POSTNATAL HISTOGENESIS AND PROLIFERATION OF CELLS OF THE PARIETAL NEOCORTEX IN NORMAL MICE AND AFTER BRAIN TRAUMA

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UDC 591.481.1:591.88+576.35

The postnatal histogenesis of the parietal region of the neocortex and the ability of the principal cell types in it to proliferate were studied in normal mice and after a stab wound of the brain by analysis of cells labeled with [^{3}H]thymidine in semithin sections. In the postnatal period no microneurons were formed in the parietal cortex of the mice either by migration of undifferentiated cells or by proliferation. Trauma to the right hemisphere caused no change in the direction of the histogenetic transformations of cells migrating into the parietal cortex toward their differentiation into microneurons.

KEY WORDS: neocortex; postnatal histogenesis; trauma; autoradiography.

During early postnatal development a high proportion of the small neurons or microneurons of the cerebellum, hippocampus, and olfactory bulbs are formed in the brain of mammals blind at birth [2, 6, 7, 12]. Data on the scale of postnatal formation of neocortical microneurons are less definite because of the difficulty of identifying microneurons and gliocytes by the use of stains compatible with the technique of autoradiography [7, 8, 10]. This same cause is responsible for the lack of clarity of views regarding the proliferative potential of the various cell types of the neocortex [3, 4, 11]. Recently, on the basis of the results of light-optical and electron-microscopic studies of the same cells, a classification of features was drawn up, with the aid of which the main types of cells of the rat neocortex can be identified in semithin sections [9]. This approach, combined with the method of autoradiography, was used in the present investigation to study the postnatal formation and proliferative potential of the principal cell types in the parietal region of the mouse neocortex during normal development and after brain trauma, inflicted in order to activate proliferative processes.

EXPERIMENTAL METHOD

Two groups of experiments were carried out on 45 male CBA × C57BL/6 mice: 1) with cumulative labeling of the mice with [^{3}H]thymidine to study the histogenesis of cells in the parietal region of the neocortex; 2) with pulse labeling with [^{3}H]thymidine to study local proliferation in the parietal cortex. In the first group of experiments [^{3}H]thymidine (specific activity 19.8 Ci/mmole) was injected intraperitoneally twice a day for 4 days. The total dose of [^{3}H]thymidine was 20 μCi/g. These 4-day courses of injections were given to mice aged 1-4, 5-8, 9-12, 13-16, and 17-20 days after birth. Injections also were given to mice aged 5-8 and 13-16 days whose right hemispheres were injured with the needle of a syringe 2 days before the beginning of the injections. All the mice in the experiments with cumulative labeling were killed on the 35th day after birth.

In the second group of experiments [^{3}H]thymidine (specific activity 19.8 Ci/mmole) was injected intraperitoneally into the mice in a dose of 10 μCi/g, and the animals were killed 1 h later. Intact mice aged 5, 13, and 35 days and mice of the same age with a stab wound of the right hemispheres inflicted 3 days before injection of the isotope, were used for the experiments (the method of injury of the brain was described previously [3]).

The brain of the animals in both groups of experiments was perfused with a 2% solution of paraformaldehyde with 2.5% glutaraldehyde in phosphate buffer (pH 7.2-7.4). Pieces of the parietal cortex were fixed in the

Laboratories of Brain Ultrastructure, Brain Cytoarchitectonics, and Functional Synaptology, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. Central Research Laboratory, 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. 85, No. 2, pp. 234-237, February, 1978. Original article submitted March 16, 1977.

0007-4888/78/8502-0242$07.50

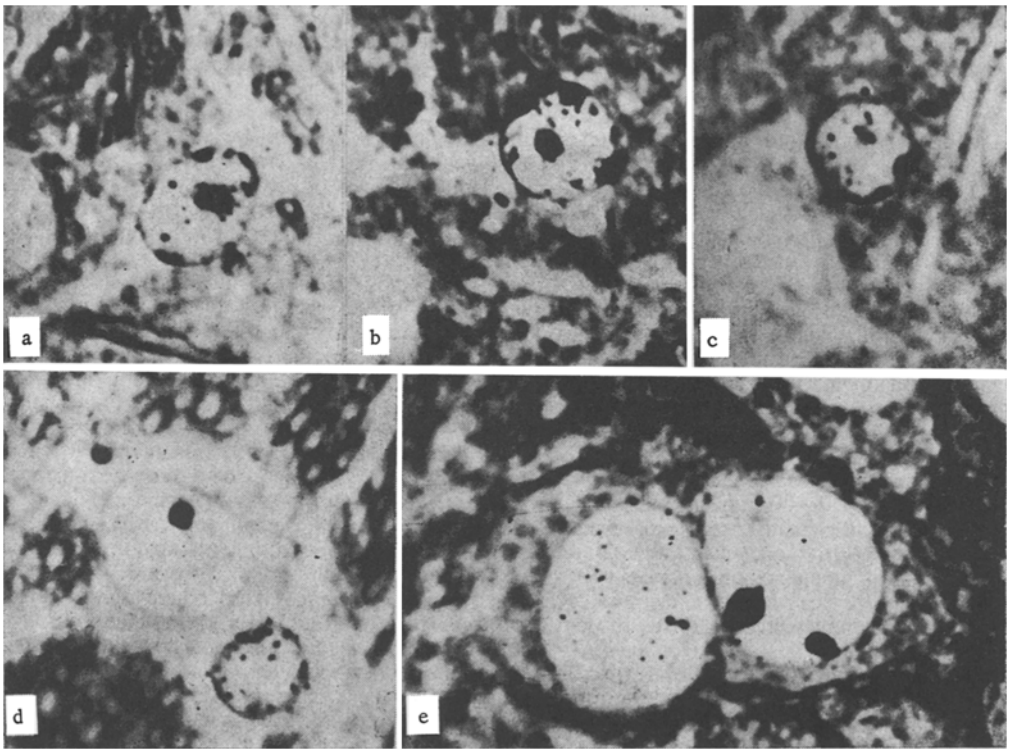

Fig. 1. Parietal cortical cells of mice, labeled with [^{3}H]thymidine, in experiments with cumulative labeling of mice aged 1-4 days with [^{3}H]thymidine (a, b, c, d) and in experiments with pulse labeling of mice aged 2 weeks (e). a, b) Labeled astrocytes; c, d) labeled oligodendrocytes; e) labeled cell with the features of a neuron. Semithin sections, toluidine blue, 900×.

same solution, postfixed in 2% OsO_4 by the standard method, and embedded in Epon. Sections 1-1.5 μ thick were cut on the LKB Ultratome, mounted on slides, and coated with type M photographic emulsion. After exposure for 35 and 60 days the sections were developed and stained with 1% toluidine blue solution in 2.5% sodium carbonate. Areas of the parietal cortex were studied by the use of an immersion objective.

EXPERIMENTAL RESULTS

In semithin sections of the adult mouse neocortex the principal cell types had the same features as were described during the study of semithin sections of the adult rat neocortex [9], and no difficulty was encountered in the differentiation between neurons and gliocytes or the identification of the main types of glial cells. In mice aged 5 days and 2 weeks the cells in the neocortex were less highly differentiated than in adult (35 days) mice, and the possibility of error in determination of the cell type was significantly greater.

Analysis of labeled cells in the neocortex of mice receiving injections of [^{3}H]thymidine from the 1st to the 20th days of life inclusive and sacrificed on the 35th day (first group of experiments) showed that [^{3}H]thymidine is incorporated into the main types of glial cells: astrocytes, oligodendrocytes, microglia, and pericytes (Fig. 1a-d). Nerve cells in the parietal cortex did not take up the isotope, and in only one of three mice receiving injections of [^{3}H]thymidine from the first through the 4th days of life were two small labeled neurons found in layers III and IV of the neocortex. Comparison of the neocortex of mice receiving [^{3}H]thymidine at the ages of 1-4, 5-8, 9-12, 13-16, and 17-20 days showed a decrease in the number of labeled glial cells with age. The sharpest decrease was observed in the number of labeled astrocytes, for hardly any of these cells were labeled in mice receiving injections of [^{3}H]thymidine from the 17th through the 20th days of life. In mice receiving [^{3}H]thymidine on the 5th-8th and 13th-16th days, the same types of labeled cells were found in the neocortex as in intact mice receiving injections of the isotope during the same periods of development. No labeled neurons were seen. The total number of labeled cells after brain trauma was greater than in the intact animals.

In the experiment with pulse labels [^{3}H]thymidine was taken up in the parietal cortex of 5-day-old and 2-week-old mice by the glial cells and by undifferentiated cells. Cells with characteristic neuron indications did not take up the [^{3}H]thymidine. With pulse labeling the labeled cells were not found in the neocortex of

adult mice, with the exception of individual cells in the vascular bed. In all studied age groups, the number of labeled cells for brain trauma was greater than the norm, whereupon all such cells were of glial or hematogen type. In one case, in layer V of a 2-week-old mouse in a brain-trauma experiment, a large labeled cell having the characteristic of a neuron was found (see Fig. 1d).

Analysis of the cells in semithin sections from the parietal region of the adult mouse neocortex thus enabled the labeled cells to be classified reliably into particular types of neocortical cells, which in many cases was impossible by the use of paraffin sections. It could accordingly be concluded from the results of the cumulative labeling experiments that practically no microneurons are formed in the parietal cortex of mice in the postnatal period. Two small labeled neurons found in a mouse receiving injections of [^{3}H]thymidine on the 1st to the 4th days of life were evidently residual phenomena of neurogenesis normally complete before birth. Brain trauma to the animals with cumulative labeling, incidentally, did not lead to the appearance of labeled neurons, i.e., the direction of differentiation of the subependymal cells migrating into the parietal cortex, and differentiating there into gliocytes, was unaffected by trauma. The absence of labeled neurons in the cumulative labeling experiments rules out the possibility not only of the postnatal formation of microneurons in the parietal cortex on account of cells migrating from the subependymal zone, but also the possibility of local proliferation of microneurons or of their undifferentiated precursors. The results of the pulsed labeling experiments confirmed those of the cumulative labeling experiments as regards inability of the microneurons to proliferate whether during normal development of the parietal cortex or after trauma. The solitary nerve cell in layer V of the cortex of a 2-week-old mouse labeled with [^{3}H]thymidine is a unique phenomenon the nature of which is not clear. The possibility cannot be ruled out that incorporation of [^{3}H]thymidine into this cell was connected with its polyploidization, for a few polyploid neurons are present in the mammalian brain [1].

This investigation accordingly showed that postnatal formation of microneurons does not take place in the parietal cortex of mice. Similar results were obtained in a study of the histogenesis of cells of the occipital region of the rat neocortex [8]. However, these workers used paraffin sections, and they therefore tentatively assumed that 0.5% of cells formed postnatally in the visual cortex could have been microneurons.

The second (postnatal) wave of neurogenesis which, according to Altman [5, 6], is characteristic of the histogenesis of microneurons in the brain of mammals blind at birth, could not therefore be found in the areas of the neocortex of mice and rats studied. The question thus arises of the justification for the use of the actual term microneuron, which applies collectively to the interneurons of the brain and the granular neurons of the cerebellum, hippocampus, and olfactory bulbs, for one of the principal criteria used to distinguish the class of microneurons, namely their predominantly postnatal origin, has been ruled out for the numerous stellate cells (interneurons) of the parietal and occipital regions of the neocortex.

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