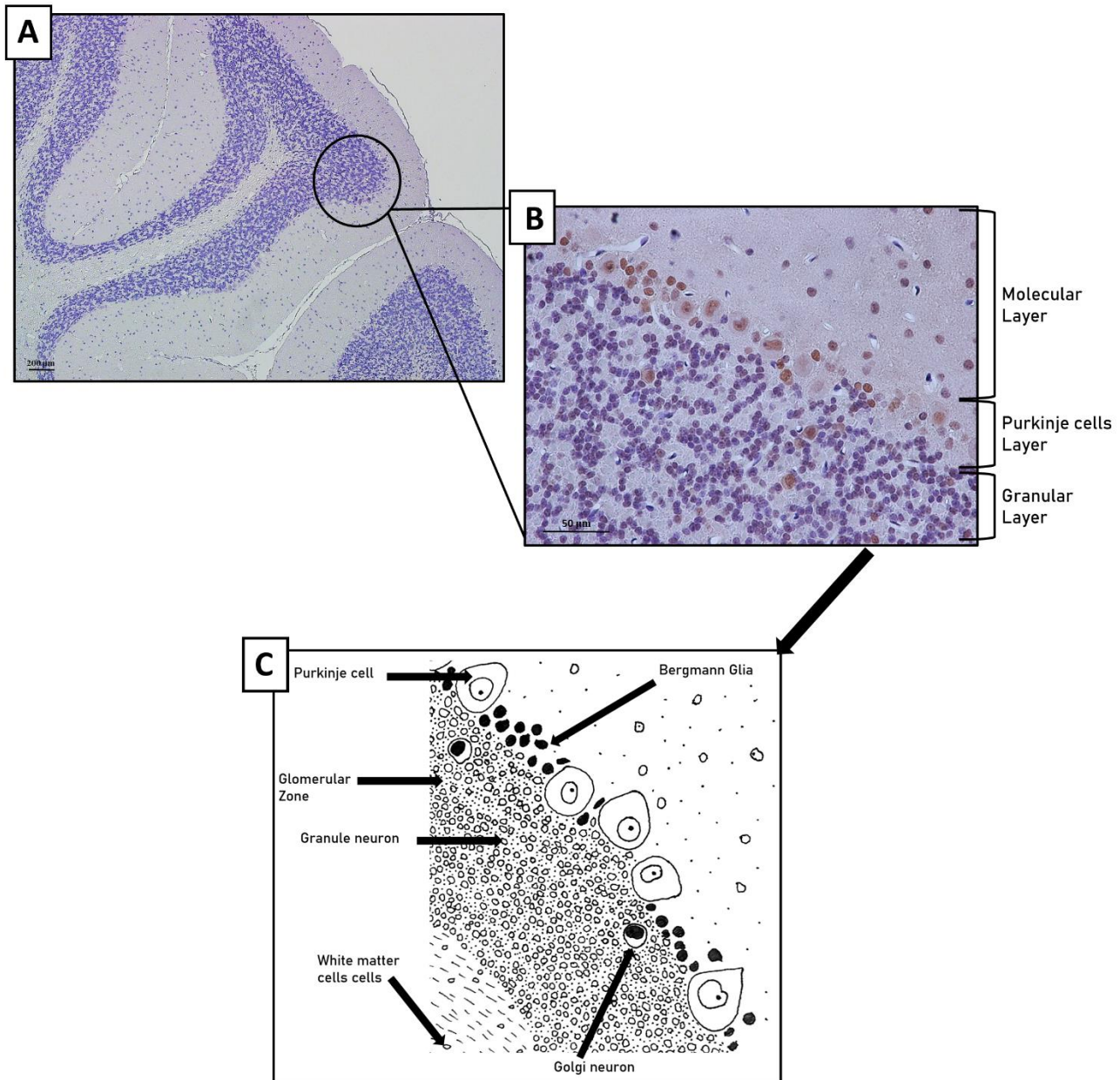


## Supplementary Material

### Cytoarchitecture of cerebellum: brief explanation

In this paper, we analyzed some specific cerebellar cell types, distributed in different layers of this organ. Here we aim to describe the location and some morphological aspects of the analyzed cells, aiming to facilitate the understanding of the immunohistochemical plates and results.



**Supplemental Figure 1.** Schematic figure of cerebellar histology. **A.** Photomicrography of cerebellar folia (4x; Hematoxylin and Eosin). **B.** Immunohistochemistry for TLR4, showing the cerebellar layers (40x; Hematoxylin). **C.** Didactic model representation of cerebellar cell types (not represented in scale).

Cerebellum consists of outer layer of grey matter, the cerebellar cortex, and inner layer of white matter. Cerebellar cortex is folded to form narrow leaf like bands called folia (Fig.1 – A). Each folium consists of central core of white matter surrounded by thin layer of grey matter. [1; 2].

Cerebellar cortex is composed of three distinct layers: (a) outer molecular layer, (b) intermediate Purkinje cell layer, and (c) inner granular layer (Fig. 1 – B).

The molecular layer consists of unmyelinated nerve fibers derived from axons of granule, stellate and basket cells, dendrites of Purkinje and Golgi cells (Fig. 1 – B). This layer content few cellular bodies from stellate and basket cells, the neurons of molecular layer [2]. The cells of this layer were not analyzed in the present article.

Purkinje cell layer consists of single layer of flask shaped Purkinje cells [2]. The large Purkinje cells corresponds to the effector pathway of the cerebellar cortex. Its axon enters the granular layer toward the central nuclei of the cerebellum, where it forms an inhibitory synapse. This cell usually presents globular cell body and dendrites in the upper pole that originates elaborated branches in the perpendicular plane to the largest axis of the cerebellar leaf (Fig. 1- C). The nucleus of the Purkinje cell is large, with granular chromatin and a prominent nucleolus [3].

In the Purkinje cells layer, another cellular element can be observed: the Bergmann glial cells (Fig. 1-C. Note that in the figure 1 – B, the Bergmann Glia presents stained nucleus, when the granule neurons do not behavior like that, facilitating the didactic differentiation of them under optic microscopy in this scheme). The nucleus of Bergmann Glia stained with Hematoxilin and Eosin is usually paler than the granule cells nearby, being first described like that by Ramón y Cajal (1911). The cell bodies of Bergmann Glia are disposed around the Purkinje cells, and extend radial fibers enwrapping synapses on Purkinje cell dendrites [4]. These cells are specialized astrocytes, which are also called 'Golgi epithelial cells' [1]. During development, Bergmann fibers display a tight association with migrating granule cells, from which the concept of glia-guided neuronal migration has been proposed. Thus, it is widely known that the Bergmann glia is associated with granule cells in the developing cerebellum and with Purkinje cells in the adult cerebellum [4].

In the granular layer, unlike the molecular layer, there are numerous nuclei, which belong, largely, to the granular cells (Figure 1 - C). The bodies of Golgi cells (another type of neuron) are also located there (Figure 1 -

C). The granular cell nuclei have an irregular distribution, and the spaces between them constitute the cerebellar glomeruli, a complex of synapses [3; 5] (Fig.1 – C).

The white matter of the cerebellum is the deepest layer in this organ. It is composed by intrinsic, afferent and efferent myelinated fibers. In this layer, glial cells can be found, being difficult to separate them under optic microscopy. In the present article, we did not classify these cells for counting and they were considered as “white matter cells”. In this layer, the intracerebellar nuclei can be observed. Also known as central nuclei, they are composed by grey matter embedded in white matter [2]. The central nuclei were not considered for counting in the present article.

#### References:

- [1] Sanford L. Palay . Victoria Chan-Palay. Cerebellar cortex: cytology and organization. *Springer-Verlag Berlin' Heidelberg*, ed.1, 1974.
- [2] Rajani Singh. Cerebellum: Its Anatomy, Functions and Diseases. *Intechopen*. 2020. doi:10.5772/intechopen.93064.
- [3] Ângelo B. M. Machado. Neuroanatomia funcional. 2 ed. São Paulo: Atheneu, 2007. 363 p.
- [4] Keiko Yamada; Masahiko Watanabe. Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. *Anat Sci Int*. 2002 Jun;77(2):94-108. doi: 10.1046/j.0022-7722.2002.00021.x.
- [5] Lazaros C. Triarhou. The significance of the granular layer of the cerebellum, by Professor Heinrich Obersteiner (English Translation). *The Cerebellum*. <https://doi.org/10.1007/s12311-020-01182-y>