SPACE AND TIME SEQUENCE AND MOSAICISM OF NEUROGENESIS IN HIPPOCAMPAL AREA CA1 IN MICE

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The study of the times and sequence of neuron formation in various structures of the mammalian brain has made substantial progress thanks to the use of autoradiographic techniques, by which the germinative precursors of neurons can be tagged with ^{3}H-thymidine and the subsequent fate of the labeled cells can be followed [4]. It has been shown that in both the archicortex and the neocortex neurons are formed in an order which corresponds to the arrangement of the architectonic layers [6-9]. Meanwhile the results so far obtained have yielded no information on correlation between neurogenesis and the vertical (radial) organization of functional neuronal modules detectable in the neocortex in the form of minicolumns and macrocolumns, and in the hippocampus in the form of narrow radial segments [1, 2]. It has recently been suggested that the germinative zones of the embryonic brain are organized as loci, or discrete proliferative units, each of which produces neurons of single radial segments of screened brain structures [12, 13]. This hypothesis is based on data on migration of young neurons along fibers of the radial glia and on observations showing that neurogenesis takes place at different times in large segments (comparable with architectonic fields) in the neocortex, hippocampus, and lateral geniculate body [12-15]. Experimental proof of the discrete character of neurogenesis, comparable in scale with the dimensions of single functional modules, was first obtained by the present writers in an investigation [5] the results of which demonstrated a mosaic pattern of alternation of groups of neurons differing in the intensity of labeling in the neocortex of day-old mice receiving ^{3}H-thymidine during embryonic development. These data indicate asynchronous production of neurons supplied to the same layer of cortex by small groups of cells in the germinative zone, each of which may give rise to a single ontogenetic column in the course of embryogenesis, on the basis of which the functional minicolumn of the neocortex is considered to be formed [2].

The question naturally arises: How clearly defined is the mosaicism of neurogenesis in other brain structures and, in particular, in structures of screened type? The hippocampus, which has a clearly defined laminar structure, and in which radial modules have been discovered by physiological methods [1], is very interesting from this point of view. Data on the order and gradients of neurogenesis in the hippocampus are largely contradictory [6, 7, 14] and require further clarification.

The aim of this investigation was to study the space and time sequence of neuron formation and to look for the presence of mosaicism of neurogenesis in area CA1 of Ammon's horn of the mouse hippocampus, one of the most regularly arranged hippocampal areas [1].

EXPERIMENTAL METHOD

Pregnant CBA mice were given a single intraperitoneal injection of ^{3}H-thymidine (10 μCi) on the 11th, 12th, 13th, 14th, 15th, 16th, 17th, 18th, and 19th days of pregnancy. The progeny of these mice was killed on the first day of postnatal life. The brain of mice receiving the isotope from the 11th through the 15th day of embryonic development was fixed in Carnoy's fluid and embedded in paraffin wax. Series of frontal sections 6 μ thick were cut at the level of the rostral hippocampus. Slides with glued-on sections were coated with type M emulsion, exposed at 4°C for 21 days and, after standard autoradiographic processing, were stained with cresyl violet. In mice receiving ^{3}H-thymidine after the 16th day of embryogenesis the

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cerebral hemispheres were fixed for 12 h in a solution of 4% paraformaldehyde with 2.5% glutaraldehyde in phosphate buffer (pH 7.2), and without subsequent postfixation in OsO_4, were dehydrated in alcohols and propylene oxide and embedded in Durcupan. Series of semithin (1 μ thick) frontal sections through the rostral hippocampus were cut on an LKB-IV Ultrotome. The sections, glued to slides, were coated with type M emulsion, exposed at 4°C for 49 days and, after development, were stained with 1% toluidine blue solution in 2.5% sodium carbonate. By using semithin sections it was possible to identify the types of nerve tissue cells more accurately than in paraffin sections [14]; however, the low labeling intensity of the cells when ^{3}H-thymidine was injected before the 16th day of embryogenesis made it necessary to use paraffin sections in the early stages of the experiment.

The sections were studied under the microscope, using an immersion objective with magnification of 100 times. With the same magnification, the arrangement of the intensively labeled cells in area CA1 of the hippocampus was mapped. For mapping, an attachment with projection screen for the nu-2E microscope was used. A cell was recorded as intensively labeled if the number of grains of silver above the nucleus ranged from maximal to half the number of grains found in cell nuclei in that particular section [12]. At each time of the experiment area CA1 from two animals was mapped. The maps were used to count intensively labeled cells in individual layers of area CA1, in order to enumerate single and paired intensively labeled cells (if these cells formed clusters, they were broken up into the corresponding number of pairs), and also to discover clusters of intensively labeled neurons.

EXPERIMENTAL RESULTS

Intensively labeled neurons were found for the first time in area CA1 of mice receiving ^{3}H-thymidine on the 12th day of embryogenesis. They were found in the suprapyramidal layers (str. radiatum, lacunosum, and moleculare), the pyramidal layer (str. pyramidale), and the infrapyramidal layer (str. oriens). At subsequent stages of the experiment the number of intensively labeled neurons increased, to reach a maximum in the suprapyramidal layers when ^{3}H-thymidine was injected on the 14th day of embryogenesis, and in the pyramidal and infrapyramidal layers when the isotope was injected on the 15th day of embryogenesis (Fig. 1). The number of intensively labeled neurons then diminished rapidly, and after the 17th day of embryogenesis injection of ^{3}H-thymidine did not lead to the appearance of labeled cells in the suprapyramidal and pyramidal layers. In the infrapyramidal layer the number of intensively labeled cells remained high until injection of the isotope on the 18th day of embryogenesis, but cells labeled in the late stages of the experiment were not neurons of the infrapyramidal layer, but migrating cells. Within individual layers of area CA1 the distribution of intensively labeled neurons was independent of the times of injection of ^{3}H-thymidine, except in the pyramidal layer, in which intensively labeled neurons in the early stages of the experiment were located mainly in the deep part of the layer, whereas after the 15th day of embryogenesis they were distributed mainly in its superficial part, nearer to the suprapyramidal layers.

The results are similar to those of autoradiographic investigations, which showed that neurogenesis in area CA1 in mice begins on the 12th day of embryogenesis, reaches a peak on the 14th-15th day, and is largely complete on the 17th-18th day of embryogenesis [14, 16]. Meanwhile our data did not confirm the presence of the sandwich gradient described by other workers [7, 10], i.e., the later beginning of neurogenesis in the pyramidal layer than in the supra- and infrapyramidal layers. According to our data, neurogenesis in the suprapyramidal layers begins rather earlier than neuron formation in both the pyramidal and the infrapyramidal layers. Finally, the tendency which we observed for a sequence of formation of neurons in the pyramidal layer in the direction from within outward, agrees with the results of earlier investigations showing gradients of neurogenesis in the pyramidal layer [6, 7].

Counting the number of single and paired intensively labeled neurons in area CA1 of mice receiving ^{3}H-thymidine between the 12th and 18th days of embryogenesis showed that in the course of the experiment the ratio between the numbers of these cells varied significantly: the proportion of paired intensively labeled neurons rose (from 40% on the 12th day to 71% on the 18th day of embryogenesis) and the proportion of single intensively labeled neurons fell correspondingly (Fig. 2). The results can be interpreted as the result of the replacement of asymmetrical critical mitoses in cells of the germinative zone in the course of neurogenesis by symmetrical, critical mitoses. In the first case, during mitosis of a germinative cell one of the daughter cells migrates into area CA1 and begins neuronal differentiation, whereas the other cell remains in the proliferative compartment of the germinative zone. In the second case the two daughter cells migrate and differentiate into neurons. These ideas

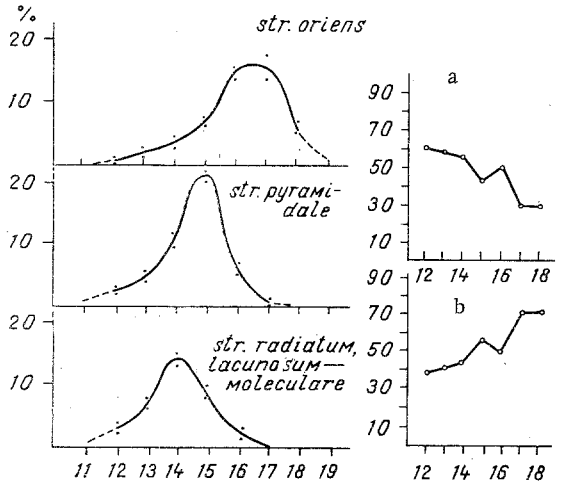

Fig. 1

Fig. 2

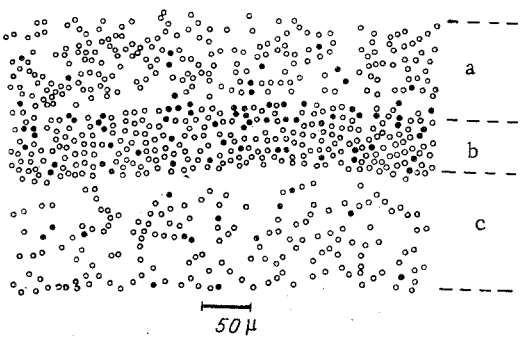

Fig. 3

Fig. 1. Number of intensively labeled cells (in % of total number of cells) in infrapyramidal (*a*), pyramidal (b), and suprapyramidal (c) layers of area CA1 of day-old mice receiving ^{3}H-thymidine on 11th-19th day of embryogenesis. Here and in Fig. 2: abscissa, days of embryogenesis.

Fig. 2. Percentage of single (*a*) and paired (b) intensively labeled neurons relative to total number of intensively labeled cells in area CA1 of day-old mice receiving ^{3}H-thymidine on 11th-19th day of embryogenesis.

Fig. 3. Mosaic character of grouping of intensively labeled neurons in area CA1 of day-old mice receiving ^{3}H-thymidine on 15th day of embryogenesis. *a*) Infrapyramidal layer, b) pyramidal layer, c) suprapyramidal layers of area CA1.

of exchange of asymmetrical and symmetrical mitoses during histogenesis, put forward by Leblond [11], have been reflected in treatises on the developing brain [3, 13]. The results of the present experiments confirm these ideas experimentally.

Finally, analysis of the distribution of intensively labeled neurons in area CA1 showed the presence of groups of intensively labeled neurons alternating with unlabeled and weakly labeled cells. These groups were found in mice receiving ^{3}H-thymidine on the 13th-16th day of embryogenesis; they were most marked when the isotope was injected on the 14th-15th day of embryogenesis (Fig. 3). The investigation thus showed that a mosaic pattern of neurogenesis exists in the hippocampus, just as in the neocortex [5], and it can be regarded as the result of asynchronous production of neurons by local areas of the germinative zone, each of which constructs a radial segment of cortex [5]. Consequently, the hypothesis that the germinative zones of screened brain structures are organized in loci [12, 13] received fresh experimental confirmation. The causes of the absence of the mosaic pattern in area CA1 in the early period of neurogenesis (12th day of embryonic development) are not yet quite clear. The possibility cannot be ruled out that it can be explained by the exceedingly small scale of neurogenesis during this period, before proliferative processes have begun to predominate in the germinative zone of the hippocampus, cells have not yet commenced neuronal differentiation, and the future neurogenic loci are only just beginning to perform their basic function.

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REACTIVITY OF MONONUCLEAR PHAGOCYTES IN THE LUNGS AND LIVER OF RATS EXPOSED TO LOW TEMPERATURES

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Resistance of the organism to stress, including cold stress, largely depends on reactivity of the mononuclear phagocyte system (MPS) [1, 4]. The MPS as a whole is a sufficiently heterogeneous cell population as regards both its morphological and functional characteristics and its role in various adaptive reactions [7]. In the investigation described below changes in some parameters of activity of MPS, and of its pulmonary and hepatic subdivisions, in the reaction of the organism to cold were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats weighing 160-180 g, divided into two groups: the animals of group 1 (n = 20) were kept at −7°C in a thermal chamber and rats of group 2 (n = 20) were kept at room temperature (20°C). The intensity of lipid peroxidation (LPO) in homogenates of the lungs and liver was determined after 2 and 24 h and after 8 days, by measuring accumulation of malonic dialdehyde (MDA) [9]. Protein was determined by Lowry's method. Alveolar macrophages were obtained by washing out the bronchopulmonary tract three times with heparinized Hanks' solution [8]; the total cell count was determined in a Goryaev's chamber. The washed out cell pool was deposited on a coverslip for differential counting, fixed with methanol, and stained with azure II-eosin. The ingestive power of the MPS was determined [5] by measuring the half-elimination time of colloidal carbon (from "Wagner") from the blood. The total number of macrophages loaded with carbon particles was counted in sections through the lungs and liver, stained with hematoxylin and eosin, under a magnification of 1000, and the total number of monocytes in the blood was counted.

EXPERIMENTAL RESULTS

After the animals had been kept for 2 h at a low temperature an almost twofold increase in the half-elimination time of colloidal carbon from the blood was observed (Table 1). Phagocytic activity of the Kupffer cells (KC) was reduced under these circumstances, whereas the phagocytic activity of the interstitial macrophages of the lungs was the same as in the control. The number of alveolar macrophages in the bronchopulmonary washings was reduced (Table 2). No LPO products were found to accumulate in the lung or liver tissues.

After exposure to cold during the next 24 h gradual disinhibition of MPS was observed, as reflected in a decrease in the half-elimination time of colloidal carbon from the blood, com-

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