Anatomical histological structure of the cerebellum in the Iraqi frog *Rana ridibunda ridibunda*

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Abstract. The current study was designed to investigate the histological structure of the cerebellum in the Iraqi frog *Rana ridibunda ridibunda*. The cerebellum was removed surgically from 20 Iraqi frogs, *Rana ridibunda ridibunda*, and preserved in fixative of 10% formalin solution for a period of 72 hr, then the histological sections were prepared by the method of paraffin. The results of the study showed that the cerebellum of the adult Iraqi frog is one of the rhombencephalon regions, and consists of three layers, molecular layer, purkinje cells layer, and granular layer. The present histological findings suggest that the cerebellum of the Iraqi frog is very similar to that of other vertebrates, Therefore it represents the basic rule in understanding the histological formation of the cerebellum.

Keywords. Cerebellum, Brain tissue, *Rana ridibunda*, Iraqi Frog.

1. Introduction

Cerebellum is the part of brain responsible for balance and movement, it is incomplete developed in amphibian, while it is well developed in other advanced vertebrates, all the sensory neurons pass through cerebellum with the exception those responsible for vision and olfaction, the reflex induces of hearing, respiration and engulfing [1]. The cerebellum is responsible the metencephalon and is histologically formed in amphibians from the corpus cerebelli and the auricular lobes [2, 3]. The corpus cerebellum presents the center of the cerebellum and consists of a transverse plate consisting of three layers: the molecular layer consisting mainly of fibers and a few neurons, purkinje cells layer which is characterized by containing large-sized purkinje cells, and granular cells layer that contains dense granular cells, auricular lobes are less developed [4].

2. Materials and Methods

A total of twenty adult Iraqi frog (*Rana ridibunda*) samples were obtained from some gardeners at Baghdad governorate during the period of 2018 to 2019. The samples were anesthetized by Chloroform. The isolation of frog brain as followed method by[5], the frog was attached at the dissection board and its back facing up, then cutting the skin crosswise behind the head and pulling forward to remove it from above the head area using the scalpel, and the edge of the scalpel was
placed above the dorsal midline of the skull, and moving the scalpel forward and backward several times with slight pressure until the cranium is opened to raise the roof cranium bones with the edge of the scalpel and cut it to reveal the brain. After the brains have been removed from the skulls, fixed in 10% formalin solution for 72 hr [6]. later, the samples washed by tap water and kept in 70% ethanol, after that the fixed tissues were processed by successive in a series of ethanol treatments, then cleared by xylene, two times was treated half an hour each, infiltrated and embedded into two changes of soft paraffin, one hour each at oven 58°C, Then paraffin blocks were obtained, serial sections of (7) μm thickness were cut and stained with the following histological stains [7, 8]. Stained with haematoxylin-van gieson stain and methylene blue stain and crystal fast violet stain for histological study [9, 10]. The sections were observed with an Olympus light microscope and were photographed with a digital camera Canon mounted on the microscope.

3. Results and Discussion

The sections view of cerebellum in adult Iraqi frog (Rana ridibunda) showed that its consists of three layers, which are from top to bottom the molecular layer, purkinje cells layer, and granular layer (Figure 1 and 2).

![Figure 1. Sagittal section of the Iraqi frog's brain passing through the cerebellum, note: molecular layer (ML), purkinje cell layer (PCL), granular layer (GL), (Haematoxylin- Van Gieson stain, 10X).](image-url)
Figure 2. Sagittal section passing through the cerebellum of the brain Iraqi frog, note: molecular layer (ML), purkinje cell layer (PCL), granular layer (GL), ependymal cell (EpC), purkinje cell (PuC), spherical neuron (SN), satellite neuron (StN), spindle neuron (SpN), granular cell (GC), parallel fiber (PrF), climbing fiber (CF), mossy fibers (MF), axon hellock (AxH), dendrite (D), axon (Ax), glial cell (GiC), cilia (Ci), (Crysel fast violet stain), A- cerebellum layers (10X), B- molecular layer (100X), C- purkinje cell layer (100X), D- granular layer (100X).

3.1. Molecular layer

In the outer surface layer, it was observed when using the (crysel fast violet stain), the presence of irregularly distributed neurons such as spherical neurons with an average diameter of 6 micrometers, and satellite neurons with an average diameter of 4 micrometers, and spindle neurons with an average diameter of 3 micrometers (Figure 2 B). All of these cells have dendrites and axons that extend towards the layer of purkinje cells and intertwine with their dendrites, in addition to the presence of diffuse glial cells, and it was also observed when using (methylene blue stain) that the molecular layer contains neurofibers that extend vertically called climbing fibers formed from the extensions of the...
dendrites of Purkinje cells through the surface layer and the dendrites of the cells of the molecular layer. These fibers represent the main component of the molecular layer (Figure 3).

3.2. Purkinje Cells Layer

In the middle layer of the cerebellum, which is a narrow region located between molecular layer and granular layer, a sagittal section showed that its consists of two to three rows of Purkinje cells, which are characterized by large cells with an average diameter of 11 micrometers, characterized by their elliptical or spherical shape. It is characterized by its elliptical or spherical shape, containing large nuclei of central location, as well as containing the Nissl's granules, and the axon hellock is evident, which is devoid of Nissl's granules, and attached to the axon which is long and extends towards the outer molecular layer (Figure 2C). Purkinje cells are also distinguished by their dendrites that intertwine with the parallel fibers and with the dendrites of the cells of the molecular layer as well (Figure 4).
3.3. Granular Layer

The inner layer of the cerebellum, which is a thick layer, is made up of granular neurons smaller than purkinje cells. They are dense in number, spread in a converging way and have a spherical shape with an average diameter of 5 micrometers. They have dense dendrites, its axons extend up towards the molecular layer and then branch into two branches, each opposite in direction to the other if it extends in a direction parallel to the outer surface of the cerebellum in the shape of a (T), forming a bundle of parallel fibers (Figure 2D and 4). It has been observed, by using the methylene blue stain, the presence of filamentous nerve fibers located between granular cells called mossy fibers, which are intertwined with granular cells, below the granular layer lies ependymal cells that lining of the cerebellum, which is made up of a single row of columnar cells ciliated towards the 4th ventricle (Figure 5).

4. Discussion

In the present result, the cerebellar wall appeared that consisting of three layers, which are from top to bottom: the Molecular layer, the Purkinje cells layer and the Granular layer and this is in agreement with what [3, 4]. In their study of different amphibians as well as similar to what was found in other vertebrates as in the study of [11] in their study on the bird Coturnix coturnix. The current result also showed that, the molecular layer represents the outer layer of the cerebellum, and it consists of irregularly scattered neurons such as spherical neurons with an average diameter of (6) µm, and satellite neurons with an average diameter of (4) µm, and spindle neurons with an average diameter of (3) µm. These cells have dendrites and axons that extend towards the purkinje cell layer and intertwine with the dendrites of purkinje cells, and this is consistent with [12] in his study of Rana esculenta frog as he indicated that the molecular layer is composed of spherical and pyramidal cells, and astrocytic cells reached (7-13) µm. The molecular layer also contains nerve fibers (climbing fibers), it extends vertically and consists of the extension dendrites of purkinje cells to the surface layer and cell dendrites in the molecular layer, this is consistent with the study of [12] on a group of frogs, Rana cateshiana, Rana pipiens and Hyla regilla. On the other hand, the current result showed that the layer of purkinje cells represents the middle layer of the cerebellum, which is a narrow region located between the outer molecular layer and the inner granular layer, consisting of rows of purkinje cells, characterized by being large cells with an average diameter of (11) µm, characterized by their elliptical
shape. Spherical, containing large nuclei in central location, and this corresponds to [13] as he indicated that Purkinje cells are distinguished by their elliptical or pear-shaped shape and have an average diameter of (16) µm in his study on the cerebellum of the frog *Rana esculenta*. The granular layer, it represents the inner layer of the cerebellum, and it consists of dense granular neurons close to each other, smaller in size, smaller than Purkinje cells of a spherical shape with an average diameter of (5) µm and have dense dendritic dendrites that extend their axons towards the molecular layer to the top and then branch to two branches, each opposite in direction to the other, if it extends parallel to the outer surface of the cerebellum forming parallel fibers a bundle of T-shaped. This is consistent with the study of [14] in their study of amphibians that the granular layer consists of small-sized granular cells with a spherical shape with an average diameter of (5-9) µm, and their dendrites and axons extend towards the molecular layer and then branch into two branches each they are opposite to each other, forming a bundle of parallel fibers T-shaped, [15] also indicates the presence of filamentous nerve fibers located between granular cells called mossy fibers, which are intertwined with the granular cells, and this is evident in the present result as well, as the mossy fibers were found intertwined with the granule cells in the form of anastomosis. The function of the moss fiber is that it is one of the incoming fibers that communicate information to the cerebellum [16]. The ependymal cells layer which lies below the granular layer, consists of a single row of ciliated columnar ependymal cells, and this is consistent with what was reported by [17] in their study of the cerebellum in the different species of frogs.

5. References

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