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QUANTITATIVE EVALUATION OF CELL PROLIFERATION AND DEATH IN AMMON'S HORN AND THE DENTATE GYRUS OF THE DEVELOPING MOUSE HIPPOCAMPUS

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UDC 519:611:612

KEY WORDS: hippocampus; development; cambial zones; mitosis; pycnosis.

Ammon's horn and the dentate gyrus of the hippocampus are known to differ significantly in the duration of their cytogenesis [4, 5, 9]. The source of cell production in both parts of the hippocampus is the ventricular zone, and after its exhaustion, the role is assumed by local proliferation of cambial cells and blioblasts [5, 8. 9]. Local cambial zones have been described and studied in the dentate gyrus [2, 5, 6, 9] but no quantitative analysis of age changes in the proliferative processes has been undertaken. Local cell proliferation has not been studied at all in Ammon's horn. Moreover, the age changes in mitotic cell death, which can have a significant effect on the over-all balance of cell production [2, 7], have not been studies in either part of the hippocampus. Yet we know that pycnosis, discovered in the cambial zones of the hippocampus, arises, as a result of mitotic cell death [1].

The aim of the present investigation was accordingly a quantitative analysis of mitosis and pycnosis in zones of cell proliferation (local cambial zones) of Ammon's horn and the dentate gyrus of the developing hippocampus.

EXPERIMENTAL METHOD

Experiments were carried out on 30 CBA mice aged 14, 16, 18, and 20 days of intrauterine and 1, 3, 7, 14, 21 and 60 days of postnatal life. Three animals were sacrificed at each time of development. The brain was fixed in Carnoy's fluid and embedded in paraffin wax. Frontal serial sections 5 μ thick were stained with cresyl violet. Histological sections for analysis of the location of the cambial zones in the rostral hippocampus were studied under the microscope (magnification 25). Indices of mitosis and pycnosis were counted in the local cambial zones thus revealed, by the use of an immersion objective. The quantitative analysis was undertaken on mice aged 18 and 20 days of intrauterine and 1, 3, 7, 14, 21, and 60 days of postnatal life. The indices were counted in Ammon's horn in the identified suprafimbrial cambial zone. In the dentate gyrus, mitoses and pycnoses were counted in

Central Research Laboratory, Medical Faculty, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 731-733, December, 1984. Original article submitted June 27, 1983.

 0007-4888/84/9812-1722$08.50

TABLE 1. Values of MI, PI, and FCL in Local Cambial Zones of Ammon's Horn and Dentate Gyrus in Mice

Age of mice, days	Period	No. of cells counted	MI, ‰	P	PI, ‰	P	FCL, %
			Ammon's horn				
18	Embryonic	20 291	3,10±0,38	<0,05	0,00	—	0,0
20		18 256	5,40±0,54	N.s.	2,57±0,36	N.s.	1,5
1	Postnatal	16 919	5,99±0,59	<0,05	3,36±0,45	<0,001	2,8
3		15 065	8,78±0,76	N.s.	13,94±0,94	<0,001	8,0
7		13 119	6,63±0,71	<0,01	3,27±0,46	<0,001	2,5
14		10 089	0,98±0,30	N.s.	1,00±0,30	N.s.	5,0
21		15 708	0,77±0,28	»	0,80±0,28	N.s.	5,4
60		16 422	0,30±0,17		0,37±0,18		6,3
			Dentate gyrus				
18	Embryonic	13 327	2,15±0,37	<0,05	0,00	—	0,0
20		14 915	6,17±0,64	N.s.	0,14±0,03	<0,001	0,1
1	Postnatal	15 358	6,84±0,66	<0,001	1,35±0,28	<0,05	1,0
3		16 378	11,05±0,81	<0,05	3,31±0,44	N.s.	0,7
7		15 407	7,32±0,67	<0,05	2,33±0,37	N.s.	1,5
14		14 297	3,41±0,48	N.s.	2,17±0,38	N.s.	3,2
21		19 009	2,93±0,39	<0,05	3,12±0,41	N.s.	5,3
60		18 480	1,48±0,32		1,85±0,37		6,2

Legend. N.s.) Differences not significant. Mean data for 3 animals given at each time of development.

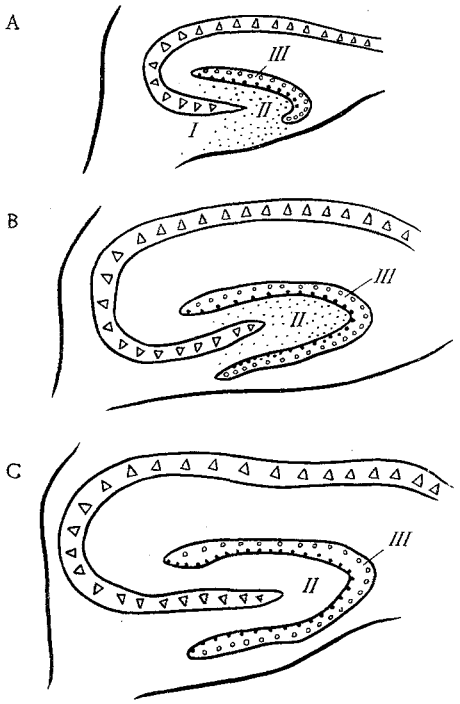

Fig. 1. Scheme of cambial zones of dentate gyrus in postnatal development of mice and rats. Animals aged: A) 1 day, B) 7 days, C) adult animals. Cambial cells indicated by dots, granular neurons of dentate gyrus by circles. I) Suprafimbrial zone, II) proliferative zone of hilus, III) subgranular zone. In this paper, until the 3rd day of postnatal life I, II, and III are combined into the general cambial zone of the dentate gyrus.

its cambial zones whose distribution and nomenclature differ in animals of different ages (Fig. 1). All phases of mitoses were counted. When counting pycnoses, criteria of pycnotic degeneration in cambial zones of the brain were used [3]. Mitotic and pycnotic indices (MI and PI respectively) were calculated in the cambial zones of each animal in 8-16 brain sections, taking every alternate section. The numerical results were subjected to statistical

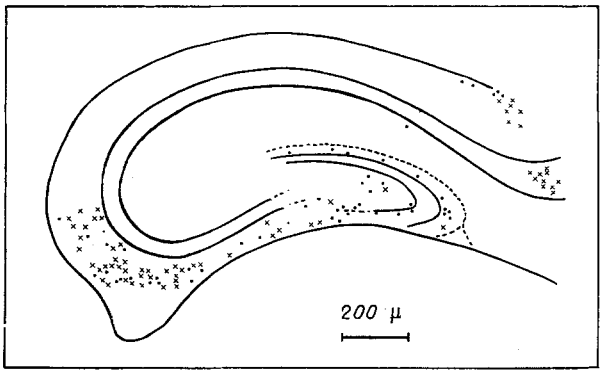

Fig. 2. Distribution of mitoses (dots) and pycnoses (crosses) in hippocampus of mice aged 3 days. 1) Suprafimbrial cambial zone, 2) general cambial zone of dentate gyrus.

analysis by standard methods. The fraction of cell loss (FCL) was calculated, as the percentage of mitotic cell death, by the equation:

$$FCL = \frac{PI \times 100\%}{2MI \times 10},$$

where 2 represents the number of pycnotic nuclei after mitosis, and 10 is a coefficient expressing the ratio of the duration of mitosis to the duration of existence of cells with a pycnotic nucleus in the brain [7].

EXPERIMENTAL RESULTS

The study of the embryonic mouse hippocampus showed that local cambial zones begin to be formed in the dentate gyrus after the 14th day, and in Ammon's horn after the 16th day of embryonic development. The local cambial zone of Ammon's horn has not been described previously. It consists of a concentration of undifferentiated proliferating cells, located in the ventral part of Ammon's horn (area CA3, immediately above the fimbria (Fig. 2). We named this zone the suprafimbrial zone. It is well defined until the 3rd day of postnatal life. By the 7th day the subprafimbrial zone has undergone considerable reduction, but individual proliferating cells can be seen in this region even at later times of development. In the dentate gyrus the local cambial zone from the 14th day of embryonic development until the 3rd day of postnatal life consists of the general cambial zone of the dentate gyrus, located between the newly formed dorsal arm of the granular layer and the surface of the hippocampus facing the brain stem (Fig. 1a). From the 7th through the 14th day of postnatal life, after formation of the ventral arm of the granular layer, proliferating cells in the dentate gyrus are found in the trigone of the hilus (the proliferative zone of the hilus) and in the subgranular zone, located in the inner part of the granular layer (Fig. 1b). After the 21st day only the subgranular cambial zone remains in the dentate gyrus, and in a reduced form it can still be traced in mice aged 2 months (Fig. 1c). The cambial zones of the dentate gyrus correspond to their descriptions given in publications devoted to the development of the dentate gyrus in mice and rats [2, 5, 6, 9].

Analysis of the distribution of mitoses and pycnoses showed that not only proliferating, but also dying cells are located in the local cambial zones of the hippocampus. However, pycnoses did not appear in these zones until the 20th day of embryonic development. The results of counting MI and PI in the suprafimbrial zone of Ammon's horn (Table 1) demonstrate a rapid increase in their values until the 3rd day of postnatal life. The values of MI and PI then fell a little on the 7th day, and later (after reduction of the suprafimbrial zone) their values were very low. In the dentate gyrus values of MI rose until the 3rd day of postnatal life (Table 1) but, unlike in Ammon's horn, the number of pycnoses became considerable only after the 1st day of postnatal life. From the 7th day values of MI and PI gradually decreased, but they were still quite high even in mice aged 60 days. Calculation of FCL showed (Table 1) that this fraction in Ammon's horn increased until the 3rd day of postnatal life, was significantly lower on the 7th day, and increased again from the 14th through the 60th day. In the dentate's gyrus a different tendency was observed: a gradual increase in the value of FCL from the 1st through the 60th day of postnatal life.

The results are evidence of differences in the age dynamics of cell proliferation and death in the local cambial zones of Ammon's horn and the dentate gyrus. Whereas in Ammon's horn these processes grow rapidly and just as rapidly decline to zero immediately after reduction of the suprafimbrial zone, in the dentate gyrus there is a comparatively smooth and lengthy increase followed by a decrease in the number of proliferating and dying cells, in harmony with the slow reduction of its cambial zones. These data agree with the results indicating earlier completion of cytogenesis in Ammon's horn than in the dentate gyrus [4, 5, 9]. The cambial zones of Ammon's horn and the dentate gyrus also show significant differences in the values of FCL, i.e., the percentage of mitoses culminating in death of the daughter cells. These data are evidence of qualitative differences in the cell composition of the proliferative compartment in the cambial zones studied. The importance of age changes in the size of the fraction of losses of proliferating cells is not yet known. However, assuming that mitotic death is connected with elimination of spontaneous mutations of precursors of glial cells, the absence of pycnoses in the cambial zones of the hippocampus in the embryonic period, when processes of neuronogenesis predominate, and the increase in mitotic mortality in postnatal life finds a satisfactory explanation.

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