GROWTH AND MATURATION OF THE BRAIN

PROGRESS IN BRAIN RESEARCH

Volume 4

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VOLUME 4

GROWTH AND MATURATION OF THE BRAIN

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Edited by M. Singer and J. P. Schadé

Volume 14: *Degeneration Patterns in the Nervous System*
Edited by M. Singer and J. P. Schadé

Preface

No single mode of attack upon problems of morphogenesis is adequate. Experimental methods yield the most decisive evidence, and these require adequate knowledge of anatomical structure. The last is the contribution of comparative anatomy and comparative embryology, and both of these must be functionally interpreted to be fruitful.

C. J. HERRICK: *The Brain of the Tiger Salamander*

Two factors have contributed to the renaissance of interest in the developing nervous system in the past decade: (1) the application of new operational methods in neurocytology, neurochemistry and neurophysiology to studies of the immature brain; and (2) the growing recognition of the value of ontogenesis as an analytical tool in neurobiological research. The expansion of investigative work in the phylogenesis and ontogenesis of the nervous system has brought into sharp focus the inevitable problem of establishing effective channels of communication within and across various disciplines. Considerable progress in this direction was made at a symposium sponsored by the Netherlands Central Institute for Brain Research. The site selected for this meeting was as much an inspiration of Dr. Schadé and his associates as was the meeting itself. Undoubtedly the all-to-few days spent at the castle 'De Hooge Vuursche' outside of Amsterdam will continue to conjure up pleasant memories for all participants long after the details of experimental data are forgotten. But that some of the record of the symposium be preserved is one of the purposes of this volume. The reports and discussions presented during 'working-sessions' of this symposium held from September 7–10, 1962 are published here in the hope that the casual reader as well as the specialist will glean from them something of the scope of the meeting. Unfortunately nothing can be done to retrieve the record of the many hours of informal discussions which greatly contributed to the success of the conference. This success is not to be measured solely in the character of formal reports. The success of any conference is to be measured by the degree to which participants allow themselves to become intellectually and emotionally involved in the work and enthusiasm of others. In this respect the Amsterdam meeting was entirely successful.

The reader will appreciate that the several topics in this volume are illustrative of the validity of Herrick's dictum that 'no single mode of attack upon problems of morphogenesis is adequate.' Thus studies of brain mitochrondria and the molecular evolution of enzyme systems are as much a part of the story of brain maturation as the migration of neurons, development of dendrites, glia, synapses or reflex activities and behavior patterns. The difference is in the level of organization. Clearly the key problem remains to define the pathways by which successive levels of organization, molecular to multicellular may be satisfactorily approached, one from the other. And when such pathways are specified, one may be flanked by trim hedges and luxurient gardens and lead to an old castle a few miles from Amsterdam.

August 1963

DOMINICK P. PURPURA

Contents

Events in the Developing Nervous System

RITA LEVI-MONTALCINI

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Ever since the most talented and perceptive of all neurologists, Ramon y Cajal, made available the silver technique for the selective impregnation of nerve cells, the nervous system of all phyla from celenterates to primates has become the object of an extensive and everlasting exploration for generations of neurologists. If the study of the different phyla provided a key for the understanding of the complexity of this system in higher forms, man included, it was the study of the developing nervous system which provided the Ariadne's thread to unraveling the labyrinthic complexity of nerve centers and nerve fiber tracts in the mature brain. Again it was the intuitive mind of Cajal which realized the enormous potentialities of this approach. To him and to his students we owe the most penetrating and rigorous series of investigations on the developing vertebrate nervous system. This analysis served as the basis for Cajal's fundamental work, *The Histology of the Nervous System*, which still represents today the most complete and authoritative study of this type (Cajal, 1909).

At the same time as Cajal and co-workers concentrated their studies on the embryonic nervous system, other brilliant scholars such as Edinger, Ariëns Kappers, Herrick, Crosby, and their students concentrated on the comparative analysis of the brain structures in the vertebrate phyla. Between the end of the past century and the first decades of this century, such a wealth of facts became known on the developing nervous system, as well as on its evolutionary changes, as to make one wonder if this field of investigation had not now been exhausted. It was with this outlook and a feeling of awe and curiosity for this most complex system that we first approached it two decades ago, shortly after the voice of the great master Cajal had become silent and while other outstanding scholars such as Ariëns Kappers, Herrick and Crosby were still actively pursuing their search on the evolution of brain structures.

The object of our first analysis was the chick embryo. As we became more and more familiar with this object and scrutinized its developing nervous system day after day and week after week, we became aware of the fact that this system had still not revealed more than a few of its many facets and that the essence of its developing mechanisms remained to be uncovered. Today, 20 years later, with the experience which comes from daily contact with the object of interest and with the dispassionate approach of the mature mind, the writer feels that we have still taken little more than a first glance at the endless complexity and intricacy of the differentiating nervous system. Thanks to the joined efforts of a large number of investigators, we have

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possibly acquired a more precise knowledge of the sequence of events which take place during the growth and differentiation of the nervous system, but little progress has been accomplished in the understanding of the mechanisms which control these events.

It is with this feeling of inadequacy and the awareness of the number of unsolved problems still ahead of us, that I propose to discuss some of the events which take place in the developing nervous system. I will limit myself to the presentation and discussion of some of the aspects which we explored and attempted to analyze in the past years. They will be considered in reference to the time pattern of their occurrence and also in reference to the opportunity they offer to obtain some information on the agents which control and direct growth and development of nerve cells and nerve centers. Two main processes which take place in early phases of the differentiation of the nervous system will be considered, namely the *migration and degeneration of differentiating nerve cells.* We will limit our presentation to these areas not because we believe that the events to be presented may be of more interest than other events taking place in the developing nervous system, but because of our personal experience and interest in these aspects of the nervous system. Most of the observations to be reported are based on the analysis of these developmental processes in the chick embryo. They were supplemented by a few observations in mammals, specifically in the mouse embryo.

Hence when not specifically stated, we refer to the chick embryo. We will then briefly consider the results of some experimental work aimed at the analysis of the above processes.

MIGRATORY PATTERNS IN THE DEVELOPING NERVOUS SYSTEM

The capacity of differentiating nerve cells to migrate during the early stages of their differentiation has been known for a long time. Little, however, has been known about the extent and magnitude of these processes and the role they play in molding nerve centers and wiring them together. We will consider here some of these migratory movements which were the object of a close inspection in the chick embryo. The first to be described take place outside the central nervous system. They concern the cells which will form some of the cephalic ganglia and have their origin in the dorso-lateral and epibranchial placodes. Other important migratory movements occur at the same time outside of the spinal cord. These consist of the migration from the neural crest of the precursors of sensory spinal and sympathetic nerve cells. These migratory movements were considered at length during the past years and are well-known in all their aspects (Van Campenhout, 1931; Hörstadius, 1950; Tello, 1925; Yntema and Hammond, 1947); for this reason they will not be further considered here. Next we will consider the active displacements which occur in the central nervous system.

Migration from dorso-lateral and epibranchial placodes

The origin of some of the cephalic ganglia from ectodermal placodes has been as-

certained through a number of investigations (Yntema, 1942, 1944; Levi-Montalcini, 1946). It is not within the aim of this presentation to report on the experimental evidence which provided the basis for the present knowledge of the origin of these ganglia. We refer the reader to the literature in this field and, in particular, to the extensive and exhaustive experimental analysis performed recently by Hamburger (1961) on the dual origin of the trigeminal ganglia in the chick embryo. Here we will consider some aspects of these migratory movements which give origin to 3 cephalic ganglia: the geniculate ganglion of the VIIth nerve, the petrosum and the nodosum which belong respectively to the IXth and Xth nerve. The observations to be reported below are based on the inspection of a large number of 2- to 6-day-old chick embryos stained with the De Castro modification of the Cajal technique (Levi-Montalcini, 1949) and sectioned serially in transversal, sagittal, and frontal sections. Although we referred many times during the past years to these studies, they were never the object of a detailed description.

The first evidence for active cell migration from the dorso-lateral and epibranchial placodes can be obtained in embryos $2\frac{1}{2}$ to 3 days of age. Since the maturation of the

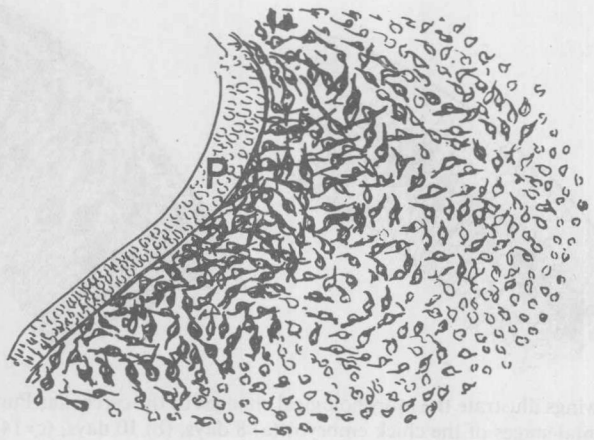

Fig. 1. Epibranchial placode (P) giving origin to the ganglion nodosum in a 3-day-old chick embryo. The migrating neuroblasts appear darkly stained and have short processes at opposite poles.

embryonic structures takes place along a rostro-caudal gradient, the migration from the dorso-lateral placode which gives origin to the geniculate ganglion precedes to some extent the migration from the 4th and 5th epibranchial placodes which give origin to the petrosum and nodosum ganglia. It was the migratory movements of these last two ganglia which offered the best condition for a close inspection.

Immediately beneath the epithelial thickening which represents the epibranchial placodes, one sees in $2\frac{1}{2}$- and $3\frac{1}{2}$-day-old embryos a large number of swarming cells which show intense silver affinity. They have two short opposito-polar prolongations which also appear intensely stained with silver (Figs. 1, 17).

Between the end of the 3rd and the 4th day, these cells move more and more away from the epithelial placodes where they were first seen and aggregate in two large

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oval-shaped agglomerates which can be easily identified as the petrosum and nodosum ganglia (Fig. 18). At the end of the 4th day the two ganglia are well-delimited and stand out against the pale background of surrounding mesenchymal cells. One gets the impression that all neuroblasts have now assembled together and that the further size increase of these ganglia results from development and growth of individual nerve

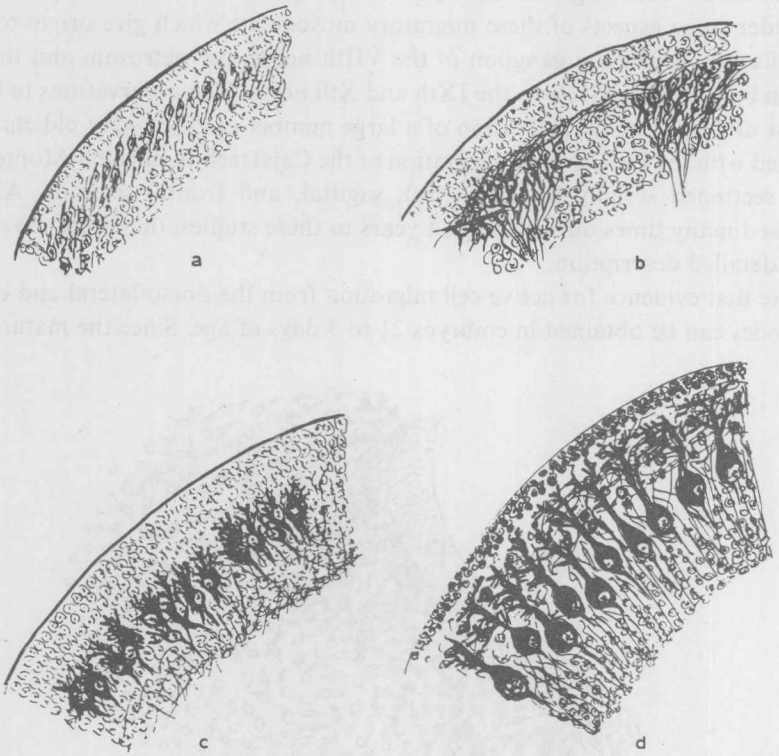

Fig. 2. The drawings illustrate the morphological changes of the cerebellar Purkinje cells in successive developmental stages of the chick embryo: (a) 8 days, (b) 10 days, (c) 14 days, (d) 17 days.

cells rather than from addition of new units. And yet all around the ganglia one sees a large number of scattered cells intensely stained with silver, with one or two short, dark filament-like prolongations. In the subsequent days the silver affinity of these cells weakens and eventually one cannot recognize them from the surrounding mesenchymal cells. The question arises as to the fate of these cells and their identity. If one has to judge on the basis of the affinity for silver, one would consider these cells as neuroblasts but this view finds no support in the observations mentioned above. Two alternatives should be considered. The first is that not all cells which react positively to silver should be identified as nerve cells. In accepting this viewpoint we would, however, deny any significance to the intense silver affinity of nerve cells as contrasted with the lack of affinity of adjacent mesenchymal cells. Also we would have to deny any value to the opposito-polar prolongations of these cells which further add to the notion that these are indeed nerve cells. The inspection of hundreds of

embryos impregnated with the Cajal–De Castro silver technique confirmed us in the belief that nerve cells, but not mesenchymal cells, react positively to silver. The second alternative would be that neuroblasts are produced in excess by the ectodermal placodes. Once the ganglia have reached a given size, they become incapsulated and the neuroblasts still present in the area no longer find access to the main ganglionic

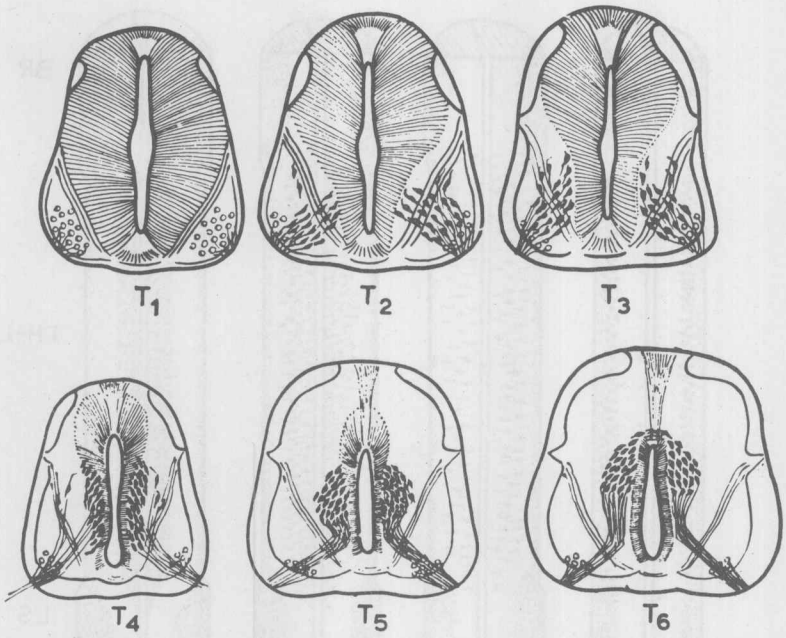

Fig. 3. Diagrammatic representation of the segregation and subsequent migration of the thoracic visceral column in the chick embryo. T_1, 3-day-old chick embryo. The somato-motor and the viscero-motor cells form a compact column. T_2, $4\frac{1}{2}$-day-old embryo. Beginning of migration of preganglionic neurons. T_3, T_4, T_5, T_6 illustrate the shift of the migrating cells from a ventro-lateral to a medio-dorsal position in embryos of 5 (T_3), 6 (T_4), 7 (T_5) and 8 (T_6) days of incubation. (From R. Levi-Montalcini, *J. Morph.*, **86**, 1950.)

agglomerates. They become dispersed and eventually die off, or possibly are otherwise utilized. Neither the first nor the second alternative were submitted to test and at present we must say that the problem of the identity and fate of these cells remains to be established.

Migratory movements in the central nervous system

Between the 4th and the 9th day of incubation, the developing nervous system of the chick embryo is the stage of the most extensive, elaborate and complex cell movements. While the brain vesicles and the spinal cord increase considerably in size, but do not undergo radical changes in shape, neuroblasts in the thousands engage in most sectors of the neural tube in long migrations which last for 2–3 days. To the student who is still not familiar with this object, these complex migratory movements appear hopelessly complicated and chaotic. It is only the hour after hour inspection of the develop-

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ing neural tube and higher brain centers in transversal, frontal and sagittal sections which persuade the observer that these movements are not at all chaotic but on the contrary are highly organized and rigidly patterned like complicated military maneuvers displacing hundreds of moving units along different routes, under the control of the central headquarters. Like soldiers, or like armies of ants or termites, the

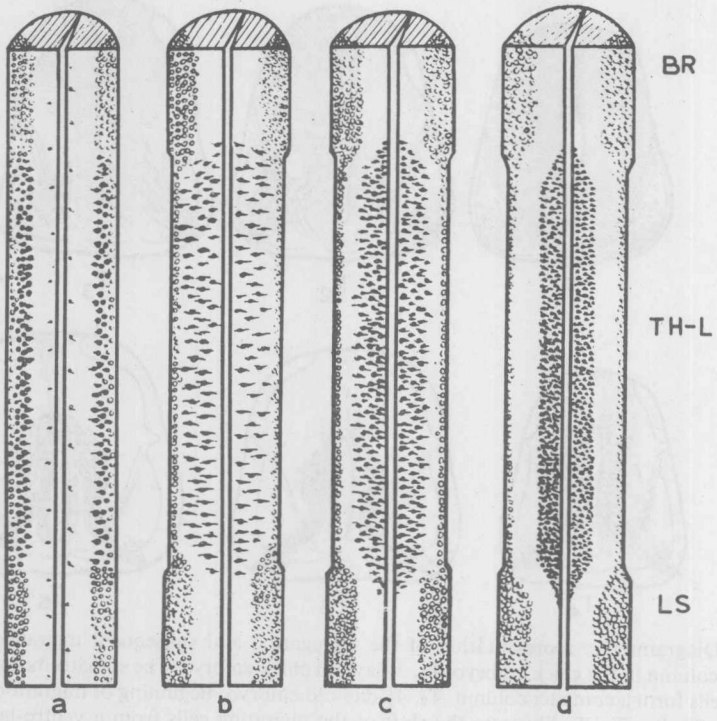

Fig. 4. Diagrammatic representation of the migratory movement of the thoracic visceral column as it appears in frontal sections of chick embryos fixed at (a) 4 days, (b) 6 days, (c) 7 days, (d) 8 days. BR, brachial segment; LS, lumbo–sacral segment; TH-L, thoraco-lumbar segment. (From R. Levi-Montalcini, *J. Morph.*, **86**, 1950.)

neuroblasts move in compact rows one after the other in long trails where each slender and spindle-shaped cell follows the other.

A characteristic common to all migrating nerve cells is their intense silver affinity and the elongated and slender shape of their bodies which stand out sharply against the pale background which forms the dense matrix of the developing nervous system. Each cell is provided with a short apical, and a long caudal filament which trails behind the body and actually represents the axon of the migrating cell. As a large number of cell units move simultaneously in different and many times opposite directions, one sees in many sectors of the brain stem or higher brain centers lines of cells crossing each other and then pursuing their own path which might bring them to entirely different areas of the developing nervous system. This intense traffic reaches its maximal expression toward the end of the 5th and the beginning of the 6th day

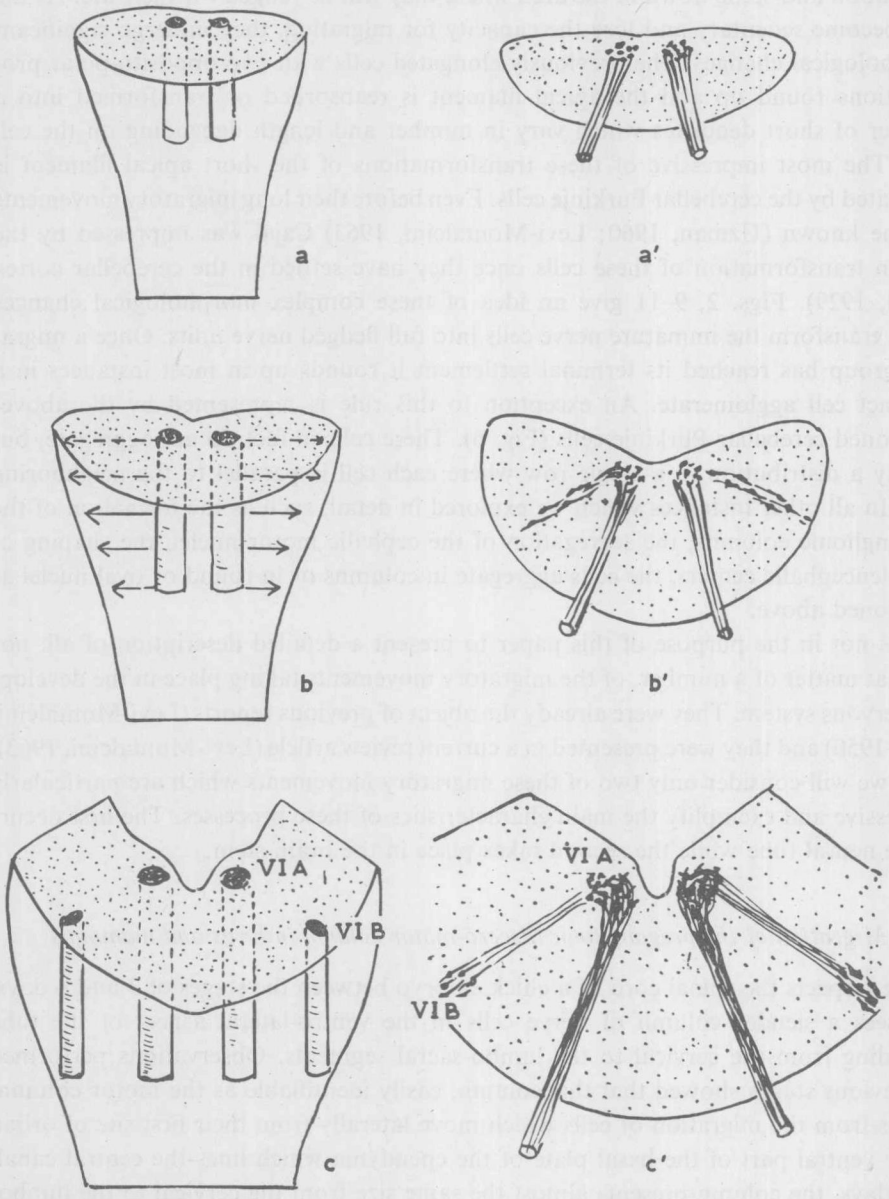

Fig. 5. Diagrammatic frontal and transversal representation of the segregation of the accessory nucleus from the main nucleus of the VIth nerve in the chick embryo. (a), (b), (c): stereographic views of the VIth nuclei at 5, 6, and 12 days. In (a), the nuclei appear as two columns; in (b), the arrows indicate the migration of neuroblasts from the main nuclei in a lateral direction; in (c), the migration is completed and the two accessory columns are formed. (a′), (b′), (c′): the same process as viewed in transversal sections. VI A, main nucleus; VI B, accessory nucleus. (From The Nature of Biological Diversity, edited by Dr. J. Allen. Copyright, 1963. McGraw-Hill Book Co. Used by permission.)

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of incubation. Immediately after, most of the migrating cells have reached their destination and settle down in the area where they will be lodged for their life. As the cells become sedentary and lose the capacity for migrating, they undergo significant morphological changes. The previously elongated cells with two opposito-polar prolongations round up and the apical filament is reabsorbed or transformed into a number of short dendrites which vary in number and length depending on the cell type. The most impressive of these transformations of the short apical filament is illustrated by the cerebellar Purkinje cells. Even before their long migratory movements became known (Uzman, 1960; Levi-Montalcini, 1963) Cajal was impressed by the sudden transformation of these cells once they have settled in the cerebellar cortex (Cajal, 1929). Figs. 2, 9–11 give an idea of these complex morphological changes which transform the immature nerve cells into full fledged nerve units. Once a migratory group has reached its terminal settlement it rounds up in most instances in a compact cell agglomerate. An exception to this rule is represented by the above-mentioned cerebellar Purkinje cells (Fig. 6). These cells in fact do not aggregate, but display a distribution in a single row where each cell is parallel to the neighboring cells. In all other instances which we explored in detail, such as the formation of the preganglionic columns, the segregation of the cephalic motor nuclei, the shaping of the telencephalic centers, the cells aggregate in columns or in round or oval nuclei as mentioned above.

It is not in the purpose of this paper to present a detailed description of all, nor for that matter of a number, of the migratory movements taking place in the developing nervous system. They were already the object of previous reports (Levi-Montalcini, 1942, 1950) and they were presented in a current review article (Levi-Montalcini, 1963). Here we will consider only two of these migratory movements which are particularly impressive and exemplify the main characteristics of these processes. The first occurs in the neural tube while the second takes place in the brain stem.

Migration of the preganglionic viscero-motor column in birds and mammals

If one inspects the spinal cord of a chick embryo between the stages of 3 and 4 days, one sees a slender column of nerve cells in the ventro-lateral aspect of the tube extending from the cervical to the lumbo-sacral segments. Observations performed in previous stages showed that this column, easily identifiable as the motor column, results from the migration of cells which move laterally from their first site of origin in the ventral part of the basal plate of the ependyma which lines the central canal. At 4 days, the column presents almost the same size from the cervical to the lumbo-sacral segment of the spinal cord (Fig. 31). It is toward the middle of the 4th day that differences become evident in the cervical, brachial, thoracic, lumbar and sacral segments of the spinal cord. The developmental patterns characteristic of each segment were the object of a detailed report in a previous paper (Levi-Montalcini, 1950). Here we will consider only the thoracic segment, while in a subsequent section we will consider the differentiation of the same column in the cervical segment of the neural tube.

Until the middle of the 4th day, the motor column in the thoracic segment of the neural tube appears to consist of a rather homogeneous cell population. If one traces, however, the peripheral distribution of motor fibers which leave the neural tube in this trunk segment, one realizes the composite nature of this apparently uniform cell population. About $\frac{3}{4}$ of the nerve fibers emerging with the thoracic ventral roots make

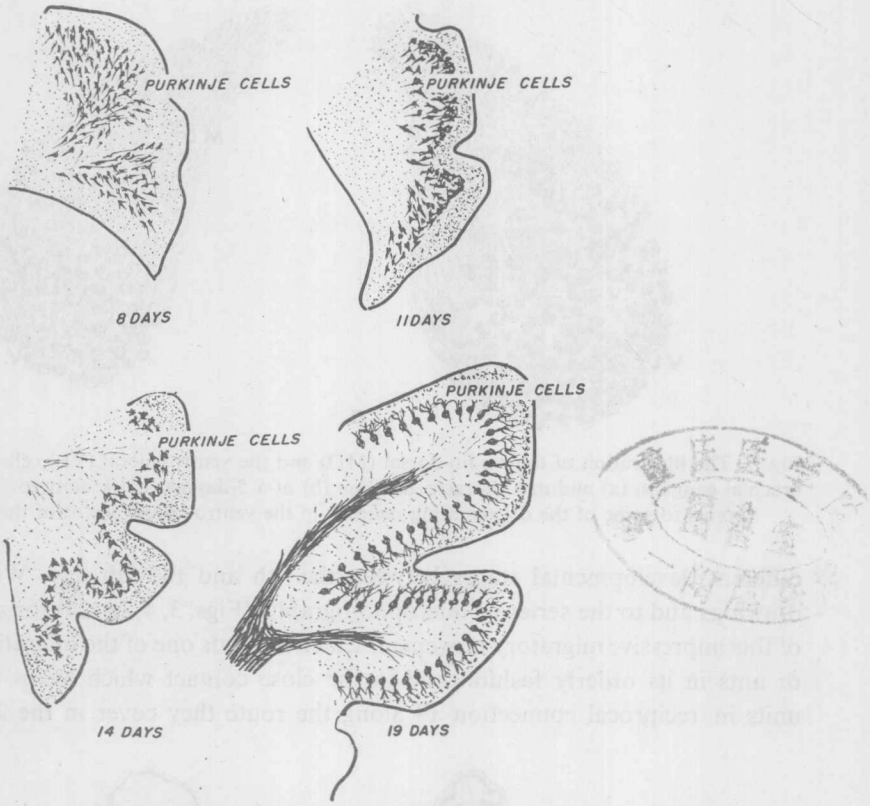

Fig. 6. Diagrammatic representation of the migratory movements of the Purkinje cells in the cerebellum of the chick embryo. (From The Nature of Biological Diversity, edited by Dr. J. Allen. Copyright, 1963. McGraw-Hill Book Co. Used by permission.)

connection with the primary sympathetic trunks which at this stage are still located in close apposition to the aorta. The residual $\frac{1}{4}$ sends fibers to the trunk muscles of the same level. Hence about $\frac{3}{4}$ of the motor nerve cells in the thoracic segment of the neural tube differ from the other $\frac{1}{4}$ in their peripheral distribution. From the above connections with the sympathetic chain ganglia they were identified as the preganglionic visceral cells. At $4\frac{1}{2}$ days these nerve cells sharply segregate from the other motor cells. The segregation of the two cell components is foreshadowed by a loosening of the compact texture of the motor column. Soon after, the $\frac{3}{4}$ of the entire group shift to a more medial position. Between the end of the 4th and the 7th day, the cells which have segregated from the residual $\frac{1}{4}$ of the motor column, move in compact cell rows in a ventro-dorsal direction. During the migration the cells are elongated

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in shape and exhibit the intense silver affinity characteristic of migrating nerve cells. It is thanks to the dark, almost black shade of the neuroblasts during their entire displacement that it is possible to inspect closely each of the thousand units during the subsequent phases of their migration. Since this process presents in fact identical features in all embryos, one can reconstruct it by examining embryos fixed at slightly

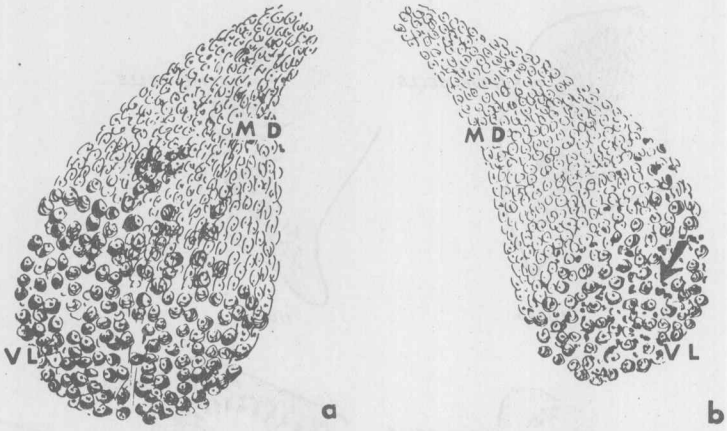

Fig. 7. The illustration of the medio-dorsal (MD) and the ventro-lateral (VL) cell populations in a brachial ganglion (a) and in a thoracic ganglion (b) of a 5-day-old chick embryo. The arrow in (b) points to some of the degenerating neurons in the ventro-lateral sector of this ganglion.

different developmental stages between the 4th and the 7th day. We refer to the drawings and to the series of microphotographs (Figs. 3, 4, 19–24) for a visualization of this impressive migratory movement which reminds one of the migration of termites or ants in its orderly fashion and in the close contact which keeps the individual units in reciprocal connection all along the route they cover in the $2\frac{1}{2}$-day period.

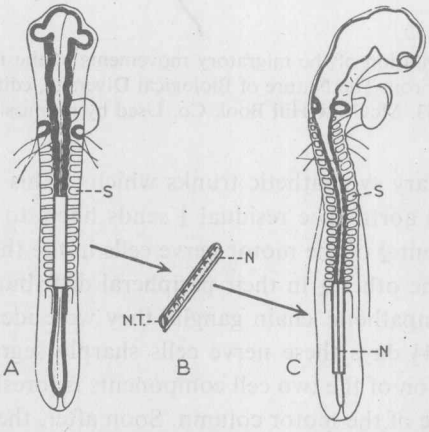

Fig. 8. The illustration of the transplantation of cervical spinal cord to the thoracic level. (A) donor with cervical spinal cord segment including the notochord removed; (B) transplant; (C) host with its thoracic segment removed and ready to receive the transplant. N, notochord; N.T., neural tube; S, somites. (From P. Shieh, *J. exp. Zool.*, **117**, 1951.)