ORIENTED MIGRATION AND DIFFERENTIATION OF NEUROBLASTS IN ORGAN CULTURES OF MOUSE EMBRYONIC SPINAL CORD

I. V. Viktorov

UDC 611.82-018.82-085.13

During cultivation of transverse sections of the spinal cord of 12-14 day mouse embryos in Maximow's chambers differentiation of nerve and glial cells took place with the formation of the organotypical structure of the explant. In some cases migration of groups of bipolar neuroblasts was observed in the 3rd-4th week of cultivation outside the explant, along bundles of nerve fibers consisting of axons of mature nerve cells located in the central fragment of the cultivated tissue. The results show that nerve fibers growing out from the explant can guide migrating neuroblasts and can evidently induce their subsequent differentiation. The hypothesis is put forward that similar processes of migration of neuroblasts along tracts formed by axons can also take place in the developing brain and that they are followed by differentiation where these tracts terminate.

KEY WORDS: *Cultivation of spinal cord; migration and differentiation of neuroblasts.*

The study of the principles and mechanisms of migration and also of the subsequent differentiation of neuroblasts in tissue culture is of great importance to the understanding of the formation of definitive structures of the brain and spinal cord. The question of migration of neuroblasts and neurons outside the explant during cultivation of nerve tissue has been discussed repeatedly in the literature [1, 2, 8, 9]. After numerous observations the view has become established that neuroblasts and, less frequently, immature neurons (for example, cells of the outer granular layer of the cerebellum [3, 15]) can migrate from the explant. Under these circumstances, as a rule solitary neuroblasts and undifferentiated neurons are found in zones of growth and migration surrounding the explant, but their identification during life in the light microscope, and also during the study of fixed cultures in the scanning microscope [5, 10], can at times be very difficult. Differentiated neurons are incapable of migration and the appearance of such neurons outside the explant is usually explained by their passive displacement as the cultivated tissue is spread out or by "sliding" along the layer of migrating neuroglial and epithelioid cells [2].

To study the factors influencing migration and differentiation of neuroblasts, the method organ culture of the spinal cord was used. It must be emphasized that the data described in this paper were obtained by means of a special neurohistological silver impregnation method, by means of which neuroblasts and neurons with their processes can be demonstrated selectively and distinguished from other types of cells. The necessity of using such methods has been stated repeatedly by Khlopin [2] and other neurohistologists [5], because it is extremely difficult to identify neuroblasts in living unstained cultures, and their distinction from migrating gliocytes and fibroblasts is at times a matter of great uncertainty.

EXPERIMENTAL METHOD

Transverse sections through the spinal cord of 12-14-day mouse embryos were cultivated for 4-6 weeks in Maximow's chambers by the method of Borstein and Murray [4]. Several ex-

Group for Culture of Nerve Tissue, Laboratory of Functional Synaptology, Institute of the Brain, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR B. N. Klosovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 8, pp. 1002-1005, August, 1976. Original article submitted February 10, 1976.

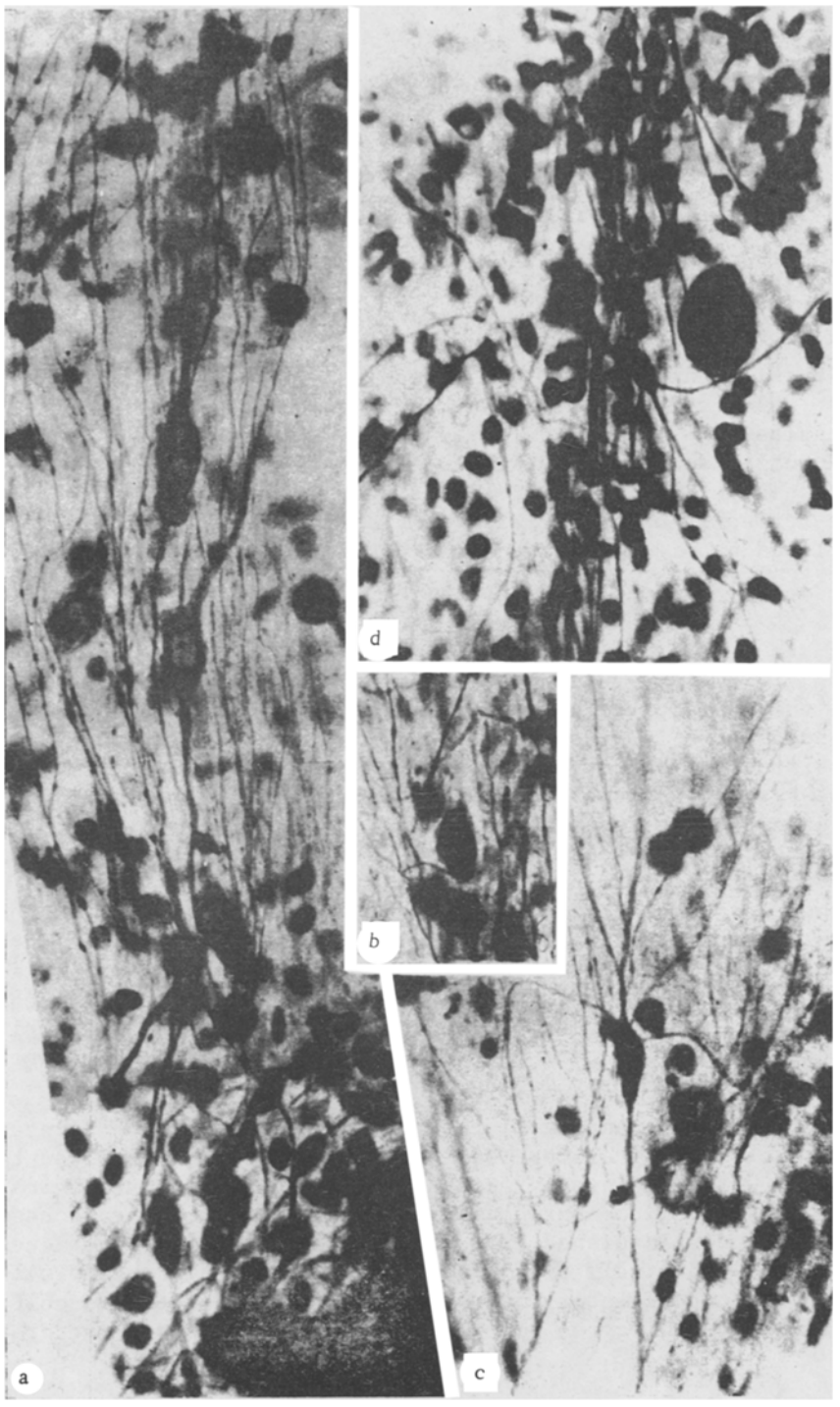

Fig. 1. Migrating neuroblasts and differentiating neurons in spinal cord cultures from 14 day mouse embryos: a) group of bipolar neuroblasts migrating along bundle of nerve fibers (32nd day *in vitro*); b) apolar neuroblast in zone of migration (22nd day *in vitro*); c) bipolar neuroblast in bundle of nerve fibers (28th day *in vitro*); d) multipolar differentiating neuron surrounded by nerve fibers in zone of migration (32nd day *in vitro*). Impregnation with silver by method of Holmes and Wolf. Scale of enlargement: 50 μ.

plants were placed on coverslips coated with collagen 1 mm apart. The nutrient medium (30% human placental serum, 30% Eagle's minimal medium, 20% Simms's salt solution, 10% extract of 9-day chick embryos, and the remaining 10% consisting of solutions of glucose and insulin in concentrations of 600 mg % and 0.2 unit/ml respectively) was changed twice a week. Intravital observations were made and the cultures photomicrographed; the fixed cultures were impregnated with silver by the method of Holmes and Wolf [14].

EXPERIMENTAL RESULTS

During the first week in culture processes of the neuroblasts were observed to grow along the edges of the explant and active migration of glial cells and fibroblasts led to the formation of a zone of migration around the central fragment of tissue in which only solitary neuroblasts were seen. Later, as a result of differentiation of neurons and neuroglial cells, the internal organotypical structure of the explant was formed and, as a rule, the intensity of migration of the neuroglia diminished. This period was characterized by intensive growth of axons of the nerve cells. In preparations impregnated with silver it was possible to see groups of multipolar neurons whose axons formed complex systems of interneuronal connections within the explant and spread partly outside its boundaries, forming bundles of nerve fibers. These bundles either spread out for a short distance from the central fragment of the tissue or grew toward other explants, forming what are called axon bridges [7].

In some cases bipolar fusiform cells with an oval nucleus, containing one or two compact nucleoli, could be seen among the bundles of nerve fibers leaving the explant. The bodies of these cells measured 15×25 μ; the nuclei were fairly constant in size, namely 10×15 μ. After impregnation with silver, thin neurofibrils could be seen in the body and processes of the cells just described (Fig. 1a, c). The whole series of morphological features, including the specific argentophilia of these cells, suggests that they were migrating bipolar neuroblasts of the spinal cord [6]. Only occasionally were argentophilic cells of the apolar neuroblast type found (Fig. 1b).

Groups of migrating neuroblasts were of the greatest interest (Fig. 1a). The peripheral zone of the explant, from which bundles of parallel nerve fibers are given off, is shown in the lower part of the photomicrograph. In the peripheral zone and among the nerve fibers migrating bipolar neuroblasts are arranged in a chain. The processes of these cells run parallel to the nerve fibers; the larger process, advancing along the course of migration, reached a length of 250-300 μ, whereas the thin process trailing behind the migrating cell was 70-100 μ in length. It is interesting to note that the long axis of the oval nuclei of the neuroblasts coincided with the direction of migration of these cells. Measurements

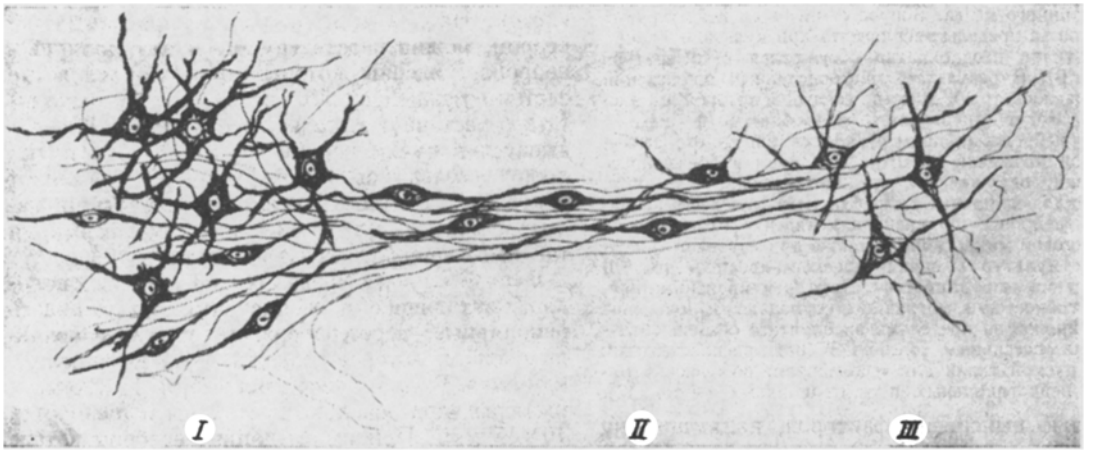

Fig. 2. Diagram illustrating the oriented migration of neuroblasts from an organotypical explant and their subsequent differentiation and formation of a nervous center: I) peripheral zone of explant containing differentiated multipolar neurons and bipolar neuroblasts; II) bipolar neuroblasts which migrate along a bundle of nerve fibers growing out from an explant; III) zone of ending of nerve fibers and site of differentiation of neuroblasts forming a neuronal structure synaptically connected with the explant.

showed that the neuroblasts could migrate along nerve fibers for 600-700 μ from the edge of the explant. Multipolar differentiated neurons with long, branching dendrites, spreading out radially from the cell body, could frequently be seen along the course of a bundle of these fibers and at the point of their termination (Fig. 1d). The nuclei of the differentiated neurons were always circular in shape (diameter of nucleus 12-15 μ) and each contained one nucleolus.

The results thus show that during cultivation of transverse sections of the spinal cord in the early stages of embryogenesis, besides maturation of neurons in the explant, oriented migration of certain populations of neuroblasts followed by their differentiation outside the explant also can take place. The processes described above take place as a rule in the 3rd-4th week of cultivation of nerve tissue, after the explant has ceased to spread and its organotypical structure has been formed. Among the conditions for active migration of neuroblasts are previous growth of axons of neurons that are already differentiated and the formation of bundles of nerve fibers which form specialized conductors determining the direction of migration. Similar processes of migration of bipolar neuroblasts along the processes of neuroglial cells have been found in the cerebellar and cerebral cortex [11, 12]. The data now described show that in some cases axons of other nerve cells can also serve as guides for the migrating neuroblasts.

It can be postulated that the subsequent differentiation of the migrating neuroblasts is caused by the action of the endings of the nerve fibers along which these cells migrated, and that the zone of endings of that system of fibers determines the region of final differentiation of the neuroblasts and the site of formation of a definitive neuronal structure, such as a nucleus (Fig. 2). In that case, synaptic connections are probably formed also between the endings of the nerve fibers guiding the migration of the neuroblasts and neurons formed as a result of the differentiation of these neuroblasts.

The hypotheses formulated on the basis of an analysis of oriented migration and subsequent differentiation of neuroblasts in embryonic nerve tissue culture thus supplement those previously expressed in the literature [13] for the purpose of explaining the formation of the neuronal structures of the brain and the development of synaptic connections between them.

LITERATURE CITED

1. S. N. Olenev, Acta Anat., 68, 68 (1967).
2. N. G. Khlopin, General Biological and Experimental Bases of Histology [in Russian], Leningrad (1946).
3. C. D. Allerand, J. Comp. Neurol., 142, 167 (1971).
4. M. B. Bornstein and M. R. Murray, J. Biophys. Biochem. Cytol., 4, 499 (1958).
5. A. Boyde, D. W. James, R. L. Tresman, et al., Z. Zellforsch. Mikrosk. Anat., 90, 1 (1968).
6. S. Ramon y Cajal, Histologie du Système Nerveux de l'Homme et des Vertébrés, Vol. 1, Madrid (1952).
7. S. M. Crain, E. R. Peterson, and M. B. Bornstein, in: Ciba Foundation Symposium on Growth of the Nervous System (ed. by G. E. W. Wolstenholme et al.), London (1968), pp. 13-31.
8. W. R. Markesbery and L. W. Lapham, J. Neuropath, Exp. Neurol., 33, 113 (1974).
9. M. R. Murray, in: Cells and Tissues in Culture (ed. by E. N. Willmer), Vol. 2, Academic Press, New York (1965), pp. 372-455.
10. A. Privat, M. J. Drian, and P. Mandon, Z. Zellforsch. Mikrosk. Anat., 146, 45 (1973).
11. P. Rakic, J. Comp. Neurol., 141, 283 (1971).
12. P. Rakic, J. Comp. Neurol., 145, 61 (1972).
13. R. Sidman and P. Rakic, Brain Res., 62, 1 (1973).
14. M. K. Wolf, J. Cell Biol., 22, 259 (1964).
15. M. K. Wolf, J. Comp. Neurol., 140, 281 (1970).