Van Vet J, 2018, 29 (2) 63-66

Van Veterinary Journal

http://dergipark.gov.tr/vanvetj

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Histological and Histometric Structure of Goose *(Anser anser)* Cerebellum

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Received: 06.06.2017 Accepted: 06.11.2017

ABSTRACT The aim of this study is to examine the goose (*Anser anser*) cerebellum histometric and histologically. Six female geese (aged between 10-12 months) were used in this study. After fixation in 10% formalin and following routine histologic processes, the cerebellar tissues of geese were embedded in paraffin blocks and the sections were taken from these blocks. The Kluver-Barrera nerve tissue stain technique was applied to the sections. The ocular micrometer was used for histometric measurements of folia length, diameter of purkinje cells and thicknesses of stratum granulosum and stratum moleculare. SPSS 16.0 was used for calculating mean values. It was observed histological that folia and sulci cerebelli were in the outermost layer of the cerebellum. As a result of Kluver-Barrera staining, the nerve cells in all layers appeared purple and myelin fibers appeared blue. It was found that the average length of the folia was 3397.88 ± 931.32 µm, the average thickness of molecular layer was 348.53 ± 72.92 µm, the average thickness of granular layer was 184.83 ± 48.77 µm and the average diameter of Purkinje cells was 24.23 ± 3.96 µm.

Keywords: *Cerebellum, Goose, Kluver-Barrera, Purkinje cell*

ÖZ

Kaz (*Anser anser*) Beyinciğinin Histolojik ve Histometrik Yapısı

Bu çalışmada, kaz (*Anser anser*) serebellum dokusunun histolojik ve histometrik yapısının incelenmesi amaçlandı. Çalışmada 6 adet dişi kaz (10-12 aylık) kullanıldı. Kazlara ait serebellum dokuları % 10'luk formalinde tespit edildikten sonra rutin histolojik işlemlerden geçirilip parafinde bloklandı ve bu bloklardan kesitler alındı. Kesitlere Kluver-Barrera sinir doku boyama tekniği uygulandı. Folia uzunluğu, Purkinje hücre çapı, stratum molekülare ve stratum granülosum katmanlarının kalınlıklarını ölçmek için oküler mikrometre kullanıldı. Ortalama değerleri hesaplamak için SPSS 16.0 programı kullanıldı. Yapılan histolojik incelemelerde serebellumun dış kısmında folia ve sulkus serebelli yapıları gözlendi. Kluver-Barrera boyaması sonucunda tüm katmanlardaki sinir hücreleri mor renkte, miyelinli lifler ise mavi renkte görüldü. Foliaların ortalama uzunluğu 3397.88 ± 931.32 µm, stratum molekülare katmanının ortalama kalınlığı 348.53 ± 72.92 µm, stratum granülosum katmanının ortalama kalınlığı 184.83 ± 48.77 µm ve Purkinje hücrelerinin ortalama çapı ise 24.23 ± 3.96 µm olarak belirlendi.

Anahtar Kelimeler: *Kaz, Kluver-Barrera, Purkinje hücresi, Serebellum*

INTRODUCTION

Cerebellum is essentially a major motor center of the avian brain and it has important functions such as maintaining equilibrium and coordinating muscle movements (Sturkie 1986; Yılmaz and Dinç 2002). The structure of cerebellum is different from animal class to another. The surface of cerebellum is smooth in some fishes but it has curved lines and lobes in mammals and birds (Butler and Hodos 2005). The cerebellum consists of a single median lobe (the corpus cerebella) with the two lateral flocculi. The surface of the cerebellum is formed into folds or folia and fissures (Erençin 1963; Hodges 1974; Voogd and Glickstein 1998). The histology of the avian cerebellum is similar to that of the mammalian cerebellum (Sturkie 1986).

Histologically, outer layer of the cerebellum consists of grey matter (a cortical layer, Subtantia grisea) and the inner is made up of white matter (medulla, Substantia alba). The cerebellar cortex consists of three layers: the molecular layer (stratum moleculare), Purkinje cell layer (stratum gangliosum) and granular cell layer (stratum granulosum) (Erençin 1963; Bolton 1971; Hodges 1974). The molecular layer has few nerve cells. This layer contains two types interneuron: The stellate cells are located on the surface while the basket cells are located deep in the molecular layer. Also unmyelinated nerve fibers and glia cells are found in this layer. The Purkinje cell layer is between the molecular and granular layers (Hodges 1974). This layer consists of single row of Purkinje cell bodies. A Purkinje cell has large and pear-shaped body. Dendritic tree grows as a single primer dendrite from one face of Purkinje cell, which is on the top

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and next to molecular layer. The cytoplasm of Purkinje cells contains Nissl bodies that are massive and abundant in the cell body. The chromatin in the nucleus of Purkinje cells appears euchromatic so it is seen lightly and its nucleolus is large and evident. Purkinje cells have a branched dendritic tree which extends to the surface of the cerebellar cortex and a myelinated axon which extends to the granular layer (Erkoçak 1984; Voogd and Glickstein 1998; Sultan and Glickstein 2007). The granular layer is composed of numerous small nerve cells (granular cells), Golgi (II) cells and many nerve fibers (Hodges 1974; Voogd and Glickstein 1998; Girgin 2008). The number of the Golgi (II) cells is fewer than granular cells. The granular layer in cerebellum has different thickness from one area to another (Erençin 1963).

The degree of folding of the cerebellum is one major difference among vertebrates (Iwaniuk et al. 2006). In birds, surface of the cerebellum is folded in all species. The degree of foliation is different in owls, chicken-like birds (Galliformes) and pigeons but the number of folia is the same in all species of birds, despite the variation in body size and brain size (Senglaub 1963; Larsell 1967; Iwaniuk et al. 2006). The size of folia is an indicator of phylogenetic history and behaviors in birds (Iwaniuk et al. 2007). It is thought that different size and number of folia in various species of mammals explain why they have dissimilar cognition and behavior (Welker 1990). Because there is no concurrence on functional organization of folia in the bird cerebellum, it is thought that suggested folia-behavior correlation is highly questionable (Iwaniuk et al. 2007).

The aim of this study is to examine the goose cerebellum (*Anser anser*) histometric and histologically.

MATERIALS and METHODS

Six female geese (*Anser anser*, aged between 10-12 months) were used in this study. Tissue samples were obtained immediately from the geese which were sacrificed to consume. After fixation in 10% formalin solution and routine histological process, the cerebellar tissues taken from geese were embedded in paraffin blocks. The sections (5 µm in thickness) were taken from these blocks for histological examination. The Kluver-Barrera stain technique was applied in the sections to examine the structure of the cells in cerebellum. Afterwards, slides were examined histologically by light microscopy (Olympus BX-51). Then, ocular micrometer was used for histometric measurements of folia length, diameter of the Purkinje cell bodies and the thickness of molecular layer and granular layer. For measuring the length of folia, 10 folia per slides; for diameter of the Purkinje cell bodies, 20 cells per slides; and for the thickness of molecular and granular layers, 20 areas per slides were randomly selected. The thicknesses of the molecular and granular layers are measured in the middle of the folia, because the thicknesses of the molecular and granular layers are very different in the fissures and in the summits of the folia. SPSS 16.0 was used for calculating mean values.

RESULTS

It was observed that folia and sulci cerebelli were in the outermost layer of the cerebellum. It was seen that geese cerebellum had two main layers which are known as substantia grisea (the grey matter) and substantia alba (the white matter) by histological examination. It was histologically observed that the grey matter or cerebellar cortex was the outer layer and the white matter or medulla was the inner layer in the cerebellum. Externally, the cerebellar cortex was covered by pia mater (Figure 1). It was seen that the cerebellar cortex had sublayers from outer to inner; the molecular layer which was made by the small cells, the Purkinje cell layer by the Purkinje cells and the granular layer by the granular cells (Figure 2).

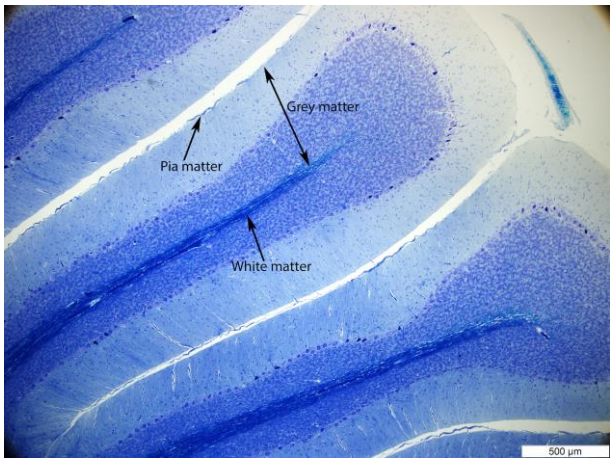

Figure 1. General appearance of the cerebellum

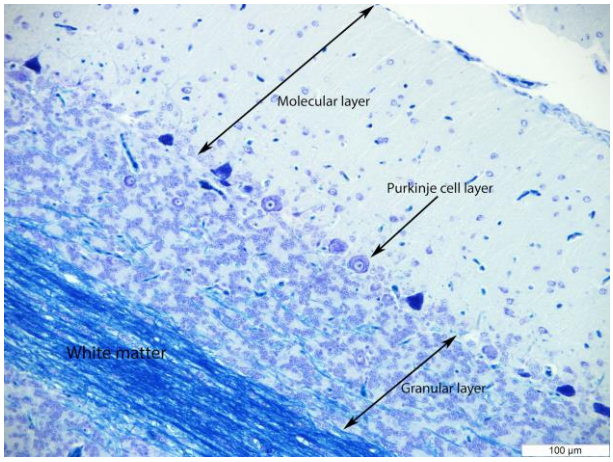

Figure 2. The Cerebellar cortex

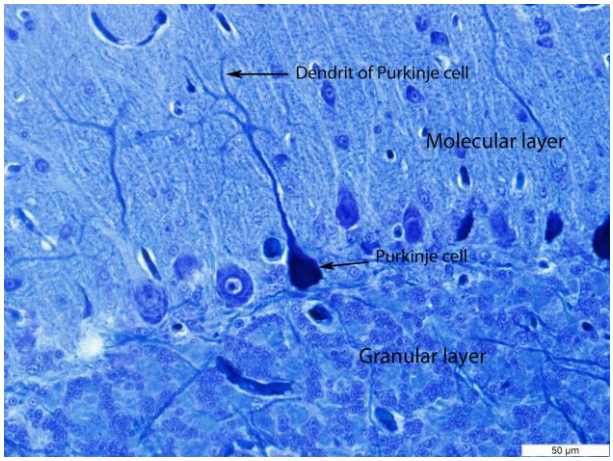

Figure 3. Purkinje cell

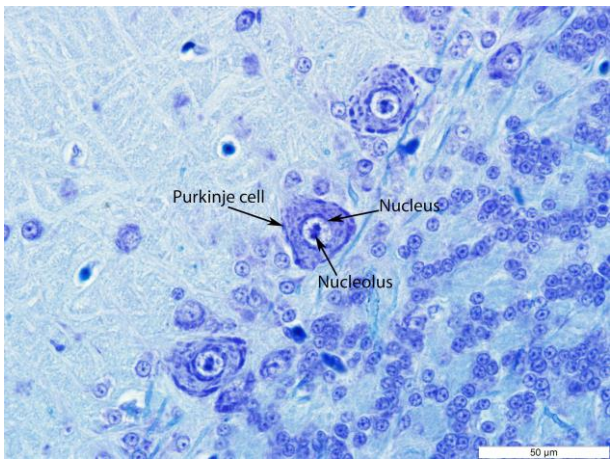

Figure 4. Purkinje cell

It was observed that there were a small number of small nerve cells in the molecular layer (Figure 2). The thickness of the molecular layer was more on the folial summit than in the fissures. It was seen that Purkinje cells evidently have clear stained nucleus with dark stained nucleolus and the dendrites of these cells reached into the molecular layer (Figure 3, 4). It was observed that the granular layer was composed of numerous granular cells and nerve fibers (Figure 2, 4). This layer was thinner in fissures and thicker in the folial summits. As a result of Kluver-Barrera staining, the nerve cells in all layers were purple and myelin fibers were blue.

It was found that the average length of folia was 3396.88 ± 931 µm, the average thickness of the molecular layer was 348.53 ± 72 µm, the average thickness of granular layer was 184.83 ± 48 µm and the average of diameter of the Purkinje cell bodies was 24.23 ± 3.9 µm by the histometric evaluation (Table 1).

Table 1. The avarage length of folia, diameter of Purkinje cells and thicknesses of molecular and granular layers. SD:Standart Deviation

	N	Min.	Max.	Mean ± SD
Length of the folia (µm)	60	1519.02	5526.09	3396.88 ± 931.32
Diameter of the Purkinje cell bodies (µm)	120	12.90	33.54	24.23 ± 3.96
Thickness of the molecular layer (µm)	120	227.70	569.25	348.53 ± 72.92
Thickness of the granular layer (µm)	120	72.45	414	184.83 ± 48.77

DISCUSSION

Pal et al. (2003) reported that the cerebellum of fowl was found to be greater in size and weight in comparison to that of human. Parker and Haswell (1963) mentioned that the greater size of the cerebellum in birds was associated with the centre of equilibrium, therefore it was important in birds.

There is variation in terms of the shape of the cerebellum among vertebrates. Cerebellar morphology is of particular interest to the degree of foliation of the cerebellum and its functional significance (Iwaniuk et al., 2006). It is indicated that differences between sizes of folia in cerebellum has an important role during the evolution (Iwaniuk et al., 2007). Variations in the degree of cerebellar foliation reflect body size in birds; larger birds have deeper folds in their cerebellum. The degree of cerebellar foliation is correlated with body, brain and cerebellum size. However, additional factors might also influence the degree of foliation of avian cerebellum (Senglaub 1963; Pearson and Pearson 1976; Iwaniuk et al. 2006). Each folia has different length. In general, there is a correlation between their lengths if a folia is longer; other is shorter (Iwaniuk et al. 2007). In this study it was found that the average length of folia was 3396.88 µm in goose.

In this study it was histologically observed that the cerebellar cortex had sublayers from outer to inner; the molecular layer which was made by the small cells, the Purkinje cell layer by Purkinje cells and granular layer which was made by small granular cells. This is in accordance with the existing literature (Hodges 1974; Voogd and Glickstein 1998; Girgin 2008).

The Purkinje cell is certainly one of the most spectacular nerve cells because of its position in the structure of cerebellar cortex. The Purkinje cells are among the largest neurons in the central nervous system and the sizes of these cells are different among vertebrates (Palay and Chan-Palay 1974). It was reported that the diameter of Purkinje cell bodies, 18.9 µm in fowl (Pal et al. 2003), 23.5-26.8 µm in ginea-pig (Rapp et al. 1994), 30-35 µm in man, 29 µm in the cats, 21 µm in the rat (Palay and Chan-Palay 1974). In our study the diameter of the Purkinje cell was found to be 24.23 µm in goose cerebellum.

The thicknesses of the molecular and granular layers are different in fissures and in the summit of the folia. Pal et al. (2003) reported that in fowl (white leg horn) the thickness of the molecular layer was 196 µm in the summit and 294 µm in the fissure. The granular layer was measured at 187 µm in the fissure and 330 µm in summit in the same study. In our study, the thicknesses of the molecular and granular layers are measured in the middle of the folia. In this study thickness of the molecular layer was measured at 348.53 µm and thickness of the granular layer was measured at 184.83 µm.

To conclude, we are investigated histological and histometric structure of goose cerebellum within this study. It was thought that this study will contribute to next similar studies about avians.

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