MORPHOGENESIS OF THE RAT FOREBRAIN

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ABSTRACT

Background and Purpose: Developmental process that leads to final forebrain shaping is a result of complex histogenetic and morphogenetic events. Comprehensions about brain development are based on observations carried out on onthogenetic successive stages. Microscopic analysis of brain together with analysis of serial sections gives information about shape the of some forebrain parts and basic relations between them. The aim of this study was to analyse morphogenesis in the earliest stages of rat's forebrain development.

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 frontal sections of rat embryonic heads from the 12th (E12) to the 16th (E16) day of gestation. Gestation was considered to have begun early in the morning when sperm was found in the vaginal smear. Histological paraffin and plastic sections were systematically inspected with regard to morphogenetic changes of the forebrain parts telencephalon and diencephalon.

Results: E12: neural tube is completely closed in its cranial part. Rostral part of forebrain shows telencephalons vesicles origins as slightly paired enlargements of neuroepithelial wall. Between telencephalic vesicles origin and in direction to caudal there is an origin of diencephalon. E13: rostral part of forebrain shows well expressed and divided areas of telencephalons vesicles as basal, basolateral, dorsal and medial telencephalon. Central area between paired vesicles is a telencephalon *impar*. In diencephalon optic vesicles appeared. Epithalamus, thalamus and hypothalamus origins are slight enlargements of its neuroepithelial wall. E14: telencephalic vesicles spread above telencephalon *impar* into rostral direction and above diencephalon in rostrodorsal direction. Their basolateral parts of are very thickened and become folded. *Sulcus telodiencephalicus* appears. E15: the main event is the appearance of the origins of *plexus choroideus* in the area of telencephalon *impar* as fingerlike processes. E16: all forebrain parts, especially telencephalic vesicles-origin of brain hemispheres and processes of *plexus choroideus*, are progressively growing and shaping.

Conclusions: Our morphologic analysis describes significant morphogenetic changes in the forebrain shape. 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.

INTRODUCTION

Neural tube formation occurs in three stages: a) formation and thickening of neural plate by elongation of neuroepithelial cells, b) bending of neural plate along the embryo midline, resulting in the elevation of neural folds and appearance of the neural groove, and c) curling over and fusion of neural folds to generate a closed neural tube. (1, 2, 3, 4)

The spectrum of shapes displayed by the developing of the neural tube of mammalian embryos is complex. In the mouse and rat, for example, the neuroepithelium exhibits rather abrupt and dramatic regional variations in size and shape. (2, 3, 5, 6, 7)

Morphogenesis and histogenesis of rat brain are much more faster than in human brain taking in consideration 21 days of rat development. Rat embryo's neural tube is already completely closed at the cranial end at 12th day of pregnancy. (8) From this time, 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. (9) Comprehensions about brain development are based on observations carried out on one after another onthogenetic successive stages. Microscopic analysis of the brain together with analysis of serial sections and model reconstruction of them gives the information about shape of some telencephalon parts and basic relations between them. (10, 11) Three-dimensional reconstructions of the normal rat embryonic (E) neocortex on days E15, E17, E19 and E21 show that the neocortical ventricular zone shrinks rapidly in the medial direction during cortical morphogenesis. (12)

The aim of this study was to analyse morphogenesis in the earliest stages of rat's forebrain development.

MATERIAL AND METHODS

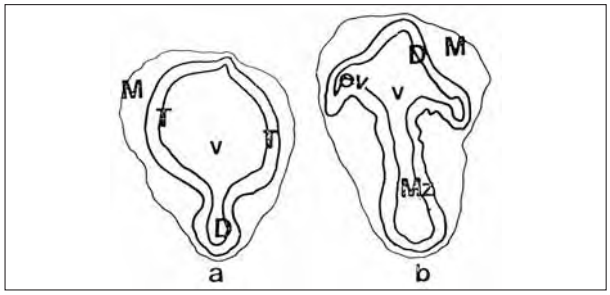
Foetal brains used in this study were obtained from "inbred" Fisher rats with accurately termed pregnancies. The day on which early in the morning sperm was found in the vaginal smear was considered as the first day of embryonic development. We have used 12 (E12) to 16 (E16) days' embryos. Each embryo was removed under anaesthesia through a separate incision in the uterus of a pregnant female. Fixation was performed by immersion of embryos in l % glutaraldehyde and l % paraformaldehyde in 0.l5M sodium phosphate buffer. Heads of each investigated embryonic day E12-E16 (n=20) were embedded in paraffin and cut into 6µm frontal serial sec-

tions through whole developing rat heads and brains. Some of E12 embryos were embedded in Epon-Araldite and cut on serial frontal 1µm plastic sections. All histological sections were stained by toluidine blue and systematically inspected with regard to morphogenetic changes of the forebrain parts telencephalon and diencephalon. The representative sections of each investigated embryonic day were projected onto a screen of microscope «Visopan» (Reichert) and brain contours were crossed out.

RESULTS

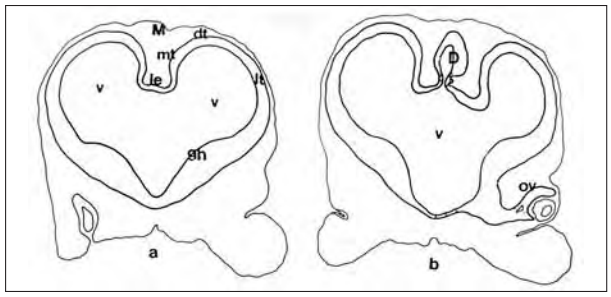
At 12th day of gestation (E12) neural tube is completely closed in its anterior cranial part. That cephalic end of the neural tube shows slight enlargements, dilatations that are primary brain vesicles origins: the forebrain (prosencephalon), the midbrain (mesencephalon) and the hindbrain (rhombencephalon). Rostral part of forebrain shows telencephalons vesicles origin as paired slightly enlargements of neuroepithelial wall (Fig. 1a). Between telencephalic vesicles origin and in direction to caudal there is origin of diencephalon. Two lateral prominences on the each side of the diencephalons neuroepithelial wall are optic vesicles (Fig 1b). Cavities inside brain vesicles are origin of the primitive ventricles.
Lateral ventricles are inside telencephalic vesicles. The cavities of the telencephalon and diencephalon contribute to the formation of the third ventricle, although the diencephalon cavity contributes more.

Figure 1. Schematic drawings of rostral (a) and caudal (b) frontal sections of the rat brain at E12



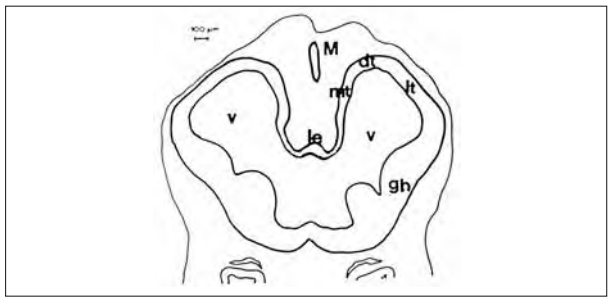
At 13th day of gestation (E13) rostral part of prosencephalon shows already well-expressed and divided areas of telencephalon. Paired lateral well-expressed prominences are telencephalic vesicles. They are primordia of the *fiuture* cerebral hemispheres (Fig. 2a). Central area between paired telencephalic vesicles is telencephalon *impar*. Neuroepithelial wall *impar* telencephalon is very thin, particularly in *lamina epithelialis* area. Some parts of neuroepithelial telencephalon wall are more thickened in comparison with the others, so they can be divided on basal, basolateral, dorsal and medial telencephalon. Particularly, basal area of telencephalon named ganglionic hill is thicken. It is *corpus striatum* origin. Origins of hypothalamus, thalamus and epithalamus appear as slight enlargements of diencephalons neuroepithelial wall. Neuroepithelial wall of diencephalon's area of optic vesicle differentiates in optic cup and *recessus opticus* (Fig. 2b).

Figure 2. Schematic drawings of frontal sections in area of telencephalon (a) and diencephalon (b) of the rat forebrain at E13.



At 14th day of gestation (E14) telencephalic vesicles are more prominent in comparison to the day before (Fig. 3).

Figure 3. Schematic drawing of frontal section of the rat telencephalon at E14.



As basolateral parts of telencephalic vesicles are very thickened, neuroepithelial wall is protruded to ventricular cavity, and lumen of ventricular becomes folded. That part of telencephalon is ganglionic hill. The neuroepithelial wall has uniform wideness in lateral and dorsal parts of telencephalon. Medial part of telencephalon, telencephalon *impar*, is elongated structure between telencephalic vesicles. Telencephalon *impar* is together with surrounding mesenchyme positioned in the front of ganglionic hill. Telencephalic vesicles spread above telencephalon *impar* in rostral direction. The neuroepithelial wall in medial telencephalon is considerably thinner than in the other parts of telencephalon. The thinnest dorsomedial part of telencephalons neuroepithelium is *lamina epithelialis*. Immediately above the *lamina epithelialis* the wall of the hemisphere is thickened, thus forming limbus, the origin of hyppocampus. From that side in caudal direction towards diencephalon thin wall of cerebral hemispheres protrudes to lateral ventricle. It is origin of the future *plexus choroideus* that is a abundantly vascularised epithelo-mesenchymal structure. It is also called *area epithelialis*. Ventricle cavity near telencephalon *impar* is still very wide. The line of demarcation between telencephalon and diencephalon becomes

complex. A groove, the telodiencephalic sulcus, divides forebrain into a ventral and dorsal region and telencephalon and diencephalon, respectively. This *sulcus telodiencephalicus* appears from ventricular side. For the first time cerebral hemispheres partially cover diencephalon in rostrodorsal direction. Neuroepithelial wall of diencephalon shows thickenings in the zone of hypothalamus origin and that a downward extension, the infundibulum, and the mammillary body which forms a distinct protuberance on the ventral surface of the hypothalamus on each side of midline. In the infundibular part of diencephalon origin of neurohypophysis appears for the first time.

At 15th day of gestation (E15) telencephalic vesicles are growing in their basal, lateral and dorsal parts. Interhemispheric groove becomes deeper and origins of brain hemispheres are better expressed because of that. Neuroepithelial wall, on the bottom of interhemispheric sulcus, is extremely thin. It is a very thin *lamina tectoria s. area epithelialis*. In area of telencephalon *impar*, on the tectorial lamina and limbus of hemisphere connecting site, mesenchyme together with thin neuroepithelial wall spread in direction to telencephalic ventricular cavity. Mesenchymal blood vessels form a rich capillary plexus that lies close against the thin neuroepithelium and push it into the ventricular lumen as a fingerlike processes. It is origin of *plexus choroideus* (Fig. 4). Basolateral part of telencephalic vesicles (ganglionic hill) is thicker than in E14. It is origin of basal ganglia. Telencephalon partially covers basomedial and posterior part of diencephalon. *Sulcus telodiencephalicus* is situated between telencephalon and diencephalon. Origins of thalamus and epithalamus are well expressed in diencephalon.

At 16th day of gestation (E16) all forebrain parts, especially telencephalic vesicles /origin of the brain hemispheres/ and processes of *plexus choroideus*, are progresivelly growing and shaping. Fingerlike shaped processes of *plexus choroideus* partially fill ventricle cavities (Fig. 5). Mesenchymal stroma with numerous blood vessels follows epithelial folds. In the area of diencephalon, thalamus, epithalamus and hypothalamus are well expressed. Thalamus is still morphologically undifferentiated.

Figure 4. Schematic drawing of frontal section of the rat telencephalon at E15

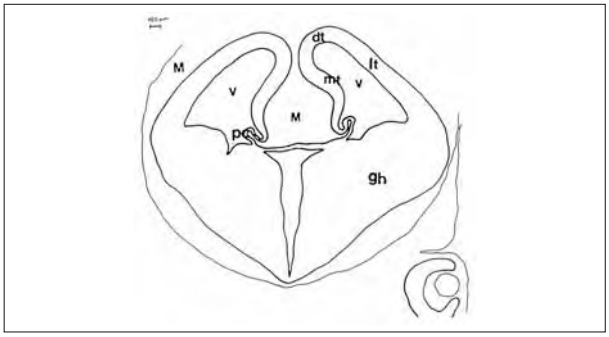
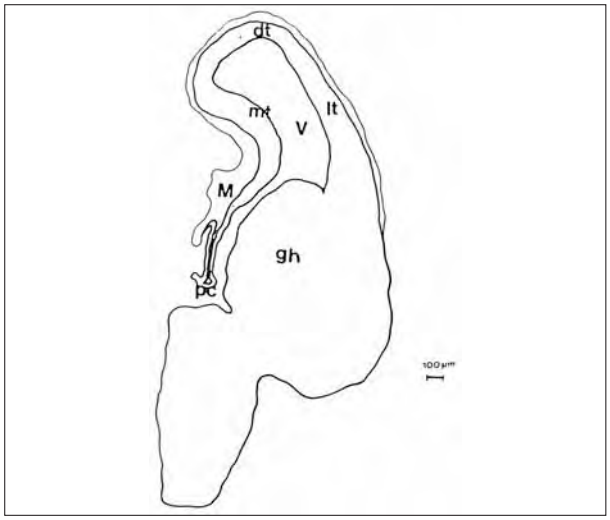


Figure 5. Schematic drawing of half frontal section of the rat telencephalon at E16.



DISCUSSION

The formation of the vertebrate nervous system during embryogenesis is a contingent in a close relation between the invaginating chorda-mesoderm and the overlying ectoderm. As a result, the ectoderm is determined to become the neuroectoderm-precursor of the nervous system. This sort of interaction, in which one tissue directs another to differentiate in a way it otherwise would not, is called induction. The induction of the neuroectoderm by chorda-mesoderm is only one link in a cascade of inductive interactions involved in determining neural structures and associated tissues. (13) In rats embryos neural tube is already completely enclosed on its cranial part on 12th day of the development. Once the neural tube is closed, its walls are subject of the pressure of contained fluid providing the formation of fluid to be greater than 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 to preserving it from collapsing, although the main contribution to growth is from mitotic activity. From three prominent vesicles on its cranial part brain develops. Rostral vesicle, prosencephalon, divide onto telencephalon and diencephalon. Primarily, telencephalon is a vesicle on the rostral end of the neural tube, but very soon, lateral prominences begin to develop on its both sides as future cerebral hemispheres. (14) During development, the structure of the brain, especially telencephalon, is dramatically transformed by region-specific proliferation and differentiation of the neuroepithelial cells. (15) Changes of the external and internal forebrain shape are results of the complex histogenetic events and internal forming of the brain vesicles' wall. (16) Our morphologic analysis describes significant morphogenetic changes in the forebrain shape. 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.

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