DEVELOPMENT OF THE RAT TELENCEPHALON - VOLUMETRIC ANALYSIS

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Abstract

With regard to intensive morphometric changes, morphometry as a method is mainly used for histogenetic studies of brain development in normal and experimental conditions. The aim of our study was to quantitatively analyse morphological parameters of the rat telencephalon during embryonic development.
The investigation was carried out on semithin serial sections of rat brain from embryonic days 12 to 15. The volume densities (VV) of the lateral ventricles, the telencephalic neuroepithelium and the surrounding mesenchyme have been analysed stereologically and compared in examined embryonic stages.
The neuroepithelial volume density was the smallest (28%) at E13 and the biggest (44%) at E15 ($p<0.0005$). The mesenchymal volume density was the smallest (32%) at E13 and the biggest (48%) at E14 ($p<0.0005$). The volume density of lateral ventricles was the biggest (40%) at E13 and the smallest (14%) at E15 ($p<0.0005$).
Neurostereological methods have been making a very valuable contribution to neuroscience over recent years. We have used unbiased stereological counting methods to obtain objective quantitative parameters which show relations between some parts of rat embryonic telencephalon examined during its normal development.
Key words: rat telencephalon, development, stereology.

Introduction

During brain development, the structure of the telencephalon is dramatically transformed by region-specific proliferation and differentiation of neuroepithelial cells (1).
In the fourth week of human embryonic development the neural tube is already formed and enclosed. The width of neural tube is almost uniform in the brain area. The rostral neuropore closes during Carnegie stage 11, when the embryo has between 13 and 20 somitic pairs. The caudal neuropore closes during Carnegie stage 12, when the embryo has about 25 somitic pairs (2). Once the neural tube has closed, its walls are subject to pressure of the contained fluid, provided that the formation of fluid is greater than the absorption. The fluid may result in rostrocaudal enlargement and widening of the brain. Such a mechanism would be expected to contribute to the shaping of the neural tube and preserve it from collapsing, although the main contribution to growth is from mitotic activity. During Carnegie stage 13 the embryo has 30 or more somitic pairs, crown-rump length (CRL) is 4-5 mm i.e. the embryonic age is approximately 28 postovulatory days. The relatively simple shape of the brain during Carnegie stage 13 is matched by a still elementary histological organization of its walls but the brain still occupies 40% of the whole neural tube (3). At this stage of development there are three prominent vesicles on its cranial part. Two weeks later rostral vesicle, prosencephalon, divides in the telencephalon (future hemispheres) and diencephalon (future tweenbrain). At first, the telencephalon is one vesicle on rostral end of neural tube, but very soon lateral prominences begin to develop on its both sides (3,4,5,6,7).
Considering that rat embryonic development occurs in 21 days, morphogenesis and histogenesis are much faster than in human. The 12th day of rat gestation (embryonic day 12, E12) corresponds to Carnegie stages 13/14 of human embryonic development (8). In rat embryo the neural tube is already completely enclosed on its cranial part at E12. The rostral part of prosencephalon shows two softly lateral widenings of neuroepithelial wall which are telencephalic vesicles. Between telencephalic vesicles in caudal direction is the diencephalon origin. Cavities inside brain vesicles are the origins of ventricules. In mesenchymal wrapper of neuroepithelium, starlike cells form a fine network with their cytoplasmatic processes. At E13 different parts of telencephalon are already well expressed and separated, and the neuroepithelial wall is not uniform. The mayor change is penetration of blood vessels from the surrounding mesenchyme to the telencephalic wall. At E14, basolateral parts of the telencephalic vesicles are much thicker, the neuroepithelial wall protrudes to the ventricular cavity and the ventricular lumen becomes folded. At E15, the telencephalic vesicles growing in their basal, lateral and dorsal parts. In the rear and basomedial side telencephalon partially covers diencephalon (9).
In modern neurobiology special attention is oriented on the mesenchymal-neuroepithelial interaction. The formation of the vertebrate nervous system during embryogenesis is contingent on a close association between the invaginating chordamesoderm and the overlying ectoderm. As a result, the ectoderm is designated to become the neuroectoderm-precursor of the nervous system.
The induction of the neuroectoderm by chordamesoderm is only one link in a cascade of inductive interactions involved in determination of neural structures and associated tissues (10). The aim of our study was to carry out morphological and quantitative analysis of rat telencephalon and its surrounding mesenchyme during embryonic devel-

opment. We examined differentiation stages of neuroepithelial wall during rat embryogenesis and volume relations between some parts of telencephalon and surrounding mesenchyme.

Materials and Methods

Rat embryonic brains used in this study were obtained from "inbred" Fisher rats with accurately termed pregnancies. Females were mated overnight. The mating day was defined as E0. E1 began 24 hours later. Five fetal animals were examined from E12 to E15. Time-pregnant females were deep-anesthesized, fetal animals were removed from their mothers and fixed by immersion in l % glutaraldehyde and l % paraformaldehyde in 0,15M sodium phosphate buffer. Animals were embedded in Epon-Araldite, sliced in serial frontal semithin sections through whole telencephalon and stained with Toluidine blue.

Stereology as a method provides meaningful quantitative descriptions of the geometry of real three dimensional glob structures from measurements that are made on two dimensional images sampled from the glob (11). Stereological measuring was done under light microscope according to the semicircular multipurpose test system L36 by Mertz, at ocular magnification x10 and objective magnification x10. Test points in all histological components of embryonic brain (the ventricular cavity, the neuroepithelial wall and the surrounding mesenchyme) were counted on the telencephalon test area. Volume densities (VV) of the ventricular cavity, the neuroepithelium and the mesenchyme were determined by a point-counting method (12). Quantitative results were obtained by the analyses of: 50 test fields in E12, 52 test fields in E13, 54 test fields in E14, and 126 test fields in E15.

We calculated basic descriptive statistic parameters (mean value -x , standard deviation - SD, standard error - SE) and used Student,s t-test to determine statistical differences amongst the examined developmental stages of rat telencephalon.

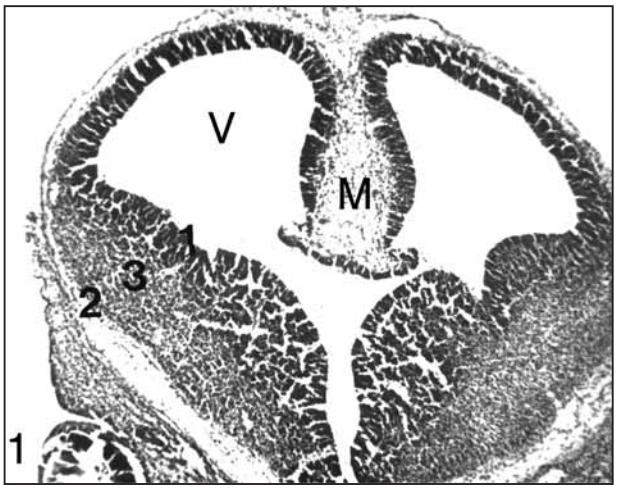

Figure 1. 15th day of gestation (E15). Telencephalic neuroepithelium consists of ventricular zone (1), marginal zone (2) and intermediate zone (3). Ventricular cavity (V), mesenchyme (M). Toluidine blue, x100.

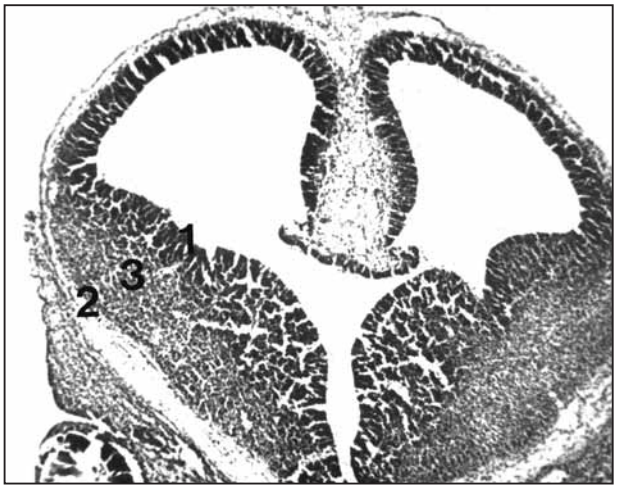

Figure 2. Volume densities (x±1SE) of the ventricular cavity (V_{VVC}), telencephalic neuroepithelium (V_{VNE}) and the surrounding mesenchyme (V_{VM}) from E12 to E15 of rat development /mm⁰/

Results

At E12 the cranial end of neural tube is closed. The neuroepithelial wall is a uniform cytoarchitectonic layer, a ventricular zone, in which columnar cells extend from ventricular to mesenchymal surface. At E13, a new cytoarchitectonic layer appears through intensive cells proliferation in the ventricular zone, a marginal zone. At E14 the neuroepithelial wall still consists of these two layers. During E15 a new cytoarchitectonic layer, the intermedi-

Table 1: Volume densities (±1SE) of the ventricular cavity (V_{Vvc}), telencephalic neuroepithelium (V_{Vn}) and the surrounding mesenchyme (V_{Vm}) from E12 to E15 of rat development /mm⁰/

Day of gestation	V_{Vvc}	V_{Vn}	V_{Vm}
E12	0.22±0.02	0.32±0.01	0.46±0.02
E13	0.40±0.02	0.28±0.01	0.32±0.02
E14	0.20±0.01	0.32±0.01	0.48±0.02
E15	0.14±0.01	0.43±.,01	0.42±0.02

ate zone ("mantle layer"), appears on the border-line between ventricular and marginal zone. This layer is the thickest in basolateral telencephalon (ganglionic eminence) - Figure 1.

The results of rat telencephalon quantitative analyses from E12 to E15 of embryonic development are presented in Table 1 and Figure 2. The volume part of neuroepithelium continually increases from E13 to E15. It is the smallest (28%) at E13 and the biggest (44%) at E15 ($p<0.0005$). In opposite, the volume part of the ventricular cavity origin continually decreases at the same time. It is the biggest (40%) at E13 and the smallest (14%) at E 15 ($p<0.0005$). The volume part of the surrounding mesenchyme is the smallest (32%) at E13 and the biggest (48%) at E14 ($p<0.0005$). From this day, when blood vessels already penetrate the neuroepithelial wall, the volume part of surrounding mesenchyme significantly decreases.

Discussion

Mammal embryo's neural tube development is extremely complex, and for better understanding of this process it is necessary to study each brain region separately. With regard to intensive morphometric changes, morphometry as a method is mainly used for histogenetic studies of brain development in normal (13) or experimental conditions (14,15). Meanwhile, the insight into normal quantitative parameters of neuroepithelium and surrounding mesenchyme during embryonic development is important for better understanding of fetal and perinatal brain damages. Comparative studies of neurogenesis (16) are more difficult because it is hard for morphometric data, obtained from analysing of each section, to be adjusted to the complex threedimensional shape of telencephalon. This can be avoided by using serial sections (17).

Bayer et al. (18) studied changes in the size and form of the rat embryonic forebrain at E12, E15, E18 and E21. During these embryonic stages authors found that "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". Due to proliferation, differentiation and maturation of neurons and glia in the forebrain as a whole (the embryonic prosencephalon) its volume continually increases from E12 to E21. In this work, attention was paid to changes in the size of the ventricles, the neuroepithelium and the mesenchyme. Volumes of the ventricles and the surrounding neuroepithelium rapidly expanded from E12 to E18 and than decreased by E21. The volume of mesenchyme continually increased.

Neurostereological methods have been clearly making a very valuable contribution to neuroscience that has grown in diversity and sophistication over recent years (19). Mayhew et al. (20) have demonstrated, using stereological methods to estimate volume, surface area and thickness of the cerebral cortex in a number of mammals, that apparent thickness (measured directly on slices) is a satisfactory estimate of true thickness (from cortical volume divided by the mean of outer and inner cortical thickness). A combination of stereology and magnetic resonance imaging has also been used to measure fetal growth and the brain volume (21,22,23). This methodology may have clinical applications in monitoring "at risk" fetuses in order to detect any abnormalities of fetal growth.

The results of our study are compatible with observation that "volume changes in the ventricles and the neuroepithelium are maintained in a "lock-step", suggesting a close relationship between the size of the ventricle and the size of the neuroepithelium" (18). As morphometric changes in the telencephalon origin are the most intensive during E13 and E14 we consider that the smallest neuroepithelial volume part at E13 corresponds to intensive growth of telencephalic vesicles in lateral direction and that leads to increase of ventricular cavity. In opposite, due to intensive cell proliferation at E15 the ventricular cavity is smaller and the neuroepithelium is thicker. In our study the mesenchymal volume ratio significantly decreased from E14 to E15. This corresponds to the statement that "the mesenchyme adjacent to the vertex of the brain becomes extremely flattened in the second month and is stretched to such a degree that its surface growth is retarded" in human embryos (24).

In our study, we examined volume changes in the ventricles, the neuroepithelium and its surrounding mesenchyme during normal rat telencephalic development. We used unbiased stereological counting methods to obtain objective quantitative parameters which show relations between parts of telencephalon during its embryonic development.

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