortex [12].

In our experiment we could observe, apart from the degeneration of cells, the changes in the stroma, particularly in the fields CA1 and CA2. The changes were displayed by a stronger affinity to the stain and by the presence of minute vacuoles. At the same time, the extension of the space around the capillaries results probably from some disturbances in the permeability of their walls, which is connected with the handicapped intra-brain circulation. This is also followed by some changes in the circulation and arrangement of ions inside and outside the cells because of the "insufficiency" of the sodium-potassium pump as a result of an energetic deficiency caused by anoxia. Similar changes in the stroma with the presence of vacuoles were observed in the hippocampus and other areas of the CNS following treating them with pilocarpine (14). Such a state was described as a glial and dendritic swelling (6). The experiment makes it evident that the most sensitive areas of the hippocampus are the fields CA1 and CA2 where the pyramidal cells particularly vulnerable to degenerating factors can be found. These data are congruent with the observations by other authors examining the changes in the hippocampus taking place in experimental anoxia (7, 11). Whereas, after the treatment with pilocarpine, damages of cells in the fields CA3 and CA4 were encountered more often (14). According to J. E. Schwob et al. (10), the degeneration of pyramidal cells in the CA1 after injection of the kainic acid is more prominent with survival times longer than 24 hrs.

In our experiment most pyramidal cells of the fields CA1 and CA2 displayed a very pronounced, apparently irreversible degree of damage. This is proved by the significantly shrunken cytoplasm and hardly visible nucleus. Around the cells, because of their decreased capacity, a bright space could be seen. In a cell altered in this manner, ordinary processes of exchange between its cytoplasm and the environment are no longer possible. In numerous cells, apparently dead, the nucleus was not visible at all.

The fields CA3 and CA4 have a similar histological form and in our experiment the cells in these areas turned out to be less sensitive to the degenerating changes caused by anoxia. These changes, transient in most cases, manifested themselves by a slight shrinking of the cytoplasm and the condensation or lack of tigroid, especially in the CA4 field. It could be claimed with a high level of probability that lower activity of a nervous

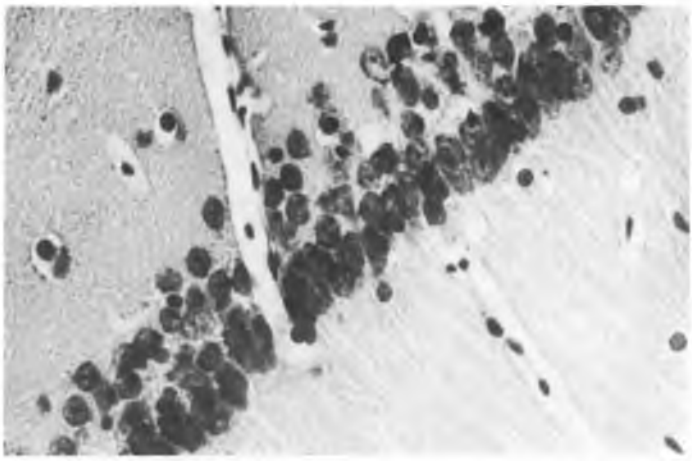


Fig. 1

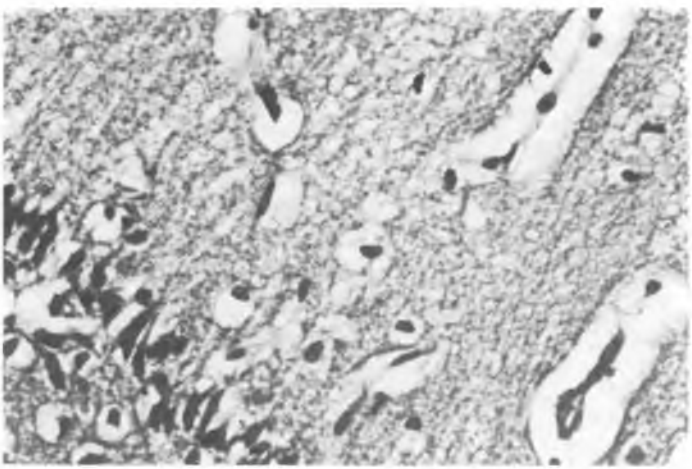


Fig. 2

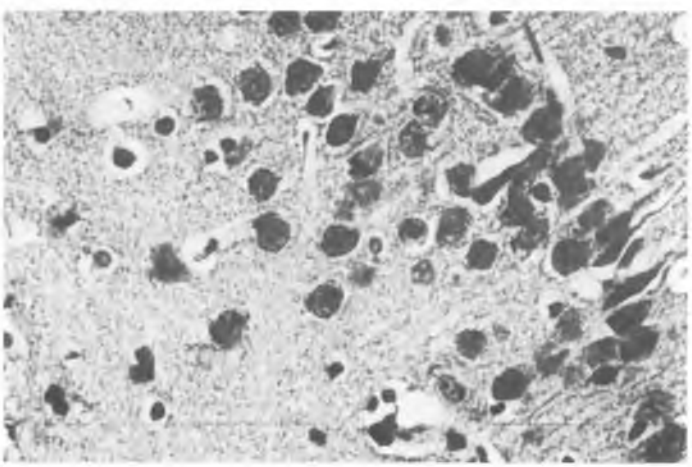


Fig. 3

Fig. 4

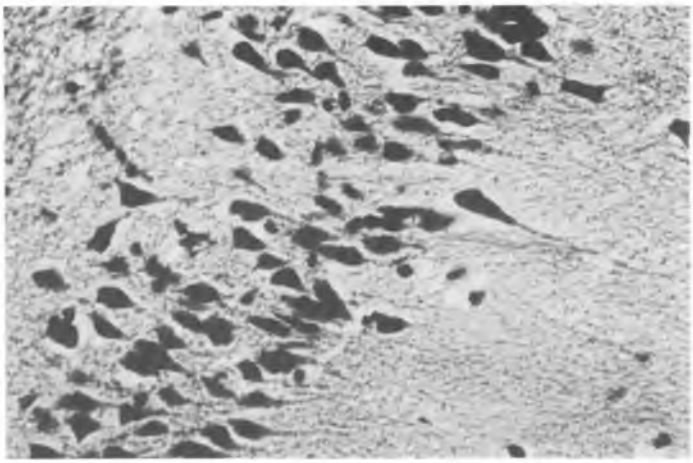


Fig. 5

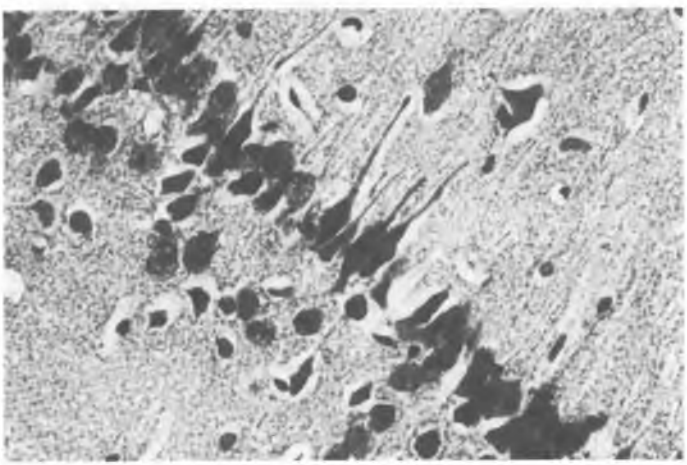
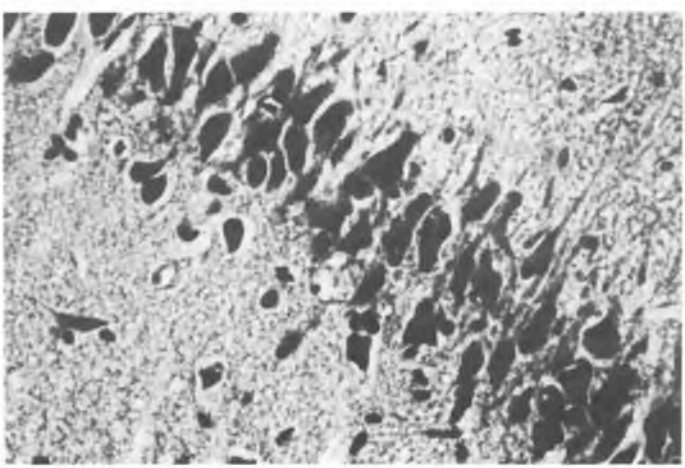


Fig. 6



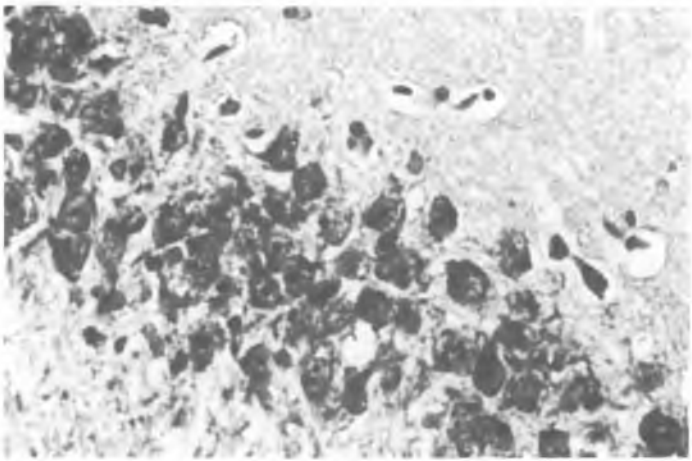
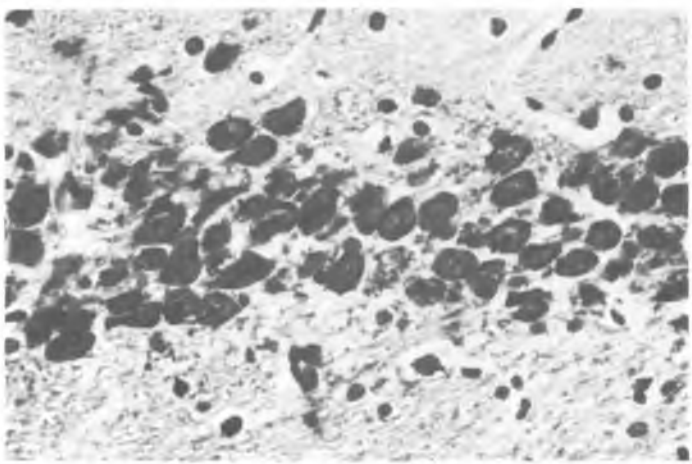


Fig. 7



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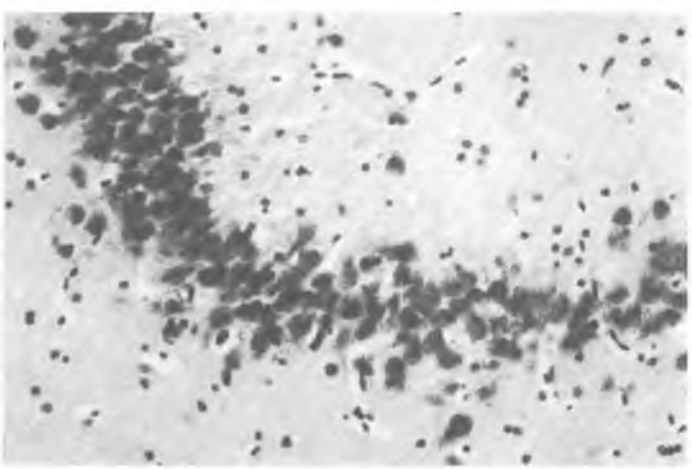

Fig. 9

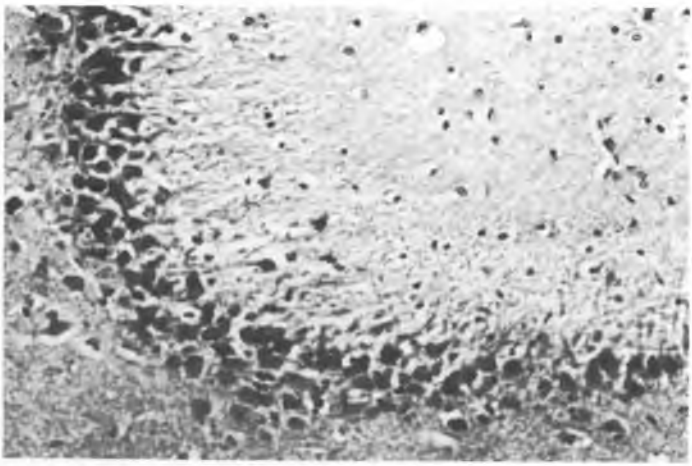

Fig. 10

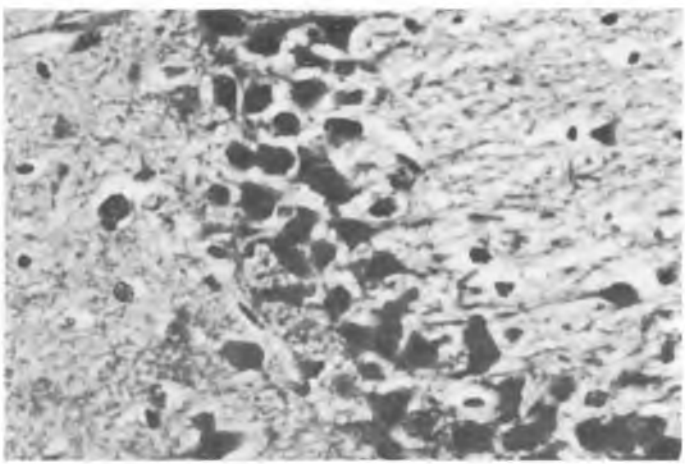

Fig. 11

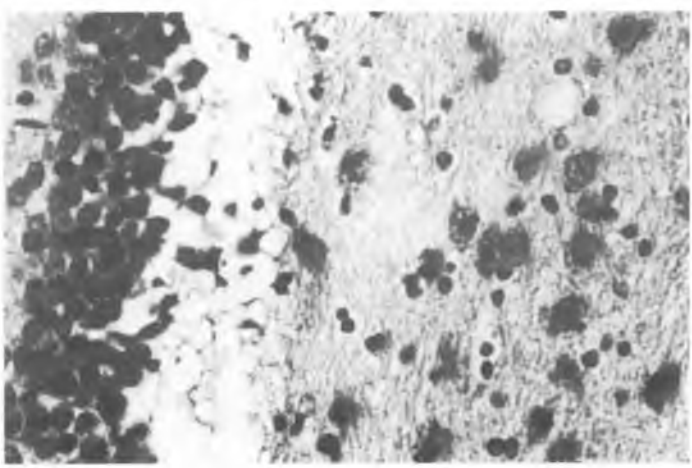

Fig. 12

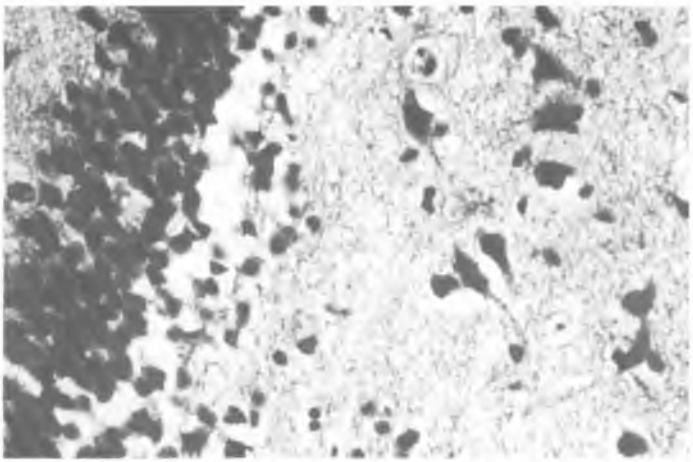

Fig. 13

cell is manifested by both the condensation of tigroid and its reduced ability to regenerate. Only in one animal the degeneration of cells in the fields CA3 and CA4 was prominent. A distinct shrinking of the cytoplasm and cellular nuclei was spotted, which was confirmed by statistical examinations in reference to the field CA3. In this field, the presence of statistically significant differences between the control and experimental animals was demonstrated. The differences concerned changes of the surface of the section area of the nuclei and the circumference of the surface of their section area. Furthermore, the changes of these parameters in one animal were so pronounced that the results varied significantly from the same type of changes in other animals. A particularly high sensitivity of cells in the fields CA3 and CA4 in one of the animals is peculiar and could be a result of an individual difference in the blood flow in the cortex vessels (12).

CONCLUSIONS

1. 20-minute complete ischemia produces the necrosis of most pyramidal cells in the hippocampus of rats.
2. The most acute fields sensitive to the factor of anoxia are the CA1 and CA2.
3. The changes of the area and circumference of the section of the nuclei in the cells of the field CA3 were statistically significant in comparison with the changes of these parameters in control animals.

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Otrz. 1996.07.10

STRESZCZENIE

Badano zmiany histologiczne neurocytów hipokampa szczurów pod wpływem całkowitej 20-minutowej ischemii, stosując koagulację tętnic kręgowych i 20-minutową okluzję tętnic szyjnych. Ponadto w polu CA3 za pomocą analizatora obrazów histologicznych wykonano pomiary obwodu i pola powierzchni przekroju jąder komórkowych. Otrzymane dane oceniono statystycznie za pomocą 1-czynnikowej analizy wariancji. Badania wykonano na skrawkach parafinowych barwionych H + E.

Stwierdzono martwicę większej części komórek piramidalnych, zwłaszcza w polach CA1 i CA2. W polu CA3 stwierdzono statystycznie istotne zmniejszenie pola i obwodu powierzchni przekroju jąder neurocytów.

EXPLANATIONS TO FIGURES

Fig. 1. Control animal. Field CA1 of the hippocampus. Note the strata moleculare, pyramidale and oriens, and capillaries in the stroma. Stained with H+E. Magn. ca. 400×.

Fig. 2. Experimental animal. Field CA1 of the hippocampus. Note the severely impaired neurocytes in the stratum pyramidale and oriens, a bright space around the cells and the extension of the space surrounding the vessels. Stained with H+E. Magn. ca. 400×.

Fig. 3. Control animal. Field CA1 of the hippocampus. Note the strata moleculare, pyramidale and oriens. Stained with H+E. Magn. ca. 400×.

Fig. 4. Experimental animal. Field CA1 of the hippocampus. Note the severely impaired cells of the strata pyramidale and oriens. Stained with H+E. Magn. ca. 400×.

Fig. 5. Control animal. Field CA1 of the hippocampus. Note the markedly narrower pyramidal layer. Stained with H+E. Magn. ca. 400×.

Fig. 6. Experimental animal. Field CA2 of the hippocampus. Severely damaged cells of the strata pyramidale and oriens. Stained with H+E. Magn. ca. 400×.

Fig. 7. Control animal. Field CA3 of the hippocampus. Note the layers of the pyramidal and basket cells. Stained with H+E. Magn. ca. 400×.

Fig. 8. Experimental animal. Field CA3 of the hippocampus. Note the condensation of the tigroid in the pyramidal and basket cells. Stained with H+E. Magn. ca. 400×.

Fig. 9. Control animal. Field CA3 of the hippocampus. The layers of the pyramidal and basked cells. Stained with H+E. Magn. ca. 200×.

Fig. 10. Experimental animal. Field CA3 of the hippocampus. Note the severely shrunken and intensely staining pyramidal and basket cells. Stained with H+E. Magn. ca 200 ×.

Fig. 11. Experimental animal. Field CA3 of the hippocampus. Note the severely shrunken pyramidal and basket cells. Stained with H+E. Magn. ca. 400×.

Fig. 12. Control animal. Field CA4 of the hippocampus. Note the layers of the pyramidal and basket cells, and the zona glomerulosa of the dentate gyrus. Stained with H+E. Magn. ca. 400×.

Fig. 13. Experimental animal. Note the severely shrunken pyramidal and basket cells of field CA4. Stained with H+E. Magn. ca. 400×.