

Macroscopic and microscopic study of the brain structure of the common kestrel (*Falco tinnunculus*)

Estudio macroscópico y microscópico de la estructura cerebral del cernícalo común (*Falco tinnunculus*)

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ABSTRACT

This study was conducted to examine the macroscopic and light microscopic structures of the brain in kestrels (*Falco tinnunculus*). In the study, brains from three adult kestrels that were brought in for treatment but could not be rescued, one from Keçiören Municipality Veterinary Affairs Directorate and two from Afyon Kocatepe University Wildlife Rescue and Rehabilitation Center were used. The brains were properly removed and subjected to macroscopic and light microscopic examination procedures. The average brain weight was measured as 3.23 g, total length as 22.5 mm, and total width as 23.5 mm. The cerebrum, cerebellum, lobus opticus, and medulla oblongata were the prominent regions of the brain. A well-developed lobus opticus was observed, and small and rudimentary bulbus olfactorius structures were present on both sides of the midline at the rostroventral part of the cerebral hemispheres. In the cerebellum, transverse protrusions called folia cerebelli, varying in number between 9 and 10, and the fissura cerebelli separating them were seen. On the sides of the cerebellum and behind the lobus opticus, auricula cerebelli were identified. At the caudal part of the brain, the pons and medulla oblongata were present and separated by a slightly prominent transverse groove. In the light microscopic examination, small neurons located beneath the pia mater in the cerebral cortex and irregularly distributed glial cells among them were observed. In the deeper cortical regions, large neurons with vesicular nuclei were identified. In the cerebellum, a typical three-layered structure consisting of molecular, Purkinje cell, and granular layers was present. In addition, numerous multipolar neurons and supporting glial cells were found in the medulla oblongata, and ependymal cells were identified in the fourth ventricle. In the optic lobe, medium-sized spherical neurons containing few glial cells attracted attention. With this study, the macroscopic and light microscopic structures of the brain in kestrels were revealed. The data obtained were compared with the morphological structures of the brains of other avian species, and similarities and differences were discussed. The findings are expected to contribute to the literature on the morphological characteristics of raptors and serve as a foundation for comparative anatomical and histological studies on wild bird species.

Key words: Brain; common kestrel; *Falco tinnunculus*; light microscopy; macroscopy

RESUMEN

Este estudio se realizó con el objetivo de investigar las estructuras macroscópicas y microscópicas del encéfalo en cernícalos vulgares (*Falco tinnunculus*). Se utilizaron los encéfalos de tres ejemplares adultos de cernícalo que no pudieron ser recuperados tras recibir tratamiento, uno procedente de la Dirección de Servicios Veterinarios del Municipio de Keçiören y dos del Centro de Rescate y Rehabilitación de Vida Silvestre de la Universidad Afyon Kocatepe. Los encéfalos fueron extraídos conforme a los protocolos anatómicos y sometidos a procedimientos de examen macroscópico y microscópico óptico. El peso cerebral medio fue de 3,23 g, con una longitud total de 22,5 mm y un ancho total de 23,5 mm. Las regiones destacadas del encéfalo incluyeron el cerebro y cerebelo, los lóbulos ópticos y la médula oblongada. Se observó un lóbulo óptico bien desarrollado, y en la parte rostroventral de los hemisferios cerebrales, a ambos lados de la línea media, se encontraron bulbos olfatorios pequeños y rudimentarios. En el cerebelo se identificaron prominencias transversales denominadas folia cerebelli (en número de 9 a 10), separadas entre sí por fisuras cerebelosas. A los lados del cerebelo y detrás de los lóbulos ópticos se localizaron las aurículas cerebelli. En la parte caudal del encéfalo se observaron el puente (protuberancia) y la médula oblongada, separadas por un surco transversal ligeramente pronunciado. En el examen microscópico óptico, se identificaron pequeñas neuronas localizadas bajo la piamadre en la corteza cerebral, con células gliales distribuidas irregularmente entre ellas; en regiones corticales más profundas, se observaron neuronas grandes con núcleos vesiculosos. El cerebelo presentaba la estructura típica de tres capas: molecular, de células de Purkinje y granular. Además, en la médula oblongada se identificaron numerosas neuronas multipolares y células gliales de soporte, así como células ependimarias en el cuarto ventrículo. En el lóbulo óptico, destacaron neuronas esféricas de tamaño mediano con escasa presencia de glía. Con este estudio se documentaron las características macroscópicas y microscópicas del encéfalo en el cernícalo vulgar, y se compararon los datos obtenidos con la morfología cerebral de otras especies de aves, discutiendo semejanzas y diferencias. Se espera que los resultados contribuyan a la bibliografía sobre las características morfológicas de las aves rapaces y sirvan de base para estudios comparativos anatómicos e histológicos sobre especies de aves silvestres.

Palabras clave: Encéfalo; cernícalo vulgar; *Falco tinnunculus*; microscopía óptica; macroscopía

INTRODUCTION

The common kestrel (*Falco tinnunculus*) is a bird of prey belonging to the Falconidae family within the order Falconiformes. The primary food sources of common kestrels are small mammals, particularly field voles and mice. Sometimes they feed on amphibians, reptiles and other birds. They can also be seen hunting small mammals and insects, especially beetles and grasshoppers, on foot [1].

The avian brain is divided into three parts, like in mammals: the prosencephalon (telencephalon, diencephalon), mesencephalon and rhombencephalon (metencephalon, myelencephalon) [2]. In the avian brain, the rostral telencephalon consists of the cerebrum, thalamus and hypothalamus; the caudal diencephalon consists of the optic vessels. The mesencephalon is where the lobus opticus is located. The rhombencephalon consists of two parts, the rostral part of the metencephalon, which forms the cerebellum, and the caudal part of the myelencephalon, which forms the medulla oblongata [3, 4].

The brain is relatively large in birds. The cerebral hemispheres, optic lobes and cerebellum are well developed, while olfactory regions such as the olfactory bulb and hippocampus are relatively small [5]. One of the most characteristic structures of the bird brain is the optic lobe pair. The optic lobes, called occipital lobes in mammals, form the roof of the mesencephalon and are large, symmetrically located structures [6].

When the macroscopic organization of the avian brain was examined, it was reported that the hemispherium cerebri were separated from each other by a prominent fissura interhemispherica and small bulbus olfactorius structures were found ventrally. At the microscopic level, it has been stated that the cerebral cortex consists of six layers in the classical sense [7, 8]. In various avian species, the surface of the cerebral hemispheres is generally smooth and without prominent gyrus or sulci formations as in mammals [3, 9, 10, 11].

The cerebellum is a central organ for balance and movement functions. Because of these functions, they are larger and more organized than other parts in birds. The arbor vitae appearance observed in mammals is similarly present in birds. As in mammals, the cerebellum of birds also consists of two distinguishable layers: gray matter and white matter [12]. The gray matter consists of three layers: the molecular layer, the Purkinje cell layer and the granular layer [12, 13].

This study to reveal the macroscopic and light microscopic structural features of the brain of the common kestrel, a diurnal bird of prey.

MATERIALS AND METHODS

Sample collection

In this study, the materials consisted of brains from three adult common kestrels. Three adult common kestrels that had been brought for treatment to the Keçiören Municipality Veterinary Affairs Directorate (1 common kestrel) and the Wildlife Rescue and Rehabilitation Center of Afyon Kocatepe University (2 common kestrels), but could not be saved, were used in the study. Permission to conduct the study on the

common kestrels used in the research was obtained from the General Directorate of Nature Conservation and National Parks of the Ministry of Agriculture and Forestry under the official document number E-21264211-288.04-10698031.

The brains were duly removed from the dead animals. The brains were fixed in 10 % formaldehyde solution for easier dissection and morphologic examination. The samples were then subjected to macroscopic and light microscopic examination procedures.

Macroscopic examination

The brain tissue of the common kestrel was examined anatomically in dorsal, ventral, lateral views and sagittal sections. Measurements were taken from the brain, cerebellum and optic lobes with a digital caliper (Carbon Fiber Composites, Made in China). After macroscopic examination, the brains were photographed using a digital camera (Canon Inc., IXUS 75, Made in Japan). Nomina Anatomica Avium [14] was used for the anatomical terminology in this study.

Light microscopic examination

The brain samples were fixed in a 10 % formaldehyde solution. The entire brain tissue was trimmed to obtain sagittal sections. For light microscopic examinations (Leica DM 2500 LED, Leica 170 HD, Manufactured in Singapore), the samples were processed in an automatic tissue processor (Leica ASP 300S), including washing with alcohol and xylene series, dehydration, paraffin infiltration, and embedding. Sections with a thickness of 5 µm were obtained from the prepared paraffin blocks using a microtome (Shandon, Finesse, Made in UK) and placed onto slides. The prepared slides were incubated in an oven at 60 °C for 40 minutes. Subsequently, the slides were stained using the Hematoxylin-Eosin staining method with a fully automatic device (Shandon, Varistain Gemini, Made in USA). The prepared slides were examined under a light microscope equipped with an attachment (Leica DM 2500 LED, Leica 170 HD, Manufactured in Singapore). The necessary examinations were performed, and microscopic images of the sections were captured.

RESULTS AND DISCUSSION

Macroscopy of the brain

The macroscopic structure of the brain in the common kestrel was examined in detail. The average brain weight was measured as 3.23 g, the total brain length as 22.5 mm, and the total brain width as 23.5 mm. The cerebrum, cerebellum, lobus opticus, and medulla oblongata were the prominent regions observed in the brain. The cerebrum constituted more than half of the brain. A fissura interhemispherica was identified between the right and left hemispheres. In the caudal region, between the cerebrum and cerebellum, there was a groove called the fissura subhemispherica, known as the transverse fissure in mammals. The anatomical features of the brain were evaluated from different perspectives in dorsal, ventral, and lateral views, as well as in sagittal sections.

Dorsal view

When the brain of the common kestrel was examined from the dorsal view, the right and left hemispheria were identified in

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the rostral region, while the lobus opticus, cerebellum, auricula cerebelli, and medulla spinalis were observed in the caudal region. A fissura interhemispherica was present between the two hemispheria, and a fissura subhemispherica was observed between the hemispheria and the cerebellum. Both hemispheria were bean-shaped in appearance and they occupy the dorsolateral surface of the cranial cavity. The average length and width of the cerebrum were measured as 15.5 mm and 11.75 mm, respectively. In the cerebrum, eminentia sagittalis structures were identified in the rostromedial region, and valliculae were observed in the rostrolateral parts. The lobus opticus structures were prominently observed, appearing quite large, located caudally to the cerebrum and laterally to the cerebellum. No gyri or sulci were present on the dorsal surfaces of the cerebrum and lobus opticus. The cerebellum was located caudal to the cerebrum and medial to the lobus opticus. The average length, width, and height of the cerebellum were determined to be 11.5 mm, 10 mm, and 10 mm, respectively. Transverse protrusions called folia cerebelli, ranging in number from 9 to 10, were observed on the surface of the cerebellum, separated from one another by fissurae cerebelli. The auricula cerebelli was observed lateral to the cerebellum and caudal to the lobus opticus. The medulla spinalis was prominently observed caudal to the cerebellum (FIG. 1).

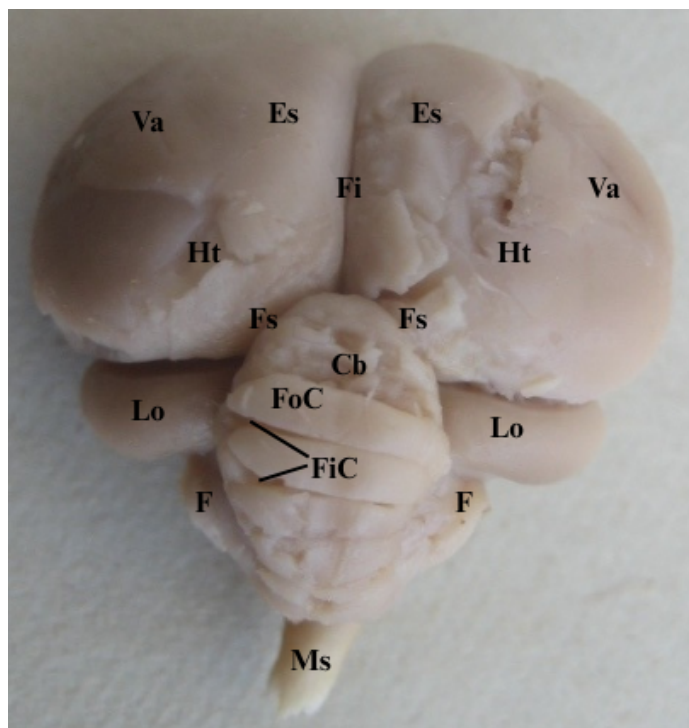


FIGURE 1. The dorsal view of the common kestrel brain. Ht: Hemispherium cerebri, Va: Vallicula, Es: Eminentia sagittalis, Fi: Fissura interhemispherica, Fs: Fissura subhemispherica, Lo: Lobus opticus, Cb: Cerebellum, FoC: Folia cerebelli, FiC: Fissura cerebelli, F: Auricula cerebelli, Ms: Medulla spinalis.

Ventral view

In the ventral examination of the common kestrel brain, bulbus olfactorius structures were present rostral to the cerebral hemispheres and on both sides of the midline. These structures were quite small and underdeveloped a difference

with mammals that have these structures more developed. A longitudinal fissure was observed just behind these structures, located between the cerebral hemispheres. The tractus opticus, chiasma opticum and lobus opticus were located caudally. The lobus opticus was divided from the cerebrum, tractus opticus and pons by a sulcus. The average length and width of the lobus opticus was 10 mm and 5 mm, respectively. There was pituitary gland in the median line caudal to the chiasm opticum and rostral to the pons. The pons and medulla oblongata were observed in the caudal part of the brain and was separated by a slightly prominent transverse groove. A longitudinally located fissura mediana ventralis was seen on the pons and medulla oblongata. On both sides of this fissure, swelling was observed on both the pons and medulla oblongata. It was determined that the fissura mediana ventralis was deeper on the medulla spinalis located behind (FIG. 2).

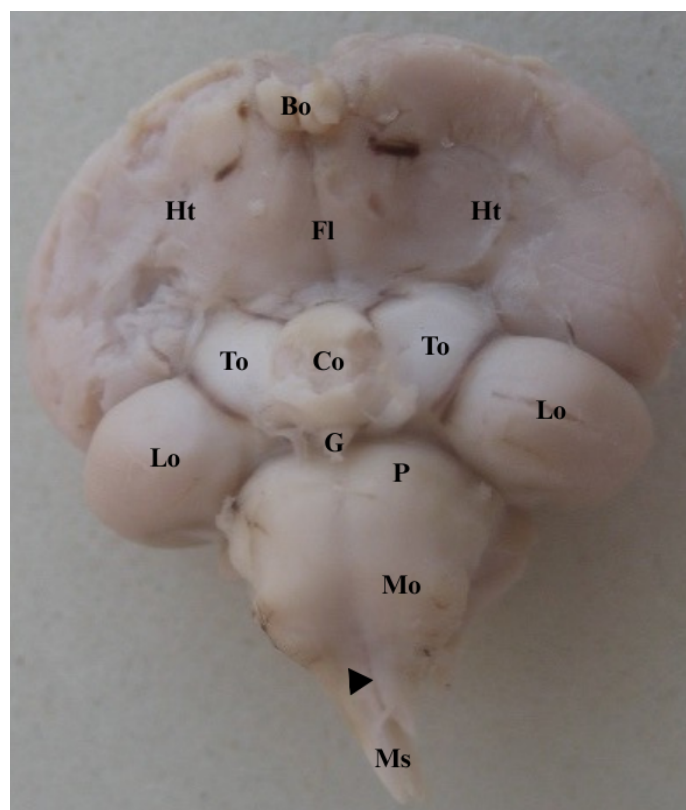


FIGURE 2. The ventral view of the common kestrel brain. Ht: Hemispherium cerebri, Bo: Bulbus olfactorius, Fl: Fissura longitudinalis ventralis, To: Tractus opticus, Co: Chiasma opticum, Lo: Lobus opticus, G: Pituitary gland, P: Pons, Mo: Medulla oblongata, Arrowhead: Fissura mediana ventralis, Ms: Medulla spinalis.

Lateral view

In the evaluation of the lateral view of the common kestrel brain, valliculae and sagittal protrusions were observed on the surface of the cerebral hemispheres. The pars frontalis cerebri, pars parietalis cerebri, and pars occipitalis cerebri were the structures identified in the lateral view from rostral to caudal. The fissura subhemispherica was observed to extend ventrally, demarcating the boundary between the hemispheria and the cerebellum. Similarly, this structure was found to continue caudoventrally between the cerebellum and the lobus opticus.

The rostral part of the cerebellum was observed to indent into the caudal aspect of each cerebral hemisphere, with its central portion elevated, and then descending as it continued toward the medulla spinalis. On the surface of the cerebellum, 9 to 10 folia cerebelli were observed, along with fissurae cerebelli located between them. The well-developed auricula cerebelli was prominently observed lateral to the cerebellum, caudal to the lobus opticus, and dorsal to the mesencephalon. The mesencephalon, pons, medulla oblongata, and medulla spinalis were the other parts of the brain visible from the lateral aspect (FIG. 3).

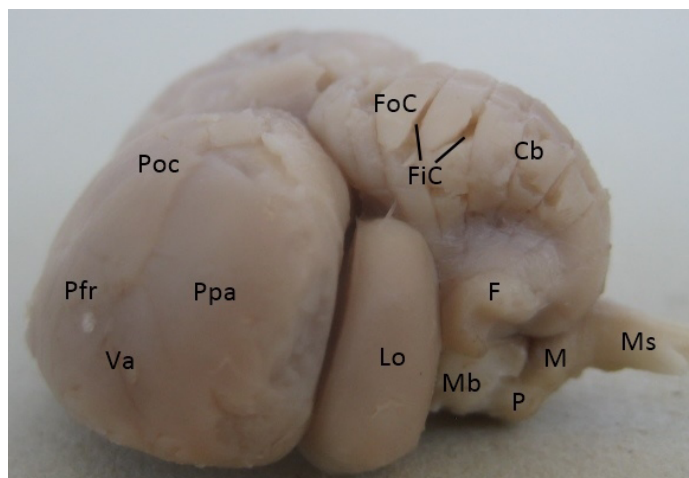


FIGURE 3. The lateral view of the common kestrel brain. Pfr: Pars frontalis cerebri, Poc: Pars occipitalis cerebri, Va: Vallecula, Ppa: Pars parietalis cerebri, Lo: Lobus opticus, Cb: cerebellum, FoC: Folia cerebelli, FiC: Fissura cerebelli, F: Auricula cerebelli, Mb: Mesencephalon, P: Pons, M: Medulla oblongata, Ms: Medulla spinalis

Sagittal section view

In the sagittal view of the common kestrel brain, the bulbus olfactorius structures were observed in the rostroventral parts of the brain. Further posteriorly, the diencephalon and mesencephalon were located. The pons and medulla oblongata were prominently observed ventral to the cerebellum. The medulla spinalis was identified caudal to the medulla oblongata. The ventriculus quartus was observed between the cerebellum and the pons and medulla oblongata. In the sagittal section of the cerebellum, gray and white matter were observed (FIG. 4).

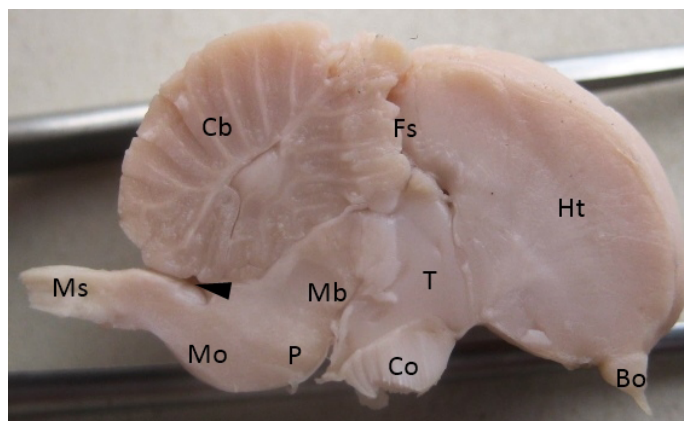


FIGURE 4. The sagittal section view of the common kestrel brain. Bo: Bulbus olfactorius, Ht: Hemispherium cerebri, T: Diencephalon, Co: Chiasma opticum, Fs: Fissura subhemispherica, Cb: Cerebellum, Mb: Mesencephalon, P: Pons, Mo: Medulla oblongata, Ms: Medulla spinalis, Ok başı: Ventriculus quartus

Light microscopy

In the light microscopic evaluation of the common kestrel brain tissue, the pia mater was observed adjacent to the surface of the cortex, enclosing the brain tissue. Immediately beneath this layer, numerous small neurons with round nuclei were observed, along with irregularly distributed glial cells among them. In the deeper cortical regions, large neurons with prominent nuclei and extensive cytoplasm were identified. The neurons were distinguished by their lightly stained cytoplasm and vesicular nuclear structure. Glial cells with dark-stained nuclei were densely observed around the neurons (FIG. 5).

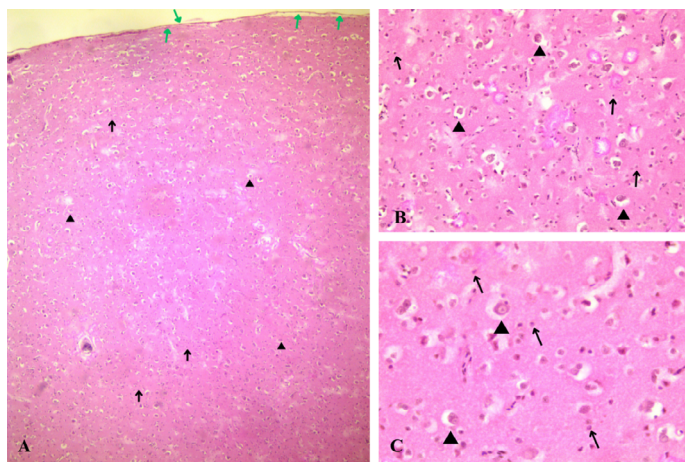


FIGURE 5. Microphotograph showing the cerebral cortex of the kestrel composed of pyramidal neurons (arrowheads) and irregularly distributed glial cells (arrows), along with the pia mater (green arrows) observed on the cortical surface, H&E, Scale bars: A, 500 μ m; B, 200 μ m; C, 50 μ m

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Histological examination of the cerebellar tissue revealed that it consisted of two main layers: the substantia grisea (gray matter) and the substantia alba (white matter). Additionally, the pia mater, one of the meningeal layers, was located external to the substantia grisea. Histologically, the substantia grisea was observed as the outer layer, while the substantia alba formed the inner layer. The substantia grisea was found to consist of three distinct layers arranged from the outermost to the innermost: the molecular layer composed of small neurons, the Purkinje cell layer characterized by prominent Purkinje cells, and the granular layer rich in granule cells. The Purkinje cell layer was observed to be located between the outer molecular layer and the inner granular layer, exhibiting a single-row arrangement composed predominantly of flask- or pear-shaped Purkinje cells, along with a smaller number of round or oval-shaped cells (FIG. 6A–B). The dendritic extensions of the Purkinje cells were observed to extend into the molecular layer (FIG. 6C).

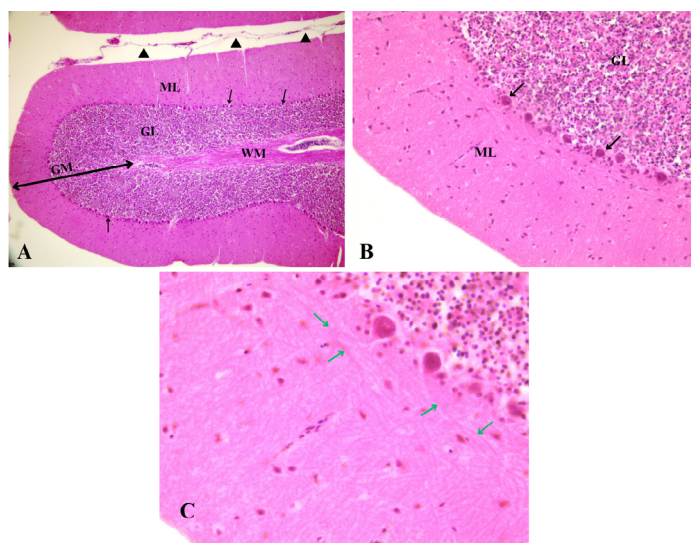


FIGURE 6. Microphotograph showing cortex and medullary regions of the cerebellum in Kestrel. GM: Grey matter, WM: White matter, ML: Molecular layer, GL: Granular layer, arrow heads: Pia mater, arrows: Purkinje cell layer, green arrows: dendrit of purkinje cell; H&E, Scale bars: A, 500 μ m; B, 100 μ m; C, 50 μ m

In the common kestrel, numerous neurons in the medulla oblongata exhibited a multipolar structure with prominent nuclei, and supportive glial cells were present among them (FIG. 7A). The ventriculus quartus was lined with ependymal cells (FIG. 7B). Histological examination revealed that the lobus opticus consisted of medium-sized spherical neurons surrounded by a small number of neuroglial cells (FIG. 7C).

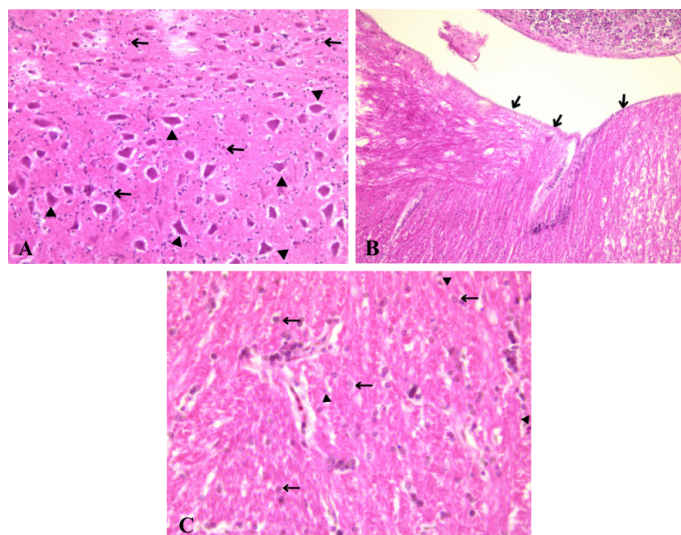


FIGURE 7. Microphotograph showing numerous multipolar neurons (arrowheads) and supporting glial cells (arrows) in the medulla oblongata of the kestrel (A), Microphotograph showing ependymal cells in the fourth ventricle of the kestrel (B), Microphotograph showing the optic lobe of the kestrel composed of medium-sized neurons (arrows) and a few neuroglial cells (arrowheads) (C), H&E, Scale bars: A, B, 200 μ m; C, 50 μ m

In this study, the structural characteristics of the brain of the common kestrel (*Falco tinnunculus*) were examined at both macroscopic and light microscopic levels. The findings revealed both similarities and distinct differences when compared with previous studies conducted on other bird species. Relative to body weight, the brain of birds is 5 to 20 times larger than that of reptiles. Within the class Aves, relative brain weight is lowest in ostriches (*Struthio camelus*), chickens (*Gallus gallus domesticus*), and pigeons (*Columbidae*), while it is highest in parrots (*Psittaciformes*) [15]. In a study conducted in sparrowhawks (*Accipiter nisus*), it was reported that the mean brain weight was 3 ± 0.2 g, the mean brain length was 24 ± 2 mm and the mean brain width was 19 ± 2 mm, which was different from the findings of the common kestrel brain in this study [16].

In the literature, it has been reported that the cerebrum exhibits varying morphological shapes in the dorsal view in different poultry species [7, 17, 18]. For example, the cerebrum has been described as triangular in the chickens [17], and obtuse triangular in the ostriches [18] and owls (*Strigiformes*) [7]. Unlike mammals it was also reported that in chickens, ostriches and owls, the brain surface was smooth and without gyri and sulci [7, 18, 19], similar to the findings of the common kestrel brain in the present study.

It has been reported that the cerebral cortex consists of six layers histologically; these layers are molecular layer, outer granular layer, outer pyramidal layer, inner pyramidal layer, inner granular layer and multi-form layer, respectively [8]. In the microscopic examinations performed in this study, a large number of pyramidal cells were observed in all layers of the cerebral cortex and these cells were similar to the pyramidal cell morphology described in previous studies [3, 20].

It is reported that the cerebellum in bird species is more developed, especially compared to reptiles, and has a large surface area thanks to the folds on its surface. This structural feature is directly related to the cerebellum's tasks of monitoring, regulating and correcting the locomotor system [20].

Consistent with the macroscopic features reported in previous studies, the cerebellum is quite prominent in the kestrel brain samples [21].

In histological examinations in geese, as demonstrated in this study, it was reported that the cerebellum consists of two main layers, substantia grisea (gray matter) and substantia alba (white matter), and the gray matter is organized as molecular layer, Purkinje cell layer and granular layer. While the molecular layer contains a small number of small nerve cells, the granular layer has been found to contain a large number of granular cells and the dendrites of Purkinje cells extend into the molecular layer [22]. Similarly, in a study in pigeons, it was reported that the cerebellar cortex consists of three layers; the outermost molecular layer consists of small neurons and glia cells, the granular layer contains dense granular cells with dark nuclei, and Purkinje cells are located in a single row between these two layers [23].

The lobus opticus is an important brain structure responsible for processing visual perception in birds, and its morphological characteristics can vary depending on the species' lifestyle, activity time and migration distance. Vincze *et al.* [24], reported that migration distance has an effect on brain size and lobus opticus volume; the optic lobes of birds migrating long distances are larger. This has been attributed to the need for high-resolution visual processing. As a matter of fact, in the common kestrel specimens examined in this study, similar to previous studies [25], it was observed that the optic lobe was large, prominent and located below the cerebrum. It has been suggested that diurnal bird species have a more advanced optic lobe structure due to their need for high vision and the capacity to process visual data [26].

This advanced optic lobe structure observed in the present study is consistent with the kestrel being a diurnally active and predatory species. A study in the African ostrich (*Struthio camelus camelus*) reported that the optic lobe is composed of medium-sized neurons with few neuroglia, similar to the kestrel brain samples examined in this study [20].

The anatomical position of the bulbus olfactorius varies significantly between species. In some species, this structure is located in front of the cerebral hemispheres and distinctly separate, while in others it is located very close together or on the undersurface of the hemispheres. In some species, the two bulbus olfactorius fuse completely in the midline to form a single structure [27]. In the kestrel brain samples examined in this study, the bulbus olfactorius structures were symmetrically located on the ventral side of the brain and were described as small and underdeveloped structures. Although this morphological feature suggests a limited reliance of the kestrel on its sense of smell during hunting, recent studies have shown that almost all bird species, regardless of the relative or absolute size of the bulbus olfactorius, are able to detect odors and that this ability is often directly linked to specific ecological functions [28]. To better understand the effect of bulbus olfactorius size on olfactory capacity, comparative studies across different bird species would be beneficial in supporting this relationship.

In studies conducted on pigeons and chicks, a thin membranous structure extending from the surface of the medulla oblongata to the subarachnoid space at the caudal end of the ventriculus quartus has been described. It was reported that this membrane is continuous with the ependymal cells

and that there is no direct anatomical connection with the subarachnoid space [29].

CONCLUSION

This study has thoroughly examined the brain structure of the common kestrel (*Falco tinnunculus*), a diurnal bird of prey, at both macroscopic and light microscopic levels, revealing morphological characteristics specific to this species. Macroscopic findings showed that the cerebrum consisted of two hemispheres with a flat surface, separated by a prominent fissura interhemispherica; the optic lobes were large and well developed, and the cerebellum was convoluted with numerous folia cerebelli. The presence of tiny and underdeveloped bulbus olfactorius on the ventral side suggests that the visual system is more dominant than the olfactory system in this species. Microscopic examination revealed the presence of pyramidal neurons and dense glial cells in the cerebral cortex, while the typical three-layered structure of the cerebellum and the histological integrity of prominent Purkinje cells were observed. In addition, numerous multipolar neurons and supporting glial cells were found in the medulla oblongata and the fourth ventricle was found to be covered with ependymal cells. These data show that the nervous system organization of the common kestrel has evolved in accordance with environmental conditions and ecological requirements, and will contribute to comparative anatomical and neurohistological studies on the brain morphology of birds of prey.

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Conflict of interest statement

The authors declare that they have no conflicts of interest concerning this article.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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