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CORRELATIVE MICROSCOPY OF CEREBELLAR BERGMANN GLIAL CELLS

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Confocal laser scanning microscopy (CLSM) techniques for double fluorescent labeling stained tissue afford the exciting possibility of studyin three-dimensional structure and cell to cell interactions at high spatial resolution in the context of three-dimensional brain tissue architecture (1,2). It is also posible to combine CLSM observations with other three-dimensional ultrastructural analysis, such as field emission scanning electron microscopy (FESEM) and freeze-fracture transmission electron microscopy. Such intermicroscopic study should improve our understanding of cell morphology and distribution, relations of different cell populations and cell-to-cell spatial relationship. Confocal laser scanning microscopy and double fluorescent labeling, transmission electron microscopy (TEM) by means of ultrathin sections and freeze-etching replicas, field emission scanning electron microscopy (FESEM) and cryofracture method were applied to the study of Bergmann glial cells of several vertebrates to obtain an in depth insight on their three-dimensional morphology.
Double fluorescent labeling of rat cerebellar cortex, using primary antibody to GFAP and Alexa fluor conjugates for secondary detection, were used for CLSM. Field emission SEM, ultrathin sectioning and freeze-etching replica methods for TEM of mouse, rat and Rhesus monkey cerebellar cortex were also examined in an attempt to obtain a new and more acurate view of three-dimensional image of Bergmann glial cells (BGC) and their topographic relationships in the molecular layer. Intense inmunopositive GFAP green staining was observed in numerous stellate, ovoid and triangular BGCs and glial limiting layer (Fig.1). Secondary antibody conjugated with Alexa fluor 488 and Alexa fluor 668-IB4 stained simultaneously red capillary endothelial cells and microglial cells. BGC morphology revealed the existence of several cell types of subpopulations of BGCs. In CLSM and FESEM, Bergmann glial fibers, in palisade arrangement, branch and rebranch forming a complex glial network in the molecular layer. Field emission SEM and freeze-fracture SEM method using chromium coating, showed the SE-I image of high mass dense Bergmann glial cytoplasm ensheathing like a veil the Purkinje cell (PC) soma (Fig.2) and denditric arborization. Bergmann glial fibers appeared completely surrounding individual parallel fibers or parallel fiber bundles, terminal climbing fiber collaterals, basket and stellate cells and capillaries (Fig.3). Figure 4 illustrates a higher magnification how BGC lamellae envelopes the tertiary spine dendritic ramifications. Freeze-etching direct replicas showed the typical orthogonal arrangement of intramembrane particles [2,3], corresponding to the large repertoire of BGC receptors. The study reveals the three-dimensional Bergmann glial cell heterogeneity and the complex network formed by Bergmann glial cells in the molecular layer and their intrincate enveloping process of all the constitutive elements of molecular layer.

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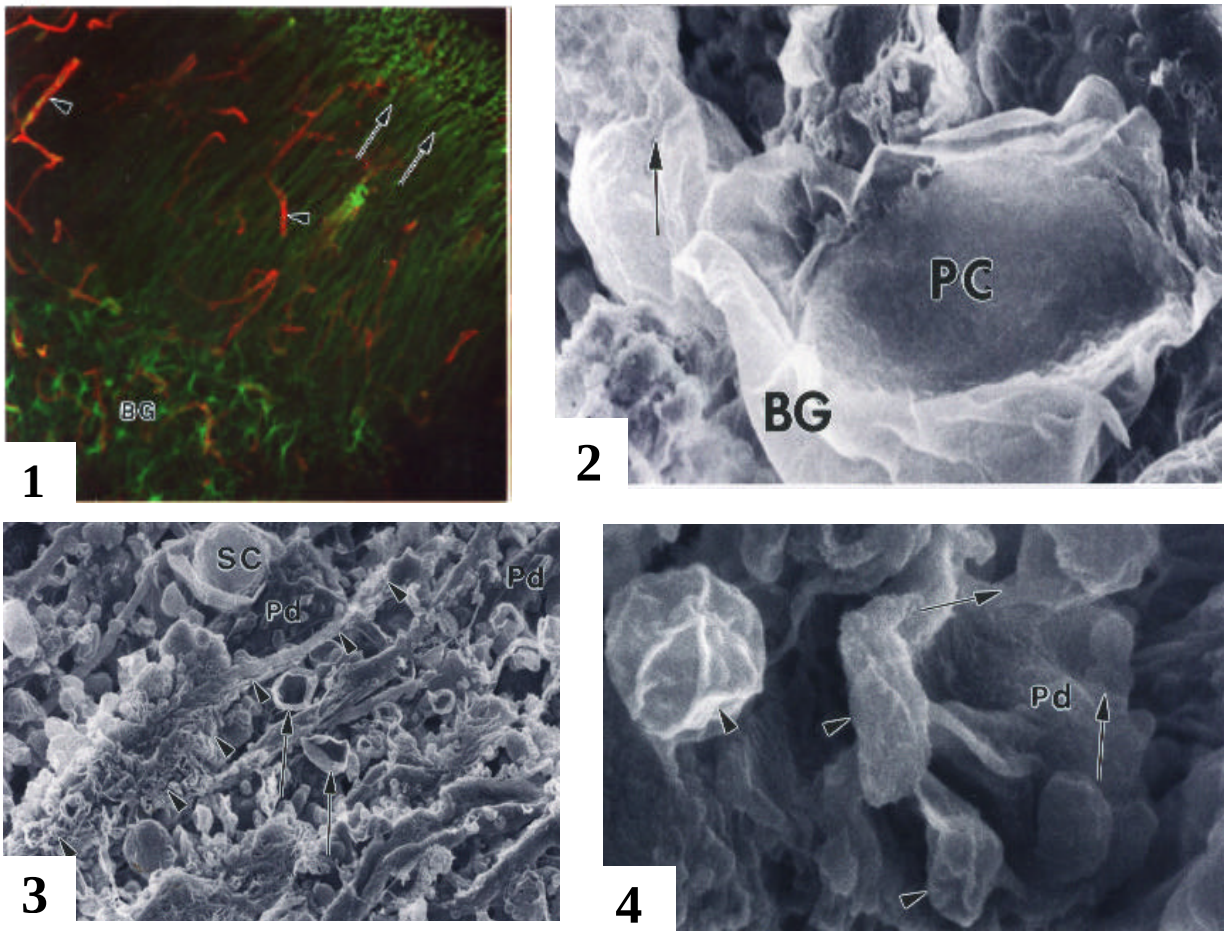

Fig. 1. Confocal laser scanning microscopy of ten day-old rat cerebellar cortex. Numerous fluorescent green coloured Bergmann glial cells (BG) exhibit an intense immunopositive GFAP expression at the cell body and radial fibers (arrows). Cerebellar capillaries (arrowheads) appear stained in red for the Alexa-fluor 668-IB4. This 2D projection image represents a stack of 12 confocal optical sections at 1 µm intervals. Note the palisade arrangement of Bergmann glial fibers and their enlarged endings (arrows) at the upper right angle of the figure, at the level of glial limiting layer. X 650

Fig. 2. Field emission scanning electron micrograph of mouse cerebellar Purkinje cell layer. The high mass density of Bergmann glial cell cytoplasm (BG) appears completely enveloping the Purkinje cell body (PC). The initial segment of ascending Bergmann glial radial fibers is also noted (arrow). X 1500

Fig. 3. Field emission scanning electron micrograph of mouse cerebellar molecular layer showing the Bergmann glial radial fibers (arrowheads) ascending throughout the thickness of molecular layer and covering Purkinje spiny dendritic branches (Pd), stellate cells (SC) and parallel fibers (arrows). X 2000

Fig. 4. Field emission scanning electron micrograph of molecular layer primate cerebellar cortex. The thin 2 nm chromium coating makes possible the visualization of the brilliant lamellar Bergmann glial cell cytoplasm (arrowheads) ensheating the Purkinje spiny dendritic ramification (Pd). Purkinje dendritic spines are indicated by arrows. X 3000

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