RELATION BETWEEN DEVELOPMENT OF FUNCTIONS AND THE STRUCTURE OF THE CEREBRAL CORTEX IN ONTOGENESIS

COMMUNICATION II. HISTOLOGICAL DIFFERENTIATION OF CORTICAL CELLS OF RABBITS DURING ONTOGENESIS.

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In Part I of this series [2] we presented data on the time required for formation of condition reflexes to various stimuli during ontogenesis in the rabbit. Histological studies were conducted parallel with the physiological ones. The histological part of these studies, which is the subject of the present communication, concerned the histological differentiation of nerve cells of the cerebral cortex and of a number of subcortical formations, covering the period from the 23rd day of intra-uterine existence up to the adult state. Some of the animals involved had been used for the physiological investigations.

EXPERIMENTAL METHODS

We followed two main lines in our research the study of the development of the structure of the cytoplasm of the cell bodies, and the study of the development of their processes, in particular of their synaptic formations. The study of cytoplasmic structures was made with the aid of Nissl's staining procedures and of staining of ribonucleic acid (RNA; staining according to Unna-Pappenheim, with Brachet's control technique). The study of changes in RNA content of nerve cells is of special interest, inasmuch as a number of authors, in particular, H. Hiden, have demonstrated the importance of RNA in the development and functioning of nerve cells. Golgi's silver impregnation method was applied to the study of cell processes and of their synaptic terminations. The results were recorded by means of photomicrographs.

EXPERIMENTAL RESULTS

The series of complex changes taking place during the process of differentiation of a nerve cell from the neuroblast stage to that of the mature neuron involves both qualitative and quantitative changes. For our purposes, special interest was attached to the qualitative changes, involving the appearance within the cells of new structures. Our object was to describe these qualitative changes occurring during the development of a nerve cell, and on this basis to arrange them in the sequence followed during differentiation of the cell.

We have, on the basis of our results and of published work, divided the process of differentiation of the internal structure of the cytoplasm of a neuron into the following 3 stages (Nissl and RNA staining; Fig. 1).

1st Stage. The cell has a deeply staining nucleus and a homogeneous nucleolus. The cytoplasm is scanty, and stains very weakly. No cell processes are evident.

2nd Stage. The cell nucleus is translucent. The nucleolus is homogeneous, but is larger than in Stage 1. The basophil cytoplasm stains diffusely (a foamy appearance in thin sections). Protuberances are evident at the sites of future processes (Fig. 2).

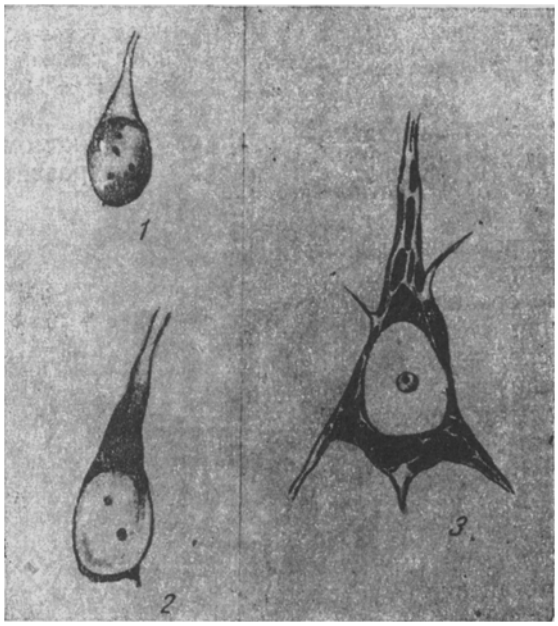

Fig. 1. Stages in the differentiation of the internal structure of the cytoplasm of a nerve cell (Nissl and ribonucleic acid staining). The figures refer to stages of differentiation.

3rd Stage. The cell nucleus is clear. The nucleolus contains crystalloid. The amount of cytoplasm shows considerable increase, and it is filled with well-defined clumps of tigroid. The cell processes are evident.

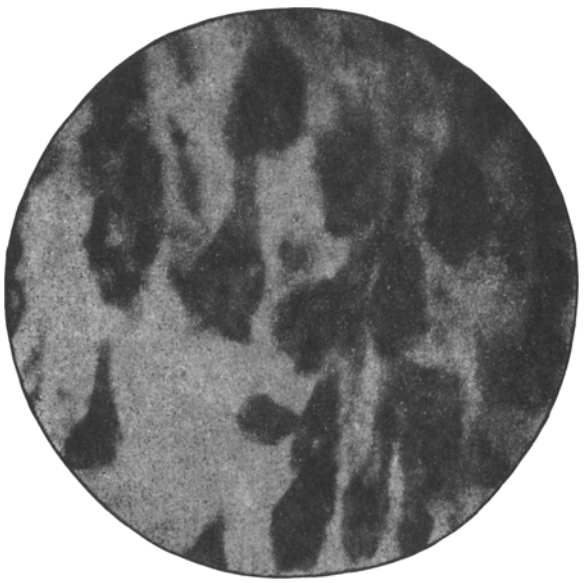

Fig. 2. Pyramidal cells from layer III of the cerebral cortex of a 6-day-old rabbit. Second stage of differentiation. Temporal region. Magnification 1200 ×.

Our data on the maturation of nerve cells of the cerebral cortex are presented in Table 1, adopting the above classification of developmental stages.

From these results we may draw the following conclusions.

1. The nerve cells of the cortex and subcortical parts of the brain all pass through the above 3 stages; our division of the process of differentiation of the neurons is therefore justified.

2. Cells belonging to different formations mature at different rates. The subcortical cells are already mature at the birth of the animal (we studied the development of cells of the striopallidus system and of the nuclei of the 7th pair of cranial nerves). Most of the cells of the rhinencephalon have achieved maturity by birth, and the remaining ones complete their development within two days of birth (Fig. 3). The cells of the neencephalon mature later than do those of the rhinencephalon. Maturity is achieved in the cells of the neencephalon by the following times: Layer I – Cajal – Retzius cells, which are found to be mature in a 23-day embryo, layer II – by the 10-13th day after birth, layer III by the 10-13th day, and layer IV by the 13th day (isolated mature cells are to be seen in 10-day-old rabbits), layer V by the 6-8th day, and layer VI by the 8th day. Differentiation of the cells of the

TABLE 1

Development of Internal Structure of the Cytoplasm of Nerve Cells During Ontogenesis (stained by Nissl's method and for RNA)

Layer and part of brain	Age, days										
	23-day embryo	0	2	5–8	8	10	13	15–16	25	40	adult
Neencephalon											
I	3	3	3	3	3	isol. 3	isol. 3	—	—	—	—
II	1	1 (2)	1 (2)	2 (1)	2 (1)	2 (1,3)	3 (2)	3 (isol.2)	3	3	3
III	1	1 (2)	1 (2)	2 (1,isol. 2)	2 (1,3)	2,3 (isol.1)	3 (isol.2)	3	3	3	3
IV	1	1	1 (isol.2)	1 (2)	1 (2)	1,2 (isol. 3)	3 (2)	3 (2)	3 (isol.2)	3	3
V	2,1	2 (1, isol. 3)	2 (1, isol. 3)	2 (3)	3 (2)	3	3	3	3	3	3
VI	2,1	2,1 (isol. 3)	2,1 (isol. 3)	2 (isol 3)	3 (2)	3 (2)	3	3	3	3	3
Rhinence-phalon	1,2	3,2	3	3	3	3	3	3	3	3	3
Subcortical	1,2	3	3	3	3	3	3	3	3	3	3

Note: The figures refer to stages of differentiation; those within parentheses signify that a minority of the cells are not the given stage; figures preceded by "isol" mean that only isolated cells are at the given stage.

neencephalon is thus basically completed by the 13th day post partum, although all layers contain mature cells by the 10th day.

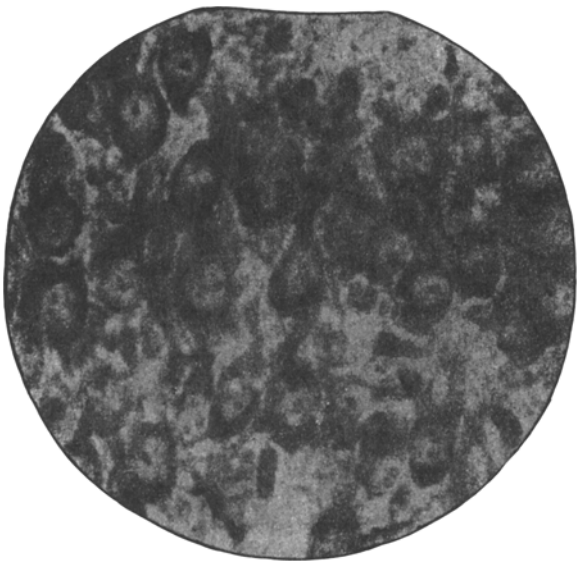

Fig. 3. Cells of the rhinencephalon of a 2-day-old rabbit. Third stage of differentiation. Entorhinal area. Magnification 600 ×.

The different times required for achievement of maturation of cells of different parts of the brain and of different layers of the neencephalon are in accordance with the general biogenetic rule that structures which are phylogenetically of more recent origin develop later during ontogenesis.

The cerebral cortex is phylogenetically more recent than are the subcortical formations, and it accordingly develops later in the course of ontogenesis.

The rhinencephalon is phylogenetically older than the neencephalon and accordingly its cells mature before those of the neencephalon (see Figs. 2 and 3). The lower layers of the neecephalon are phylogenetically older than the upper layers [2], and during ontogenesis their cells mature earlier than do those of the latter. The differences between the times of maturation of different parts of the neencephalon are insignificant. The greatest difference is in the somewhat later time of maturation (by 2-3 days) of the occipital regions.

Certain deviations from the biogenetic rule governing the general sequence of maturation of the cells of different parts of the brain do, however, occur, some individual formations maturing earlier than would be expected.

3. Individual nerve cells belonging to the same part and the same layer of the neencephalon may mature at different times. These differences are, however, much smaller than are the differences between times of maturation of cells of different types.

4. We have, on the basis of the data of Table 1, attempted to make a rough estimate of the duration of the process of maturation of a neuron. Since a cell may remain in its 1st stage of development for a considerable time without showing any significant structural changes, we took the duration of the differentiation process as being the time interval between entry of a cell into the 2nd stage of development and completion of differentiation (transition to the 3rd stage), i.e., as being equal to the duration of the 2nd stage of development. We thus obtained the following approximate values for the duration of the process of differentiation of a neuron (the data relate only to cells of the neencephalon): layer II 4-7 days, layer III 4-7 days, layer IV 3 days, layer V 16 days, layer VI 16 days.

A correlation can be perceived between the duration of the maturation process and the length of the axon of a given cell; thus it is known that the axons of layer IV cells are confined to the cortex, those of layers II and III form the association pathways, and those of layer V and VI are descending projection fibers, and are hence of considerable length.

Our conclusions apply equally to sections stained according to Nissl and for RNA. However, RNA staining permits one to draw a number of additional conclusions. Although staining of RNA does not give as fine detail of the internal structure of the cytoplasm as does staining according to Nissl, it has the advantage of giving greater contrast; it is of particular value in the study of the process of cell differentiation, since the difference in deepness of staining between mature and immature cells is quite considerable.

This staining procedure is also convenient for the study of processes taking place within the cell nuclei.

We found that the RNA content of the cells rose steeply during the process of differentiation. Calculations based on published biochemical data [5] and on our data for increase in the weight of the brain showed that

from the 23rd day of intra-uterine life to adult maturity there is a 40-fold increase in the average RNA content of a cell. There is a particularly large increase in the RNA content of the cytoplasm at the time of transition of a cell into its 3rd stage of maturity. Changes in the distribution of RNA in the cytoplasm also take place during the process of differentiation. During the 2nd stage of maturity RNA is diffusely distributed throughout the cytoplasm, whereas in the fully mature cell it is concentrated into clumps of tigroid.

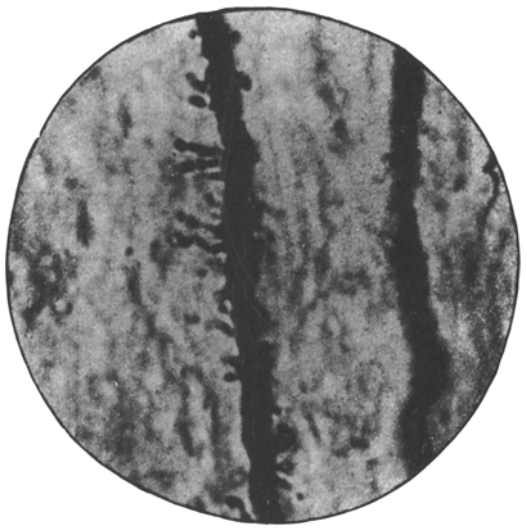

Fig. 4. Lateral outgrowths (spines) on a dendrite of a cortical cell of a 19-day-old rabbit. Third stage. Magnification 1200 ×.

The staining of the nucleus changes during the process of differentiation. The nucleus of an immature cell has a high chromatin content, with diffusely distributed RNA. The intensity of staining then diminishes, until in the mature cell it is practically colorless.

Due to the distribution of nucleolar chromatin, particularly striking changes take place in the nucleolus, which develops from the stage of being a small, homogeneous body to that of a large, RNA-rich nucleolus with a well-defined structure. The number of nucleoli falls during the process of differentiation from 3-4 to 1. A connection may be discerned between the changes in nucleolar structure and the rise in their RNA content with the rise in the RNA content of the cytoplasm, and the formation of Nissl bodies.

In studying the development of the dendrites of nerve cells and of their synaptic terminations we paid special attention to the development of the so-called "dendritic spines," which are the morphological substrate of axodendritic synaptic connections [3, 4, 5]. The dendritic spines are most highly developed in the pyramidal cells of the neencephalon, and somewhat less so in the cells of the olfactory cortex; the cells of subcortical formations possess far fewer spines than do those of the cortex. We also divided the process of cell differentiation as shown by Golgi staining into 3 successive stages. For this purpose we made use of A. D. Zurabashvili's scheme [1] for cells of the spinal cord. We introduced a few modifications into this scheme, so as to adapt it to the cells of the cerebral cortex.

We divided the process of cell differentiation into the following stages:

Stage I – cells with a weakly developed system of processes; in pyramidal cells only the apical dendrite is evident.

Stage II – appearance of branches of the 2nd or 3rd order in the apical dendrite, of basal dendrites, and of dendritic spines (subdivided into stage IIa, in which the spines are present only in the proximal part of the processes, and stage IIb, in which they are seen in the terminal arborizations of the dendrites).

Stage III – the mature nerve cell, with the arborization of a large number of processes; the dendritic formations are practically covered with spines (Fig. 4).

As was shown by further observations, these stages of differentiation of cell processes coincide largely with those of the cell body, as described above.

Our results are presented in Table 2, in which this scheme for the subdivision of the process of cell differentiation is incorporated. The following conclusions may be drawn from these results:

1) subcortical cells, and cells of the rhinencephalon have achived maturity before the termination of pregnancy, while those of the neencephalon achieve it within 10-15 days of birth;

2) a certain correlation is evident between the stages of differentiation of the cell body and of its processes; this may be an indication of the role of the cell body in the formation of its processes and synaptic terminations.

Thus the maturation of the cytoplasmic structures of the cell body and the maturation of its processes, with their synaptic terminations, proceed concurrently.

TABLE 2

Scheme of Development of the Processes of Cortical Cells and of their Synaptic Terminations

Layer and region	Age, days					
	1	5	10	15	30	adult
Neencephalon III	I (IIa)	IIa	IIb	III	III	III
Neencephalon V	IIa	IIa	IIa –III	III	III	III
Rhinencephalon	III	III	III	III	III	III
Subcortical	III	III	III	III	III	III

Note: The Roman numerals represent stages of differentiation.

The following conclusions emerge from our results:

1) We have subdivided the process of ontogenetic differentiation of neurons into 3 stages, each of which is distinguished by qualitative differences in the structure of the neuroplasm and of the cell processes.

2) The process of differentiation of a neuron is associated with a fall in the intensity of staining of the nucleus, with increase in RNA content and size of the nucleolus, with the accumulation of large amounts of RNA in the cytoplasm, with formation of clumps of tigroid, into which cytoplasmic RNA is concentrated, with increased arborization of dendrites, and with formation of dendritic spines.

3) The cells of different parts of the brain mature at different times. Most of the cells of subcortical formations and of the rhinencephalon are mature at birth; those of the neencephalon achieve maturity within 10-13 days of birth. The cells of different parts of the brain mature in a sequence which is in accordance with the biogenetic rule of ontogenesis.

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