Embryogenesis of the Rat Telencephalon - a Morphologic and Stereologic Analysis

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Abstract

Comparative researches of borderline between telencephalon neuroepithelium and its surrounding mesenchyme in successive early developing stages lack in literature. The aim of this investigation was to carry out systematic morphologic and stereologic analyses of rat telencephalon in early developmental stages. We analysed semithin (1μm) serial sections of rat embryonic brain from the12th (E12) to the15th (E15) day of gestation. The surface densities (SV) of an external mesenchymal surface, an internal mesenchymal surface and a neuroepithelial (ventricular) surface were examined stereologically and compared. The surface density of the external mesenchymal surface was the biggest at E12 (4,05mm^{-1}) and the least at E15 (1,87mm^{-1}) - p<0,0005. The surface density of the internal mesenchymal surface was the biggest at E12 (4,02mm^{-1}) and the least at E15 (2,69mm^{-1}) - p<0,0005. The surface density of the internal neuroepithelial surface was the biggest at E12 (3,31mm^{-1}) and the least at E15 (2,01mm^{-1}) - p<0,0005. Our stereological examines give objective numerical proof of significant morphogenetic changes in telencephalon shape described by morphologic analyses. The major advantage of stereological methods is the possibility to carry out the estimation procedures in specified, well-defined brain regions or layers.

KEY WORDS: rat telencephalon, development, stereology.

Introduction

Development of the human telencephalon begins by enclosing of rostral neuroporus at the cranial end of embryonic neural tube (24 days of development; crown-rump length 2,5-4,5 mm). It is the beginning of cortex development. Developmental process that leads to final forming of telencephalic hemispheres could be named as morphogenesis of the brain cortex. Changes of external and internal telencephalic shape are results of complex histogenetic events and internal forming of telencephalic vesicles wall (1). Morphogenesis and histogenesis of rat brain are much more faster than in human with regard of 21 days rat development. Rat embryonic neural tube is already completely closed at the cranial end at 12th day of pregnancy (E12) (2,3). The forebrain changes from a relatively simple tubular structure with thin walls surrounding a large ventricular system to a thick-walled brain with a highly convoluted but reduced ventricular system (4). Comprehensions about brain development are based on observations carried out on ontogenetic successive stages. Microscopic analysis of brain together with analysis of serial sections and model reconstruction of them gives information about shape of some telencephalon parts and basic relations between them (5,6). Three-dimensional reconstructions of the normal rat embryonic neocortex on days E15, E17, E19 and E21 show that the neocortical ventricular zone (VZ) shrinks rapidly in the medial direction during cortical morphogenesis (7). Cell proliferation and migration, growth of immature neuronal processes (dendrites and axon) and forming of citoarchitectonic layers may be light microscopic analysed. Altman and Bayer (8) studied cellular compartmentation in the germinal matrices (a primary neuroepithelium and a subventricular zone - SVZ) of the rat cerebral cortex at embryonic developmental stages. Between E12 and E15 the rat cortical germinal matrix consists only of the primary neuroepithelium. SVZ has formed in the early ventrolateral aspect of the cortex by E16. It grows in depth for several days but the neuroepithelial depth decreases. In order to understand the overall organization of the neocortex Marin-Padilla (9) studied cortical development of various mammalian embryos using the Golgi method and introduced a new citoarchitectonic theory of the phylogenetic evolution of the mammalian neocortex. The neocortex starts its development with a primary plexiform layer in the telencephalon, which precedes and is essential for the formation of the cortical plaque. Layer I and the sublayer derived from this primary plexiform layer which represents the primitive cortical organization shared with reptiles and amphibians. The other cortical layers (II-VI) derived from cortical plaque which is an innovation in mammals. During the development of the cortical plaque migration, early differentiation and morphological/functional maturity of the neurons occur. Light microscopic researches incompletely showed subsequent events in pia mater development and relation of some layers of mesenchymo-neuroepithelial interface. There are doubt opinions about origin of delicate layer on the brain surface called pia mater (1). Some authors maintain pia develops from mesodermal layer which surrounds telencephalon (10), while the others consider that the origin of pia mater is neuroectoderm developing from rostral part of neural groove (11). However, most authors agree with claim that each tissue taking part in forming meninges have to pass mesenchymal stage without regard to its origin.It is necessary to collect data from different fields of research for complete analysis of every single stage of brain development. Modern methods of quantitative analysis are from stereology field. Stereology is the methodology which provides meaningful quantitative descriptions of real three-dimensional glob structure from measurements of two-dimensional images sampled from the glob (12). Efficient unbiased stereological methods of quantitative analysis of different regions in CNS have been developed since 1984. and are superior to the conventional basic methods previously used (6). The usefulness of stereological principles is established for estimation of neuron and synapse number and size, synapse gradients, neuron point patterns in three-dimensional space and capillary surface area (13,14,15,16,17,18,19,20,21,22). Neuronal migration occurs early during embryonic development (23). Control of neuronal migration involves different cell populations and multiple molecular mechanisms (cell-cell adhesion, interaction with extracellular matrix protein, neurotransmitter release, growth factors availability). Various forms of cell migration in the embryonic telencephalon of mammals were investigated using a combination of several molecular biological techniques (24,25). Systematic comparative stereological researches of borderline surfaces of mesenchyme and neuroepithelium in early developing stages of rat telencephalon lack in literature. The aim of this investigation was to analyse the earliest stages of the developing rat telencephalon morphologically and quantitatively and to compare sizes of neuroepithelial and mesenchymal surfaces.

Material and Methods

Rat brains used in this study were obtained from Fisher inbred rats with accurately timed pregnancies. The investigation was carried out on serial sections of five embryonic rat heads in each examined day of development. Gestation was considered to have begun early in the morning when sperm was found in the vaginal smear. The following 24 hours were designated E1. Embryos were isolated from the uterus of a pregnant female under anesthesia on E12 to E15. Fixation was performed by immersion of embryos in 1% glutaraldehyde and 1% paraformaldehyde in 0,15M sodium phosphate buffer. The whole telencephalon was embedded in Epon-Araldite. Serial 1 μm plastic sections of telencephalon were stained with toluidine. The stereologic analysis was performed using the semicircular test system L 36, at objective magnification x10. The sections were used systematically and intermittently. Number of samples for each embryo was calculated according to DeHoff (12). By means of standard stereological procedures were calculated surface density (Sv) of external mesenchymal surface (m/ext), surface density of the mesenchymo-neuroepithelial borderline (m/int) and surface density of neuropeithelial ventricular surface (e/int). Referent space was telencephalon area of the forebrain (Fig.1). The results are presented as the mean ± SD. Statistical differences between two means were determined by Student's t-test. $P< 0,05$ was considered statistically significant.

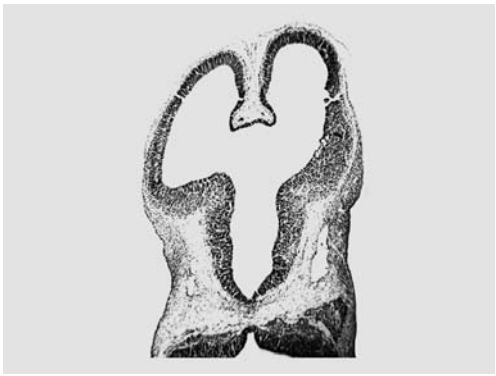

FIGURE 1. Photomicrograph of telencephalon area referent space in rat forebrain at E14. Toluidine stain, x40.

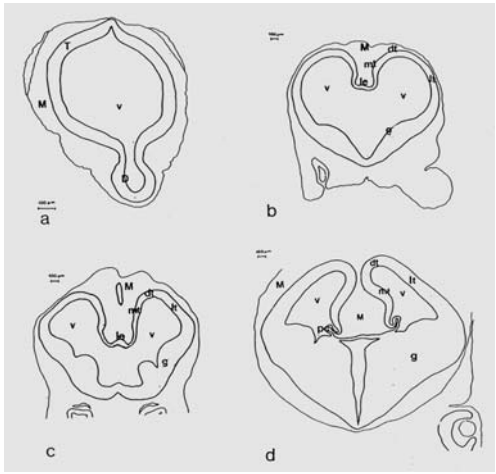

FIGURE 2. Morphometric changes of rat developing telencephalon: a) E12: paired telencephalon vesicles origin (T) on the rostral part of forebrain, its ventricular cavity (v), surrounding mesenchyme (M) and diencephalon origin behind; b) E13: lamina epithelialis (le), medial (mt), dorsal (dt) and lateral (lt) telencephalon and ganglionic eminence (g); c) E14; d) E15: plexus choroideus origin (pc).

Results

E12: neural tube is completely closed in its cranial part. Rostral part of prosencephalon shows slightly widenings (telencephalons vesicles origin) which appeared as paired, slightly expressed lateral prominences of neuroepithelial wall (Fig.2a). Between telencephalic vesicles origin and in direction to caudal there is origin of diencephalon. Cavities inside brain vesicles are origin of primitive ventricules. Lateral ventricules are in telencephalic vesicles area. In mesenchymal wrapper of neuroepithelial origin starlike cells partly touching each other with their cytoplasmatic processes. Primitive blood vessels are in parallel position with the neuropeithelial surface. Surface density of mesenchymal external surface was $(4,05 \pm 0,37)mm^{-1}$, surface density of mesenchymo-neuroepithelial borderline was $(4,02 \pm 0,15)mm^{-1}$ and surface density of ventricular neuroepithelial surface of was $(3,31 \pm 1,07)mm^{-1}$.

E13: rostral part of prosencephalon shows already well expressed and divided areas of telencephalon. Paired lateral well expressed prominences are telencephalic vesicles –origin of future hemispheres (Fig.2b). Central area between paired telencephalic vesicles is telencephalon impar. Its neuroepithelial wall is very thin, particularly in lamina epithelialis area. Some parts of neuroepithelial telencephalon wall are more thicken in comparing with the other, so they can divide on basal, basolateral, dorsal and medial telencephalon. Particularly, basal area of telencephalon named ganglionic eminence is

thicken. It is corpus striatum origin. At E13 there is already penetration of blood vessels from surrounding mesenchyme to neuroepithelial wall of telencephalon. All examined surface densities were significantly higher at E12 comparing to E13 ($p<0,0005$).

E14: telencephalic vesicles are more prominent in comparing to day before (Fig.2c). As basolateral parts of telencephalic vesicles are very thicken, neuroepithelial wall protrudes to ventricular cavity, and lumen of ventricule becomes folded. Telencephalon impar and surrounding mesenchyme are positioned in front of ganglionic eminence. Telencephalic vesicles spread above telencephalon impar to rostral direction. There aren't any statistic differences between surface densities examined at 13th and 14th day of development ($p>0,05$).

E15: telencephalic vesicles are growing in their basal, lateral and dorsal parts. In area of telencephalon impar mesenchyme and a thin neuroepithelial wall spread in direction to telencephalic ventricular cavity in fingerlike shape processes. It is the origin of plexus choroideus (Fig.2d). Surface density of external mesenchymal surface is significantly lower ($p<0,005$), internal neuroepithelial surface is significantly lower ($p<0,01$) but surface density of mesenchymo-neuroepithelial borderline is not different in comparing to a previous day ($p>0,05$). Average surface densities of mesenchymal external surface, mesenchymo-neuroepithelial borderline and ventricular neuroepithelial surface from E12 to E15 of rat telencephalon development are shown on Table 1. and Figure 3.

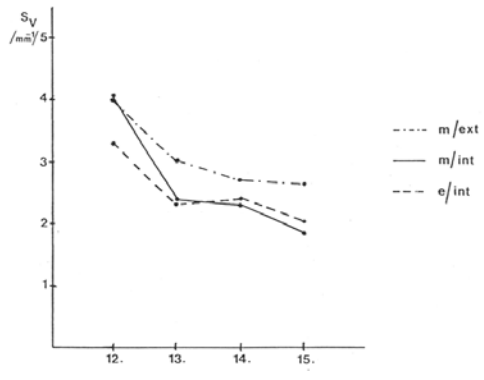

FIGURE 3. Surface densities (SV) of neuroepithelial internal surface (e/int), mesenchymal external (m/ext) and internal (m/int) surface from E12 to E15 of rat embryonic development.

Discussion

Unbiased stereological methods have been applied for determining real changes in cell number because of disparity between a density and the total number as a quantitative measure (23). Numerous investigators of CNS by means of data about the number of neurons and their synapses developed meaningful animal models of normal development and aging, neuronal connectivity and neurodegenerative diseases (24,25,26,27,28,29,30,31,32,33). The major advantage of stereological methods is the possibility to carry out the estimation procedures in specified, well-defined brain regions or layers (34,35,36,37). Quantitative results of our research of rat telencephalon development show that sizes of mesenchymal and neuroepithelial bordering surface decrease with increasing of telencephalons origin and its surrounding mesenchyme. Surface density of mesenchymal external borderline is the biggest at E12. At E13 it suddenly decreases and is the smallest at E15. Surface density of internal (ventricular) neuroepithelial surface also is the biggest at E12 and the smallest at E15 but it shows minor increase at E14. Surface density of mesenchymo-neuroepithelial borderline is the biggest at E12 and suddenly decreases at E13. It reaches the smallest value at E15 similar to external mesenchymal borderline surface. With regard that the surface density is quantitative indicator of surface size in unit of organ volume, we consider that our stereological examines objectively, numerically give proof of significant morphogenetic changes of external and internal shape of telencephalon described by morphologic analyses. At E12 all examined surfaces are relatively the biggest in compare to the later developmental stages, with regard to that telencephalic neuroepithelial wall and surrounding mesenchyme are the smallest at that time. There is an increase of neuro-

EMBRYONIC DAY	$S_{Vm/ext}$	$S_{Vm/int}$	$S_{Ve/int}$
E12	4,05 ± 1,37	4,02 ± 1,15	3,31 ± 1,07
E13	2,38 ± 1,15	2,99 ± 0,96	2,32 ± 1,09
E14	2,33 ± 0,85	2,76 ± 0,82	2,39 ± 0,84
E15	1,87 ± 1,18	2,69 ± 1,02	2,01 ± 1,30

TABLE 1. Quantitative proportions between some pigments

epithelial quantity by progressing of morphogenesis and histogenesis. Our morphologic findings are consistent suggest that rat cortical germinal matrix consists only of a primary neuroepithelium between E12 and E15 (8). At E15 a thick ventricular zone is a mayor component of the neocortex (7). Mesenchymo-neuroepithelial borderline is of a special significance because of their inductive interaction. At E13 when blood vessels penetrated from mesenchyme to neuroepithelium, the size of their borderline suddenly decreases. From E13 to E14 slightly increase of ventricular neuroepithelial surface density is possible to explain with the most intensive telencephalic morphogenetic changes observed at these stages of development. The elongated medial structure of telencephalon impar appears at E13. With thickening of basolateral telencephalon some parts of neuroepithelial wall protrude to the ventricular cavity and ventricular lumen becomes folded at E 14. The result is the increasing of internal neuroepithelial surface . One of the early events in the establishment of regional diversity in brain is the subdivision of the forebrain into the cerebral cortex and underlying basal ganglia. Fishell et al. (38) visualized cell movement within the VZ of the dorsal and basal regions of the E15 murine telencephalon. Cell dispersion was restricted at the border between the cortical VZ and the lateral ganglionic eminence, the basal telencephalic VZ. Neyt et al. (39) examined a short range signal that restricted movement of cells between the proliferative zones of the dorsal and basal telencephalon. They found the boundary which isolates these respective environments through either a contact dependent or a short-range diffusible mechanism. The dramatic shrinkage of the neocortical part of VZ is caused by a rapid growth of the basal ganglia in dorsomedial direction from primordia in the ventrolateral telencephalon which obliterates the contiguity between the lateral ventricle and neocortical primordium. As the result the neocortical VZ is displaced dorsomedially (7). We concluded that the ventricular lumen and internal surface decrease at E15 because of sudden growth of ganglionic eminence.

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