

CEREBELLAR SYNAPTOGENESIS: WHAT WE CAN LEARN FROM MUTANT MICE

By CONSTANTINO SOTELO

*Laboratoire de Neuromorphologie, INSERM U. 106, Hôpital de la Salpêtrière,
75013 Paris, France*

Summary

Examination of perturbations in the adult cerebellar connectivity, that follow well-defined lesions produced by gene mutations in the mouse, reveals a few of the numerous and intricate cellular interactions taking place during synaptogenesis. In *weaver* and in the central ectopia of *reeler*, Purkinje cells form innumerable dendritic spines, despite the absence of parallel fibers. Only a small proportion of these spines are innervated, and their presynaptic partners are mossy fibers (heterologous synapses) originating from spinal cord, but not from pontine nuclei. Hence, the early phase of membrane recognition is based more on a hierarchical choice between a wide range of graded preferences, than on the complementarity of a narrow range of synaptic affinities. The comparative analysis of *weaver*, *reeler*, *staggerer* and *hyperspiny Purkinje cell* has allowed us to establish that the late phase of synapse stabilization or elimination, leading to the numerical matching of one climbing fiber per Purkinje cell, is not based on climbing fiber translocation. Conversely, this regression appears to be the result of a process of competition between climbing fibers and parallel fibers. Whatever the mechanisms of the competition are, the results obtained with the mutants suggest that activity of the forming cerebellar circuitry is involved in their regulation. Finally, a new mutation is reported, the *nodding* mouse, to illustrate the fact that the ultimate morphology of presynaptic boutons results from an interplay between intrinsically regulated factors (features of presynaptic organelles) and the morphogenetic influence of postsynaptic partners. This accounts for the size and shape of the boutons as well as for the class of synaptic junction. Furthermore, this morphogenetic influence is not restricted to early life but occurs whenever the originally established balance between pre- and postsynaptic elements is upset.

Introduction

The essential feature in the organization of the central nervous system is the precision and order exhibited by nerve cell connections. The genesis of the specificity of these connections is the ultimate goal of the complex sequence of developmental events that, proceeding with strict and defined kinetics through neuronal migration and differentiation together with oriented axonal outgrowth, will end in the establishment of functional neuronal networks. Therefore, the final

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choice between pre- and postsynaptic partners (synaptogenesis) results from the coherence of all previous events. Furthermore, synaptogenesis is achieved not only when contacts between partners are permitted but when they are stabilized and maintained (Changeux and Danchin, 1976). Thus, the development of a synapse is a relatively late event, that lasts for days or even weeks and results from a cascade of complex molecular processes, starting from membrane recognition between eventual partners and ending with the stabilization of some of the immature contacts and the regression of the others.

The mouse cerebellum, especially its cortex, offers an optimal material for the study of some of the cellular mechanisms involved in the different phases of the synaptogenic process. The relative simplicity of the circuitry, owing to the low number of neuronal populations and afferent systems and their precise geometrical distribution, has greatly facilitated the understanding of adult cerebellar synaptology, both electrophysiologically (Llinas, 1981) and morphologically (Palay and Chan-Palay, 1974). More importantly, the cerebellum of the mouse is the target of numerous mapped gene mutations (Lyon and Searle, 1989). These offer a means, through well-defined lesions, of disrupting the normal process of synapse formation and, by the analysis of the consequences of the perturbations, of revealing a few of the numerous complex interactions involved in the sequential phases of cerebellar synaptogenesis.

The work that I will discuss in this paper concerns studies carried out during the last 16 years on cerebellar mutant mice. I will only attempt to emphasize some general points emerging from this material, as well as some of the most recent results.

Differentiation of pre- and postsynaptic elements: an independent or an induced process?

In adult cerebellum, as elsewhere in the central nervous system (CNS), synaptic contacts are characterized by a subset of common features (see Peters *et al.* 1976) that allow the ultrastructural identification of pre- and postsynaptic elements by the specialization of the patches of neuronal membranes involved in synaptic transmission. During development, it is also possible to correlate changes in membrane structure with phases in the synaptogenic process (Landis and Weinstein, 1987). What is the sequence in the acquisition of these features? Do they differentiate simultaneously in both pre- and postsynaptic membranes? Can they be formed in the absence of their respective partner? The answers to these questions will provide indications about the mechanisms governing the differentiation of synaptic membranes, and the mutant material can partially answer these questions.

Are presynaptic elements needed for the formation of postsynaptic specializations?

Purkinje cells are the pivotal elements of the cerebellar cortex around which all

the cortical circuitry is organized. Over 90 % of the synaptic inputs received by these neurons originate from parallel fibers, the axons of granule cells. The parallel fibers synapse on the numerous spines emerging from the distal compartment of Purkinje cell dendrites, the spiny branchlets. Granule cells are congenitally absent in the *weaver* mutant (Rakic and Sidman, 1973; Sotelo and Changeux, 1974b; Goldowitz and Mullen, 1982; Hatten *et al.* 1986) and in the massive ectopia of Purkinje cells created by the *reeler* in the central cerebellar mass (Mariani *et al.* 1977). Purkinje cells, in both mutations, develop atrophic and disorganized dendrites that are studded with spines, despite their extreme denervation. These spines, that bear all the common features of normal spines (Hirano and Dembitzer, 1973; Rakic and Sidman, 1973; Sotelo, 1973, 1975a; Landis and Reese, 1977) are, however, devoid of presynaptic elements, being directly apposed to thin astrocytic processes. Therefore, in these two mutant mice, in which the only common bond is the absence of parallel fibers, deafferented Purkinje cells are able, by an independent mechanism, to complete spinogenesis and differentiation of postsynaptic specializations, the morphological correlate of receptor surfaces. Moreover, deafferented Purkinje cells can also produce ectopic postsynaptic differentiations along the smooth surface of their membranes (Sotelo, 1975a; Mariani *et al.* 1977). Their existence testifies not only to the capacity of Purkinje cells to develop receptor surfaces in an autonomous manner, but also permits the hypothesis that these neurons could determine the total amount of acceptable synaptic inputs by elaborating an almost constant amount of postsynaptic receptive surface, as also suggested in another situation by Hillman and Chen (1981). These kinds of results are unique, and are difficult to extrapolate to other regions of the CNS.

Can presynaptic elements differentiate and survive in the absence of postsynaptic partners?

Studies on cultured neurons indicate that presynaptic differentiations are another intrinsic feature of developing neurons. Indeed, presynaptic elements can differentiate even on artificial surfaces, such as polylysine-coated beads (see references in Burry, 1987). Is it possible to reproduce these results in the *in vivo* mouse cerebellum? Or, dealing with parallel fiber–Purkinje cell synapses, are parallel fibers able to differentiate presynaptic varicosities on their own, or are they under the inductive effect of specific postsynaptic targets? The answer to this question was obtained by analyzing the development of granule cells and their parallel fibers in a situation in which almost 90 % of their normal postsynaptic targets never form, i.e. that of the *staggerer* mutation (Sidman *et al.* 1962).

The cerebellum of *staggerer* lacks most Purkinje cells before granule cell proliferation and migration (Herrup and Mullen, 1981). Moreover, the remaining cells (less than 25 % of the whole population) are abnormal since, among other deficiencies, they have undeveloped spiny branchlets with a consequent absence of distal spines, the postsynaptic targets of parallel fibers. In this cerebellum, parallel fibers are able to establish junctional complexes with the shafts of Purkinje cell

dendrites, as in control mouse cerebellum (Landis and Weinstein, 1987). Nevertheless, owing to the lack of dendritic spines, these initial contacts do not evolve into immature synapses. Despite this aberrant development, parallel fibers succeed in forming varicosities (Fig. 1) that resemble axon terminals bearing presynaptic vesicular grids (Pfenninger *et al.* 1969) and establish pseudosynaptic contacts with thin astroglial processes (Sotelo, 1973, 1979; Sotelo and Changeux, 1974a; Landis and Sidman, 1978). These results indicate that granule cells follow an intrinsic developmental program leading to the differentiation of presynaptic elements. Of course, the maturation of parallel fibers in the *staggerer* cerebellum occurs in a complex cellular environment and other explanations are possible. For instance, either the initial junctions with Purkinje cell dendritic shafts or the adhesive surface of the astrocytic process could contribute to the differentiation of presynaptic elements. In this respect, it is worth mentioning that transient axoglial synaptoid contacts have been encountered in the mouse spinal cord during the early synaptogenic period (Henrikson and Vaughn, 1974). These contacts have been interpreted as favoring a low-affinity stage in the initial phases of synapto-

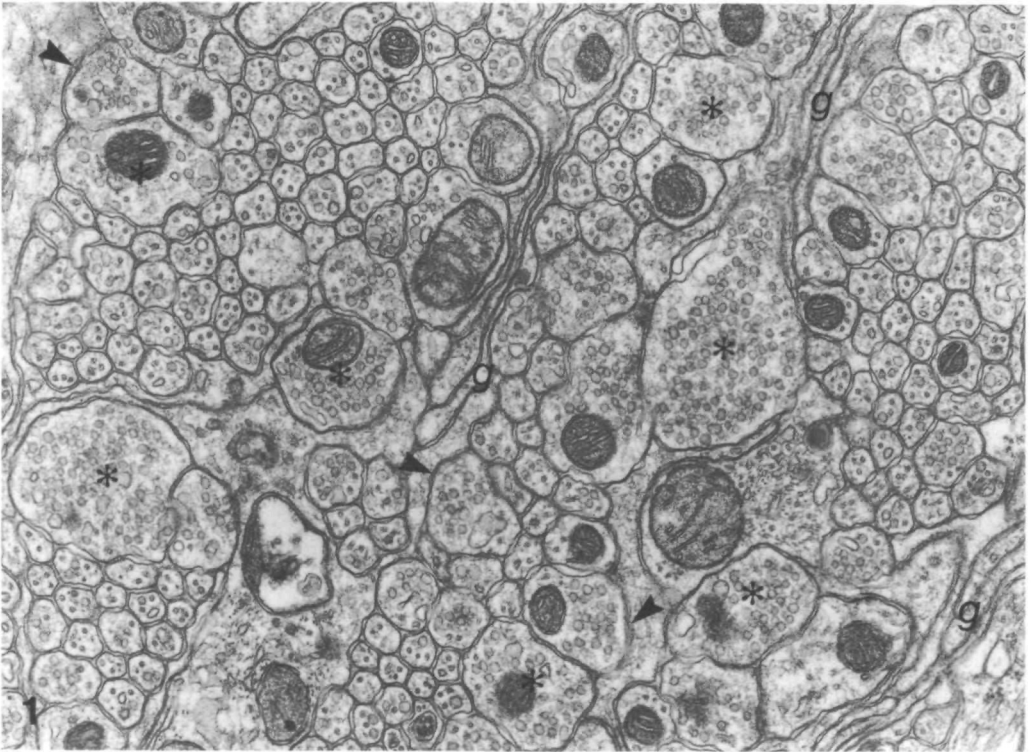


Fig. 1. Molecular layer in a 28-day-old *staggerer* mouse. The neuropil contains bundles of parallel fibers separated by thin protoplasmic lamellae of astrocytes (g). Note the presence of abundant parallel fiber varicosities resembling presynaptic boutons (asterisks), directly apposed to or facing the glial processes (arrowheads). $\times 31\,000$.

genesis that could contribute to the maintenance of presynaptic elements for further synaptogenesis with more specific postsynaptic targets.

Whatever the mechanisms leading to the formation of parallel fiber varicosities, in the *staggerer* cerebellum it is clear that they are formed in the absence of their specific postsynaptic elements. More importantly, the *staggerer* cerebellum has allowed us to conclude (Sotelo and Changeux, 1974a) that granule cells are dependent on their synaptogenesis for survival. Hence, in the molecular layer of P26–P43 *staggerer* cerebellum, the presynaptic varicosities facing astroglial processes degenerate, provoking the death, through lack of retrograde synaptic signaling, of their neurons of origin. Thus, during the third and fourth postnatal week, there is a rapid and massive granule cell death, resulting in an almost completely agranular cerebellum. This leads to reactive gliosis of the molecular layer and preservation of those parallel fibers that have successfully formed synapses with local circuit neurons (Fig. 2). These findings suggest that the early presynaptic boutons are labile structures, which need the presence of the postsynaptic neuron and/or synaptic function to become stabilized and survive.

The question that arises from the above conclusion is: can parallel fiber

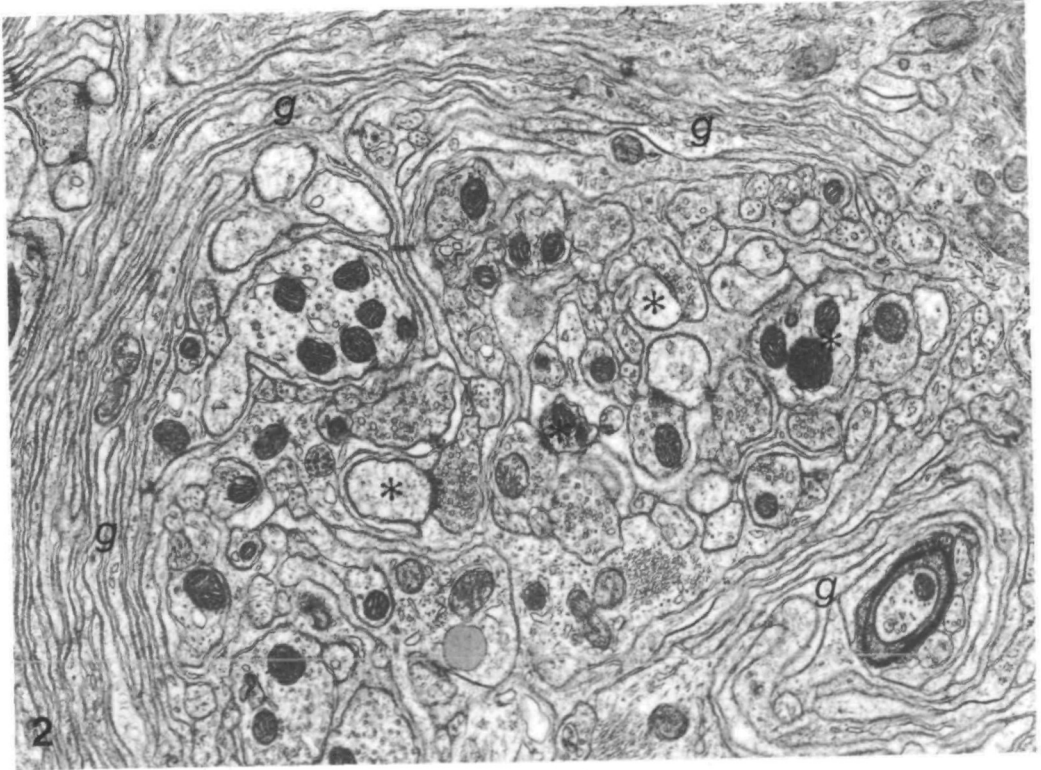


Fig. 2. Molecular layer in a 6-month-old *staggerer* mouse. The neuropil is mostly filled by thin astrocytic processes (g) that surround a small island containing dendritic profiles of several sizes. Some of the latter are postsynaptic (asterisks) to axon terminals belonging to parallel fiber varicosities. $\times 17\,000$.

varicosities survive after removal of their postsynaptic targets, once they have been synaptically active? This question can be answered, at least partially, by studying the cerebellar hemispheres of the *nervous* mutant mouse (Sidman and Green, 1970). In this mutant, Purkinje cells develop normally until P20 (Landis, 1973), when the majority of synaptic connections with parallel fibers have been formed. Between P20 and P60, over 90 % of the Purkinje cells degenerate in the hemispheric cortices. In the adult *nervous* mouse there is a slow, progressive degeneration of parallel fibers and their granule cells. In mice aged over 1 year, this loss has been estimated to be about 50 % of the granule cell population (Sotelo and Triller, 1979). The most plausible interpretation of these findings is that once the parallel fibers have been stabilized through functioning, they are able to survive for long periods independently of their postsynaptic targets. A retrograde signaling, most probably concomitant with synaptic activity, seems necessary for the passage of labile presynaptic elements to permanent ones.

Synaptic specificity: the mossy fiber system

The cerebellum is characterized by a strict selectivity of the intercellular connections between axon afferents and cerebellar neurons, as well as by a large topographic precision in the organization of afferent and efferent projections (topographical maps). In the adult cerebellum, mossy fibers establish synaptic contacts with granule cell dendrites and with Golgi cells, but not with the other categories of cerebellar neurons, especially Purkinje cells (Palay and Chan-Palay, 1974), although transient immature contacts have been reported between extremely long filopodia arising from mossy fiber primary swellings and Purkinje cells during the late developmental period (second postnatal week, Mason and Gregory, 1984). That the axons of an afferent system form synapses with neurons of one class but not with those of an adjacent class supports the concept that axon to neuron conjunction is the result of a mechanism of specific chemical affinity between matched identification labels in the growing presynaptic axons and in their target postsynaptic neurons (Sperry, 1963: the chemoaffinity hypothesis). Hence, membrane recognition, adhesion and formation of an initial synaptic contact would only occur between axons and targets provided with complementary cytochemical affinities.

A strict application of this specificity principle cannot be readily reconciled with some kinds of synaptic interrelationships, occurring mainly in mossy fiber afferents in the cerebellar cortex of mutant mice, where Purkinje cells develop in the absence of granule cells (in the *weaver* mouse and the central cerebellar mass of the *reeler* mouse). Under these conditions, some of the mossy fibers can establish synaptic contacts on inappropriate target neurons, especially on Purkinje cell distal spines (Fig. 3) (Sotelo, 1975a; Mariani *et al.* 1977). These heterologous synapses are physiologically active since over 15 % of tested Purkinje cells, in the central cerebellar mass of the *reeler* cerebellum, respond with monosynaptic simple spikes after juxtafastigial stimulation (Mariani *et al.* 1977). Therefore, the

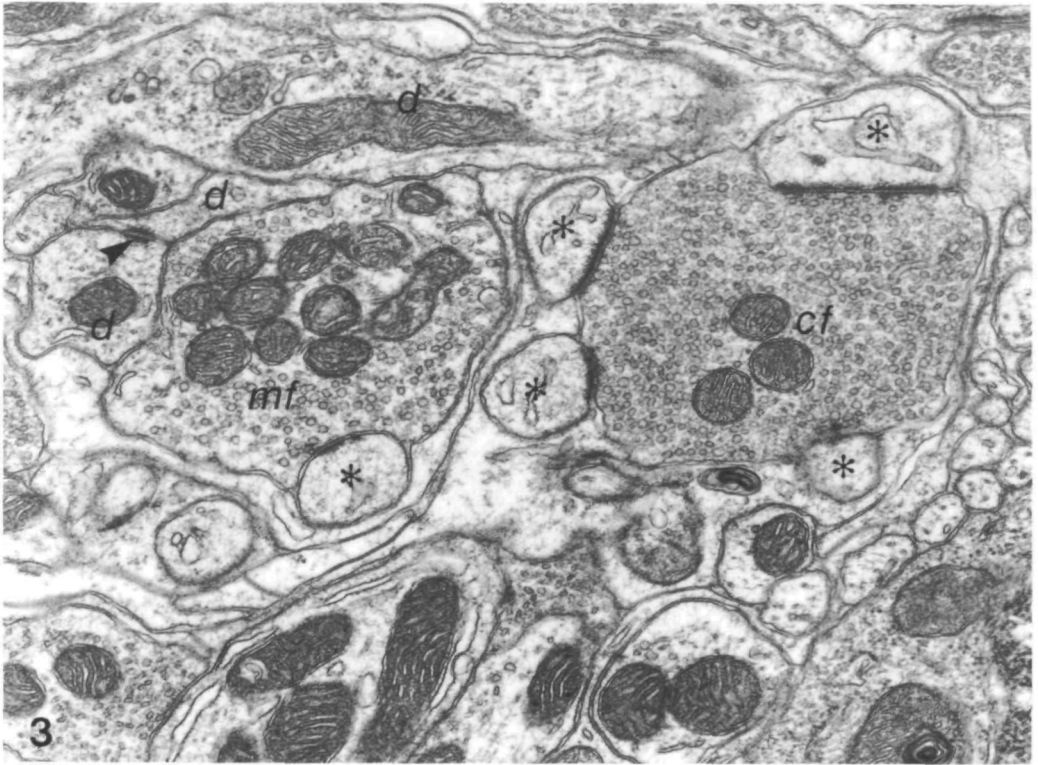


Fig. 3. Two presynaptic elements in the granular layer of the *reeler* mouse synapse on Purkinje cell dendritic spines (asterisks). One of the terminals (*mf*) is apposed to dendritic profiles of granule cells (*d*), joined by an attachment plate (arrowhead), and shares intrinsic features with mossy fibers. The second axon terminal (*cf*) is only apposed to the dendritic spines, and it shares intrinsic features with those of climbing fibers. (Micrograph taken from Wilson *et al.* 1981.) $\times 28\,000$.

local cellular environment seems to play an important role in the regulation of the synaptic specificity.

Mossy fibers constitute a heterogeneous afferent system originating from many different sources, particularly the spinal cord and the pontine nuclei. Axonal tracing methods have allowed us to show that most of the heterologous mossy fiber synapses in the *reeler* belong to the spinocerebellar system (Fig. 4) (Wilson *et al.* 1981). Furthermore, Goffinet *et al.* (1984) have autoradiographically traced the pontine projections, and shown that they are distributed within the granular layer of the hemispheres, preventing them from being engaged in the heterologous synapses of the central cerebellar mass. Although a variety of mechanisms could explain these findings, an attractive explanation could be based on the existence of a certain degree of chemoaffinity, as inferred from the hypothesis of a hierarchy of choices. The early steps of membrane recognition may not require a complementarity of identification tags as exquisite as that generally assumed to account for

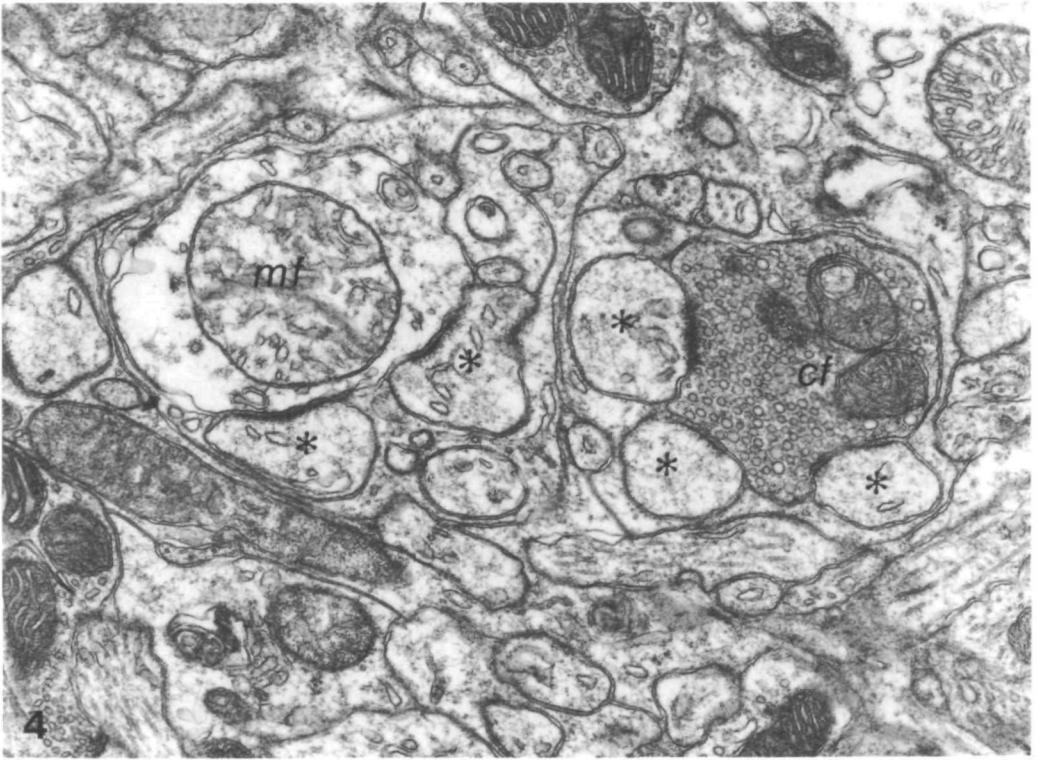


Fig. 4. Neuropil of the medial region of the central cerebellar mass in the *reeler* mouse, fixed 24 h after ipsilateral hemicordotomy. In this neuropil two presynaptic elements are in synaptic contacts with Purkinje cell spines. One of the terminals (*mf*) is a spinal mossy fiber, exhibiting the 'clear' pattern of degeneration indicated by electron-lucent axoplasm and swollen mitochondria. The second axon terminal (*cf*) belongs to a climbing fiber varicosity. (Micrograph taken from Wilson *et al.* 1981.) $\times 28\,000$.

synapse specificity (Sperry, 1963). A compatibility of chemical labels based on a hierarchy of preferences could account for the relative degree of specificity deduced from the hodological results. Thus, the synaptic recognition molecules at Purkinje cell branchlet spines will have preferential compatibility for a similar marker in parallel fiber varicosities; in the absence of these inputs, the spines could accept a second or even a third choice of afferent. In any case, the study of mossy fiber synaptogenesis in the mutants indicates that the intrinsic specificity for afferent–efferent coupling can be more or less overridden by local factors, conferring to the system a certain degree of adaptation to abnormal situations.

Synaptic rearrangement: the climbing fiber system

The numerical relationship existing between climbing fibers and Purkinje cells, each Purkinje cell being synaptically contacted by a single climbing fiber, provides

an optimal situation in the CNS for examining mechanisms regulating the quantitative matching between synaptic partners during development. The possibility that, during maturation of climbing fiber synapses, a transient phase of multiple innervation could occur has been analyzed with electrophysiological methods. Crepel *et al.* (1976) have shown that the most important difference between immature and adult climbing fiber responses is that the immature responses are not all-or-none in character but are graded in parallel with increasing stimulus intensities, similar to 'compound' EPSPs recorded from immature neuromuscular junctions (Redfern, 1970; Bennett and Pettigrew, 1975). These graded responses have been considered to correspond to a transient stage of multiple innervation, and have permitted the determination of its chronological limits. At P3 there is initiation of synaptogenesis, at P5 multiple innervation reaches a peak, between P7 and P10 there is an abrupt decline in the number of climbing fibers impinging on single Purkinje cells and by P14 only mono-innervated Purkinje cells remain (Crepel *et al.* 1981; Mariani and Changeux, 1981).

One of the mechanisms most widely implicated in the process of synaptic rearrangement and numerical matching has been that of competition between the different axons innervating a common postsynaptic target (Betz *et al.* 1980, in the case of motor unit size). Axons seem to compete for some kind of trophic or stabilizing factor that is produced by the target neuron and is necessary for the survival of the afferent inputs. A similar hypothetical explanation can be applied to the withdrawal of the multiple innervation of Purkinje cells by climbing fibers, although evidence in favor of such a factor and of its nature are missing. Two, out of many, potential processes can be evoked to explain the competition leading to the loss of supernumerary climbing fiber synapses: (1) priority of translocation from their initial perisomatic to their ultimate peridendritic location, so the first climbing fiber to be attracted to the dendritic domain will win the contest and become stabilized; (2) the presynaptic reward could result from 'resonance' between intrinsic activity of one of the climbing fibers and that of the postsynaptic Purkinje cell, determined by the general activity of the developing network. The cerebellar mutations have, here again, been extremely useful for obtaining information concerning the validity of such mechanisms.

Climbing fiber translocation and the withdrawal of multiple innervation

The peak of the multiple innervation of Purkinje cells by climbing fibers coincides with the 'nest stage' (Ramón y Cajal, 1911) of climbing fiber development. It is during the period of abrupt decline of the multiple innervation that climbing fibers start their peridendritic translocation (Larramendi, 1969). This coincidental timing suggests that there could be some correlation between these two apparently independent processes. The verification or invalidation of such an explanation can be obtained through the study of the *staggerer* (Sidman *et al.* 1962) and the *hyperspiny Purkinje cell (hpc)* cerebellum (Guénet *et al.* 1983). In these two mutants, synaptogenesis between climbing fibers and Purkinje cells begins on perisomatic spines (Sotelo, 1975b; Landis and Sidman, 1978), but translocation is

almost nonexistent. Indeed, the study of old mutant mice has shown that adult Purkinje cells retain most of their somatic spines with their attached climbing fibers (Figs 5, 6). Moreover, climbing fibers on the dendrite are rare and are only present on the proximal third of the main dendritic segments (see Fig. 8), suggesting that in these mutants the sequential process of climbing fiber synaptogenesis does not evolve further than the 'capuchon' stage (Ramón y Cajal, 1911). Electrophysiological analysis of climbing fiber–Purkinje cell synapses in these adult mice has revealed that in the *staggerer* the Purkinje cells remain multiply innervated (Crepel *et al.* 1980; Mariani and Changeux, 1980), whereas in the *hpc* they are monoinnervated (Guénet *et al.* 1983). These findings clearly indicate that the translocation of climbing fibers *per se* is not a determinant of the numerical rearrangement of these synaptic connections. As discussed below, the main difference between these two mutant mice is that in *staggerer* parallel fiber–Purkinje cell synapses do not form, whereas in the *hpc* synapses of this class are established not only on distal spines but also on ectopic spines emerging from

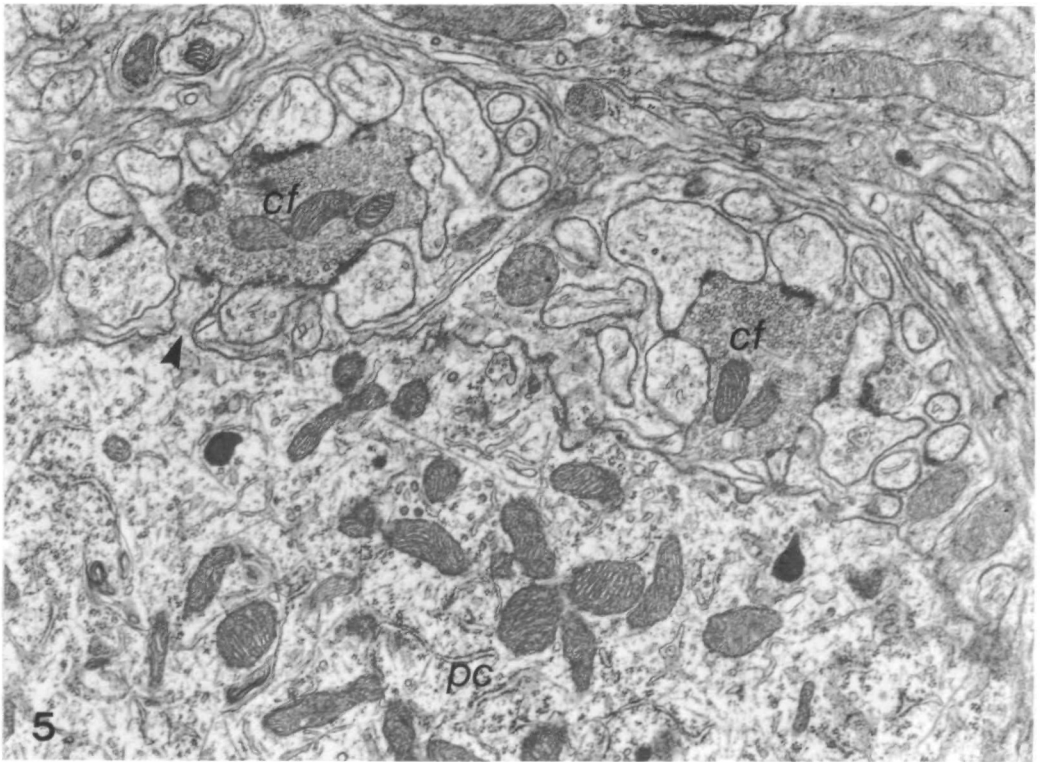


Fig. 5. Purkinje cell perikaryon (*pc*) in a 6-month-old *staggerer* mouse. Numerous perikaryal spines, one of them (arrowhead) still in continuity with the Purkinje cell, are postsynaptic to two axon terminals belonging to climbing fiber varicosities (*cf*). Note that these perisomatic climbing fibers establish rosette-like terminals with the spines, as in the stage of pericellular nest formation, testifying to their impaired translocation. $\times 17000$.

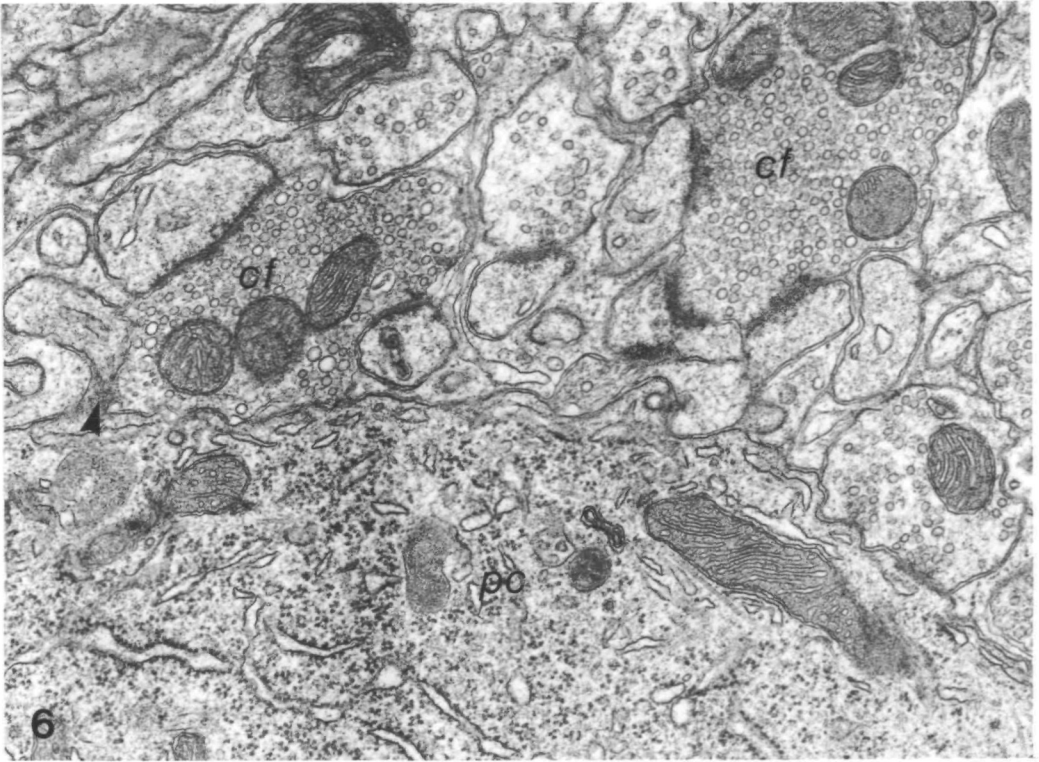


Fig. 6. Similar situation to that in Fig. 5, but in a 45-day-old *hyperspiny Purkinje cell* mouse. The climbing fibers (*cf*) also retain their perisomatic location. Arrowhead indicates point of contact between Purkinje cell and climbing fiber. $\times 28\,000$.

proximal dendritic segments. Indeed, one of the constant features of the *hpc* cerebellum is that its Purkinje cells have spines all over their somata as well as on their proximal and distal dendrites (for this reason, we decided to call this mutant *hyperspiny Purkinje cell*, although it is not certain that the total number of spines is greater than in control Purkinje cells). The ectopic spines could be the result of the impaired translocation of climbing fibers, as is the case in experimentally lesioned rats with perinatal transection of the olivocerebellar pathway (Sotelo and Arsenio-Nunes, 1976; Bradley and Berry, 1976). Parallel fibers, which contribute from the beginning of their synaptogenic period to the innervation of these spines, can modify their size and shape to form large rosette-like terminals covered by postsynaptic spines (see Fig. 9). The importance of this observation will be discussed in the context of findings obtained from the *nodding* cerebellum.

Furthermore, the study of the *hpc* cerebellum has also revealed that the absence of climbing fiber translocation does not prevent basket cell axons from proceeding with their developmental program. The axons of these inhibitory interneurons establish normal synaptic contacts on the smooth perikaryal surface (Fig. 7) and on the initial segment of the axon of Purkinje cells constituting, respectively,

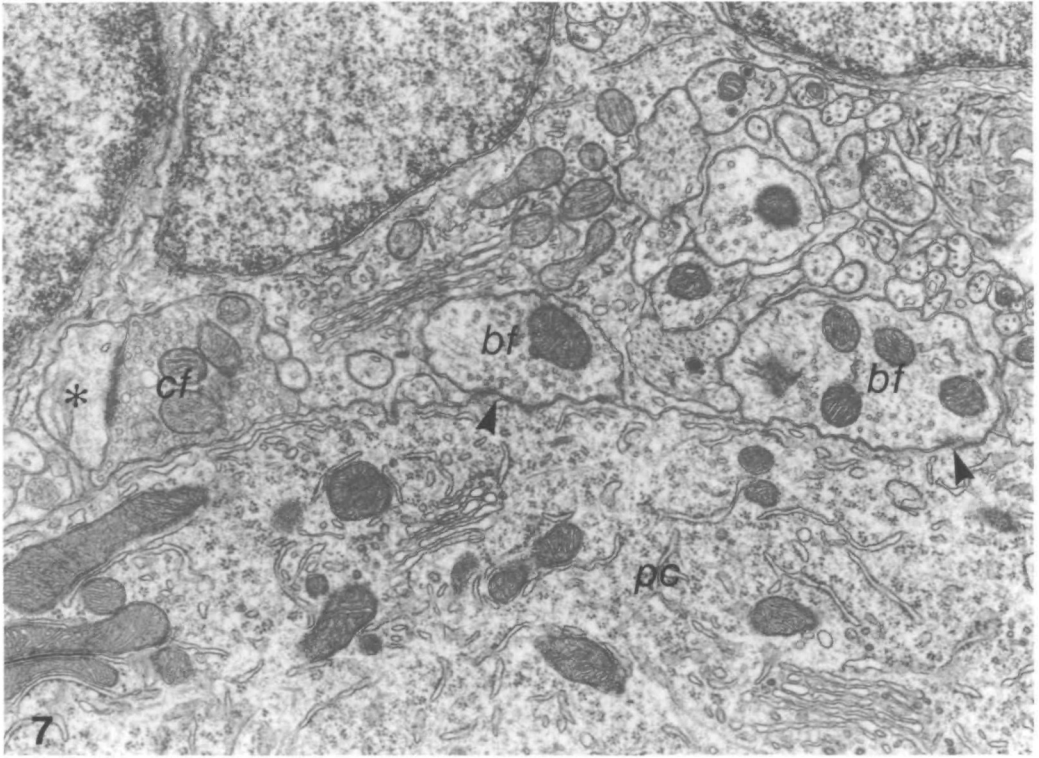


Fig. 7. Purkinje cell perikaryon (*pc*) in a 6-month-old *hyperspiny* Purkinje cell mouse. Three presynaptic boutons are apposed to this perikaryon. Two of them (*bf*) belong to axons of molecular layer local circuit neurons, and they establish synaptic contacts (arrowheads) on the smooth surface of the Purkinje cell. The third bouton (*cf*) belongs to a climbing fiber varicosity, and synapses on a somatic spine (asterisk). $\times 20\,000$.

pericellular baskets and 'pinceaux' formations. These observations give a new insight on the mechanisms involved in the late synaptogenic process between basket cell axons and Purkinje cells. During normal development, it is when climbing fibers abandon their somatic location that basket cell axons begin to replace them, suggesting a competition for postsynaptic space between these two categories of inputs. The *hpc* cerebellum proves that, since both inputs coexist on the same perikaryal surface, such steric competition cannot explain the sequential changes of axosomatic synapses, i.e. that climbing fiber nests evolve into pericellular basket axonal formations.

Parallel fiber–Purkinje cell synapses during climbing fiber synaptic rearrangement

Studies on adult granulooprival cerebella (*weaver*, and the central cerebellar mass of *reeler*) have determined that there is an anomalous numerical matching between climbing fibers and Purkinje cells. Indeed, electrophysiological data from *weaver* (Crepel and Mariani, 1976; Siggins *et al.* 1976; Puro and Woodward, 1977) as well as from *reeler* (Mariani *et al.* 1977) indicate that Purkinje cells deprived of

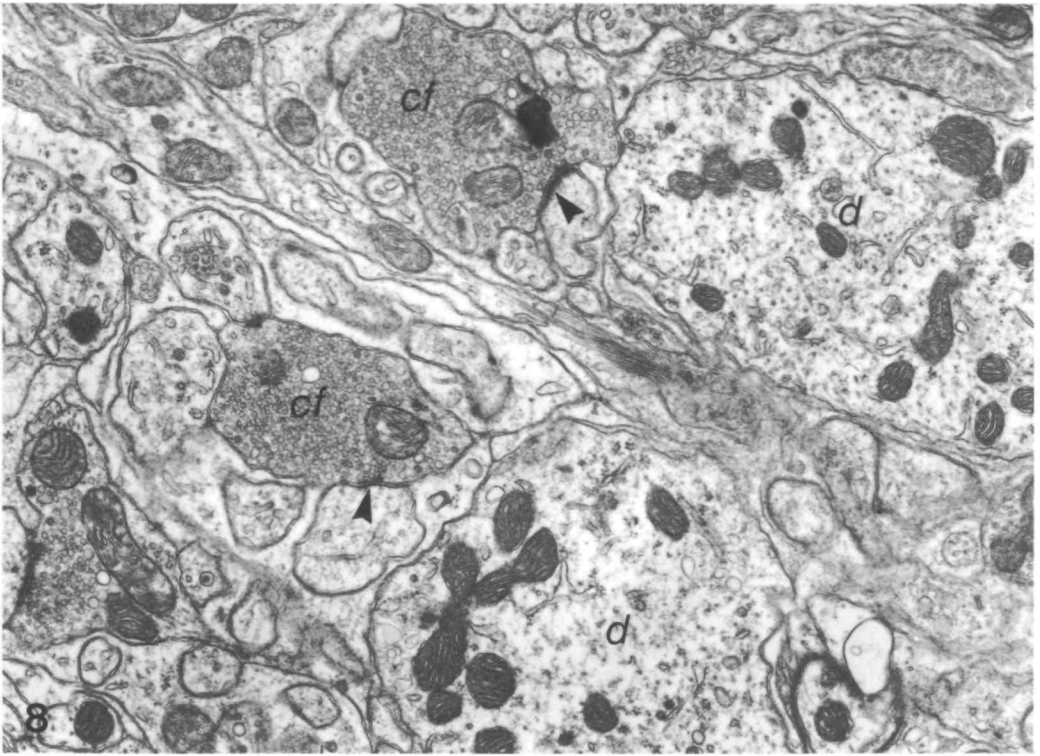


Fig. 8. Two thick, primary dendritic segments (*d*) of Purkinje cells, in a 6-month-old *staggerer* mouse, receive climbing fiber varicosities (*cf*) synapsing on dendritic spines (arrowheads). $\times 20\,000$.

their parallel fiber input retain multiple innervation. Thus, the numerical rearrangement seems to be dependent upon the maturation of postnatally generated granule cells. Nevertheless, these two mutations do not indicate whether granule cells participate in the regression of supernumerary climbing fibers either through synaptic activity or through other kinds of cellular interactions.

At variance with the situation in the above-mentioned mutants, granule cells are present in the developing *staggerer* cerebellum but, owing to the absence of branchlet spines, the synaptogenesis between climbing fibers and Purkinje cells takes place in the presence of granule cells and in the absence of parallel fiber–Purkinje cell synapses. As stated above, in the adult *staggerer* cerebellum, Purkinje cells retain multiple innervation (Crepel *et al.* 1980; Mariani and Changeux, 1980), indicating that granule cells must exert their regressive influence through a synaptic mechanism. Hence, the lack of synaptic rearrangement in this mutant does not depend on partial climbing fiber translocation but on the absence of parallel fiber–Purkinje cell synapses. In this way the apparent paradox between

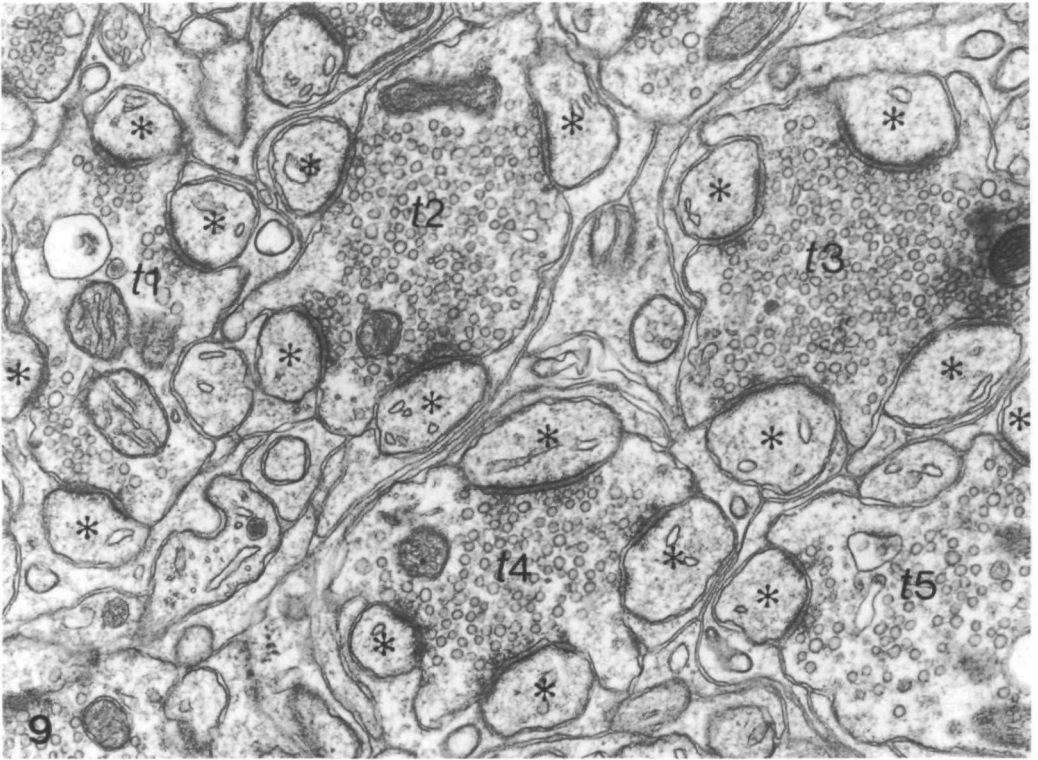


Fig. 9. Molecular layer of a 25-day-old *hyperspiny Purkinje cell* mouse. The neuropil contains five large, rosette-like boutons (t1–t5) belonging to parallel fiber varicosities. Note that each rosette is synapsing on several (average of four) Purkinje cell dendritic spines (asterisks). $\times 33\,000$.

staggerer and *hpc* cerebella discussed in the preceding section is explained, since in *hpc* parallel fiber synaptogenesis does occur.

In the adult stage, the presence of supernumerary axon terminals at sites which, under normal conditions, receive only one may be interpreted in terms of the theoretical suggestion proposed by Changeux and collaborators (Changeux *et al.* 1973; Changeux and Danchin, 1976; Changeux and Mikoshiba, 1978), that processes of selective stabilization take place during the formation of neuronal networks. At critical stages a limited 'redundancy' and 'fluctuation' of the connectivity would exist, and the early activity of the circuits in reducing the transient redundancy would refine the synaptic specificity, bringing additional order to the system. Some of the labile, early-formed contacts would become selectively stabilized while, in a concomitant manner, others would regress. As far as climbing fiber regression and the involvement of granule cell–Purkinje cell synapses in this regression is concerned, a tempting assumption is that the activation of early-formed parallel fiber–Purkinje cell synapses is necessary for the functioning of the developing network that will generate proper Purkinje cell

activity. Thus, when these synapses are not formed, the ensuing modifications of the Purkinje cell activity might perturb the regulatory mechanisms that account for the selective stabilization of their climbing fiber inputs. Whatever the ultimate mechanism is, the idea that synaptic activity is involved in the regulation of the synthesis of a trophic Purkinje cell factor, for which climbing fibers need to compete, still remains an excellent working hypothesis.

Remodeling of the cerebellar circuitry by local circuit neurons

The distinct perturbations of the cerebellar cellular milieu produced in the mutant mice have also allowed the dissection of some of the mechanisms involved in the variability of the synaptogenesis between axons of local circuit neurons and Purkinje cells, during the plastic remodeling of the deficient cerebellar circuitry.

Local circuit neurons and their potential for developing presynaptic somata and dendrites

In many regions of the CNS from the spinal cord to the neocortex, especially in the thalamus, local circuit neurons can have presynaptic properties at axonic as well as at somatic and dendritic levels (see in Rakic, 1976). The cerebellar cortex, despite its plethora of local circuit neurons, is an exception; this neuronal network is totally lacking in unconventional synaptic connections. It is of interest to notice that somato-dendritic and dendro-dendritic synapses have been encountered in those mutant cerebella in which granule cells are extremely reduced in number, the *weaver* (Sotelo, 1975a) and its phenocopy obtained in the rat by neonatal X-irradiation (Sotelo, 1977), as well as the *reeler* (Mariani *et al.* 1977; Wilson *et al.* 1981). Some of these unconventional synapses, although 'ectopic', respect their specificity [granule cell somata and dendrites and Purkinje cell branchlet spines (Fig. 10); stellate cell somata and Purkinje cell branchlet spines], whereas some others in addition to their 'ectopic' location enter into the category of 'heterologous' synapses (Golgi cell somata and dendrites and Purkinje cell branchlet spines).

These observations plead in favour of a pluripotentiality of cerebellar local circuit neurons to transfer information within neuronal networks through axon terminals as well as through presynaptic somata and dendrites. This pluripotentiality is repressed during normal synaptogenesis and is revealed in the agranular conditions. It is characterized by the presence of innumerable free postsynaptic receptor sites in Purkinje cells calling for innervation. Hence, lack of competition between afferent inputs and the presumptive attraction exerted by non-innervated postsynaptic sites seem to be responsible for the phenotypic expression of these unconventional synaptic connections. Hamori and Somogyi (1982) have reported that, after isolation of the cerebellar cortex in adult rats, local circuit neurons of the granular layer respond to mossy fiber denervation by developing presynaptic sites on their somata and dendrites. These findings corroborate the above-mentioned hypothesis and, furthermore, indicate that cerebellar local circuit neurons retain their pluripotentiality during their life-span.

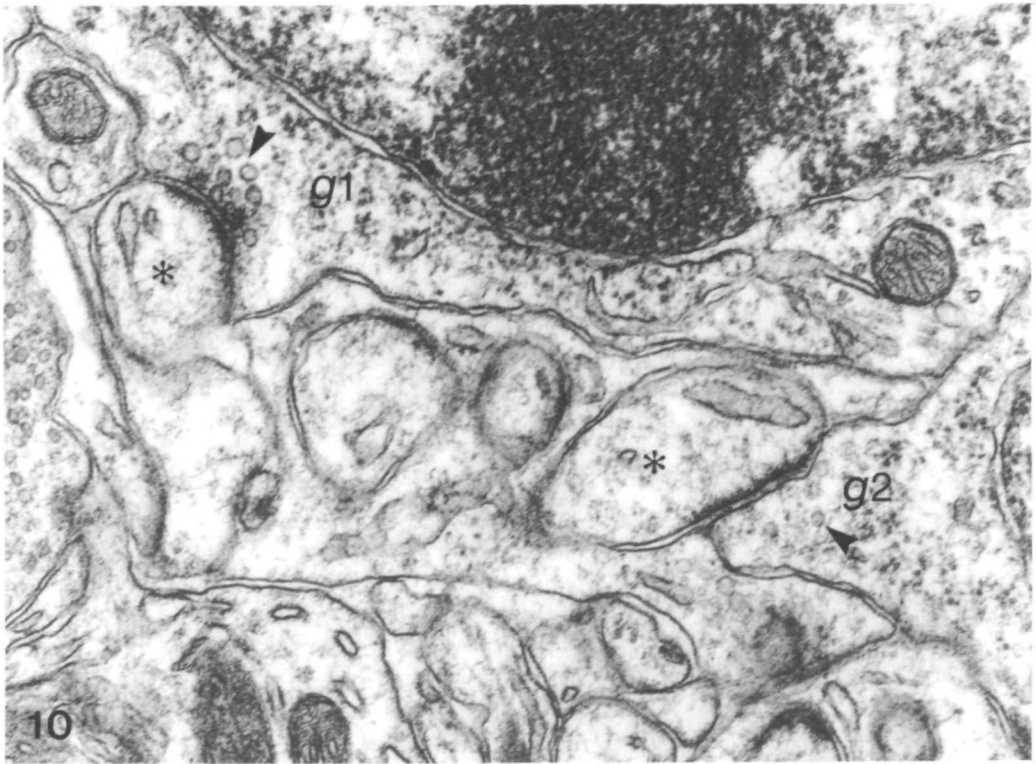


Fig. 10. Granular layer of an adult *reeler* mouse. Two perikarya of granule cells (g1, g2) are apposed to Purkinje cell dendritic spines (asterisks). The arrowheads point to presynaptic vesicles facing the postsynaptic spines. $\times 48\,000$.

Morphogenetic influence of postsynaptic targets on presynaptic elements with the nodding cerebellum, a new neurological mutation of the mouse, as an example

An ultrastructural survey of classes of axon terminals present in the cerebellar cortex provides evidence that size, geometrical complexity and inner features of presynaptic boutons are essential criteria for the identification of the different categories of axons synaptically involved in the cerebellar network (Palay and Chan-Palay, 1974). The features of presynaptic organelles (types, size, shape and density of vesicles, cytoskeletal elements, mitochondria, smooth profiles of endoplasmic reticulum and axoplasmic matrix) are most probably the result of the phenotypic expression of the parent neuron (the principle of axoplasmic congruity of Palay and Chan-Palay, 1975). Conversely, the size and geometrical complexity of the terminals, together with the kind of synaptic junction (Mugnaini, 1970), could result from the interplay between intrinsic and environmental factors, especially the morphogenetic influence of postsynaptic elements. Evidence in favour of the reality of such mechanisms, by which one neuron affects another, has been obtained through the investigation of the cerebellar cortex of the *nodding* mutant mouse.

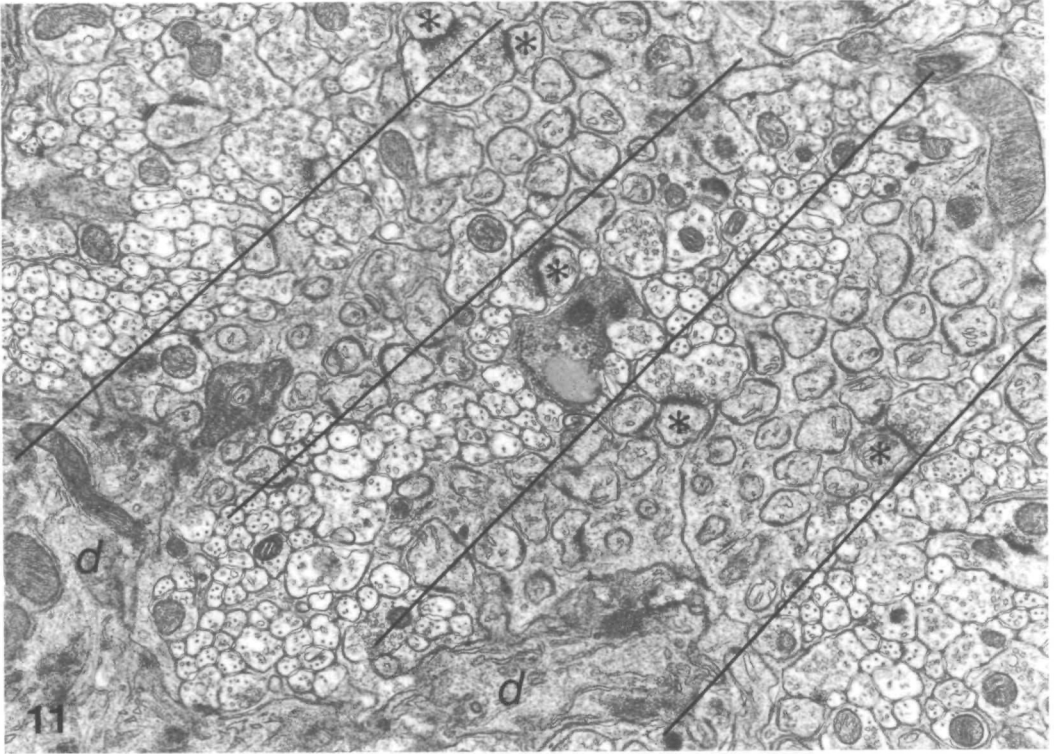


Fig. 11. Deeper one-third of the molecular layer in a 28-day-old *nodding* mouse. The neuropil is mostly formed by bundles of parallel fibers. Some of their varicosities establish synaptic contacts with Purkinje cell dendritic spines (asterisks). Two profiles of proximal dendrites, belonging to a Purkinje cell (*d*), are present in the lower portion of the micrograph. The sectional plane passes tangential to the thick dendrites (regions between the solid lines) revealing numerous ectopic spines arising from these dendrites. The ectopic spines bear postsynaptic differentiations facing glial processes, but are free of innervation. $\times 16000$.

Nodding (*nd*) is an autosomal recessive mutation affecting the cerebellum. It appeared spontaneously, almost 10 years ago, in the 129/Sv strain (Sotelo and Guénet, 1983) and it has never before been reported *in extenso*. The homozygous mice become ataxic and spastic (the hind limbs being the most affected). Their heads nod continuously in the anteroposterior axis. Locomotion does not improve with age. Although we do not have direct proof that Purkinje cells are the primary target of the mutation, Golgi impregnation, immunocytochemical studies and ultrastructural observations strongly suggest that this category of neurons is the target of the *nd* gene.

Purkinje cell dendrites are hypotrophic, and their compartmentalization is not completed since, in addition to distal spiny branchlets, the dendritic segments of the proximal compartment are studded with long-necked spines. Ultrastructural analysis in mice aged between 25 and 35 days confirms that the cerebellar circuitry

develops in a qualitatively correct manner. However, numerous spines arise from the normally smooth proximal dendritic branches (Fig. 11). These spines exhibit anatomical maturity (each one bears a patch of membrane coated with a postsynaptic differentiation), despite being embedded in a neuropil containing abundant parallel fibers, and remain covered by astroglial processes and free of innervation (Fig. 11). Conversely, the spines emerging from distal branches are synaptically connected with parallel fibers. These observations indicate that parallel fibers are able to engage in synapse formation and, therefore, the absence of innervation of the ectopic spines points to an anomaly in the Purkinje cells.

Similar ultrastructural analysis performed in older mice (between 6 and 14 months) reveals that the ectopic spines ultimately receive synaptic innervation (Fig. 12). Most of the presynaptic elements are giant boutons, up to five times larger than those encountered in the molecular layer of control cerebellum. According to the cytological features of the organelles within the giant boutons, all the classes of presynaptic axons of the molecular layer have participated in the protracted innervation of the ectopic spines. The majority of the giant boutons resemble parallel fibers that, instead of forming a small varicosity synapsing on

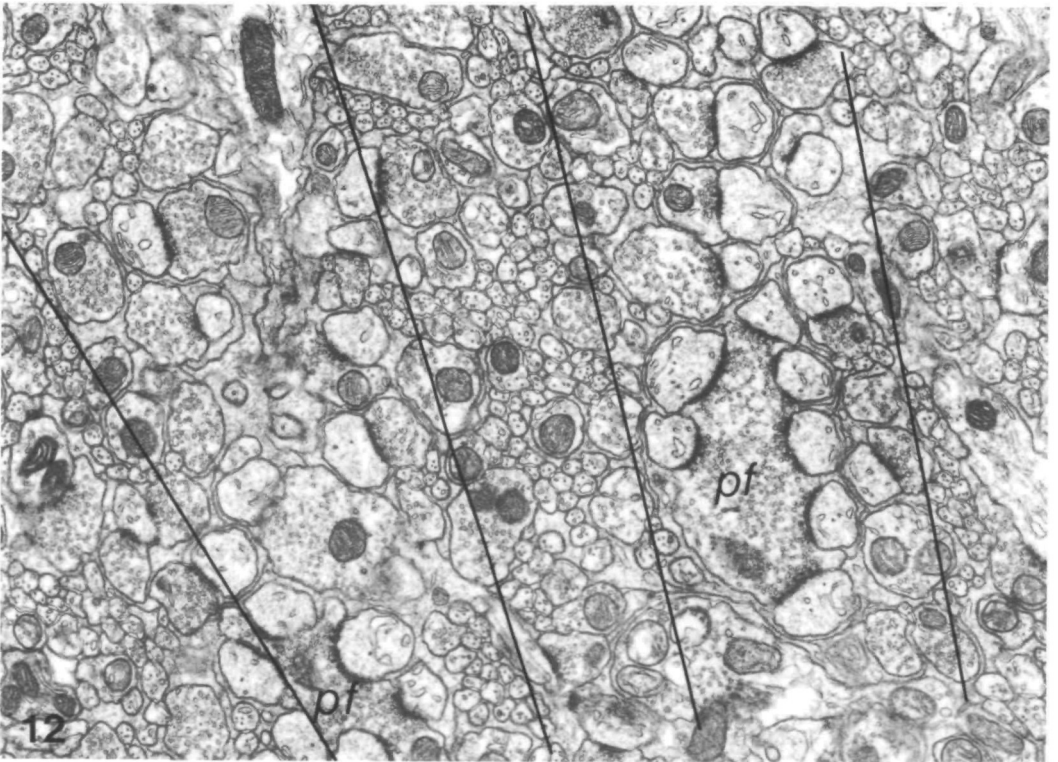


Fig. 12. Similar field to that in Fig. 11 but from the cerebellum of a 230-day-old *nodding* mouse. The large majority of the ectopic spines (regions between the solid lines) are innervated by parallel fiber varicosities. Some of the latter (*pf*) are hypertrophic. $\times 21\,000$.

one Purkinje cell spine, form large rosette-like structures covered by up to 7–10 spines (Fig. 13). The second class of giant boutons, less numerous, resembles axon terminals of molecular layer local circuit neurons (Fig. 14). The third and last class, the least frequent, shares cytological features with those of climbing fibers

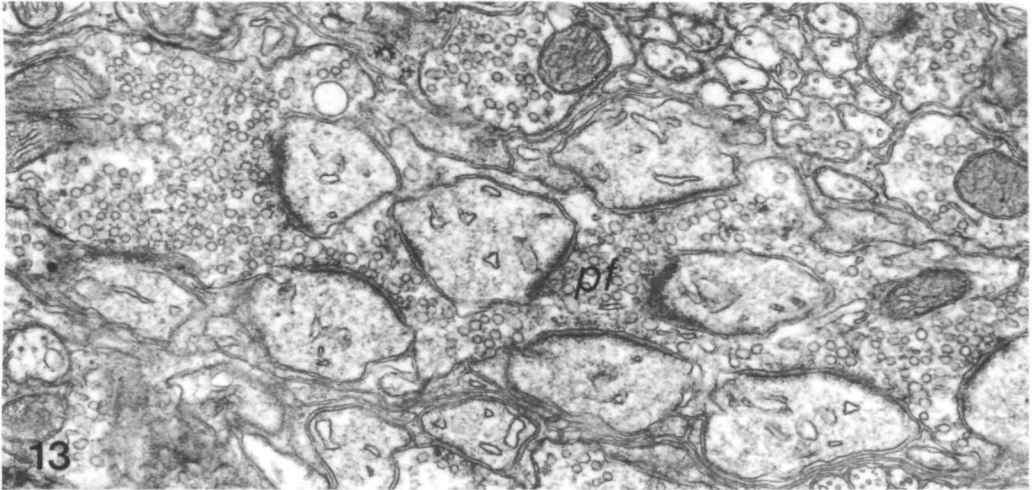


Fig. 13. Molecular layer in a 6-month-old *nodding* mouse. A hypertrophic parallel fiber varicosity (*pf*), shaped as an elongated rosette, establishes synaptic contacts on nine spines of Purkinje cell dendrites. $\times 27\,000$.

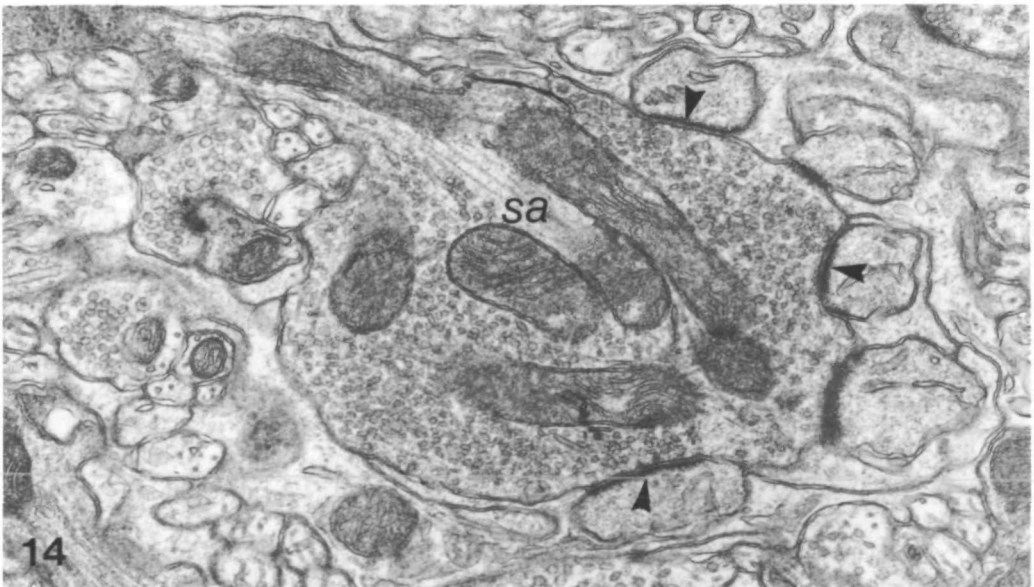


Fig. 14. Molecular layer in a 9-month-old *nodding* mouse. A hypertrophic presynaptic bouton (*sa*), sharing its inner features with those of a stellate axon, establishes synaptic contacts on five spines of Purkinje cell dendrites. Note that the synaptic junctions (arrowheads) are of the asymmetric type. $\times 27\,000$.

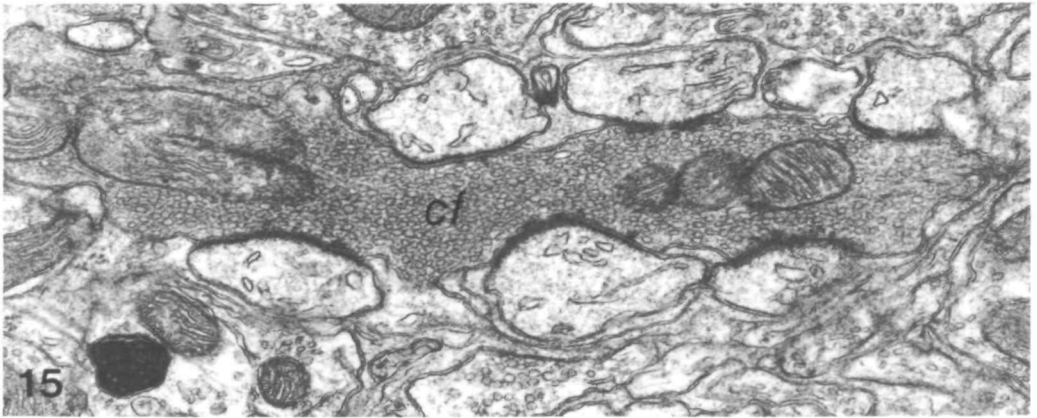


Fig. 15. Molecular layer in a 6-month-old *nodding* mouse. A hypertrophic climbing fiber (*cf*) varicosity establishes synaptic contacts on six spines of Purkinje cell dendrites. $\times 27\,000$.

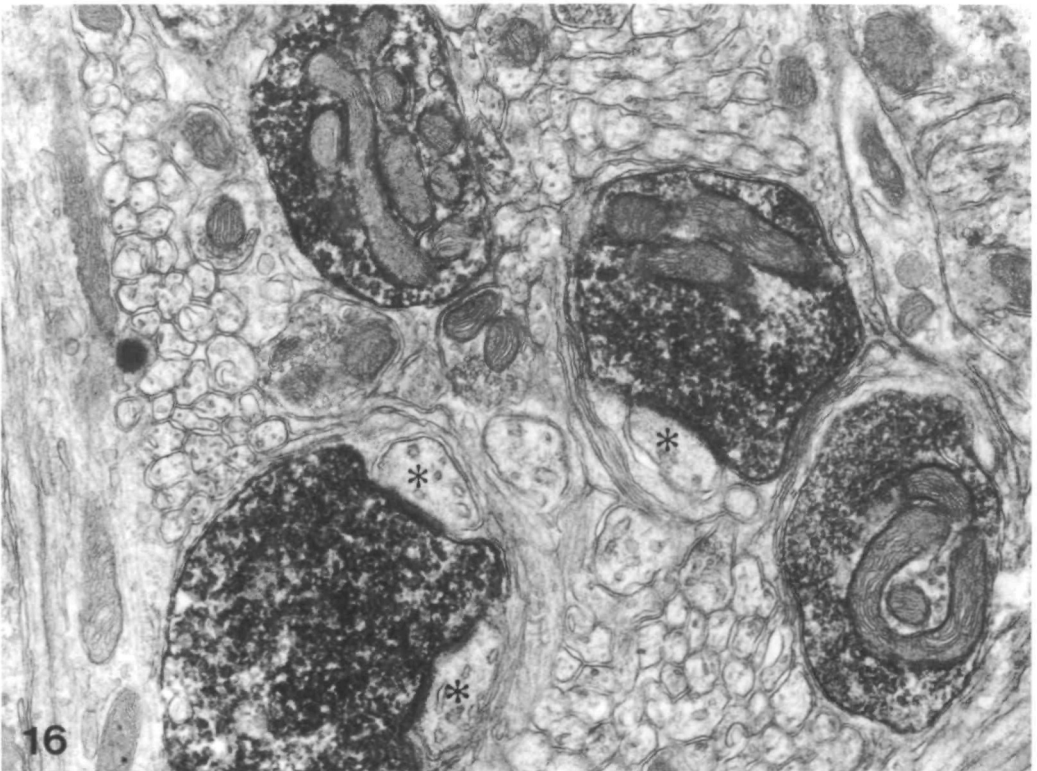


Fig. 16. Molecular layer in a 6-month-old *nodding* mouse. This electron micrograph illustrates four glutamic acid decarboxylase (GAD)-immunoreactive boutons of large size. Note that two of these boutons establish synaptic contacts on spines of Purkinje cell dendrites (asterisks). $\times 22\,000$.

(Fig. 15). Since all these classes of axons establish synaptic connections with Purkinje cell spines in control cerebellum, despite the striking change in size of the terminals, the synaptic specificity of the circuitry is preserved.

Immunocytochemical studies of the *nodding* cerebellum, with anti-glutamic acid decarboxylase (GAD, gift of Dr Tappaz) and anti-neurofilament antibodies (Nf, gift of Dr Dahl) have been helpful in identifying one category of giant boutons, and disclose further alterations in the Purkinje cell synaptic investment. The giant boutons of the second class, filled with pleomorphic vesicles, are indeed of GABAergic nature, as shown by their GAD-immunoprecipitation (Fig. 16), and they belong to molecular layer local circuit neurons. Moreover, basket cell axons, visualized by their GAD- (Fig. 17) and Nf-immunoreactivity (Fig. 18), establish 'pincaux' formations much longer than in control animals. Thus, local circuit

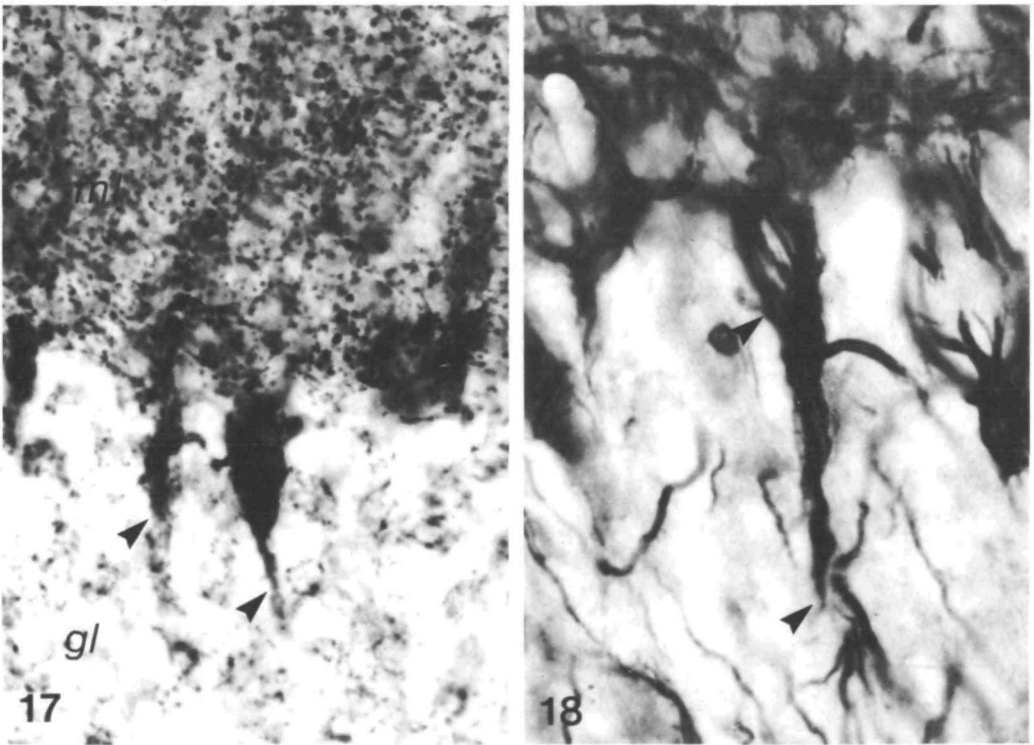


Fig. 17. Light micrograph of the cerebellar cortex, in a 9-month-old *nodding* mouse, immunostained with an anti-GAD antiserum. The arrowheads point to the hypertrophic 'pincaux' formations between GABAergic basket fibers and the initial segment of Purkinje cell axons. Note the presence of GAD-immunopositive dots in the molecular layer (*ml*) as well as the GAD-immunostaining of Golgi cell axons at the periphery of the glomeruli in the granular layer (*gl*). $\times 800$.

Fig. 18. Light micrograph of the cerebellar cortex, in a 6-month-old *nodding* mouse, immunostained with an anti-neurofilament antiserum. The arrowheads point to a hypertrophic 'pincau' formation, deeply penetrating the granular layer. $\times 1000$.

neurons of the molecular layer are involved in the remodeling of the synaptic investment of *nodding* Purkinje cells at all their postsynaptic locations.

One possible explanation of the sequential events accounting for the observed abnormalities in the *nodding* cerebellum is based on a delayed maturation of ectopic Purkinje cell spines. In young mice, despite their apparently normal appearance, the spines are not mature enough to be engaged in synaptogenesis. In adult animals, the ectopic spines reach maturity and, through an inductive process of axonal sprouting, become innervated. The presence of free postsynaptic sites would, therefore, influence nearby axon terminals, attracting them to establish new synaptic connections in a process that we have named 'terminal sprouting' (Sotelo, 1975c), to differentiate it from collateral sprouting. The end result will be an increase in the multiple synaptic index (Raisman and Field, 1973) of the affected boutons, leading to overexaggerated growth. The morphogenetic influence of postsynaptic elements is not restricted to early life, as is the case in the *hpc* cerebellum, but may occur whenever the originally established balance between pre- and postsynaptic elements is overturned, as seems to happen in the *nodding* cerebellum. In particular, the findings in both mutations suggest an important morphogenetic role for postsynaptic elements in the acquisition of the size and shape of the presynaptic boutons.

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