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EARLY DEVELOPMENT OF THE RAT PRECEREBELLAR SYSTEM: MIGRATORY ROUTES, SELECTIVE AGGREGATION AND NEURITIC DIFFERENTIATION OF THE INFERIOR OLIVE AND LATERAL RETICULAR NUCLEUS NEURONS. AN OVERVIEW.

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Last year, Neuroscience lost one of its most outstanding investigators, Professor Alf Brodal. For those of us, working with morphological tools in the field of the Development of Cerebellar Systems, Professor Brodal has not only been a distinguished pioneer, but a remarkable guide and example. The senior author was invited, for his first international meeting, to the 1965 Summer School of Brain Research in Amsterdam, devoted to "The Cerebellum". This chance was the opportunity to meet Professor Brodal, and to hear his splendid review on "Anatomical Studies of Cerebellar Fibre Connections with Special Reference to Problems of Functional Localization" (13). I was fascinated by the clearness and modesty of the presentation. I really believe that my interest in the organization of cerebellar systems was reinforced at that meeting. Two months later, I started my postdoctoral training with Professor Sanford L. Palay on the synaptic organization of the lateral vestibular nucleus. There I spent a delightful time reading Professor Brodal's papers on the vestibular systems. In a sense, as it is the case for many other neuroscientists, I consider myself one of his students, and I am most grateful. During these last 20 years, I had several occasions for discussions with him (not as often as I would have liked). I was always surprised by his knowledge and interest in the work done by younger investigators, and I will never forget his kindness and encouragements.

INTRODUCTION

The amazingly complex architecture of the adult CNS is elaborated through a sequence of developmental events that begins with cell proliferation in the neuroepithelium and ends with synaptic stabilization of the neuronal circuits.

After leaving the mitotic cycle, most if not all the young neurons undergo a phase of migration which leads them to their ultimate domain. In most instances, this latter is located near the primitive neuroepithelium. The formation of neuronal connections is then almost entirely due to axonal elongation, the axonal growth cone being guided along its pathway by positional cues (6, 17, 26, 32, 38) or by gradients of chemoattractant substances (43).

Rarer situations are however documented where postmitotic neurons have to migrate over considerable distances, and sometimes in a direction opposite to their target, in order to reach their final territory. Hence, the neuronal perikarya are translocated along precociously emitted leading processes, which are guided by a growth cone and are meant to become the axons of the adult cells. In these

cases, in addition to the above-mentioned cues required for the navigation of the axonal growth cones, another set of signals must be provided to the neuronal cell bodies, in order to instruct them where to stop, aggregate to form nuclei, and begin dendritic differentiation.

In the mammalian CNS, the neurons of the brainstem precerebellar nuclei provide good examples of this complex behaviour: originating in the primary precerebellar neuroepithelium, located dorsally in the wall of the IVth ventricle, they migrate ventrally, away from their cerebellar target, by circumnavigating the ventrolateral aspect of the medulla oblongata. Therefore, this system furnishes a valuable material for the study of such general developmental problems as pathway selection or selective aggregation in the CNS. Indeed, the neurons of these nuclei are generated in the same place, largely at the same time, migrate in the same direction, albeit choosing different pathways, and finally send their projections to the same target, but according to different patterns. We shall summarize and discuss here data obtained in our laboratory (9, 10) about the early ontogenesis of the inferior olivary nucleus (ION) and lateral reticular nucleus (LRN), with remarks about the development of the external cuneatus nucleus (ECN) and caudal raphe nuclei (raphe obscurus and raphe pallidus). In our studies, we used mainly the HRP *in vitro* axonal tracing method, which furnishes Golgi-like pictures of retrogradely filled neurons, allowing us therefore to analyze the changes in shape and orientation of their perikarya and processes. This can provide an indirect evidence of the existence of local or secreted factors in the guidance of growth cones and in the selection of the final territories. Moreover, the initial stages of the dendritic differentiation of these cells can be examined.

I. Date and place of birth of the brainstem precerebellar neurons.

The ION is a major precerebellar nucleus, whose importance lies in the fact that it is the only proven source of the cerebellar climbing fibers (16, 20, 42). The LRN, on the other hand, has long been known as one of the numerous sources of the cerebellar mossy fibers (12).

A great deal of studies have been devoted to the analysis of the structure and connections of these nuclei in adult mammals, as well as, in the case of the ION, to the analysis of its postnatal development, especially in the rat (7, 8, 9, 11, 14, 15, 41). However, despite the fact that many events in this system are obviously prenatal, as examplified by the fully mature cytoarchitecture of these nuclei at birth, their intrauterine ontogenesis is much less documented.

The exception to this is the large amount of data concerning the place of birth of the ION neurons. It has indeed been long recognized to be part of the dorsolateral recess of the IVth ventricle. His (27) named this place the rhombic lip (*Rautenlippe*) in human embryos, and later investigators (24, 25) agreed with this localization, although Ellenberger *et al.* (23) claimed that the term "rhombic lip" is inappropriate in the rat. More recently, Altman and Bayer (1, 3) confirmed,

by means of short-term tritiated thymidine (^{3}HThy) autoradiography, that the ION neurons are generated in the most caudal part of the precerebellar primary neuroepithelium (*ppn*), that is, in the dorsal aspect of the wall of the IVth ventricle just rostral to the spinal cord.

These same authors also examined the birthplace of the neurons of the LRN and ECN (1, 2, 3) and found it *to be the same as that of the ION neurons.*

Finally the ^{3}HThy method allowed the determination of the date of birth of these precerebellar neurons. Ellenberger *et al.* (23) claimed that in the rat, the ION neurons are generated during the embryonic days 13 (mostly) and 14 (E13 and E14), while Altman and Bayer (1, 4) established that this event takes place at E12 (over 70%) and E13 (less than 30%). In our own ^{3}HThy experiments (9), injections of the radioelement at E14 never labelled neurons in the adult ION, thus confirming the results of Altman and Bayer. It should be noted that in this paper, we consistently consider the day of mating as E0, not E1.

The only studies devoted to the determination of the birthdate of the reticular precerebellar neurons are those of Altman and Bayer (1, 2, 4, 5). These authors reported that the LRN cells are generated at E12-*E13*-E14 and the ECN cells at E12-E13-*E14*, the peak of production being italisized in each case.

The general conclusion that can therefore be drawn from these studies is that the neurons of the ION, LRN and ECN are generated *in the same place* (the primary precerebellar neuroepithelium) and *during overlapping periods.*

II. Migration and axonogenesis of the precerebellar neurons.

The migratory route or routes that the precerebellar neurons take to reach their final location in the ventral (for the ION and LRN) or dorsal (for the ECN) brainstem have been, at least in the case of the ION, a controversial matter.

Two streams of cells have long been described in the ventral aspect of the embryonic medulla oblongata of vertebrates: a superficial one, running just under the pial surface, and a deeper one extending into the medullary parenchyma. Essick (24) believed that the ION, in human embryos, originated from the "superficial migration over the ventral surface of the medulla". Harkmark (25), studying the chick embryo, assumed that "the deep cell strand in the chick gives rise to the dorsal lamella, the superficial strand to the ventral lamella of the olive". Ellenberger *et al.* (23) and Altman and Bayer (1), using the rat embryo as material, agreed with the concept of a dual origin of the ION. The former authors called the two migrations the pial or marginal migratory stream (*mms*) and parenchymal or submarginal migratory stream (*smms*) respectively, a terminology that we shall consistently use in this review.

However, all these opinions were based on evidence obtained from Nissl-stained material. Recently, Altman and Bayer (4, 5) re-investigated this problem using sequential ^{3}HThy autoradiography, and claimed that the *smms only* (that they called "intramural migratory stream") is responsible for the formation of the

ION, while the *mms* (their "posterior extramural migratory stream") conveys neurons that will form the LRN and ECN anlagen.

We approached again this question with a different methodology, namely the HRP *in vitro* axonal tracing method, used in rat embryos aged E15 to E20. Our results (9, 10) can be briefly summarized as follows.

At E15, both the ION (Fig. 3) and LRN presumptive territories are devoid of cell bodies. Two streams of cells can be recognized in the ventral and lateral aspects of the brainstem: just lateral to the ION domain, within the medullary parenchyma, a stream that can be identified as the *smms* (Fig. 3); and close to the pial surface, laterally and dorsally to the LRN domain, the *mms*. Both of these streams are composed of typical migrating neurons: bipolar, elongated cells, with two short processes of irregular diameter, bristling with filopodia and protrusions; from the shorter of these neurites emerges a long and thin process of regular diameter, often exhibiting a beaded appearance, which is the axon (Figs. 6, 7, 8). A point of special importance is that, in this precerebellar system, *the axonogenesis is an extremely precocious event*. Actually, emergence of an axon or axonal-like process is likely to be the first event in the postmitotic life of the young neurons, although these early stages have not been directly examined. Nevertheless, at E15, when no ION or LRN neuron has yet reached its adult location, their axons have already crossed the ventral midline and are on their way to their target, the hemicerebellum contralateral to their proliferation site.

At E16, the olivary neurons have begun to invade their ultimate territory. The ION at that stage appears as a club-shaped lamella (Fig. 1). Lateral to and connected with this lamella, the *smms* can clearly be recognized (Figs. 1, 4). An important point to notice is that *no* olivary neuron *crosses* the interolivary commissure, or "floor plate" (see below), either at this stage or later (Figs. 2, 5, 6, 12). In other words, *all* ION cells do settle *ipsilaterally* to their proliferation side. The adult, crossed pattern of the olivo-cerebellar projection is thus achieved by means of an axonal crossing of the midline at the "floor plate" level.

At this stage, the future LRN domain is still devoid of cell bodies, with the exception of some neurons belonging to the *smms* present at its peripheral aspect (Fig. 16). It is impossible to determine if these neurons are to stay within the LRN territory, or if they are merely crossing it on their way to the ION anlage. The *mms* is quite prominent in the ventral medulla of E16 embryos (Figs. 2, 6); it appears thickest at the midline (Fig. 2) and extends laterally up to the ventro-medial aspect of the presumptive LRN domain. Examination of HRP labelled neurons, as well as comparison with former and subsequent stages, indicate that, contrary to the *smms* neurons, most if not all the *mms* cells are *crossing* the midline at the "floor plate" level (Figs. 2, 6, 7, 8). There are two exceptions to this rule: i) a small contingent of neurons can be seen, at E16 and at the subsequent stages (E17, E18 and E19) leaving the *mms* and "climbing" dorsally along the "floor plate" on both sides of the midline (Figs. 2, 6, 14, 15). In previous studies of this system (1, 23), these cells were believed to be olivary

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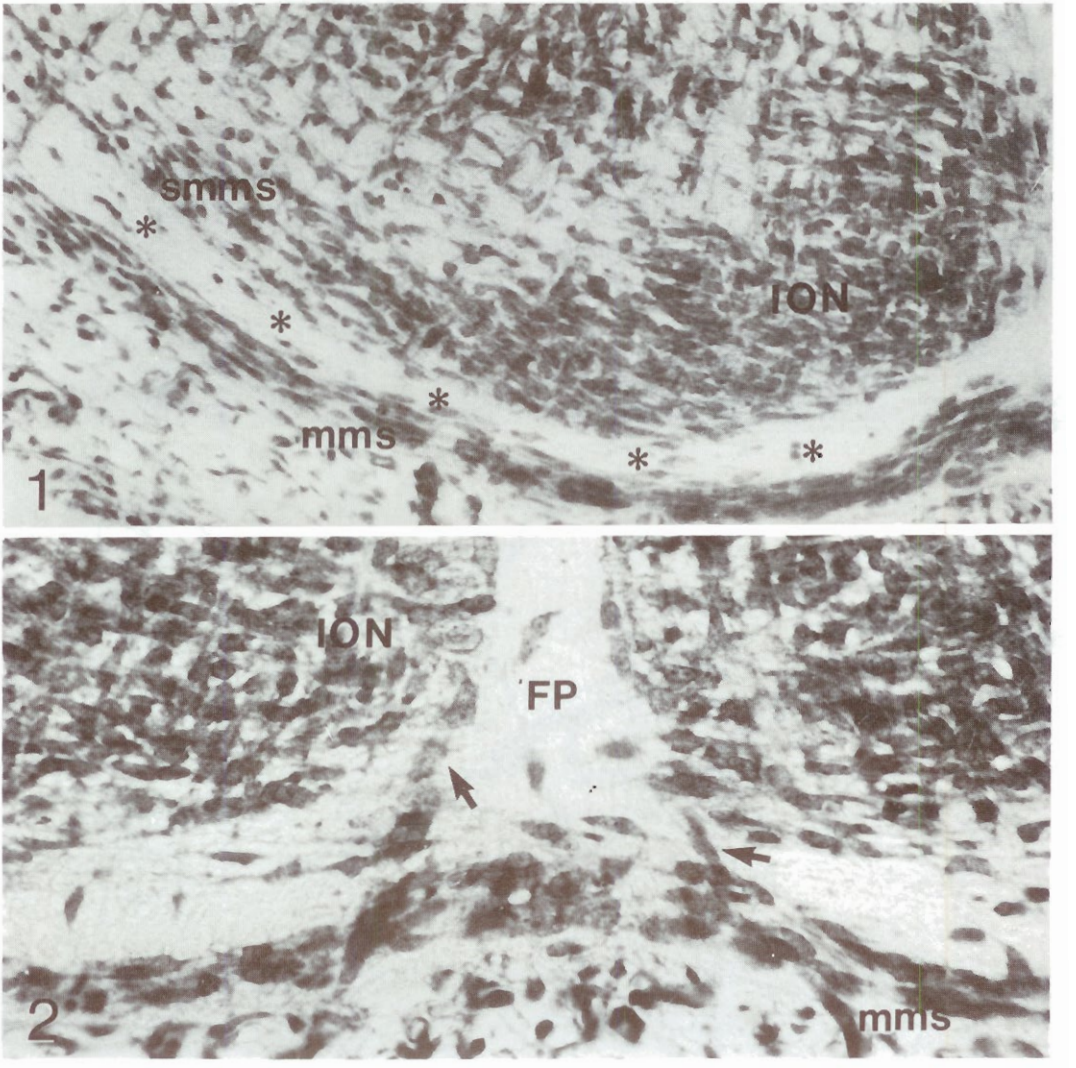
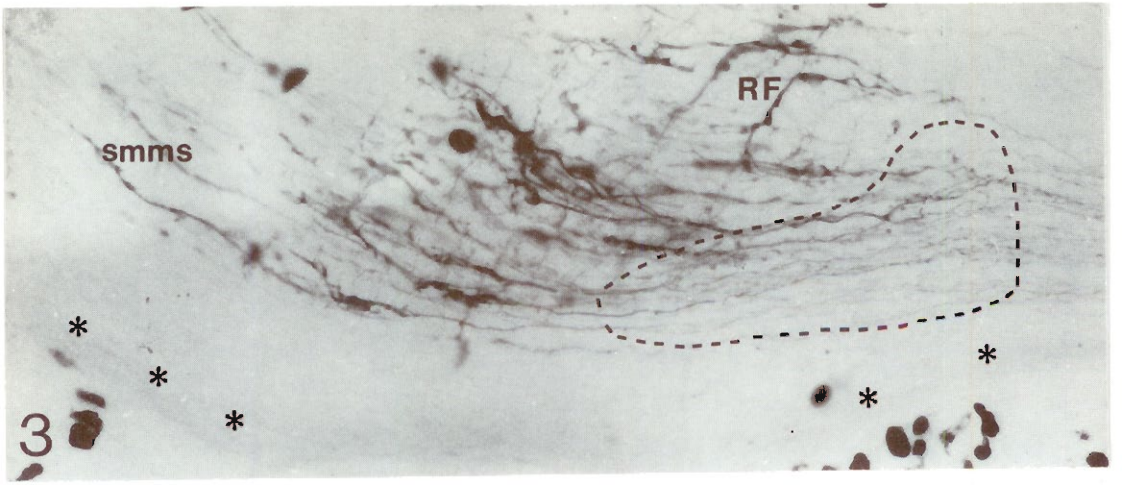


Plate I. Nissl-stained paraffin sections cut in the frontal plane through the caudal brainstem of E16 rat embryos.

Fig. 1. – The ION anlage results from the aggregation of the neurons of the submarginal migratory stream (*smms*). The marginal migratory stream (*mms*) does not provide cells to the ION. It is separated from the *smms* by an acellular strip (asterisks). X 260.

Fig. 2. – The midline interolivary commissure, or "floor plate" area (FP), is crossed by the *mms* neurons, but not by the ION cells. The arrows point to cells that leave the *mms* and "climb" on both sides of the "floor plate". They probably represent raphe neurons (see also Figs. 14 and 15). X 400.

cells contributing to the medial accessory olive (MAO). However, they much more likely represent serotoninergic neurons on their way to settle in the raphe obscurus and raphe pallidus domains. ii) Whereas the *smms* and *mms* are clearly separated,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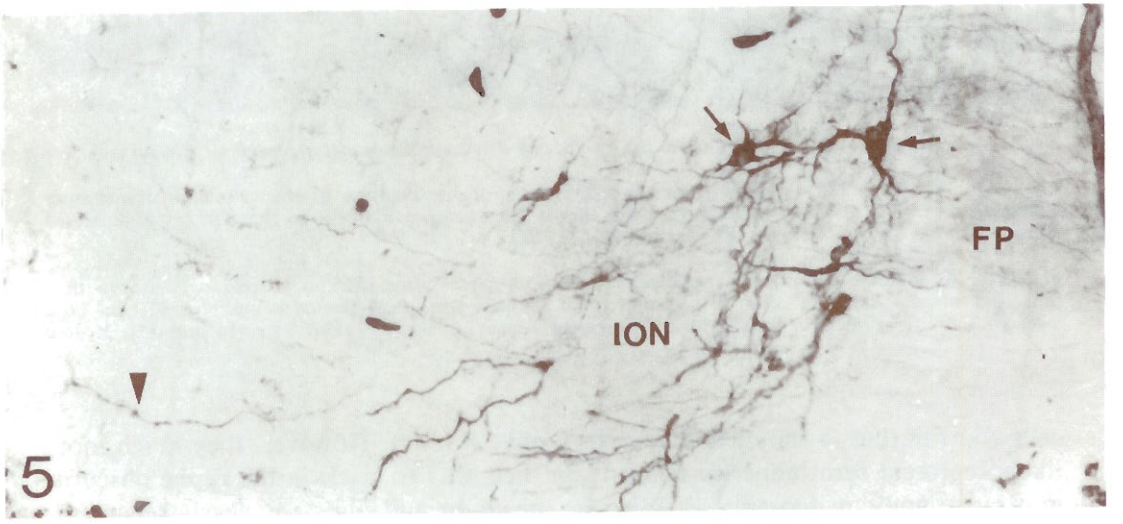
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at the level of the ION primordium and laterally up to the future LRN territory, by a largely acellular strip (Fig. 1), a *small* number of cells can be seen, at E16 and E17, leaving the *mms* to invade the ION anlage. This contribution of the *mms* to the olivary formation is, however, minimal, and it can safely be assumed that over 95% of the ION neurons migrate via the *smms*.

At E17, almost all olivary neurons have been committed to the olivary primordium (Fig. 5): the *smms* appears as a thin and short strand of neurons just lateral to the still club-shaped olivary lamella. The LRN anlage is visible at this stage as a dorsal condensation of the *mms* (Fig. 9). The two main subdivisions of the LRN (the ventro-lateral parvocellular region -LRNp- and dorso-medial magnocellular zone -LRNm-) are already distinguishable. The *mms* is still present, but greatly reduced, at the midline, and it continues beyond the LRN domain dorsally (Fig. 16). It is thus clear that not all the *mms* neurons aggregate in the LRN anlage. However, it is likely that all the LRN neurons come from the *mms*, with the possible exception of a few *smms* cells having stopped at E16 in the presumptive LRN domain (see above).

This pattern of aggregation of the LRN cells has important consequences for the establishment of the LRN-cerebellar projection. In adult mammals, this projection is largely ipsilateral, with a small contralateral component (12, 18, 19, 21, 28, 31, 35). From our experiments, it is therefore clear that the bulk of this projection is established by a "double crossing" of the midline at the brainstem level, first of the axons, then of the cell bodies. However, one point remains obscure: how do the neurons forming the small contralateral component settle? Two hypotheses can be proposed: *i*) a "cell body choice", in which a small group of migrating LRN neurons stops on the side ipsilateral to its proliferation; in this case the midline crossing is achieved by the axons at the bulbar level, and *ii*) an "axonal choice" where *all* the LRN somata cross the midline, and the contralateral projection pattern derives from a further axonal decussation in the cerebellum, that is, from a *triple* crossing (axons as leading processes, then somata in the brainstem, then axons again in the cerebellum).

It is impossible, with our methodology, to answer this question, which has

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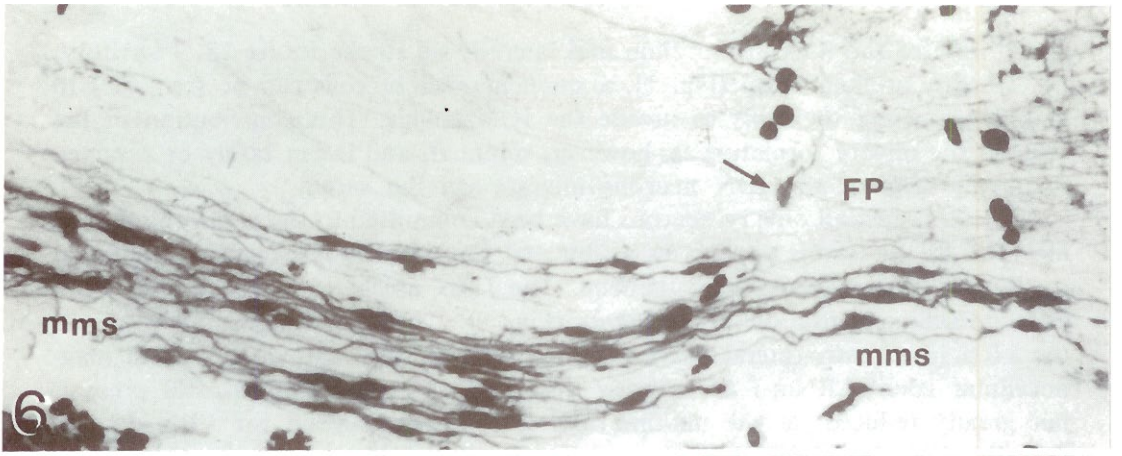
Plate II. Aggregation of the olivary neurons between E15 and E17.

Fig. 3. – At E15, the presumptive ION territory (dots) is still devoid of cell bodies. Laterally, the *smms* is apparent. Notice that the *mms* has not yet reached this ventral aspect of the brainstem (asterisks). Some neurons belonging to the reticular formation (RF) were labelled by the injection. HRP *in vitro* technique. X 290.

Fig. 4. – Camera lucida drawing of HRP-filled neurons within the ION anlage in an E16 embryo. Laterally, the *smms* is still present (see Fig. 1). It is composed of bipolar, elongated cells. Medially, the neurons begin to settle in their ultimate position, but are still very immature. X 470.

Fig. 5. – The ION domain at E17 contains more mature neurons, especially medially (arrows). Remnants of the *smms* can still be seen laterally (arrowhead). The ION neurons stay ipsilateral to their proliferation and migration side, and do not cross the "floor plate" (FP). X 290.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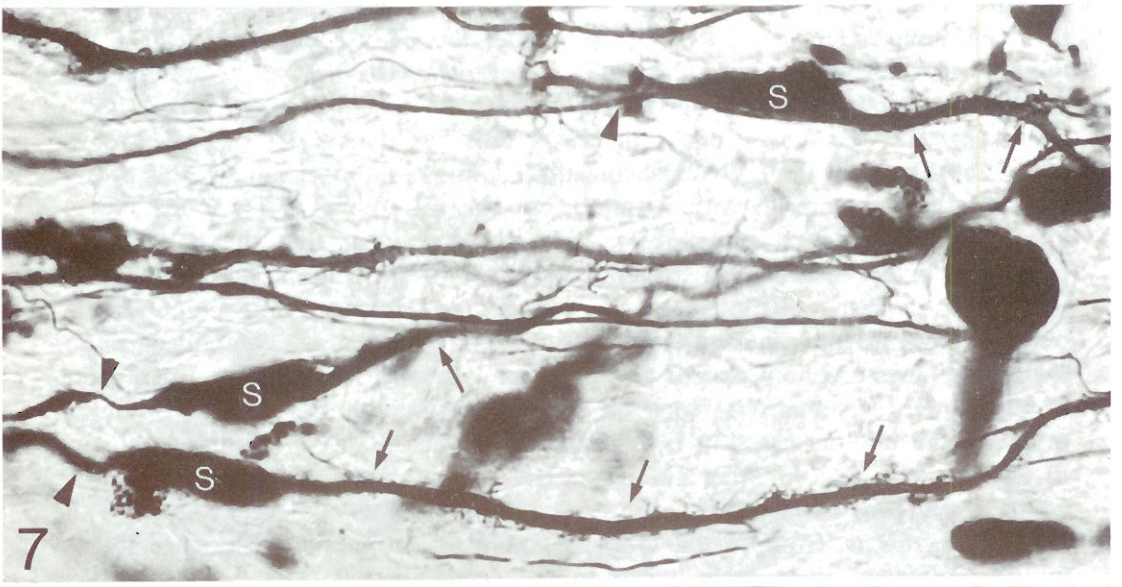
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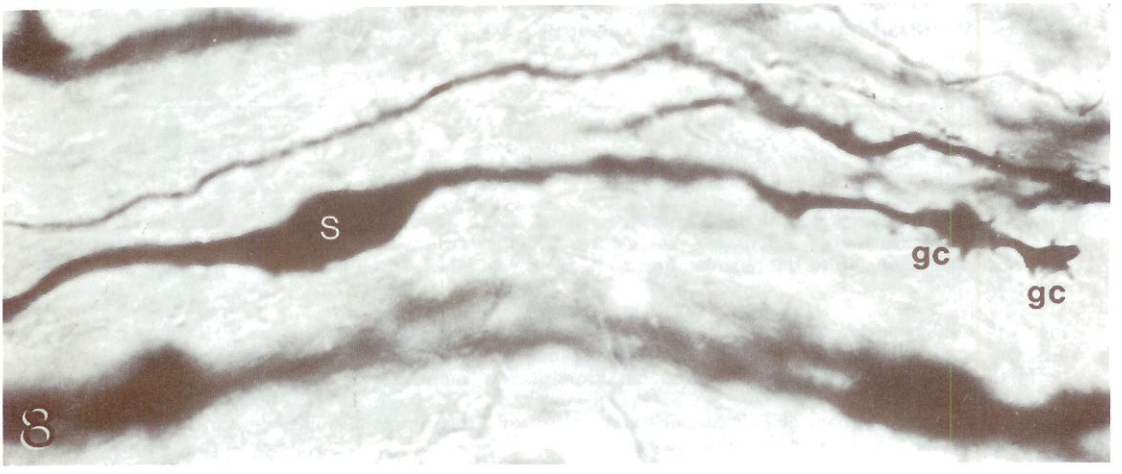
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important implications (discussed below) for the aggregation pattern of the LRN neurons: indeed a few cell bodies can be seen at E16 (see above) in the LRN domain ipsilateral to the proliferation side, but one cannot tell if they are to settle there. On the other hand, midline HRP injections made at later stages do not lead to an unambiguous labelling of LRN neurons, which seems to indicate that the LRN-cerebellum axonal projection does not decussate at the brainstem level. Furthermore, the data concerning the adult projection are equally unclear: most of the authors (12, 18, 19, 21, 28, 31, 35) were chiefly concerned with the topographical organization of this projection, regardless of its crossed or uncrossed character, and there is a conflict between the two papers which explicitly deal with this matter: whereas Künzle (31) claims that at least part of the LRN fibers travel through the ION and decussate at the ventro-bulbar level, Chan-Palay *et al.* (18), using the same autoradiographic method, report that "unilateral injections in the LRN produced heavy, uncrossed, ipsilateral and light, crossed, contralateral projections with the crossing occurring in the cerebellum". Obviously, this point requires further clarification.

At E18, the *smms* has vanished, and all the ION neurons are within their ultimate domain (Fig. 12). The ION subnuclei (medial accessory olive -MAO-, principal olivary nucleus -PON- and dorsal accessory olive -DAO-) begin to be recognizable, at least at the ventral pole of the olive.

E18 is the stage of massive commitment of the *mms* neurons to the LRN territory, which appears packed with numerous cell bodies (Figs. 10, 11), much more densely in the LRNp than in the LRNm. The *mms* has almost completely disappeared at the midline, but can be traced dorsally to the LRN primordium up to the ECN, where a cell condensation begins to form (Fig. 16). Thus, the ECN is formed from cells generated in the *ppn* that have circumnavigated around the entire brainstem, and finally settled contralateral to their proliferation side, next to the site of proliferation of their contralateral homologues. As Altman and Bayer (4) pointed out, this is a unique situation in the mammalian CNS ontogenesis.

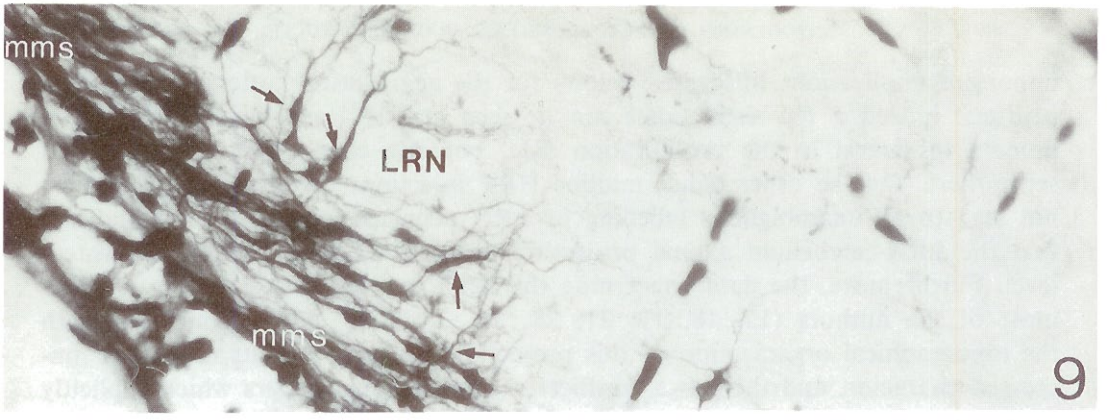
At E19-E20 (these two stages are grouped because they do not show obvious differences), the ION is achieving its cytoarchitectural maturation. All the olivary subnuclei, and even the fine subdivisions of the MAO, are thus fully recognizable before birth in the rat.

Plate III. Cytology of the mms *neurons, as seen with the HRP* in vitro *technique.*

Fig. 6. – The *mms* is most prominent at E16 near the midline. Notice the neurons (arrow) leaving the *mms*, probably to settle in the raphe territory. X 300.

Fig. 7. – Higher magnification of *mms* neurons at E16. They have bipolar, elongated somata (S), from which emerge leading processes (arrows), bristling with numerous protrusions and filopodia, and smoother trailing processes (arrowheads). X 1260.

Fig. 8. – High magnification of a *mms* neuron at E17. This neuron is just crossing the midline. From the soma (S) emerges a dendritic process provided with a dual growth cone. X 1510.



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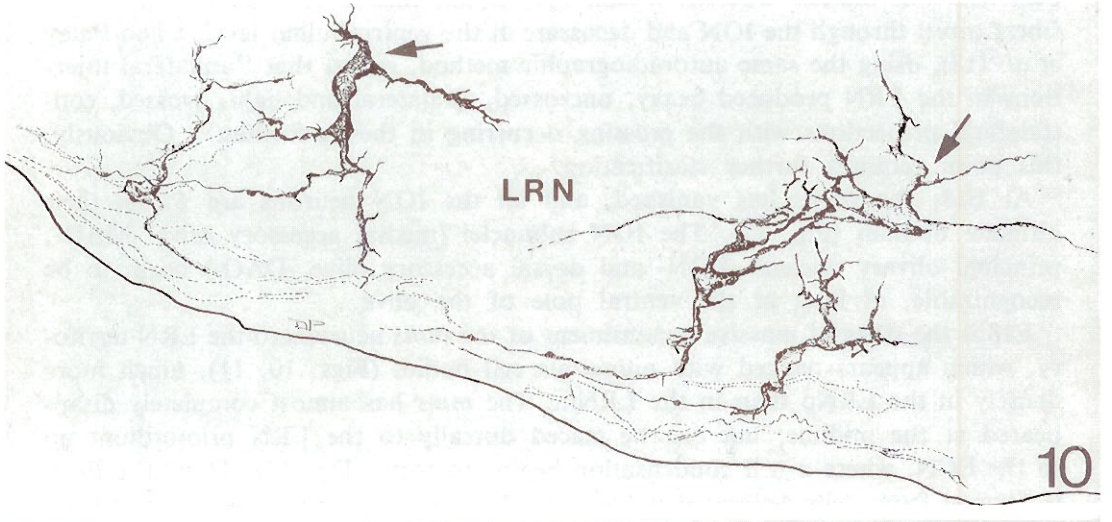

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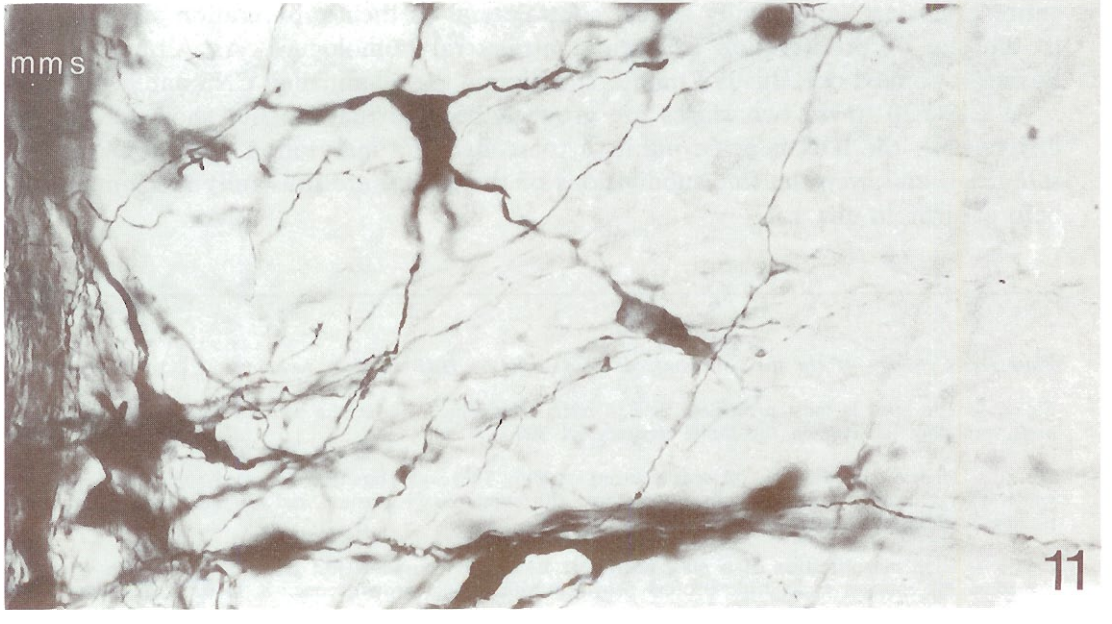

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The *mms* has completely vanished at the LRN level, indicating that all the LRN neurons have settled in their ultimate territory. These late stages are mainly characterized by a rapid dendritic differentiation of the LRN and ION neurons (Figs. 11, 13).

III. Dendritic maturation of the precerebellar nuclei neurons.

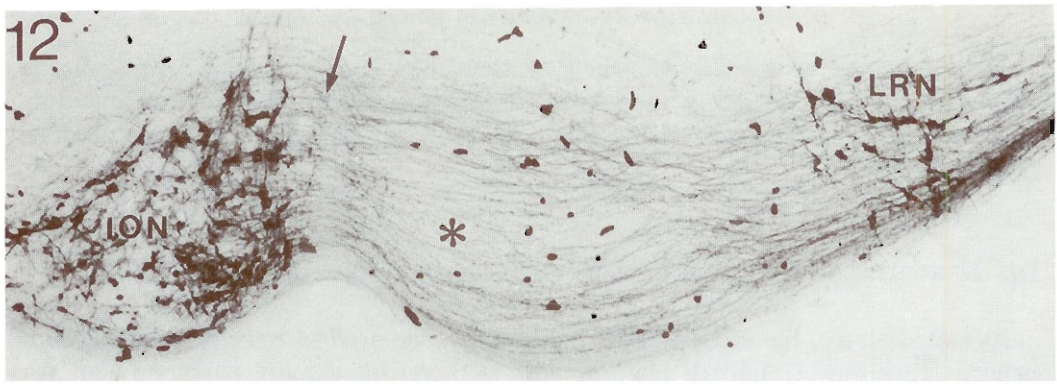
As stated above, the *in vitro* HRP method that we used to trace the precerebellar neurons furnishes Golgi-like pictures and is therefore a most adequate tool for the analysis of the tridimensional maturation of nerve cells. Two main phases can be distinguished during the formation of the ION dendrites: *a*) *The stage of the fusiform neurons*, predominant until E16, that characterizes cells during migration, has been described above (Figs. 4, 6, 7, 8). The neuronal migration is accomplished by mean of a continuous forward translocation of the cell nucleus within the leading process, while the trailing process constantly regresses. *b*) *The stage of the stellate neurons with long and straight dendrites* predominates from E17 until E20 (Fig. 13). This stage characterizes the ION neurons which have reached their final destination and most likely persists during the first steps of postnatal life. In these cells, the axon emerges from the cell body, which has acquired a more or less spherical shape. These neurons exhibit long, straight and irregularly thick dendrites (Fig. 13); they have short and thin appendages, sometimes branching away from the soma without turning backwards as they normally do in the mature state (37). In our studies we did not observe any neuron with the complex shape that Ramon y Cajal (36) compared to a "ball of wool", characteristic of the adult dendritic tree. It is therefore obvious that the most critical steps of the tridimensional maturation of the ION neurons take place postnatally in the rat, coinciding with the period of maximal synaptogenesis. Another noticeable feature is the existence of a clear latero-ventral to medio-dorsal gradient of maturation within the olivary primordium. Whereas the most laterally situated cells (close to the *smms*) exhibit the characteristic aspect of migrating neurons, those which are close to the midline tend to belong to the second type described above (with long and straight dendrites).

Plate IV. Aggregation of the LRN neurons at E17 and E18. HRP in vitro *method.*

Fig. 9. – At E17, some neurons leave the *mms* and invade the LRN domain. Notice that some of them do so by extending a dendritic process in a radial direction (arrows). X 450.

Fig. 10. – Camera lucida drawing of 5 neurons leaving the *mms* (dotted cells) to invade the LRN territory. The arrows point to 2 cells that have already begun to acquire the dendritic pattern characteristic of LRN neurons. X 530.

Fig. 11. – At E18, while some neurons (bottom) are just leaving the *mms*, others are rapidly differentiating their dendritic trees (top). Their multipolar shape is already close to that of adult cells. X 600.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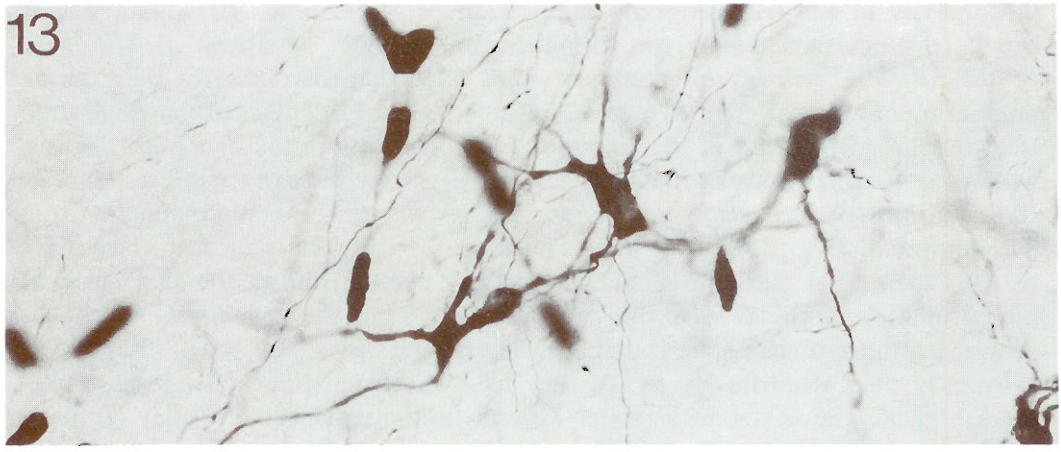
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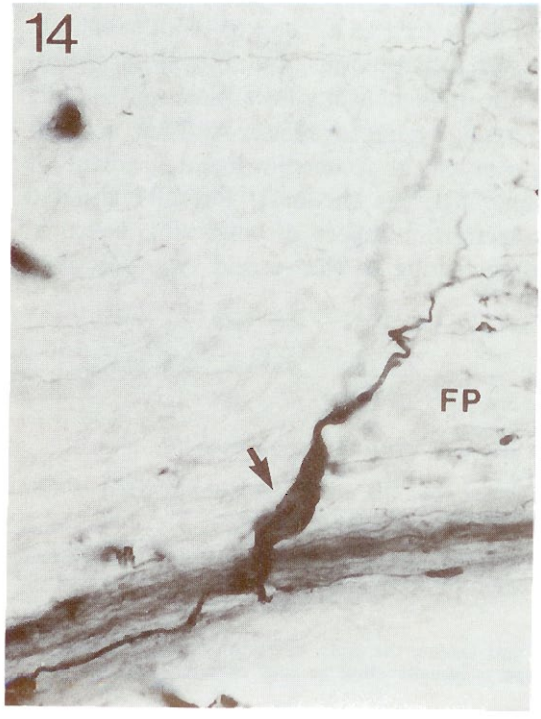
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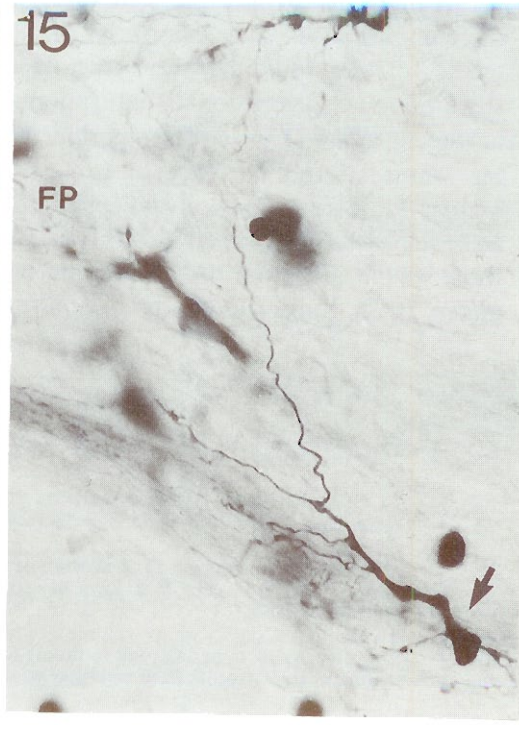
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A similar gradient can be observed in the maturation of the LRN cells at E17-E18. The LRN area is invaded following a ventro-lateral to medio-dorsal gradient: the more ventrally and laterally situated cells (roughly, those belonging to the LRNp domain) differ very little from the migrating neurons described above. On the other hand, the more dorsally and medially positioned neurons are already, at E18, much more mature: these are bigger cells, multipolar (3-5 primary dendritic trunks being usual, with straight secondary and tertiary dendrites), with axons which emerge either directly from the soma, or from a very short dendrite-like appendage.

The various intermediate neuronal conformations observed within the LRN domain at E18 (and to a lesser degree at E17 and E19) provide some insights into the patterns of aggregation of the LRN neurons (Figs. 9, 10, 11): *i*) the majority of the LRNp neurons pull off from the *mms* and invade their terminal domain while keeping a bipolar shape; they seem to stack on top of each other in this area, the more recently arriving neurons pushing the previously arrived ones away from the *mms*. *ii*) The greater part of the LRNm neurons behaves differently: when they reach the level of their terminal domain ventrally, they emit a process perpendicular to the direction of their migration in the *mms*, and, changing their polarity to a radial one, they "climb" to their final location, where they immediately begin to differentiate into more mature, multipolar cells (Figs. 9, 10, 11).

At E19-E20 the LRN neurons are rapidly differentiating into mature forms. However, the latero-ventral to medio-dorsal maturational gradient described above is still quite apparent: the cells of the LRNm exihibit a multipolar shape, with 4-6 primary dendritic trunks ramifying in secondary branches, and further in tertiary long and thin appendages. Their appearance is actually very similar to that of the adult neurons of the LRNm in the rat, as described by Kapogianis *et al.* (30), and the different types that these authors distinguished (multipolar, piriform, fusiform, etc...) can already be identified in the LRNm of E20 embryos. Although no quantitative assessments were attempted, the main difference lies in the length of the terminal branches, which often appear much longer in the embryos than in their adult counterparts. On the other hand, the cells of the LRNp seem much more immature, a considerable proportion of them still keeping a fusiform and bipolar shape similar to that of migrating neurons, although one

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Plate V. Olivary and presumptive raphe neurons in E_{18}-E_{20} embryos. HRP in vitro method.

Fig. 12. – At E18, after a HRP injection in the right inferior cerebellar peduncle, the contralateral ION is labelled. The ION domain ipsilateral to the injection site (asterisk) is devoid of cell bodies. Thus, the crossed pattern of the olivocerebellar projection is achieved by the olivary axons crossing the interolivary commissure (arrow). In the LRN, only neurons ipsilateral to the injection site are labelled. X 110.

Fig. 13. – At E20, the ION neurons have predominantly reached the "stage of stellate cells with long and straight dendrites". X 550.

Figs. 14 and 15. – High magnifications of two neurons (arrows) leaving the *mms* and "climbing" along the "floor plate" (FP). They are likely to be raphe neurons (see also Figs. 2 & 6). X 680.

of the dendritic trunks (usually the one opposite to the pole of emergence of the axon) is often fairly well ramified. The adult conformation of the LRNp neurons has received very little attention in the rat. Thus, it is difficult to determine if they are actually more immature than those of the LRNm, or if their undeveloped appearance at E19-20 only reflects a comparatively uncomplicated adult shape.

IV. Selective aggregation and cytoarchitecture maturation of precerebellar neurons.

The problem of the aggregation of the ION cells is two-fold: firstly, a problem of "laterality", or why do the ION neurons stop their migration on the side ipsilateral to their proliferation, while the other precerebellar neurons cross the midline? This question, which is related to the problem of pathway selection, will be approached in the next paragraph. Secondly, how is the complex, multilamellar organization of the ION realized?

A first point to be noticed is that this shaping of the ION lamellae take place at E17-E19, that is, *after* the commitment of the migratory neurons to their ultimate domain. Neurons are indeed added to the olivary primordium at E16-E17, which, at the end of this process, exhibits the simple form of a club-shaped lamella. Thus, the hypothesis of a progressive cytoarchitecture formation by addition of laterally arriving *smms* neurons can be discarded. Moreover, long-survival autoradiography experiments of Altman and Bayer (4), as well as our own preliminary results using a similar methodology (5'bromodeoxyuridine immunocytochemistry) indicate that the distribution of the olivary neurons among the lamellae is complex: one would indeed expect in the adult ION the more medially situated cells (schematically, those of the MAO) to have been generated earlier that the more laterally situated ones (those of the DAO), since the neurons are added laterally to the ION primordium.

This is far from being the case — for example, the most lateral part of the DAO is entirely composed of *early* generated cells —, and the distribution of the ION cells is in fact so peculiar that it prompted Altman and Bayer (4) to abandon the classical subdivision of the ION in favor of a new, "neurogenetic" classification. It is thus quite evident that in a 2-day critical period (E17-E19) a major reorganization, involving complex cell movements, and maybe an important neuronal death, takes place within the inferior olive. The cues that "tell" a neuron to settle in a precisely defined position, thus creating an olivary cartography, are unknown and deserve further studies.

The problems of neuronal aggregation and cytoarchitectural maturation are rather different in the case of the LRN. As previously stated, the cytoarchitecture of this nucleus is indeed recognizable from the very beginning of the cellular aggregation. Thus, unlike the ION, the simple mechanisms that the neurons of the *mms* use to invade the LRN domain (see above) can account for the formation of the adult cytoarchitecture. The main problem in this case is how and when do

the migrating neurons of the *mms* receive a signal instructing them to stop and settle in the LRN anlage?

It is clear that the bulk of the *mms* neurons *bypass* the LRN domain ipsilateral to their proliferation side, cross the midline and settle contralaterally (Fig. 16). But do *all* the LRN neurons do so? This question can be linked with the problem of the establishment of the LRN-cerebellar crossed projection exposed previously. We formulated two hypotheses that have different implications for the question of the LRN neuronal aggregation: if the crossed component results from a "cell body choice", this implies that the presumptive LRN domain becomes "attractive" for the cells of the *mms* very early (at E15-E16), so that some LRN neurons leave this stream when they first bypass it, on the side ipsilateral to their proliferation. It becomes in this case rather difficult to understand why only a few neurons are attracted ipsilaterally. On the other hand, the "axonal choice" hypothesis means that the LRN territory is not recognized as such by the LRN neurons of the *mms* when they first skirt it, but becomes "attractive" only after these neurons have crossed the midline. This could be due to a later change in the biochemical composition of the LRN territory itself, or to a change in the LRN neurons, giving them the ability to recognize the LRN territory, when they traverse the "floor plate" region. This later possibility is not unlikely, in view of recent work on spinal cord axons and growth cones in the rat embryos (22), which clearly demonstrates that important modifications in surface axonal glycoproteins (loss of TAG-1 and initiation of L1 expression) occur at the "floor plate" region in a subset of these axons. Indeed, as will be discussed now, the brainstem "floor plate", at the interolivary commissure level, seems to play a major role in the migration of the various precerebellar nuclei.

V. Selection of migration pathways in relation to the "floor plate" in the brainstem precerebellar system.

The three main nuclei of this system (LRN, ION, ECN) provide valuable material for the analysis of some mechanisms of early neurogenesis. They are generated at the same plate, the precerebellar primary neuroepithelium, and during overlapping periods of time: ION, *E12*-E13; LRN, E12-*E13*-E14; ECN, E12-E13-*E14*, the peak of production being italisized (1, 2, 3). Immediately after leaving the mitotic cycle, the young neurons are faced with a first choice: while the future ION cells "translocate to the inferior olivary premigratory zone" (4), penetrating the medulla parenchyma where they migrate as the *smms*, the LRN and ECN neurons keep close to the pia and form the *mms* (Fig. 16). In many places in the developing CNS, the sub-pial zone, and more specifically the radial glia endfeet, has been shown to be used as a preferential substrate for initial axonal elongation and cell migration (33, 34, 39, 40). In this respect, the LRN and ECN cells are making the most usual choice. However, examples have been recently provided

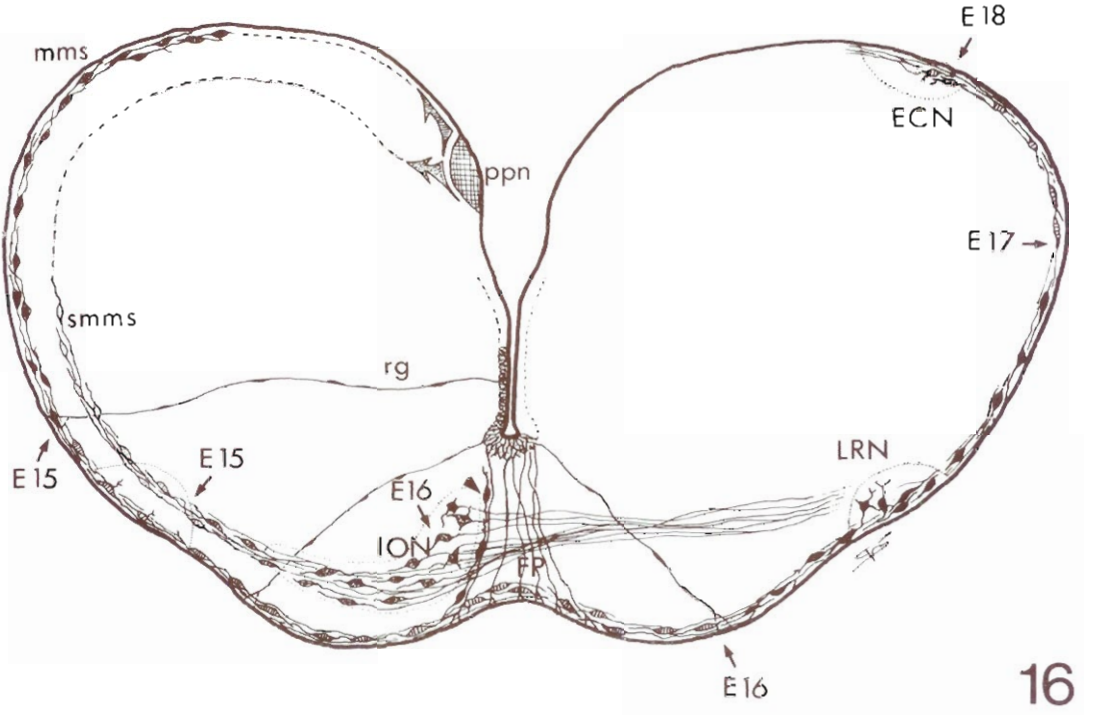

Plate VI. Schematic representation of the migration and aggregation events in the brainstem precerebellar system.

Fig. 16. – Generated together in the primary precerebellar neuroepithelium (*ppn*), the ION, LRN, and ECN neurons migrate differently. The ION cells take the submarginal pathway (*smms*), whereas the LRN and ECN neurons migrate via the marginal stream (*mms*), at the level of the radial glia (rg) endfeet. The position of the fronts of the migration waves are indicated between E15 and E18. The ION neuronal somata do not cross the "floor plate" (FP) and begin to settle at E16 in their ultimate domain. The LRN and ECN cells cross the FP and begin to aggregate at E17 and E18, respectively, within their final territories. The arrowhead points to a raphe neuron, which migrated via the *mms* and "climb" along the FP.

of intraparenchymal guidance of axons, as seems the case for the ION neurons (22, 43).

In both instances, the two migratory streams convey neurons toward the midline territory occupied by the "floor plate" (Figs 2, 6, 16). It is therefore tempting to see the "floor plate" as an attracting zone, producing diffusible factor(s) (43) that drive the growth cones of the ION, LRN and ECN neurons toward the medulla ventral midline.

However, the "floor plate" must clearly play a more complex role in this system: indeed, after this initial choice of the migration pathway, the neurons of the precerebellar nuclei are faced with a second decision when their cell bodies reach the "floor plate" area. Whereas the LRN and ECN somata cross it, the ION cells stop ipsilaterally to their proliferation side. In addition, neurons of the raphe obscurus and raphe pallidus nuclei also migrate via the *mms*, and have a still different behaviour: they stay ipsilateral to their migration side and "climb" along

the "floor plate" (Figs. 2, 6, 14, 15, 16). Leaving aside these still incompletely studied neurons, it should be noted that the speculation of Altman and Bayer (5): "all precerebellar neurons are specified to send their axons to the opposite side" is validated by our studies (9, 10). This holds true even if, as discussed above, a few LRN neurons stay on the ipsilateral side, or if a small contingent of ION neurons actually cross the midline by the *mms* (see discussion in ref. 9). Thus, the mechanism that determine the choice of an ipsilateral or contralateral settling for the precerebellar neurons, and consequently for the establishment of a crossed or uncrossed projection to the cerebellum, are to be sought at the somatic rather than axonal level, and the choice is done in the "floor plate".

We propose therefore that the "floor plate" plays an important role in this sorting of neuronal cell bodies, being not only an attracting zone for the growth cones (see above), but a decision-making region. We postulate that the precerebellar neurons are specified to send not only their axons to the contralateral side, but their somata as well. Contrary to the speculation of Altman and Bayer (5) ("... this is modified in the case of the ipsilaterally projecting lateral reticular an external cuneate neurons by the cell bodies following their neurites to the opposite side"), it would then be the ION that is an exception to the general rule of migration and settling of the precerebellar neurons. Therefore, the fate of the neurons in this system would be first governed by the initial choice of the parenchymal (*smms*) or subpial (*mms*) pathway of migration, and then by a second decision ("stop or go on") in the "floor plate" area.

Although a detailed ultrastructural study of this area is obviously necessary to confirm or reject this conjecture, several indirect arguments can be put forward in favor of the chemical, and possibly physical, peculiarity of this area: *i*) at the ages studied, and wherever the HRP injection was made, the only neuronal cell bodies that we observed crossing the midline were those of the *mms*, *ii*) when viewed immunocytochemically with an anti-vimentin antibody, the brainstem "floor plate" is very strongly immunoreactive, and appears to be made of neuroepithelial cell processes much more closely packed than anywhere else in the medulla; moreover, and contrary to the radial glia in the vicinity, these fibers strongly react with an anti-calbindin antibody, a further indication of their specificity (M. Wassef, personal communication), and *iii*) Joosten *et al.* (29), in a study of the developing cortico-spinal tract in the rat embryo, have indicated that in this system there is a midline barrier that interrupts at the pyramidal decussation level, and have suggested "a physical role of this glial septum in the guidance of outgrowing corticospinal axons in preventing them from decussation". Such a role could be proposed for the analogous "floor plate" in the interolivarly commissure, although via a probably different mechanism: in view of the "in vitro" experiments of Tessier-Lavigne *et al.* (43), the "floor plate" is more likely a zone producing a variety of "chemo-attractant" molecules rather than a physical barrier.

This precerebellar system provides valuable material for the analysis of some problems related to neuronal migration and aggregation; as Altman and Bayer (5) pointed out, it offers a situation "presently unknown in any other region

of the mammalian brain''. Indeed, if one considers the route followed by the LRN neurons, and still more intriguingly, by the ECN cells, one realizes that these neurons circumnavigate the entire brainstem, to finally reach a position very close to the place where they have been generated, but on the opposite side (Fig. 16). The rationales underlying such a long detour, and the cellular as well as molecular mechanisms involved, merit further analyses.

SUMMARY

The migration, cytoarchitectonic segregation and neuritogenesis of the inferior olive (ION) and lateral reticular (LRN) neurons are described in the rat. Generated in the same primary precerebellar neuroepithelium, at embryonic days 12-13 (E12-E13) for the ION and E12-E14 for the LRN, the postmitotic cells take either the intraparenchymal (*smms*, for ION neurons) or the subpial migratory streams (*mms*, for LRN neurons and other populations, as those of the external cuneate nucleus, ECN). The ION neurons settle in their ultimate domain from E16 to E18, ipsilaterally to their proliferation side. The LRN (and ECN) neurons cross the midline at the «floor plate» (FP) level, and settle contralaterally to their birthplace between E17 and E19. In both cases, the acquisition of a mature dendritic tree is a late event when compared to the precocious axonogenesis. The FP structure may play a major role in i) attracting the axons of the precerebellar neurons, and ii) instructing these neurons whether to cross the midline or not. Thus, ultimately the FP may govern the pattern (crossed or uncrossed) of the projections of the ION and LRN to their common cerebellar target.

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