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العدد الثالث  
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### دراسة نسجية للمخيخ في الحمامة الضاحكة (*Spilopelia senegalensis*)

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#### المستخلص:

اجريت الدراسة للتعرف على الوصف الشكلي والفحص النسجي والقياسي للمخيخ في طائر الحمامة الضاحكة (*Spilopelia senegalensis*) ولتحديد طبقاته النسجية. تم الحصول على ١٥ طائراً بالغاً من كلا الجنسين من الاسواق المحلية في بغداد، العراق. تضمنت الدراسة التشريحية اجراء شق تشريحي في الجزء الخلفي من الدماغ وفصل عظام الجمجمة بعناية للحصول على المخيخ. أظهرت نتائج الدراسة ان المخيخ كان الجزء الاكبر من الدماغ الخلفي واحتوى على عشر وريقات مخيخية كما احتوى على ثلاث طبقات نسجية هي الطبقة الجزيئية بلغ معدل سمكها من ناحيه سمك الورقة ( $0.870 \pm 42.337$ ) مايكرومتر، والطبقة خلايا البركنجي تقع بين الطبقتين بلغ معدل سمكها ( $1.07 \pm 23.6$ ) مايكرومتر والطبقة الحبيبية تقع اسفل طبقة خلايا البركنجي بلغ معدل سمكها ( $1.04 \pm 27.101$ ) مايكرومتر، حيث ظهرت اكثر الطبقات سمكاً. كما احتوت الطبقات النسجية على خلايا عصبية متعددة منتشرة داخل المادة السنجابية Gray mater والمادة البيضاء White mater.

الكلمات المفتاحية: المخيخ، خلايا البركنجي، القشرة المخيخية، الحمامة الضاحكة.

#### Histological Study of the Cerebellum in (*Spilopelia senegalensis*)

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**Abstract:**

The study was conducted to identify the morphological description as well as the histological and standard examination of the cerebellum of the laughing dove (*Spilopelia senegalensis*) and to determine its histological layers. 15 adult birds of both sexes were obtained from local markets in Baghdad, Iraq. The anatomical study included making an anatomical incision in the posterior part of the brain and carefully separating the skull bones to obtain the cerebellum. The results of the study showed that the cerebellum was the largest part of the hindbrain and contained ten cerebellar folia and three layers: the molecular layer, the average thickness of which in terms of folia thickness was  $(42.337 \pm 0.870) \mu\text{m}$ , the Purkinje cell layer located between the two layers, the average thickness of which was  $(23.6 \pm 1.07) \mu\text{m}$ , and the granular layer located below the Purkinje cell layer, the average thickness of which was  $(27.101 \pm 1.04) \mu\text{m}$ , where it appeared to be the thickest layer. The tissue layers also contained multiple nerve cells spread within the gray matter and the white matter.

**Keywords:** Cerebellum, Purkinje cells, cerebellar cortex, *Spilopelia senegalensis*.

**Introduction:**

The laughing dove (*Spilopelia senegalensis*) is one of the types of birds found in all regions of Iraq, it belongs to the order Columbiformes, family columbidae, it is distinguished by its pink head (a mixture of red and brown) and wide dotted collar below the neck and above the chest, consisting of split feathers with black bases and their tips are gunmetal (light brown). The back is brown with a gray tinge in the wing coverts and gray in the rump, the chin is whitish, the chest is reddish brown, the abdomen with white undertail, the tail is long and it contains two middle feathers are grey-brown, the next four feathers are gray and the rest are black with a white tip. The female is darker in color than the male, and her neck collar is less clear, the iris is reddish-brown, the beak is black, the feet are crimson, and the length



is about 27 cm (Allouse, 1961). The neural tube which includes brain and spinal cord formed by neurulation in which the hollow tube formed during this process to form the ventricles of the brain and the spinal cord, brain cavity gives rise three cavities, cranio-caudally and are labelled as: Prosencephalon, mesencephalon and rhombencephalon (Kadasine, 2011). Prosencephalon further gets subdivided in to the telencephalon and the diencephalon, telencephalon forms the cerebral vesicles, diencephalon forms the cavity of the thalamic region, rhombencephalon gets subdivided in the cranial part is called the metencephalon and in the caudal part is called the myelencephalon (Hodge, 2010). The nervous system is divided anatomically into the central nervous system (CNS) and peripheral nervous system (PNS). The CNS comprises the brain and spinal cord, the PNS all nerve fibers axons and dendrites and collections of their cell bodies that lie outside the CNS (Kardong, 1997). The cerebellum is a bilaterally symmetric part of the brain with an extensively folded surface that has thin transverse folds Known as folia, which resemble leaves of a tree. It consists of a surface layer of cortex of gray matter and a medullary center of white matter (Treuting, 2017; Hammodi & Al Aamery, 2022). Based on surface appearance, three lobes can be distinguished in the cerebellum: the flocculonodular lobe, anterior lobe (above the primary fissure), and posterior lobe below the primary fissure (Finlay et al., 2001; Khadhim et al., 2021). The cerebellar cortex has a remarkably uniform trilaminar organization: an outer molecular layer, an inner layer of granule cells, and a middle monolayer of large pear-shaped neurons Known as purkinje cells. The molecular layer is a pale-stained zone with relatively few neuron bodies. It contains a network of profusely branching dendrites of purkinje cells and represents mainly a synaptic field (Ashour et al., 2019). Cerebellar purkinje cells have a unique flask-like shape and one of the largest neurons in the CNS. At 15-30 million, they are also among the most numerous in the brain, they form a single row of uniformly arranged large neuron bodies on the outer surface of the granule cell layer, light microscopy show a single, vascular nucleus with prominent Nissl substance in surrounding cytoplasm, by electron microscopy, primary and secondary dendrites are smooth surfaced small tertiary branches have



short, stubby spines (Paulsen, 2000; Mahmoud et al., 2018). Each purkinje cells has more than 100,000 dendritic spines that markedly increase its surface area for synaptic contact. A single myelinated axon projects from the base of each purkinje cells and descends to the underlying medullary white region. Granular cells are densely packed round to oval, small neurons. Only the nucleus is readily seen, as there is very little surrounding cytoplasm, several short dendrites project from the base of each granular cells and one apical axon extends in to the molecular layer, loses its myelin sheath and bifurcates up to 3 mm in each direction, because of their orientation parallel to the surface unmyelinated axon are Known as parallel fibers, they establish multiple synaptic contacts with dendritic spines of purkinje cells (Ovalle & Nahirney, 2013; Abid, & Hussain, 2018). This study aims to identify the morphological .description, histological and measurement examination, and identify the histological layers in the cerebellum.

#### **Materials and Methods:**

In the current study, 15 laughing doves (6-10 months old, weighting about 80.7-95.3 g) were collected from local markets of the city of Baghdad, Iraq. The animals were anesthetized (Chlorophorm, HiMedia, Indea). The birds were sacrificed through an anatomical incision in the area where the upper jaw connects to the lower jaw, and the skull bones were carefully separated to obtain the brain. The brain was carefully removed from the cranial cavity and examined using the following tools to describe and measure the brain: a ruler, a digital camera (Samsung / ES70-5X0). The brains were then examined using a dissecting microscope and fixed in a 10% formalin Solution comprising of 10 ml formaldehyde with 90 ml tap water, after 24 h they were stored in 70% ethyl alcohol unital further use for experiments as mentioned by (Suvarna et al., 2018). Then, the histological technique stain was conducted. Then 7 micrometer thick sections were cut using a rotary microtome, and then the sections were staining with hematoxylin - eosin, methylene blue and crystal violet to illustrate the cellular structure of the cerebellum as well as the branches of nerve cells (Humason et al., 1962).

#### **Statistical Analysis:**



Then the eight selected histological sections from eight birds were photographed using a compound light microscope is equipped with an imaging camera to illustrate the results of the current study, the Image J program was used to measure the layers of the cerebellar cortex. Statistical data analysis was performed using statistical software (SPSS version 23.0, SPSS Inc., Chicago, IL). Morphometric data were analyzed using t-test at the significance level  $P \leq 0.05$ .

### Result and Discussion:

The results of the study included the cerebellum of the laughing dove (*Spilopelia senegalensis*) is show in figures.

#### 1- The morphological structure of the cerebellum

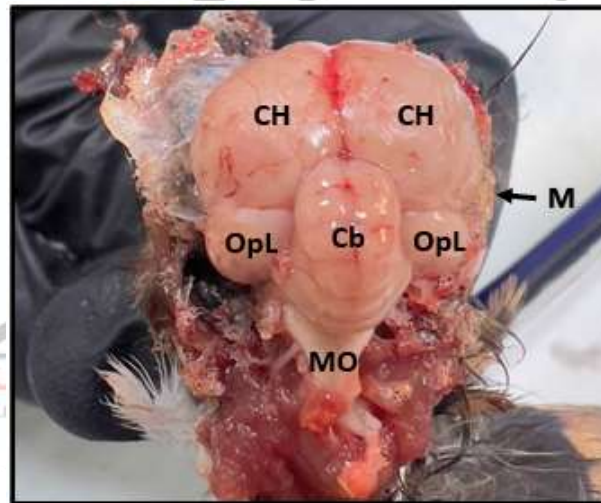
The whole brain of the laughing dove was broad in the front and tapered in the back and shaped as pear like (Figure 1). Its average length is ( $17.3 \pm 0.3$ ) mm average width is ( $15.3 \pm 1.2$ ) mm, and average weight is ( $1.3 \pm 0.02$ ) gm, As for the cerebellum, the average weight was ( $0.97 \pm 0.13$ ) grams, the average length was ( $1.01 \pm 0.07$ ) mm, and the width rate was ( $0.88 \pm 0.09$ ) mm, the result is consistent with a study (Gupta et al., 2019) on the domestic chicken bird *Gallus domesticus* indicated that the average cerebellar length was ( $11.80 \pm 0.25$ ) mm, and the width rate was ( $8.53 \pm 0.21$ ) mm, and study (Kumar et al., 2020) on *Numida meleagris guinea* fowl indicated that the average cerebellum length was ( $11.101 \pm 0.0206$ ) mm, and the width rate was ( $7.773 \pm 0.191$ ) mm. (Table 1).

**Table 1:** The average weight, length and width of whole brain and cerebellum.

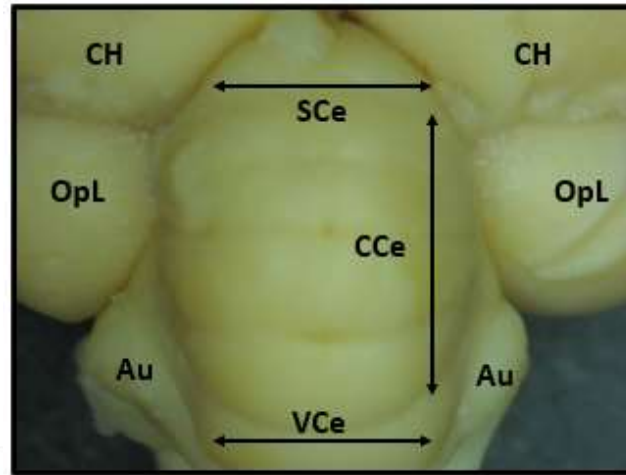
Anatomical part	Weight (gm) (M±S.E)	Length (mm) (M±S.E)	Width (mm) (M±S.E)
Whole brain	$1.3 \pm 0.02$	$17.3 \pm 0.3$	$15.3 \pm 1.2$
Cerebellum	$0.97 \pm 0.13$	$1.01 \pm 0.07$	$0.88 \pm 0.09$

The cerebellum appears as a well-growing, broad structure of large size, as it is the largest part of the posterior brain, with a convex shape and covers the interstitial brain and extends back to the oblong medulla, and the cerebellum is located behind the hemispheres of the brain, the current result

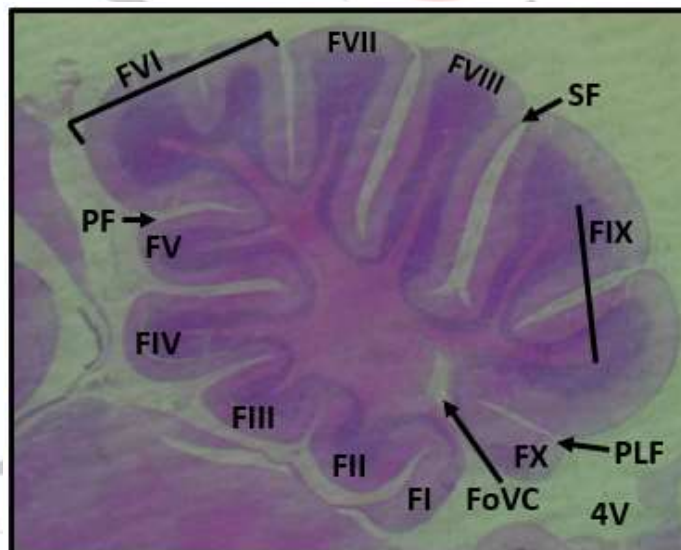
is consistent with a study (Abd-Alrahman & Mahmoud, 2008) on the Gold-capped parrot, the study (Abdullah, 2010) on the diurnal birds, and the study (Gupta et al., 2016) on the *Vencobb broiler* chicken. It is many folds, its top is wide, and corresponds to the transverse median fissure, The result is consistent with the study (Azeez et al., 2023) on the white egret *Bubulcus ibis*. The cerebellum consists of three divisions: Spino cerebellum, cerebro cerebellum and Vestibulo cerebellum, which contains two small lateral protrusions, Auriculae (Fig. 2). these three sections contain three cerebellar lobes, namely the anterior lobe of the cerebellar, the posterior lobe and the flocculonodular lobe, and the body of the cerebellum Corpus or the worm Vermis includes the front lobe and the posterior lobe, and it is noted on its outer surface that there are many folds representing the ten cerebellar folia (Fig. 3), and this result consistent with (Abd-Alrahman, 2012) on the bird of the barn owl *Tyto alba*.



**Figure 1:** Photostereomicrography of the neuroanatomy of the laughing doves (*Spilopelia senegalensis*) brain (dorsal view) showing: Cerebral hemisphere (CH), Optic tectum (OpL), Cerebellum (Cb), Medulla oblongata (MO), Meninges (M).



**Figure 2:** Dorsal view of the whole brain showing: Cerebral hemisphere (CH), Optic tectum (OpL), Auriculae (Au), Cerebro cerebellum (CCe), Spino cerebellum (SCe), Vestibulo cerebellum (VCe).



**Figur 3:** longitudinal section of the cerebellum in laughing dove showing : primary fissure (PF), secondary fissure (SF), Posterior lateral fissure (PLF), First folia (FI), Second folia (FII), Third folia (FIII), Forth folia (FIV), Fifth folia (FV), Sixth folia (FVI), Seventh folia (FVII), Eighth folia (FVIII), Ninth folia (FIX), Tenth folia (FX), Fourth ventricle (4V), Fourth ventricle cavity (FoVC), (H & E,2x ).

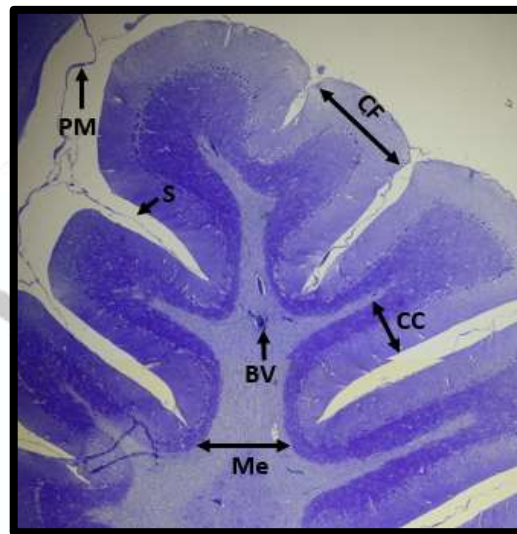
## 2- Histological description of the cerebellum

Histologically, the cerebellum consists of two regions includes:

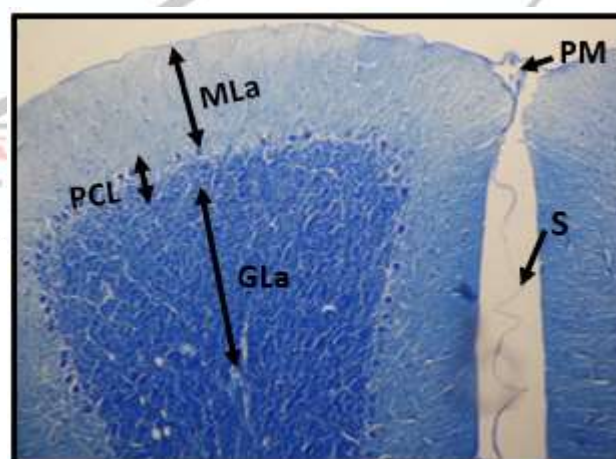
The cerebellar cortex, which represents the gray matter, and the Medulla, which represents the white matter, and the cerebellar cortex contains three layers: the molecular layer, the purkinje cells layer and the granular layer,



this is consistent with study (Maulana et al., 2021) on the *Gallus gallus domesticus* chicken, study (Sur et al., 2011) on different species of birds, and with the study (Abid, 2022) on the white-cheeked bulbul bird *Pycnonotus leucotis*, and study (Al-Bakri & Al-Zuheri, 2021) on the Iraqi frog *Rana ridibunda ridibunda* (Fig. 4-6).

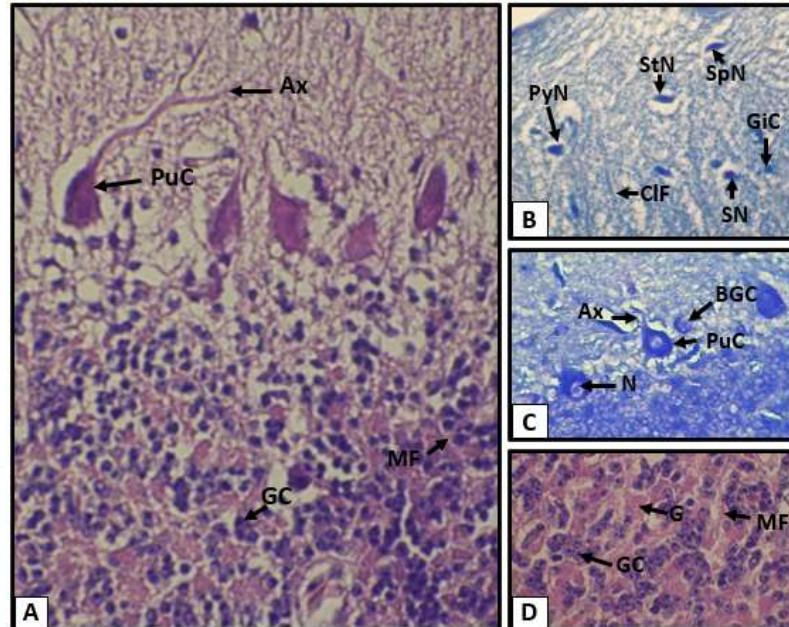


**Figure 4:** Longitudinal section through the cerebellum of the laughing dove showing the histological structure of the cerebellar folia: Pia mater (PM), Cerebellar folia (CF), Cerebellar cortex (CC), Sulci(S), Blood vessels (BV), Medulla(Me), (Crystal violet, 4x).



**Figure 5** Longitudinal section through the cerebellum of the laughing dove showing the histological structure of folium: Pia mater (PM), Molecular layer (MLa), Purkinje cells layer (PCL), Granular layer (GL), Sulci (S) (Methylene blue, 10x).





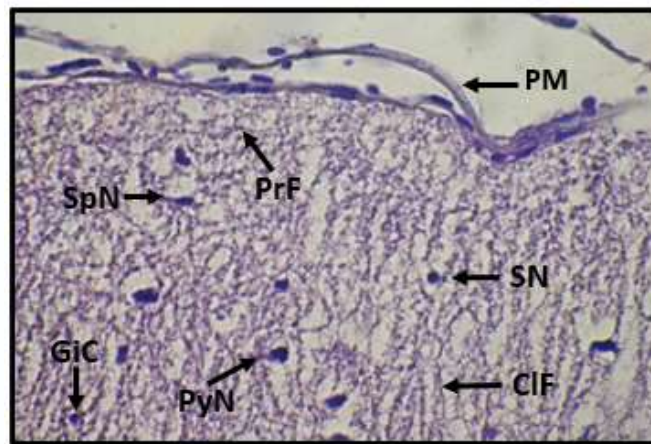
**Figure 6:** Sagittal section of the hindbrain of the laughing dove (*Spilopelia senegalensis*) showing the histological structure of the layers of the cerebellar cortex: fusiform neuron (SpN), Spherical neuron (SN), Piriform neuron (PyN), Astrocyte neuron (StN), Bergman glial cell (BGC), Purkinje cell (PuC), Granular cell (GC), Glial cell (GiC), Mossy fiber (MF), Climbing fiber (CIF), Axon (Ax), Dendrite (D), Nucleus (N), A- (H & E, 40X), B- Magnified section of the molecular layer (methylene blue stained, 100X), C- Magnified section of the Purkinje cell layer (Cresyl violet fixed stained, 100X), D- Magnified section of the granular layer (H & E, 100X).

The histological description of each layer from the outside to the inside is as follows:

- Molecular layer

It represents the superficial layer of the cortex and is surrounded by a thin membrane of connective tissue known as the pia mater, which is rich in blood vessels. The molecular layer is characterized by being a thick layer, and its average thickness at the apex of the cerebellar folia reached  $(2.210 \pm 76.980) \mu\text{m}$ , while its average thickness in the sulci region  $(0.870 \pm 42.337) \mu\text{m}$  (table 2). Histological examination of the molecular layer showed a density of nerve fibers, including climbing fibers and parallel fibers, called neuropile. Neurons were few in number and randomly scattered within this layer. Pyramidal neurons with an average diameter of  $(1.6) \mu\text{m}$ , as well as fusiform neurons with an average diameter of  $(2.4) \mu\text{m}$ , astrocytes

with an average diameter of (1.14)  $\mu\text{m}$ , spherical neurons with an average diameter of (1.1)  $\mu\text{m}$ , as well as the presence of small (glia) cells, this result is consistent with the study (Abid & Al-Bakri, 2016) on the Quail *Coturnix coturnix coturnix*, and study (Alzubaidi et al., 2023) on *Mus musculus* Embryo. The result of the current study is also consistent with the study (Kadhim, 2014) on the diurnal birds of *Falco tinnunculus tinnunculus*, as he indicated that this layer appeared spotted due to the density of dendrites and axons of nerve cells. (Fig. 7).



**Figure 7:** Cross section through cerebellar cortex of cerebellum showing histological structure of molecular layer: Pia mater (PM), Parallel fibers (PrF), Spherical neuron (SN), fusiform neuron (SpN), pyramidal neuron (PyN), climbing fibers (CIF), Glial cells (GiC), (Crystal violet, 100x).

#### • Purkinje cells layer

It represents the middle layer of the cerebellar cortex, and its average thickness at the apex of the cerebellar folia reached (1.2±28.08)  $\mu\text{m}$ , while its average thickness in the sulci region (1.07±23.6)  $\mu\text{m}$  (Table 2). This layer contains large neurons with a round or pyramidal shape, with locations close to each other, and they spread in a linear manner within the layer, with an average diameter of (6.8)  $\mu\text{m}$ . The cells of this region contain have large, flat, branched dendrites that extend to the upper layer of the cerebellar cortex, each cell has one long axis that extends to the lower layer of the cerebellar cortex. Histological examination of this layer also revealed the presence of Bergmann glial cells, which are unipolar glial cells, these cells are located around purkinje cells, and Bergman glial cells contain radial

nerve fibers that pass through the molecular layer and have an average diameter of  $(1.5) \mu\text{m}$ , The result of the current study was consistent with the study (Sur et al., 2011) on different species of birds, and the study (Taşçi, 2018) on the geese *Anser anser*. The study (Pal et al., 2003), (Abdullah, 2010) on Diurnal Raptor Species (Buzzard) showed that this layer contained branched branches that extended to the upper layer (molecular layer) and axons that extended to the granular layer, and this is consistent with the result of the current study (Fig. 8).

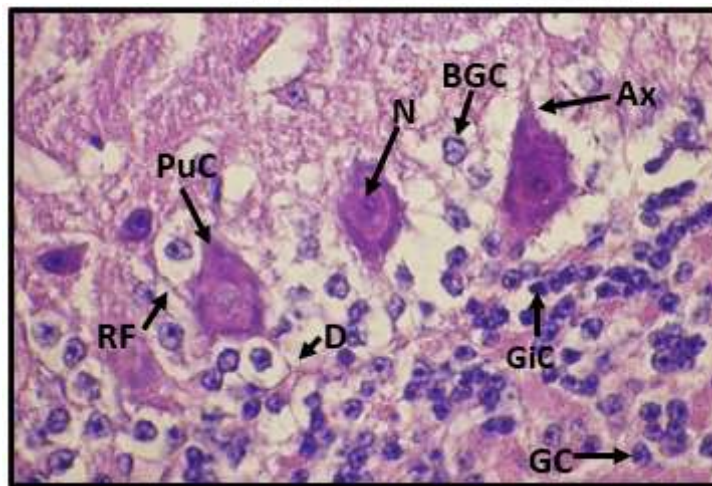
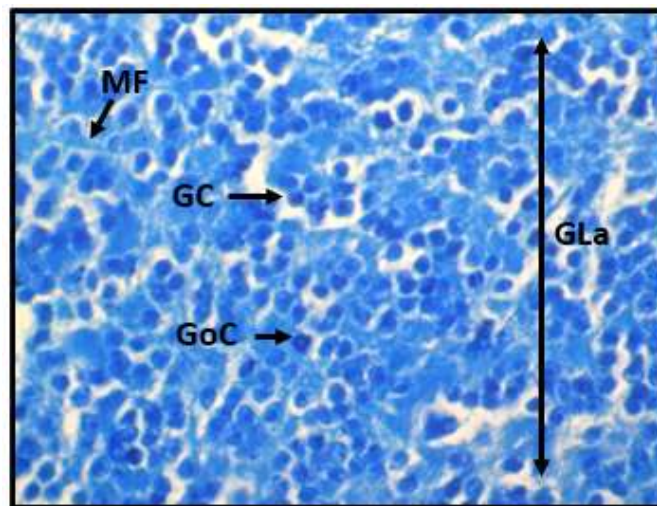


Figure 8: Longitudinal section through the cerebellar cortex of the cerebellum of the laughing dove showing the histological structure of the purkinje cells layer: Purkinje cell (PuC), Nucleus (N), Axon (Ax), Dendrites (D), Bergman glial cell (BGC), Radial fiber (RF), Granular cell (GC), Glial cell (GiC), (H & E,100x).

#### • Granular layer

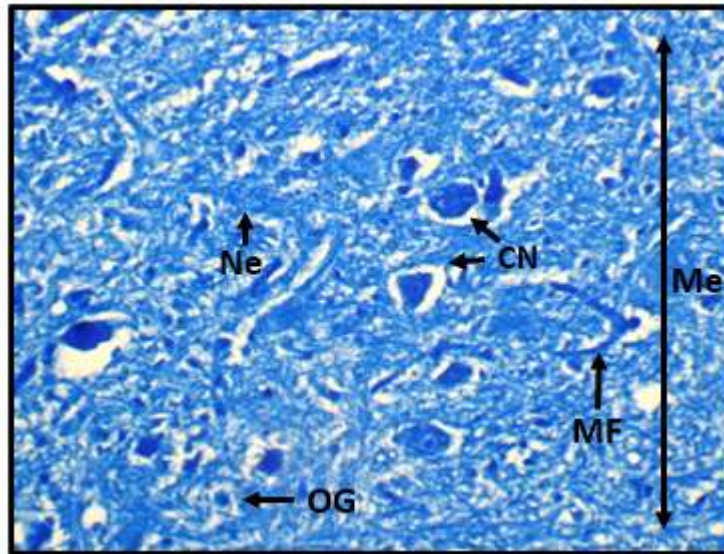
It represents the inner layer of the cerebellar cortex and is located below the Purkinje cell layer. This layer is thick at the apex of the folia, where its average thickness reached  $(5.830 \pm 106.85) \mu\text{m}$ . As for the sulci region, it was thin, with its average thickness reaching  $(1.104 \pm 27.101) \mu\text{m}$  (Table 2). This layer contains many tightly packed granular cells, circular to oval in shape, and small in size, with an average diameter of  $(1) \mu\text{m}$ . In this layer, Golgi II cells were found, which are stellate-shaped nerve cells with short axons, with an average diameter of  $(1.5) \mu\text{m}$ . Histological examination of this layer showed the presence of mossy fibers coming from the medulla and communicating with the granule cells in a light-colored area called the

glomeruli, which represent the areas of nerve synapses, and climbing fibers whose branches reach the molecular layer, This is consistent study (Kadhim, 2014) on diurnal raptors *Falco tinnunculus tinnunculus*, study (Abid, 2022) on *Pycnonotus leucotis*. The study (Abid & Abid, 2017), (Abed, 2020) indicated that the granular layer contained mossy fibers coming from the medulla and containing glomeruli representing synaptic sites, as each glomerulus represents the site of communications for more than one granular cell, and this corresponds to the result of the current study (Fig. 9).



**Figure 9:** Cross section through cerebellar cortex of cerebellum showing histological structure of granular layer (GLa): Granular cell, (GC), Golgi cell II (GoC), Mossy fiber (MF), (Methylene blue, 100x).

The Medulla represents the inner region of the cerebellum, which is made up of myelinated axons. Histological examination showed the presence of many blood vessels and a number of glial cells, which appear as large, dark-pigmented cells known as deep cerebellar nuclei with an average diameter of (5.07)  $\mu\text{m}$ . We also note the presence of oligodendroglia with an average diameter of (1.2)  $\mu\text{m}$ , spread inside the medulla, in addition to the presence of astrocyte neuron with a diameter of (1.8)  $\mu\text{m}$ , and this corresponds to the study (Abid & Abid, 2017) on pigeon *Columba livia gaddi*, and study (Abid and N.A. Al-Bakri, 2017) on Quail *Coturnix coturnix* (Fig. 10).

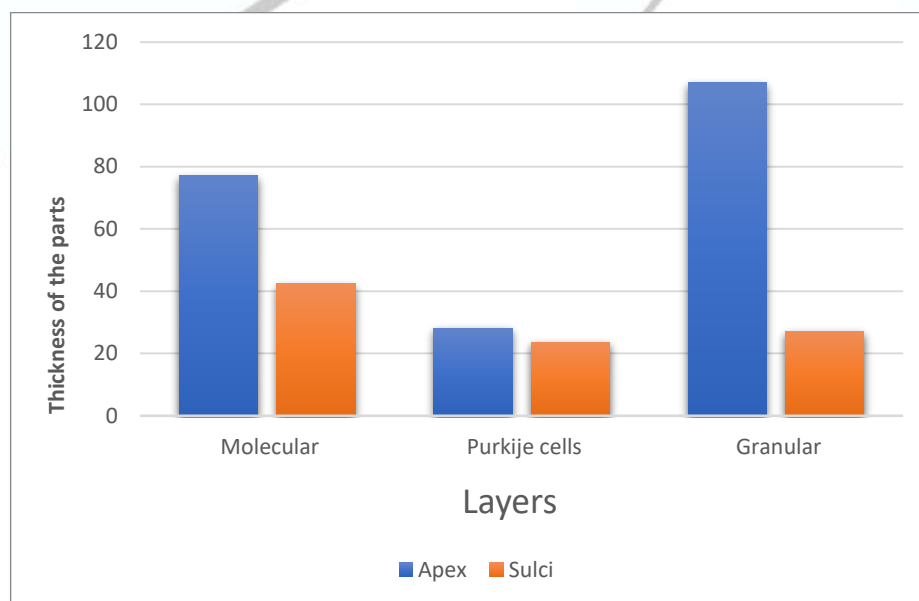


**Figure 10:** Cross section through the cerebellum showing histological structure the medulla in the laughing dove: Cerebellar nuclei (CN), Oligodendroglia (OG), Mossy fiber (MF), Neuropile (Ne), Medulla (Me), (Methylene blue, 100x).

**Table 2:** Thickness of the apex of the folia and sulci of the cerebellar cortex.

Thickness of parts	Thickness of the apex (M±S.E) $\mu\text{m}$	Thickness of the sulci (M±S.E) $\mu\text{m}$
<b>Molecular</b>	<b>76.980±2.210</b>	<b>42.337±0.870</b>
<b>Purkinje cells</b>	<b>28.08±1.2</b>	<b>23.6±1.07</b>
<b>Granular layer</b>	<b>106.85±5.830</b>	<b>27.101±1.104</b>

High-significant differences at probability value ( $p \leq 0.001$ ).



**Figure 11:** Thickness of the cerebellum layers.

**Conclusion:**

The cerebellum contained ten cerebellar folia interspersed with Sulci. The granular layer appeared as the thickest layer in the cerebellar layers. The layers contained multiple nerve cells spread within the gray matter and white matter.

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