Etlik Vet Mikrobiyol Derg, 2026; **37 (1):** 45-53
doi: https://doi.org/10.35864/evmd.1957767

Original Article
Özgün Araştırma

Anatomical and histological characterization of brain in the Long-legged buzzard (*Buteo rufinus*)

Burhan Toprak[1*], Aylin Bilen Çoban[2]

[1] *Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Anatomy, Yozgat, Türkiye*
[2] *Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Yozgat, Türkiye*

Geliş Tarihi / Received: 23.05.2026, **Kabul Tarihi** / Accepted: 18.06.2026

Abstract: This study was conducted to determine the macroscopic and light microscopic characteristics of the brain of the long-legged buzzard (*Buteo rufinus*), a diurnal bird of prey. The study utilized five adult long-legged buzzards that were admitted to the Yozgat Directorate of Nature Conservation and National Parks and the Afyon Kocatepe University Wildlife Rescue and Rehabilitation Center after sustaining injuries that could not be treated successfully. The brain tissues of the long-legged buzzards were subjected to macroscopic and light microscopic examinations. Macroscopically, the pear-shaped brain was observed to have a smooth surface characteristic of birds. The olfactory bulbs were identified as reduced structures in the rostroventral region. A prominent optic lobe was observed. It was determined that the cerebellum was large and laterally flattened, with 11-13 cerebellar folia separated by the cerebellar fissure. During light microscopic examination, it was observed that the cerebral cortex is enveloped externally by the pia mater and does not exhibit the typical laminar organization seen in mammals. The cerebellum was found to consist of three characteristic layers: the molecular layer on the outside, the Purkinje cell layer in the middle and the granular layer on the inside. In the medulla oblongata, the pia mater, pyramidal cells and glial cells were distinguished, while the optic lobe was found to consist of medium-sized spherical neurons and pyramidal cells, containing a small number of glial cells. The findings provide comprehensive neuroanatomical data on the brain structure of the long-legged buzzard and contribute to the comparative brain morphology and histology of diurnal birds of prey.

Keywords: Brain; *Buteo rufinus*; Light microscopy; Long-legged buzzard; Macroscopy

Kızıl Şahinde (*Buteo rufinus*) beynin anatomik ve histolojik karakterizasyonu

Özet: Bu çalışma, gündüz yırtıcı kuşlardan kızıl şahin (*Buteo rufinus*) beyninin makroskobik ve ışık mikroskobik özelliklerini belirlemek amacıyla gerçekleştirildi. Araştırmada, Yozgat Doğa Koruma ve Milli Parklar Şube Müdürlüğü ile Afyon Kocatepe Üniversitesi Yaban Hayatı Kurtarma ve Rehabilitasyon Merkezi'ne yaralı olarak getirilen ancak kurtarılamayan beş adet erişkin kızıl şahin kullanıldı. Kızıl şahinin beyin dokuları makroskobik ve ışık mikroskobik incelemelere tabi tutuldu. Makroskobik olarak armut şeklindeki beynin, kuşlara özgü pürüzsüz bir yüzeye sahip olduğu gözlendi. Bulbus olfactorius yapıları, rostroventral bölgede küçülmüş yapıda tanımlandı. Oldukça gelişmiş bir lobus opticus tespit edildi. Cerebellum'un büyük ve yanlardan basık olduğu, üzerinde fissura cerebelli ile birbirinden ayrılan 11 ila 13 adet folia cerebelli bulunduğu belirlendi. Işık mikroskobik incelemelerde, cortex cerebri'nin dıştan piamater ile sarılı olduğu ve memelilerde görülen tipik laminer organizasyona sahip olmadığı gözlendi. Cerebellum'un dışta moleküler katman, ortada Purkinje hücre katmanı ve en içte granüler katman olmak üzere üç tipik tabakadan oluştuğu görüldü. Medulla oblongata'da piamater, piramit benzeri hücreler ve glia hücreleri ayırt edilirken, lobus opticus'un az sayıda glia hücresi içeren, orta büyüklükte küresel nöronlar ve piramidal hücrelerden oluştuğu gözlendi. Elde edilen bulgular, kızıl şahin beyin yapısına ait kapsamlı nöroanatomik veriler sunmakta ve gündüz yırtıcı kuşların karşılaştırmalı beyin morfolojisi ile histolojisine katkı sağlamaktadır.

Anahtar kelimeler: Beyin; *Buteo rufinus*; Işık mikroskop; Kızıl şahin; Makroskobi

Introduction

The genus Buteo, belonging to the Accipitridae family which represents the group of diurnal birds of prey, includes twenty-nine species (Hołówka et al., 2025). Long-legged buzzards are found in mildly arid, steppe, rocky and open areas. They feed on small to medium-sized mammals, birds, reptiles and occasionally carrion and large insects (Karataş et al., 2024).

The bird brain consists of three main parts: the cerebrum, the cerebellum and the medulla oblongata. The bird brain is composed of two hemispheres and lacks the cerebral sulci and gyri cerebri on its surface (Batah et al., 2012). One of the most

Yazışma adresi / Correspondence: *Burhan Toprak, Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Anatomy, Yozgat, Türkiye e-mail: burhan.toprak@bozok.edu.tr*

ORCID IDs of the authors: *[1]0000-0003-1082-4559 • [2]0000-0002-6220-8240*

characteristic features of the bird brain is the pair of optic lobes, which are symmetrical parts of the midbrain roof and are located laterally and slightly ventrally (Cobb, 1960). In birds, the olfactory bulbs is usually very small and underdeveloped (Vishen et al., 2023). A study examining brain volumes in birds reported that the proportional structures of brain regions are directly related to both bird genetics and their environment and feeding habits (Iwaniuk and Hurd, 2005). Another study reported that the olfactory bulbs in birds exhibit morphological differences among themselves; these differences are related to habitat, migration and feeding strategies (Corfield et al., 2015).

The neocortex of mammals has a highly laminated structure consisting of six layers, from superficial layers at the surface to deeper layers beneath. Each layer has its own unique cell types, connection patterns and neurochemical composition. In contrast, the telencephalon of birds has a nuclear (non-laminar) with little or no laminar organization (Emery and Clayton, 2005). Microscopic examinations have indicated that the cerebellar cortex has three typical layers, with the cerebellar medulla being the innermost. The study states that the outermost layer, the molecular cell layer, consists of small neurons and neuroglia and the Purkinje cell layer, located between the outer molecular and inner granular layers, is predominantly composed of a single row of bottle/pear-shaped cells (Azeez et al., 2023).

Macroscopic and light microscopic studies were conducted on the brain tissue of domestic and wild bird species, including the common barn owl (*Tyto alba*) (Abd-Alrahman, 2012), white-cheeked bulbul (*Pycnonotus leucotis*) (Abid et al., 2022), pigeon (*Columba livia domestica*) (Al-Nakeeb and Jasim, 2018), cattle egret (*Bubulcus ibis*) (Azeez et al., 2023), sparrowhawk (*Accipiter nisus*) (Balkaya and Toprak, 2018) and chicken (*Gallus gallus domesticus*) (Batah et al., 2012).

Although studies on the brain anatomy of different bird species exist in the literature, information on the macroscopic and light microscopic structure of the brain of long-legged buzzard is quite limited. The long-legged buzzard, belonging to the order of diurnal birds of prey (*Accipitriformes*), has a wide geographical distribution and holds an important place in the ecosystem with its morphological characteristics and hunting abilities. The aim of this study was to examine the macroscopic and light microscopic structure of the long-legged buzzard brain in detail, to reveal the neuroanatomical features of this species and compare the obtained data with those of other bird species

Materials and Methods

Collection of material

In this study, five adult long-legged buzzards that could not be rehabilitated due to severe injuries or mortality and were brought to the Yozgat Directorate of Nature Conservation and National Parks (3 birds) and the Afyon Kocatepe University Wildlife Rescue and Rehabilitation Center (2 birds) were used. The necessary permission to carry out the study was granted by the General Directorate of Nature Conservation and National Parks of the Ministry of Agriculture and Forestry, with reference number E-72784983-288.04-22460315. Brains obtained from dead animals following standard anatomical procedures were fixed in a 10% neutral formaldehyde solution to facilitate dissection and morphological examination. Subsequently, the brain samples were examined macroscopically and using light microscopy.

Macroscopic Analysis

The brain tissue of the long-legged buzzard was examined macroscopically in dorsal, ventral, lateral and sagittal views and then photographed with a Canon IXUS 75 digital camera. In this study, where morphometric measurements were taken from the cerebrum, cerebellum, medulla oblongata and optic lobe using a digital caliper, the Nomina Anatomica Avium (Baumel et al., 1993) was used as the basis for anatomical terminology.

Light Microscopic Analysis

Brain tissue samples were fixed in 10% neutral formaldehyde solution and then trimmed sagittally. Samples were embedded in paraffin after passing through routine histological series on an automated tissue processing device (Leica ASP 300S) for light microscopic analysis. 5 µm thick sections were taken from the paraffin blocks using a microtome (Shandon/Finese), placed on slides and incubated at 60°C for 40 minutes. Subsequently, the Hematoxylin-Eosin protocol was applied using a fully automated staining device (Shandon/varistain gemini). Microscopic examination and imaging of the resulting preparations were performed using a digital light microscope (Leica DM2500 LED). After the necessary examinations were completed, microscopic images of the sections were taken.

Results

Macroscopic Morphology of the Brain

When the macroscopic structure of the brain was examined in the long-legged buzzard, the total weight, length and width of the brain were measured as 7.86 g, 29 mm and 31 mm, respectively. The brain of the long-legged buzzard had a flattened structure; the cerebral hemispheres had a smooth surface and lacked prominent gyri and sulci. The cerebrum was found to be approximately 18.5 mm long on average. When viewed dorsally, the brain resembled a pear. A distinct interhemispheric fissure was present, separating the two cerebral hemispheres. A vallecula, appearing as a shallow groove oriented caudolaterally, was observed in both cerebral hemispheres. A bilateral projection known as the sagittal eminence was observed between the interhemispheric fissure and the vallecula. A transverse groove, the subhemispheric fissure, was identified between the most caudal part of the cerebral hemispheres and the rostral part of the cerebellum, which it borders. The optic lobes were observed caudally to the cerebral hemispheres, on either side of the rostral aspect of the cerebellum. The average length of the optic lobe was measured at 13 mm and its width at 7.5 mm. The cerebellum was observed to be large and laterally flattened. Its length was measured at 16.5 mm and its width at 14 mm. It contained cerebellar folia and fissures. The cerebellar auricula was located caudal to the optic lobe, on either side of the cerebellum. The spinal cord was located caudal to the cerebellum (Fig. 1).

When the brain was examined in the ventral view, the olfactory bulb was significantly reduced and located rostral to the cerebral hemispheres. Immediately below the olfactory bulb, the ventral longitudinal fissure, separating the cerebral hemispheres, was observed. The optic chiasm was located in the rostroventral area, while the optic tracts were present laterally to this structure and on either side. The optic lobes were observed as paired oval projections protruding laterally and ventrally from the midbrain surface. The cerebellar auricula structures were observed caudally to the optic lobes, on either side of the pons. The pons and medulla oblongata were located on the ventral surface of the cerebellum, while the spinal cord was caudal to it. In the ventral view of the brain, the cerebellar auricula were observed on either side of the pons and medulla oblongata. The medulla oblongata was square-shaped, with average length and width of 10 mm and 10.5 mm, respectively. The median ventral fissure was found to extend along the pons, medulla oblongata and spinal cord (Fig. 2).

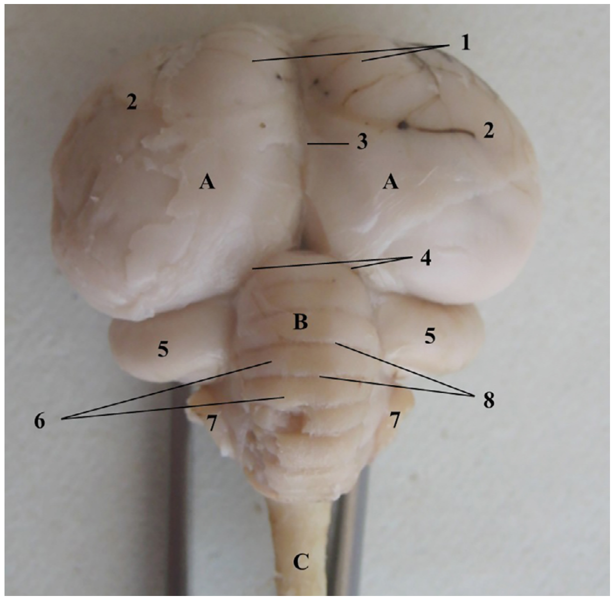

Figure 1. Dorsal view of the brain of long-legged buzzard. A: Hemispherium cerebri, B: Cerebellum, C: Medulla spinalis, 1: Eminentia sagittalis, 2: Vallecula, 3: Fissura interhemispherica, 4: Fissura subhemispherica, 5: Lobus opticus, 6: Folia cerebelli, 7: Auricula cerebelli, 8: Fissura cerebelli.

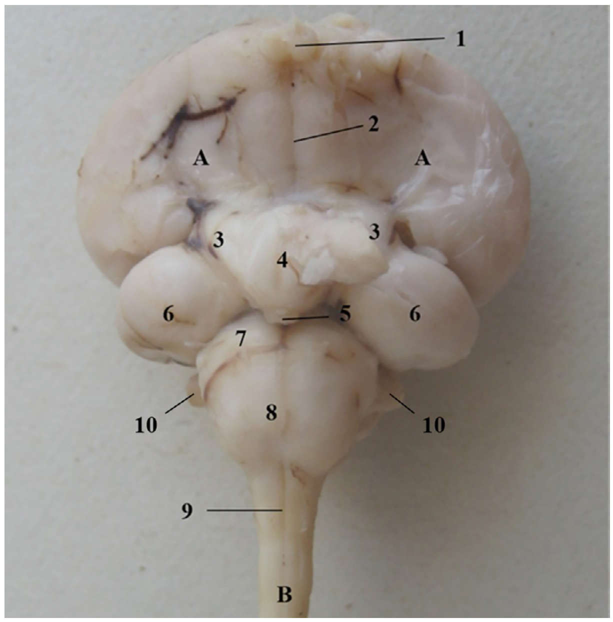

Figure 2. Ventral view of the brain of long-legged buzzard. A: Hemispherium cerebri, B: Medulla spinalis, 1: Bulbus olfactorius, 2: Fissura longitudinalis ventralis, 3: Tractus opticus, 4: Chiasma opticum, 5: Glandula pituitaria, 6: Lobus opticus, 7: Pons, 8: Medulla oblongata, 9: Fissura mediana ventralis, 10: Auricula cerebelli.

When the brain was examined laterally, the frontal cerebrum, occipital cerebrum and parietal cerebrum regions were distinguished on the surface of the cerebral hemispheres. A small olfactory bulb was observed in the rostroventral region of the brain. The vallecula was prominently located laterally in the cerebral hemispheres, while the prominent optic lobe was noticeable laterally in the caudal region. The cerebellum, located superior to the optic lobe, contains the cerebellar folia separated by cerebellar fissures, while the optic chiasm is visible inferiorly. The number of cerebellar folia on the cerebellum was observed to be 11-13. The cerebellar auricula, mesencephalon, medulla oblongata and pons were located ventrally on the cerebellum, while the spinal cord was visible caudally (Fig. 3).

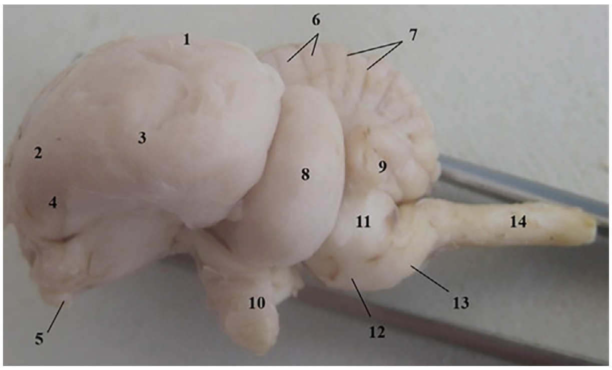

Figure 3. Lateral view of the brain of long-legged buzzard. 1: Pars occipitalis cerebri, 2: Pars frontalis cerebri, 3: Pars parietalis cerebri, 4: Vallecula, 5: Bulbus olfactorius, 6: Folia cerebelli, 7: Fissura cerebelli, 8: Lobus opticus, 9: Auricula cerebelli, 10: Chiasma opticum, 11: Mesencephalon, 12: Pons, 13: Medulla oblongata, 14: Medulla spinalis.

Examination of the sagittal section of the brain revealed that the cerebral hemispheres occupied a large area. The small olfactory bulb was located rostroventral to the cerebrum, while the cerebellum, separated from the cerebrum by the subhemispheric fissure, was observed caudally. The sagittal section of the cerebellum clearly showed the arbor vitae and the gray and white matter structures were visible. The diencephalon was located in the center of the section, with the mesencephalon caudal to it. Caudal to the brainstem, the pons and medulla oblongata were identified. The dorsal surface of the pons, along with the medulla oblongata, formed the ventral wall for the fourth ventricle and their ventral surfaces formed the lower boundary of the brainstem. The section was found to terminate caudally at the spinal cord (Fig. 4).

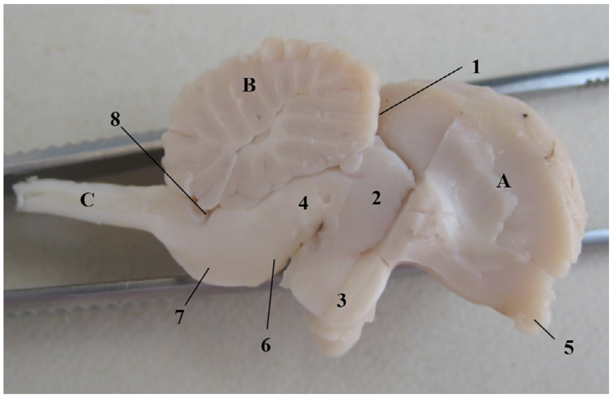

Figure 4. Sagittal section through the brain of long-legged buzzard. A: Hemispherium cerebri, B: Cerebellum, C: Medulla spinalis, 1: Fissura subhemispherica, 2: Diencephalon, 3: Chiasma opticum, 4: Mesencephalon, 5: Bulbus olfactorius, 6: Pons, 7: Medulla oblongata, 8: Ventriculus quartus.

Light Microscopic Structure

In the light microscopic structure of the long-legged buzzard, the cerebral cortex was observed to be surrounded externally by the pia mater. Here, a high concentration of large, pyramidal neurons with extensive cytoplasm and prominent nucleoli was noted. Among these pyramidal neurons, irregularly arranged glial cells with small, dark nuclei were distinguished (Fig. 5). The cerebellum was observed to be surrounded externally by the pia mater. Three layers were distinguished in the cerebellum: the molecular layer, the Purkinje cell layer and the granular layer. Irregularly arranged glial cells were present in the molecular layer. Purkinje cells were observed arranged in a pear shape and in a single row between the two layers. Numerous glial cells were seen in the granular layer (Fig. 6). The medulla oblongata was covered externally by the pia mater. In the histological section of the medulla oblongata, glial cells were found in the lower layers of the pia mater, while pyramid-shaped cells and glial cells were present in between towards the interior (Fig. 7A).The optic lobe was observed to consist of medium-sized spherical neurons, generally containing a small number of glial cells. It was noted that the neurons had a pyramid-like morphology (Fig. 7B).

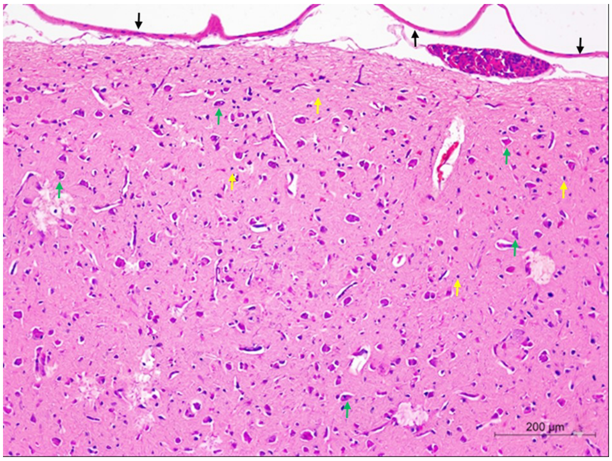

Figure 5. Cross section of the cerebral cortex. Black arrow: Pia mater, Green arrow: Neurons, Yellow arrow: Glial cell. Scale bar: 200 µm, H&E.

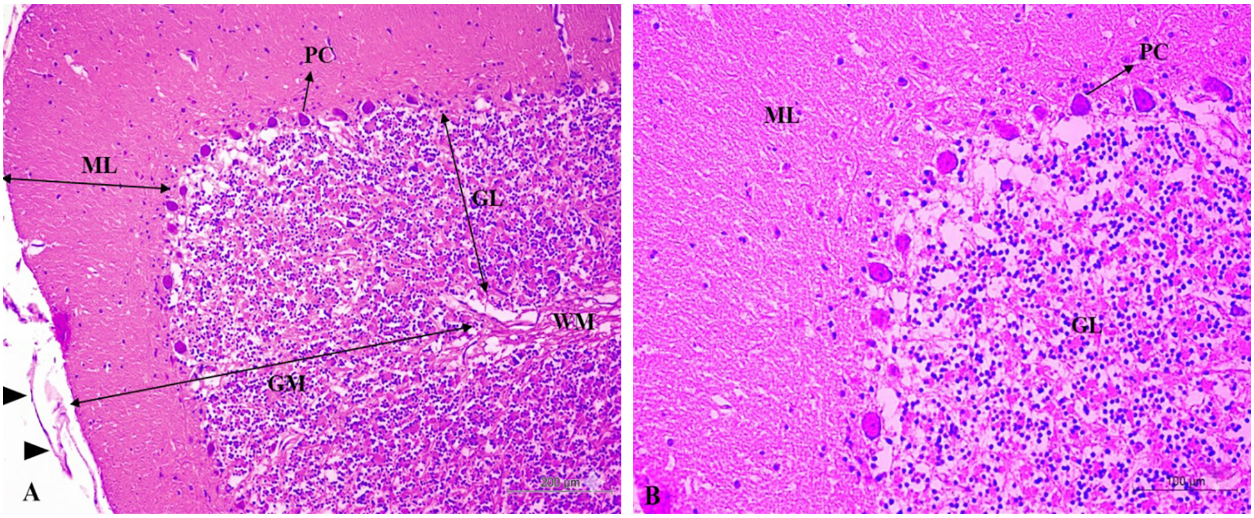

Figure 6. Cross section of the cerebellum. ML: Molecular layer, PC: Purkinje cell layer, GL: Granular layer, GM: Gray matter, WM: White matter, arrowhead: Pia mater. Scale bar: A: 200 µm, B: 100 µm, H&E.

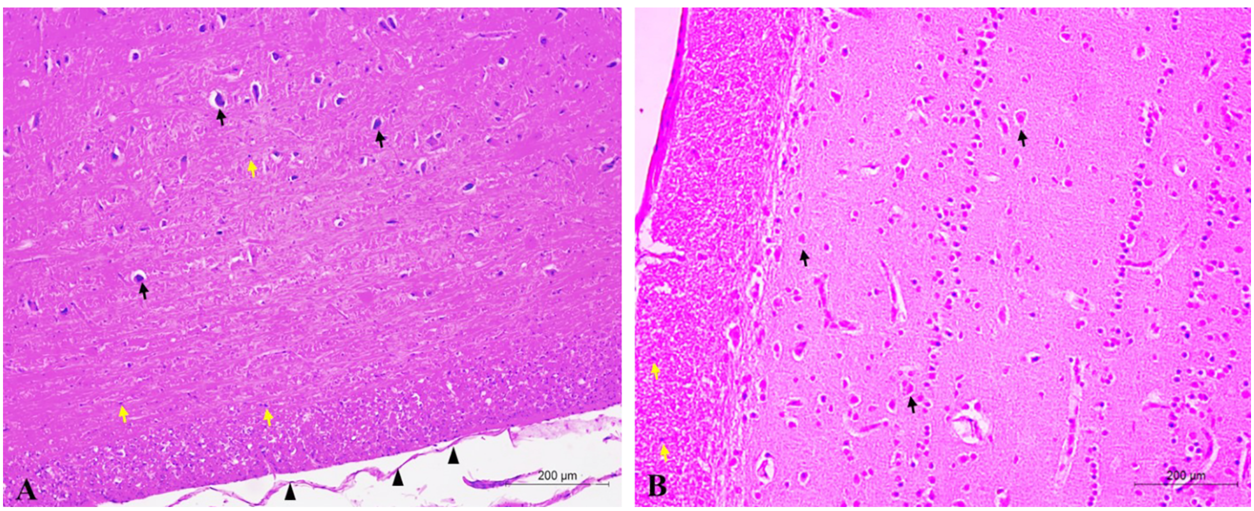

Figure 7. Cross section of the medulla oblongata and lobus opticus. Arrowhead: Pia mater, Black arrow: Neurons, Yellow arrow: Glial cell. Scale bar: A,B: 200 µm, H&E.

Discussion

Various data exist regarding macroscopic brain measurements of domestic and wild birds. Previous studies have reported that the average brain weight of the African ostrich (*Struthio camelus*) (Peng et al., 2010) is 26.34 g, its length is 59.26 mm and its width is 42.30 mm; the brain weight of the diurnal sparrowhawk (*Accipiter nisus*) (Balkaya and Toprak, 2018) is 3±0.2 g; the brain weight of the common kestrel (*Falco tinnunculus*) (Toprak et al., 2025) is 3.23 g, its total length is 22.5 mm and its total width is 23.5 mm; the brain of the laughing pigeon (*Spilopelia senegalensis*) (Muhammed and Abdul, 2023) has an average weight of 1.3 g, a length of 17.3 mm and a width of 15.3 mm and the brain length of the white-cheeked bulbul (*Pycnonotus leucotis*) (Abid et al., 2022) is 2.5 cm. The average weight of the quail (*Coturnix coturnix*) (Abid and Al-Bakri, 2016) brain is reported to be 0.6 g, its length 2.5 cm and its width 2 cm. In this study, the total weight of the brain in the long-legged buzzard was determined to be 7.86 g, its length 29 mm and its width 31 mm.

In terms of shape, the brain is pear-shaped, oval, or even spherical in the Vanaraja chicken (Panigrahy et al., 2017), in the laughing pigeon (Muhammed and Abdul, 2023) the entire brain is broad anteriorly, narrowed posteriorly and pear-shaped; in the African ostrich (Peng et al., 2010) the brain and in the barn owl (Abd-Alrahman, 2012) the cerebrum are shaped like a broad triangle and in the neotropical parrot (Carril et al., 2016) it appears walnut-shaped when viewed dorsally and has a smooth surface due to the absence of gyri and sulci. Similar to previous literature, this study also found that the long-legged buzzard's brain had a flattened structure; the cerebral hemispheres exhibited a smooth surface and lacked prominent gyri and sulci. Viewed dorsally, the brain resembled a pear shape, similar to that of the Vanaraja chicken (Panigrahy et al., 2017) and the laughing pigeon (Muhammed and Abdul, 2023).

Initial studies correlated birds' sense of smell with the size of the olfactory bulb (Bang and Cobb, 1968). However, later research revealed that small olfactory bulb structures do not necessarily indicate a poor sense of smell and that olfactory ability is linked to ecological function rather than size (Corfield et al., 2015;Abankwah et al., 2020). Morphologically, the olfactory bulb is located anterior to the cerebral hemispheres in quail (Abid and Al-Bakri, 2016). In the barn owl (Abd-Alrahman, 2012), white-crested polish chicken (Frahm and Rehkämper, 1998) and neotropical parrot (Carril et al., 2016) the roundness of the cerebral hemispheres means that the olfactory bulb is visible only on the ventral surface rather than the dorsal surface. Similar to the literature, the olfactory bulb, which can be seen ventrally in the long-legged buzzard, was considerably reduced and located rostral to the cerebral cortex.

Vincze et al. (2015) showed that relative brain mass decreases as migration distance increases in birds, but the relative size of the optic lobe increases due to the critical role of visual cues in migration. Interspecies comparisons revealed that emus and pigeons have significantly larger optic lobes, while kiwi, owl, hummingbird and parrot species have relatively small and oval structures (Martin et al., 2007; Wanmi et al., 2018). In Vencobb broiler chickens, the optic lobes, located in the ventrolateral portion of the cerebral hemispheres, have been reported as distinct spherical structures (Gupta et al., 2016). In Kadaknath (*G. gallus domesticus*) birds, the optic lobe is located in the ventral and lateral parts of the brainstem, with an average length of 7.10±0.16 mm and a width of 4.88±0.12 mm (Sharma et al., 2022). In the present study, the length of the optic lobes, located laterally on the midbrain surface, was measured as 13 mm and their width as 7.5 mm.

The cerebellum is reported to be located caudally in the brain, dorsal to the diencephalon and medulla oblongata, between the cerebral hemispheres and the optic lobes (Abdullah, 2010; Elnegiry et al., 2022). In the golden-headed parrot, the cerebellum has been reported to be a large, protruding structure, connected rostrally to the midbrain and medulla oblongata. Ten folia cerebelli structures were observed in a sagittal section of the cerebellum and it was noted that they surround a small, centrally located cavity connected to the fourth ventricle of the brain (Abd-Alrahman and Mahmoud, 2008). In this study, it was determined that the cerebellum is macroscopically large and laterally flattened and has folia cerebelli separated by the cerebellar fissure. The number of folia cerebelli on the cerebellum was found to be 11-13.

In birds, the cerebral cortex cannot be histologically distinguished as having five to six layers as in mammals (Aslan et al., 2018). A study on African ostriches revealed that the brain cortex consists of a small number of neurons whose boundaries are not precisely defined, with the most common appearance being a small number of small neurons supported by neuroglia (Karkoura et al., 2015). Similarly, this study's findings did not differentiate between layers in the cerebral cortex, but identified numerous pyramidal neurons.

It is reported that the avian cerebellum is surrounded externally by the pia mater and histologically it is divided into a cortex consisting of gray matter externally and a medulla consisting of white matter internally (Abd-Alrahman and Mahmoud, 2008; Taşçı and Bingöl, 2018; Elnegiry et al., 2022). The cerebellar cortex has been reported to consist of molecular, Purkinje and granular layers from the outside inward (Abdullah, 2010). It has been reported that in the white-eared Arabian bulbul (*Pycnonotus leucotis*) (Abid, 2022) and the barn owl (*Tyto alba*) (Abdulla, 2023) small pyramidal cells are located in the light-colored molecular layer, Purkinje cells are arranged in a single row in the narrow Purkinje layer and a thick granular layer is located in the innermost part. In the present study, light microscopic examination of cerebellar sections of the long-legged buzzard revealed a clearly discernible three-layered structure, consistent with the literature, allowing for the identification of cell types and the determination that the tissue is surrounded by pia mater. Consistent with the current study, the avian optic lobe has been reported to consist of medium-sized spherical neurons containing a small number of neuroglia in its light microscopic structure (Karkoura et al., 2015). Another study reported that during embryological development, the optic nerve differentiates into complex layers (stratum opticum, stratum griseum periventriculare, stratum album centrale) containing pyramidal neurons, spindle neurons, neuroglia and granule cells (Nathaniel et al., 2016). Interspecies comparisons using silver staining have reported that hawks have a much higher density of brown-black neurofibrils and dark-colored oligodendrocytes in their optic lobes compared to owls (Hussain and Al-taee, 2022). It has been noted that the medulla oblongata is covered externally by the pia mater, a loose connective tissue composed of simple squamous epithelium and that multipolar neurons are located within the tissue, supported by a few neuroglia (Karkoura et al., 2015). In this study, similar to the literature, the histology of the medulla oblongata revealed pyramidal cells covered externally by a pia mater, with glial cells interspersed between them.

Conclusion

In conclusion, this study comprehensively revealed the macroscopic and histological organization of the brain of the long-legged buzzard (*Buteo rufinus*), a diurnal raptor. The smooth cerebral structure and rudimentary olfactory bulbs observed in macroscopic examinations reflect the general characteristics of avian anatomy. The prominent optic lobes and cerebellum with 11-13 folia cerebelli observed in the long-legged buzzard suggest a correlation with the visual capacity and high motor coordination ability required for the species' hunting strategies. At the histological level, the presence of pyramidal neurons in the cerebellar cortex, the characteristic three-layered structure of the cerebellum and the specific Purkinje cells arrangement were confirmed through detailed cellular analysis. This study provides a detailed description of the macroscopic and histological organization of the long-legged buzzard brain. The findings contribute to expanding the current knowledge of the brain structure of diurnal raptors and provide a valuable reference for future comparative anatomical and histological studies involving other wild avian species.

Financial support: No financial support was received for this investigation.

Declaration of competing interest: The authors would like to declare that there are no competing interests.

Ethics Committee Approval: Article 8, paragraph k/2 of the Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees states that studies conducted on dead animals and their tissues are not subject to ethics committee approval. Therefore, although ethics committee approval was not obtained, written permission for the study was received from the General Directorate of Nature Conservation and National Parks because it is a study on wildlife.

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