Histogenesis of Hippocampus and Neocortex Isolated from Postnatal Rats in Organotypic Roller Tube Cultures of Floating Brain Sections

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Floating sections of the hippocampus and neocortex isolated from 6-10-day-old rats retained their spatial cell organization after 2-week roller-tube culturing. Cell structure in CA1, Ca2, and CA3 fields, polymorphic layer of the fascia dentata and its medial and lateral limbs were revealed in sections of the dorsal hippocampus. In neocortical sections, cortical neurons and subcortical structure were preserved, however, some cortical fragments changed their configuration and formed spherical structures, where cortical neurons were located in the external layer without forming typical of neocortex 6-layer structure.

Key Words: *postnatal rat brain; hippocampus; neocortex; roller culturing; floating sections*

Sections of the postnatal brain obtained from 7-8-day-old rats cultured on glass coverslips in roller tubes [5,7] and on semipermeable polycarbonate membranes in multiwell plates [6,8,11,15] are now widely used for obtaining organotypic cultures. Organotypic embryonic brain sections cultured in roller tubes were used for subsequent intracerebral transplantation [10, 13,14]. Culturing in a horizontal high-speed miniroller previously used for obtaining dissociated and reaggregated embryonic brain cultures [2] was adapted for culturing of rat brain sections [14]. The data on histogenesis of the neocortex (NC) and hippocampus were obtained using a new method elaborated at our laboratory and consisting in roller-tube culturing of floating sections from postnatal rat brain.

MATERIALS AND METHODS

The brain of 6-10-day-old rats decapitated under ether anesthesia was isolated under sterile conditions and placed in cold (4°C) Dulbecco's phosphate buffered saline (DPBS, Sigma). The hippocampus and NC isolated in a fresh portion of DPBS were cut into 350-400-μ sections. The obtained sections were cultured for 2 weeks in 50-ml flasks containing 10-15 ml nutrient medium in a horizontal roller (60 rpm, 35.5°C) [2]. Twenty or more sections can be simultaneously cultured in one flask. The nutrient medium consisted of 70% Eagle minimum essential medium, 5% fetal calf serum, 5% human placental serum, 2 mM glutamine, 0.8% glucose, 0.2 U/ml insulin, 25 mM HEPES (pH 7.2), and 0.0015% (15 mg/l) phenol red. The medium was not changed during the whole period of culturing. After 2-week culturing the tissue fragments were washed with DPBS, fixed in Telessnitskii—Lillie fluid [3] containing 38% FAE (20% formaldehyde, 10% acetic acid, 70% ethanol) mixture and embedded in paraffin. Serial 10-μ paraffin sections were stained by the method of Nissl with fast cresyl violet on 0.1 M acetate buffer (pH 3.4) [1].

RESULTS

Hippocampus is a complex structure with various spatial relationships between the hippocampal fields and fascia dentata (FD) at different cross-section levels

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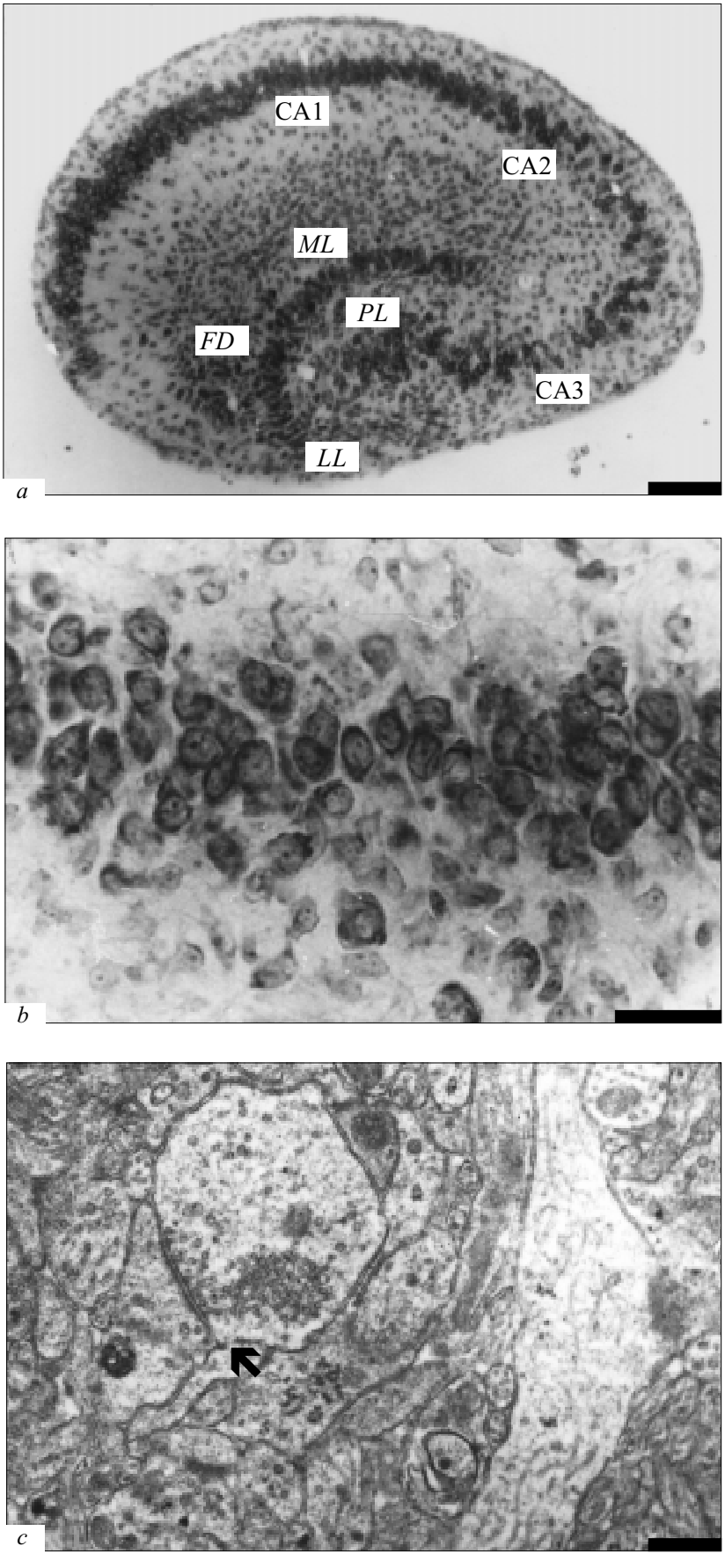

Fig. 1. Roller organotypic hippocampal culture from 8-day-old rat, 2-week culturing. Fast cresyl violet staining according to Nissl (*a, b*). *a*) cells of CA1, CA2, CA3 fields, polymorphic layer (*PL*) of fascia dentata (*FD*) and its medial (*ML*) and lateral (*LL*) limbs are clearly distinguished in dorsal hippocampus section; *b*) CA1 field neurons; *c*) synaptic axodendritic contact. Here and in Fig. 2: scale bars 200 μ (*a*), 50 μ (*b*), 5 μ (*c*).

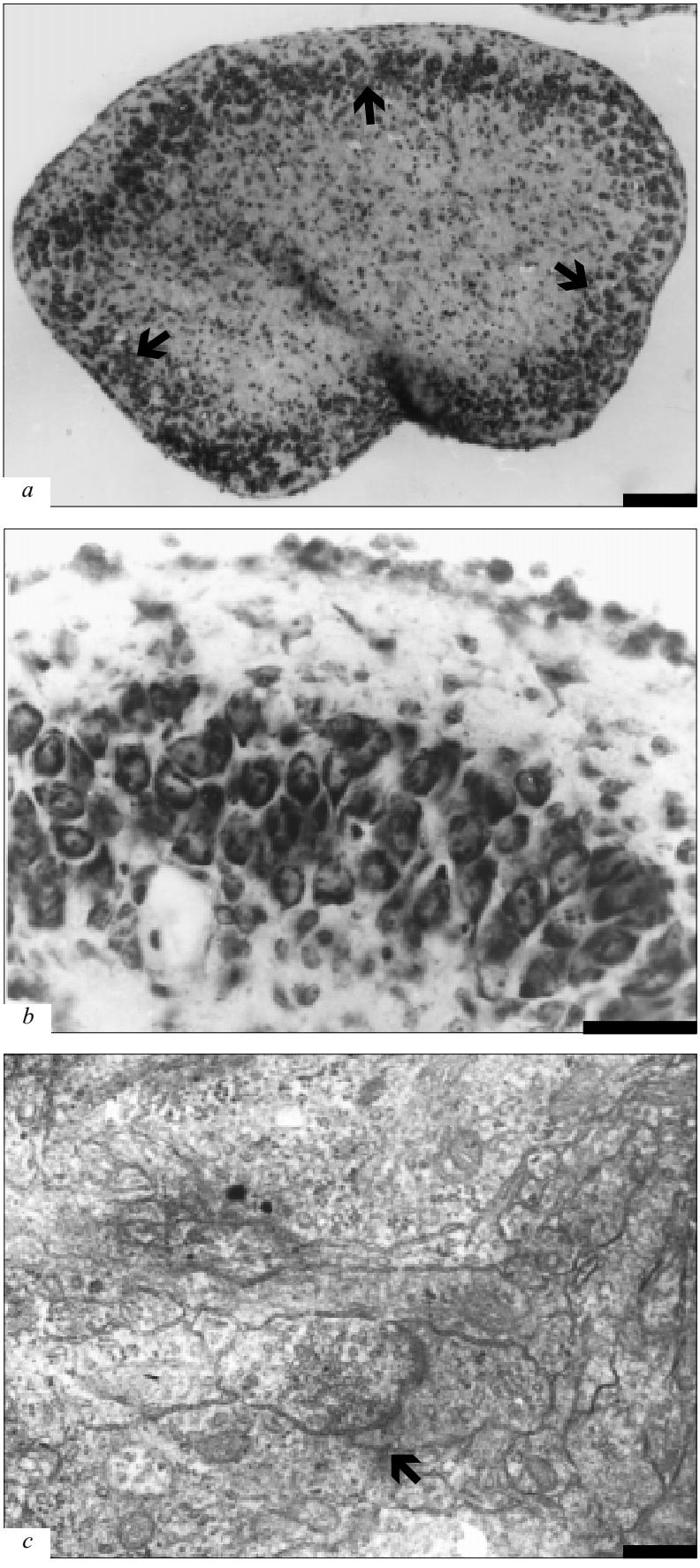

Fig. 2. Roller organotypic culture of the neocortex from 8-day-old rat brain, 2-week culturing. Fast cresyl violet staining according to Nissl (*a, b*). *a*) section of a fragment with preserved cortical plate (arrows) surrounding the subcortical formation; *b*) neurons of the cortical plate and superficial glial cell layer (arrow); *c*) synaptic axodendritic contact (arrow).

[4,9]. Organotypic architectonics of the dorsal hippocampus obtained from 6-8-day-old rats was most pronounced after 2-week culturing, which agrees with the data obtained by other methods of organotypic culturing of postnatal rat hippocampus [5,11].

Differentiated neurons of CA1, CA2, CA3 fields, and FD formations, including medial and lateral limbs and the hilus containing polymorphic neurons are clearly distinguished on Nissl stained sections (Fig. 1, *a, b*). Hippocampal sections obtained at earlier postnatal stages were characterized by incomplete formation of FD lateral limb and partial degeneration of its neurons in cultures. Electron microscopic studies of these cultures revealed differentiated nerve and glial cells in hippocampal layers. Neuropil formed by processes of these cells contained axodendritic contacts with synaptic vesicles (Fig. 1, *c*).

Histological examination of NC sections obtained from 8-day-old rats revealed a clear-cut layer of cortical neurons after 2-3-week roller culturing. Some NC fragments formed spherical structures due to bending and closing of fragment edges. This resulted in the formation of continuous circular layer of cortical neurons surrounding the white matter (Fig 2, *a*). Subcortical formation of these sections consisted of glial cells. Neuroglial cell layer was also formed on the surface of cultured NC fragments (Fig. 2, *b*). Similar structures were formed in dissociated and reaggregated roller cultures of NC cells [2].

Microscopy with higher magnification (Fig. 2, *b*) revealed radially oriented pyramidal and fusiform neurons located at different levels of the cortical plate, the superficial layers of this plate were formed by small oval neurons. This cell composition of roller NC fragments attests to only partial survival of neurons in the initial cortical plate during culturing and the absence of typical 6-layer architectonics. At the same time, electron microscopy demonstrated high degree of cell differentiation and formation of interneuronal synaptic contacts in these cultures (Fig 2, *c*).

The advantage of culturing of postnatal brain sections consists in using more phenotypically and neurochemically differentiated nervous tissue compared to embryonic and perinatal brain tissues. Moreover, completely or partially preserved organotypic structure of cultured brain sections with typical interneuronal and neuroglial connections allow to obtain more comprehensive data on *in vitro* neuron development and pathology corresponding to the parameters of the adult brain [5-7,10-12,15].

Thus, our experiments demonstrated that roller culturing of floating hippocampus sections from 6-8-day-old rats does not disturb their ogranotypic architectonics and interneuronal and neuroglial connections. However, some NC fragments change configuration and form spherical structures consisting of preserved superficial cortical plate without clear-cut neuronal stratification and deeply located subcortical glial core.

In conclusion, it is should be emphasized that one of the advantages of roller culturing system is the possibility of culturing a large number of postnatal brain sections in one flask, which provides identical development conditions and yields significant volumes of cerebral tissue for examination of neuronal development and pathology induced by hypoxia, neurotoxicity, autoimmune factors, or neuroinfection.

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