Anatomicaland Histological study of the cerebellum in Tytoalba (barn owl) by using Giemsa stain method

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Abstract

Morphological and histological aspects on the cerebellum of(barn owl) were made in order to find out the cerebellum size, design, lobal, folial variation and the description of the cerebellar regions components at the light microscopical level by using Giemsa stain method . The results of the morphological aspectswere revealed that the cerebellum was large and spade in shape .Sagittal sections revealed the three folded lobes , the arrangement and the nomenclatures of the ten primary folia . The results of the microscopic examinationswere indicated that the three distinct layers of the cortex were more clearly appeared with Giemsa stain methods .The thicker outer light molecular layer were mostly occupied by axons and dendrites . The star shaped stellate cells and the basket cellswere found scattered between them. The single middle purkinje cells layer had large flask shaped cells , the high linear density was in the posterior lobe of the cerebellum . The thick inner dark granular layer had a numerous different neurons,the white matter which formed the medulla was the region of the myelinated fibers and the deep cerebellar nuclei .

Introduction

The cerebellum is a region of the hindbrain which occupy a position in the rostral roof of the 4th ventricle in vertebrates [1] . The cerebellum is responsible for the integration of sensory perception, cognitive abilities and muscle movement coordination [2,3] . The cerebellum controls movement by collecting sensory nerve inputs, it receives 200 million sensory input from the spinal cord ,midbrain ,and forebrain ,in contrast with the optic nerve which composed of one million fibers [4] . The cerebellum characterized by the great prominence of the neurons , it contains more than all of the rest of the brain [5] . Birds have large , sophisticated and highly folded cerebellum that does not scale uniformly . The structure of this highly conserved organ has been undertaken by [6,7,8,9] . This study has been made on the cerebellum of (barn owl)Tytoalbathe only member of the Family Tytonidae which belonges to the class

Aves and Order Strigiformes . It is a medium sized owl . As seen in (Fig. 1) it has a distinctive heart shaped facial disc , relatively small eyes and lacks ear tufts [10] . In this study we use Giemsa stain method instead of routine Heamatoxyline andEosine stain , in order to give better description for each cerebellar region and its neuronal elements and fibers at the light microscopical level .

Material and Methods

Five healthy adult (barn owl) were utilized in this investigation ,the brains were obtained from the skull by careful dissection , the whole brain and the cerebellum were submersion fixed in 10% buffered formalin , the brains were bisected in sagittal plane and pieces of cerebellum made by a sharp knife were fixed in 10% buffered formalin , washed, dehydrated through ascending grades of alcohol , cleared and embedded in paraffin wax . Five-Six microns thick sections were cut by using rotary microtome . The paraffin wax was removed by immersing the slides in xylene . The slides were passed through descending grades of alcohol , distilled water , the sections were stained with Giemsa stain The slides were rinse in 0.5% aqueous acetic acid , tap water , ascending grades of alcohol , cleared with xylene and mounted with D.P.X. as per usual method[11] .

Result

The barn owl cerebellum : Morphology

The large cerebellum in barn owl is located in the inferior posterior portion of the head , dorsal to the pons and inferior to the occipital lobe of the cerebrum. The cerebellum is connected with the midbrain rostrally and with the medulla oblongata caudally by the peduncles . The cerebellum is spade in shape externally its surface exhibits a transverse parallel grooves with finely spaced named sulci which divide the cerebellum into large foldes named gyri (Fig.2) . The cerebellum consists of a vermis(the main central part) , and the auricles . As seen in(Fig. 3) the vermis divided by two deep fissures primary (x) ,Secondary (y) into three lobes (anterior , posterior and vestibulocerebellum or pars auricularis) with ten primary folia number (I-X) . The anterior lobe consists of the Folia (I-V) which named as follows : Folium I Lingula ; Folium II and Folium III Central lubules ; Folium IV Culmen ; Folium V Declive . The posterior lobe consists of the Folia (VI-IX) which named as follows : Folium VI Folium vermis; Folium VII Tuber vermis ; Folium VIII Pyramis ;Folium IX Uvula . The subfoliumIX the Ventral uvula and the Folium X Nodulus comprise the vestibulocerebellum lobe or parisauricularis . The extensions of Ventral uvula and the Nodulus laterally continues to be the paraflocculus and flocculus , they merge to form the auricle on each side . There is a clear expansion in the folia I Lingula ,II and III Central lubules . The folia V

Declive ,VI Folium vermis and IX Uvula are large and subfoliated , while the folia VII Tuber vermis and VIII Pyramis are long . The folium X Nodulus was obliquely oriented and contains small ventral lamella . The cerebellum enclosed centrally placed cavity continuous by a small passage with the fourth ventricle of the brain.

The barn owl : Histology

The cerebellum is divided histologically into an outer cortex and inner medulla . Externally the cortex was covered by two meninges , the outer duramater and the inner pia arachnoid mater , the later invaginated into the fissures between the cerebellar folia (Fig.4) . The cerebellar cortex (gray matter) forms a series of deeply convoluted folds (folia) supported by a branching central medulla of white matter . (Fig .4) shows the three distinct layers of the cerebellar cortex from outside to inside , they are : the molecular layer (ML) , the Purkinje cell layer (PL) , and the granular layer (GL). Thickness of molecular layer (ML) was more in the fissures than on the folialsummit . The molecular layer characterized by the less dense and the light appearance , it is mostley occupied by axons and dendrites (Fig.5) . Stellate cells and basket cells found scattered between a horizontally directed unmylinated fibers and parallel fibers .The stellate cells are the superficially located neurons . It has several dendrites radiating from the cell body giving them a star shaped appearance , along its dendrites surface there are many dendritic spines . The basket cells are the more deeply placed neurons , they form a connection around the Purkinje cells in a basket – like arrangement(Fig.6) illustrates the black stained fibrils of the basket cells axons that surrounding the Purkinje cell bodies . A dense network of fibers that fills the interneuronal spaces named the neuropil (Fig. 7).The Purkinje cell layer is the middle of the cerebellar cortex (Fig.4,5,8) . It is a narrow zone (one cell thick) . Purkinje cells are the largest neurons , they a ligned like dominos stacked one in front of the other , it is a multipolar cell , it has a flask shaped body with large oval nucleus and dark stained nucleolus . Its relatively fine axon extending down through the granular layer and its multiple branching dendrites ramify throughout the molecular layer , each dendrites has a small branches covered with dendritic spines (Fig. 6) . The high linear density of the Purkinje cells was in the posterior lobe of the cerebellum(Fig.8). The granular layer (GL) is thicker on folial summits than in the fissures . It is extremely cellular and it has dark appearance . The granular cells are small , numerous and tightly packed . It has dark stained nucleus with small amount of cytoplasm . The larger Golgi cells found near the Purkinje cell layer , it has a typical vesicular nucleus with more cytoplasm There are a nuclei of different neuroglia , microglia and fibrous astrocytes , the later found near the blood vessels, they have dark stained nucleus with long thin and smooth radiating process

extending from the cell body . Throughout the granular there are small irregularly dispersed clear spaced named the cerebellar islands or glomeruli (Fig. 9) .The nonmyelinated axons of the granular cells pass outwards to the molecular layer where they end in a T-shape and run parallel tothe surface , so they were named parallel fibers . (Fig. 5) shows the parallel fibers that synapses with the huge dendritic arrays of Purkinje cells .The medulla (Fig. 4,8) is the region of internal white matter , it was deep to the granular layer and it is occupied by myelinated fibers and associated deep cerebellar nuclei (Fig.10) . The later are clusters of multipolar neurons lying within the white matter at the core of the cerebellum(Fig.11)

Discussion

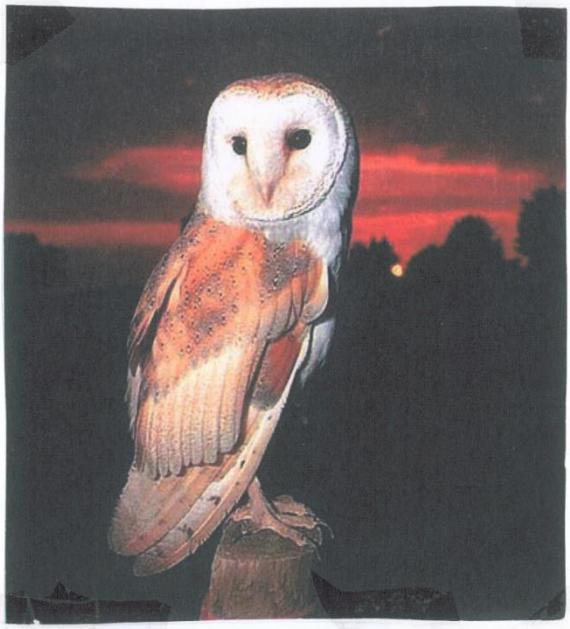
Barn owl had large and spade shaped cerebellum , in comparison to that of other birds . It was large and transversely narrow in <u>Bullockornisplanei</u> [4] ; large and dorsally protrudes in African ostrich [12] ; small and rounded in domestic pigeon [13] . In sagittal sections it was found that the cerebellum consists of the vermis and the auricles . The vermis divided by two deep fissures into three lobes with ten primary folia , this finding is in accordance with the statement of [8] in biras and [9] in fowl- white leghorn . [6] stated that the pigeon cerebellum consists of a vermis without evident hemisphere, there is no prominent cerebellar hemisphere in comparison to mammalian cerebellum which consists of two cerebellar hemisphere each of them divided into three lobes . The vermis is highly developed in birds which fly compared to the flightless one [9] . The importance of the vestibulocerebellum is for integrating visual and balance signals during bird flying [4] . The arrangement of the ten folia within the three lobes of the barn owl cerebellum and its nomenclature were in accordance with the statement of [6] in pigeon and [9] in domestic fowl . One of the most prominent differences among birds cerebellum is the variation in shape and size of the individual folia [14,15,16] . In this study the folia I lingua ,II and III central lubules is similar to that of the pigeon , while it is cleary different to that of the spotted nighjar , they are very much reduced [7] . The folia V declive and X nodulus are resembled to that in oilbirds <u>Steatorniscaripensis</u> and the tawny frogmouth <u>Podargusstrigoides</u> , unlike (grey potoo) <u>Nyctibiusgriseus</u> folium V declive was single and not subfoliated , also folium X nodulusdose not have ventral lamella and it is vertically oriented . The enlargement of the individual folium VI foliumvermis , VII tuber vermis and VIII pyramis that observed in this study in barn owl cerebellum was related to their specific behavior . [15] stated that it reflects the increased visual processing requirements with rapid flight especially in species classified as strongfliers . Histologically barn owl cerebellum is the Intermediate state in regarded to the degree of the folding of the entire cerebellar cortex . The cockatoo and magpie had the highly folded cerebellum In comparison to that

of the dove [14] . The more thickness of the molecular layer In the fissures that observed in this study is in accordance with that in fowl [9] . In Giemsa stained slides the molecular layer of barn owl was clearly appeared while in fowl it was featureless with Heamatoxylin and Eosin stains as statedby [9] . The small and the star shaped stellate cells found in barn owl molecular layer had variable shape and size in mammals as they stated by [17], both stellate and basket cells are inhibiter interneurons they synapses onto Purkinje cell dendrites[4] . The large flask shaped Purkinje cells found in barn owl cerebellum was different in fowl , it was pear shaped as stated by [9] . Purkinje cells distinguished by the shape of its extraordinary dendritic branches [17]. In pigeon they appears like a fan shaped as stated by [4] . It was found that the high linear density of the Purkinje cells in barn owl was in the posterior lobe . Similar to that in black vulture and unlike to that in dungcook [18] , this difference was concerned with the type of locomotion as stated by [19]. The small , numerous, tightly packed and dark stained nucleus cerebellar granule cells in barn owl are about 60-80 billion in human cerebellum as stated by [4] . The endind of granule cell dendrites are sites of excitatory input from mossy fibers and inhibitory input from Golgi cells [4] . The cerebellar islands or the glomeruli was clearly apparent with the use of Gimesastain . [20] stated that the use of routine Heamatoxyline and Eosine staining does not allow it to stand out well . This region contain only synaptic complexes [21] . The deep cerebellar nuclei in barn owl found lying within the white matter at the core of the cerebellum , similar observation was made by [22] in pigeon , they stated that these nuclei were homologus with that of mammals . They acts as the main centers of communication and balance also they receive and send information to other specific parts of the brain [4] .

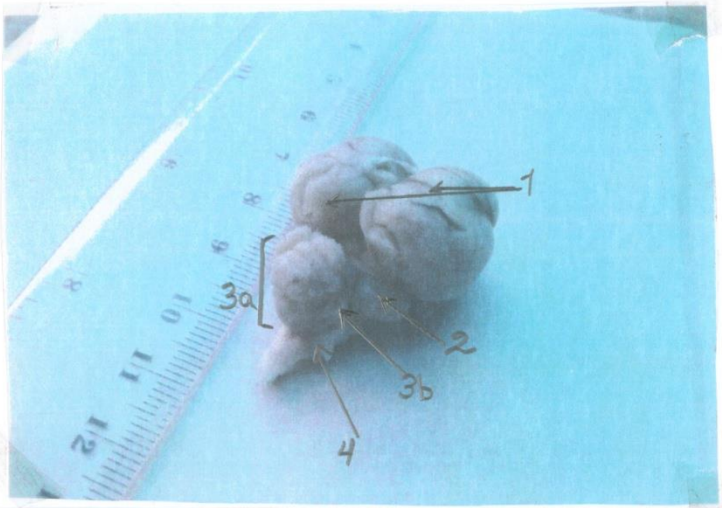
References

1- Voogd, J. and Glickstein , M. (1998) .The anatomy of the cerebellum . Neurosciences Vol. 21, Issue 9, pp. 370-375 .
2- Sultan ,F. and Glickstein, M. (2007). The cerebellum ; comparative and animal studies . The cerebellum Vol. 6 ,No. 3,pp. 168-176 .
3- Loionde, R. and Botez, M. I. (1990). The cerebellum and learning process In animals . Brain ReasearchReviews , Vol. 15 ,pp. 325-332 .
4- Mccaffrey, P. (2008). The cerebellum ,Neuroanatomy from Wikipedia, The free encyclopedia , pp. 1-30 .
5- Glickstein, M. (2007). What dose the cerebellum really do . Current Biology Vol. 17, Issue 19, R: 824-827 .
6- Pakan, JM. ; Iwaniuk , AN. ; Wylie , DR. ; Hawkes, R. and Marzban, H. (2007).
7- Purkinje cell compartmentation as revealed by zebrin II expression in the cerebellum cortex of pigeon Columbalivia . The Journal of Comparative Neurology , Vol. 501 (4) , pp. 619- 630

8- Iwaniuk , AN. ; Hurd, P.L. and Wylie, D.R.W. (2006). The comparative morphology of the cerebellum in caprimulgiform birds : Evolutionary and Functional Implication . Brain Behev.Evol. , Vol. 67, pp. 53-68 .
9- Sultan, F. (2005). Why some bird brains are larger than others. Current Biology , Vol. 15 , Issue 17, pp. R649- 650 .
10- Pal, B.; Chowdhury, S. and Ghosh, R,K. (2003) . Comparative anatomical study of the cerebellum of man and fowl . Journal of the Anatomical Society of India, Vol. 52, No. 1, pp. 1-8 .
11- Cholewiak, D. (2008) .General description of owls from Wikipedia animal diversity web. The regents of the university of Michigam and its licensors . pp. 1-20 .
12- Sood, R. (2009). Medical Laboratory Technology : Methods and Interpretatios 6th edition , Ch. 27, New Delhi ,India, pp. 1436- 1450 .
13- Peng, K.; Feng, Y. ; Zhang, G. ; Liu, H. and Song, H. (2010). Anatomical study of the brain of the African ostrich . J. Vet. Anim. Sci. , 34 (3), pp. 235-241.
14- Rehkmpera, G. ;Frahma, H. D. ; Cnotkaa , J. (2008). Mosaic evolution and adaptive brain component alteration under domestication seen on the back ground of evolutionary theory , Brain Behavior and Evolution , Vol. 71, pp. 115-126 .
15- Iwaniuk, AN. ; Lefebvre, L. and Wylie, D.R. (2009) . The comparative approach and brain behavior relations: Atool for understanding tool use , Canadian Journal of Experimental Psychology , Vol. 63 (2) , pp. 150-159 .
16- Iwaniuk, AN. ;Hurda, P.L. ; Wylie, D.R.W. (2007). Comparative morphology of the avian cerebellum :II size of folia . Brain Behavior ,Vol. 69: pp. 196-219.
17- Iwaniuk, AN. (2003). The evolution of brain size and structures in birds , Unpublished Ph D thesis Monash university , Clayton, Australia, pp. 1-9 .
18- Bevelander, G. and Ramaley, J. (1979). Essentials of Histology 8th edition, by the C.V. Mosby Company , United States of America .
19- Lemos, VP. ; Fronta, V. and Crisostomo, L. (1976). Cerebellum of birds, Comparative study of the density of the Purkinje cells in the anterior lobe. Rev. Bras. Pesqui. Med. Biol. , Vol. 9 (5-6) , pp. 243-250 .
20- Lemos, VP. And Crisostomo, VF. (1975). Cerebellum of birds quantitative And comparative study of the Purkinje cells . Rev. Bras. Pesqui. Med. Biol., Vol. 8 (5-6), pp. 369- 371.
21- Mescher, A.L. (2010) . Junqueiras basic histology : Text and Atlas , 12th ed. ,ch. 1 and 9, the McGrow –Hill Companies , pp. 1-16 and 140-166 .
22- Eroschenko, V.P. (2005) .Difiores atlas of histology with functional correlations. 10th ed. Chap. 7, university of Idaho, Moscow .
23- Arends, JJ. And Zeigler, HP. (1991). Organization of the cerebellum in the pigeon : II projections of the cerebellar nuclei . J. Comp. Neurol. , Vol. 8:306 (2), PP. 245-272 .



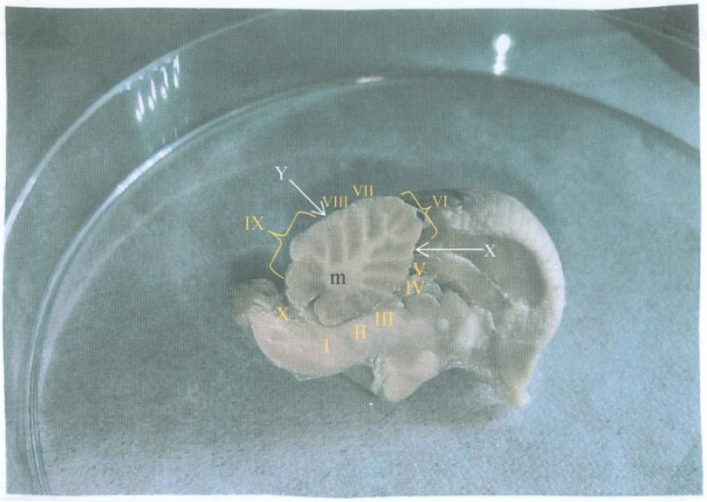
(Fig. 1) Tyto alba (barn owl) .



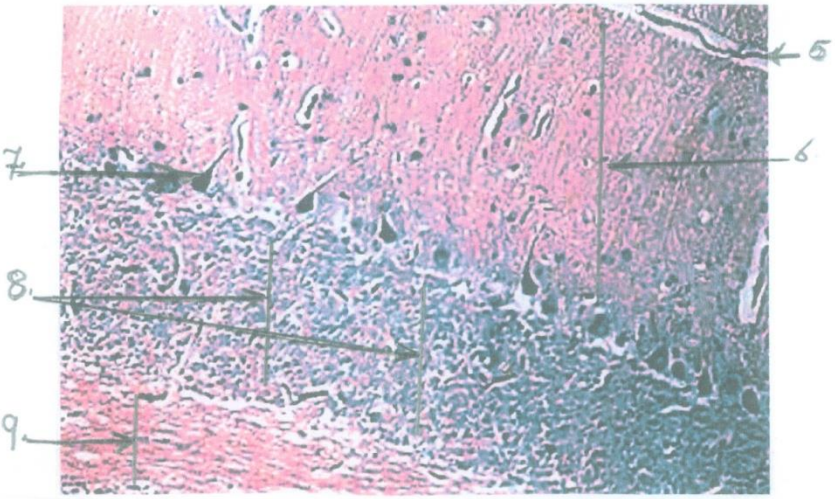
(Fig.2) Dorsal view of barn owl brain showing :

1- cerebral hemisphere 2- optic lobe 3- cerebellum 3a- the vermis 3b - the auricles

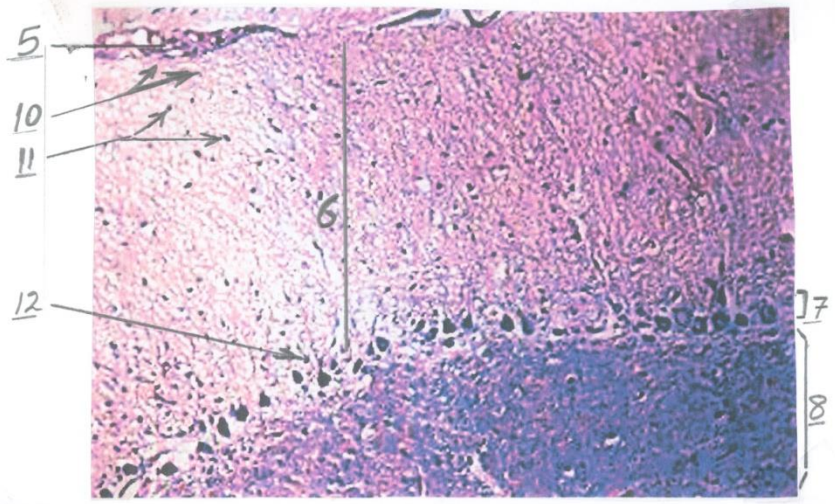
4- medulla oblongata .



(Fig.3) Sagittal section in barn owl brain showing the two deep fissures (X,Y) and the ten primary folia number (I-X) , m. medulla

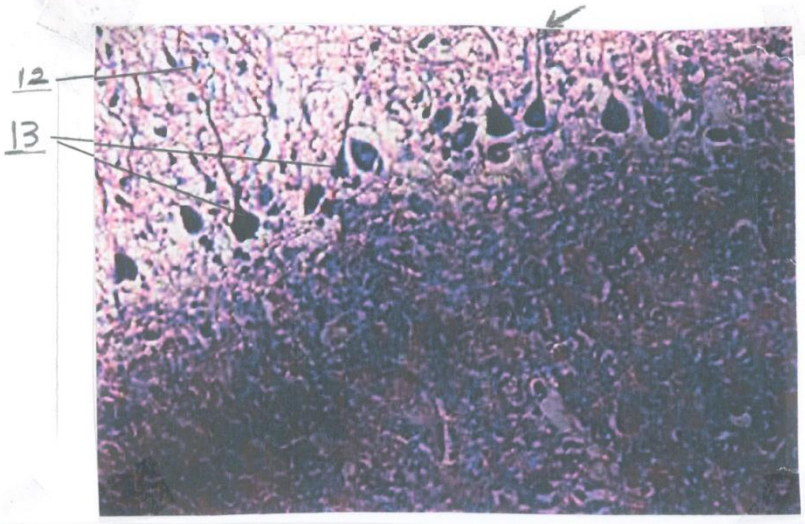


(Fig.4) Longitudinal section in Part of one folium of the barn owl cerebellum (10X) : 5- inner Pia- arachnoid 6- the molecular layer (ML) 7- the Purkinje Cell layer (PL) 8- the granular layer (GL) 9- the inner medulla .

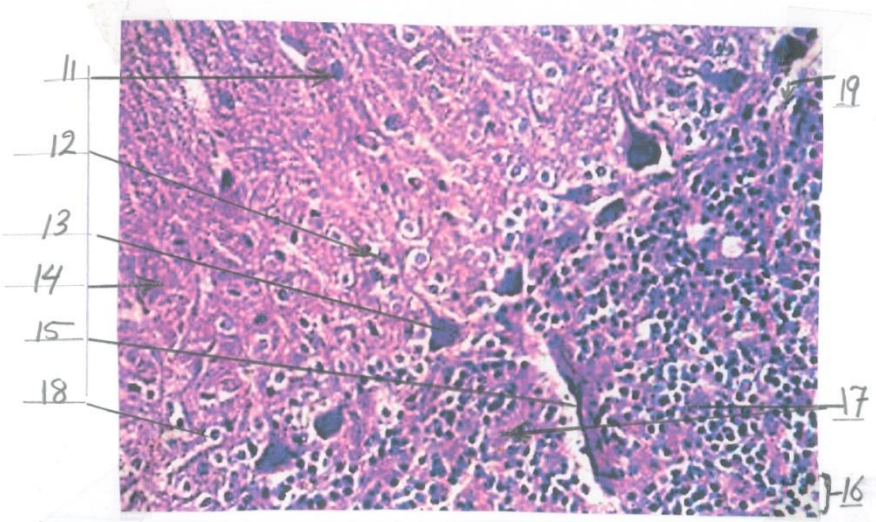


(Fig.5) Longitudinal section in Part of cortical strip (4X) :

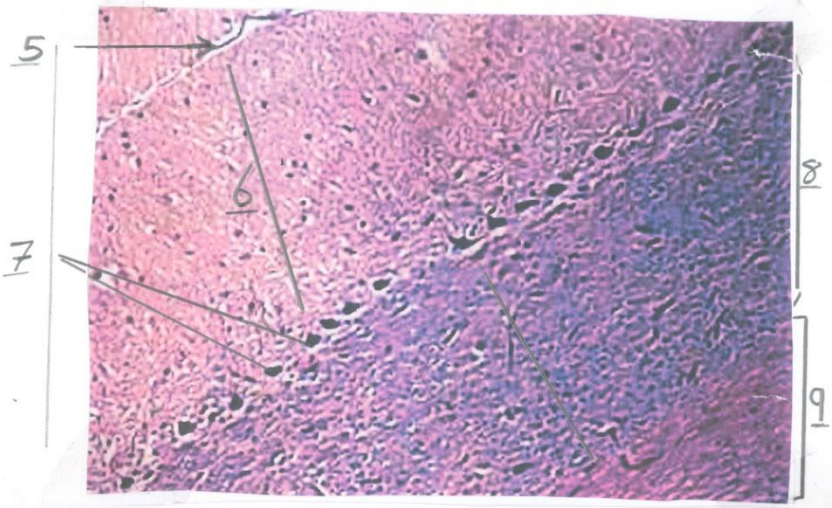
5- inner Pia- arachnoid 6- the Molecular layer (ML) 7- the Purkinje cell layer (PL) 8- the granular layer (GL) 10-the Parallel fibers 11- stellate cell 12- basket cells .



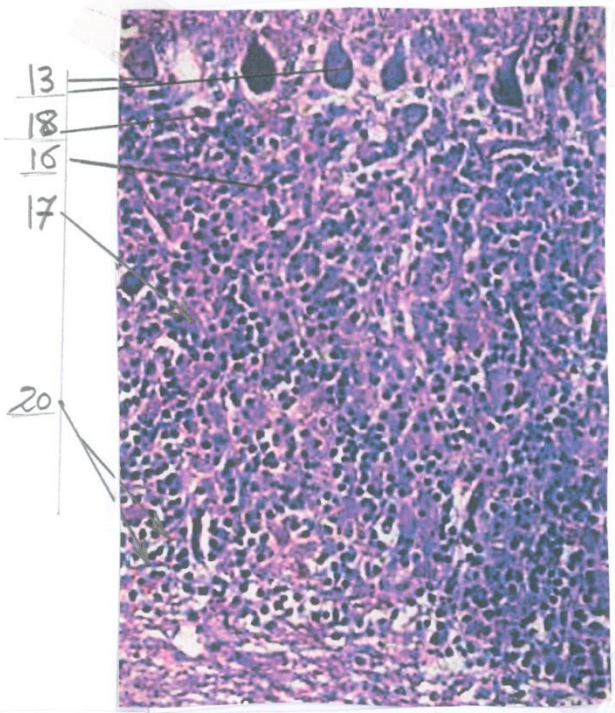
(Fig. 6) longitudinal section in Part of cortical strip (20x) : 12- basket cells 13- the Purkinje cell with its branching dendrites .



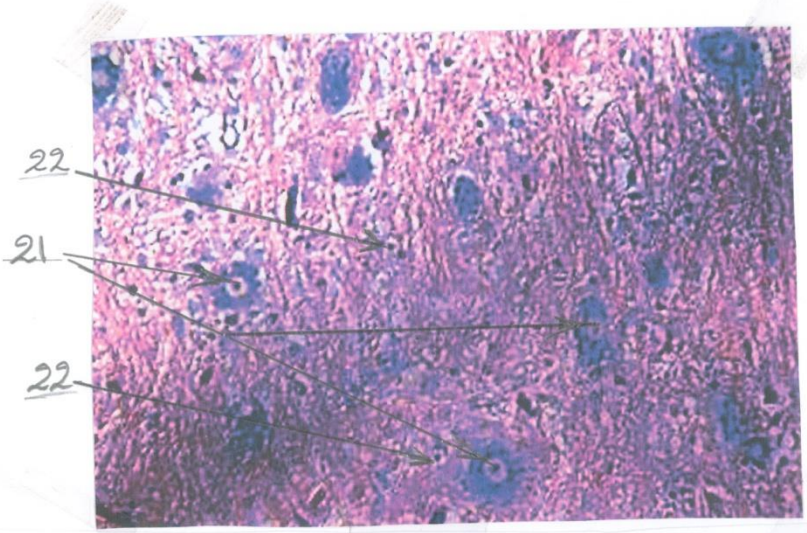
(Fig. 7) Longitudinal section in Part of cortical strip (20X) : 11- stellate cell 12- basket cell 13- Purkinje cell with its dendrites 14- neuropil 15- blood vessel 16- granule cell 17- glomeruli 18- Golgi cell 19- fibrous astrocytes .



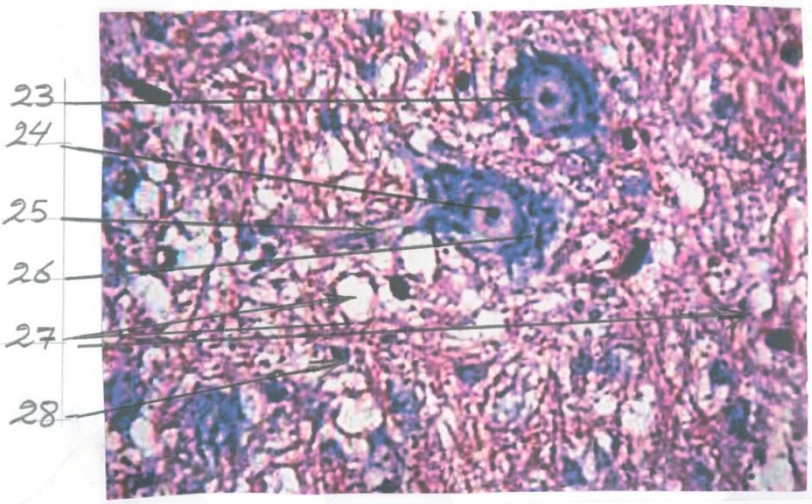
(Fig. 8) Longitudinal section in Part of one folium of the posterior lobe of the cerebellum (4X) : 5- inner Pia- arachnoid 6- the molecular layer (ML) 7- the Purkinje layer (PL) 8- the granular layer (GL) 9- the inner medulla .



(Fig. 9) Longitudinal section in part of cortical strip (20X) : 13- Purkinje cell.
16- granule cell 17- glomeruli 18- Golgi cell 20- different neuroglia



(Fig. 10) The inner medulla(20X) : 21- deep cerebellar nuclei 22- myelinated fibers



(Fig. 11) The deep cerebellar nuclei (40X) : 23- nucleus 24- nucleolus 25- dendrites 26- cytoplasm 27-myelinated fiber 28- axon .

الخلاصة:

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تناولت الدراسة الجوانب الشكلية والنسجية لمخيخ طائر البوم (barn owl) للتعرف على حجم المخيخ وشكله وتغايرات الفصوص والوريقات كما وتم وصف مكونات الطبقات المخيخية عند فحصها بالمجهر الضوئي وبأستعمال تقنية التلوين بصبغة كميزا . اظهرت نتائج الشكلياء ان المخيخ كبير الحجم وذو مظهر بستوني . اظهرت المقاطع السهمية الفصوص الثلاثة المطواة والمتضمنة ترتيب وتسميات الوريقات الاولية العشرة . اظهرت نتائج الفحص المجهري ان الطبقات الثلاث المنفصلة للقشرة كانت واضحة المعالم تماما عند استخدام تقنية التلوين بصبغة كميزا . تميزت الطبقة الجزيئية السميكة الخارجية الفاتحة بكون معظمها ممتلئ بالتغصنات والمحاور ،كما وتنتشر بينهم الخلايا النجمية وخلايا السلة . تميزت الطبقة المفردة الوسطية لخلايا بركنجي بكونها خلايا كبيرة الحجم ودورقية الشكل ، وتنتشر بكثافة خطية عالية ضمن الفص الخلفي للمخيخ . تمتلك الطبقة السميكة الداخلية الغامقة للخلايا الحبيبية اعداد كبيرة ومختلفة من الخلايا العصبية . تعد المادة البيضاء التي تشغل اللب منطقة الالياف العصبية المغمدة ومجاميع الانوية العميقة المخيخية .