Variations versus Similarities in the Cerebellar Structure of Different Vertebrates: A Comparative Anatomical Study الاختلافات مقابل التشابهات في تركيب المخيخ لفقاريات مختلفة: دراسة تشريحية مقارنة

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Short running title

Cerebellar Variations in Vertebrates

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ABSTRACT

Aim of work: To compare morphology of anterior cerebellar lobe's lobules: lobule III for hindlimb and lobules IV-V for forelimb movements among different vertebrates with emphasis on cerebellar structural-functional relationship. Material and Methods: Different adult vertebrates were used; from mammals: rat, cat, rabbit, cow, from avian: pigeon, bat and duck, from reptiles: snake, and from primates: human. Cerebella of all animals were examined for gross morphology and microscopic structure. In lobules III-IV-V, thicknesses of cerebellar cortical layers, Purkinje cell surface area, and Purkinje cell count were measured. Obtained data were statistically analyzed. Results: Snake's cerebellum didn't show foliations. Pigeon, duck and bat had large vermis with rudimentary cerebellar hemispheres. Rat, cat, rabbit and cow showed large complex foliations of cerebellar hemispheres and vermis. The human had massive cerebellar hemispheres and small vermis. Pigeon, duck and bat had well developed lobules IV-V and so did the cat. In contrast, rat and rabbit had well developed lobule III in comparison to lobules IV-V. In cow and human, lobules III-IV-V were well developed. Order of cerebellar cortical layers in different animals was similar. Significant differences were between lobule III and lobules IV-V in Purkinje cell count and cortical layers thickness following animals' behavior. In animals using hindlimbs more than forelimbs, mean numbers of those data showed significant increase in lobule III compared to lobules IV-V and vice versa. Conclusion: V ariations in cerebellar morphology-structure are related to behavioral differences among animals. Degree of structural complexity of cerebellar lobules III-IV-V is related to limbs' function.

Keywords: Cerebellum; Vertebrates; Comparative Anatomy

INTRODUCTION

The cerebellum is the large folded structure wedged between the top of the brainstem and the posterior part of the cerebral cortex. The bottom of the cerebellum forms the roof of the 4th ventricle. Though apparently smaller than the cerebral cortex, the cerebellar cortex contains double the number of the neurons. The cerebellum is somewhat ovoid in shape and constricted in its median part. It consists of two hemispheres joined by a narrow median part; the vermis¹.

The smallest folds, forming the ridges visible on the outside of the cerebellum are called the folia. The groups of the adjacent folia form the lobules that are divided by fissures. They are numbered using the Roman numerals; I to X. The lobule just rostral to the 4th ventricle is I and the one just caudal to it is IX. Between lobules V and VI, the primary fissure separates the posterior lobe from the anterior lobe. Between lobules VIII and IX, the posterolateral fissure separates the posterior lobe from the flocculonodular lobe².

The vertebrates vary in their movement characteristics. As a result of their anatomical structures, the balance and equilibrium differ in the bipedal and quadruped. In the bipedal group, variable extremity movements are observed between the flying and walking vertebrates. The movements of the forelimbs (the hands in the case of human and the wings in the case of the birds) and the hind limbs are interestingly variable. These differences are reflected on the anatomical and histological organization of the organs in the central nervous system³. Previous researchers stated that since the cerebellum is responsible for the balance, timing and coordination of the movements of the extremities⁴.

The vertebrates are divided into 5 classes: fish, amphibians, reptiles, avian and mammals. Although all vertebrates have three main brain regions; the forebrain, midbrain and hindbrain, they also have different adaptations of the brain⁵. The cerebellum controls the movements while acquiring information about the environment⁶. The cerebellum; its overall shape and cortical gross pattern varies significantly among the vertebrate classes from sheet-like structures in some fish, amphibians and reptiles to folded and lobulated structures in birds and mammals⁵.

The comparative anatomists Glickstein & Voogd derived structural/functional correlations by comparing the size of a cerebellar region with the characteristics of the species to which it belonged⁷. There is an upside-down somatotopic representation in the anterior lobe: the leg and foot sensorimotor areas are localized in lobule III, the hand in lobules IV and V and the orofacial movements and sensory stimulation activate the medial regions of lobule VI⁸.

The gait ataxia, arm and leg dysmetria and cerebellar dysarthria arise following strokes in the anterior lobe and lobule VIII. Voxel-based lesion-symptom mapping confirms that lesions in the vermal and paravermal regions of lobules II, III and IV cause an impaired posture and ataxic gait. Lesion in lobule III produces a lower limb ataxia, in lobules IV and V produce an upper limb ataxia and dysarthria results from the paravermal lesions in lobule VI⁹.

MATERIAL AND METHODS

The study included a variety of vertebrates as follows: from the mammalian group; the rat (the albino race of Rattus Norvegicus), cat (Felis Domesticus), rabbit (the albino

race of Lepus Capensis) and cow (Lolium Bos Taurus), from the primates; the human, from the avian; the pigeon (Columba Livia), Egyptian fruit bat (Rousettus Aegyptiacus) and duck (Anas Platyrhynchos) and from the reptiles; the African house snake (Lamprophis Aurora) were selected.

Source of the Animals: All the animals were purchased from different sources in Assiut and Cairo. The human cerebellar specimens were obtained from the Human Anatomy and Embryology Department, Faculty of Medicine, Cairo University whereas the cow cerebellar specimens were from the Anatomy and Histology Department, Faculty of Veterinary Medicine, Assiut University. They were handled gently and housed under the normal room temperature with a 12:12 hour light: dark cycle. Food and water were available ad libitum. The dealing of the animals was approved by Ethical Committee of Assiut Faculty of Medicine.

Age of the Animals: The used animals were in the adult age.

Number of the Animals: Fifty-four animals belonging to the examined nine vertebrates were taken; six for each.

Methods: The animals were anaesthetized by Ether inhalation. Each animal was fixed in the dissecting dish. The chest was opened to expose the heart for the intracardiac perfusion. The scarification was done by the intracardiac perfusion with heparinized isotonic saline 100 mg/kg body weight to prevent the coagulation¹⁰. The perfusion was performed through the left ventricle and Bouin's fixative was injected. The skull was opened to obtain the cerebellum for the following:

1- Gross Morphological Study: As soon as the brain specimens were collected, they were photographed by using a hand-held digital camera (Nicon D2500) except that of the snake which was photographed under the binocular gross microscope (Leica S6D) at x 20

magnification. It should be noticed that the actual dimensions of the cerebella were measured and indicated in the photographs to compare the structural variations among the different animals. In addition, the individual cerebella were weighed.

2- Morphological Labeling and Histological Study: Using Gallocyanin Chrom- Alum Stain¹¹: The specimens were put in Bouin's fixative solution for 12-24 hours according to the size of the specimen. Bouin's fixative was used as it penetrates the brain tissue rapidly and causes a little shrinkage. For the morphological labeling of the cerebellar lobules, 6 Gallocyanin stained sections from each animal were examined by the binocular gross microscope (Leica S6D) and the images were captured at x 20, x 32, x 40 and x 63 magnifications then, the labeling was done¹². For the histological study, the slides were examined by Olympus Cx31 microscope and the images were captured at x 200 and x 400 magnifications.

3- Morphometric Study¹³:

The image analyzer (soft imaging system-Olympus Company) in the Histology Department, Faculty of Medicine, Assiut University was used to estimate the following parameters of the cerebellar lobules, III, IV and V in a non-overlapping field from each slide per a fixed area of 12360 μ^2 :

- the thickness of the cortical layers,
- Purkinje cell surface area and
- > Purkinje cell count.

The statistical analysis was done by using the one-way analysis of variance (ANOVA) with Tukey's posthoc test.

The statistical significance was considered as follows:

>0.05: non-significant

< 0.05: significant

< 0.01: highly significant

RESULTS

1- Gross Morphology of the Cerebellum (Fig.1):

The Snake: The cerebellum was a small simple rounded mass resting on the top of the pons and medulla oblongata and separated from them by the cavity of the fourth ventricle. It showed the simplest form with no foliations on its surface.

The Avian Group (bat, pigeon and duck): had a characteristic common external form composed of a central ovoid well-developed vermis and two rudimentary cerebellar hemispheres.

The Mammalian Group (rat, cat, rabbit and cow): had a cerebellum with two large lateral hemispheres as well as a large central vermis.

The Primates (human): had a characteristic form of a narrow central vermis and two lateral very large cerebellar hemispheres.

2- Morphological Labeling and Comparison of the Cerebellar Lobules (Fig.2):

The cerebellum of the adult African house snake represented the most primitive form in the study. The lobulation was absent. It was composed of a single flat lamella separated from the pons and medulla oblongata by the cavity of the fourth ventricle.

The cerebellum of the adult pigeon had three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe included the anterior part of the rostral surface of the cerebellum and was formed of lobules I, II, III, IV and V. It was separated from the posterior lobe by the primary fissure. The posterior lobe formed the whole of the dorsal surface of the cerebellum and the caudal part of the ventral surface. It included lobules

VI, VII, VIII and IX. The flocculonodular lobe was separated from the posterior one by the posterolateral fissure. It included lobule X. Lobules I and II were separated from each other by the precentral fissure a (f.prc.a). Lobule III was separated from lobule II by a deep furrow; the precentral fissure (f.prc.) and had a short stalk with two small surface folia; IIIa and IIIb. Lobule IV was well developed, had a long stalk with two large surface folia; IVa and IVb and was separated from lobule III by the preculminate fissure (f.pc.). It was separated from lobule V by the intraculminate fissure (f.icul.). Lobule IV was followed from the deepest part of the preculminate fissure to the depth of the intraculminate folia; Va and Vb and vb and represented the cortex from the deepth of the intraculminate fissure to the deepest part of the primary fissure (f.pr.)

The cerebellum of the adult duck was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe formed the anterior part of the rostral surface of the cerebellum. It included lobules I, II, III, IV and V. The posterior lobe formed the whole of the dorsal surface of the cerebellum and the caudal part of the ventral surface. It contained lobules VI, VII, VIII and IX. The flocculonodular lobe contained lobule X. Lobule III was small. It a single short stalk with no secondary foliation. Lobule IV had a long stalk. Lobule V was further subdivided into two large surface folia; Va and Vb.

The cerebellum of the adult Egyptian fruit bat was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe almost formed the rostral surface of the cerebellum. It contained lobules I, II, III, IV and V. It was separated from the posterior lobe by the primary fissure. The posterior lobe made the whole of the dorsal surface of the cerebellum. It included lobules VI, VII, VIII and IX. The

flocculonodular lobe was separated from the posterior one by the posterolateral fissure and included lobule X. Lobule III was composed of a small simple lamella. Lobule IV was well developed and had a long stalk with a small secondary folium. Lobule V was well formed and had a long stalk with two large surface folia.

The cerebellum of the adult cat was formed of three lobes; an anterior, a posterior and a folcculonodular. The primary fissure divided it into anterior and posterior lobes that were more or less equal in size. The anterior lobe contained lobules I, II, III, IV and V. The posterior lobe included lobules VI, VII, VIII, and IX. The folcculonodular lobe was formed of lobule X. Lobule III was divided into two lamellae; IIIa and IIIb. Lamella IIIa was further subdivided into two surface folia. Lobule IV was formed of two large lamellae with secondary foliations. Lobule V had a complicated form. It was composed of the large anterior sublobule referred to as VA and the two superficial lamellae; Vb and Vc, in addition to the characteristic lamellae Vd, Ve and Vf that were present in the anterior wall of the primary fissure.

The cerebellum of the adult rat was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe almost formed the whole of the rostral surface of the cerebellum. It was formed of lobules I, II, III, IV and V. The posterior lobe formed the whole of the dorsal surface of the cerebellum. It was composed of lobules VI, VII, VIII, and IX. The flocculonodular lobe had contained lobule X. Lobule III was well developed. It was further subdivided into the two folia; IIIa and IIIb. Lobules IV and V did not have any secondary foliations and were served by one medullary ray. Lobules IV and V together, were indicated as the culmen.

The cerebellum of the adult rabbit was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe made almost the whole of the

rostral surface of the cerebellum and was composed of lobules I, II, III, IV and V. The posterior lobe formed the whole of the dorsal surface of the cerebellum and the caudal part of the ventral surface. It was formed of lobules VI, VII, VIII and IX. The flocculonodular lobe contained lobule X. Lobule III had a complicated form. It was divided by the intracentral fissure (f.ic.) into the two large sublobules; IIIA and IIIB which were further subdivided into multiple secondary foliations. Lobules IV and V had a single medullary column. Each one was formed of a short stalk with two surface folia.

The cerebellum of the adult cow was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe comprised the whole of the rostral surface of the cerebellum. It was formed of lobules I, II, III, IV and V. The posterior lobe included the whole of the dorsal surface of the cerebellum. It contained lobules VI, VII, VIII, and IX. The flocculonodular lobe included lobule X. Lobule III was well developed. It was divided into the sublobules; IIIA and IIIB which had multiple secondary foliations. Lobule IV was prominent in the median sagittal section and was divided into the two large sublobules IVA and IVB with multiple secondary folia. Lobule V was also well formed. It had multiple long lamellae.

The cerebellum of the adult human was formed of three lobes; an anterior, a posterior and a flocculonodular. The anterior lobe formed the anterior part of the rostral surface of the cerebellum. It contained lobules I, II, III, IV and V. The posterior lobe formed the whole of the dorsal surface of the cerebellum and the caudal part of the ventral surface. It had lobules VI, VII, VIII and IX. The flocculonodular lobe had contained lobule X. Lobule III was well formed. It terminated into two large lamellae. Lobule IV had an elongated large segment that divided into two large superficial lamellae. Lobule V was well formed and had large multiple lamellae.

3-The Comparative Microscopic Study of the Cerebellar Structure (Figs. 3,4,5):

- a. The present study showed that all the studied vertebrates had the same cerebellar cortical architecture.
- b. The cerebellum of the adult snake was formed of a single flat lamella. The lamella had three layers; the molecular layer, Purkinje cell layer and the granular cell layer, in that order of arrangement from outside inwards. Purkinje cells were arranged in a diffuse zone between the molecular layer and the granular cell layer. They were small in comparison to those of the other studied vertebrates. The granular cells were small, packed together and deeply stained.
- c. In the animals using the hind limbs more than the forelimbs; the rat and rabbit, lobule III had a more condensation of Purkinje cells than in lobules IV and V in the constant measured area.
- d. In the animals using the forelimbs more than the hind limbs; the pigeon, duck and bat, lobules IV and V showed a more condensation of Purkinje cells than in lobule III in the constant measured area.
- e. Regarding the human and cow, no marked differences in the numbers of Purkinje cells were observed among lobules III, IV and V.

4- Morphometric Results:

I-Purkinje Cell Count:

In the adult snake, the mean number of Purkinje cells per an area of 12360 μ^2 was (29 ± 1.9). In the animals using the forelimbs more than the hind limbs ; the pigeon, duck, bat and cat, the mean numbers of Purkinje cells per the same surface area in lobule III were (31 ± 0.79), (54 ± 1.4), (59 ± 0.71) and (26 ± 0.62), respectively with a significant decrease in comparison to the mean numbers of Purkinje cells in lobules IV and V of the same animals

which were (62 \pm 0.75 and 66 \pm 1.5), (73 \pm 1.6 and 98 \pm 1.5), (67 \pm 0.84 and 78 \pm 0.62) and (33 \pm 0.73 and 33 \pm 0.48), respectively.

In the animals using the hind limbs more than the forelimbs; the rat and rabbit, the mean numbers of Purkinje cells per the same surface area in lobules IV and V were (36 \pm 1.4 and 27 \pm 1.4) and (31 \pm 1.3 and 29 \pm 1.3), respectively with a significant decrease in comparison to the mean numbers of Purkinje cells in lobule III which were (62 \pm 0.95) and (52 \pm 1.2), respectively. In the animals using the forelimbs and hind limbs equally; the human and cow, the mean numbers of Purkinje cells per the same surface area in lobule III were (17 \pm 0.79) and (23 \pm 7.6), respectively with a non-significant difference in comparison to the mean numbers in lobules IV and V of the same animals which were (17 \pm 0.85 and 17 \pm 0.40) and (21 \pm 0.87 and 21 \pm 1), respectively (Table 1 and Graph 1).

Table (1): Mean numbers of Purkinje cells in lobules III, IV and V per an area of 12360 μ^2 in the studied animals and in the single lamella of the snake.

	Lobule III				Lobule	IV		Lobule	P III	P III	
	Ν	Mean	SE	Ν	Mean	SE	Ν	Mean	SE	vs. IV	vs. V
Pigeon	6	31	0.79	6	62	1	6	66	1.5	**	**
Duck	6	54	1.4	6	67	0.84	6	78	0.62	**	**
Bat	6	59	0.71	6	73	1.6	6	98	1.5	**	**
Cat	6	26	0.62	6	33	0.73	6	33	0.48	*	*
Rat	6	62	0.95	6	36	1.4	6	27	1.4	**	**
Rabbit	6	52	1.2	6	31	1.3	6	29	1.3	**	**
Cow	6	23	0.76	6	21	0.87	6	21	1	Ns	Ns
Human	6	16	0.79	6	17	0.85	6	17	0.40	Ns	Ns
Snake	6	29 ± 1	.9 in tl	ne s	ingle la	nella					

SE: standard error of the mean

*: a significant difference (p < 0.05)

**: a highly significant difference (p < 0.01)

Ns: a non-significant difference (p > 0.05)



Graph (1): Comparison of the mean numbers of Purkinje cells in lobules III, IV and V per an area of 12360 μ^2 in the studied animals and in the single lamella of the snake.

II- Purkinje Cells Surface Area (in μ^2):

In the adult snake, the mean surface area of Purkinje cell was (99 ± 3.4 μ^2). In the animals using the forelimbs more than the hind limbs; the pigeon, duck, bat and cat, the mean surface areas of Purkinje cells in lobule III were (179 ± 3.4 μ^2), (324 ± 4.5 μ^2), (149 ± 4.1 μ^2) and (437 ± 9.8 μ^2), respectively with a significant decrease in comparison to the mean surface areas of Purkinje cells in lobules IV and V of the same animals which were (290 ± 2.2 μ^2 and 295 ± 3.7 μ^2), (206 ± 3 μ^2 and 199 ± 3.3 μ^2), (389 ± 2.4 μ^2 and 398 ± 5.1 μ^2) and (501± 7.3 μ^2 and 578 ± 5.6 μ^2), respectively.

In the animals using the hind limbs more than the forelimbs; the rat and rabbit, the mean surface areas of Purkinje cells in lobules IV and V were (176 ± 5.2 μ^2 and 168 ± 4.8 μ^2) and (225 ± 9.4 μ^2 and 234 ± 8.1 μ^2), respectively with a significant decrease in comparison to the mean surface areas of Purkinje cells in lobule III which were (218 ± 6.2 μ^2) and (303 ± 5.5 μ^2), respectively.

In the animals using the forelimbs and hind limbs equally; the human and cow, the mean

surface areas of Purkinje cells in lobule III were (607 ± 10 μ^2) and (615 ± 8.9 μ^2), respectively with a non-significant difference in comparison to the mean surface areas of Purkinje cells in lobules IV and V in the same animals which were (621 ± 7.6 μ^2 and 597 ± 9.1 μ^2) and (618 ± 8.1 μ^2 and 606 ± 9.5 μ^2), respectively **(Table 2 and Graph 2).**

Table (2): Mean surface areas of Purkinje cells (in μ^2) in lobules III, IV and V in the studied animals and in the single flat lamella of the snake.

	Lobule III				Lobule	IV		Lobule	P III	P III	
	Ν	Mean	SE	Ν	Mean	SE	Ν	Mean	SE	vs. IV	vs. V
Pigeon	6	179	3.4	6	290	2.2	6	295	3.7	**	**
Duck	6	324	4.5	6	389	2.4	6	398	5.1	**	**
Bat	6	149	4.1	6	206	3	6	199	3.3	**	**
Cat	6	437	9.8	6	501	7.3	6	578	5.6	*	*
Rat	6	218	6.2	6	176	5.2	6	168	4.8	**	**
Rabbit	6	303	5.5	6	225	9.4	6	234	8.1	**	**
Cow	6	615	8.9	6	618	8.1	6	606	9.5	Ns	Ns
Human	6	607	10	6	621	7.6	6	597	9.1	Ns	Ns
Snake	6	99 ± 3	.4 in tl	he s	ingle la	mella					

SE: standard error of the mean

*: a significant difference (p < 0.05)

**: a highly significant difference (p < 0.01)

Ns: a non-significant difference (p > 0.05)



Graph (2): Comparison of the mean surface areas of Purkinje cells in lobules III, IV and V in the studied animals and in the single lamella of the snake.

III- Purkinje Cell layer Thickness (in microns):

In the adult snake, the mean thickness of Purkinje cell layer was $(87 \pm 2.4\mu)$. In the animals using the forelimbs more than the hind limbs; the pigeon, duck, bat and cat, the mean thicknesses of Purkinje cell layer in lobule III were $(27 \pm 1 \mu)$, $(33 \pm 0.66 \mu)$, $(22 \pm 0.90 \mu)$ and $(34 \pm 0.18 \mu)$, respectively with a significant decrease in comparison to the mean thicknesses of Purkinje cell layers in lobules IV and V of the same animals which were $(34 \pm 1.3 \mu \text{ and } 39 \pm 0.42 \mu)$, $(38 \pm 0.79 \mu \text{ and } 39 \pm 0.51 \mu)$, $(46 \pm 1.2 \mu \text{ and } 47 \pm 1.6 \mu)$ and $(43\pm 1.2 \mu \text{ and } 49 \pm 0.33 \mu)$, respectively.

In the animals using the hind limbs more than the forelimbs; the rat and rabbit, the mean thicknesses of Purkinje cell layers in lobules IV and V were ($21 \pm 0.66 \mu$ and $21 \pm 0.54 \mu$) and ($28 \pm 1.1 \mu$ and $28 \pm 2.1 \mu$), respectively with a significant decrease in comparison to the mean thicknesses of Purkinje cell layer in lobule III which were ($29 \pm 0.39 \mu$) and ($43 \pm 1.2 \mu$), respectively.

In the animals using the forelimbs and hind limbs equally; the human and cow, the mean thicknesses of Purkinje cell layer in lobule III were $(33 \pm 0.59 \mu)$ and $(39 \pm 0.33 \mu)$, respectively with a non-significant difference in comparison to the mean thicknesses of Purkinje cell layers in lobules IV and V in the same animals which were $(36 \pm 0.91 \mu$ and 36 \pm 1.1 μ) and $(39 \pm 0.26 \mu$ and 41 \pm 0.40 μ), respectively **(Table 3 and Graph 3)**.

Table (3): Mean thicknesses of Purkinje cell layers (in microns) in lobules III, IV and V in the studied animals and in the single flat lamella of the snake.

	Lobule III				Lobule	IV		Lobule	P III	P III	
	Ν	Mean	SE	Ν	Mean	SE	Ν	Mean	SE	vs. IV	vs. V
Pigeon	6	27	1	6	34	1.3	6	39	0.42	**	**
Duck	6	33	0.66	6	46	1.2	6	47	1.6	**	**
Bat	6	22	0.90	6	38	0.79	6	39	0.51	**	**
Cat	6	34	0.18	6	43	1.2	6	49	0.33	*	*
Rat	6	29	0.39	6	21	0.66	6	21	0.54	**	**
Rabbit	6	43	1.2	6	28	1.1	6	28	2.1	**	**
Cow	6	39	0.33	6	39	0.26	6	41	0.40	Ns	Ns
Human	6	33	0.59	6	36	0.91	6	36	1.1	Ns	Ns
Snake	6	87 ± 2	.4 in tl	ne s	ingle la	mella					

SE: standard error of the mean

*: a significant difference (p < 0.05)

**: a highly significant difference (p < 0.01)

Ns: a non-significant difference (p > 0.05)



Graph (3): Comparison of the mean thicknesses of Purkinje cell layers in lobules III, IV and V in the studied animals and in the single lamella of the snake.

IV- The Molecular Layer Thickness (in microns):

In the adult snake, the mean thickness of the molecular layer was (79 \pm 4 $\mu)$. In the

animals using the forelimbs more than the hind limbs; the pigeon, duck, bat and cat, the mean

thicknesses of the molecular layer in lobule III were (122 ± 1.7 μ), (240 ± 4.5 μ), (124 ± 7.9 μ)

and (220 \pm 3.9 $\mu)$, respectively with a significant decrease in comparison to the mean

thicknesses of the molecular layers in lobules IV and V of the same animals which were (178 ± 12 μ and 219 ± 4.3 μ), (182 ± 4.8 μ and 199 ± 4.8 μ), (314 ± 15 μ and 346 ± 14 μ) and (290± 8.3 μ and 295± 16 μ), respectively.

In the animals using the hind limbs more than the forelimbs; the rat and rabbit, the mean thicknesses of the molecular layers in lobules IV and V were (146 ± 7.6 μ and 132 ± 9 μ) and (146 ± 6.8 μ and 166 ± 5.4 μ), respectively with a significant decrease in comparison to the mean thicknesses of the molecular layer in lobule III of the same animals which were (186 ± 5.1 μ) and (287 ± 13 μ), respectively.

In animals using the forelimbs and hind limbs equally; the human and cow, the mean thicknesses of the molecular layer in lobule III were $(362 \pm 12 \mu)$ and $(337\pm 7.2 \mu)$, respectively with a non-significant difference in comparison to the mean thicknesses of the molecular layers in lobules IV and V in the same animals which were $(364\pm 12 \mu \text{ and } 368 \pm 7.7 \mu)$ and $(338 \pm 6.2 \mu \text{ and } 340\pm 9 \mu w)$, respectively **(Table 4 and Graph 4)**.

Table (4): Mean	thicknesses	of the	Molecular	layers (ir	n microns)	in	lobules	III, IV	and '	V in	each
studied	l animal a	nd in the sing	gle flat	lamella of t	the snake							

		Lobule III			Lobule	IV		Lobule	P III	P III	
	Ν	Mean	SE	Ν	Mean	SE	Ν	Mean	SE	vs. IV	vs. V
Pigeon	6	122	1.7	6	178	12	6	219	4.3	*	*
Duck	6	240	4.5	6	314	15	6	346	14	*	*
Bat	6	124	7.9	6	182	4.8	6	199	4.8	*	*
Cat	6	220	3.9	6	290	8.3	6	295	16	*	*
Rat	6	186	5.1	6	146	7.6	6	132	9	**	**
Rabbit	6	287	13	6	146	6.8	6	166	5.4	**	**
Cow	6	337	7.2	6	338	6.2	6	340	9	Ns	Ns
Human	6	362	12	6	364	12	6	368	7.7	Ns	Ns
Snake	6	79 ± 4	in the	sin	gle lame	ella					

SE: standard error of the mean

*: a significant difference (p < 0.05)

**: a highly significant difference (p < 0.01)

Ns: a non-significant difference (p > 0.05)



Graph (4): Comparison of the mean thicknesses of the molecular layers in lobules III, IV and V in the studied animals and in the single lamella of the snake.

V- Granular Cell Layer Thickness (in microns):

In the adult snake, the mean thickness of the granular layer was (81 ± 3.9 μ). In the animals using the forelimbs more than the hind limbs; the pigeon, duck, bat and cat, the mean thicknesses of the granular cell layer in lobule III were (154± μ), (341 ± 11 μ), (163 ± 4.3 μ) and (258 ± 9.1 μ), respectively with a significant decrease in comparison to the mean thicknesses of the granular cell layers in lobules IV and V of the same animals which were (270 ± 4.9 μ and 331 ± 2.2 μ), (196 ± 4.4 μ and 212 ± 4.1 μ), (409 ± 2.2 μ and 505± 24 μ) and (314± 5.8 μ and 331± 22 μ), respectively.

In the animals using the hind limbs more than the forelimbs; the rat and rabbit, the mean thicknesses of the granular cell layers in lobules IV and V were $(239 \pm 6.1 \mu \text{ and } 233 \pm 15 \mu)$ and $(250 \pm 3.9 \mu \text{ and } 244 \pm 3.3 \mu)$, respectively with a significant decrease in comparison to the mean thicknesses of the granular cell layer in lobule III which were $(299\pm 18 \mu)$ and $(388 \pm 4.9 \mu)$, respectively.

In the animals using the forelimbs and hind limbs equally; the human and cow, the mean thicknesses of the granular cell layer in lobule III were (615 \pm 3.9 μ) and (635 \pm 13 μ),

respectively with a non-significant difference in comparison to the mean thicknesses of the

granular cell layers in lobules IV and V in the same animals which were (619 \pm 9.4 μ and 618 \pm

3.8 μ) and (633 ± 8.8 μ and 637 ± 10 μ), respectively (Table 5 and Graph 5).

	Lobule III				Lobule	IV		Lobule	P III	P III	
	Ν	Mean	SE	Ν	Mean	SE	Ν	Mean	SE	vs. IV	vs. V
Pigeon	6	154	6.3	6	270	4.9	6	331	22	*	*
Duck	6	341	11	6	409	2.2	6	505	24	*	*
Bat	6	163	4.3	6	196	4.4	6	212	4.1	*	*
Cat	6	258	9.1	6	314	5.8	6	331	7.4	*	*
Rat	6	299	18	6	239	6.1	6	233	15	**	**
Rabbit	6	388	4.9	6	250	3.9	6	244	3.3	**	**
Cow	6	615	3.9	6	619	9.4	6	618	3.8	Ns	Ns
Human	6	635	13	6	633	8.8	6	637	10	Ns	Ns
Snake	6	81 ± 3	.9 in tl	he s	single la	mella					

Table (5): Mean thicknesses of the granular layers (in microns) of lobules III, IV and V in each studied animal and in the single flat lamella of the snake.

SE: standard error of the mean

*: a significant difference (p < 0.05)

**: a highly significant difference (p < 0.01)

Ns: a non-significant difference (p > 0.05)



Graph (5): Comparison of the mean thicknesses of the granular layers of lobules III, IV and V in the studied animals and in the single lamella of the snake.

DISCUSSION:

The cerebellum is known to play an important role in the control of the muscle tone, posture and skilled motor activity¹⁴. There is a scarcity of the literatures studying the microscopic structure and morphometry of the various cerebellar lobules in the different vertebrates. This is a comparative research studying the cerebellar gross morphology, microscopic structure of the cerebellar lobules controlling the limb movements and their morphometry.

1- Gross Morphology of the Cerebellum:

Regarding the snake, this study showed that the cerebellum is composed of a small rounded mass on the top of the fourth ventricle. This comes in agreement with previous researchers who reported that, the simplest cerebellum in the reptiles was found in the snakes¹⁵. This could be due to the absence of the limbs.

This study observed that the cerebellum of the avian group; the pigeon, duck and fruit bat is composed of a large median vermis and two rudimentary cerebellar hemispheres which agrees with the findings of other researchers¹⁶. These researchers stated that the avian cerebellum had a central region that was highly developed for flying¹⁶.

Regarding the rat, cat and rabbit, the present study declared that, the cerebellum was formed of two relatively large lateral hemispheres as well as a large central vermis. The results are in line with earlier observations that found that, in the rat, cat and rabbit the cerebellum had a relatively broad vermian region and extended hemispheres due to the importance of the axial trunk as well as the limbs in the quadruped¹⁴.

The present study showed that, the cow's cerebellum was formed of two large

lateral hemispheres as well as a large central vermis which is concomitant with the descriptions of scientists who attributed those findings to the large trunk and well-formed limbs¹⁷. Regarding the human, the present study showed that, the cerebellum was formed of two large hemispheres and a rudimentary central vermis. That variation could be attributed to the structural/behavioral differences of the human in comparison to the other mammals¹⁸. This is also in agreement with Demaerel who found that, in primates, especially the human; the large cerebellar hemispheres were because those regions received signals from the distal parts of the limbs and were commonly supposed to be involved in controlling the independent skillful movements of the fingers, hands and feet¹⁹. The rudimentary vermis in the human's cerebellum was attributed to the decreased importance of the trunk muscles.

Many researchers reported that considering the topography of the cerebellar cortex it was divided into three functional zones; the cortex of the vermis controlled the movements and equilibrium of the trunk, the intermediate zone controlled the muscles of the limbs especially of the hands and feet while the most lateral part of each hemisphere was concerned with the planning of the sequential movements, learning skills and cognition^{5,16}. Therefore, it could be emphasized that there were obvious variations in the gross structure of the different parts of the cerebellum among the vertebrates used in the study. This could be attributed to the different classes of the vertebrates.

2- Comparative Evaluation of the Structural/Functional Relationship of the Cerebellar Lobules III, IV and V:

In the present work, the cerebellar structure was investigated on the basis of the lobular theory of earlier researchers that considered each cerebellar lobule as a separate functional unit²⁰. This is in agreement with Le Roy-Duflos who concluded that, each folium of the mammalian cerebellum represented a discrete structural and functional unit that mediated specific sensorimotor projections resulting in a specific behavior²¹. This also agrees with previous researchers who found that there was an upside-down somatotopic representation in the anterior lobe; the leg and foot sensorimotor representations were localized to lobule III and those of the hand in lobules IV and V^{8,22}.

The present work showed that, the cerebellum of the adult African house snake represented the most primitive form. It was composed of a single flat lamella. This comes in agreement with other scientists who reported that, the simplest cerebellum in the reptiles was that of the snakes¹⁵. This was due to the absence of the limbs.

Regarding the pigeon and duck, the present work showed that lobules IV and V were well developed compared to lobule III. These results coincide with earlier observations reported that, lobules IV, V controlled the forelimb muscles in birds^{20,23}. Thus, the structural complexity of lobules IV and V in the pigeon and duck could be attributed to the importance of the forelimb muscles on expense of the hind limb muscles due to flying function.

The present study declared that in the bat, lobule III did not give either sublobules or folia in comparison to the well-developed lobules IV and V. This is in accordance with other studies suggested that the size of lobule III was correlated to the hind limb musculature and walking ability¹⁶. Many investigators reported that the small size of lobule III in the fruit bat could be attributed to the weakness of the hind limbs as

they were only used in hanging to the caves^{23,24}. On the other hand, the well-developed lobules IV and V were due to the strong large wings used in flying.

Regarding the domestic cat, the present study revealed that lobules IV and V were well developed. This could be attributed to the importance of the forelimbs in the cat using them in climbing the walls and playing²⁰.

Regarding the rat and rabbit, the present work showed that lobule III had a complicated form compared to those of lobules IV and V. This could be attributed to the fact that, the hind limbs of the rat and rabbit were stronger and larger than the forelimbs as the hind limbs were used in jumping²⁰.

Regarding the cow, the present study revealed that lobules III, IV and V were well developed. This could be due to the importance of both the forelimbs and hind limbs in the cows being large and heavy quadruped animals supported by the forelimbs and hind limbs in an equal manner. This explanation agrees with previous investigators¹⁷.

Regarding the human, the present study showed that lobule III was well formed, subdivided and foliated. This is in agreement with researchers who concluded that in the higher primates that took an erect posture such as the human, lobule III reached its highest complexity that could be due to the fact that the primates used the hind limbs in supporting the body weight and in walking²⁵.

The present work also showed that, lobules IV and V were well formed in man. This is in line with recent investigators who stated that in the higher primates such as rhesus monkey, chimpanzee, gorilla and man, lobules IV and V were large, subdivided and foliated compared to other species which could be attributed to the highly skillful and fine movements of the hands and fingers²⁶.

These results are concomitant with an earlier study indicated that one major difference in the cerebellar morphology among the major classes of the vertebrates was the degree of folding or foliation of the cerebellar cortex⁵. At one end of the spectrum were reptiles that had a curved cerebellum but no actual folds. At the opposite end of that spectrum were birds and mammals that all possessed cerebella with numerous folds. Lying in between those two extremes were the species with few simple folds, such as lungfish. The differences in the degree of foliation among those vertebrates were attributed to behavioral specializations, such as the complex motor behaviors in birds and mammals.

From the previously mentioned data an association between the degree of the complexity of the limb function and the degree of foliation of the corresponding cerebellar lobule could be deducted. The more the complexity of the limb function, the more the degree of foliation of the corresponding lobule. This is in accordance to a previous study reported that the increase in the amount of folding of a structure was a means of increasing the surface area within a constrained volume²⁷. This also agrees with other scientists who concluded that for laminar structures, such as the cerebellar cortex, increasing the surface area had an important role in improving the function of the cerebellar cortex^{28,29}.

3- Histological and Morphometric Analysis:

The present work revealed that the cerebellar cortex of the studied vertebrates had the same three cell layers; the outer molecular, the middle Purkinje cell layer and the inner granular cell layer. This agrees with earlier researchers who stated that the three cell layers overlaid the white matter tracts that contained the afferent and efferent cerebellar projection axons were found similar throughout the vertebrates³⁰. This

study also showed the differences in the layers' thickness and in the number of Purkinje cells in lobules III, IV and V of the studied animals. The Purkinje cell count and surface area and the thicknesses of the granular cell layer, Purkinje cell layer and the molecular cell layer in the region of the folial crown of lobules III, IV and V in each animal were measured. In the present work, the folial crowns were selected for performing the morphometric measurements based on previous observations³¹. They gave attention to the differential development of the cerebellar lobules dividing each folium into three parts; the top (folial crown), the sides (the wall) and the stalk (the fundus). They studied the morphological differences between the crowns, walls and fundi of folia as was seen in sections at right angles to the long axis of any folium. The granular cell layer was very thick in the folial crown, thinner in the walls, and very thin in the fundi. Purkinje cells were closer together in the folial crowns and anguli than in the walls, and were few in the fundi. The myelinated fibre bundles were more numerous and prominent beneath the crown than in the folial walls or fundi.

The mean number of Purkinje cells in the adult human cerebellar lobules was 17 ± 2 and the mean thickness of the molecular layer was 370 ± 12 . Our results are in line with those of a recent study reported that the Purkinje cell count was 12 ± 5 and the molecular layer thickness ranged from 309 to 779 microns including the different lobules in the human cerebellum³¹.

Regarding the cat, the present work revealed that the mean thickness of the molecular layer ranged from 223 to 311 and that of the granular layer from 260 to 353. The obtained results are in agreement with an earlier study that calculated the mean thickness of those layers to be 307.7 ± 43 and 293.19 ± 49 , respectively³².

Regarding the albino rat, the present work found that the mean number of

Purkinje cells ranged from 26 to 62, the mean thickness of Purkinje cell layer was from 21 to 29 and that of the molecular layer was from 121 to 191. The obtained results are concomitant with those of another study whose measured values were 27 ± 1.4 , 20.9 ± 2 and 230.7 ± 43 , respectively³³. So far, regarding the morphometric measurements performed for the other studied animals, there is a scarcity in the researches to compare with.

The present study revealed that in the animals using the forelimbs more than the hind limbs as the pigeon, duck, bat and cat, the data recorded had a significant decrease in lobule III than in lobules IV and V. On the other hand, in the animals using the hind limbs more than forelimbs as the rat and rabbit, the data recorded showed a significant decrease in lobules IV and V than in III. In the animals using the forelimbs and hind limbs more or less equally, like human and cow, no significant differences among lobules III, IV and V were observed.

The results of the present study are in agreement with a previous research reported that an increase in the degree of the foliation was functionally translated into a greater number of Purkinje cells per unit volume of the cerebellum²⁰. Purkinje cells provided the sole output of the cerebellar cortex and a 'bottleneck' on the information entering the cerebellar nuclei from the cortex. In considering the degree of foliation, researchers stated that increasing the cerebellar foliation and thereby increasing the number of Purkinje cells as well as the cerebellar cortex layers' thickness, resulted in a concomitant increase in the processing capacity of the cerebellar cortex that, in turn, led to an increase in the behavioral complexity and/or cognitive ability³⁴. Cerebellar foliation was defined as an increase in the number of the cells and depth of the cerebellar layers³⁵. Foliation and fissuration was discriminated by the lengths of the

molecular layer and of the Purkinje cell layer³⁶. Therefore, the differences in the layers' thicknesses and numbers of Purkinje cells among lobules III, IV and V in the different vertebrates studied could be attributed to the variations in their behavioral complexity.

The present study indicated that the position of the animal in the phylogenetic scale had a direct relationship with the mean surface area of Purkinje cells, and an inverse one with their mean number in lobules III, IV and V in the animal's cerebellum. Also, regarding the complexity of Purkinje cells, the present study revealed variations starting from just cell primordia in the snake's cerebellar single flat lamella to highly branching cells in the cerebella of the higher vertebrates which comes in agreement with an earlier research stated that there was an increase in the complexity of the structure of Purkinje cells when one ascended in the phylogenetic scale³⁷. Moreover, it was noticed that, there is a correlation between the relative size and histological and morphological complexity of the cerebellum and the agility of the animal³⁸.

Conclusion:

The present study concludes that the variations in the cerebellar morphology and histological structure are related to behavioral differences among animals. The position of the animal in the phylogenetic scale has a direct relationship with the mean surface area of Purkinje cells, and has an inverse relationship with the mean number of Purkinje cells in lobules III, IV and V in the animal's cerebellum. In addition, the degree of structural complexity of cerebellar lobules III-IV-V is related to limbs' function.

Conflict of Interest: There is no conflict of interest.

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Figure Legends:

Fig. (1): A plate of photographs showing the gross picture of the different studied vertebrates' cerebella in the dorsal view:(1A) snake, (1B) pigeon, (1C) duck, (1D) bat, (1E) cat, (1F) rat, (1G) rabbit, (1H) cow and (1I) human, where the variations in the size of the central vermis and of the hemispheres are observed.

Fig. (2): A plate of photomicrographs showing the studied vertebrates' cerebella at the sagittal section: **(2A)** snake, **(2B)** pigeon, **(2C)** duck, **(2D)** bat, **(2E)** cat, **(2F)** rat, **(2G)** rabbit, **(2H)** cow and **(2I)** human, where the variations in the degree of foliation and in the sizes of lobules III, IV and V among the different vertebrates are observed. Gallocyanine, (2A) X40, (2B) X40, (2D) X40, (2F) X 40, (2C) X32, (2E) X40, (2G) X20, (2H) X6.3, (2I) X6.3

Fig. 3: A plate of photomicrographs of lobule III in the studied vertebrates' cerebella: (3A) snake, (3B) pigeon, (C) duck, (3D) bat, (3E) cat, (3F) rat, (3G) rabbit, (3H) cow and (3I) human, showing the condensation of Purkinje cells in the case of the rat and rabbit. Variations in the sizes of Purkinje cells among the different studied vertebrates are also observed. Gallocyanine X400

Fig. 4: A plate of photomicrographs of lobule IV in the studied vertebrates' cerebella:(4A) snake, (4B) pigeon, (4C) duck, (4D) bat, (4E) cat, (4F) rat, (4G) rabbit, (4H) cow and (4I) human, showing the condensation of Purkinje cells in the case of the pigeon, duck, bat and cat. Gallocyanine X400

Fig. 5: A plate of photomicrographs of lobule V in the studied vertebrates' cerebella:**(5A)** snake, **(5B)** pigeon, **(5C)** duck, **(5D)** bat, **(5E)** cat, **(5F)** rat, **(5G)** rabbit, **(5H)** cow and **(5I)** human, showing the condensation of Purkinje cells in the case of the pigeon, duck, bat and cat. Gallocyanine X400

الملخص العربى

الاختلافات مقابل التشابهات في تركيب المخيخ لفقاريات مختلفة: دراسة تشريحية مقارنة محمد نبيل محمود صالح ، منال محمود سامي احمد المليجي ، أيمن صلاح الدين عامر ودعاء حامد عبد الحميد قسم التشريح الآدمي وعلم الأجنة، كلية الطب، جامعة أسيوط

الهدف من البحث: مقارنة شكل فصيصات لفص المخيخي الأمامي (الفصيص الثالث المختص بحركة الأطراف الخلفية والفصيين الرابع والخامس المختصين بحركات الأطراف الأمامية) بين فقاريات مختلفة، مع التركيز على العلاقة التركيبية الوظيفية للمخيخ.

المواد والطرق: تم استخدام فقاريات بالغة مختلفة؛ من الثدييات: تم اختيار الفأر، القط، الأرنب، البقرة، من الطيور: تم اختيار الحمامة، البطة والخفاش (حيوان ثديي طائر)، من الزواحف: تم اختيار الثعبان، بالإضافة إلى الإنسان. لقد تم فحص مخيخات كل الحيوانات من حيث الشكل العام والتركيب النسيجي.ولقد تم قياس سمك طبقات قشرة المخيخ والمساحة السطحية لخلايا بوركينج كما تم عد خلايا بوركينج في الفصيصات ثلاثة وأربعة وخمسة وتحليل البيانات التي تم الحصول عليها إحصائيًا.

النتائج: لم يظهر مخيخ الثعبان أي فصيصات. الحمامة والبطة والخفاش كان لديهم الجزء الدودي المنتصف كبير ونصفي كرة المخيخ ضامرتين. الفأر والقط والأرنب والبقرة كان لديهم الجزء الدودي المنتصف معقد وكبير وكذلك نصفي كرة المخيخ. كان لدى الإنسان نصفي نصف كرة مخيخ كبيران وجزء دودي منتصف صغير. كان لدى الحمامة والبطة والخفاش تركيبا متطور اللفصيصين الرابع والخامس وبالمثل كان الحال بالنسبة للقط. في المقابل، كان لدى الفأر والأرنب فصيص ثالث اكثر تطورا تركيبيا بالمقارنة مع الفصيصين الرابع و الخامس. في البقرة والإنسان كانت الفصيصات ثلاثة وأربعة وخمسة متطورة تركيبيا. كان هذاك تشابه في ترتيب طبقات المخيخ في الحوانات المختلفة. كان هذاك فوارق معنوية بين الفصيص الثالث والفصيصين الرابع و الخامس. في البقرة والإنسان كانت الفصيصات ثلاثة وأربعة وخمسة متطورة تركيبيا. كان هذاك تشابه في ترتيب طبقات المخيخ في الحيوانات المختلفة. كان هذاك فوارق معنوية بين الفصيص الثالث والفصيصين الرابع و الخامس من حيث عدد خلايا بوركينج وسمك طبقات الفصيصات ثلاثة وأربعة وخمسة متطورة تركيبيا. كان هذاك تشابه في ترتيب طبقات المخيخ في الحيوانات المختلفة. المنوع المؤيخ تبعا لسلوك الحيوانات الذي الفصيصين الرابع و الخامس من حيث عدد خلايا بوركينج وسمك طبقات المتوسطة لتلك البيانات المقاسة زيادة معنوية في الفصيصين الرابع و الخامس من حيث عدد خلايا بور كينج وسمك معقات المتوسطة لتلك البيانات المقاسة زيادة معنوية في الفصيصي الثالث مقار نها الخلفية أكثر من الأمامية أظهرت الأرقام وترتبط درجة تعقيد تركيب المام والتركيبات المخيخات لها علاقة بالاختلافات السلوكية بين الحيوانات المختلفة وترتبط درجة تعقيد تركيب المعام والتركيبات المخيخات لها علاقة بالاختلافات الماتية مين الحيوانات المختلفة