EXPERIMENTAL BIOLOGY

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

PART III. ON CERTAIN FINE HISTOLOGICAL STRUCTURES PARTICIPATING IN THE ACCOMPLISHMENT OF THE FUNCTION OF CLOSING (OF REFLEX ARCS)

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Physiological and histological data on the ontogenesis of the cerebral cortex of rabbits were presented in our preceding communications [4, 5].

In the present paper, the ages at which conditioned reflexes can be elaborated are considered in conjunction with histological data, with the object of ascertaining what histological picture corresponds with the state of functional maturity of the cortical cells.

It should be noted that the establishment of a correlation between function and structure, based on their synchronous appearance in the course of ontogenesis, does not constitute direct evidence that these events are casually interrelated. It may never the less provide some indications as to the significance of certain histological structures for the accomplishment of (reflex arc) closing functions, especially if the correlations established prove to be applicable to a number of analyzers.

For convenience, the physiological and the histological data are presented side by side in the Table.*

It is of interest, for the establishment of any correlations between physiological and histological data, to establish the existence of a connexion between the origination of any function and the appearance of certain new structures, i.e. the achievement of a definite stage in the development of the neuron. Only in such a case is it possible to attribute a function to a given structure, and the conclusions drawn assume a general nature. Conclusions as to the significance of cell structures are more conveniently derived separately for the structure of the cytoplasm, and separately for the cell processes with their synaptic apparatuses.

In making the comparisons, it should be borne in mind that olfactory stimuli are projected to the region of the rhinencephalon, whereas tactile, auditory, and visual stimuli are projected to various regions of the neencephalon.

We shall first consider which internal cytoplasmic structures of nerve cells of the cerebral cortex are essential for the formation of a conditioned reflex. We shall begin with the neencephalon, the development of which is completed during extra-uterine life. By the age of 10 days, when conditioned reflexes to auditory and tactile stimuli first begin to make their appearance, we first began to find, in all the layers of the

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*The data relating to the histological differentiation of the cells are presented in a shotened form. More complete data were recorded in Part II of this series (Tables 1 and 2) [5].

Comparison of the Results of Physiological and Histological Study of the Cortex and Subcortical Region of Rabbit Brain, at Different Stages of Postnatal Development

Age, in days after birth	Physiological data	Histological data										
		structure of cytoplasm							structure of processes and synapses			
		subcortex cent. high	olfact. cortex —	neencephalon (skin, auditory, visual, and motor analyzers)					sub-cortex	olfactory cortex	neencephalon	
		unconditioned reflexes	olfactory analyzer	layer II	layer III	layer IV	layer V	layer VI			layer III	layer V
0—1	Unconditioned reflexes											
	Conditioned food reflexes to smell	3	3,2	1 (2)	1 (2)	1	2	2,1	III	III	I(IIa)	IIa
5—6	Ditto	3	3	2 (1)	2	1 (2)	2 (3)	2	III	III	IIa	IIa
8	» »	3	3	2 (1)	2 (1,3)	1 (2)	3 (2)	3 (2)	—	—	—	—
10	The same, and unstable conditioned reflexes to sound, touch, and heat	3	3	2 (1,3)	2,3	1,2(single cells 3) visual region 1,2	3	3 (2)	III	III	II a	II b III
13	The same, and more stable conditioned reflexes to the specified stimuli	3	3	3 (2)	3	3 (2)	3	3	—	—	—	—
	Conditioned reflexes to light											
15—16	Ditto	3	3	3	3	3 (2)	3	3	III	III	III	III
Adult animals	» »	3	3	3	3	3	3	3	III	III	III	III

Designations: Arabic numerals — stages of differentiation (see footnote on p. 1241); Roman numerals — stages of differentiation of cell processes, the figures in parentheses indicating the stage reached by the minority of the cells; IIa) synaptic knobs at the basal parts of the dendrites; IIb) synaptic knobs on the terminal ramifications of the dendrites; III) large numbers of knobs on the dendrites.

neencephalon, cells which are at the third developmental stage (mature nerve cells).* At an earlier age, for example in an 8-day-old rabbit, stage 3 cells are absent from layers II and IV of the cortex. It has been shown that formation of conditioned reflexes is not possible at this age. It is hence natural to associate functioning of the cell with the 3rd stage of development. This assumption is confirmed by the results of study of a number of analyzers, and this largely excludes the possibility of a fortuitous coincidence of the times of functional maturation and of the cells entering into the 3rd stage of differentiation.

Third stage cells are absent from the occipital cortex of a 10-day-old rabbit, and it has accordingly not been found possible to form reflexes to light at this age. Reflexes to visual stimuli can first be formed at the age of 13 days, by which time mature nerve cells are to be found in all the layers of the occipital cortex.

Most of the nerve cells of the rhinencephalon and of the subcortical areas are mature at birth. Accordingly, if the assumption that cell function is contingent on the cells having achieved the third stage of maturity is justified, these parts of the brain should be functional from birth. Physiological experiments have, in fact, shown that the formation of conditioned reflexes to olfactory stimuli projected to the olfactory cortex is possible from the moment of birth, as is the accomplishment of unconditioned reflexes (subcortical region).

Thus the appearance of the closing function can be associated only with the third stage of maturation of the cells. Cells which are at the second, and a fortiori at the first, stage of maturity cannot be regarded as being fully functional. The functioning of the neencephalon requires the presence of mature nerve cells, even though in small numbers, in all the layers of the cortex. Admittedly, in such cases the conditioned reflexes lack stability, as is exemplified in 10-day-old rabbits. As the number of cells achieving maturity increases, however, the conditioned reflexes become more stable.

Thus we find full consonance between the physiological and the histological data.

With which structural changes in the cell may we associate development of function ? The most important qualitative change concerned with transition of the cells to the third stage of maturity is the formation of Nissl bodies in the cytoplasm. Thus the most important change in the structure of the neuroplasm is the appearance of Nissl bodies, on which the function of the cells depends. It may hence be assumed that the Nissl bodies are the morphological formations directly concerned with the specific function of the cells.

The application of histochemical methods permits of a somewhat more profound approach to the nature of the processes taking place within nerve cells during their maturation. We have observed [5] that the total ribonucleic acid content of the cells increases considerably during their maturation. This must point to the importance of the part played by ribonucleic acid in the accomplishment of its function by the mature cell. However, the mere presence of ribonucleic acid in the cytoplasm is not in itself sufficient to ensure the development of cell function. It is essential that the ribonucleic acid should enter into the composition of special cytoplasmic structures, represented by the Nissl bodies. Ribonucleic acid, which is present in high concentration in the cytoplasm of second stage cells, fulfills a different role from that in third stage cells; it appears to be concerned in the synthesis of protein for formation of the growing axon. This hypothesis is in agreement with the findings of a number of foreign authors [9]. The direct proportionality between the duration of the second stage of maturation and the length of the axon (Part II) also supports this hypothesis.

The function of the ribonucleic acid changes when the cells have achieved the third stage of maturity, and it now takes part in metabolic processes connected with specific nerve function.

It might, from our general knowledge of the role of ribonucleic acid in living organisms, be supposed that a characteristic feature of the specific processes of nervous activity is the high rate of protein turnover.

Apart from the appearance of Nissl bodies, a second important histological change associated with entry of the cells into the third stage of maturity is the development of the large, differentiated nucleolus, rich in ribonucleic acid. The conclusion may hence be drawn that the nucleolus plays an important part in the accomplishment of cell functions, and this would be in accordance with the findings of a number of authors [10].

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*First stage – dark nucleus and clear cytoplasm; second stage – clarification of nucleus, increase in size of nucleolus, accumulation of ribonucleic acid in the cytoplasm; third stage – clear nucleus with a large, differentiated nucleolus, formation of clumps of tigroid in the cytoplasm (Part II [5]).

Comparison of physiological data with those on the development of synaptic apparatuses of cortical cells (see Table) shows that the appearance of the closing function coincides in time with the appearance of bottons on the terminal ramifications of the apical dendrites of the cortical cells. At the age of 10 days, when the number of such bottons is small (stage IIb), conditioned reflexes to stimuli projected to the neencephalon are only formed after a large number of associations, and are of low stability. With increase in the number of bottons (stage III) conditioned reflexes are formed more rapidly, and are more stable. Conditioned reflexes are not formed before the synaptic knobs develop (first 10 days of life). Conditioned reflexes to olfactory stimuli are formed from the first day after birth, and this corresponds with the presence of synaptic knobs on the dendrites of the cells of the rhinencephalon of a new-born rabbit. Synaptic knobs are the morphological substrate of the axodendritic synaptic links [8], and appear to be essential for the formation of the temporal link. The coincidence in time of the origination of the closing function and the formation of bottons on the terminal ramifications of dendrites is also understandable, since the study of the cytoarchitectonics of the cortex has shown that it is in the external layer that the most favorable conditions for establishment of contact between cells are to be found. It may be supposed that closing of the temporal link requires the active participation of the axodendritic synaptic links of the marginal layer of the cortex.

Our data on the times of maturation of the cerebral cortex, obtained by physiological and histological methods, are in agreement with those of other authors, who used other methods for the study of this question: biochemical [6], electrophysiological [1, 3], and other methods, and with the findings of A. A. Volokhov [2] and G. A. Obraztsova [7] as to the times of formation of the twitching conditioned reflex to sound.

We conclude from the results of our experiments that the presence within the cortical cells of the following histological structures is essential for their functioning: 1) formed Nissl bodies; 2) differentiated nucleoli; 3) synaptic knobs on the terminal ramifications of dendrites. The presence of these formations is evidence of the functional maturity of the cells.

In associating the given structures with the possibility of accomplishing the closing function we do not, of course, claim that the mere presence of these structures is all that is needed for the establishment of conditioned reflexes: a function of the complexity and perfection of the conditioned reflex function of an organism must undoubtedly depend on the functioning of large complexes of nerve cells. This is the more probable inasmuch as the specified structures appear, to a greater or lesser degree, in all nerve cells, and not only in those of the cerebral cortex.

The methods at our disposal do not, as yet, permit of the demonstration of any structural peculiarities characteristic of the cortical cells, such as may be done in relation to such of their properties as is the specific closing function. This peculiarity of the functioning of cortical cells may perhaps be explained on the basis of the cytoarchitectonics of the cortex, i.e. on the way in which the cells are interconnected.

Thus, in speaking of the functional significance of the structures in question, we may say that their presence is an essential condition, although not, of course, the only one, for the origination of the closing function, and that in the absence of these structures this function cannot be accomplished.

Notwithstanding our reservations, we believe that our findings may possess some theoretical and practical interest, since they establish the significance of certain important intracellular structures for the accomplishment of the conditioned reflex function; in particular, they may assist in the elucidation of disturbances in cortical function encountered in various morbid conditions in which morphological investigations have shown changes in certain structural elements of cells. They may also be of assistance in determining the times of functional maturation of different parts of the cerebral cortex, using histological methods of investigation.

SUMMARY

The age at which conditioned reflexes are first able to be formed coincides in rabbits with presence of third stage (mature) neurons in all the layers of the cerebral cortex. The morphological criteria of maturity are: appearance of formed Nissl bodies, differentiation of the nucleoli, and appearance of synaptic knobs on the terminal dendrites.

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*In Russian.

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