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Neuronal Differentiation in The Cerebral Cortex of The Rat: A Golgi-Cox Study

Abstract: The development of cerebral cortex neurons has been studied in different stages of pre- and postnatal periods of rats. In order to show the neuronal processes, the Golgi-Cox impregnation technique was used. The first neuronal process development was observed in the cranial and caudal poles of the cerebral cortex during the 15th prenatal day and continued spreading from the lateral regions and poles towards the dorsocentral regions of the hemispheres. The cells which showed the first impregnation in the cerebral cortex, were bipolar cells and their bodies were located in the deep regions of the hemisphere wall. Primitive multipolar cells, which were seen in the superficial regions on the 17th prenatal day, dispersed to the deeper regions during the following days of gestation. The neurons showing impregnations formed cell groups during the 19th prenatal day. These cells were arranged on an axis; whose perikaryons were touching each other. At the beginning of the postnatal period, they often appeared to be in the deeper regions, laminae V-VI of the cortex, later they were observed in the upper regions. Probably, a "starter cell" created cellular inductive effects, resulting in these celular accumulations. After birth, cells separated from these groups according to the maturation level of their cellular and neuropilic contents, cerebral growth and the increase in number and length of neuron processes.

As a result, in the course of the prenatal development of the cerebral cortex, consisting of cell proliferation and migration, we observed differentiation and maturation activities. The periods, in which differentiation and maturation occur, are predominantly late prenatal and postnatal periods. In addition, functionally related cells differentiate and mature in the same cell groups.

Key Words: Development, cerebral cortex, neuron, rat.

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Introduction

The central nervous system (CNS) is one of the most interesting subjects for researchers because during embryonic development it differs a lot in shape and in content. In recent years, studies have focused on the early embryonic stages, researched the kinetic behaviours of neural epithelium (1), the ontogenesis of cranial neuromeres (2), the ontogenesis of the neural segment (3, 4), the development stages of neurons (5, 6), migration of cells and the differentiation of neurons (7-9) in detail.

The neuroepithelial cells in the neural plate constitute a pseudostratified epithelium, and primary neurolation occurs due to the cell shape variety, which is formed by the intracellular filaments and microtubules (10).

Embryonic CNS in composed of four zones: ventricular, subventricular, intermediate and marginal. Although mature CNS derives from these zones, it does not exhibit the same organization (11). When cerebral vesicles emerge, their walls consist of a pseudostratified columnar epithelium (12). On the other hand, the neural elements of the cerebral cortex derive from a germinal layer via mitotic division, and these elements are located in their permanent places by means of a superficial migration (13).

The formation of a functional system from postmitotic cells requires, a) neuronal migration, b) differentiation and growth of dendrites and axon, c) synapse formation with other neurons (11). Cells, situated near the centricular lumen, are called "germinal" or "matrix" cells and their external cytoplasmic processes form a "marginal zone" (10). These mitotic cells show only nuclear division close to the ventricular face. While
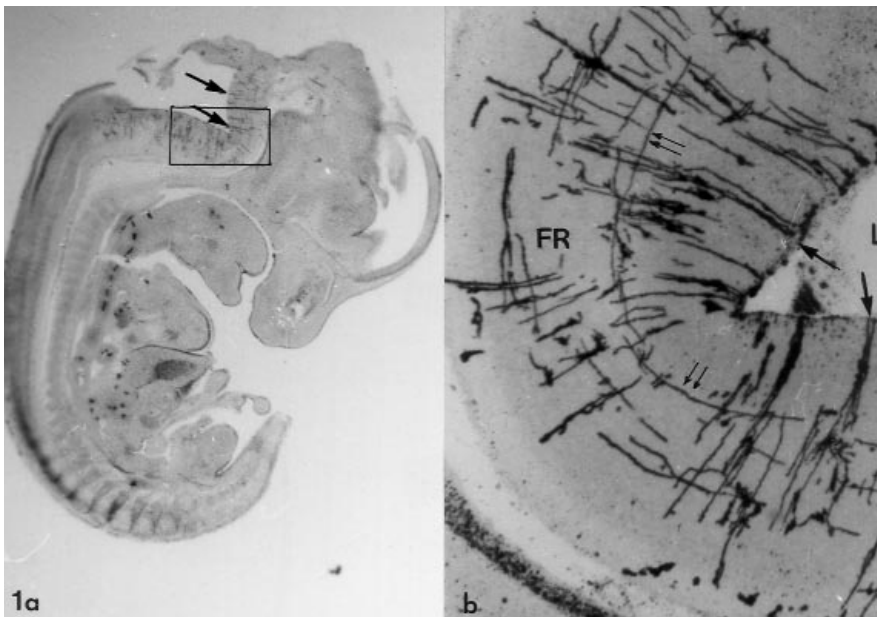
Figure 1. a) This micrograph shows impregnated cells and processes on the floor of the rhombencephalon (single arrows) of a 13 days old embryo (adapted from Demir and Demir 1994 (24)). b) magnified area of (1a), most of the impregnated cells have extended perpendicularly (single arrows) and parallel in direction (double arrows) towards the lumen. L: lumen, FR: floor of the rhombencephalon, original magnification (OM): a,X10; b, X60, Golgi-Cox.

one of the daughter nuclei migrates towards the pial surface along the superficial processes, the others stay in the same location. Also cytoplasmic division occurs on the floor of the marginal zone (13). Germinal or matrix cell proliferation and migration towards the external direction forms subventricular, subplate and cortical plates (11, 14).

Smart and Smart (12) reported that the neuron production showed two peaks during the formation of the cerebral cortex. The neuron production in the forebrain is limited to a short time period and there is a stage of rapid transformation from proliferation to differentiation in the ventricular germinal layer (6). It has been reported that young neurons, migrating on the radial glia, are guided to their exact destinations in the cortical plate (15). In addition to these studies, the organization of the cerebral cortex in the postnatal period has been explained by different studies (16-22). However, there not enough studies answering the following questions: where do the neurons develop and in which periods are differentiation and maturation seen intensively?

Concerning the reports mentioned above, this study has been performed with the aim of describing the development and the structure of neuron processes in the cerebral cortex, in the pre- and postnatal developmental periods in rats.

Materials and Method

In this study, rats of the Rattus norvegicus strain were used. The rats were examined on the 11th, 13th, 15th, 17th and 19th prenatal days; at the newborn stage; and the 5th, 10th, 15th and 20th postanatal days (n=4, for each group). In order to produce these embryos or pups, males were caged overnight with two virgin females. When sperms were seen in the vaginal smear, that day was designated as the day (0) of pregnancy. To obtain embryos from the pregnant animals, they were anaesthetised using an intraperitoneal injection of 1ml of 30% urethan aqueous solution. Then the pregnant animals were laparotomied and the uterine horns were taken. The embryos were removed from the uterus under a Nikon stereomicroscope.

The embryonal specimens were dissected in an impregnation solution (23) at room temperature and fixed in the same solution. The animals which were in postnatal periods were decapitated, their brains were removed from the cranium and the brains were processed according to the Golgi-Cox impregnation technique, was described by Rammon-Molliner (23). Celloidin embedded brains were cut in serial sections of 80-100 μm thickness from the coronal and sagittal plan. The sections were examined and photographed using a Nikon Optiphot model light microscope.

Results

I- Prenatal period

a) on the 11th and 13th days:

No cell processes were seen in the cerebral hesmisphere walls of the serial sections. There was an
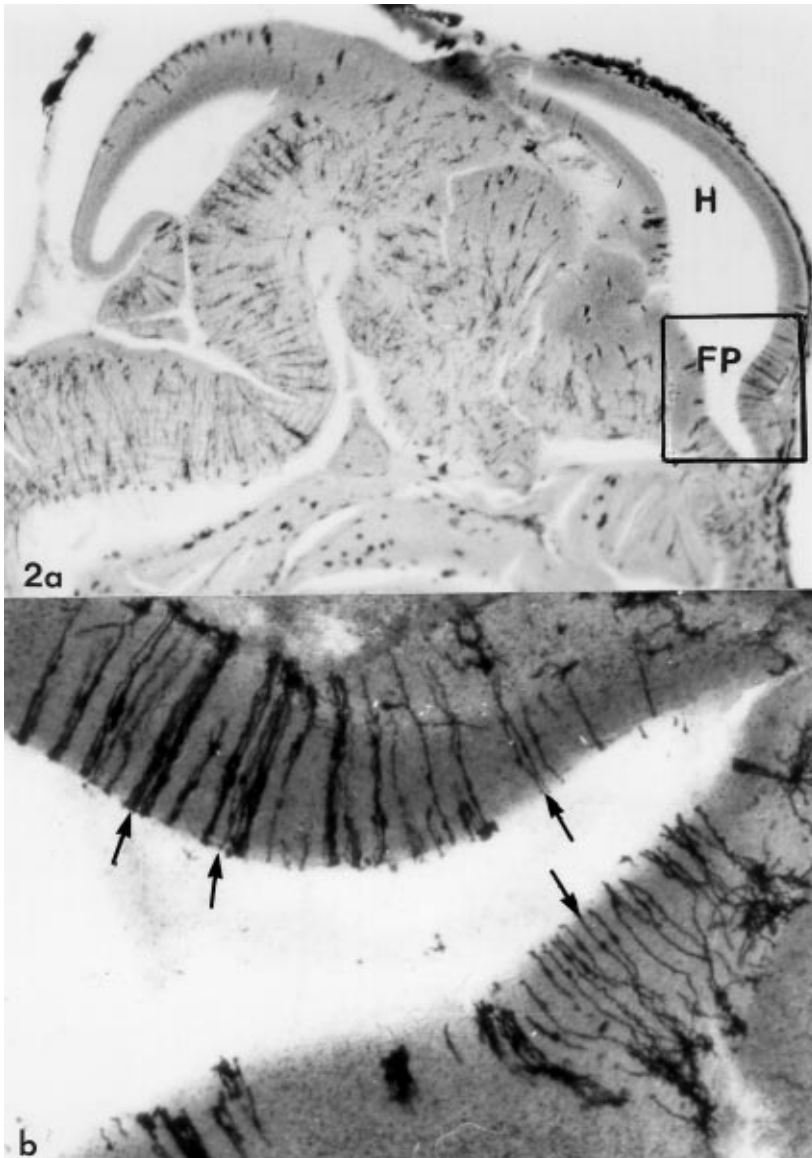
increase in the thickness of the ventricle walls depending on the cell proliferation and extracellular matrix accumulation. When the other parts of the CNS were examined, the impregnation patterns were observed only on the floor of the rhombencephalon and the cervical region of the spinal cord on the 13th embryonic day (Figs. 1a, b). In both of these areas, while most of the impregnated cell processes had extended vertically to the lumen and the external surface, few processes had extended in a horizontal direction to the lumen, crossing vertical processes at a right angle (Fig. 1b).

b) 15th day:

The impregnation had extended in the rostral direction and had reached the mesencephalon and telencephalon. The impregnation in the hemisphere walls was intense in the frontal and caudal poles in contrast to the central region (Fig. 2a). The impregnated cells, appearing in the hemisphere walls, were frequently in bipolar forms and cell bodies were located in the deeper region. While one of the cell processes was extending in the ventricular direction, the other was extending in the external direction from the deep region to the upper zone of the wall (Fig. 2b). Meanwhile the impregnated cells formed cell groups.

c) 17th day:

The impregnated cells were observed along the hemispheres. In addition to bipolar cells, new impregnated multipolar cell forms were seen. These cells
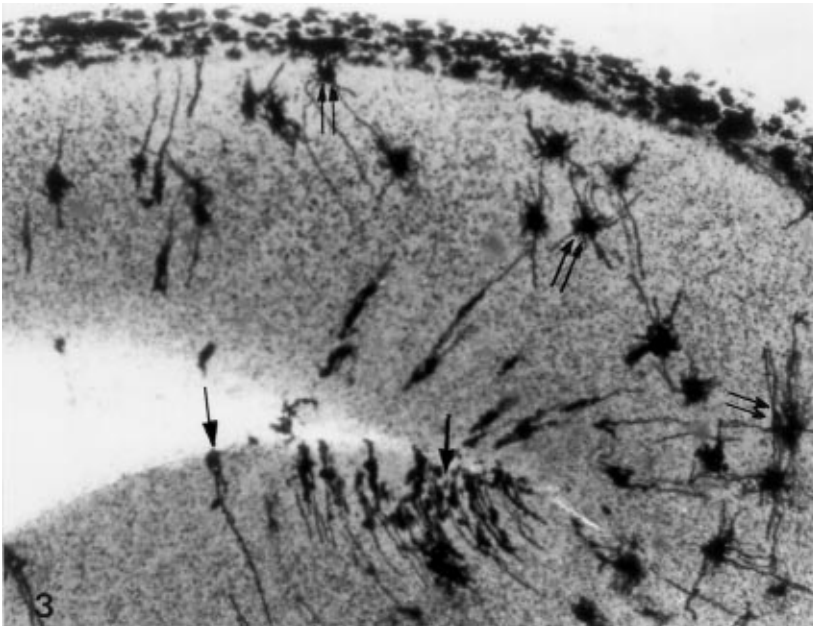
Figure 3. The bipolar (single arrows) and multipolar cell impregnations (double arrows) are seen in the frontal pole of the brain hemisphere in a prenatal 17-day-old embryo. OM: X40, Golgi-Cox.
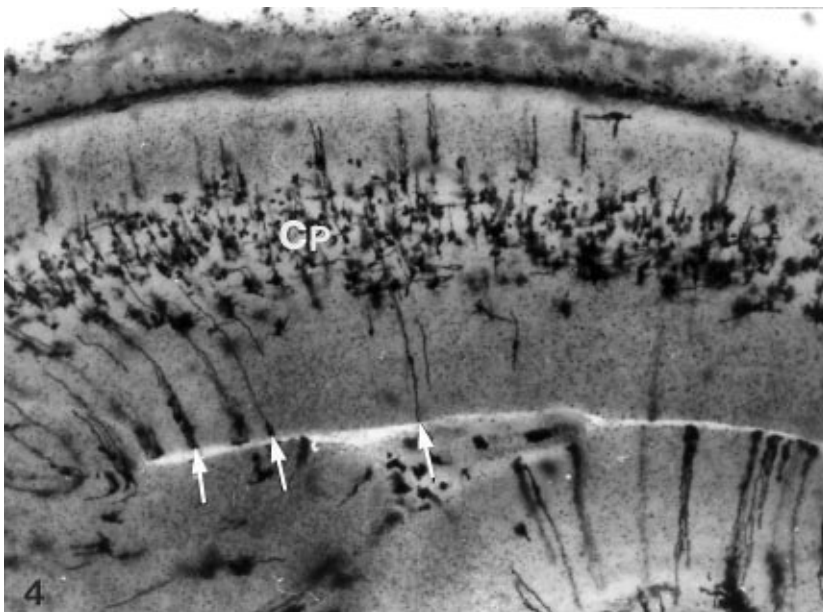

Figure 4. 19th day of gestation, various impregnated cells in the cortical plate (CP) and bipolar cells (single arrows) extending from the ventricular layer to the cortical plate are seen in the cerebral hemisphere wall. OM: X40, Golgi-Cox.

had numerous processes, which extended in every direction, and were seen first in the frontal region of the brain and their bodies were localised in the upper level of the hemisphere walls. No bipolar cells were observed. The long process of bipolar cells had some connections with the multipolar cell processes or perikaryons (Fig. 3).

d) 19th day:

Cortical plate, which consists of various impregnated cells, was definitely seen in the brain hemispheres. Showing different polarity, cells located along the cortical plate and bipolar cells still extended from the ventricular to the upper layers (Fig. 4).

II. Postnatal period

a) newborn (offsprings)

The cerebral cortex exihibited a very intensive impregnation response in newborn rats. The impregnated cell groups were visible at this stage, too (Fig. 5a-c). The cell number of each group was different, including primitive cells. Pyramidal cells showed an arrangement on an axis parallel to the hemisphere surface. However, it
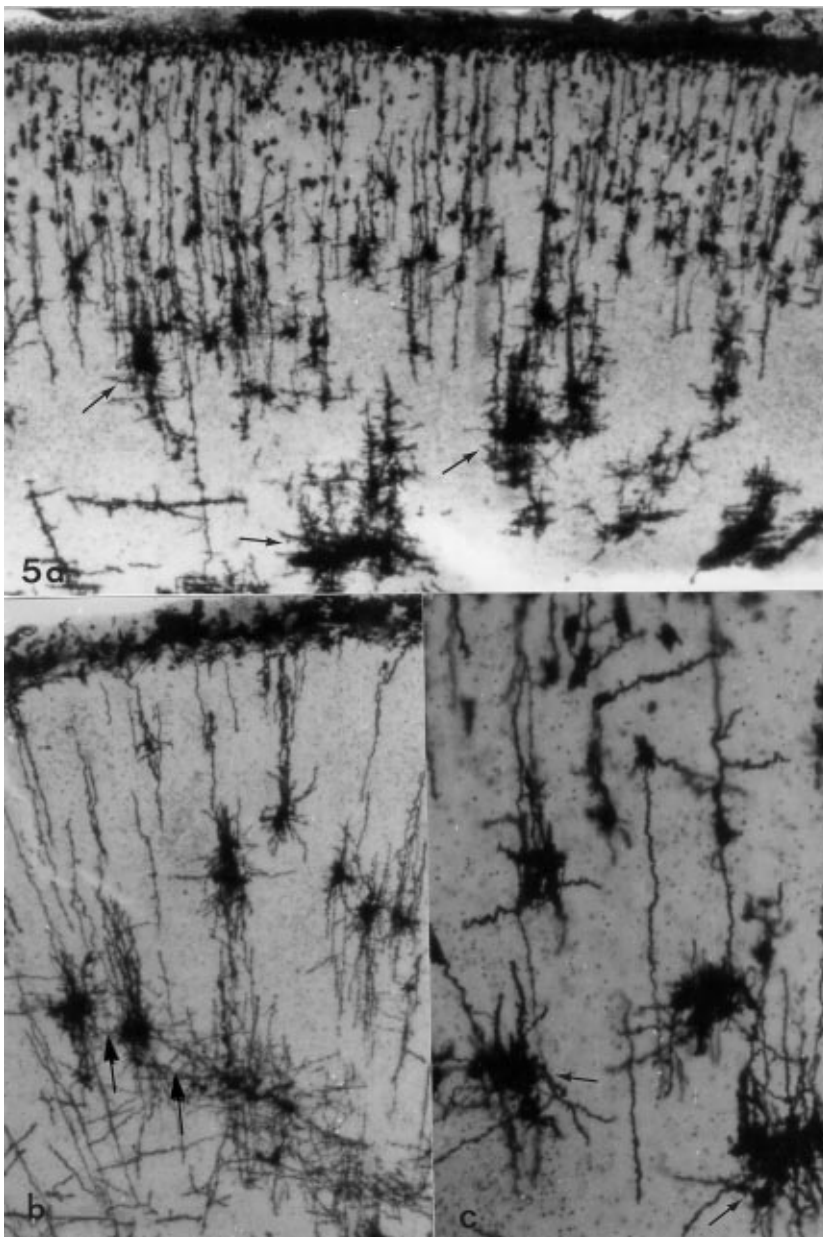
Figure 5. a) The dorsocentral region of the cerebral cortex of neonatal rats has many impregnated cells and cell groups. Large groups (single arrows) are seen in the deeper areas. However in the superficial regions, the cells did not exhibit group impregnations; b) magnificated ICGs are seen larger in the frontal area and they have many connections (single arrows) with the others; but c) they have few processes (single arrows) connecting to each other in the precentral region. OM: a,X40; b,X50; c,X100, Golgi-Cox.

was very difficult to determine the cell types for a clear identification, because the processes had not developed enough and cell bodies were not seen clearly. At this stage, the neurons' apical dendrites had reached the level of lamina I (Figs. 5a-c). The apical and basal dendrites were short and did not exhibit any branches. The cells, which were situated in laminas V-VI, showed horizontal processes contrary to the other cells, which were localised in the superficial regions. The neuronal processes, which established the connection with other groups, were absent in the dorsocentral region during this stage; thus, many of the groups were isolated. On the other hand, the impregnated cell groups (ICG) were larger in the frontal regions and established interconnections with each other (Fig. 5b). These connections had not developed yet in the cells of the Precentral region, next to the frontal region. Dendritic spines were observed rarely at this stage.

b) 5th day:

A definite increase in the number of ICG and small cell groups composed of few cells were observed. This observation has been regarded as an original result. The connections between the larger groups and the others had not developed. The prevalence of ICG ascended from
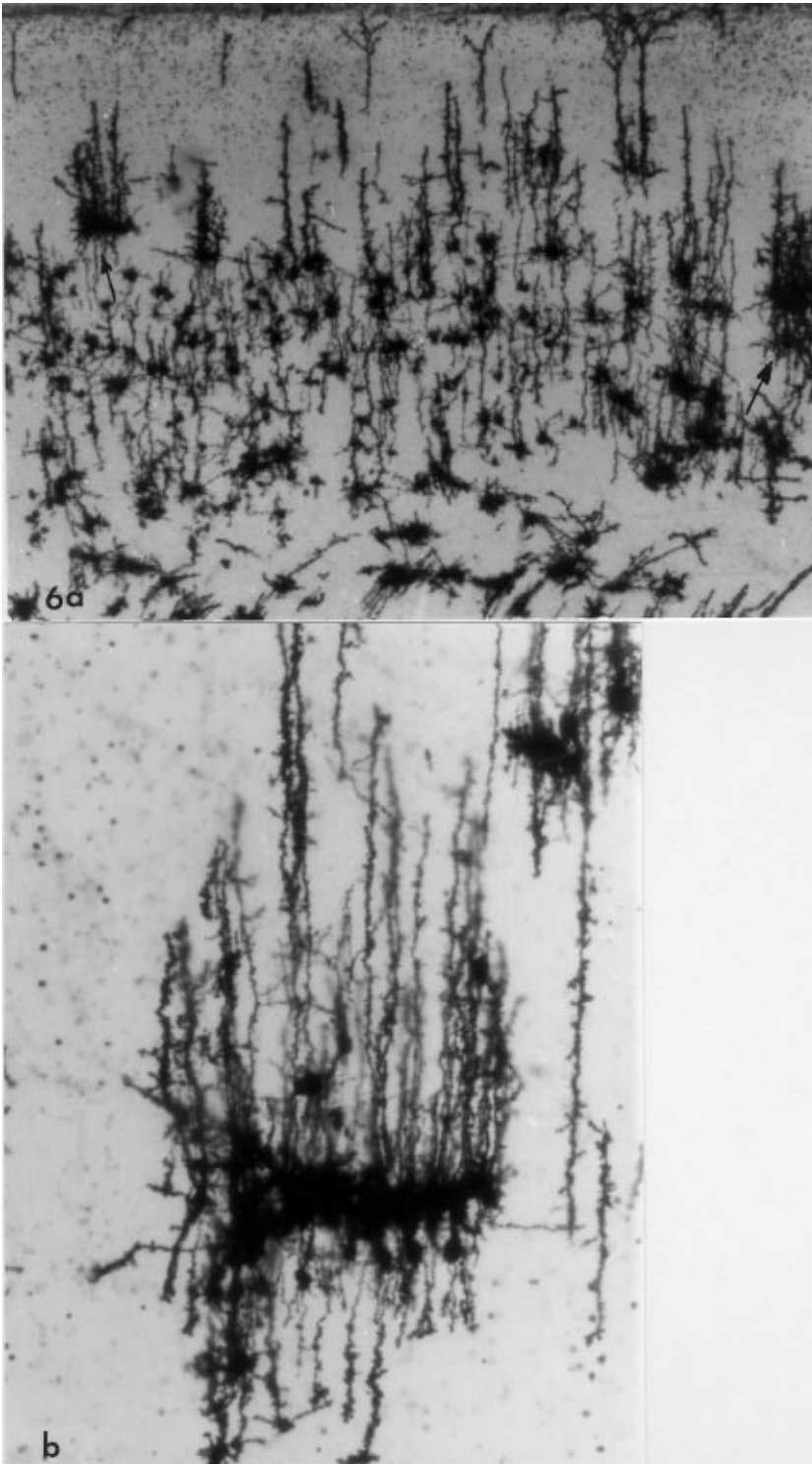
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Figure 6. a) On the 5th postnatal day, large ICGs (single arrows) localised at the superficial region of cortex are seen; b) the cell bodies connected to each other, lying on a parallel axis to the surface of cortex, are seen. OM: a,X40; b,X100, Golgi-Cox.

the deeper region to the upper zone was revealed in lamina III (Fig. 6a). Also the connections mentioned above were not developed between the large cell groups and the others. At this stage, the cells forming large groups were arranged on an axis parallel to the surface (Fig. 6b). All of the impregnated cells of the cerebral cortex had many spines and their apical dendrites showed an irregular secondary ramification in lamina II (Fig. 6a).

Figure 7. This micrograph shows the neuronal structure in the cerebral cortex of a postnatal 10-day-old rat. The cells forming ICGs before this stage, are separate, and more developed neuronal processes are seen (single arrows). OM: X40, Golgi-Cox.

c) 10th day:

At this stage, apical and basal dendrites of neurons had grown. Cells of ICG began to separate from each other, so we were able to distinguish various neuron types in the cerebral cortex at this stage (Fig. 7).

d) 15th day:

Rats at this stage exhibited a cortical structure with a developed network as well as the following stage in the cerebral cortex. The cells, once arranged in cell groups, were now separated. The connections between these cells were somewhat complete and the ramification processes reached the tertiary level (Fig. 8).

e) 20th day:

We observed that the cerebral cortex development of 20-day-old rats was somewhat complete (Fig. 9a). When we compared the cerebral cortex of 20-day-old rats with mature animals (Fig. 9b), it was noted that there was no significant difference.
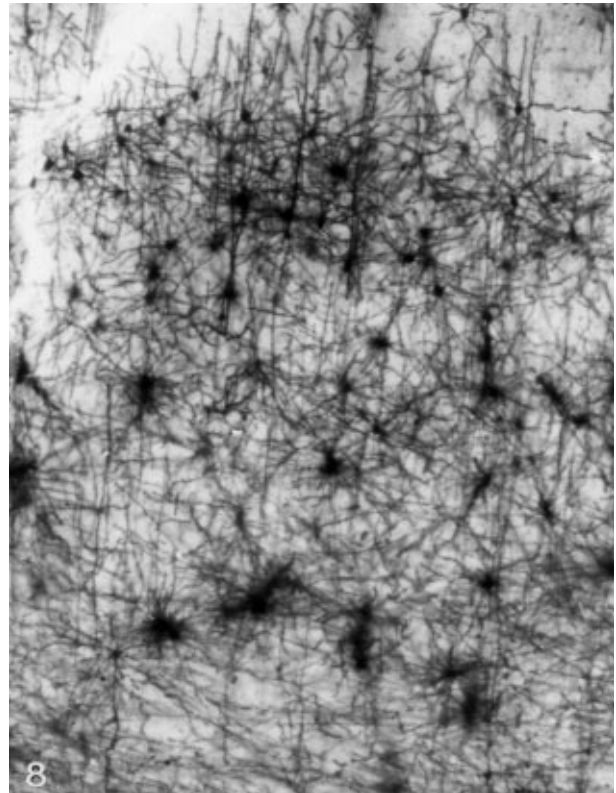

Figure 8. The micrograph shows the increase in the number of processes in the cerebral cortex on the 15th postnatal day. OM: X40, Golgi-Cox.

Discussion

As the cell proliferation and differentiation occur in the CNS during prenatal life, the development, in other words growth and cell maturation, also takes place in the late fetal and the early postnatal stages (11). The cranial neural epithelium shows high mitotic activity during the early prenatal period (1). The neural tube is formed by an epithelium, composed of an array of columnar cells, and eventually this arrangement imposes a radial geometry on the processes of production and migration (6). In general, proliferation and migration occur during prenatal life, so the maturation time has not yet been definitely identified.

In our study, the beginning of differentiation was seen on the 15th prenatal day. The observation of the first cell processes in the spinal cord and in the floor of the rhombencephalon, the lack of impregnation in other regions, and the rising of impregnation to the upper parts (e.g. mesencephalon and subsequently telencephalon) in the following days can be considered clear evidence of the functional forward development from the spinal cord to the cerebral cortex.

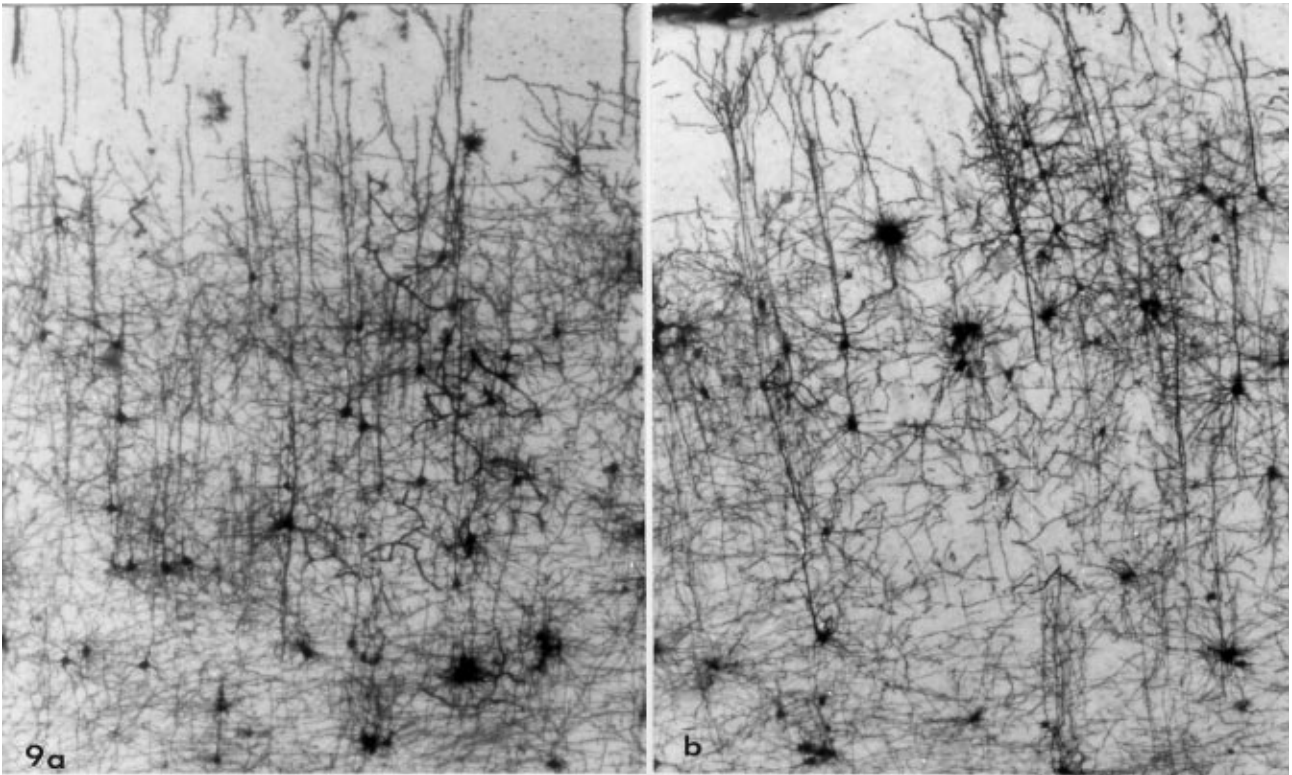Compared with the brain stem and spinal cord, the

Figure 9.   The organization of impregnated neurons of the cerebral cortex of a) postnatal 20-day-old rat and b) mature rat. OM: a,X40; b, X40, Golgi-Cox.

differentiation and the maturation of the cerebral cortex is observed in relatively later stages, even extending into the postnatal period (10). Our results, indicating the initial presence of the impregnation in the spinal cord and the floor of rhombencephalon on the 13th prenatal day and in the cerebral cortex on the 15th prenatal day, are consistent with previous reports (24).

The impregnation of cell processes were seen at the poles of the frontal, caudal and lateral regions of the brain hemispheres on the 15th prenatal day. It had extended to the dorsocentral region of hemispheres by the 17th prenatal day. Similar results were obtanied in studies of the mouse (12) and ferret (5), showing that the cell production of the cortical plate started in the lateral regions, extending in the dorsomedial direction.

The cortical plate in the rats appeared in the lateral walls of the brain hemispeheres on the 15th day of pregnancy; the cells belonging to I and VIb layers of the neocortex are the first developed cells (7). In our observations, the maturation of cell processes occurred in the same way as the development of cells in the cerebral cortex.

In the embryonic cerebral cortex, the initial

connections between the cells were established on the 17th prenatal day. These connections occurred between the bipolar and the differentiating multipolar cells. This situation was the most interesting observation of the prenatal 17-day-old rat's cerebral cortex. Another interesting occurrence was the ICG observed in the early postnatal period. While the ICG was seen in newborn and the 5th postnatal day, it was not seen in the 10th postnatal day in the cerebral cortex. The cells in impregnated groups began to separate, depending on the growth of the cerebral cortex after the 5th postnatal day. Localization of the ICG in deeper laminas (in the V. and VI.) in the newborn and in upper laminas (III. and IV. laminas) in postnatal 5-day-old rats, suggests that the differentiation and maturation extend from one cell to another by an inductive action of "starter cells". This pattern leads us to believe that the cells in the same group might be the elements which are concerned with the same function.

Development of the cerebral cortex on the 10th postnatal day was suitable for distinguishing neuron types, and at that time the cerebral cortex showed a definitive neuropil and a clear structure. Previous investigations have stated that cortical (22) and

hippocampal neurons (25) of rats complete their maturation in the 2nd and 3rd postnatal weeks.

Mathers (16) reported that postnatal dendritic development of pyramidal and stellate neurons was considerable and their total dendritic length trebled and the number of dendritic branches doubled during the period from birth to adulthood in rabbits. Also, in our study, the 1st postnatal week is a period in which a densely dendritic arborization takes place in rats.

We suggest that in the early postnatal period the impregnation group shows the primitive forms of dendritic bundles, as referred to by Peters and Walsh (26) in rabbits. Although cell bodies of specific groups separate, dendrites remain as members of the same dendritic bundle. Cordero et al. (27) reported that in rats, the first month after birth was very important for the development of dendritic arbores. Our finding, concerning the rat's cerebral cortex maturation at the end of the 1st month, is consistent with their observation.

As a result, in addition to the suggestions indicating that the prenatal development of the cerebral cortex consists of cell proliferation and migration, we could further observe differentiation and a little maturation as well. The periods in which differentiation and maturation occur are predominantly late prenatal and postnatal periods. In addition, functionally related cells differentiate and mature in the same cell groups.

Acknowledgement

References

1. Tuckett F, Morris-Kay GM. The kinetic behaviour of the cranial neural epithelium during neurolation in the rat. J. Embryol. Exp. Morp. 85: 111-19, 1985.

2. Tuckett F, Lim L, Morriss-Kay GM. The ontogenesis of cranial neuromeres in the rat embryo: I. A scanning microscope and kinetic study. J. Embryol. Exp. Morp. 87: 215-28, 1985.

3. Sakai Y. Neurolation in the mouse. I. The ontogenesis of neural segments and the determination of topographical regions in a central nervous system. Anat. Rec. 218: 450-57, 1987.

4. Sakai Y. Neurolation in the mouse: Manner and timing of neural tube closure. Anat. Rec. 223: 194-203, 1989.

5. McSherry GM. Mapping of cortical histogenesis in the ferret. J. Embryol. Exp. Morp. 81: 239-52, 1984.

6. McSherry GM, Smart IHM. Cell production gradients in developing ferret isocortex. J. Anat. 144: 1-4, 1986.

7. Valverde F, Facal Varverde MV, Santacana M, Heredria M. Development and differentiation of early generated cells of sublayer VIb in the somatosensory cortex of the rat: A correlated Golgi and autoradiographic study. J. Comp. Neurol. 290: 118-40, 1989.

8. Altman J, Bayer SA. Vertical compartmentation and cellular transformations in the germinal matrices of the embryonic rat cerebral cortex. Exp. Neurol. 107: 23-35, 1990.

9. Bayer SA, Altman J, Russo RJ. Simmons JA, Dai X. Cell migration in the rat embryonic neocortex. J. Comp. Neurol. 307: 499-516, 1991.

10. O'Rahilly R, Muller F. The development anatomy and histology of the human central nervous system. In Vinken PJ, Bryn GV, Klawans HL, (Eds). Handbook of clinical neurology, Chapter I. New York. Elsevier Science Pub. Co. Inc., pp: 1-17, 1987.

11. Noback CR, Demarest RJ. The human nervous system basic principles of neurobiology. Chapter 4; Development and growth of the nervous system, Fong at Sons Printers Pte. Ltd. Singapur, pp: 124-49, 1984.

12. Smart IHM, Smart M. Growth patterns in the lateral wall of the mouse telencephalon: I. Autoradiographic studies of the histogenesis of adjacent areas. J. Anat. 134: 273-98, 1982.

13. Berry M, Rogers AW. The migration of neuroblast in the developing cerebral cortex. J. Anat. 134: 273-98, 1982.

14. Rakic P. Specification of cerebral cortical areas. Science 241: 170-6, 1988.

15. Rakic P. Organising principles for development of primate cerebral cortex. In Sc Sharma (Ed.) Organising principles of neural development. New York, Plenum Press. 1984.

16. Mathers Jr. LH. Postnatal dendritic development in the rabbit visual cortex. Brain Research. 168: 21-9, 1979.

17. Schmolke C, Fleischhauer K. Morphological characteristic of neocortical laminae when studied in tangential semithin sections throught the visual cortex of rabbit. Anat. Embryol. 180: 125-132, 1984.

18. Peters A, Kara DA Harriman K.M. The neuronal composition of area 17 of rat visual cortex. III. Numerical considerations. J. Comp. Neurol. 238: 263-74, 1985.

19. Peters A, Kara DA. The neuronal composition area 17 of rat visual cortex. II. The nonpyramidal cells. J. Comp. Neurol. 234: 242-63, 1985.

20. Ferrer I, Martinez MJA. Development of nonpyramidal neurons in the rat sensory motor cortex during the fetal and early postnatal periods. J.für Hirnforschung 22:555-62, 1981.

21. Ferrer I, Fabregues I, Condom E. A Golgi study of the sitxth layer of the cerebral cortex. II. The gyrencephalic brain of carnivora, Artidactyla and primates. J. Anat 146: 87-104, 1986.

22. Ferrer I, Fabregues I, Condom E. A Golgi study of the sixth layer of cerebral cortex. III. neuronal changes during normal and abnormal cortical folding. J. Anat. 152: 71-82, 1987.

23 Rammon-Molliner E. The Golgi-Cox technique. Neatua W.J.H. Ebson S.O.E. (Eds) Contemporary research methods in neuroanatomy. New York, NY Springer Verlag, pp: 32-50, 1970.

24. Demir N, Demir R. The areas of first neuronal development in the central nervous system of rat embryo. Tr. J. Med. Sci. 22: 157-62, 1994.

25. Lang U, Frotscher M. Postnatal development of nonpyramidal neurons in the rat hippocampus (areas CA1 and CA3): Combined Golgi/electron microscopy study. Anat. Embr. 181: 533-45, 1990.

26. Peters A, Walsh TMA. A study of the organization of apical dendrites in the somatic cortex of the rat. J. Comp. Neurol. 144: 253-68, 1971.

27. Cordero ME., Trejo M, Garcia E, Barros T, Colombo M. Dendritic development in the neocortex of adult rats subjected to postnatal malnutrition. Early Human Develop. 12: 309-21, 1985.