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MORPHOLOGICAL STUDY OF ORGANOTYPIC CEREBELLAR CULTURES*

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Organotypic cerebellar cultures from 8-days-old (P8) mouse pups were studied following 11 days of *in vitro* (11DIV) culturing. The cerebellar cytoarchitectonic structure was maintained in most parasagittal cerebellar cortical slice cultures (also containing the deep cerebellar nuclei). The two main extrinsic excitatory inputs (the climbing and the mossy fibers) seem to be replaced by other axonal types: in the molecular layer mostly by parallel fibers (for climbing fibers) and in the granular layer by intrinsic mossy fiber collaterals of local excitatory interneurons, the unipolar brush cells. However, in a few organotypic cultures, which (although preserving the trilaminar cerebellar cortical structure) were "granuloprival" but also contained some of the deep cerebellar nuclei, the participation of extracortical axons from the deep cerebellar nuclei in the replacement of the missing afferents is suggested.

Keywords: Cerebellum – organotypic culture – ultrastructure – synaptic plasticity

INTRODUCTION

Organotypic cerebellar slice cultures derived from mouse pups exhibit many of the structural and functional aspects of the cerebellum *in vivo* [41, 42, 43] and provide a useful model with which the developmental, morphological, biochemical, electrophysiological and pharmacological properties of this CNS structure are studied [9, 20, 31, 40]. Parasagittally oriented explants from the central cerebellar vermis have both cortical and subcortical regions, the latter consisting of deep nucleus neurons and Purkinje cell axons that project to these neurons [43]. Besides Purkinje cells, the main cerebellar cortical neurons such as granule cells, basket, stellate and Golgi cells [8, 21, 42, 43] and also unipolar brush cells (UBCs) [33, 34] are present in organotypic cultures of the cerebellar cortex [35]. Synaptic relationships among these neurons develop in culture and reproduce the characteristic cerebellar cortical circuitry [43]. The synaptic development of organotypic cultures of cerebellum from newborn mice *in vitro* faithfully follows the *in vivo* development despite substantial disruption of the cell migration patterns, although there is an initial lag in synaptic development

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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at days 5 and 8 *in vitro* (DIV), followed by an accelerated development resulting in a close *in vivo* and *in vitro* correspondence by DIV 19 [20]. Granule cells project their axons to all other cortical cells, and form synapses with Purkinje cells exclusively on dendritic spines, although the development of the Purkinje dendritic tree is stunted *in vitro*, and tertiary branchlets, the sites of dendritic spines *in vivo* [8, 14, 37], are not elaborated in culture [31, 43, 44]. The main difference between the *in vivo* and the *in vitro* cerebellar circuitry is that the two main extracerebellar afferents, the olivocerebellar climbing fibers [5, 21, 27, 28, 38, 51] and the mossy fibers [8, 12, 15, 21, 28, 37, 38], are not present in standard cerebellar cultures [43] separated from the pons and brainstem. Glutamatergic mossy fiber-like terminals which originate from the UBCs and form a large system of intrinsic mossy fibers in the postnatal vestibulocerebellum [35, 36] are characteristic of long-term cerebellar organotypic cultures [36].

The aim of the present study was to investigate the cerebellar cortical circuitry by light (LM) and electron microscopy (EM) in order to clarify possible rearrangements and compensatory synaptic plasticity in the absence of the two main extracerebellar excitatory inputs in organotypic cerebellar cultures. In order to simplify the identification of neuronal elements, metabotropic glutamate receptor mGluR1a immunocytochemistry was applied, which (except for granule cells) is expressed virtually in all of the neurons of the cerebellar cortex [10, 18, 22, 30, 39, 49, 53].

MATERIALS AND METHODS

Organotypic slice cultures of mouse cerebellum

The preparation of cerebellar slices was performed after a protocol from [45] and modified as recently described [31, 40]. In brief, 8-day-old (P8) C57/Bl6 mouse pups were decapitated and the brain was aseptically removed. The cerebellum was dissected in an ice-cold preparation medium (MEM containing 2 mM glutamax I, pH 7.3) and the meninges were carefully removed. Sagittal slices of 400 μm were cut with a McIllwain tissue cutter, separated with fine forceps and transferred onto humified transparent membranes (Millicell-CM, Millipore). They were cultured on a liquid layer of serum-free Neurobasal medium containing glutamax I (2 mM) and B27 supplement (all from Life Technologies) in a humified atmosphere with 5% CO_2 at 37 °C. The medium was changed every 2–3 days. Cultures were maintained for 11 DIV.

Immunocytochemistry

The cultures were rinsed several times in phosphate-buffered saline (pH 7.3, PBS), fixed in 3.5% paraformaldehyde and 0.5% glutaraldehyde (pH 7.3 in 0.1 M phosphate buffer, PB) for 2 hours and stored at 4 °C in fixative diluted 1 : 5 in PB. The slices were washed several times in PB at 4 °C, cryoprotected with 10, 20 and 30%

sucrose in PB, frozen and thawed twice in liquid N_2, washed several times in PB and incubated with monoclonal mGluR1a antibody, previously characterized [23] with purified IgG (Pannonia Kutatási Park Kft., Pécs, Hungary) (1 : 10000). The immunohistochemical protocol was as described previously [10, 11, 23, 46] with 3,3'-diamino benzidine (DAB) as chromogen (0.0125%, and 0.005% hydrogen peroxide; time of incubation 10–15 min at room temperature). After the incubation, half of the slices were osmicated (1% OsO_4 in PB for 1 hour), dehydrated in alcohol (including "en block" contrasting in 1% uranyl acetate dissolved in 70% ethanol for 1 hour) and flat-embedded in Durcupan ACM (Fluka). The remaining sections were air-dried overnight, coverslipped with Paramount and photographed with an Olympus DP 50 digital camera equipped with an Olympus BX51 microscope. Small pieces from parasagittal vermal lobules X and IX of the flat-embedded slices were glued onto the surface of "blank" Durcupan blocks with cyano-acryl nitrite glue and sectioned with a Reichert Ultracut ultramicrotome at 0.5 μm and at 60 nm thickness. Semithin sections were stained with toluidine blue, and ultrathin sections were collected on Formvar-coated single slot Ni grids, and examined with a Jeol JEM 100B electron microscope at 80 kV without further contrasting. LM and EM pictures were processed with CorelDraw 9 and Corel PhotoPaint 9 (Corel Corporation) programs.

RESULTS

LM observations

Organotypic cerebellar cultures derived from parasagittal vermal slices of mouse pups at P8 and maintained in culture for 11 DIV preserved the main cytoarchitectonic characteristics of the cerebellar cortex (Fig. 1A). Following the mGluR1a immunoreaction (IR) on the organotypic culture slices, the outline of the cerebellar lobules can be recognized; it which seems to be more or less intact (Figs 1A, B). Because of the very high expression of mGluR1a receptor in Purkinje cell dendritic spines, the immunostaining of the molecular layer is the strongest, whereas the granule cell layer (and the white matter) is only faintly stained. At this stage (altogether 19 days postnatally), the outer granule cell layer has disappeared. At higher magnification, numerous UBCs can be recognized in the (internal) granular layer of the vestibulocerebellum (lobules X and IX) (Fig. 1B). The LM morphological features of the cerebellar cortex can be well recognized on semithin sections at lower (Fig. 1C) and at higher (Fig. 1D) magnifications. There is a notable difference, however, in some cultures (or in smaller areas of the cultures), where a certain degree of morphological damage can be observed (Figs 1E, F) in the form of numerous empty holes and small clumps of intensively staining apoptotic figures (mainly in the granular layer), although the (tri)laminated cerebellar cortical structure is preserved. More severely damaged cortical structures, where Purkinje cells could only be seen in smaller groups without building up their lamina (Fig. 2A) or located out of their lamina with

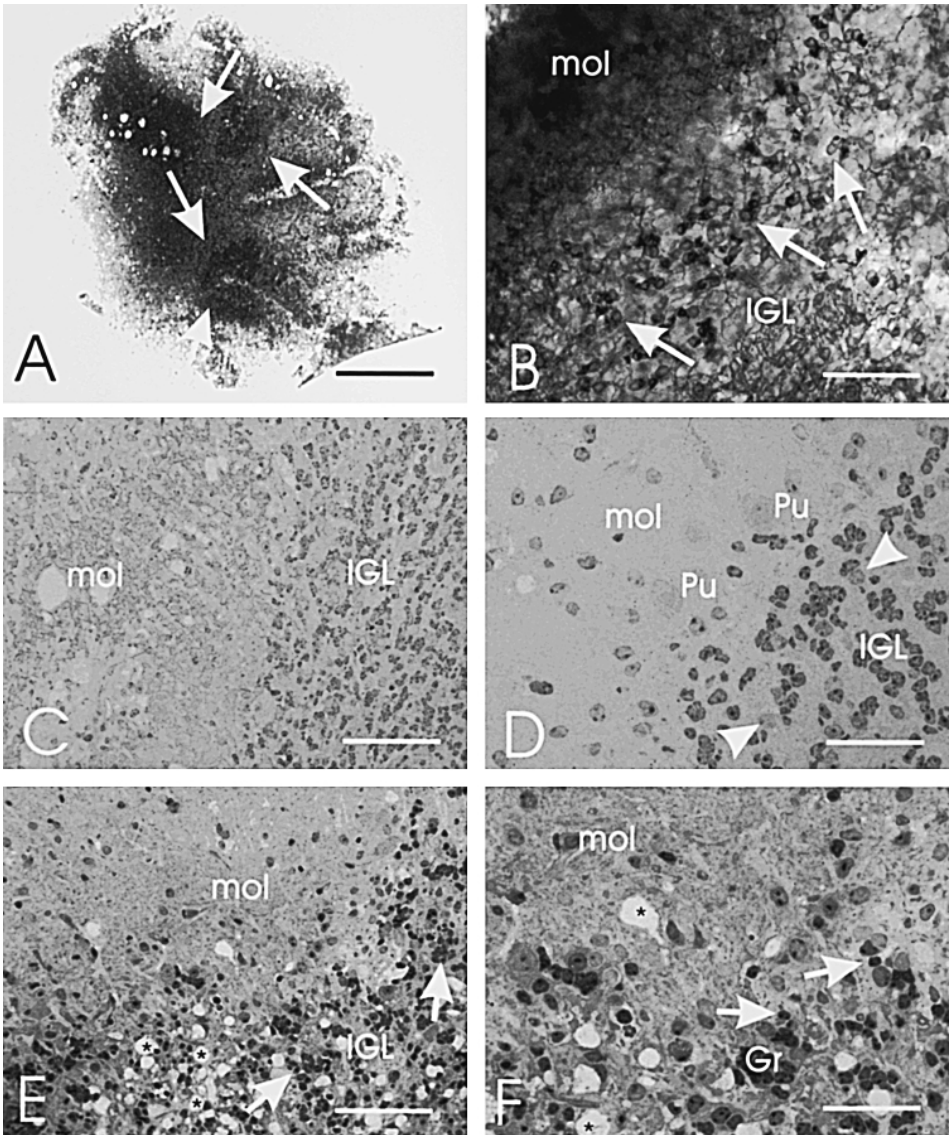

Fig. 1. A, B: Organotypic cerebellar culture established from P8 mouse pups at 11 DIV, following mGluR1a IR. – C–F: Azur II-toluidine blue-stained semithin sections from lobule X of a parallel culture. A. Most of the cerebellar lobules and sub-lobules can be recognized on this parasagittal vermal slice, showing (at least partly) preserved cytoarchitectonic characteristics of the laminated cerebellar cortex (arrows). At the periphery the slice is much thinner; smaller and larger "empty holes" are also seen in some areas (nonlabeled). Scale bar: 1 mm. – B. Detail of parasagittal vermal section of lobule X. In the IGL numerous mGluR1a IR unipolar brush cells (UBCs) can be distinguished (arrows), most of them corresponding to "stage 3" or the "intermediate brush stage" of postnatal differentiation (described in rat pups by Morin et al. [32]). Scale bar: 100 μm. – C. This picture shows a part of the "healthy-looking" cerebellar cortex: the mol and IGL can be clearly recognized. Scale bar: 150 μm. – D. At higher

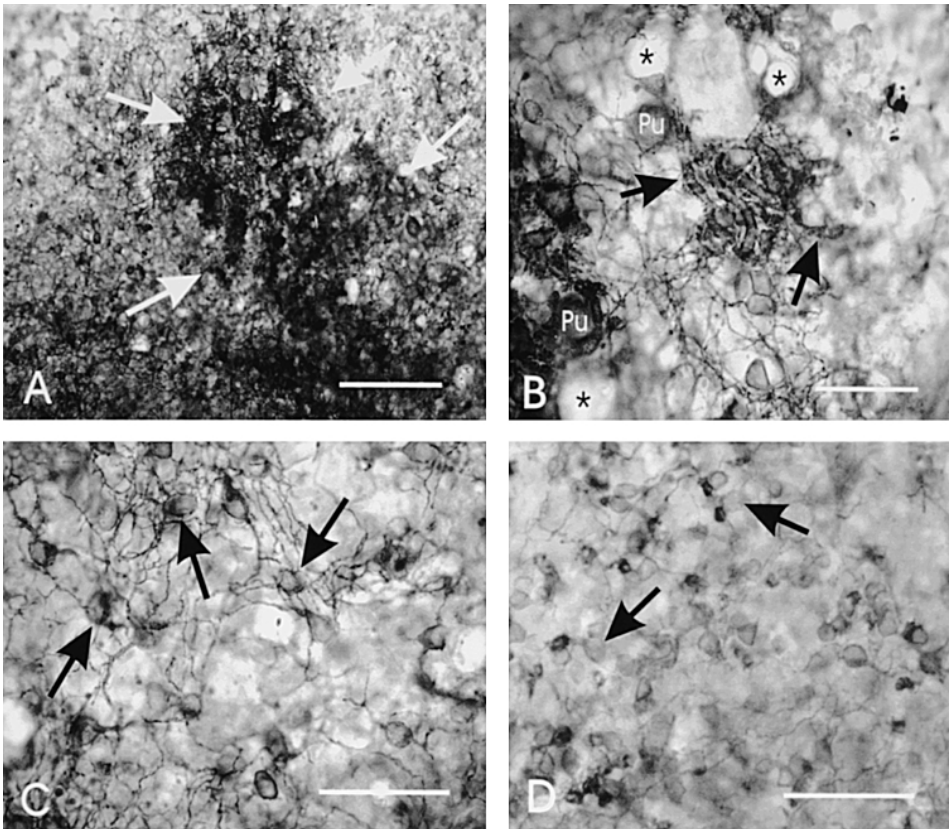

Fig. 2. Details from different lobules (and sub-lobules) of organotypic cerebellar cultures following pre-embedding mGluR1a IR. – A. In this area the cerebellar cortical structure is not well preserved; the darkly immuno-stained Purkinje cells (including dendrites) form a small island (arrows) instead of a continuous layer. Scale bar: 150 µm. – B. A small group of Purkinje cells with a "misoriented" dendritic tree (arrows) appear in the granular layer in lobule VII. Pu: Purkinje cell bodies. Small stars label capillaries. Scale bar: 75 µm. – C. Detail of lobule IV, near the periphery of the slice. Medium-sized, predominantly multipolar neurons (arrows) are surviving cells, resembling stellate cells and/or small Golgi cells. Scale bar: 100 µm. – D. Detail of the granular layer in lobule X. Numerous UBCs (arrows) with different LM morphology can be seen, corresponding predominantly to the postnatal developmental "stage 3" (or "intermediate brush"). Scale bar: 50 µm

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magnification, somewhat larger and less stained neuronal somata (most probably corresponding to UBCs) in the IGL can be distinguished (arrowheads) from smaller and darker groups of granule cells. Pu: somata of Purkinje cells. Scale bar: 75 µm. – E. Semithin section at about 15 µm depth from the upper surface of an organotypic culture. Numerous empty holes (asterisks) appear in the granular layer (IGL). Arrows show smaller groups of granule cells. Scale bar: 150 µm. – F. At higher magnification, the mol seems to be relatively intact, whereas the IGL shows definite signs of damage: numerous shrunken, apoptotic figures can be recognized (arrows), and "empty holes" (small stars) are also seen. Pu: somata of Purkinje cells; Gr: granule cells; mol: molecular layer; IGL: internal granule layer. Scale bar: 75 µm

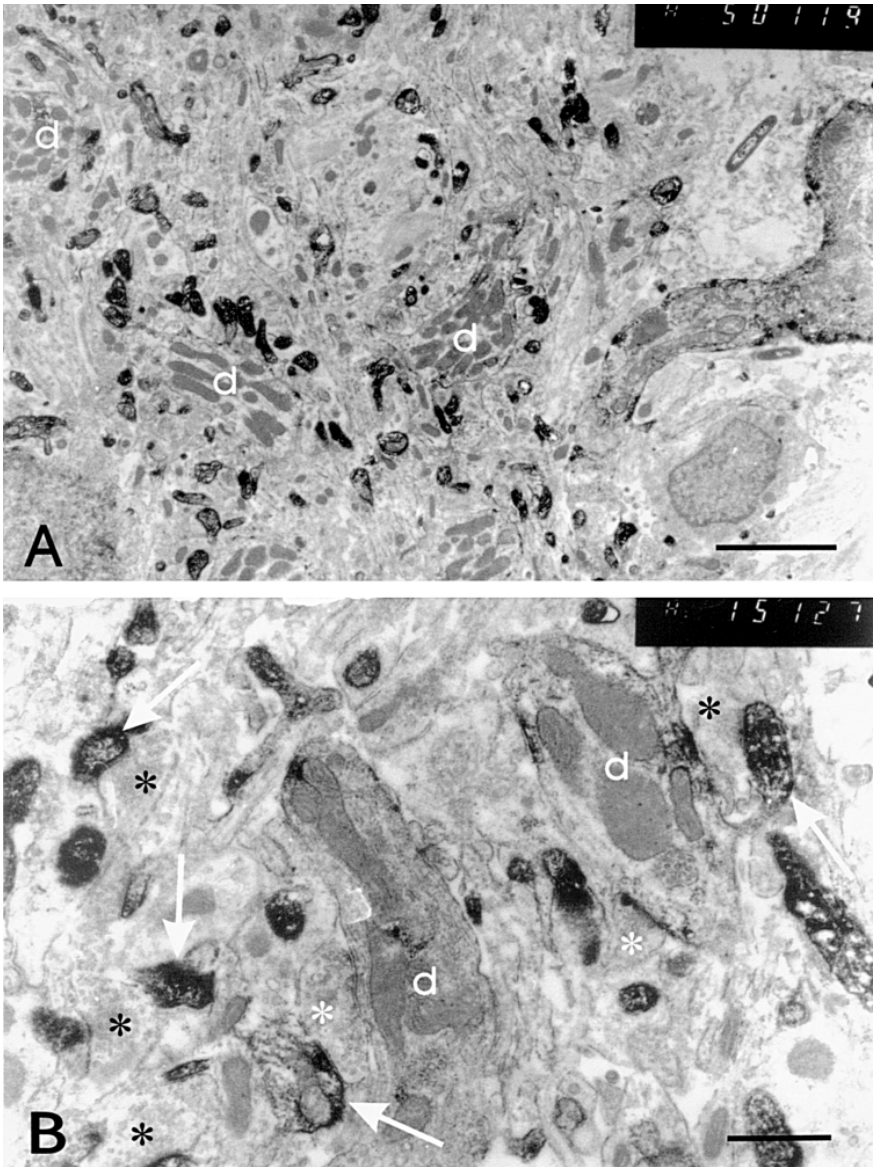


Fig. 3. EM pictures of the molecular layer neuropil from an organotypic culture (lobule X) following pre-embedding mGuR1a IR. – A. Fine, electrondense DAB reaction product can be seen beneath the plasma membrane of Purkinje cell dendrites (d). However, the heaviest accumulation of the DAB reaction product is more characteristic of Purkinje cell dendritic spines (on the right side, a thicker dendrite/soma of a strongly IR basket cell can be seen). Scale bar: 5 µm. – B. Higher magnification picture of the mGluR1a IR Purkinje dendritic spines (white arrows), in synaptic contact with small caliber parallel fibers (black asterisks) filled with numerous round synaptic vesicles. Smaller axonal profiles similar to those of parallel fibers (white asterisks) are also in contact with the dendritic stems of Purkinje cells (d), without forming synapses in the plane of the section. Scale bar: 1 µm

dendrites upside-down, were also observed occasionally (Fig. 2B). In these cultures, the accumulation of multipolar mGluR1a-immunoreactive (IR) neurons resembling stellate cells (Fig. 2C) and a high accumulation of UBCs (Fig. 2D) were visible.

EM observations

For EM analysis, cultures from parasagittal vermal slices were used, following pre-embedding mGluR1a IR at 11 DIV. Exclusively vermal lobules X and IX (receiving vestibular afferents *in vivo*) were used in this study; these seemed (at least by LM) to be well preserved as regards the laminated structure characteristic of the cerebellar cortex. In the neuropil of the molecular layer, cross-sections of Purkinje cell dendrites and numerous intensively immunolabeled Purkinje dendritic spines could be seen (Fig. 3A) synapsing with parallel axons (Fig. 3B). Besides the small-diameter parallel fibers, containing round synaptic vesicles in a pale axoplasmic matrix, there were also a few large axonal endings synapsing with Purkinje cell dendritic spines (Fig. 4A). Not infrequently, these enlarged axon terminals containing round synaptic vesicles established synaptic contacts both with dendritic spines and with the dendritic shaft of Purkinje cells (not shown). A third type of large axonal endings containing numerous, pleomorphic synaptic vesicles in a dark axoplasmic matrix also built up synapses with several (5–7) Purkinje cell dendritic spines (Fig. 4B). The ultrastructural features of apoptotic granule cells in the (internal) granular layer with condensed chromatin arranged in smaller groups could be well differentiated from those of the "healthy-looking" granule cells (Fig. 5A). In the cytoplasm of some Purkinje cells with persisting somatic spines, multilaminated bodies were seen near the plasma membrane (Fig. 5B). Mossy fiber-like axon terminals formed various synaptic glomeruli in the granular layer. Some glomeruli were smaller and were formed by the participation of relatively few postsynaptic elements, including granule cell dendrites (or dendritic digits) which are nonlabeled with mGluR1a IR and moderately immunolabeled dendrites, most probably belonging to Golgi neurons (Fig. 6A). Simple glomeruli composed of large mossy terminals containing round synaptic vesicles and pale mitochondria in an electronlucent cytoplasmic matrix and of intensively immunolabeled (mGluR1a) UBCs were frequently seen in organotypic cerebellar cultures (Fig. 6B). In these glomeruli, "giant" synapses (characteristic of UBCs) were found either on the cell body or on the short main dendrite of these neurons. Besides this cerebellar glomerular type, a more "complex" form of mossy fiber-UBC contacts was also numerous in these cultures, in which the dendriolar brush was intermingled with the large mossy terminal, but bearing fewer synaptic contacts (Fig. 7). In those cultures which (because of apoptotic neuronal loss) had fewer granule cells in the granular layer, two unusual axon terminals (and synaptic glomeruli) were visible: One mossy fiber-like axon terminal is large, and contains smaller, pleomorphic synaptic vesicles dispersed in a pale axoplasmic matrix and darker mitochondria (Fig. 8). This axon terminal is the central element of "incomplete" glomeruli, synapsing only with a few dendrites (or dendritic digits) of granule

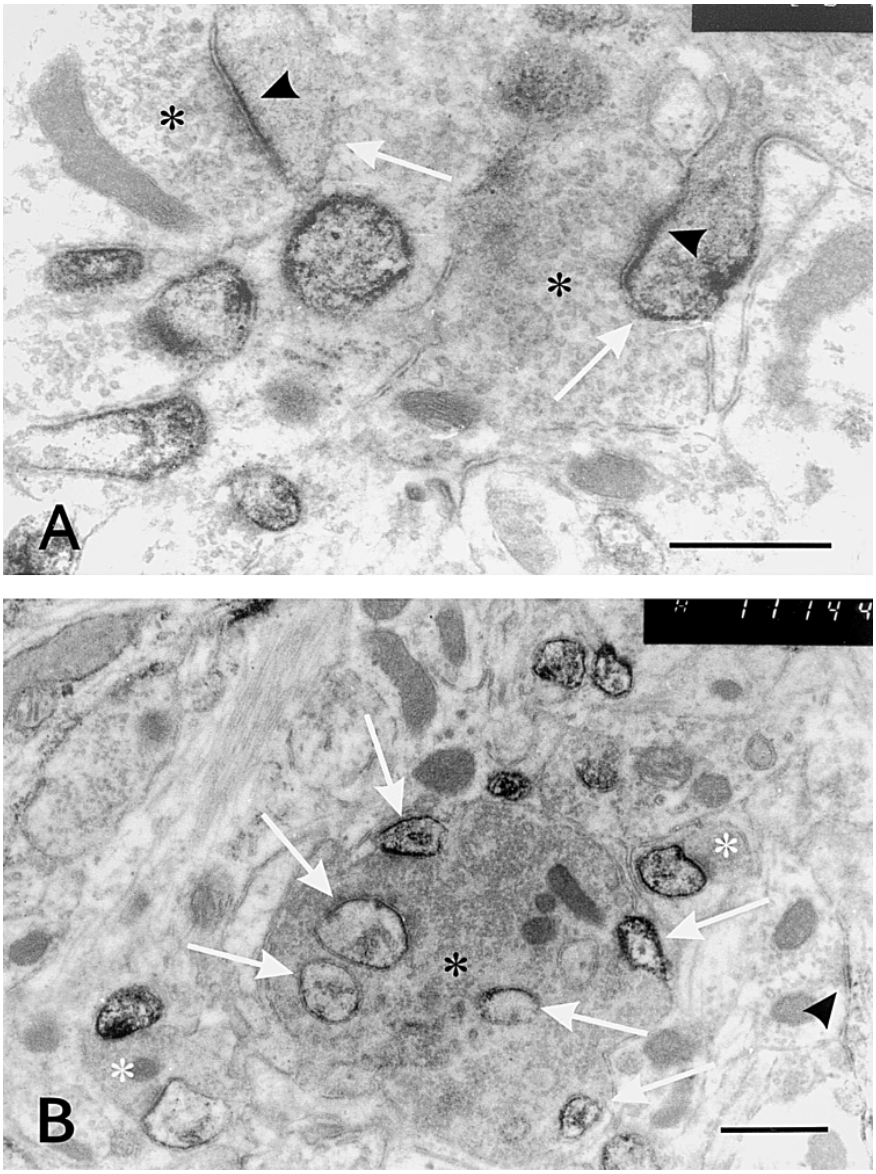


Fig. 4. Different heterotypical axon terminals synapsing on Purkinje cell dendritic spines in the lower part of the molecular layer of an organotypic cerebellar culture. – A. Synaptic contacts (black arrowheads) built up by larger axonal elements (black asterisks) and Purkinje dendritic spines (white arrows). The synaptic vesicles are pleomorphic in these axons, which could replace the missing climbing fibers. Scale bar: 1 µm. – B. Extremely large axon terminal (black asterisk) with pleomorphic synaptic vesicles and a darker axoplasm forms synaptic contacts with numerous Pu-dendritic spines (white arrows). Some homotypical parallel axons (white asterisks) near the large (heterotypical) axon terminal are also visible. On the right side of the picture, an inhibitory axon forms a synaptic contact of symmetric type (black arrowhead) with a Purkinje cell dendritic shaft. Scale bar: 1 µm

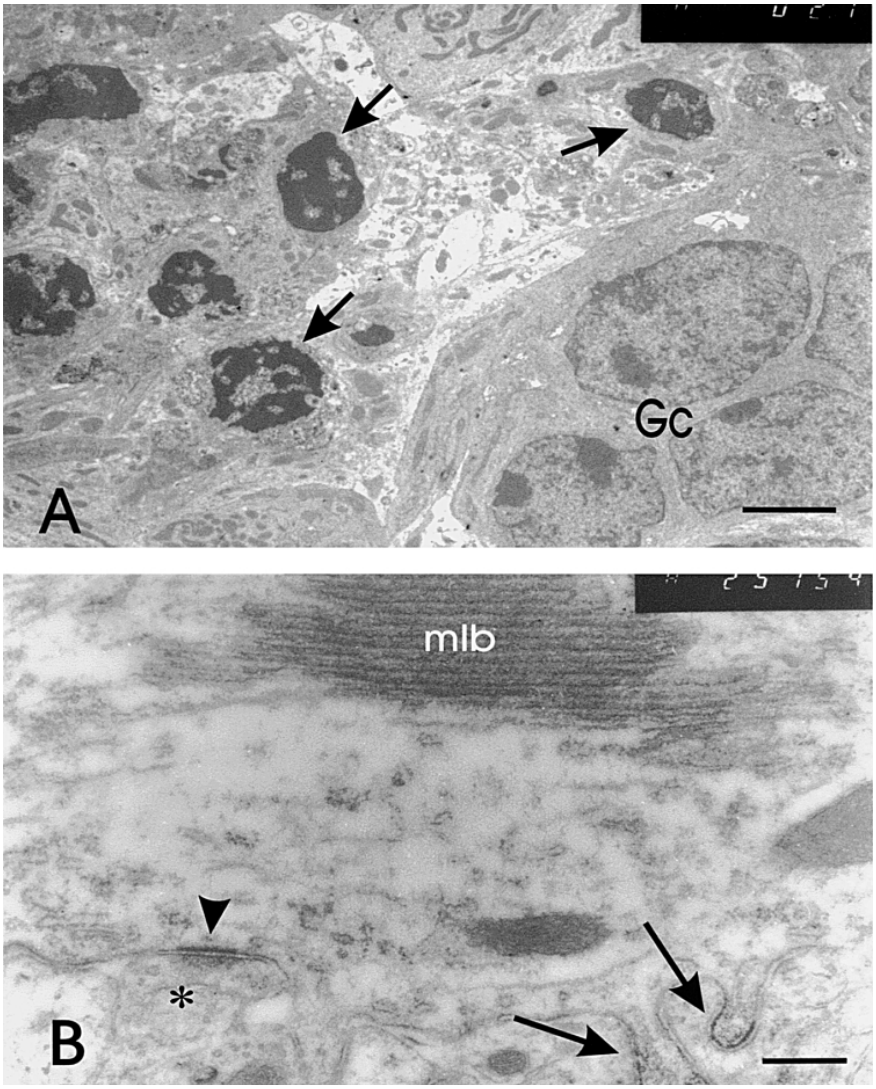


Fig. 5. EM pictures of "granuloprival" areas of the granular layer (A) and of a Purkinje cell (B). – A. Numerous dark, apoptotic figures (black arrows) representing mostly granule cells are seen near some "healthy-looking" ones (Gc). Scale bar: 10 µm. – B. At higher magnification, two somatic spines (black arrows) can be seen with fine DAB deposits decorating the cytoplasmic side of the plasma membrane of a Purkinje cell. To the left of the spines, a small parallel fiber (black asterisk) forms an asymmetric axo-somatic synaptic contact (black arrowhead). A multilamellar body (mlb) can also be observed somewhat deeper in the cytoplasm, which is characteristic of anoxic/hypoxic cells. Scale bar: 0.5 µm

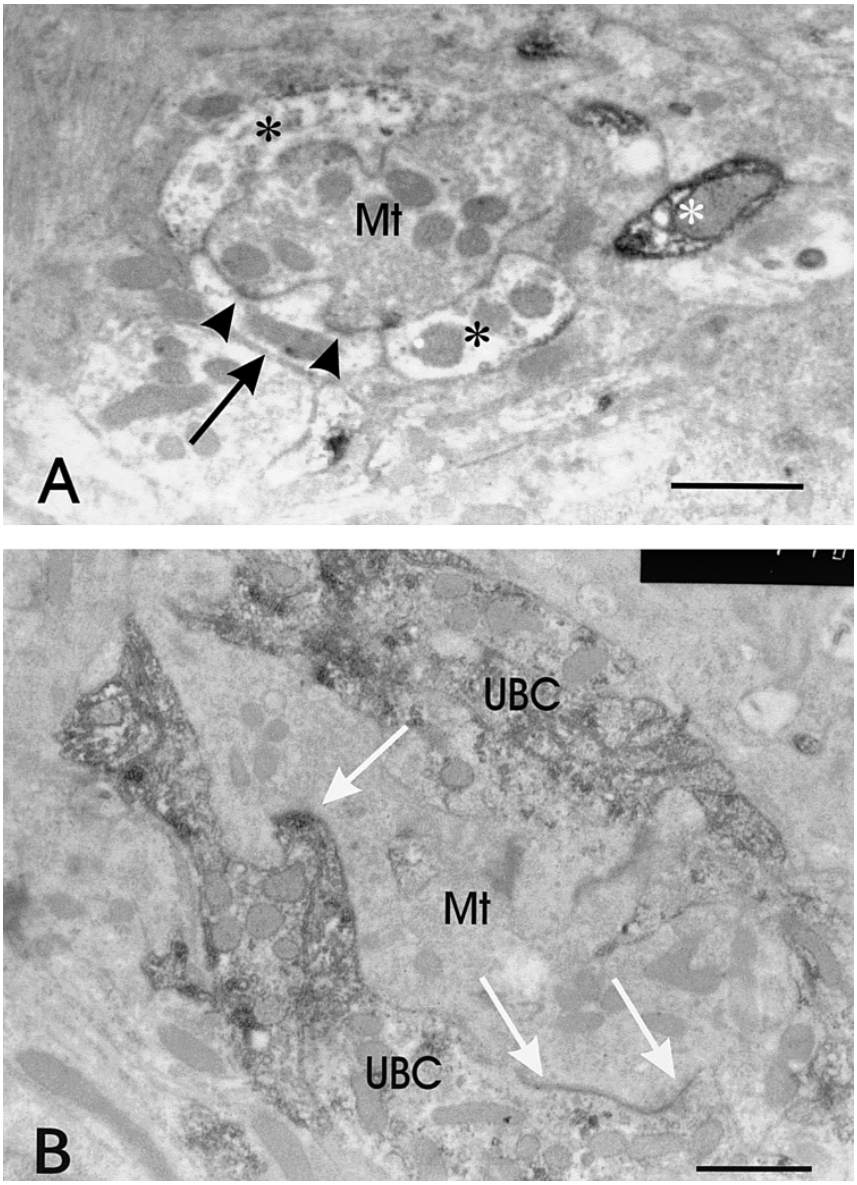


Fig. 6. Different types of "simple" glomeruli in the granular layer of an organotypic cerebellar culture (lobule X). – A. A relatively small Mt is the central element of this glomerulus surrounded by two moderately labeled (black asterisks) dendritic profiles (most probably corresponding to Golgi dendrites) and a nonlabeled (black arrow) dendrite of a granule cell, receiving two small asymmetric synapses (arrowheads) from the Mt. A more strongly mGluR1a-IR dendritic fragment (white asterisk) could belong either to a Golgi cell or to a UBC. Scale bar: 1 μm. – B. A large Mt is completely engulfed by the main dendritic and/or somatic domains of a UBC, forming multiple, large synapses (white arrows) of asymmetric type. Mt: mossy fiber terminal; UBC: unipolar brush cell. Scale bar: 1 μm

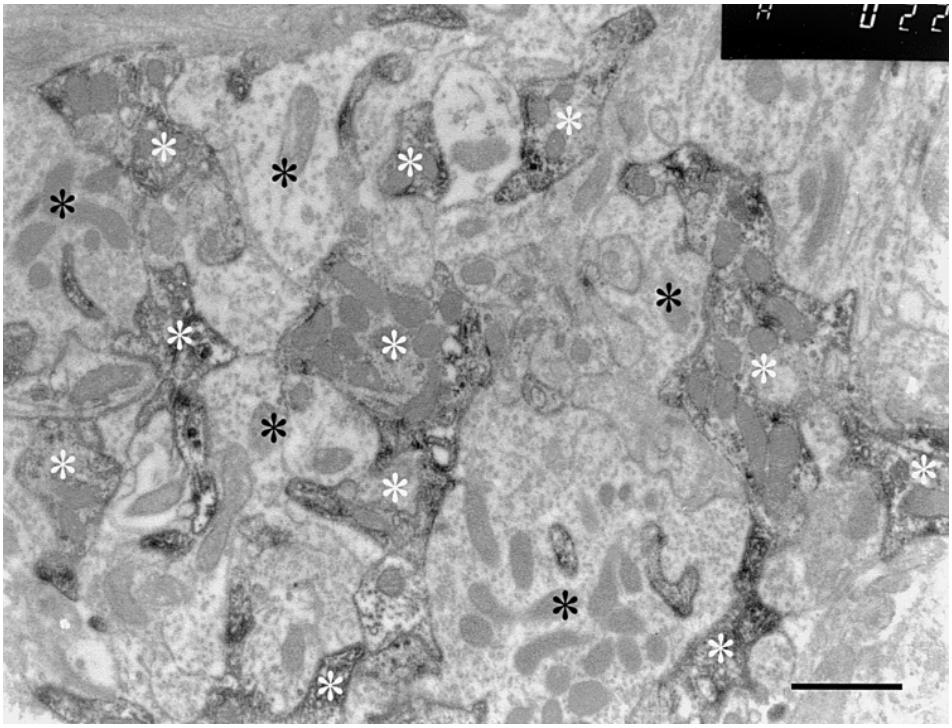


Fig. 7. Mossy fiber terminal–UBC synapses in the granular layer of an organotypic cerebellar culture. Heavily immunolabeled dendriolar brush of UBC cell (white asterisks) forming complex contacts with dispersed mossy terminal(s) (black asterisks) containing small synaptic vesicles in a pale axoplasmic matrix. Only a few real synapses are to be seen either with UBC "nonsynaptic appendages" or with sporadically occurring granule cell dendritic digits. Scale bar: 1 μm

cells, and with moderately labeled dendrites of Golgi neuron(s), also facing either the somata of granule cells, or glial profiles without forming synaptic contacts (Figs 8 and 9A). In addition to the above-mentioned mossy (and mossy fiber-like) axon terminals, a second type of large axonal endings filled with numerous small, ovoid-pleomorphic synaptic vesicles, but clustered in a dark axoplasmic matrix, were seen to build larger synaptic contacts with UBCs, and smaller synaptic contacts either with granule cell dendrites (and dendritic digits) or with moderately immunolabeled (most probably Golgi) dendrites (Figs 9A, B) too. Occasionally, the dark, "clustered" axon terminal and the mossy fiber-like axon terminal participated in the formation of the same, rather large glomerular complex (Figs 9A, B).

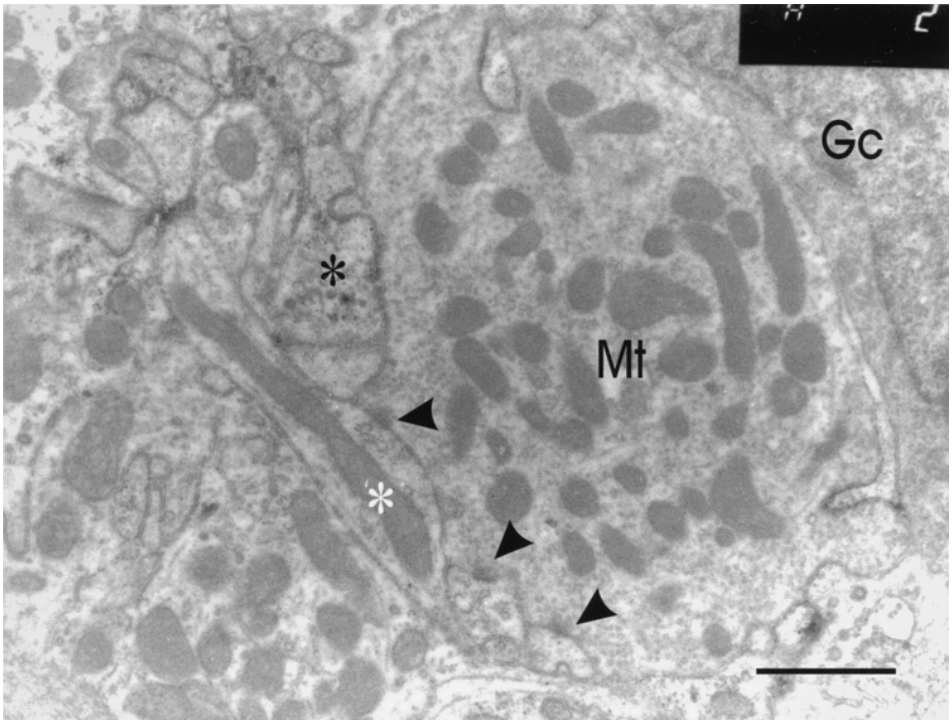
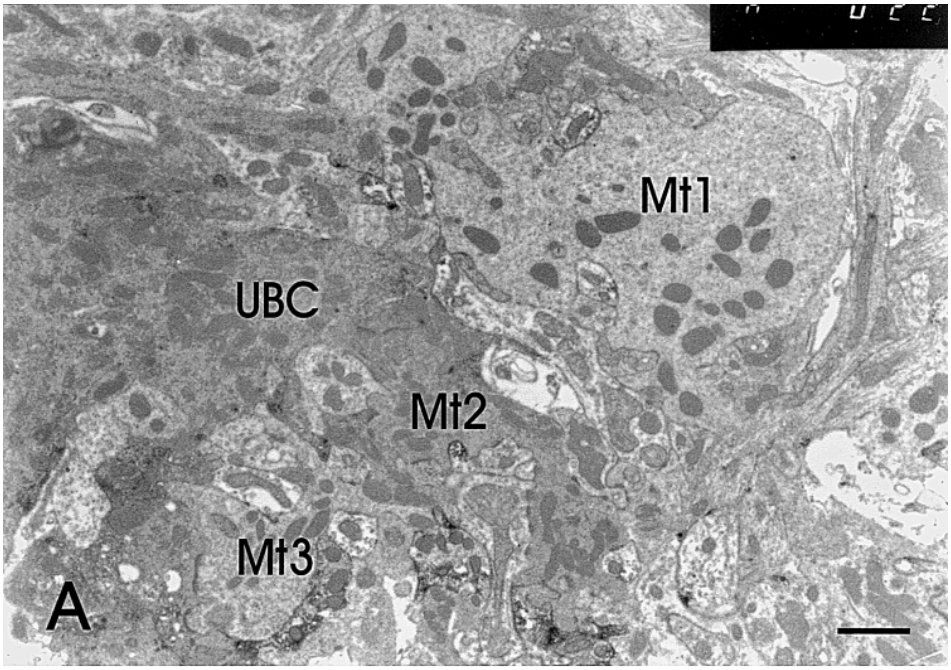
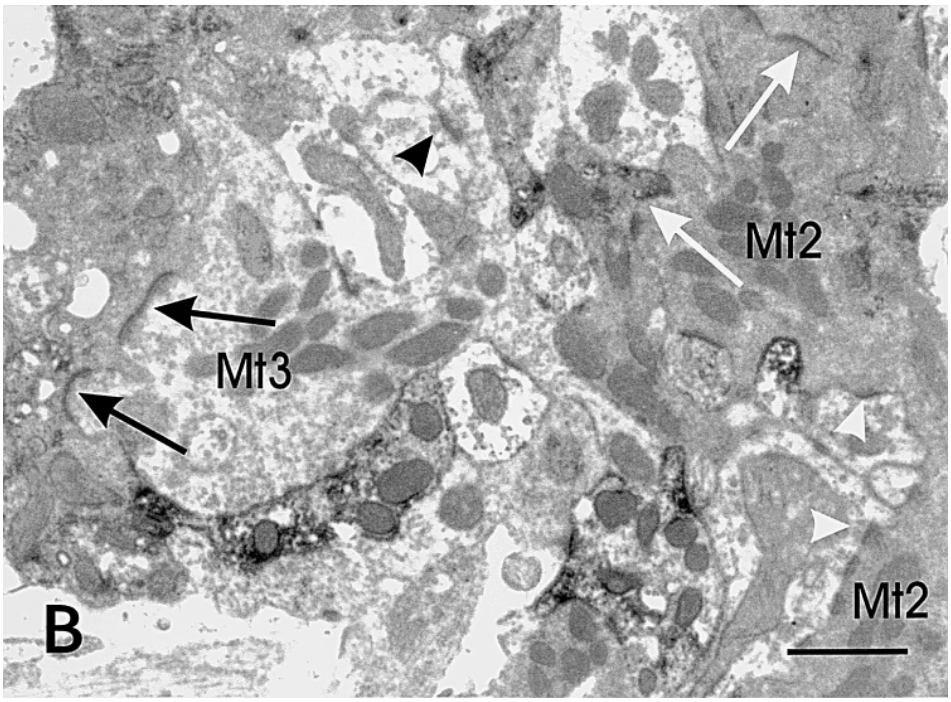


Fig. 8. Incomplete glomerulus built up by the participation of a mossy terminal of "dispersed" type (Mt). This axon terminal is in close apposition with the soma of a typical granule cell (Gc) on the right side of the picture, and with a short, moderately labeled (most probably Golgi) dendrite (black asterisk) and forms three synapses of asymmetric type (arrowheads) with a granule cell dendrite (white asterisk) and dendritic digits. Scale bar: 1 μm

Fig. 9. Different types of mossy terminals form synapses with various postsynaptic elements. – A. A large, "dispersed" mossy fiber (Mt1) containing sparsely distributed, small, pleomorphic synaptic vesicles and dark mitochondria in an electronlucent axoplasmic matrix. This axon terminal is the central element of an "incomplete" glomerulus. Another, "clustered" mossy terminal (Mt2) which has a dark axoplasm and contains numerous small synaptic vesicles, and a third type of mossy terminal (Mt3), which has a pale axoplasmic matrix and contains numerous synaptic vesicles and pale mitochondria. Scale bar: 1 μm. – B. At higher magnification, the postsynaptic elements can be distinguished more easily. Mt2 forms large synapses with the somata and/or thick dendritic stem of a UBC (white arrows), and builds up multiple, smaller synapses with enlarged granule cell dendrites and dendritic digits (white arrowheads). Mt3 is synapsing with strongly immunolabeled UBC dendrites (black arrows) and with a moderately labeled Golgi dendrite (in the midddle of the picture) and also with nonlabeled dendrites of granule cells (black arrowhead). Scale bar: 1 μm


Mt1
UBC
Mt2
Mt3
A


Mt2
Mt3
Mt2
B

DISCUSSION

There are convincing data that in dissociated cerebellar cultures mossy and olivocerebellar climbing fibers are missing [43]. In co-culturing experiments, some of the extracerebellar afferents were observed to be present and functionally integrated in these cerebellar tissue cultures [4, 24, 25, 52]. In the present study we investigated those synaptic elements which are able to replace the missing extrinsic afferents of the cerebellar cortical circuitry in regular organotypic cerebellar cultures. The cerebellar explants were taken from P8 mouse pups. At this stage, both mossy and climbing fibers are present in the differentiating cerebellar cortex [1, 7, 13, 26, 29]. As far as the climbing fibers are concerned, a transitory multiple innervation of Purkinje cells has been observed by these afferents during the early postnatal period [6]. Climbing fibers at P8 synapse exclusively on the somata of Purkinje cells, and the regression of multiple climbing fiber innervation is followed by the translocation of olivocerebellar inputs from the soma to the dendritic tree, beginning at the end of the second postnatal week [6, 19] in the rat. On the basis of the similarities in the progression of postnatal developmental events in rat and mice, it seems possible that there is no climbing fiber synapsing on the Purkinje cell dendrites either in the cerebellar cortex of P8 mice. On the other hand, the axosomatic olivocerebellar synapses (and all extrinsic afferents) undergo degeneration in a few days following the set-up of the cultures, similarly to axonal degeneration *in vivo*, following undercutting the cerebellar cortex [17]. In the present study we analyzed the ultrastructural features of the organotypic cultures at 11 DIV (at 19 days of age), by which time olivocerebellar climbing fibers have been eliminated completely, practically before their translocation onto proximal Purkinje dendritic spines (and dendritic shafts), characteristic of in *vivo* cerebellar cortices [21]. In spite of the completely missing olivocerebellar afferents, however, there were only very few empty Purkinje cell spines on thick dendrites (the original location of climbing fibers *in vivo*) in the cultures studied. An ample number of empty Purkinje cell dendritic spines were found in both the lower and upper molecular layers in the developing cerebellar cortex of the rat *in vivo* [48]. On the other hand, the tertiary spiny branchlets of Purkinje cell dendritic trees normally receiving only parallel axons were underdeveloped in organotypic cerebellar cultures [3, 9, 31, 43, 50] resulting in a relative excess of parallel axons without their postsynaptic dendritic targets (the spiny branchlets). It is possible, therefore, that the excess parallel fibers also present in our cultures might replace the missing climbing fiber input to the thicker, proximal Purkinje cell dendrites. Likewise, interneurons of the cerebellar cortex may also occupy vacant synaptic sites, giving supernumerary (although inhibitory) synapses, especially onto the Purkinje cell dendritic shaft. In "granuloprival cultures" (where many granule cells underwent apoptotic cell death), larger axon terminals containing pleomorphic synaptic vesicles (and a few dense-core vesicles) were also seen to synapse with the dendritic stem (and simultaneously with dendritic spines) of Purkinje cells. These terminals may correspond to Purkinje cell recurrent collaterals. Indeed, the plastic changes of recurrent Purkinje cell axon collaterals have been studied in detail in cerebellar cultures in which cere-

bellar granule cells (and oligodendrocytes) were destroyed: in these cultures the collaterals sprout enormously and hyperinnervate the unsheathed somata of Purkinje cells, and form heterotypical synapses with Purkinje cell dendritic spines, normally occupied by homotypical excitatory parallel fibers [43]. In the present study, extremely large axon terminals (containing pleomorphic synaptic vesicles and a dark axoplasmic matrix) connected numerous dendritic spines in granuloprival cultures. It is possible that these axon terminals are the endings of recurrent Purkinje cell axons. Another possibility is that they originate from the deep cerebellar nuclei present in our organotypic cultures (obtained from slices of parasagittal planes). The participation of axonal elements with similar morphology, probably originating from the cerebellar nuclei, has also been observed in cerebellar transplants grafted in the anterior eye chamber [16].

The degeneration and disappearance of extrinsic mossy terminals in organotypic cultures have recently been reported [36], with a degeneration peak at 2 DIV in mouse cerebellar cultures. In long-term cultures (15–30 DIV), the same authors described that the granular layer appeared well-preserved and the UBC axons formed an extensive system of mossy fiber collaterals. In our cultures (in the granular layer), various forms of mossy fiber glomeruli have been observed at DIV 11: i) a simple glomerulus, comprised exclusively of mossy terminal and UBC dendrioles, ii) mixed glomeruli in which besides UBC dendrites, several granule cell dendrites and some Golgi cell dendrites received synaptic contacts from mossy fiber terminals (described also by Morin et al. [32] in the postnatal rat cerebellum *in vivo*). In the present granuloprival cultures, in addition to the above-mentioned two types of glomeruli, iii) the observed "incomplete" glomeruli can be regarded as suppressed ones, in which the characteristic glomerular structure has been altered because of the loss of the original extrinsic mossy fiber input, and the following incomplete reconstruction by dispersed axon terminals most probably originating from the deep cerebellar nuclei. Heterotypical (GABA-containing) and homotypical glutamate-containing axon terminals from the deep cerebellar nuclei exhibiting fine structural features similar to those of the regular mossy-endings and participating in the build-up of cerebellar glomeruli have been observed in the cat [17] and in the rat [2]. Similarly, in our granuloprival organotypic cerebellar culture, iv) the large axon terminals containing numerous smaller synaptic vesicles in a dark axoplasmic matrix can be considered to be either special forms of sprouting intrinsic mossy terminals of UBCs, or axon terminals coming from the cerebellar nuclei.

In conclusion: Organotypic cerebellar cultures from P8 mice at 11 DIV have been studied. In most of these cultures, the cerebellar cytoarchitectonic structure was preserved, at both LM and EM levels. In these cultures, the two main extrinsic excitatory inputs (the climbing and the mossy fibers) of the cerebellar cortex are missing. These inputs seem to be replaced by other axonal types: in the molecular layer mostly by parallel fibers (for climbing fibers) and in the granular layer by intrinsic mossy fibers of local excitatory interneurons (the UBCs). In this compensatory mechanism, the participation of the local neuronal elements (granule cell and UBCs) seemed to be overwhelming. However, in those organotypic cultures, which (although preserv-

ing the trilaminar cerebellar cortical structure) were "granuloprival" but still contained some of the deep cerebellar nuclei, the participation of extracortical axons from the deep cerebellar nuclei in the replacement of the missing efferents is probable.

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