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VERTICAL ORDER AND MOSAIC PATTERN OF NEUROGENESIS IN THE MOUSE NEOCORTEX

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The mammalian neocortex is characterized by a combination of horizontal and vertical orderliness in the organization of its cells. Horizontal orderliness is manifested as the layers of cortical cells and fibers, vertical orderliness by a number of vertically organized functional neuronal assemblages (minicolumns, macrocolumns) and by morphologically detectable vertical groupings of neuron bodies and their apical dendrites and synapses [1, 2]. The most conspicuous morphological features of the columnar organization are to be found in the developing neocortex [9]. Many autoradiographic studies of the order of formation of neurons in the mammalian neocortex have shown how the times of formation of neurons depend on their arrangement in layers of the cortex, i.e., on the horizontal orderliness of the arrangement of the neurons in the neocortex [3, 5]. Meanwhile, no conclusions have been drawn regarding the connection between the times of formation of neurons and the vertical organization of neocortical structures.

Meanwhile, we know that in the tectum of amphibians and mammals, which also possesses horizontal layers and columnar neuronal assemblages, vertical orderliness of neurogenesis is quite clearly defined [4, 6, 8, 10]. Since the tectum and neocortex are based on similar principles of organization of their cells, the present writers postulated that a vertical orderliness of neurogenesis also exists in the neocortex and has remained unnoticed as a result of the superposition of a more marked laminar sequence of neuron formation on it. It was also postulated that the summation of these two tendencies in neurogenesis may lead to a mosaic order of neuron formation in the structure. To test these hypotheses the investigation described below was undertaken.

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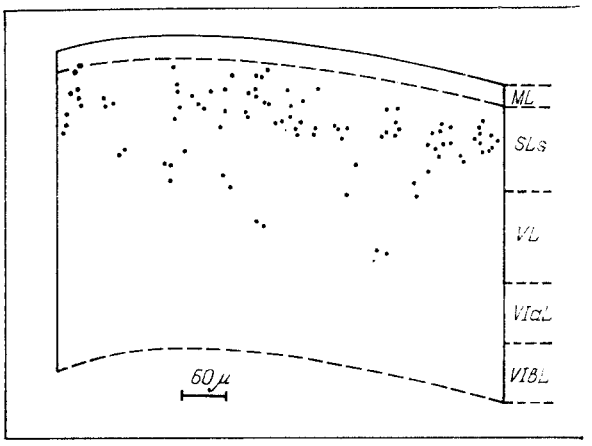

Fig. 1. Distribution of intensively labeled neurons in area 6 of neocortex of a day-old mouse receiving [^{3}H]thymidine on the 15th day of embryogenesis. ML) Molecular layer, SLs) superficial layers (II-IV), VL) layer V.

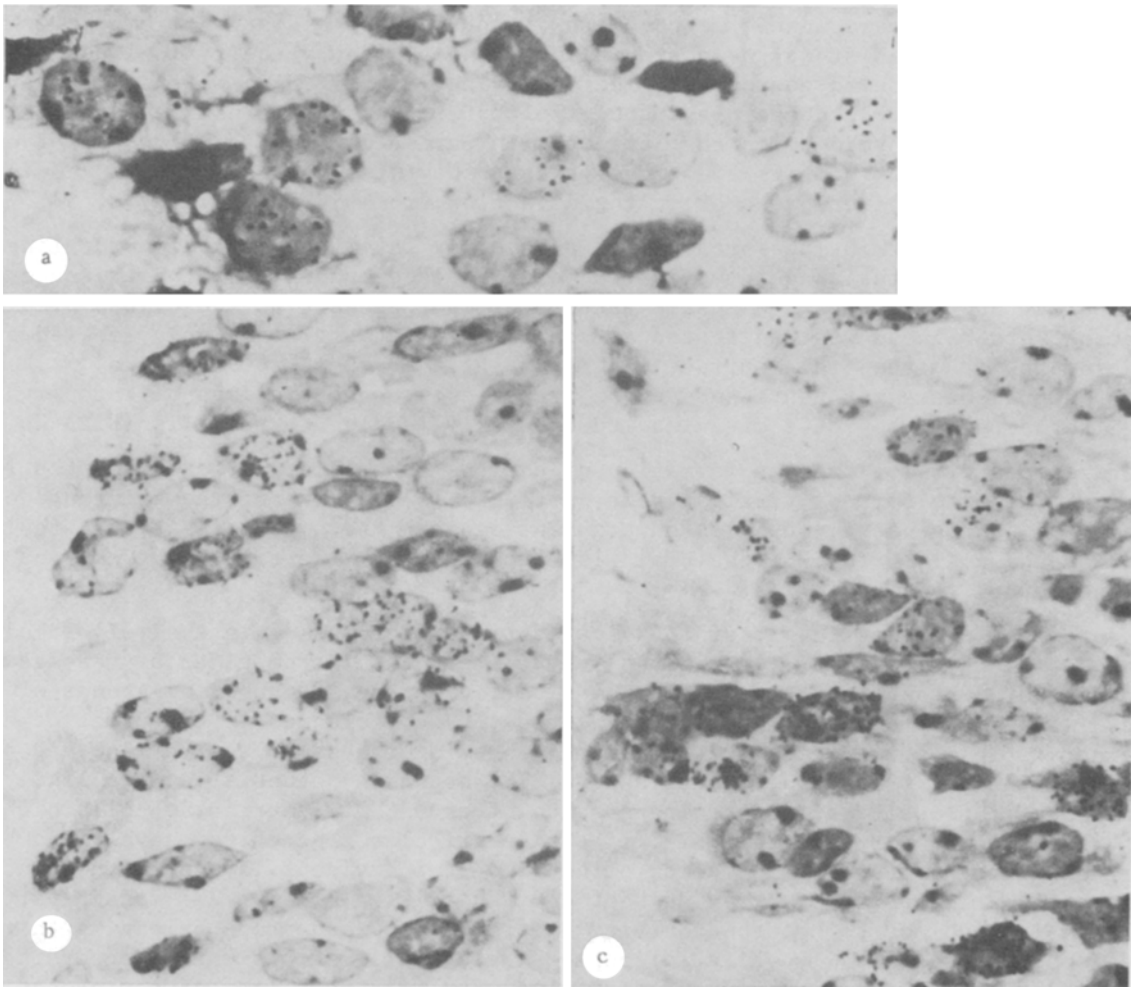

Fig. 2. Vertical orderliness and mosaic pattern of grouping of neurons labeled intensively with [^{3}H]thymidine in neocortex of day-old mice receiving [^{3}H]thymidine on 15th (a) and 16th (b, c) days of embryogenesis. a) Arrangement of intensively labeled neurons in vertical column in layers III-V; b, c) concentrations of intensively labeled neurons in layers II-III. Frontal (b) and sagittal (c) sections through neocortex. Semi-thin sections. Stained with toluidine blue. Magnification 1250 ×.

EXPERIMENTAL METHOD

Pregnant CBA mice were given a single intraperitoneal injectin of [^{3}H]thymidine (10 μCi/g) on the 15th, 16th, 17th, 18th, and 19th days of pregnancy. The progeny of these mice was killed on the 1st day after birth. Three animals were killed at each time of injection of the isotope during embryonic development. The 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 with OsO_4 they were dehydrated in alcohols and propylene oxide and embedded in Durcupan. Series of semi-thin frontal and sagittal sections (1μm) through the hemisphere in those regions of the neocortex where vertical columns were parallel to the plane of section were cut on an LKB-IV ultramicrotome. Slides on which the semi-thin sections were glued were coated with type M emulsion, exposed at 4°C for 49 days, and after standard autoradiographic processing were stained with 1% toluidine solution in 2.5% sodium carbonate. The preparations were examined under a microscope with immersion objective giving a magnification of 100 times. Under the same magnification the arrangement of neurons intensively labeled with [^{3}H]thymidine was mapped in frontal sections of area 6 of the frontal neocortex. The mapping was done by the use of graph paper with a coordinate grid and ocular micrometer with grid.

EXPERIMENTAL RESULTS

A study of the autoradiographic preparations showed that neurons labeled intensively with [^{3}H]thymidine occupied their final positions in the upper stratum of the neocortex of mice receiving [^{3}H]thymidine on the 15th-17th days of embryogenesis. If the isotope was injected later, labeled neurons were unable to complete the process of migration from the ventricular zone of the brain by the 1st day of postnatal life and to occupy their final positions in the cortex. Intensively labeled neurons in mice receiving [^{3}H]thymidine on the 15th day of embryogenesis were located in deeper layers of the cortex than the intensively labeled neurons in mice receiving [^{3}H]thymidine on the 16th and, in particular, on the 17th day of embryogenesis. These observations confirm the previous data showing a tendency toward the formation of neocortical neurons layer by layer in the direction from within outward [3, 5].

Besides data confirming the presence of a layer by layer (horizontal) orderliness of neurogenesis in the neocortex, the investigation also yielded evidence of the presence of phenomena of vertical orderliness and a mosaic pattern of neurogenesis not described previously (Fig. 1). Vertical orderliness was manifested as the arrangement of intensively labeled neurons at different depths from the brain surface within a single vertical row (Fig. 2a). The mosaic pattern of neurogenesis was expressed as the presence of concentrations of intensively stained neurons alternating with groups of unlabeled or weakly labeled nerve cells (Fig. 2b, c).

The presence of simultaneous formation of neurons located at different depths from the brain surface, in the form of vertical rows, not only is evidence of the existence of vertical orderliness of neurogenesis, but also an argument against the hypothesis that establishment of contact with the pial surface of the brain is important for arresting young neurons migrating into the neocortex [5].

The presence of a mosaic pattern of distribution of groups of intensively labeled neurons in the neocortex may arise for two reasons: 1) differences in the times of passage through the mitotic cycle by an amount exceeding the S period between neighboring groups of cells in the ventricular zone producing neocortical neurons; 2) differences in the times of completion of the final divisions of precursors of neurons entering the same layer of the neocortex between neighboring groups of ventricular cells. The second hypothesis is more likely, for it does not contradict observations showing absence of groups of ventricular cells highly synchronized in respect of their mitotic cycle, in the ventricular zone of the developing brain [7] and it agrees with data on the formation of neurons of individual layers of the neocortex in mice and rats in the course of a few days of embryonic life [3, 5]. The mosaic pattern of neurogenesis is thus due to heterochronicity of production of neurons belonging to the same layer by local regions of the ventricular zone. These small regions which, according to our approximate estimate, contained five or six cells, are evidently loci of neurogenesis, producing neurons for the separate ontogenetic columns of the neocortex, from which functional minicolumns may be formed [2].

The phenomenon of a mosaic pattern of neurogenesis also suggests that the basic units of neocortical neogenesis are not single neurons, but groups of neurons or neuronal blocks, evidence of the presence of which is given by the concentrations of intensively labeled neurons.

Successive addition to the structure of these partly intersecting blocks, taking place in the direction from within outward, may ultimately give rise to the finished ontogenetic columns on the basis of which the functional minicolumns of the neocortex are considered [2] to be formed.

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