DEVELOPMENT OF INTERNEURONAL SYNAPSES BETWEEN ANTERIOR HORN NEUROBLASTS OF THE HUMAN SPINAL CORD IN EARLY PRENATAL DEVELOPMENT (SEVENTH TO NINTH WEEKS OF GESTATION)

A. A. Milokhin, S. S. Reshetnikov,
and I. V. Chernova

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Synaptogenesis in presumptive anterior horn cells of the spinal cord (C4-T3) was studied by electron microscopy in early human prenatal development. The morphological stages of synaptogenesis were traced and the minimum structural organization of the synapse corresponding to the onset of its functional activity was identified. The possible mechanisms of synaptogenesis are postulated.

KEY WORDS: *synaptogenesis*; *spinal cord*; *human prenatal development*.

Among the many problems which face the investigator studying the developing human nervous system that of synaptogenesis is undoubtedly one of the most radical. From the time of appearance of the first synapses between differentiating neurons in the ontogenetic development of nerve tissue a most crucial period arises: the period of formation of the nervous system as such.

This argument motivated us to make a systematic investigation of the newly formed interneuronal connections in the human nervous system during prenatal ontogeny.

For the purposes of this investigation differentiating neurons were chosen from the presumptive anterior horns of the cervico-thoracic portion (C4-T3) of the human spinal cord inthe transition period from embryonic to fetal of intrauterine development (7-9 weeks). At that time these neurons in the developing human embryo begin to exhibit the first definite signs of their functional competence and for the first time they are able to participate in some reflexes [1-3, 8, 9].

EXPERIMENTAL METHOD

Material for the investigations was taken after abortions performed in Moscow gynecological clinics. Altogether 16 human embryos and fetuses were examined electron-microscopically: 4 aged 7 weeks, 7 aged 8 weeks, and 5 aged 9 weeks.

EXPERIMENTAL RESULTS

The first synaptic contacts on neuroblasts in presumptive anterior horns of the developing human spinal cord were found at the level C6-8 and T1 at the seventh week of prenatal development , i.e., at the time when the first spinal reflexes appear. In this and during the next 2 weeks of prenatal development a concentration of neuroblasts at different stages of differentiation can be seen in the presumptive anterior horns in the chosen region of the spinal cord. Besides undifferentiated neuroblasts, others now fully formed also were seen. In the cytoplasm of the latter there was a well developed endoplasmic reticulum whose membranes were thickly studded with ribosomes; the number of mitochondria in them was sharply increased and the Golgi complex was now multiple in character and, to judge from its morphological picture, in a state of intensive function. Nerve fibers, forming synapses with their dendrites, grew toward the more highly differentiated neuroblasts. These nerve fibers were characterized by small expansions on their growing end, distinguishing them in several respects from the so-called

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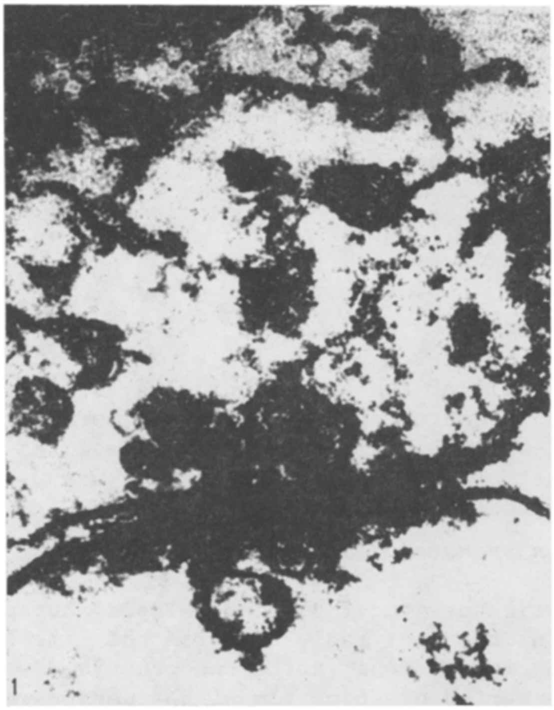

Fig. 1 Fig. 2

Fig. 1. Developing synapse. Anterior horns of brachial region of spinal cord of 8-week human embryo, 163,300×.

Fig. 2. Initial stages of development of interneuronal synapse in neuropil of presumptive anterior horns of cervico-thoracic region of human spinal cord. Digital projections on postsynaptic membrane can be seen. First half of 8th week of prenatal development 162,400×.

classical cones of growth. It would therefore be more correct to call them presumptive synaptic endings. Growing toward the neuroblasts, they were in close contact with the surface of their body or processes. However, at the point of contact appreciable changes in the structure of the adjacent membranes could not always be found. These morphological pictures can be taken evidently to reflect the most initial stages of synaptogenesis, when contact between the presumptive synaptic ending and the neuroblast has only just taken place and no functional synaptic connection between them has yet been formed.

Simultaneously with pictures such as these (and sometimes in the same sections) much more mature forms of connection were found between the terminal of the nerve fiber and the neuroblast, which had now acquired all the basic features of a true interneuronal synaptic contact (Fig. 1). Among its most characteristic features were: the parallel arrangement of the opposite membranes, bounding a well-developed synaptic space, in which columns (or septa) of electron-dense material could be seen to alternate with a definite sequence.

Specialization of the membranes at the site of contact was now sufficiently well marked. In particular, this applies to the postsynaptic membrane, in contact on its cytoplasmic side with a layer of well-developed electron-dense material, beginning to transform itself into distinctive conical or funnel-shaped structures so characteristic of the synapses of the mature organism.

The presynaptic membrane also was a little thickened on account of the loose osmiophilic layer formed on its inner aspect. Compared with the postsynaptic side, however, it was much less well developed and, consequently, even in this period of its development, the synapse appeared distinctly asymmetrical. As a rule, several pale synaptic vesicles could always be seen at this time adjacent to the membrane (Fig. 1).

The appearance of these synaptic structures in the neuropil of the presumptive anterior horns of the spinal cord was observed originally in the seventh week of human prenatal development; during the eighth week and, in particular, toward its end their number increased sharply

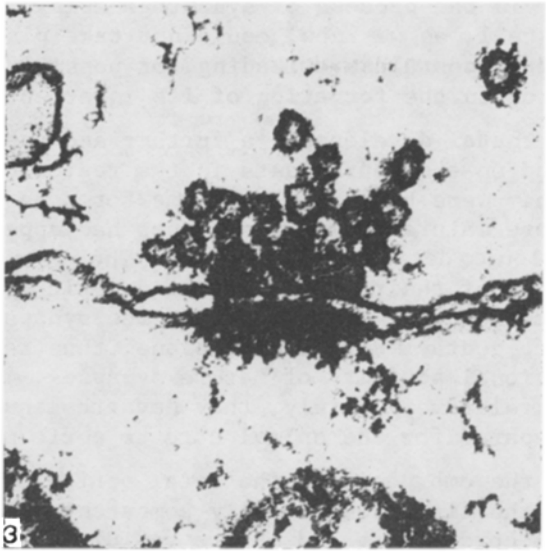

Fig. 3. Axo-dendritic synapse from neuropil of anterior horn of brachial region of spinal cord of 9-week human fetus, 115,000×.

although at this time other more mature forms of synaptic contact were not found. Since at this period of development of man clearly defined reflexes to adequate stimulation of the upper limbs are beginning to arise, it is reasonable to suppose that the forms of maturing synapses described above were functionally competent and that, consequently, their structure was the basic essential minimum for the structural organization of a synapse that would enable it to transmit information between nerve cells.

This conclusion, based on observations of developing synapses in the human spinal cord, is in full agreement with the results of investigations into synaptogenesis in the spinal cord of vertebrates [4, 5] and on spinal neurons cultured in vitro [6, 7].

To understand the mechanisms of synaptogenesis, it is of the utmost importance to make a detailed investigation of its stages that immediately precede the one just described, for it is in that period that it will be possible to ascertain how and in what order the whole of the structural ensemble necessary for functioning of an interneuronal synapse is laid down.

Unfortunately, these stages are extremely rarely found in electron micrographs, evidently because of their short duration. However, one of these moments so extremely important for the understanding of early synaptogenesis is recorded in the photomicrograph in Fig. 2. Here it can be seen that an axon terminal (so-called presumptive synaptic ending) has grown up against a thin dendritic branch and grown around it in a semicircle; at the point of their contact the membranes are curved and parallel to one another. However, most important of all, it is possible to see clearly with our own eyes that the degree of maturation of the two poles of the interneuronal contact is different. Whereas the presynaptic membrane appears morphologically almost completely unchanged and differs only a little from other parts of the plasma membrane of the axon terminal, the postsynaptic membrane and the adjacent layer of cytoplasm of the dendrites has already undergone considerable specific differentiation.

Special attention is directed to the distinctive type of digital projections which arise at this moment on the outer surface of the postsynaptic membrane in the direction of the approaching terminal. There are good grounds for suggesting that these projections, which occupy a definite area of the cell membrane in the region of the future active zone of the synapse, can serve as a unique indicator to guide the growth of the presumptive synaptic endings toward it. Later, they evidently connect with the presynaptic membrane and form the characteristic septal striation of the synaptic space, which appears in the later stages of synaptogenesis.

These observations suggest that during the development of interneuronal synapses their specific differentiation begins from the subsynaptic zone and that the postsynaptic neuron evidently acts as the leader in the process of synaptogenesis. Since differentiation of the subsynaptic zone of a nerve cell, as we know, could not take place without the participation of its genome, it is natural to postulate a leading, or possibly decisive role of the genetic mechanisms of that same neuron in the formation of its interneuronal synapses.

In the ninth week of prenatal development a further and appreciable enlargement of the synaptic contact was observed on the neuroblasts in the region of the spinal cord. Most of them lay on dendrites and they were basically the same forms as characterized the eighth week of development. However, more mature forms of synapses had appeared by now, much more similar in their structural organization to definitive synapses. They were characterized not only by a high degree of differentiation of their pre- and postsynaptic membranes, but also by a definite accumulation of vesicles in the active zone of the synapse (Fig. 3). These vesicles in some places were in direct contact with the membrane, thus recalling the morphological picture of well-marked functional activity of mature synapses. Although in this period these forms of synapses are found relatively rarely, they nevertheless justify the conclusion that they are relatively well prepared for the spinal cord to perform its reflex functions.

In the transition from the embryonic to the fetal period of human development, these observations thus show that the first functionally competent synapses in the presumptive anterior horns of the spinal cord appear and mature rapidly (at the level C4-T3). The fact that they develop so early in man is undoubtedly responsible for the early appearance and rapid development of the functional system of the upper limbs, which play such an important role throughout man's later life.

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