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QUANTITATIVE ANALYSIS OF NEURONAL MOSAIC FORMATION IN THE MOUSE NEOCORTEX AND HIPPOCAMPUS

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UDC 611.813.14-018.82-013-019

KEY WORDS: neocortex; hippocampus; neurogenesis; autoradiography; computers.

The mosaic pattern of neurogenesis, reflected in the arrangement of concentrations of neurons intensely labeled with ^{3}H-thymidine, was described as a result of the writers' investigations with injection of ^{3}H-thymidine into mice at different times of embryogenesis, followed by analysis of the arrangement of the labeled cells in the neocortex and hippocampus of day-old mice [4, 5, 13]. It was concluded that the mosaic pattern of neurogenesis is determined by the nonsynchronized course of differentiation of cell groups in the ventricular zone of the embryonic brain, i.e., evidence was obtained of its discrete organization in the form of loci of neurogenesis.

The aim of the present investigation was to obtain mathematical confirmation of the nonrandomness of mosaic formation of neuronal groups in the cerebral cortex, to characterize this process quantitatively, and to compare its scale with the formation of the modular organization of the cortex.

EXPERIMENTAL METHOD

Pregnant female CBA mice were given a single intraperitoneal injection of ^{3}H-thymidine (10 μCi/g) on the 13th-19th day of pregnancy (E13-E19). At the age of 1 day, two or three mice from each mother were killed by decapitation. The cerebral hemispheres of mice receiving ^{3}H-thymidine from E15 to E19 were fixed in Karnovsky's fixative and embedded in Durcupan. When the isotope was injected during the period E13-19 the cerebral hemispheres were fixed in Carnoy's mixture and embedded in paraffin wax. One hemisphere from each animal was cut into frontal sections, the other into sagittal. Preparations with glued semithin (1 μ) or paraffin (6 μ) sections were covered with type M emulsion and, after standard autoradiographic processing, were stained with 1% toluidine blue solution in 2.5% sodium carbonate solution or with 0.1% cresyl violet. The arrangement of the centers of the nuclei of the intensely labeled neurons in area 6 of the frontal region of the neocortex and in area CA1 of the dorsal hippocampus was mapped on an NU-2E microscope with projection screen. Mapping in the neocortex was carried out on frontal semithin brain sections from mice receiving ^{3}H-thymidine from E15 through E17, whereas mapping in the hippocampus was carried out on paraffin and semithin frontal brain sections from animals receiving the isotope from E13 through E17. Cells were recorded as intensely labeled if the number of grains of silver above the nucleus varied from maximal to half that number of grains per section. Maps of areas 6 and CA1 from two animals at each time of the experiment studied were analyzed mathematically to discover nonrandom groups of intensely labeled neurons. For this purpose an approach was used which enables the regularity of neurogenesis to be assessed in brain structures organized on the principle of a rec-

Department of Autoradiography, Central Research Laboratory, Medical Faculty, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6, pp. 735-738, June, 1987. Original article submitted May 12, 1986.

 0007-4888/87/0006-0840$12.50

tangular grid, formed by horizontally and vertically oriented cells. These criteria are satisfied by frontal sections of areas 6 and CA1 if the plane of section runs parallel to vertically oriented cells of these structures. A series of coordinates of projections of intensely labeled neurons on the horizontal axis of the maps was processed by DZ-28 computer, programmed for $U(\rho^2)$-grouping, by means of which a nonhomogeneous set can be split up into several significantly homogeneous parts [3]. The nonrandom groups obtained were again analyzed by computer, using the chi-square test. The numerical characteristics of the isolated groups of neurons were computer-processed to determine their standard biometric parameters.

EXPERIMENTAL RESULTS

Mathematical analysis of the maps showing the arrangement of neurons intensely labeled with ^{3}H-thymidine in the neocortex and hippocampus showed that labeled neurons were arranged on the horizontal coordinate of the maps not in random fashion, but in relatively close groups, isolated from each other. This phenomenon corresponds to the reliably observed orderliness of arrangement of labeled neurons in the form of vertically oriented concentrations (Figs. 1 and 2). Of the 16 maps analyzed by computer, significant differences from a randomly uniform distribution were found in 14 cases at the highest level of significance $p < 0.001$, for one of the two maps of the neocortex of mice receiving ^{3}H-thymidine on E17 the level of significance was $p < 0.01$, and only for one of the two maps of the hippocampus of mice receiving the isotope on E13, were the differences not significant. In area 6 of the neocortex, when ^{3}H-thymidine was injected on E15-E16, the mean number of neurons in the group was 4.44 ± 0.25, the width of the group was 14.2 ± 0.53 μ, and its height 80.8 ± 4.23 μ for E15 and 30.6 ± 5.10 μ for E16; the width of the intervals between the groups was 16.4 ± 0.77 μ. In hippocampal area CA1 in mice receiving the isotope from E14 through E16 the number of neurons in the group was 4.35 ± 0.16, the width of the group was 10.8 ± 0.73 μ, its height 108.0 ± 3.36 μ, and the width of the intervals between groups was 11.1 ± 0.44 r.

The results of this investigation not only confirmed previous data showing the mosaic formation of cerebral cortical neurons in mice, but they also enabled this process to be characterized quantitatively. In this connection it becomes possible to estimate the size of the separate loci of the ventricular zone producing cortical neurons asynchronously. Since, as was shown previously [5, 13], concentrations of intensely labeled neurons are not clearly distinguishable in frontal and sagittal sections through the cortex, it can be concluded that they are cylindrical in shape. Consequently, a group of four or five cells visible in a section 1 μ thick corresponds in the volume of the cylinder to a concentration of 6.28-7.85 (i.e., 6 to 8) cells. Using the criterion which we adopted, identifying intensely labeled neurons as cells containing between the maximal number of grains of silver above the nucleus and half of that number, it can be postulated that in mice receiving ^{3}H-thymidine on E14-E16 the groups of intensely labeled neurons represent the production of two mitotic cycles from ventricular precursors. This follows first, from the results of an experiment using double labeling with ^{14}C- and ^{3}H-thymidine, which showed that labeled precursors of neocortical neurons in mice at the E14-E16 period pass through two divisions in the course of 24 h; the intensity of labeling above nuclei of neurons of the 1st and 2nd generations, moreover, may differ at most by twice [1], and second, from calculations based on data for parameters of the mitotic cycle of the ventricular cells of the mouse telencephalon in the E14-E16 period [14]. Under these circumstances the fraction of cells labeled with ^{3}H-thymidine as the result of a single injection, compared with the total number of proliferating cells, ought to be equal to the ratio of the duration of the period of DNA synthesis (tS) plus the circulation time of ^{3}H-thymidine in the body (30 min) to the total duration of the mitotic cycle (T) [2]. Correspondingly, in mice on E15, when T = 16 h and tS = 7.1 h [14], ^{3}H-thymidine is incorporated by 47% of proliferating ventricular cells; some of them, moreover, must be either at the beginning or at the end of the S period and must incorporate only a negligible quantity of the isotope.

Thus to estimate the number of neurons produced by a locus of the ventricular zone during one mitotic cycle in the period E14-E16, two corrections have to be introduced into the value obtained for the number of intensely labeled neurons in a three-dimensional concentration (6-8 cells): 1) this number must be divided by 2 to obtain the number of neurons formed during one mitotic cycle; 2) it must be multiplied by 2.2, to allow for neurons whose precursors did not incorporate the isotope, for they were in the pre- and postsynthetic period of the mitotic cycle. By introducing these corrections we find that a single locus in the ventricular zone produces, in the course of one mitotic cycle, 7-9 cells commencing neuronal differen-

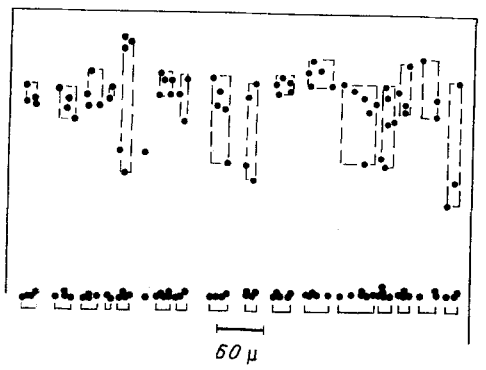

Fig. 1

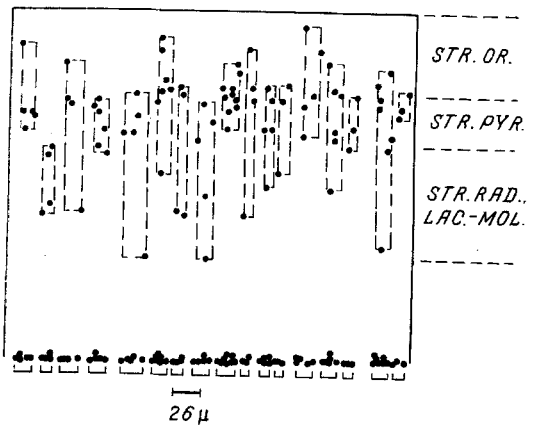

Fig. 2

Fig. 1. Nonrandom groups (broken line) of intensely labeled neurons (dots) in area 6 of the neocortex of a day-old mouse receiving ^{3}H-thymidine on E15. Here and in Fig. 2, projections of intensely labeled neurons (dots) and the width of the groups are given on the horizontal axis of the map. Here and in Figs. 2 and 3, the level of significance of the groups distinguished is $p < 0.001$.

Fig. 2. Nonrandom groups (broken lines) of intensely labeled neurons (dots) in hippocampal area CA1 of a day-old mouse receiving ^{3}H-thymidine on E15.

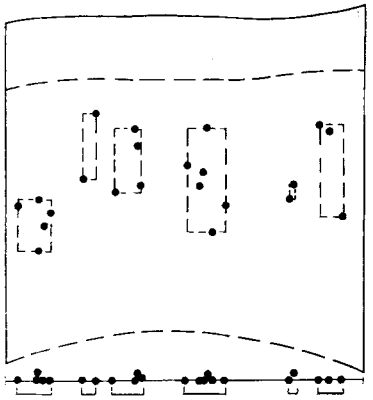

Fig. 3. Nonrandom groups (broken line) of intensely labeled neurons (dots) in the occipital cortex of a 66-day-old rhesus monkey receiving ^{3}H-thymidine on E56. An area of a map was taken from [12] for computer processing.

tiation. This means that fewer than 10 ventricular cells take part in their formation so that the order of the number of cells in a locus of neurogenesis can be estimated. These results can also be used to compare the scales of processes of neurogenesis in individual loci with the formation of the modular organization of the cerebral cortex. According to the calculations, a locus of the ventricular zone produces 7-9 neurons in one mitotic cycle. In view of evidence that the mouse neocortex is formed by 12 generations of neurons [1], it can be concluded that a single locus, during this period, produces a column containing 84-108 neurons. This is quite close to the number of neurons in a minicolumn of the neocortex, which contains about 110 cells [10]. These calculations give only an approximate estimate, but they are evidence that processes of neurogenesis comparable with the formation of the cellular composition of neocortical minicolumns and of the recently discovered hippocampal micromodules do exist [15]. The mosaic pattern of neurogenesis we have described can be found in laminar strain structures of other species of mammals. Evidence of this is given by maps showing the arrangement of thymidine-labeled neurons in the neocortex and paleocortex, tectum, and thalamic nuclei in rats and monkeys [6, 7, 9, 12]. An example of our mathematical analysis of an area of a map of the occipital cortex of the rhesus monkey [12] will illustrate this state of

affairs (Fig. 3). The discreteness of organization of the ventricular zone of the brain shows similarity with the organization of the germinative zone of the olfactory epithelium and epidermis of the skin, which consists of proliferative loci, each of which forms a vertical column of cells [8, 11].

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MORPHOLOGICAL CHARACTERISTICS OF HIPPOCAMPAL NEURONS DEVELOPING IN CELL CULTURE

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UDC 612.825.26-014.2-085.23+611.813.14-018.82-085.23

KEY WORDS: cell culture; hippocampus; morphogenesis of neurons.

Dissociated and reaggregated cultures of cells from different parts of the CNS of mammalian embryos are widely used nowadays to study the principles governing morphogenesis of neurons, glioneuronal interactions, oriented growth of axons, and the formation of interneuronal connections. Objects widely used for research of this kind are cell cultures of the embryonic hippocampus [3, 6, 7, 9, 10].

The aim of this investigation was to study the morphogenesis of neurons in cell cultures from the hippocampus of mouse embryos.

EXPERIMENTAL METHOD

A homogeneous cell suspension obtained by enzymic and mechanical dissociation of the hippocampal tissues of 18-19-day C57BL mouse embryos was applied to collagen-coated coverslips (about 3000-4000 cells/cm^2 of glass) and cultured in Maximow's chambers [1] at 35°C. The nutrient medium was changed once after the 3rd-4th day of culture. The cultures were investigated intravitally at various times in vitro by photomicrography and by time-lapse motion picture filming in a light field and in phase contrast, stained by Nissl's method, and impregnated with silver by the method of Holmes and Wolff.

EXPERIMENTAL RESULTS

Most cells immediately after application of the suspension to collagen were spherical in shape, and only a few were observed to give off short single processes. In the course of a

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6, pp. 738-741, June, 1987. Original article submitted June 13, 1986.

0007-4888/87/0006-0843$12.50