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Cerebellar Development: Afferent Organization and Purkinje Cell Heterogeneity

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Cerebellar development: afferent organization and Purkinje cell heterogeneity

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SUMMARY

Olivo- and spinocerebellar maps in the adult cerebellum of small rodents are discontinuous, with sharp boundaries. Cortical Purkinje cells constitute a heterogeneous population, organized into parasagittal, mutually exclusive compartments. The boundaries of the intrinsic cortical compartments and those of the projectional maps are congruent.

During development; (i) The incoming olivary fibres, once they penetrate in the cerebellar parenchyma, are attracted toward their ultimate terminal fields, without passing through a stage of random dispersion. (ii) Migrating Purkinje cells and inferior olivary neurons begin, asynchronously, to express cellular markers in an independent manner, giving rise to a transient compartmentation of the cerebellar cortex and the inferior olivary complex respectively. In both instances, the biochemical heterogeneity disappears during the first postnatal week, simultaneously with the acquisition of adult-like cerebellar maps. (iii) The formation of the maps is an early event, prior to the establishment of the synaptology of the cerebellar cortical circuitry. Moreover, the organization of the spinocerebellar projection in adult mutant mice does not depend on the presence of granule cells (staggerer) but on the presence of normal Purkinje cells (weaver), indicating that synaptogenesis with their target neurons is not involved in the process of map formation. The matching of region specific chemical labels between incoming afferent fibres and heterogeneous sets of Purkinje cells is the most appealing mechanism for the formation of cerebellar maps.

1. INTRODUCTION

In the cerebellum, as in other regions of the brain, afferent projections are arranged according to a precise spatial order, resulting from axonal segregation into different compartments distributed in adjacent parasagittal zones. Segregation of incoming fibres into distinct compartments is one of the most common used way of building up neural maps. This is the case of the cerebral cortex and its modular columnar organization, the dorsal lateral geniculate nucleus and its laminar arrangement, or the striatum with its two compartments (named the patches and matrix) arranged as a mosaic. The understanding of the mechanisms by which the organization of projectional maps is acquired during development remains one of the major goals of developmental neurobiology. The formation of projectional maps is a long-lasting process, and a variety of mechanisms can be tentatively involved in the sequentially timed stages leading to the acquisition of adult neuronal networks. The invasion of the terminal domains may be oriented by matching of positional cues between the ingrowing axons and their transient and/or ultimate target neurons (Sperry 1963). The process will end by synaptogenesis and the refinement of the final connectivity by the activity of the forming network. Although the relative importance of these normal influences still remains unknown, it is quite clear that their combination in a cooperative way is necessary for success (Fraser & Perkel 1990; Udin & Fawcett 1988).

The aim of this paper is to review evidence, mostly obtained in our laboratory, of the essential role of Purkinje cells in organizing broad projectional maps during cerebellar ontogeny. We will argue that the segregation of incoming extracerebellar afferents results from a previous subdivision of the cerebellar cortex into small subsets of biochemically different Purkinje cells. We favour the hypothesis, that the acquisition of neuronal identities in precerebellar nuclei and in Purkinje cells (chemoaffinity hypothesis of Sperry (1963)) will allow, by matching of positional cues, the formation of cerebellar maps, without precluding that neuronal activity could later refine the ultimate order of the maps.

2. ORGANIZATION OF PROJECTION FIELDS AND HETEROGENEITY OF PURKINJE CELLS IN ADULT CEREBELLUM

The striking uniformity of the neural elements constituting the cerebellar cortex, with its monotonous cytoarchitectonic arrangement (five neuronal classes: Purkinje, basket, stellate, granule and Golgi cells) and the constant presence of climbing, mossy and mono-

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aminergic afferent fibres, is in apparent contradiction with the well known functional parcellation of this cortex into narrow sagittal zones (Oscarson 1979). This parcellation, or longitudinal-zonal organization, is based upon differences in the arrangement of afferent and efferent connections (see references in Ito (1984)), that is to say upon the disposition of cerebellar projection maps.

Although molecular correlates of this parcellation have been known for almost 30 years (see references in Gravel *et al.* (1987)), it is only recently that increasing evidence has been obtained showing that Purkinje cells, the pivotal elements of the cerebellar cortex and its unique output, are a biochemically heterogeneous population. This evidence has been mainly acquired by the use of monoclonal antibodies (mab), against still not completely characterized antigens, that are expressed according to a parasagittal band pattern, with subsets of Purkinje cells either possessing or lacking the respective antigens. Several mabs sharing these staining properties are known: mab B1 (Ingram *et al.* 1985); mab Q113, now named zebrin I (Hawkes *et al.* 1985); mab zebrin II (Brochu *et al.* 1990); and mab B30 (Stainier & Gilbert 1989). The B30 epitope, carried on a minor ganglioside, is the only one present on the Purkinje cell surface. It is important to note, that zebrin I, zebrin II and B30 are carried by the same subsets of Purkinje cells, indicating that these molecular markers reveal a common compartmentation of the cerebellum. Incidentally, the mab 192 IgG, specific for the rat NGF receptor, also shares in adult cerebellum this common staining pattern, with alternating bands of positive and negative Purkinje cells (Koh *et al.* 1989; Pioro & Cuello 1990; Keep and Sotelo, unpublished data). The heterogeneity of the Purkinje cell population has also been shown by the study of neurological mutations affecting this category of neurons in the cerebellum of the mouse. In some of these mutants (*pcd*, *nr*, *tbl*), the heterogeneity is unmasked by variations of topographically determined subsets of Purkinje cells in their vulnerability to the mutation (Wassef *et al.* 1987).

How is the parcellation revealed with mabs zebrin I and II and B30 correlated with projectional maps? Hawkes and collaborators (Gravel *et al.* 1987; Gravel & Hawkes 1990) have addressed this question in a series of experiments tracing anterograde projections either from the inferior olive (a unique source of climbing fibres) or from the thoraco-lumbar spinal cord (one of the several sources of mossy fibres), together with the zebrin I staining of Purkinje cells. The organization of the olivocerebellar projection is such that, although covering the entire surface of the cerebellar cortex, adjacent clusters of inferior olivary neurons do not necessarily project to adjacent parasagittal bands of cerebellar cortex (Azizi & Woodward 1987). Thus, a random injection in the inferior olive results in the labelling of several sagittal bands, which boundaries coincide with the real boundaries of the olivocerebellar projection. This important property in the organization of the projection is meaning with the approach followed by Hawkes and collaborators. Their results indicate that climbing fibre compartments coincide with antigenic boundaries between zebrin I positive and negative clusters of Purkinje cells. Furthermore, and rather surprising, the climbing fibre bands never subdivide an antigenic compartment, despite the low probability that random injections in the olive could repeatedly result in the complete filling of determined cortical bands by labelled climbing fibres.

However, the choice by Hawkes and collaborators of WGA-HRP as axonal tracer is not without danger. Indeed, the comparison between boundaries of climbing fibre stripes and shifts in zebrin I-immunoreactivity must be done in successive sections, that have different shrinkage ratios, owing to the use of acidic solutions needed to reveal the axonal tracer. Thus to be certain that Purkinje cell antigenic compartments and the parcellation of olivocerebellar projection are superimposed, we have repeated Hawkes' experiments, by using tritiated leucine as an anterograde axonal tracer, double labelling the same cerebellar sections with anti-zebrin I staining, and by using statistical analysis to determine the significance of the results. Under these favourable conditions we have shown (Wassef *et al.* 1990*a*) that: the boundaries of autoradiographically labelled climbing fibre bands and immunoreactive Purkinje cell subsets are strongly correlated. Furthermore, as expected from random olivary injections, we observed a high number of Purkinje cell shifts in immunoreactivity occurring inside an autoradiographically labelled band.

The results obtained by Gravel & Hawkes (1990) on the organization of the spinocerebellar projection are more difficult to interpret. These authors concluded that: whereas, some boundaries of the spinocerebellar terminal fields in the granule cell layer correlate well with shifts in Purkinje cell immunoreactivity, some others do not. For Gravel & Hawkes (1990), each antigenic compartment may be further divided into subzones, increasing the resolution of the projectional topography to small areas compatible with the size of microzones and patches identified by electrophysiological methods. In any case, these experiments show that there is also some degree of correlation between spinal mossy fibre distribution and zebrin I Purkinje cell compartments, strongly suggesting a possible functional significance for this compartmentation.

3. THE COMPARTMENTATION OF THE CEREBELLAR CORTEX BY ZEBRIN I ANTIGENIC EXPRESSION IS INDEPENDENT OF EXTRACEREBELLAR AFFERENT INNERVATION

The expression of zebrin I immunoreactivity is developmentally regulated. It begins to appear late, at post-natal day 6 (P6), by P12 all Purkinje cells are positive (Leclerc *et al.* 1988), and the adult pattern of sagittal parcellation gradually comes about during the third and fourth post-natal weeks, as parasagittally distributed populations of Purkinje cells lose expression of the molecule. The mechanisms for the organization of zebrin I + and − Purkinje cells into mutually exclusive compartments are not known. The possibility

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exists that post-natal synaptic connections with differential afferent inputs could impose upon a still homogeneous population of Purkinje cells the expression of the two phenotypes, thus explaining the tight correlation observed between zebrin I antigen compartmentation and the organization of cerebellar afferent projections.

Cerebellar grafts, taken from rat embryos (E12–E15) before the arrival of extracerebellar afferents, and implanted either in the anterior chamber of the eye or in a cavity within the neocortex, were used to evaluate the possible role of afferent fibres (Wassef *et al.* 1990*b*). These grafts mature into a minicerebellar structure consisting of a foliated trilaminated peripheral grey matter and a central deep nuclear zone. Double labelling with anti-zebrin I and anti-calbindin antibodies (the latter being a universal marker for Purkinje cells) have verified that both the zebrin I+ and the zebrin I− phenotypes are expressed in the minicerebellar structures. Moreover, their distribution pattern resembles that of control cerebellum. Thus two different environments (*in oculo* and *in cortico*) having a common feature, the absence of extracerebellar specific inputs, can support an apparent normal induction of zebrin I antigen compartments. The most reasonable conclusion is that the zebrin I phenotype matures independently of afferent influences. It could result either from clonally restricted heterogeneity in Purkinje cell precursors, or be developmentally acquired from differential phenotypic regulation of homogeneous young post-mitotic Purkinje cells.

The lack of influence of afferent inputs on zebrin I antigen compartmentation, as well as the congruity of the latter with projectional maps provide strong support for our working hypothesis of the essential role of Purkinje cells in the segregation and ordering of afferent fibres. However, the late development of zebrin I phenotypes, and the confinement of this antigen to the cytoplasm (Hawkes *et al.* 1985) argue against the possibility that this Purkinje cell marker could be involved, as a positional cue, in the formation of the maps. Nevertheless, in adult rats, zebrin I antigen is co-expressed with other markers of Purkinje cell heterogeneity, revealing an intrinsic compartmentation of the cerebellar cortex of presumed functional significance. In our hypothesis, Purkinje cell heterogeneity implies more than the presence or absence of zebrin I in Purkinje cell clusters. It also implies an early differential expression of additional cell surface molecules. So far, such cell surface markers remain to be discovered.

4. TRANSIENT PURKINJE CELL HETEROGENEITY DURING EMBRYONIC DEVELOPMENT: EARLY FORMATION OF AN INTRINSIC CORTICAL COMPARTMENTATION

A number of proteins and glycoproteins have been described that, although expressed by different neuronal populations in several regions of the adult brain, within the cerebellum they are exclusively present in Purkinje cells, and in all of them (these antigens are referred as 'Purkinje cell markers'). Among these 'Purkinje cell markers', we have used in our study (Wassef & Sotelo 1984; Wassef *et al.* 1985) four of them (cyclic GMP-dependent protein kinase, cGK; calbindin, a 28 kDa vitamin D-dependent calcium binding protein, CaBP; Purkinje cell specific glycoprotein, PSG and PEP 19), that have nothing in common besides being highly concentrated in these neurons.

Despite differences in the onset of expression (in rats, CaBP appears first at P16, cGK is the second at P17, and PSG and PEP19 are the latest appearing about E20) for each of these markers, there is a transient period in which only a subset of Purkinje cells is immunostained. They are arranged in alternating bilaterally symmetric positive and negative clusters. The pattern is reproducible for each marker, and for each embryonic age but it changes progressively with age, as the Purkinje cells develop. An important observation is that each antibody reveals a different cortical pattern, although some boundaries are common to two or more markers. This observation indicates that the acquisition of each marker is not just tied to a simple delayed expression by maturing Purkinje cells, but they follow different developmental pathways. The organization of the cerebellar cortex in alternating immunopositive and negative clusters of Purkinje cells is still observed after birth, until P5 when adjacent clusters become indistinguishable.

The results obtained with Purkinje cell markers, unmasking a transient biochemical heterogeneity among this neuronal population and their segregation into tight homogeneous clusters, strongly suggest the early establishment of an intrinsic cortical compartmentation. According to Wassef *et al.* (1985), the basic compartment is not the immunopositive or negative cluster of Purkinje cells by itself but the intersection of such clusters where, by definition, all these neurons are identical. A basic compartment is characterized by a combination of proteins or glycoproteins expressed at high or low levels. This combination will constitute the 'label' of the Purkinje cell compartment. Because the position in the cerebellar cortex of each basic compartment is characteristic and reproducible with age, the Purkinje cell heterogeneity builds up a reproducible internal 'map' of this cortex.

5. FORMATION OF THE OLIVOCEREBELLAR MAP

The congruity encountered in adult rat cerebellum between the olivocerebellar map and the cortical compartments of heterogeneous Purkinje cells suggests, as already discussed, that this molecular heterogeneity is implicated in the process of segregation of incoming inferior olivary axons. But, other mechanisms such as axon-to-axon interactions and pathway selection through specific environmental guidance cues in the cerebellar white matter might also produce ordered projections. Therefore, the formation of the olivo-

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cerebellar projection from its onset to its adult organization must be known to distinguish whether the axons grow directly to their eventual termination sites, or grow elsewhere and find their termination sites after a wider search.

The difficulties in using axonal tracing method in intra-uterine fetal rats and mice have been overcome by the introduction of the carbocyanine dye (DiI), applied to fixed tissue (Godement *et al.* 1987). Before the application of this technique, almost all studies involved post-natal animals and produced conflicting descriptions of the relationships between climbing fibres and Purkinje cells. Autoradiographic labelling of the projection 20 h after tritiated amino acid injections in the inferior olive of newborn rat pups (Sotelo *et al.* 1984) has shown that at this age the projection is heavy and properly organized into broad parasagittal bands. Therefore, the cerebellar arrival of these fibres and their organization must occur during fetal life. Moreover, the conclusion was reached that the climbing fibres were confined to the white matter, in a waiting compartment before invading the Purkinje cell zone. This interpretation could be due to the difficulty of discriminating autoradiographic labelling of thin fibres in the Purkinje cell layer from background. Recently, the use of a more refined axonal marker, rhodamine isothiocyanate (Mason *et al.* 1990) has disclosed that, at birth, climbing fibres are very thin and already in the cortical zone occupied by rows of Purkinje cells, discarding the existence of a waiting period.

The analysis of the embryonic development of the olivocerebellar projection has been recently carried out by Wassef *et al.* (1990*c*), mostly with DiI in fixed brains. Climbing fibres reach the cerebellar plate at E16, without entering it, ruling out any involvement of these fibres in the differential calbindin expression observed at this age. During the succeeding days (E17–E18), and following an unexpectedly slow growth, the olivary bundles grow over the cerebellar surface attaining the midline. Simultaneously, puffs of defasciculated fibres begin to advance deep, invading the cortical anlage according to a stereotyped and reproducible pattern. At E19–E20, the cerebellar cortex is filled with a dense network of labelled fibres, although reproducible topographic differences in density are still maintained. Calbindin immunolabelling of the same sections containing the DiI tracing has been extremely useful in correlating the progressive development of the olivary projection with the heterogeneity of Purkinje cells. Defasciculated puffs of climbing fibres first avoid the regions of tightly clustered Purkinje cells, travelling through raphes of lesser cell density. However, the fibres seem to be attracted by the Purkinje cell clusters, because at E18 they accumulate between and around these clusters. At E19 the variations in density of the labelled climbing fibres are at least partially correlated with the calbindin compartmentation of Purkinje cells.

The slow pace of climbing fibre growth, the fact that they begin to invade the prospective cerebellar cortex through stereotyped pathways apparently unrelated to the Purkinje cell heterogeneity, and later follow a differential development related to the Purkinje cell clustering suggest that a recognition phenomenon, which could involve timing and specific Purkinje cell marking, is operating in the E17–E20 cerebellum.

6. TRANSIENT BIOCHEMICAL HETEROGENEITY OF INFERIOR OLIVARY NEURONS DURING DEVELOPMENT

For the formation of cerebellar maps, within the context of the chemoaffinity hypothesis (Sperry 1963), the early compartmentation of the cerebellar cortex needs as a counterpart a similar acquisition of neuronal identities in precerebellar neurons, also based upon biochemical heterogeneity. The study in perinatal rats of the pattern of immunostaining with antibodies that either stain all neurons in the adult olive (CaBP) or do not stain any of them (parvalbumin, CGRP) has revealed a transient biochemical heterogeneity of olivary neurons. CaBP immunoreactivity (Wassef *et al.* 1990*d*) is first expressed by E16 when, in the rat, inferior olivary neurons are still in their migratory period and their axons have not penetrated the cerebellum. From this age till P14, CaBP positive neurons are confined to part of the ventral lamella of the principal olive (PO), and to a dorsomedial column of neurons comprising the beta nucleus, the caudal MAO and the dorsomedial cell column (dmcc). From P14, the immunostaining begins to spread the previously negative regions of the inferior olive, and at about one month, all neurons in this complex are CaBP positive. Parvalbumin immunoreactivity (Wassef *et al.* 1990*d*) begins to be observed at E18. From E19 until P7-P10, a small column of intensely immunoreactive neurons extends rostrocaudally covering most of the subnucleus named the dorsal cap of Kooy. Antibodies against calcitonin gene related peptide (CGRP) have also provided transiently immunoreactive neurons in restricted regions of the inferior olive (Morara *et al.* 1989). From the day of birth until P13, CGRP immunoreactive neurons are present in the medial sector of the caudal MAO, in the caudal part of the beta nucleus and dorsal cap of Kooy, as well as in a very restricted medial region of the caudal part of the dorsal accessory olive (DAO). By P13–P15 the immunoreactive neurons are no longer detectable. All these observations demonstrate that, during development, restricted parts of the inferior olivary complex are transiently individualized by their different biochemical properties, defining a compartmentation of this complex nuclear system.

Although the markers used to visualize the olivary compartmentation are not involved in the process of target recognition, they show that in subgroups of otherwise similar neurons, there is a coregulation of the expression of specific proteins. The existence of this coregulation marks the identity of the subgroup and can be used to particularize its response to external cues. Moreover, the first indication of a biochemical heterogeneity among inferior olivary neurons is observed at E16, before their axons, the climbing fibres, penetrate into the cerebellar parenchyma (see above). Hence, the acquisition of both, cerebellar and olivary

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compartments, takes place independently one from another.

7. THE SPINOCEREBELLAR PROJECTION IN AGRANULAR CEREBELLA OF MUTANT MICE: PURKINJE CELLS AS PRESUMPTIVE ORGANIZER ELEMENTS

Spinal neurons projecting to the cerebellum terminate as mossy fibres, mostly in the cortex of the anterior vermal lobe, where they form longitudinal bands or columns. In the adult cerebellum, the post-synaptic targets of these axons are the granule cell dendrites and, to a lesser extent, the Golgi cells. However, during development, mossy fibres of unknown origin can establish transient contacts with Purkinje cells (Mason & Gregory 1984). The anatomical analysis of the formation of the spinocerebellar projection in the rat (Arsenio-Nunes & Sotelo 1985) has disclosed that, although the axons reach the cerebellum during foetal life, the adult topographic pattern is acquired during the first post-natal week. From P3, when the axons begin to invade the cortical gray matter of preferential places, with important fibre dispersion between the nascent columns (protocolumnar stage), until P7 when the axons are already organized with the adult columnar pattern. There is a temporal correlation between the protocolumnar stage, the formation of the incipient inner granule cell layer, and the appearance of earliest-maturing mossy rosettes. This suggests that the synaptic interactions between spinal fibres and their granule cell targets could be involved in the organization of the topography of the spinocerebellar projection. The hodological analysis of the terminal arrangement of thoracolumbar spinocerebellar projection in cerebella of some mutant mice does not support this interpretation.

Weaver (*wv*) is a recessive mutation, mapped on chromosome 16, principally affecting postmitotic granule cells (Sotelo & Changeux 1974*a*; Goldowitz & Mullen 1982). In this mutant, all or most of the premigratory, post-mitotic granule cells degenerate. Thus spinocerebellar neurons will organize their projections in the absence of their main post-synaptic targets, and in the presence of normal Purkinje cells. Conversely, the agranularity in the staggerer (*sg*) cerebellum (recessive mutation, mapped on chromosome 9) is secondary to Purkinje cell abnormalities (Herrup & Mullen 1981). Their number is reduced to less than 25%, and the remaining ones have ectopic positions and lack distal spiny branchlets (Sidman 1968). In the adult *sg* cerebellum, there is an almost complete absence of granule cells, because of a reduced mitotic rate of their precursors, and to the massive death of post-migratory granule cells during the first four postnatal weeks. Both alterations are provoked by the failure of Purkinje cells to regulate granule cell proliferation and to offer postsynaptic targets to granule cell axons. (Sotelo & Changeux 1974*b*). Hence, in the *sg* cerebellum, spinocerebellar neurons will organize their projection in the presence of a small contingent of granule cells but in the absence of normal Purkinje cells.

In the *wv* cerebellum, the spinal fibres succeed in organizing terminals zones, occupying most of the vermal cortex, that are segregated in parasagittal columns, mimicking the projectional pattern found in controls. Whereas, in the *sg* cerebellum, spinal fibres are distributed more or less homogeneously throughout the vermal cortex, without the columnar segregation characterizing control animals (Arsenio-Nunes *et al.* 1988). Hence, other factors that the intensity and precocity of the degranulation should be involved in the organization of the spinocerebellar map.

The most obvious difference between the *wv* and the *sg* cerebellum resides in their Purkinje cell composition. In the *wv*, although the vermis only contains half of the normal amount, Purkinje cells seem genetically normal (Goldowitz & Mullen 1982). Even the typical heterogeneity and compartmentation revealed with the anti-zebrin I antibody is preserved (unpublished observations). Conversely, in the *sg* the remaining Purkinje cells, in addition to the lack of spiny branchlets, bear important biochemical alterations affecting either some of their cell surface proteins (Hatten & Messer 1978; Edelman & Chuong 1982) the calcium membrane conductances (Crepel *et al.* 1984), the turn off of the normal expression of the calmodulin gene (Messer *et al.* 1990) or, more importantly, the expression of markers for adult Purkinje cell heterogeneity and compartmentation (none of the *sg* Purkinje cells have immunohistochemically detectable zebrin I antigen, unpublished observations). All these results point to the appealing hypothesis that Purkinje cells are the central elements in the organization of spinocerebellar maps.

8. MECHANISMS UNDERLYING THE ARRANGEMENT OF CEREBELLAR MAPS

The observations summarized in this review allow us to propose that the cerebellar cortex is constructed by the assembly of small sets of Purkinje cells that are labelled by a particular selection of a large collection of molecules. By a combinatory mechanism, as discussed by Wassef *et al.* (1990*a*), the number of sets with different identities can be as large as needed for the physiology of the cerebellar cortex (the sets can reach the high number needed for the specification of the microzones, see Oscarson (1979)). The diversity of sets and their distribution, either in adjacent stripes or in patches, can be involved in the segregation of incoming afferent fibres and, thus, in the organization of their projection map. According to the mechanism proposed by Meinhardt (1983) for the organization of subfields during development, the common boundary between two sets of Purkinje cells can be at the origin of gradients of positional information. Indeed, if we assume the two different sets cooperate for the production of a label, this cooperation imposes that the synthesis of the label takes place at their boundary. Hence, by diffusion and decay, the generated gradient of positional information will attain its highest con-

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centration at this boundary. The hypothesis we favour (Sotelo 1987; Wassef *et al.* 1990*a*) is that the precise matching between the organized afferent inputs and pre-defined cerebellar microzones occurs directly, by the matching of identification cues in growth cones of incoming afferent axons and in Purkinje cells. Such an identification mechanism could allow the establishment of cerebellar maps with strict topographic order, even in absence of synaptogenesis, as inferred from one of the models proposed by Malsburg & Willshaw (1976).

Hawkes and collaborators (Leclerc *et al.* 1988) have proposed an alternative model to our direct matching hypothesis. For these investigators the matching can be achieved indirectly by both the Purkinje cells and the afferent axons recognizing the cerebellar nuclei as a common third party. To consider the cerebellar nuclei as the candidate to match cerebellar cortex afferent and efferent topographies is an attractive proposition, as Purkinje cell projections and extracerebellar afferent axons share, in these nuclei, common target territories. Unfortunately, this proposition does not take into consideration that, during the onset of afferent fibre penetration into the cerebellar parenchyma, these axons rapidly and directly enter in cortical territories occupied by Purkinje cells, and it is only later that collaterals reach the cerebellar nuclei (Wassef *et al.* 1990*c*).

In conclusion, cerebellar maps are organized by matching topographic chemical labels between incoming afferent fibres and Purkinje cells. This mechanism appears to be able to construct maps to the level of small modular cerebellar zones. It remains an open question if the fine tuning of the projection to the single cell level still requires an additional mechanism based upon activity of the developing implying redundancy of the forming synapses (there is a transient period of multiple innervation of Purkinje cells by climbing fibres, Crepel *et al.* (1976)) and regression of supranumerary ones, as postulated by Changeux & Danchin (1976) in their theory of selective stabilization of synapses.

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