CORRELATIVE MICROSCOPY OF CEREBELLAR AFFERENT AND EFFERENT FIBERS IN THE WHITE MATTER. A REVIEW

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ABSTRACT

The afferent fibers (mossy and climbing fibers), and the efferent fibers represented by Purkinje cell axons of the cerebellar white matter have been described by means of conventional scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) and SEM cryofracture technique, transmission electron microscopy conventional (TEM), and confocal laser scanning microscopy. At SEM level the three-dimensional characterization of mossy and climbing fibers has been made according to their thickness and branching pattern. The mossy fibers appear as thick parent fibers, up to 2.5 μm in diameter with a characteristic dichotomous bifurcation pattern. The climbing fibers are thin fibers, up to 1 μm in diameter, with a typical crossing-over bifurcation pattern. The efferent Purkinje cell axons labeled with calbindin and imaged with confocal laser scanning microscopy appear as red fibers exhibiting recurrent collateral processes.

Key words: Cerebellum, microscopy, afferent fibers, efferent fiber.

MICROSCOPÍA CORRELATIVA DE LAS FIBRAS AFERENTES Y EFERENTES CEREBELOSAS EN LA SUSTANCIA BLANCA. REVISIÓN.

RESUMEN

Las fibras aferentes (fibras musgosas y trepadoras), y las fibras eferentes representadas por los axones de la célula de Purkinje a nivel de la substancia blanca se describen por medio de la microscopía convencional de barrido y la microscopía de emisión de campo y por la técnica de criofractura para microscopía scanning, y la microscopía confocal de rayos láser. En la microscopía convencional de barrido la caracterización tridimensional de las fibras musgosas y trepadoras se describen de acuerdo con el grosor y su patrón de bifurcación. Las fibras musgosas son gruesas (2.5 μm de diámetro), las fibras trepadoras son finas (1μm en diámetro), con un patrón de bifurcación arborescente. Las fibras eferentes representadas por los axones de las células de Purkinje marcadas con calbindina y observadas con la microscopía confocal de rayos láser exhiben prolongaciones colaterales recurrentes.

Palabras clave: Cerebelo, microscopía, fibras aferentes, fibra eferente.

INTRODUCTION

The afferent and efferent fibers of cerebellar cortex were firstly described by Golgi [1], Ramón y Cajal [2-6]; Kolliker [7], Dogiel, Retzius, Van Gehuchten, Held, Bielschowski, Athias and Lugaro (cited by Ramón y Cajal [4]. In his monumental treatise, Ramón y Cajal formerly described the connections of the cerebellum with another centers of central nervous system through the cerebellar peduncles (Fig. 1).

A detailed descriptions of afferent and efferent pathways were later reported by Eccles et al. [8], Brodal and Drablos [9], Brodal [10], and Larsell and Jansen [11].

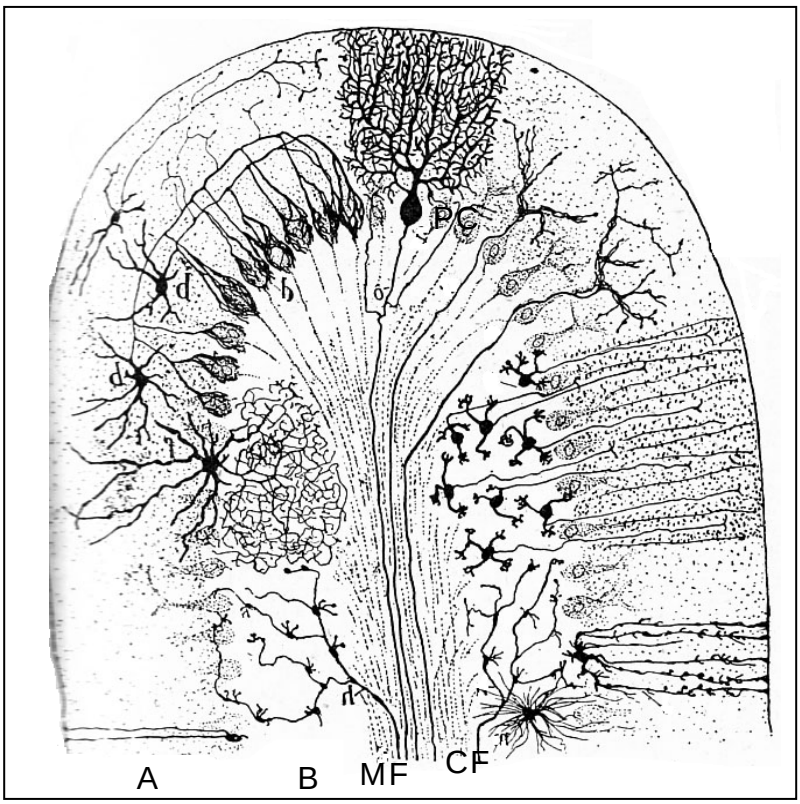

Fig. 1. Transversal section of cerebellar folia drawn from Ramón y Cajal (1911) showing the mossy fibers (MF) entering the granular layer (GL), and the climbing fibers (CF) ascending to the Purkinje cell layer (PC). [Reprinted from Ramón y Cajal, 1911].

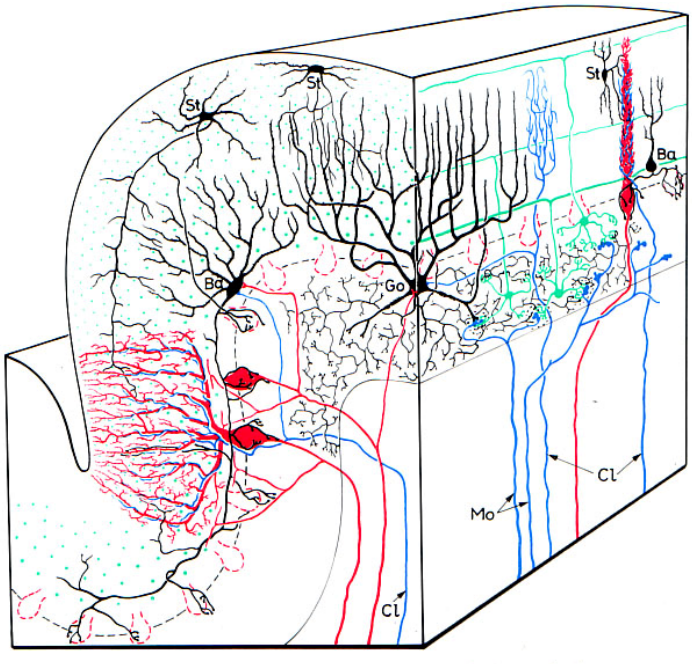

Fig. 2. Stereodiagram illustrating the two main afferent fibers: mossy (Mo) and climbing fibers (Cl) and their main connections with the five types of neurons of the cerebellar cortex [Reprinted from Eccles, Ito and Szentagothai, 1967].

An elegant description of the two main afferent and efferent pathways were subsequently published by Mugnaini [12], and Palay and Chan-Palay [13]. Rivera-Domínguez et al. [14] reported the origin of climbing fibers of Rhesus monkey cerebellum. Ito [15] published an excellent monograph describing in detail the origin of afferent fibers and their topographical distribution in the cerebellum. Feirabend et al. [16] made a quantitative light and electron microscopic analysis of myelinated fibers in the white matter of chicken cerebellum. Shinoda et al. [17] described the entire trajectory of single climbing and mossy fibers in the cerebellar nuclei and cortex. Castejón and Caraballo [18,19]; Castejón and Valero [20], Castejón [21,22]; Castejón and Castejón [23]; Castejón and Sims [24,25]; Castejón and Castejón [26]; and Castejón et al. [27-31] reported the scanning electron microscopic and confocal scanning laser microscopic characterization of mossy and climbing fibers in the white matter of cerebellar cortex, and the intracortical course in the cerebellar cortex of several vertebrates.

According to Ito [32], the afferent and efferent fibers of the cerebellum are localized in the cerebellar peduncles that connect the cerebellar cortex with the rest of the central nervous system. The inferior peduncle contains the vestibular afferents, spinal afferents, lateral reticular nucleus, reticular formation, which form some sources of mossy fibers. The afferents from the inferior olive represent the climbing fibers. The efferent fibers are mainly the axons of Purkinje cells and from the neurons of interpositus, dentate and fastigial nuclei. The middle cerebellar peduncle contains mainly mossy fiber afferent from the pontine nuclei. The superior cerebellar peduncle contains afferent mossy fibers from spinal afferents, and spinocerebellar tract. Pijpers et al. [33] (2006) examined the detailed pattern of collateralization of both types of cerebellar afferent using small injections of the bidirectional tracer cholera toxin b subunit into the posterior cerebellum. The cortical and zonal location of these injections was characterized by mapping climbing fiber field potentials, the distribution of retrograde labeled olivary neurons, and the intrinsic zebrin pattern of Purkinje cells. Labeled climbing fiber collaterals were distributed as longitudinal strips, and were always accompanied by clusters of labeled mossy fiber rosettes in the subjacent granular layer.

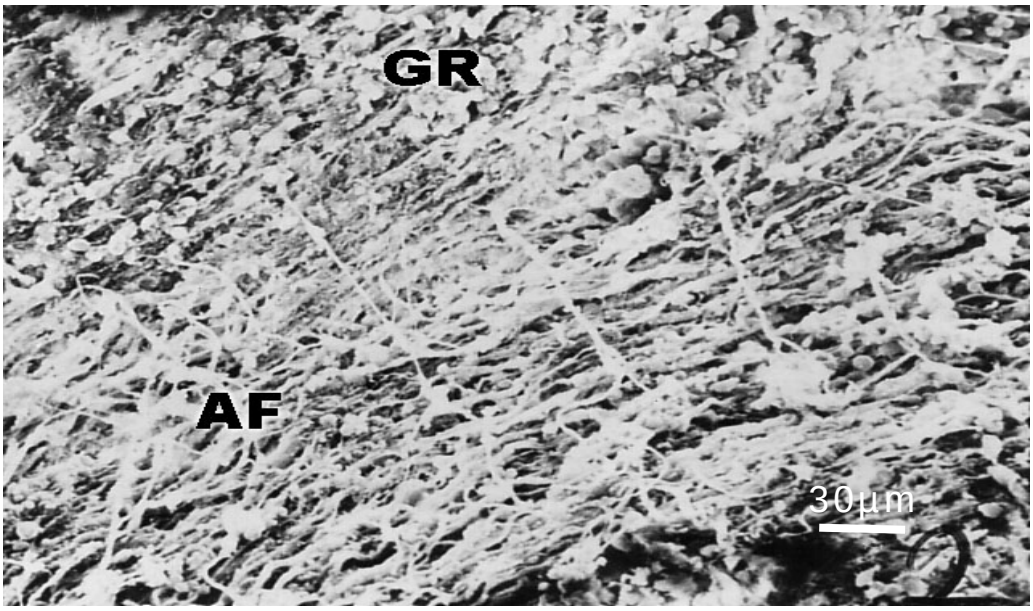

Fig. 3. Low magnification (X 1,000), conventional SEM micrograph showing the afferent fibers (AF) toward the inside of the granule cell layer (GR). [Castejón, 1988].

Diffusion tensor imaging (DTI) color mapping and fiber fractography was used to study the white matter within the cerebellum along with the afferent and efferent tracts associated with the cerebellum in 24 normal human subjects. The most prominent structures that can be readily identified using these DTI techniques are the middle, inferior and superior cerebellar peduncles [34].

The scanning electron microscopy (SEM) demonstration of afferent fibers.

At the center of each cerebellar folium lies a thin layer of white matter composed of the myelinated afferent and efferent fibers connecting the cerebellar cortex with other central nervous system centers. By means of SEM low magnification, the mossy and climbing fibers can be identified according to differential caliber and branching pattern (Fig. 3). Exploration of teleost fish of cerebellar white matter with the scanning electron probe at low magnification, in samples coated with gold-palladium, shows the longitudinal bundles of thick mossy parent fibers intermingled with the bundles of thin afferent climbing fibers. At higher magnification, both types of afferent fibers can be clearly distinguished by their different thickness (Fig. 4). The mossy fibers are up to 2.5 μm in diameter and the climbing fibers up to 1 μm in diameter, measured in cross sections of these fibers in conventional SEM fractographs. At the level of the entrance site to the granular layer, the afferent mossy and climbing fibers are additionally distinguished by their branching pattern. The mossy fibers exhibit a characteristic dichotomous pattern of bifurcation, whereas climbing fibers displayed a typical arborescence or crossing-over type of bifurcation [21-26].

These criteria for identification at SEM level are in agreement with previous Golgi light microscopic studies [12]. The cross-over which follows climbing fiber branching was firstly described by Athias [35], and lately by O' Leary et al. [36].

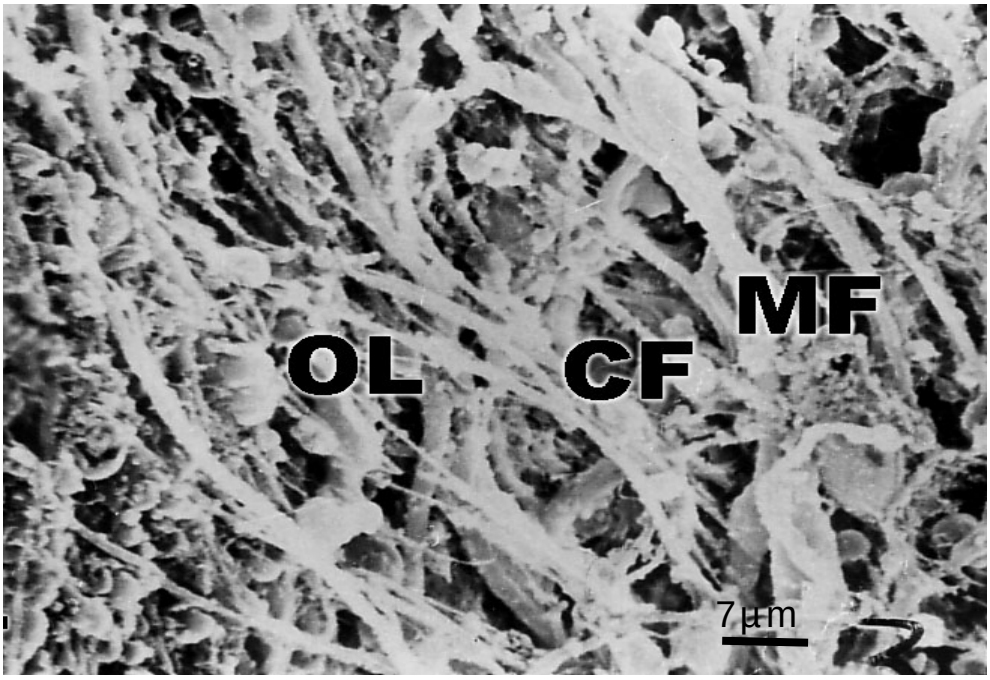

Fig. 4. Higher magnification (X 1,500) conventional SEM image showing the thick mossy fiber (MF), and the thin climbing fibers (CF). The associated interfascicular oligodendroglial cells (OL) also are distinguished. [Castejón, 2003].

At higher magnification the thick mossy fibers, the thin climbing fibers, and the oligodendroglial cells can be clearly distinguished.

Oligodendrocytes or myeling forming cells are characterized by round or spindle shaped cells associated to the afferent fibers (Fig. 4).

Using the freeze-fracture method for SEM [18,22] the cross section of bundles of myelinated axons can be imaged at the white matter (Fig. 5). With the field emission SEM and the freeze fracture method [26], the fractured myelinated axons in the longitudinal axis also exhibit the myelin layer, axoplasm, axonal cytoskeleton, and the relief of mitochondria (Fig. 6).

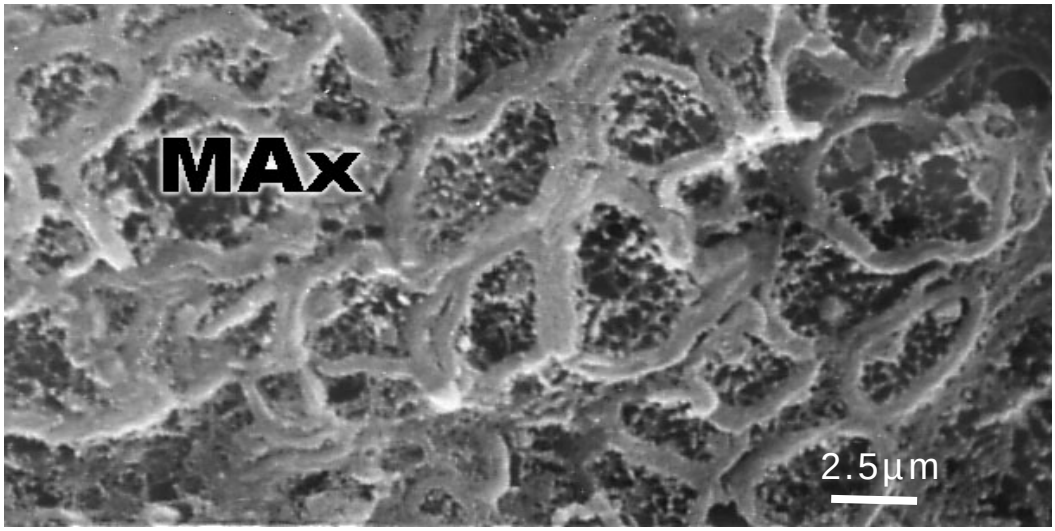

Fig. 5. Freeze-fractured SEM image (X 3,800) showing the cross sections of closely packed myelinated axons (MAx) in the white matter. The low depth of focus and resolution power of scanning microscope do no allow to resolve the periodic structure of myelin sheath. [Castejón, 2003].

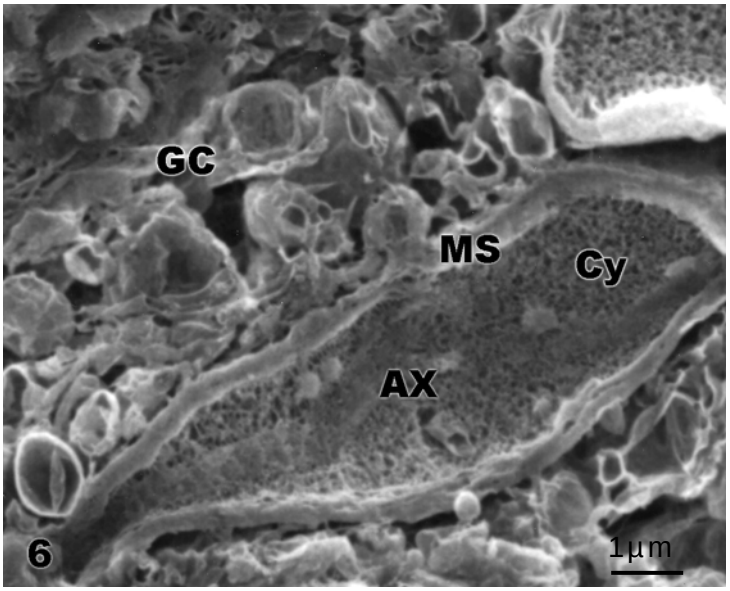

Fig. 6. Rhesus monkey cerebellar cortex. Field emission SEM showing the longitudinal section of a myelinated axon (AX) which display the axoplasmic cytoskeleton (Cy). Note the dense packing of myelin sheath (MS), and the neighboring glial cell cytoplasm (GC) [Castejón and Castejón, 1997].

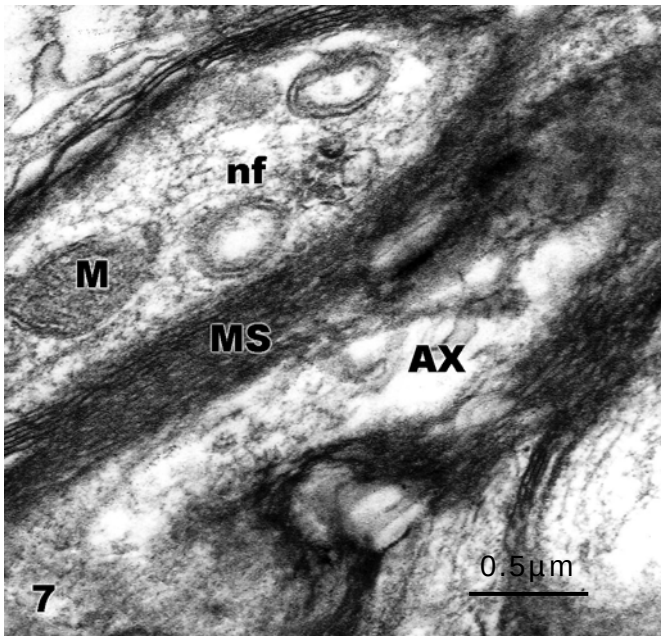

Fig. 7. Mouse cerebellar cortex. Electron micrograph of myelinated axons (AX) showing the periodic structure of myelin sheath (MS). The axoplasm contains the mitochondria (M) and neurofilaments (nf).[Castejón, 2003].

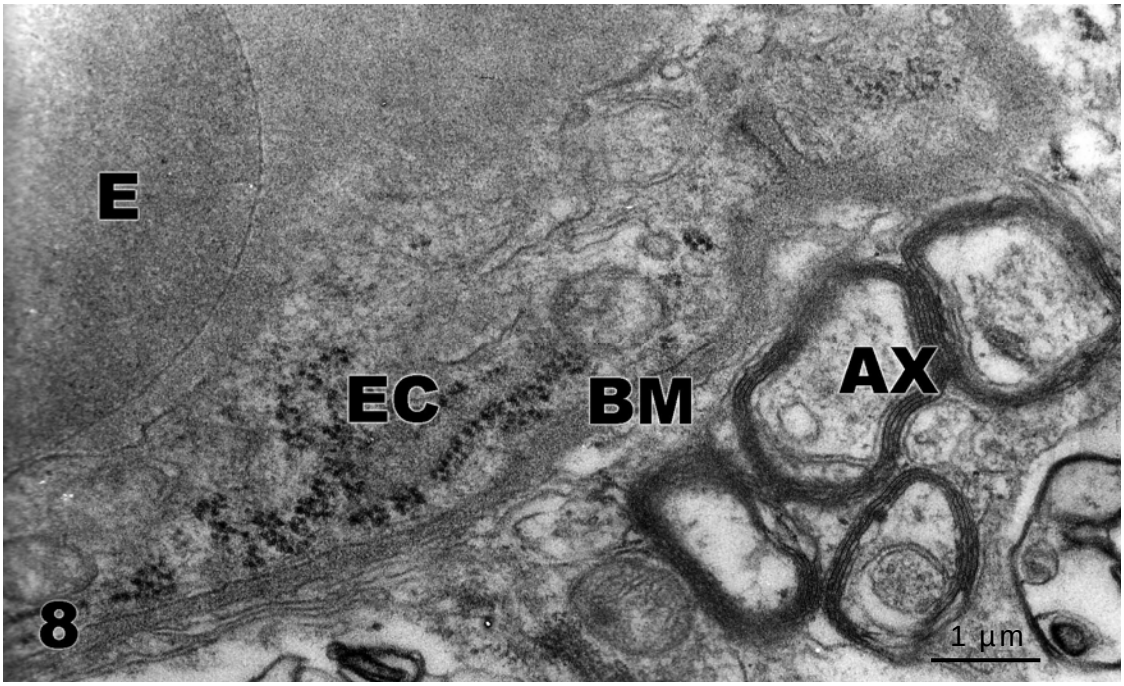

Fig. 8. The myelinated axons (AX), in the close neighborhood of a capillary wall, mouse cerebellar cortex showing a group of the endothelial cell (EC), the basement membrane (BM), and an eritrocyte (E) can be distinguished [Castejón, 2003].

Transmission electron microscopy (TEM).

Close examination of ultrathin sections by means of transmission electron microscope shows the fine structure of myelinated and undifferentiated afferent fibers (Fig. 7).

A group of thin myelinated axons are observed surrounding the capillary wall, apparently providing vascular innervation (Fig. 8).

Confocal laser scanning microscopy.

Optical sections of confocal laser scanning microscopy, taken at the level of the cerebellar white matter of rat, and labeled with Synapsin-I and FM-64 [30, 31], show undifferentiated afferent nerve fibers (mossy and climbing fibers) as compact bundles of myelinated axons with high red fluorescence signal at the level of myelin sheath (Fig. 9).

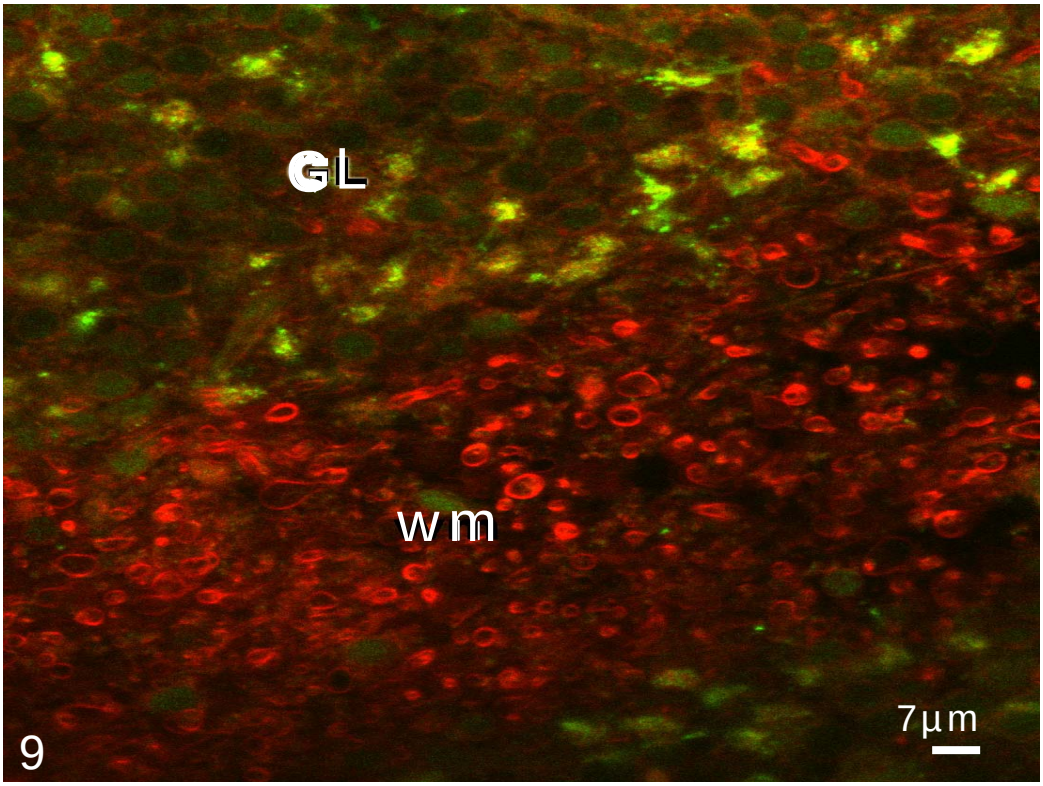

Fig. 9. Confocal laser scanning micrograph of white matter labeled with Synapsin I and FM-64. The cross optodigital sections of myelinated afferent fibers in the white matter appear in red. The green spots at the granule cell layer (GL) correspond mainly to the distribution of mossy fiber presynaptic endings [Castejón and Dailey, 2009].

Serotoninergic and catecholaminergic cerebellar afferent fibers.

In addition to the afferent mossy and climbing fibers, there are also a catecholaminergic and a serotoninergic innervation in the cerebellar cortex. The monoamine innervation of rat cerebellum was earlier studied by Hökfelt and Fuxe [37], both in vivo and in vitro techniques using the histochernical fluorescence method for demonstration of catecholamines (CA) and certain tryptamines. By way of a pharmacological approach using inter alia protriptyline, which acts mainly by blocking the membrane pump of the noradrenaline (NA) neurons, evidence was obtained that CA nerve terminals in the cerebellum mainly represent NA nerve terminals. These were found to innervate practically all parts of the cerebellar cortex with a patchy innervation pattern and with an innervation of especially the anterior and posterior lobes. The terminals mainly seem to make axodendritic contacts in the molecular and granular layers without any strict localization of the terminal

plexus to any special plane of the cerebellar folia. The fibers enter the cerebellum via the inferior cerebellar peduncle and run in the white matter of the cortex cerebelli. Incubation studies with 6-hydroxytryptamine indicate that there exists also a 5-hydroxytryptamine (5-HT) innervation of the cortex cerebelli, although not as pronounced as the NA innervation. The 5-HT nerve terminals are very fine, varicose fibers and innervate mainly the molecular layer, especially of the anterior lobe. The terminals run mainly in the transverse plane of the folium parallel to the surface. Serotoninergic afferent fibers from the caudal brainstem form a fine beaded plexus that is confined almost exclusively to the granular and Purkinje cell layers, as demonstrated by double-labeling paradigm combining retrograde transport of horseradish peroxidase (HRP) with serotonin immunohistochemistry [38]. The catecholaminergic innervation is of the nonjunctional modality and appears as labeled varicosities apposed to dendritic profiles of granule, Purkinje, stellate and basket cells. Abbott and Sotelo [39] suggested that the modulatory function of noradrenergic afferent fibers is exerted through paracrine interaction.

The presence of corticotrophin-releasing factor (CRF) in the cerebellar afferent fibers.

Cummings et al. [40] showed the presence of corticotrophin-releasing factor (CRF) in the cerebellar afferent fibers. The flocculus and paraflocculus of cat and sheep cerebellum were studied with immunohistochemical methods, using antisera to corticotropin-releasing factor. CRF immunoreactivity was present within three populations of varicose nerve fibers. One population of CRF-immunoreactive (CRFIR) fibers appeared to appose Purkinje cell somata and to follow their dendrites into the molecular layer. This arrangement suggested that they are climbing fibers. A second group of CRF-IR profiles reminiscent of mossy fibers was widely distributed throughout the granule cell layer. A third population of CRF-IR fibers was present as a beaded plexus lying parallel to the pial surface, above and subadjacent to the Purkinje cell layer.

Cholinergic afferent fibers.

Multiples origens of cerebellar cholinergic afferents have been described by Lan et al. [41] (1995). Jaarsma et al. [42, 43] have demonstrated immunocytochemically by LM and TEM the presence of ChAT-immunoreactive mossy fibers, mainly those originating in the vestibular nuclei. These Authors showed the autoradiographic distribution of muscarinic and nicotinic receptors in the cerebellar cortex.

Dopaminergic afferent fibers.

According to Ikai [44], it has been suggested that dopamine in the cerebellum not only acts as a precursor for noradrenaline in afferent fibers supplied by locus coeruleus neurons, but also subserves an independent transmitter role in a separate neural system. Employing anterograde and retrograde axonal tracing with cholera toxin and a combination of fluorescent retrograde axonal tracing with Fluoro-Gold, and tyrosine hydroxylase immunofluorescence histochemistry, found in the rat that the ventral tegmental area, containing the A10 dopaminergic cell group, sends projection fibers to the cerebellum bilaterally with a slight contralateral predominance. The projections from the ventral tegmental area to the cerebellum are segregated into the dopaminergic one to the cerebellar cortex, and the non-dopaminergic one to the deep cerebellar nuclei. Dopaminergic fibers projecting from the ventral tegmental area to the cerebellar cortex terminated mainly in the granular layer, additionally in the Purkinje cell layer, but not at all in the molecular layer. They are distributed predominantly in the crus I ansiform lobule and paraflocculus, and to a lesser extent in the crus II ansiform lobule. The projections from the ventral tegmental area to the cerebellum revealed in this study might exert limbic influences upon the cerebro-cerebellar loops subserving the execution and co-ordination of voluntary movements.

Cerebellar efferent fibers.

The main efferent fibers from the cerebellar cortex are represented by Purkinje cell axons. As seen with the confocal laser scanning microscopy and calbindin labeling [31], these axons originate from the lower pole of Purkinje cell soma at the Purkinje cell layer, proceed directly through the granular layer and enter into the white mater. Rat cerebellar slices labeled with calbindin show the entire course of Purkinje cell axon toward the granular layer in their way to the white matter (Fig.10). These axons are thicker than mossy and climbing fibers, and exhibit varicosities and collaterals that return upward to the granular layer, hence their name as recurrent collaterals. [4]. These collaterals distribute in the granular layer, and participate in the formation of Purkinje cell infraganglionic plexus [30].

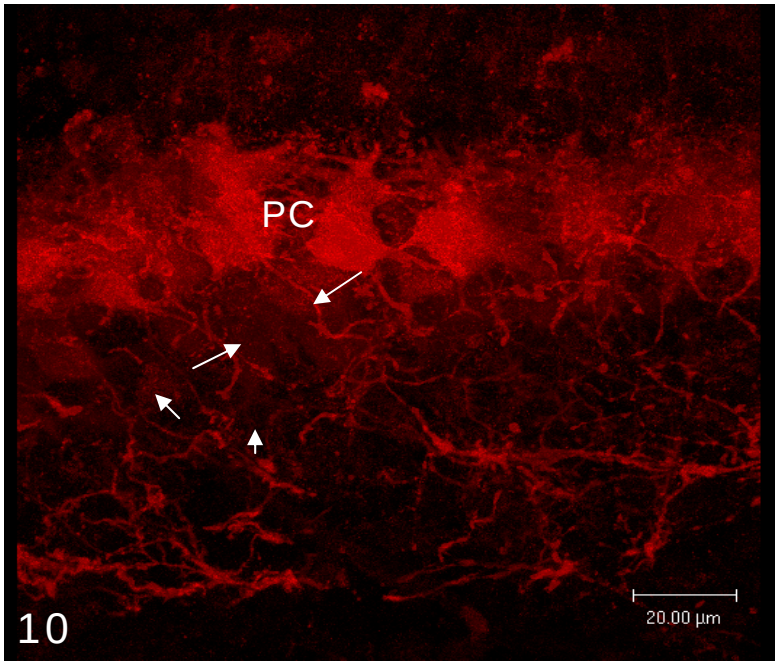

Fig.10. Slice of rat cerebellar cortex labeled with calbindin showing the Purkinje cell bodies row (PC), and the origin of Purkinje cell axons (long arrows) directed toward the granular layer, exhibiting their recurrent collaterals (short arrows). [Castejón and Dailey, 2009].

CONCLUSIONS

The afferent fibers, mossy and climbing fibers, and the efferent fibers represented by Purkinje cell axons of the cerebellar white matter have been described by means of conventional SEM and field emission SEM, and the SEM cryofracture technique, conventional TEM, and confocal laser scanning microscopy. At SEM level the three-dimensional characterization of mossy and climbing fibers has been made according to their thickness and branching pattern. The mossy fibers appear as thick parent fibers, up to 2.5 µm in diameter with a characteristic dichotomous bifurcation pattern. The climbing fibers are thin fibers, up to 1 µm in diameter, with a typical crossing-over bifurcation pattern. The efferent Purkinje cell axons labeled with calbindin and imaged with confocal laser scanning appear as red fibers exhibiting recurrent collateral processes.

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