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Do Oculomotor Neuroblasts Migrate across the Midline in the Fetal Rat Brain?

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Summary. Toluidine blue-stained semithin sections and Cajal-Castro preparations are used to study in rat fetuses whether oculomotor neuroblasts migrate across the midline at a certain period of development. In confirmation of previous studies, a group of oculomotor neuroblasts was detected which first grow cytoplasmic processes into the mesencephalic midline, and afterwards translocate their somata towards the midline, between the 12th and the 15th days of gestation. At this moment a midline mass of neuroblasts characterizes the meeting at this landmark of both left and right migrating neuroblastic groups. No crossing oculomotor axons yet are demonstrable with reduced silver techniques.

In further stages of development the neuroblasts continue their migration until they arrive at the contralateral nucleus at the 16th and 17th day of gestation. At the midline the mass of neuroblasts disappears gradually and crossed oculomotor axons become visible.

The electron microscope was then used to study ultrastructurally the migrating motoneurons. It was discovered that no preexisting structure guides their movement by contact. Their leading processes show no filopodial activity, and contain abundant microtubules and thick bundles of neurofilaments in eccentric position. The neuroblasts carry their axon across the midline as a trailing process.

Key words: Migration – Neuroblast – Oculomotor.

Introduction

Recent work (Puelles *et al.*, 1975), has given further support to the old statements of Biondi (1910) regarding the crossed migration of the oculomotor neuroblasts in the chick embryo. Using a modified Cajal-Castro silver impregnation, it was clearly shown that a portion of the oculomotor primordium migrates massively towards the midline, where the cells from both sides intermingle, and then reach the opposite side.

Before the migration across the midline is complete, no crossed oculomotor axons are demonstrable with the same technique. At subsequent stages of development, where the transitory midline cell mass disappears, crossed oculomotor axons become apparent.

The aim of the present investigation was first to study with the light microscope whether the same phenomenon occurred in the rat embryo, and subsequently to extend this study to the ultrastructural level; such a study was felt to be particularly necessary as information on the ultrastructural substratum of basal plate migrations is lacking (Levi-Montalcini, 1964); comparatively, alar plate migrations have been more extensively worked out (Rakic, 1971; 1972; Sidman, 1973; Das *et al.*, 1974). As a rule, it has been concluded that radially disposed astrocytic, ependymal or neuronal processes guide by contact the migrating cells.

Methods

Rat fetuses were obtained from anesthesized pregnant females in the 12th, 14th, 15th and 17th day of gestation (E12, E14, E15, E16, E17). Five or six fetuses from each litter were treated with the Cajal-Castro technique after 24 hours fixation in 60% pyridine. For electron microscopy, an equal number of fetuses were fixed by immersion in 2.5% glutaraldehyde in phosphate buffer. Different osmolarities were used. The best results were obtained with a 0.08 M buffer solution supplemented with 0.01% $CaCl_2$. Fixation lasted overnight at 4° C. After washing with the same buffer, dissected mesencephalic rings were postfixed in 2% osmic acid (2 or 3 hours) in the same phosphate buffer, washed, and in most cases block-stained with uranyl acetate. After dehydration in ethanol and a passage through acetone the blocks were embedded in Araldite. Semithin sections, 1 micron thick, were stained with toluidine blue in borax; ultrathin sections were stained on the grid with uranyl acetate and lead citrate. Several series of semithin sections were obtained in order to reconstruct the tridimensional extension of a few cells[1].

Results

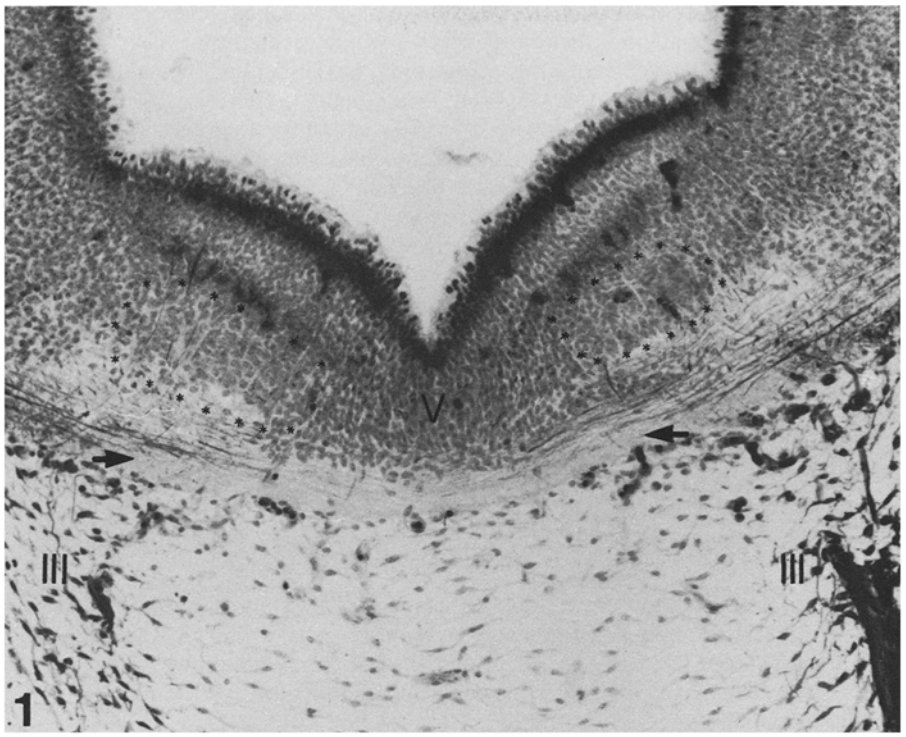
E12 Fetuses

In these embryos the mesencephalon appears as a thin-walled round vesicle, where neuronal differentiation has already started. In the tectum sizeable fascicles of nerve

[1] The terminology recommended by the Boulder Committee (1970) is used, with the only exception of the term "neuroblast", which is taken as synonymous of "immature neuron" of that terminology. We consider that the prefix *neuro-* overrides the connotation of "proliferating cell" attributed to the suffix *-blast*, because no immature neuron has yet been shown to be able to proliferate. Neuroblasts are therefore postmitotic; non-proliferating, immature cells which on various grounds are supposed to be differentiating into neuronal cells.

Fig. 1. E12 fetus. Transverse section through the oculomotor primordia. Cajal-Castro preparation counterstained with cresyl violet. Oculomotor primordia are outlined with a dotted line; ventricular zone (**V**), tectotegmental fibers (arrows) and the rootlets of the oculomotor nerve (**III**) are marked out. No mixing of oculomotor neuroblasts and ventricular cells has yet occurred. × 300

Fig. 2. E14 fetus. A comparison of this section with that shown in Fig. 1 reveals the development of the marginal zone; Arrow: light-staining transverse bundle of leading process within the ventricular zone. Notice the darkly staining small cells sorting themselves out of the ventricular zone into the marginal zone. × 400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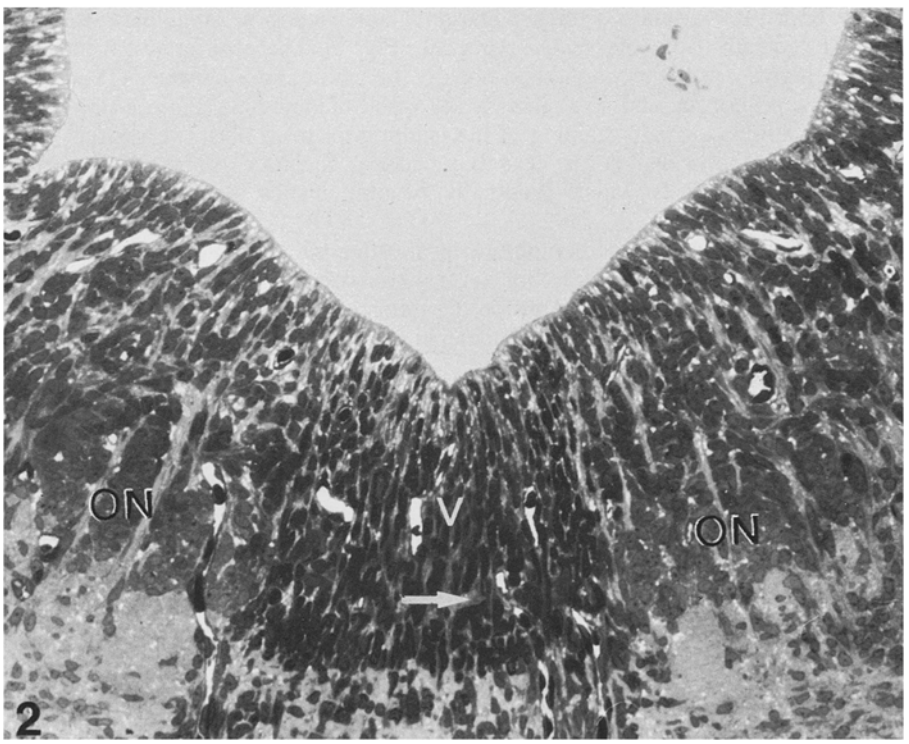
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fibers run in the transverse plane through the marginal zone. Development is more advanced ventrolaterally in the basal plates. where oculomotor neuroblasts accumulate. Immediately ventral to this primordium a well-defined medial longitudinal fasciculus separates it from the very few tectal fibers which already traverse the floor plate marginal zone. The different rootlets of the oculomotor nerve enter the mesenchyme fairly distant one from the other, clustering shortly thereafter. At the floor plate, the ventricular zone extends practically from the ventricular surface down to the external limiting membrane (Fig. 1). Neuroblasts of the oculomotor primordia have a round or oval light nucleus and little cytoplasm. Many darkly staining cells were found mixed with them in semithin preparations. This last type of cell was not found in older fetuses.

The enlarged ventricular zone which separates the oculomotor primordia medially from the ventral midline displays on its ventral border several strings of small dark cells which seem to be entering into the marginal zone (Fig. 1). The latter is occupied by transversely running axons, probably of tectal origin.

E14 Fetuses

Oculomotor primordia are clearly distinguishable at this stage. On semithin sections (Figs. 2, 3, 4) they appear as consisting only of cells with light cytoplasm and nucleus. These cells are tightly packed, and the whole population is subdivided into two or three cellular groups by the passage across the nucleus of blood vessels and fascicles of radially oriented ventricular cell processes.

The floor plate displays a thicker marginal zone, and more small dark cells have detached ventrally from the ventricular zone (Fig. 2). The proliferating ventricular cells which give rise to this population occupy the whole space between left and right oculomotor primordia, and also stain darkly with toluidine blue (Fig. 2). In toluidine blue preparations, the light staining of the oculomotor neuroblasts versus the darker shade of all other cells, at or near the midline, facilitate the tracing of those oculomotor neuroblasts which eventually become displaced from their original primordium.

At the caudal pole of the oculomotor primordia, all the oculomotor neuroblasts are still confined to the anlage, but several transversely oriented bands of clear cytoplasmic processes are found inside the midline ventricular zone. These can be shown to originate in the oculomotor nuclei in serial semithin sections (Fig. 3). These cytoplasmic processes must be fairly long, as some of them may be seen crossing the midline at this stage. The course of each process within the fascicles is sinuous. Even at low magnification, the clear bands traversing the ventricular zone have a wavy appearance (Fig. 3). These cytoplasmic processes were never seen contacting the floor plate marginal zone; two or three cell layers of ventricular or small dark cells were always found in-between.

Rostral levels show more advanced stages of this growth of the oculomotor nucleus towards the midline: there, the same bands of optically light processes are found crossing the midline inside the ventricular zone; in a triangular area whose base is in the oculomotor nucleus and whose apex is at the midline zone occupied by these light processes, cell somata with the same staining properties as the oculomotor neuroblasts are seen interspersed with ventricular cell somata (Fig. 4). These lightly stained somata have their long axis transversely oriented, whereas the ventricular

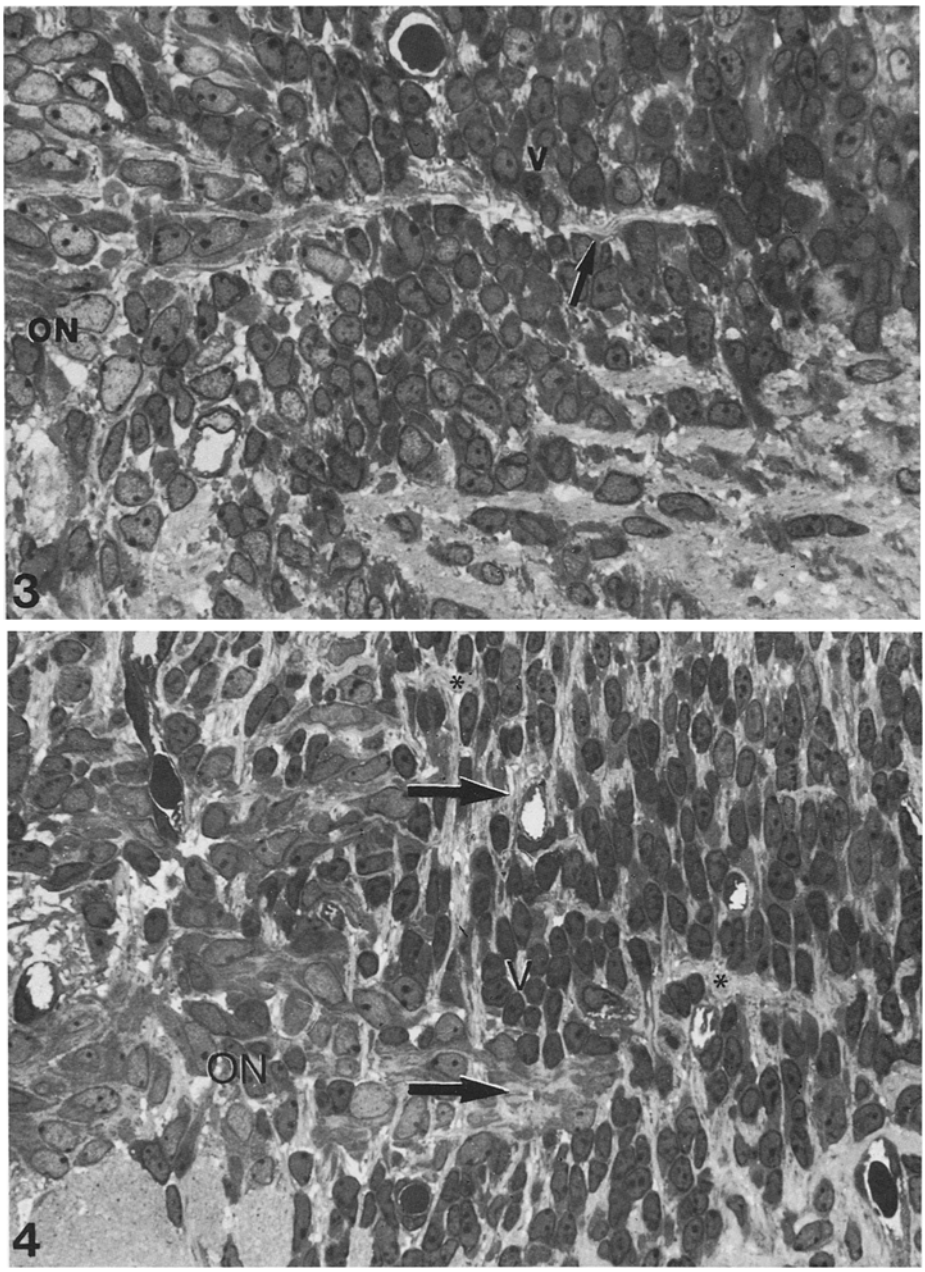


Fig. 3. E14 fetus. Caudal level. A higher magnification of border zone between oculomotor nucleus (**ON**) and ventricular zone (**V**) reveals the outgrowth of light-staining cytoplasmic processes of oculomotor neuroblast medially into the midline ventricular zone (arrows). Notice that these are not in contact with the floor plate marginal zone. ×800

Fig. 4. E14 fetus. Rostral level. The border between the oculomotor nucleus (**ON**), and the ventricular zone (**V**) is seen to be crossed by numerous light-staining neuroblasts. Two zones of massive penetration are shown in this figure (arrows). Several bundles of light staining leading processes are also evident (asterisks), at right angles to the radial structure of the ventricular zone. × 800

cells are always perpendicular to the floor plate. We assume that these cells are migrating oculomotor neuroblasts[2].

As only an occasional migrating oculomotor neuroblast was found approaching the midline, these fetuses were thought to provide the most useful information on the ultrastructure of these cells: first, they are identified in ultrathin sections as migrating oculomotor neuroblasts thanks to their location medial to the oculomotor primordium; second, as none of these neuroblasts has crossed the midline, the medial extension of each one of them represents the leading process, and their lateral extension, the trailing process. It is usually more difficult to ascertain the polarization of each cell at later stages, when right and left populations mix together at the midline.

In the following ultrastructural description the denomination "leading process" refers to the medially oriented cytoplasmic outgrowth of the migrating oculomotor neuroblasts. Like other immature neurons, the migrating oculomotor neuroblasts are fusiform, have an oval or elongated nucleus with dispersed chromatin and a clear nucleoplasm, and display a high concentration of free polyribosomes in the cytoplasm (Figs. 5, 6, 7, 8). The nucleus lies eccentrically in the cytoplasm. At variance, the smaller nuclei of the ventricular cells show a more irregular outline, with occasional deep notches, dense chromatin aggregates along the nuclear membrane, and a denser nucleoplasm (Figs. 5, 12a). Mitochondria, rough endoplasmic reticulum and especially the Golgi apparatus are well developed in the oculomotor cells. Occasionally, multivesicular bodies, centrioles and dense core vesicles were also found. Extending from the trailing process to the leading process, neurotubules form a lattice of variable density (Figs. 5, 8). Neurofilaments are scarce in the soma, but increase in number in the trailing process, assumed here to be the axon, as indicated by the Cajal-Castro preparations. In the axon, the polyribosomes adopt a position just below the axonal membrane. The axonal neurotubular skeleton continues into the perinuclear cytoplasm as an intraperikaryal fascicle exactly as described by Hinds and Ruffett (1973) in the olfactory bulb mitral cells. Puncta adhaerentia were often observed between adjacent migrating cells or cell processes. Various stages in the formation of dense coated pinocytotic vesicles were also encountered.

Incipient leading processes appear as simple cytoplasmic extensions whose neurotubular elements are longitudinally oriented towards the mesencephalic midline, and whose tips contain a more or less dense filamentous lattice with or without smooth endoplasmic reticulum vesicles (Figs. 6, 7, 8).

Longer leading processes show a marked increase in the neurotubular formation. The whole area between the leading pole of the cell soma and the growing tip appears filled uniformly with neurotubules. Mitochondria and endoplasmic reticulum vesicles are seen interspersed along the whole leading process. The leading pole of the cell soma is always thicker than the trailing pole, and it is particularly rich in mitochondria and Golgi flattened vesicles (Fig. 7). Neurotubules are not as uniformly stacked at this level as distally in the leading process. The width of the leading process diminishes progressively, and a gradual tapering away of the neurotubules leads to the filamentous lattice of the growing tip of the leading process. No filopodia were detected. The leading processes each end up with a fingertip-like profile (Figs. 6, 7, 8).

[2] For simplicity's sake we shall speak of migrating oculomotor neuroblasts, anticipating our conclusions, whenever cell somata with staining properties equal to those shown by oculomotor neuroblasts are detected within the midline ventricular zone. The proper logical fundamentation of this terminology is to be found in the discussion.

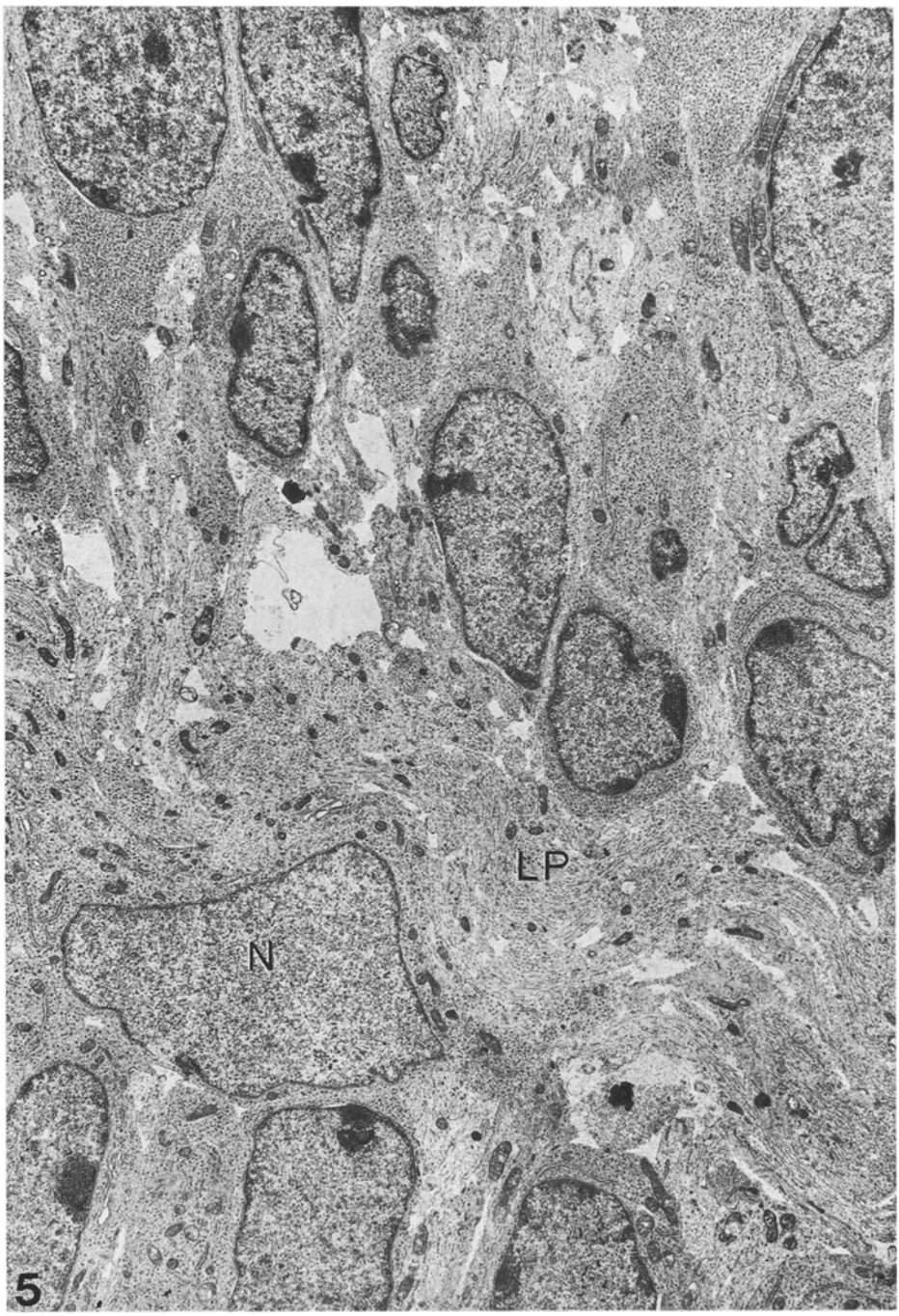


Fig. 5. E14 fetus. This transverse ultrathin section shows a migrating oculomotor neuroblast (**N**) surrounded by a bundle of leading processes (**LP**) at right angles to the radially oriented ventricular cells of the floor plate. Notice the wavy appearance of the leading processes and their microtubular cytoskeleton. × 4,000

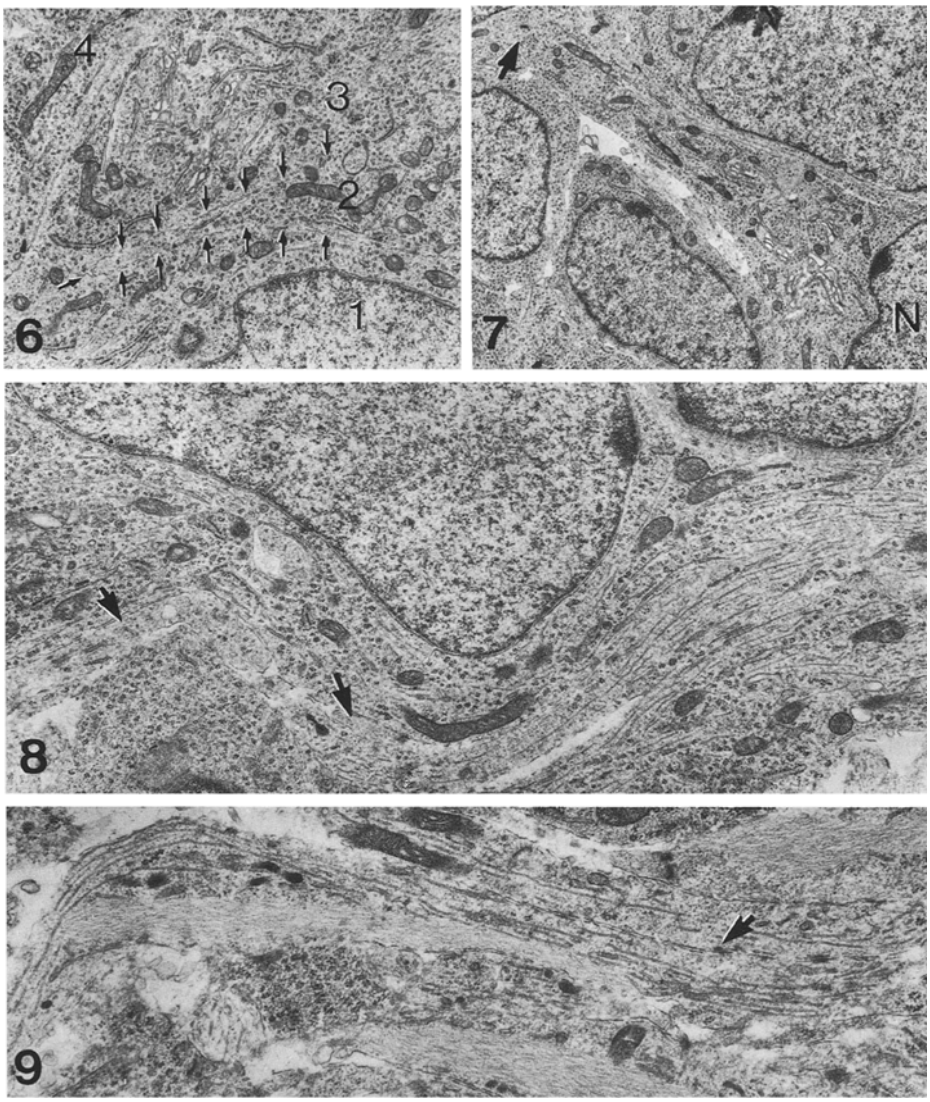


Fig. 6. E14 fetus. At the less-developed caudal pole of the oculomotor nucleus neuroblasts with an incipient leading process are present. These end up as a thin tip containing a filamentous matrix (arrows). No specialized contacts exist between the tightly packed oculomotor cells (**1, 2, 3, 4**). × 9,000
Fig. 7. E14 fetus. Caudal level. At the medial border of the oculomotor nucleus one of the neuroblasts (**N**) exhibits a broad incipient leading process in continuity with the organelle-rich pole of the cell. An attachment plate (arrow) links it with another neuroblast. × 5,400
Fig. 8. E14 fetus. This figure illustrates the morphology of one of the migrating oculomotor neuroblasts, which has already entered the ventricular zone, at a rostral level. It displays a sinuous outline. At the right lies the microtubule-rich trailing process (assumed to be axonal) whereas at the left is the organelle-rich leading pole of the soma. Just below the latter, note the fingertip-like endings of other leading processes. (arrows). × 8,400
Fig. 9. E14 fetus. Rostral level. Detail of a bundle of leading processes crossing the ventricular zone. They exhibit both microtubules and fascicles of thin microfilaments. The latter are disposed just beneath the cell membrane. There is a tiny attachment plate between two leading processes (arrow). × 16,000

In a small number of leading processes, but in all our preparations, we found a thick bundle of filaments localized eccentrically in the leading process (Fig. 9). Those segments of leading process which displayed the bundle of filaments had only very few neurotubules adjacent to the bundle. The rest of the cytoplasm at this level consists of a granular matrix with some smooth vesicles, mitochondria and occasional polyribosomes. These filamentous portions of the leading processes could generally be shown to continue on both sides with other portions with abundant neurotubules. In favourable cases (Fig. 9) the filamentous bundle was found to extend between both arms of one of the sinuous curves which leading processes trace on their course, in the fashion of the chord of an arc. This structure, not described till now, to our knowledge, in neuroblasts migrating *in vivo*, may be the equivalent of the cortically located "sheath microfilamentous bundles" believed to be involved in the contractile phase of cell locomotion *in vitro* (Luduena and Wessells, 1972).

Individual leading processes are stacked together constituting the clear transverse bands found across the midline ventricular zone in semithin sections (Figs. 3, 4, 5, 9). Puncta adhaerentia and pinocytotic dense coated vesicles are often found along their apposed membranes.

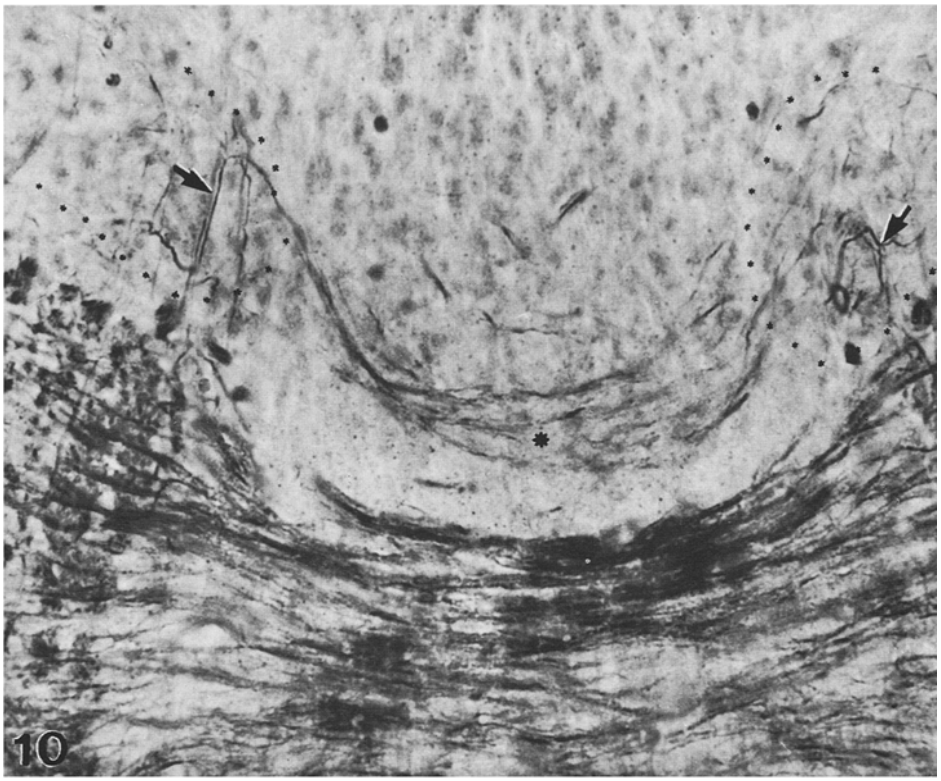
Finally, it remains to be said that fingertip-like profiles, filled by an amorphous filamentous lattice, were detected at this stage of development between the packed-up perikarya in both oculomotor primordia. Although we have no useful serial sections to demonstrate that these growing tips belong to the contralateral oculomotor migrating neuroblasts, we hold this possibility to be the most likely one. They proceed from the same general direction, and we know that some of the leading processes have already crossed the midline.

E15 Fetuses

In these embryos the occurrence of a midline cellular tangle of transversely oriented fusiform neuroblasts, as typically observed in chick embryos, indicates the meeting at the mesencephalic ventral midline of both migrating oculomotor populations (Figs. 10, 11). The oculomotor migrating neuroblasts have an elongated nucleus with very clear karyoplasm (Fig. 11). At the midline it is now difficult to distinguish the clear bands of leading processes. On the lateral borders of the midline cellular mass, strands of nerve fibers diverge towards the oculomotor anlage. In material impregnated with the Cajal-Castro technique those fibers can be shown to be axons. On reaching the oculomotor nucleus they sharply change their course and enter the rootlets of the oculomotor nerve (Fig. 10).

The migrating cells do not contact at this stage, or at any other, the transversely running fascicles of the floor plate marginal zone (Fig. 10). Inside the ventricular zone, the neuropil is very complex, due to the accumulation of oculomotor neuroblasts, leading processes, trailing axonal processes, blood vessels and ventricular cell somata with their radial processes (Fig. 11). The peripheral processes of the ventricular cells are now detectable in semithin sections as vertical clear bands of translucent fibers, which also follow regular sinuous incurvations along their passage across the midline mass of migrating neuroblasts (Fig. 11).

With the electron microscope, migrating oculomotor neuroblasts are seen apposed one to the other in the midline mass. The only differentiation observed along the contacting surfaces is an occasional punctum adhaerens. The cells are ultrastructurally similar to those described on the 14th day of gestation. Cytoplasmic organelles still are most abundant in the leading pole of the cells; a distinct decreas-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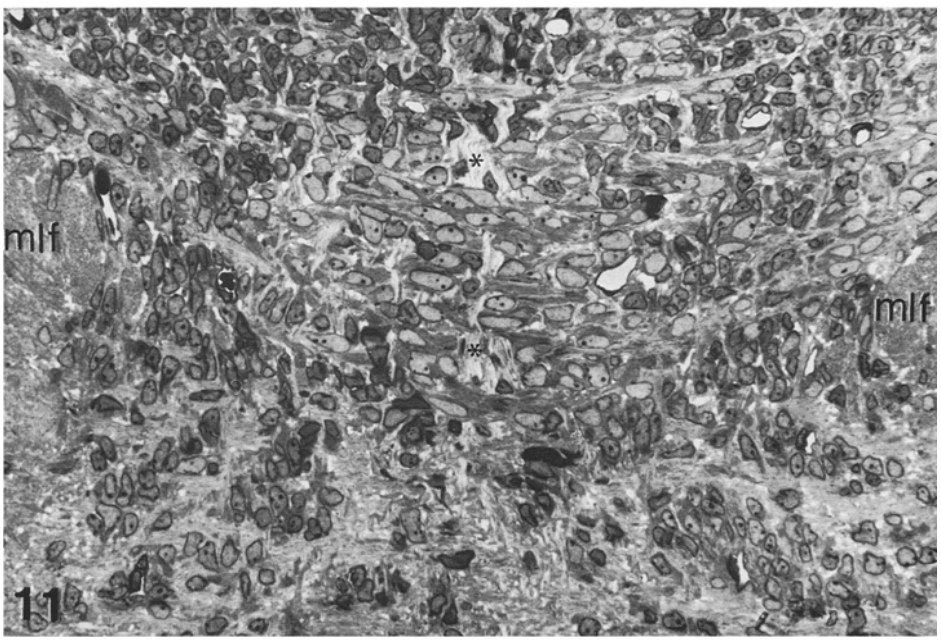
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ing gradient towards the trailing pole of the soma is evident in the concentration of polyribosomes (Fig. 12a). Both leading and trailing processes are rich in neurotubules, but the former are much wider due to the accumulation of organelles and cytoplasmic matrix (Fig. 12a).

Those leading processes which could be followed for a certain distance showed the characteristics already described. Eccentric bands of filaments were also present in some of these processes.

E16 Fetuses

At this stage of development the midline neuroblastic tangle has lost its compact configuration. Both in semithin (Fig. 13) and in Cajal-Castro preparations (Fig. 14), the oculomotor nuclei have a more mature aspect. Efferent axons course in an oblique ventrolateral direction. The oculomotor nuclei are still connected across the midline by several small groups of light staining neuroblasts. The ventricular zone at this level has thinned, but small dark cells are still seen at the interface between ventricular and marginal zones (Fig. 13).

The axonal processes of the oculomotor neuroblasts which have already completed their migration now constitute the crossed projection of the oculomotor nuclei. The complexity of the midline neuropil makes it difficult to follow these axons individually in semithin or ultrathin sections. However, in Cajal-Castro preparations the crossed axons are clearly evident (Fig. 14).

At the ultrastructural level, postmigratory cells, on arriving at the contralateral nucleus, are found to lose their characteristic fusiform shape and their rich neurotubular content. In fact, they become indistinguishable from those oculomotor cells which did not participate in the migration, and never left their original primordium. The rough endoplasmic reticulum becomes prominent in the cytoplasm while mitochondria, polyribosomes and Golgi vesicles are still abundant. We often detected a pair of centrosomes and sometimes a cilium. Leading cell processes are gradually transformed into dendritic processes (Fig. 15), this latter change is marked by a more translucent matrix, more dispersed neurotubules, mitochondria and endoplasmic reticulum, and polyribosomes which gradually become attached to the canalicular system. Eccentric bands of neurofilaments also are present in this material. No synapses are detectable at this stage in the oculomotor nucleus.

E17 Fetuses

An essentially adult configuration of the oculomotor nuclei was observed in this group of embryos. Oculomotor neurons are bigger and rounder than before. Very

Fig. 10. E15 fetus. This Cajal-Castro preparation shows the presence of a midline mass of neuroblasts (asterisk) which laterally is related to oculomotor axons (arrows). Note the separation between these cells and the tectal fibers occupying the marginal zone. The outline of the oculomotor nucleus is marked with a dotted line. × 700

Fig. 11. E15 fetus. In a toluidine blue preparation the midline between both oculomotor nuclei is occupied by the mass of migrating neuroblasts. At right angles to the predominantly transverse orientation of these cells, radially coursing clear processes of ependymal cells are evident (asterisks) (**mlf**: medial longitudinal fascicle). × 490

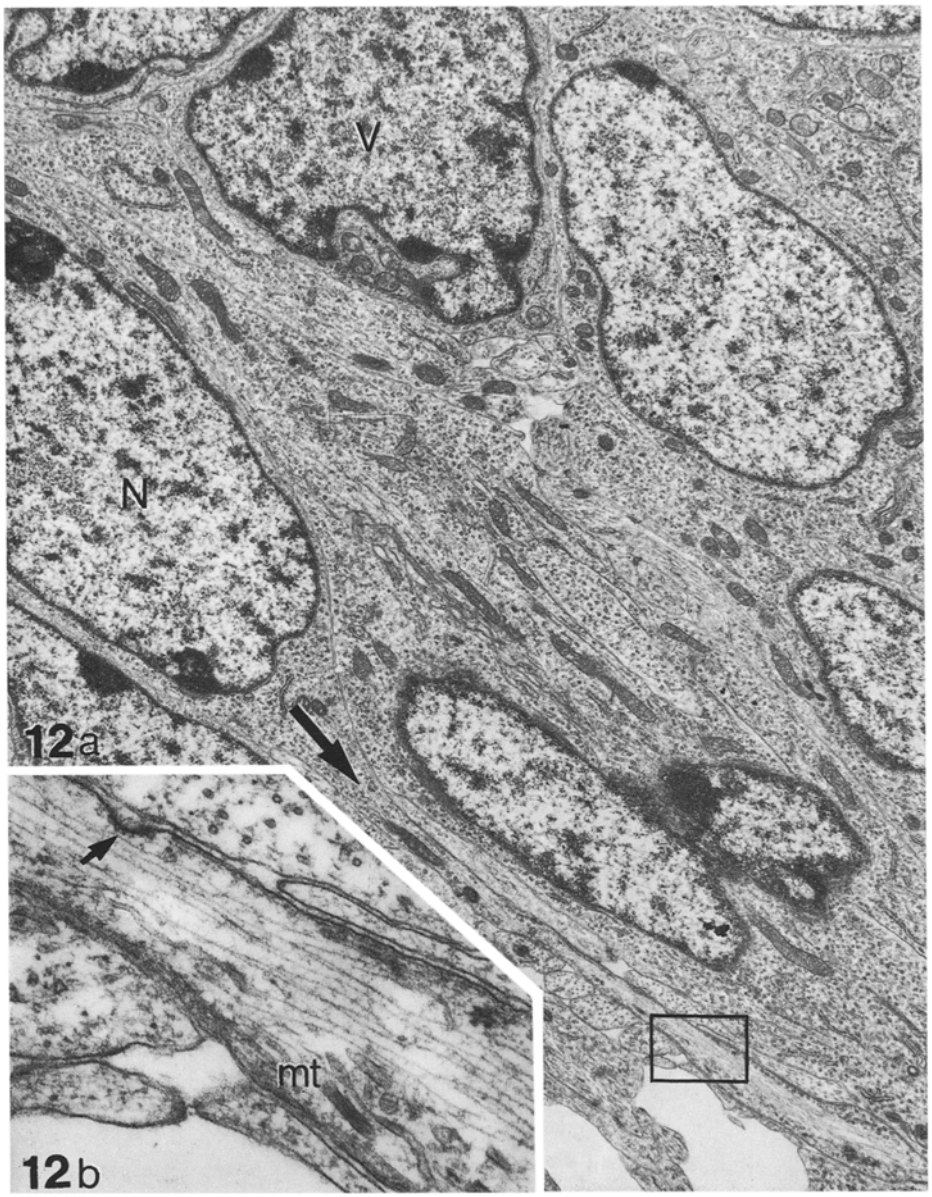
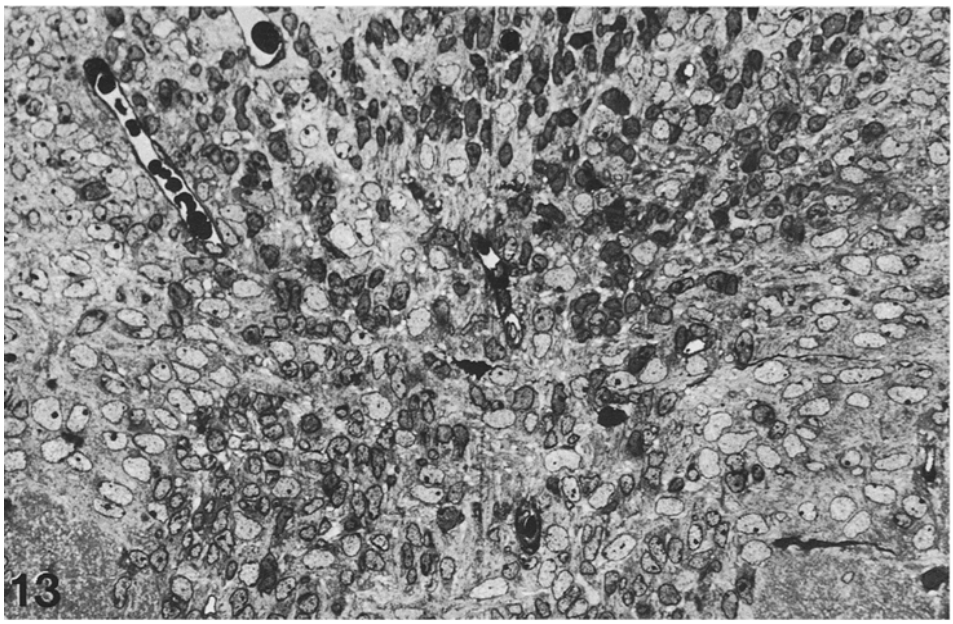


Fig. 12 a. E15 fetus. A migrating oculomotor neuroblast (**N**) within the midline cellular mass exhibits a typical axonal trailing process (arrow), which appears as a regular cylinder containing mostly neurotubules and microfilaments. Notice the organelle-poor trailing pole of the cell and the different nuclear characteristics of oculomotor and ventricular cells (**V**). × 10,000. **b** Enlargement of the framed area of Fig. 12a. There is some evidence of undercoating of the plasma membrane, as well as fasciculation of microtubules (**mt**) and an initial stage in the formation of a coated pinocytotic vesicle (arrow). × 46,000



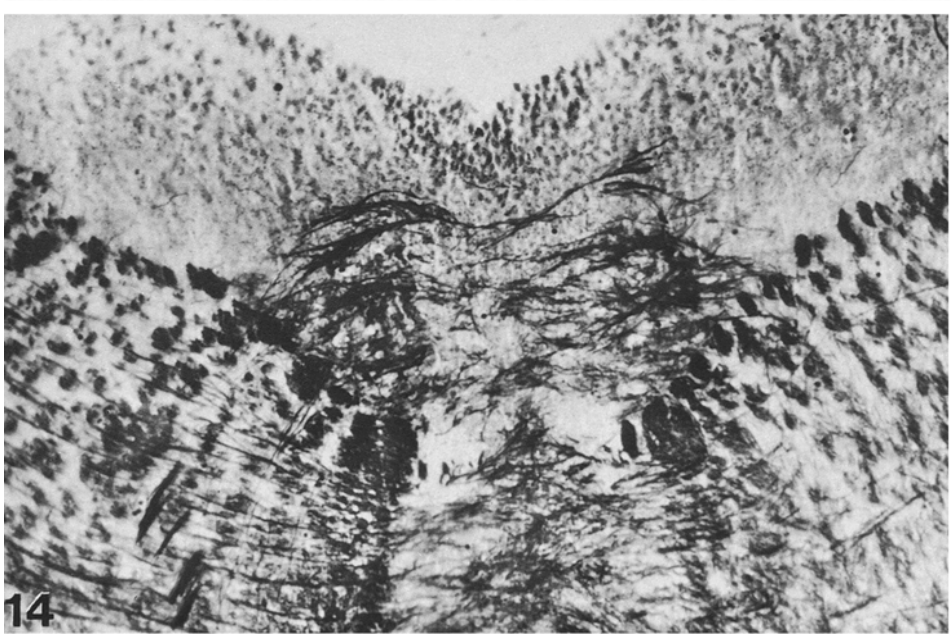


Fig. 13. E16 fetus. A small number of oculomotor neuroblasts remain at the midline, where the neuropil shows a more mature appearance. The nuclear characteristics of ventricular cells and oculomotor neuroblasts are still very distinctive. × 375

Fig. 14. E16 fetus. In Cajal-Castro preparations many migrating oculomotor neuroblasts have crossed to the other side, so that crossed oculomotor axons now become evident. Most of these neuroblasts still have a fusiform configuration; some of them are still seen near the midline. Compare this figure with Fig. 10. A separation between the oculomotor crossing elements and the marginal layer is still evident. × 150

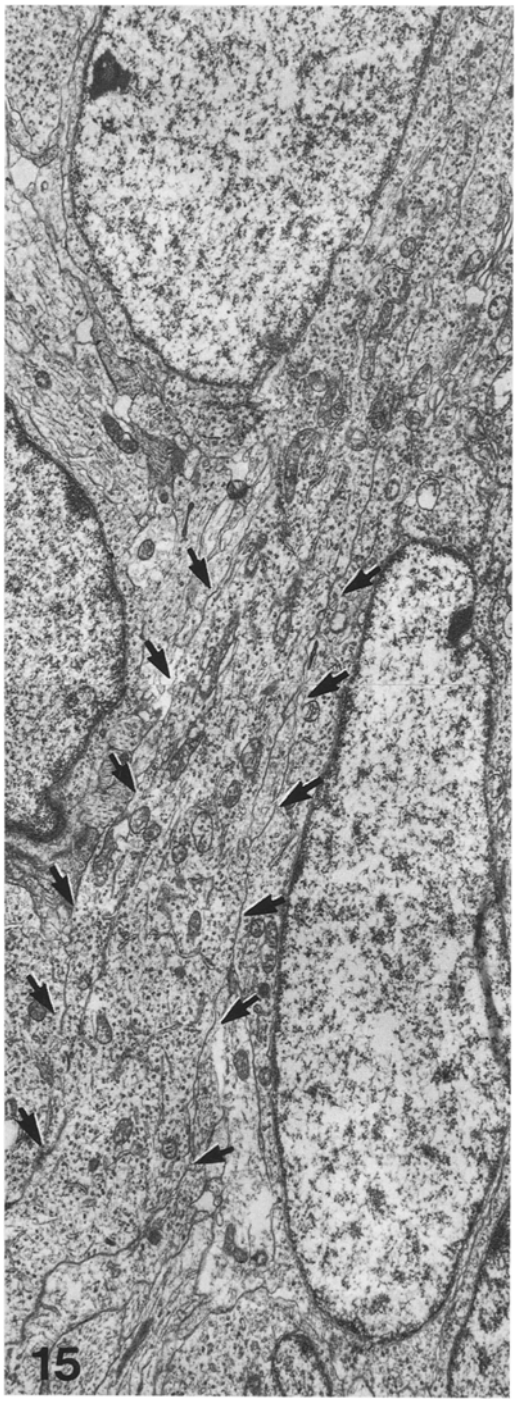


Fig. 15. E17 fetus. This figure shows a migrating oculomotor neuroblast approaching the contralateral nucleus, as found in E16 and E17 fetuses. The nucleus lies in an eccentric position. The leading process displays a less dense microtubular array, indicating its gradual transformation into a dendrite. × 8,400

few cells remain near the midline. Cajal-Castro preparations show that many cells already possess two or three dendrites. The crossed axons are clearly evident (Fig. 16).

With the electron microscope, dendritic configurations appear more numerous in the neuropil (Fig. 15). However no typical synaptic structure was detected.

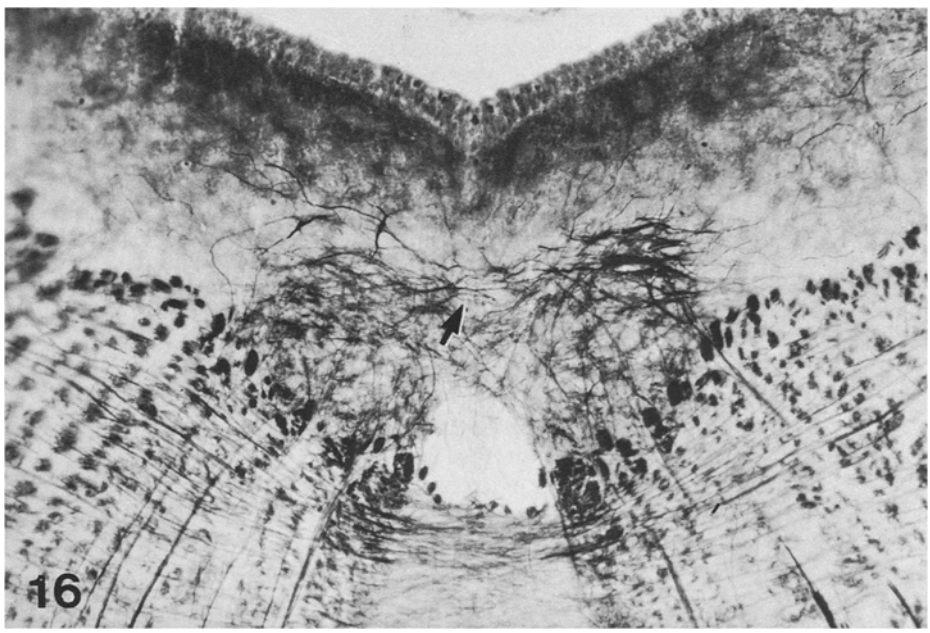


Fig. 16. E18 fetus. On this Cajal-Castro preparation oculomotor axons are seen crossing the midline (arrow) with an adult configuration. × 150

Discussion

It is the thesis of the present investigators that the temporal series of morphological changes observed at the mesencephalic midline finds a simple and total explanation in the hypothesis of the migration across the midline of oculomotor neuroblasts. Every other hypothesis considered by us either does not explain all the reported observations or beomes unnecessarily complex in the attempt to do so.

Throughout the close series of embryos (E12 to E17 fetuses), the following invariant parameters related to the studied phenomena were detected:

A) Oculomotor nuclei anlagen, medial longitudinal fasciculi, ventricular and marginal zones mutually display a constant topological relationship and represent the environment of the observed phenomena (Figs, 1, 2, 10, 11, 13, 14, 16).

B) Cells identified as neuroepithelial ventricular cells because of their location within the ventricular zone, their radial disposition and their small dimensions, constantly appeared darkly stained in semithin Araldite sections stained with toluidine blue (Figs. 2, 3, 4, 11, 13).

Two variables become apparent throughout this same series: the *location* of light staining somata and cellular cytoplasmic processes in toluidine blue-stained semithin

sections, particularly in relation to the midline sagital plane, and, secondly, the *presence* of oculomotor axons across the midline, as detected in Cajal-Castro preparations.

The successive "values" adopted by the first variable are as follows: E12 fetuses – Some oculomotor neuroblasts have grown light staining cytoplasmic processes across the topological boundary between oculomotor primordium and ventricular zone. These processes point towards the midline.

E14 fetuses – light cytoplasmic processes laterally related to the oculomotor primordia cross now at several depth levels the midline ventricular zone (Figs. 2, 3, 4). Several light staining somata are detected within the ventricular zone, lying transversely within the bands of light cytoplasmic processes. Their number decreases in a lateromedial gradient (Figs. 2, 4).

E15 fetuses – A great number of transversely oriented light staining somata occupy a considerable space within the midline ventricular zone (Fig. 11).

E16 fetuses – Transversely oriented light staining somata are not as numerous and packed together at the midline as in the previous day (Fig. 13).

E17 fetuses – Only very few light staining somata remain within the midline ventricular zone.

The second variable (silver impregnated oculomotor axons) evolves temporally as follows:

E12, E14 fetuses – Oculomotor axons can be followed centrally from the rootlets to the oculomotor primordium (Fig. 1).

E15 fetuses – Many oculomotor axons can be followed centrally across the oculomotor primordium, where they suddenly incurvate entering the lateral border of the midline cellular mass within the ventricular zone (Fig. 10). Most of these axons, however, do not cross the midline.

E16, E17 fetuses – Many oculomotor axons are seen crossing the midline within the ventricular zone (Figs. 14, 16).

The interpretation of these facts centers on the transitory existence of the midline cellular mass. These cells either have been generated *in situ*, or they have come from elsewhere. The local generation hypothesis finds the following difficulties: a) no intermediate cells are detected between the small, dark, radially disposed ventricular cells and the large light, transversely disposed somata of the midline mass (Figs, 2, 3, 4, 11); b) it is not evident why young postmitotic cell should differentiate within the ventricular zone, adopting a transverse disposition, when normally such cells sort themselves out of the ventricular zone entering the peripheric layers of the neural wall; c) young postmitotic cells (small and darkly staining) actually do sort themselves out of the midline ventricular zone and enter the marginal zone (*cf.* Figs. 1 and 2).

If the midline transverse cells come from elsewhere, the observations on E12 and E14 fetuses point strongly to an origin in the oculomotor primordia (Figs. 3, 4). These same observations constitute accessory problems for any hypothesis which rejects an oculomotor migration. In this sense it is to be noted that our ultrastructural findings exclude an identification of the light staining leading processes as growing axons.

The simplest hypothesis, therefore, is that oculomotor neuroblasts initiate the growth of leading processes towards the midline in the 12th day of gestation and subsequently translocate the cell nucleus and the perinuclear cytoplasm medially within these processes. In the 15th day of gestation, the right and left populations meet at this midline. An oculomotor migration towards the midline seems evident.

The disappearance of the midline mass in the E16 and E17 fetuses might be accounted for in three ways: a) the oculomotor neuroblasts continue their migration, crossing to the other side; b) their number is drastically reduced by cell death; c) the neuroblasts migrate again, but *backwards* towards their original primordium.

Cell death phenomena are not observable in our light and electronmicroscopic material.

The findings in Cajal-Castro preparations on E15 fetuses indicate that the migrating oculomotor neuroblasts already possess an axon when they arrive at the midline (Fig. 10). In these circumstances, a backwards migration to the initial side, apart from being bizarre, would not explain the formation of the oculomotor commissure in the following days of gestation.

The foregoing considerations finally lead us to the conclusion that the simplest interpretation of our results is that oculomotor neuroblasts migrate between the 12th and 17th days of gestation across the mesencephalic midline, laying down the oculomotor commissure with their trailing axonal processes. This migration occurs within the floor plate ventricular zone, without any contact with the marginal zone.

Together with the descriptive work we attempted to characterize radioautographically the oculomotor migration. This approach, however, entailed considerable difficulties of interpretation due to the fact that the migrating cells cross a zone which itself displays proliferative activity.

In the literature, the only papers which have supported the migration are those of Biondi (1910), Bok (1915), Hogg (1961) and Puelles *et al.* (1975). Recently obtained Golgi impregnations of chick embryos confirm the results given by the Cajal-Castro technique (Puelles, in preparation). Only Hogg's work describes rat fetuses, and, being centred on other issues, does not place much emphasis on the oculomotor migration. On the other hand, Tello (1923), Mann (1927), Windle (1932, 1933), Windle and Baxter (1936), Windle and Austin (1936), Zahajszky (194), Cooper (1946), Niimi *et al.* (1959) and Cowan and Wenger (1968) do not report oculomotor migratory phenomena. A study of these papers reveals that none of these authors expected or even considered the possibility of a migration. Biondi's 1910 paper, in Italian, possibly was not known to them, although Kappers, Huber and Crosby (1960) mention it (including figures) in their treatise. Mann (1927), Zahajszky (1940) and Cooper (1946), who specifically studied oculomotor development, did not use a sufficiently close series of fetuses, and thus possibly failed to detect the intermediate stages of the migration. Other authors, using conventional Nissl staining techniques, generally identified the embryonic midline cellular mass with the adult midline nucleus of Perlia. The identity of this nucleus as an oculomotor motoneuron pool seems doubtful in the light of recent experimental work (Gacek, 1974; Naita *et al.*, 1974). The studies of Tello (1923) and the group of Windle, being of wider scope, do not properly examine the oculomotor material.

In our electron microscopic observations we have dedicated special attention to the ultrastructure of the leading processes, considering these structures to be the possible sites for the mechanism and directional control of the neuroblastic migration. Leading cell processes of migrating oculomotor neuroblasts are thicker than growing axons, and do not have a terminal enlargement with filopodia as the latter do. Their ultrastructural characteristics are similar to those described in migrating cortical neuroblasts (Butler and Caley, 1972; Peters and Feldman, 1973; Rakic, 1972), although their cytoplasm and organelles seem to be more abundant and the nuclear ultrastructure more mature. It is plausible that the more mature aspect rests on the fact that oculomotor migrating neuroblasts already have grown on axon

towards the periphery. The fingertip-like ending of the leading processes is filled by a microfilamentous lattice with few or no organelles. Luduena and Wessells (1973), in tissue culture studies, correlated this configuration with the extension phase of cellular locomotion.

The density of neurotubules, which are known to facilitate intracytoplasmic transport (Schmitt, 1966), and the existence of a gradient of organelles throughout the cell soma and leading process, partially support the assumption that there exists an intense transport of cytoplasm and organelles from the soma towards the leading process. A similar abundance of neurotubules was observed by Rakic (1971) in the leading processes of migrating cerebellar granule cells in developing monkeys.

The occasionally detected fascicles of filaments within the leading processes are more difficult to explain (Fig. 9). Considering their morphology and the present-day knowledge on the biology of such cytoplasmic structures in various systems *in vitro*, we estimate that they cannot be other than contractile formations. (Wessells *et al.*, 1971.) They are strikingly similar to the "sheath filamentous fascicles" described by Luduena and Wessells (1973) in tissue cultures of dorsal root ganglia; those filamentous fascicles present in that *in vitro* system bound heavy meromyosin and were assumed by the authors to be responsible for the contractile phase of cellular locomotion. There is no indication that the fascicles detected by us can be artifactual in nature (Fig. 9). Their presence in only a small proportion of the leading processes may be due to their lability, or possibly to their extreme eccentric position within the processes, so that only nearly tangential sections show them. If true, this circumstance may induce other investigators to search in serial sections for these filamentous fascicles in other migrating neuroblasts. The fact that such filaments exist *in vivo* in actively migrating neuroblasts of the central nervous system would support Luduena and Wessels' (1973) assumption of a locomotive nature of these cytoplasmic structures. We conclude here, that both intrinsic mechanisms – the "filling up" of the leading process through neurotubule action, and the contractile activity of the filament fascicles – may account for the motile force which advances the migrating neuroblast into the midline ventricular zone.

Apart from the motile force of the migration, guidance mechanisms need to be considered: it is remarkable that leading processes are always perfectly oriented towards the midline; in fact, they do not use the theoretically easier pathway at the interface between the ventricular and marginal zones, where abundant crossing axons (possibly tectotegmental fibers) provide directional guidelines. Migration occurs straight across the ventricular zone, as if selecting the shortest way (Figs. 3, 4).

Migrating oculomotor neuroblasts clearly display contact relationships between themselves. Most cells contact at least another migrating cell as seen in the E14 and E15 fetuses. The puncta adhaerentia are the only specialized contacts present. Coated pinocytotic vesicles are also frequent. The mutual help between fasciculated leading processes, however, does not fully explain the guidance of the whole migrating population. Indeed, we observed in the E14 material that there were several bundles of leading processes (Fig. 4). Assuming that there is at least one leading cell at the font of each group of leading processes, each one of them may guide a number of the following cells thanks to their mutual contacts and their trailing axonal process. As no pre-existing fibrillar structure whatsoever has been found some other non-fibrillar guiding mechanism must be active at the mesencephalic midline, at least to orientate the foremost leading cells. In contrast to the observations published on cortical migrations (Rakic, 1971, 1972; Sidman and Rakic, 1973) our results indicate that some types of neuroblasts (in this case immature

motoneurons) are able to migrate at right angles to the predominantly radial organization of their microenvironment.

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