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Spatial Orientation of Cells and Intercellular Interactions in Neuro-ontogenesis

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The dynamics of cell processes of the sensorimotor cortex of newborn rats in neural tissue culture revealed that individual processes had two types of growth: directed (rectipetal) and adaptive (twisting). The replacement of one type of growth with another can take place all along one neuroblast process. The adaptive and directed growths, regulated by different levels of protein synthesis have different chemical requirements.

The cell body transfer in the periods of migration and stabilization of cell position, the number of processes in pericellular zones, the relative constancy of the process number per cell, and the individual variability of neuropil density in the pericellular zone were observed.

The redistribution of cytoplasm among different parts of the network with hourly rhythm reflected neuroblasts interaction. The daily rhythms reflected glial elements activity. The many day rhythms reflected age waves of cell migration.

Cells with identical rhythms of fluctuation process number occupied a definite zone in the field of culture growth.

The question of cellular biorhythms being able to determine the spatial dispostion of cellular groups and the interaction among cells as well as between the nervous tissue transplants and the hosts tissue is discussed.

Key Words: Neuroontogenesis, Neuropil, Biorhythm, Cell, Topography

Introduction

The generally known pattern of basic stages of neuro-ontogenesis, including cell division, their migration and the systems orientation within the organism and relative to each other (Maximova 1979, Kokina 1980) has not so far explained the factors determining the final morphology of brain regions providing the finite function implementation.

It is known that during the structure formation, cells actively transfer and react to the environmental changes. However, the main principle underlying cellular systems development is not known so far, which could have otherwise thrown some light in solving problems facing neuroontogenesis as well as for the central nervous system regeneration and for studying the interaction of a transplant with the host's tissue.

On observing the amazing processes of forming cellular systems out of heterogenous elements in tissue cultures, we decided to carry out a thorough characterization of individual cells out of a set. This was aimed to find cellular parameters responsible for the spatial grouping of cells

Materials and Methods

One of the suitable methods of studying cellular behaviour in neuroontogenesis is the *in vitro* nervous tissue culture method (Veprintsev 1988). The sensorimotor cortex of newborn rats of Wistar line was

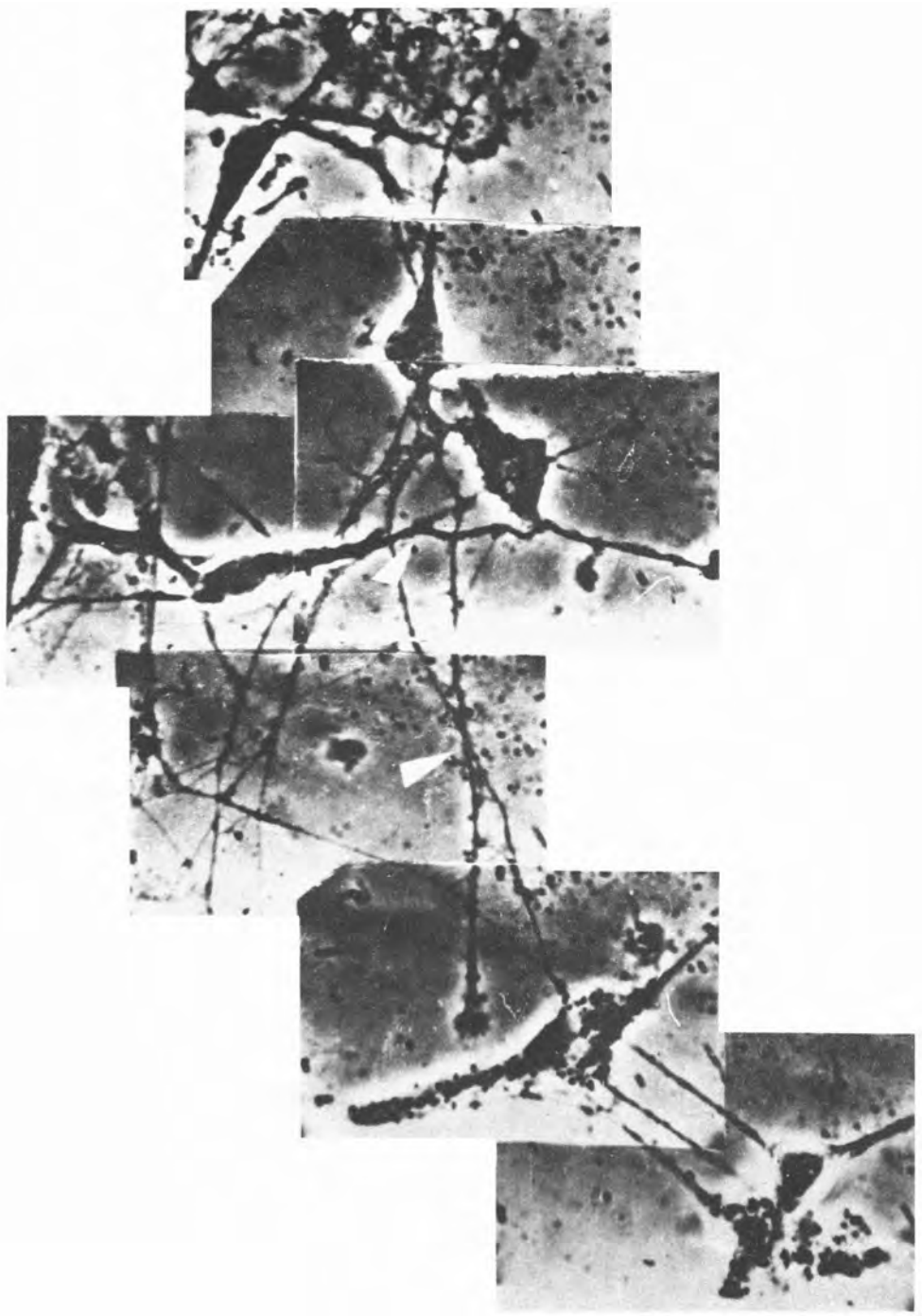

Figure 1 Phase contrast micrograph showing the neuronal-glail network in culture *in vitro* White arrows mark two types of cellular processes growth (20 × 40)

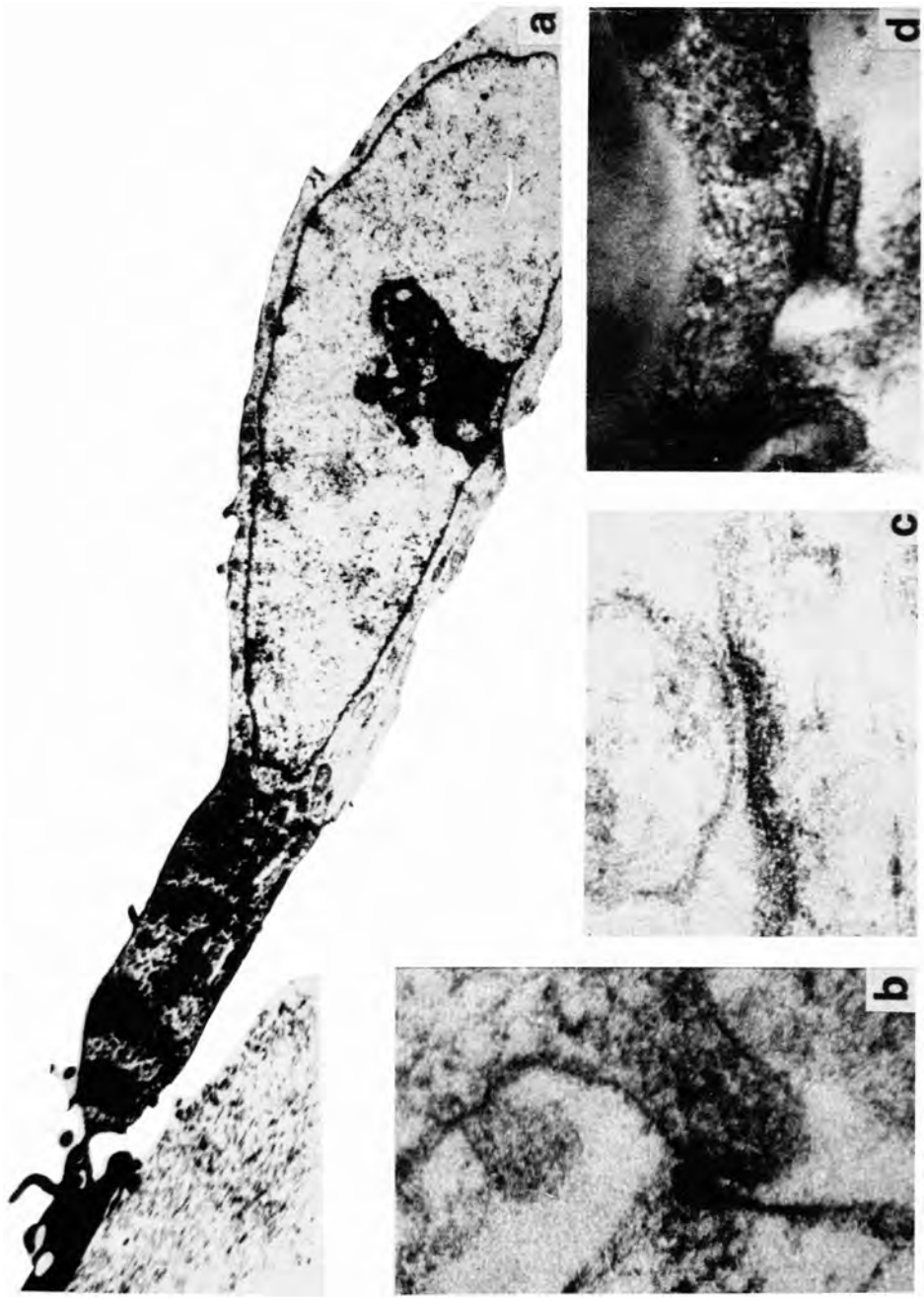

Figure 2 a-d Neuroblast (a × 250) and immature contact membrane (b, c × 9000; **d** × 1500). Election microscopy data.

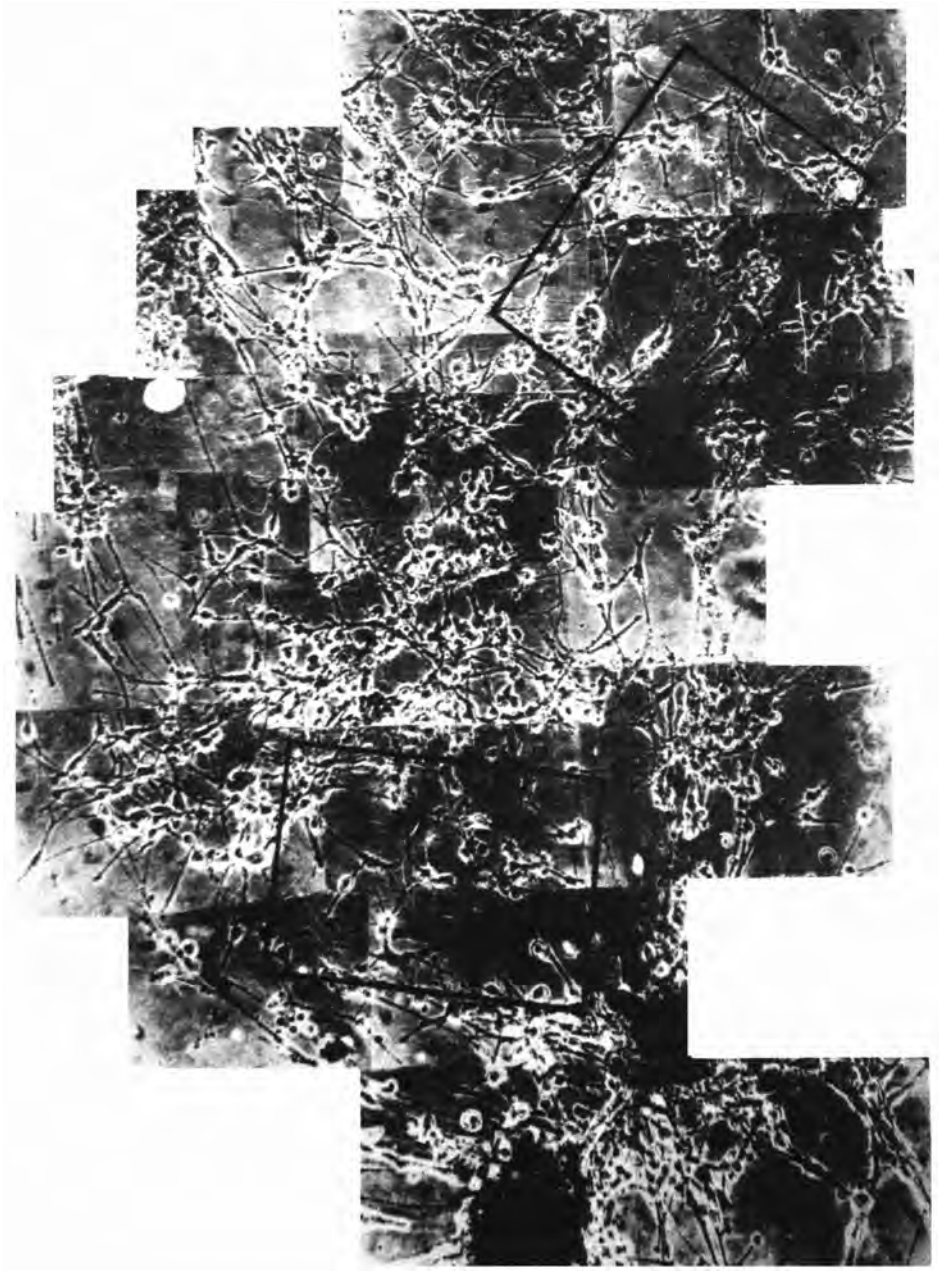

Figure 3 Phase contrast micrograph showing Photomontage of cellular network. Standard cell fields are shown in frames (10 × 20)

studied. Dissociated culture diluted by a weak (0.025%) trypsin solution was prepared to obtain single cells and small conglomerates (micro explants). It is known that the sensorimotor cortex in the intact brain of newborn rats consists of immature neuroblasts. The contacts among neuronal cells are respresented as single synapses (Maximova 1985).

In the tissue culture one can observe cell transfer and assortment by selective neighbourhood principle. In this case a neuronal-glial network preceding the consolidation of cells into more localized clusters called "units of migration" or into denser multilayer conglomerates will be the most primitive form of cellular organization. Thus, various stages of neuronal organization can be observed in tissue culture (Victorov 1985). However, it is the neuronal-glial network that, thanks to its sparse nature, will give the best possibilities for studying cellular behaviour and intercellular interactions (figure 1). It is known that such a form of nervous tissue organization is immature ontogenetically and the most ancient phylogenetically (Koshtoyants 1957).

Electron microscopic data show that in the networks of the cortical tissue culture of newborn rats the ratio of neuronal and glial elements is 1:10. The contact membranes are immature. There are single symmetric and asymmetric contacts with a few synaptic vesicles (figure 2).

The main parameter which was taken into consideration for different cells of the network was the number of processes per cell.

To take into account the dynamics of pericellular processes, the original count method developed in our laboratory by Makhmutov was applied. After taking pictures of a vast area of culture a photomontage was made (figure 3). A field of standard size including 20-30 cells with the ratio of neuronal and glial elements 1/10 was chosen for analysis. Then the middle of the greatest chord of the cell was taken for a center of a circle and was drawn with the radius calculated according to the equation

$$R = \frac{2(d_1 + d_2 + d_3)}{3}$$

where d_1, d_2, d_3 are the three chords of the cell body oriented on the basis of processes. All the processes crossing the circle and belonging to the cell itself as well as coming to the pericellular zone from other cells were counted (figure 4).

Alongside the number of processes in the pericellular zone, changes in cell body disposition during 24 hrs, the form of cell body, the presumable neuronal or glial nature of cells and their density per unit of the cover glass area in the given field of neural tissue culture were taken into account.

The observations were made round the clock beginning from the 3rd to the 14th day of cultivation.

Results

Beginning with the 3rd day of cultivation the organization of neuronal-glial network seemed to be relatively stable. However, slight transfer of cell bodies and changes in the form of processes were constantly taking place in the network. To investigate cellular mobility, the trajectories of cell body transfer in reference to a fixed point were taken into account (figure 5). The cells with a stable position continued to move relative to a center along the broken line with an amplitude not exceeding the size of four cell diameters and at the speed of not more than 15 μm/hr. The second group included cells (43%) with the same type of transfer but

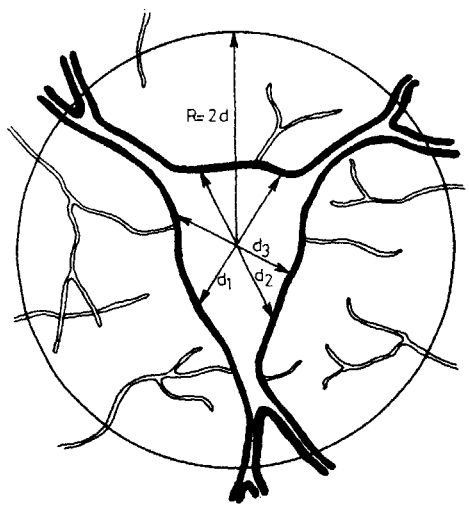

Figure 4 The scheme of the process number count in the pericellular zone R Ya. Makhmutov's method

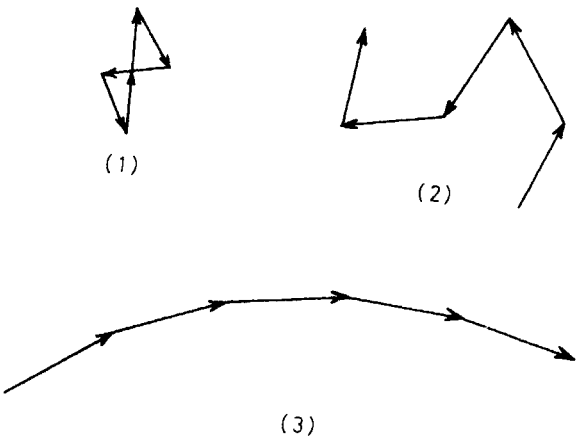

Figure 5 Different trajectories of cell migrations

with an indefinite trajectory and without a visible orientation in relation to a constant center. There was also a third type of cell (7%), moving along an arc.

The transfer speed in the second and the third cases was twice as much as that of the first type. The movement had a progressive nature. The cells of the first type which took the most stationary location continued to perform oscillatory movements. Hence, the conclusion that stabilization of cellular arrangement did not mean movement termination.

Cells with a stable arrangement were interconnected through their processes. The growth of individual processes was of two types: rectipetal and twisting. In the first case, the growth cone moved along the right line. In the second case, it performed searching movements by changing the direction, thus showing a twisting trajectory. The periods. of rectipetal and twisting growth alternated all along one process (figures 1 & 6). At the same time the cell could develop processes of one type or another (figure 7). Difference in the response to various chemicals of these two types of growth, their different functional significance and the reciprocal nature of interdependence were identified.

Ribonuclease in the concentration of 1 mg/ml added into the pericellular medium inhibited the formation of processes of both types. Desoxyribonclease in the concentration of 1 mg/ml inhibited the direction and branching of the processes. The actinomycin in the concentration of 25 μg/ml activated the formation of searching processes inhibiting the nucleus-dependent protein synthesis. The addition of ATP in the concentration of 0.1% to the pericellular medium resulted in the appearance of clusters of rectipetal processes not requiring targets. The observations compell us to assume different chemical regulation of growth of the two types of cellular processes and their potential functions.

The analysis of neuropil dynamics showed that beginning with the 3rd day of cultivation the average number of processes found in relation to one cell seemed to be constant and equalled 10. However, in the individual pericellular zones, the number of processes increased

Table 1 *The changes according to age in the dynamics of processes*

1	Cultivation (days) 3	6	8	14
Average number of processes per one cell	10	10	8	10
The total dynamics of processes per one cell for 4 hr	1·0.4	0.4-0.1 $P \geq 0.999$	1.3-1.0 $P \geq 0.99$	2.5-1.2 $P \geq 0.95$
% cells with.				
no change in the proceses' number	38	70	43	0
decrease in the processes' number	27	15	43	63
increases in the processes' number	35	15	14	37

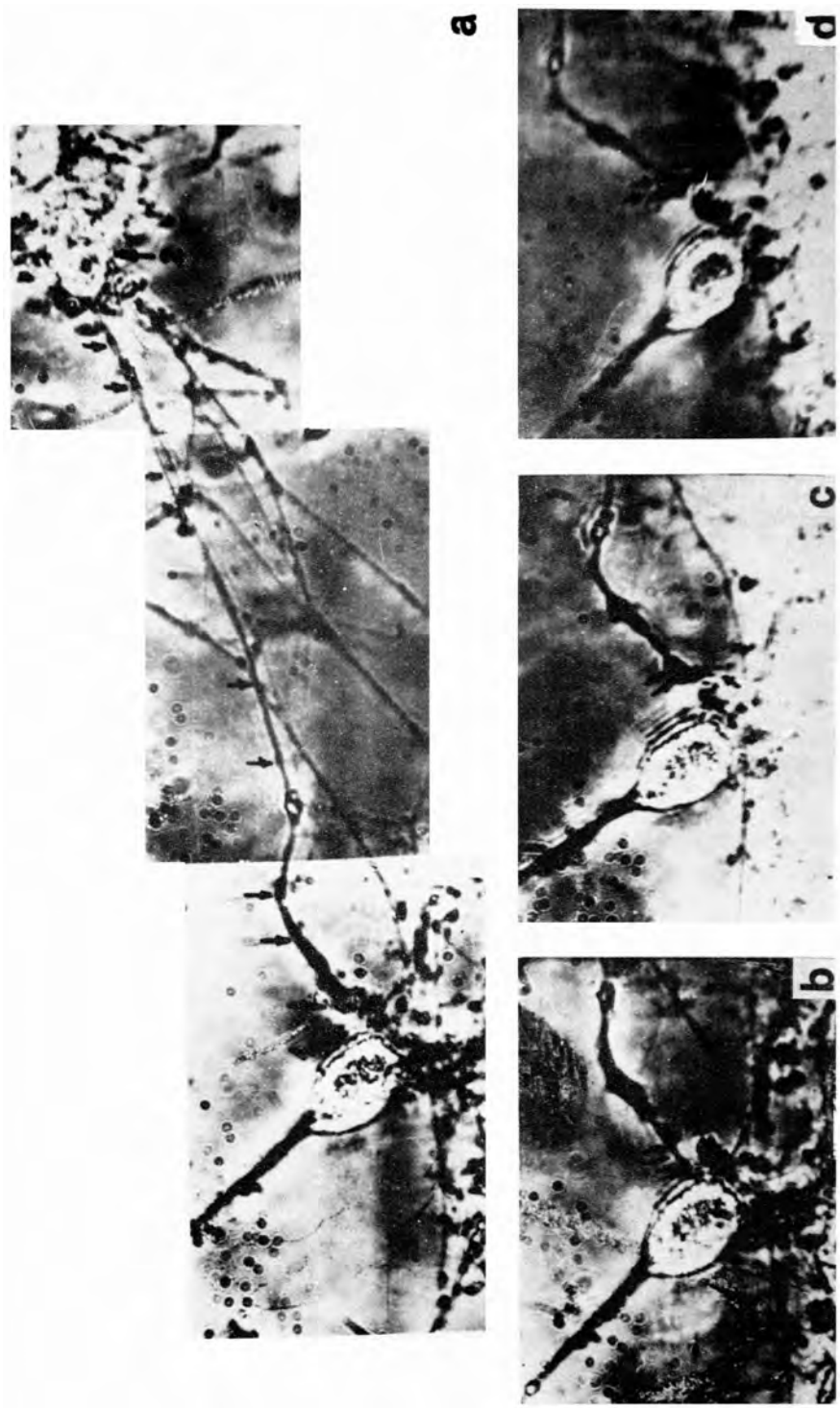

Figure 6 Interaction of two neuroblasts (1 : 2) in the tissue culture. **a**, General picture; **b**, **c**, **d**, Successive pictures show interaction between the process of Cell 9 and another cell (1) body (15 × 40); Phase contrast micrograph.

Figure 7a. The directed growth of cell processes in the nervous tissue culture stimulated by ATP, **b,** The adaptive cellular processes in the nervous tissue culture (15 × 40) Phase contrast micrograph

or decreased by 2-8. The degree of their activity changed with age. In a 3-day culture, one-third of cells did not manifest neuropil dynamics and in a 6-day culture there were already two-thirds of them. In an 8-day culture, the neurpil activity increased again and the cells changed the number of processes with time (table 1). The dynamics of activation in the neuropil is influenced by both appearance and disappearance of processes. The following regularity was observed: with the constant number of processes in the neuronal-glial network the cytoplasm transfer among different parts of neuropil took place. It manifested itself in the appearance of cellular processes and their disappearance from the pericellular zones of individual cells of the given network.

The dependence of neuropil dynamics on cellular density was observed—the higher the cellular density the less active the dynamic processes in the pericellular zone (table 2). One hundred and twenty individual cells were analysed for the changes in the number of process within 4 hr period. It showed that the change in the number of processes in the pericellular zone of individual cells had an oscillatory nature. The semiperiod

Table 2 *The dependence of the processes' dynamics on the cell density*

The number of cells in a standard field (n)	The change in the number of processes per cell for 5 hr
$n < 20$	$1,6 \pm 0,8 > 1$
$n > 20$	$0.8 \pm 0,2 \geq 1$ $P \geq 0.99$

of changes in the majority of cells was 4 hr, after which the number of processes in the neuropil decreased and then increased again. The second group included the cells whose process either increased by $6{\cdot}4 \pm 4.3$ or decreased by 5.3 ± 2.0 during 24 hr (figure 8). The analysis of the curves showed that there were cells having fluctuations in neuropil density with a full period of 8,10 20, 40 and 80 hr.

If we correlate the disposition of cellular elements the changes in the neuropil density at different periods with reference to a stable microexplant, one would see that these elements had a definite topography of their distribution over the area with the radius of 250 μm. Cells with neuropil dynamics in the pericellular zone lasting many days were the closest to the explant. Cells with hourly dynamics were farther. And the farthest were the cells with daily pericellular neuropil dynamics. Therefore, there was a distinct intercellular interaction of individual cell groups, determining their topographical disposition. The cells concentrated within

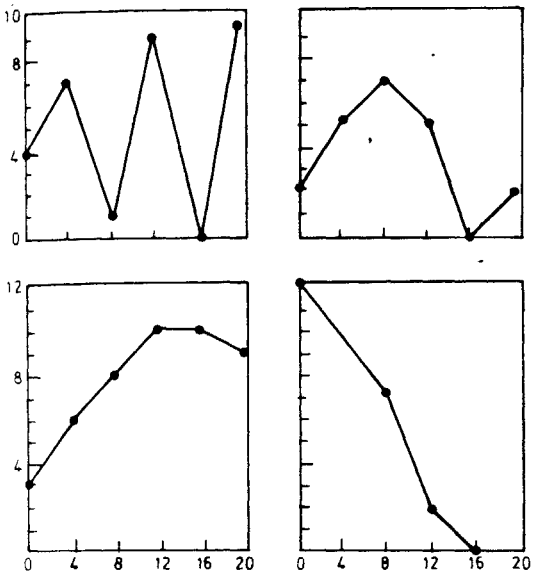

Figure 8 Different forms of changes in the process number in the pericellular zone of individual cells

X-axis—time in hours
Y-axis—the number of processes

a definite zone had common biorhythms (figure 9). As stated above, this could be judged by rhythmical fluctuations of neuropil density in the pericellular zone.

Discussion

All such events as are connected with active transfer of cells or their processes can be defined as cellular behaviour. What factors determine the cellular behaviour at a given moment? Some of them are well known. They are: mechanical tension caused by the substrate, the adhesive property of cells towards the substrate and chemical gradients of different nature. Some of the gradients are produced by neurosecretion of individual cells or cellular groups and can cause positive or negative hemotaxis of other cells and their processes. The third important factor is energetic fields existing in a living structure. First of all, they are electric fields but there can also be energetic fields of practically unknown nature, acting, for instance, by holographic principle (Shuleikina 1985, Jacobson 1978).

Besides, there is also genetic potency of a cell determining its morphology and the direction of cell transfer (Kokina 1974). Interacting by Vector Principle, intracellular and extracellular factors create a resultant determining the final position of a cell and its links with other cells (figure 10). At present comparison of external and internal factors in neuroontogenesis loses its significance and makes way for the cognition of mechanisms of their resulting interaction.

The resulting behaviour from moment to moment depends not only on the whole complex of factors but on their distribution in time. The growth of individual processes of cells of the neuronal-glial network is regulated in different periods of time from different levels of protein synthesis. The rectipetal processes are to a greater degree connected with the nucleus-dependent syntehsis, they are relatively indifferent to

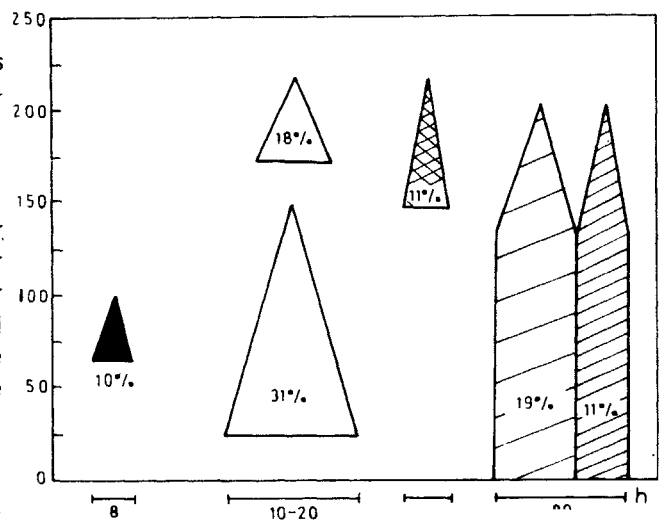

Figure 9 Grouping of cells in the growth zone of the explant in X = axis: the length of fluctuation period of neuropil density in hours; the number of cells with a given length period is expressed in percent. Y = axis: Distance from the micro-explant (μm)

external influences and to the presence of the target. They have considerable length and require high energy. The blocking of the route of cellular behaviour regulation activates the membrane-ectoplasmatic level of regulation and results in the appearance of twisting processes highly sensitive to the substrate, gradients and to the presence of adjacent cells. The change in growth type of one process means that the period of directed behaviour is followed by that of its adaptive correction.

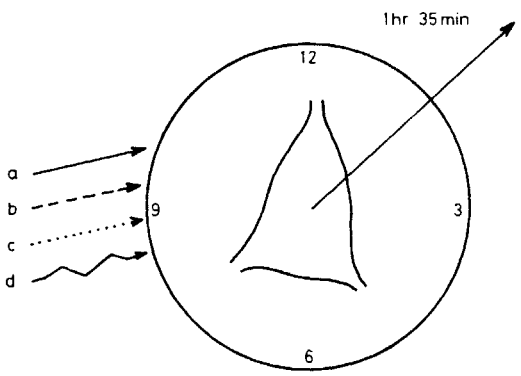

Figure 10 The vector principle of cellular behaviour determination. The vector forces influencing the cell and determining the cellular behaviour are indicated by the arrows on the left: **a**, external gradients; **b**, cell competence; **c**, cell genetic programme; **d**, cellular biorhythms. The arrow on the right indicates the resulting vector of cellular behaviour for a given moment. The process is considered relative to time.

The significance of rhythm factor for nervous tissue organization also becomes apparent following total analysis of neuropil activity in the pericellular zone. Three different intervals of neuropil pulsation are singled out: hourly rhythms (8 hr), daily rhythms (20 hr) and manyday rhythms (upto 80 hr). Hourly rhythms coincide in their characteristics with periods of change of directed adaptive growth of neuroblasts processes (figure 6). At the speed of the process growth upto 40 μm/hr during a period of several hours one can watch the development of the process into the zone of cell recipient influence, the process orientation and its leaving the zone in case the contact is not formed (figure 6). Thus, the hourly rhythms of neuropil pulsation should be related to the contact formation among neuroblasts. Cells with hourly rhythms of neuropil fluctuations are localized in a definite area of the growth zone. These cells as well as other cellular clusters can be surrounded by cells with daily rhythms. Daily variations of process activity can be characteristic of glial cells. Many day-rhythms are close to the age changes of process dynamics and they can reflect the alternation of migration and stabilization phases of cell bodies.

As the fluctuations in the number of and the type of growth are determined by the protein synthesis, the cell becomes the regulator of its own neuropil. The genetically determined biorhythm of a given cell specifies its spatial disposition in relation to other cells of the given cerebral area and the approach of other cell processes to it.

Table 3 *The distribution of cellular groups in with reference to the microexplant according to the process dynamics in the pericellular zone*

The period length of process fluctuations in the pericellular zone (hr)	Total cell No. (%)	Distance from the microexplant in arbitrary units (2.5 μm)	
		all cells within the groups	the majority of cells
80	35	0-70	0-30
40	13	60-90	
10-20	42 a) 27	10-50	
	b) 15	60-80	
8	10	23-45	

The interaction of individual biorhythms of cells is shown as one of the factors of spatial neurogenesis. In case of neural tissue transplantation the preliminary analysis of biorythms of the transplant and the host's brain in conditions of tissue culture may be used for slection of the transplanted tissue.

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