Research Article

Neuroanatomical and Immunohistochemical Insights into the Cerebrum of Myna Birds (*Acridotheres* Spp)

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Abstract | The aim of this study is to know the morphological and histological structure of the brain of the Myna bird in order to explain the behaviour of this bird, which indicates a higher intelligence than that found in most birds "Eight myna birds with broken bones in different parts of their bodies were obtained from local clinics in Diyala and Baghdad cities.". "The brain of the myna bird is relatively large compared to the body size, with the brain's relative weight and relative length being about 0.02% and 0.06% of the total body mass and length, respectively." . "There are two types of cells in the myna's central nervous system: the large cells, called neurons, and the small cells, called glial cells." "The number of neurons and glial cells per micrometer is very high in mynas compared to other birds in previous studies.", Myna bird show a lot of blood vessels per micrometer. "In this study, NSE was predominantly and strongly expressed in the cytoplasm of cortical pyramidal neurons."

Keywords | Brain, Cerebrum, Myna bird, Neuroanatomical, Immunohistochemical, Broken bones

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INTRODUCTION

The common myna (*Acridotheres tristis*) belongs to the family Sturnidae. It is a bird species indigenous to Asia, with populations ranging from Iran, Pakistan, India, and Kazakhstan to Malaysia and China (Ray *et al*., 2023). Nestling and juvenile birds, especially hand-reared chicks, exhibit innate curiosity and often consume strange objects, including toys, bedding, and seeds. The common myna (*Acridotheres tristis*) is a notably successful invader (Heinrich, 2010). The brain comprises three primary components: the cerebrum, cerebellum, and medulla oblongata. "Avian species possess comparatively large cerebral hemispheres and cerebella, along with substantial optic lobes and diminutive olfactory bulbs (Azeez *et al*., 2023).". "The avian brain hemispheres are prominent.". "Examinations ". of the brains of flying birds (swifts and falcons) reveal that they possess a small cerebellum"The optic lobe is very large in birds due to the significance of vision, while the olfactory lobes are relatively small owing to their limited olfactory capability (Gunturkun, 2000)."The avian brain comprises two components: grey matter, located externally, and white matter, situated inside (Emery and Clayton, 2005). The cerebral cortex consists of six layers: the molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer, and multiform layer. "In contrast, the medulla contains a dense bundle of fibers and glial nerve cells (Pal et al., 2003)."The Cerebral cortex comprises three layers: the molecular layer, the Purkinje cell layer, and the granular layer (Bunyamin *et al*., 2001). The medulla oblongata of birds comprises three zones of grey matter, characterized by irregularity. This study aims to elucidate the anatomy and histology of mynas birds, given the little research on the anatomy and histology of the mynas brain.

MATERIALS AND METHODS

Eight myna birds suffering from broken bones in different parts of their bodies were obtained from local veterinary clinics in Diyala and Baghdad between November 2024 and February 2024." "The birds were weighed and euthanized using an overdose of ketamine and xylazine." "Morphometric analysis was conducted to determine the length and weight of the bird's body and brain, as well as the relative weight and relative length." "The specimens were fixed in 10% neutral buffered formalin for 3 days, then washed with tap water to remove the fixative. Dehydration was carried out using different concentrations of ethanol (70%, 80%, 90%, 100%), followed by clearing with xylene and embedding in paraffin blocks." "The sections were cut to a thickness of 5-6 µm using a rotary microtome and then stained with hematoxylin and eosin (H and E).

Immunohistochemistry

- The sections were mounted on positively charged slides immediately, deparaffinized with xylene three times for 3 minutes each, and then washed with ethanol three times for 3 minutes each.
- The slides were washed three times in PBS for 3 minutes each, after which the slides were removed from PBS, and each section was covered with 3% H2O2 for 10 minutes at room temperature.
- The slides were removed from PBS, gently wiped around each section, and the tissues were covered with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining "Do not wash."
- The blocking buffer was tipped off, and the tissues were gently wiped around each section and covered with anti-NSE monoclonal antibody (ready for use).
- The sections were incubated for 1 hour at room temperature and then washed twice in PBS for 5 minutes each.
- The tissues were gently wiped around each section and covered with Histostar™ (Ms + Rb), incubated for 30 minutes at room temperature, and washed as in step 5. Visualization was achieved by reacting for 5 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). The slides were then washed in water for five minutes.
- The slides were counterstained with hematoxylin for 1 minute, washed three times in water for five minutes each, and then immersed in PBS for 5 minutes. Dehydration was carried out by immersing the slides in ethanol three times for 3 minutes each, followed by immersion in xylene three times for 3 minutes each, and then examined.

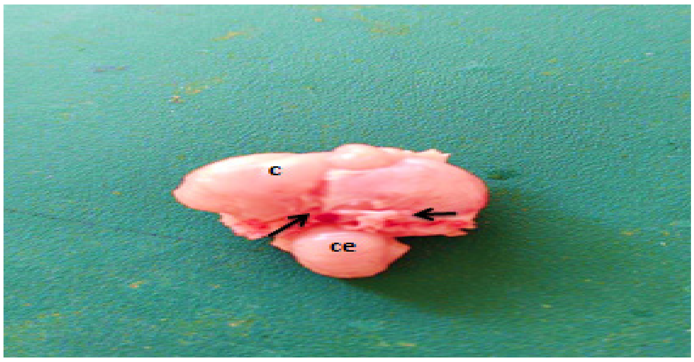

Figure 1: brain of myna bird showing the cerebrum (C), cerebellum(ce), meninges (black arrows).

RESULTS AND DISCUSSION

Brain Weight and Gross Morphometric Results

In the current study, the brain of the myna bird is relatively large compared to the body size, reflecting the bird's advanced behavioral and cognitive abilities (Hamza *et al*., 2021). It was noted that "It was noted that the anatomical brain structure and cytoarchitecture did not reveal specific characterizing features except for the hooded crow with large-sized soma. "It is enclosed within a strong, thin bony cranium and surrounded by three layers of meninges, which support and protect the brain"In general- the brain is composed of two large cerebral hemispheres, separated by a longitudinal fissure (Figure 1).

Table 1: Ensure the table is properly formatted and aligned. The current table is difficult to read due to inconsistent spacing and alignment.

parameters	Weight (G) Mean ± SEM	Cerebrum Weight (G) Mean ± SEM	Relative weight	Length (cm) Mean ± SEM	Cerebrum Length (cm) Mean ± SEM	Relative length
Myna bird	70± 0.03 SE	1.9± 1.15 SE	0.02	21.5± 0.02SE	1.5± 0.10 SE	0.06

The myna bird had a body weight of 70 ± 0.03 g, while the brain weight was 1.9 ± 1.15 g, with the brain's relative weight being about 0.02% of the total body mass."The length of the cerebral cortex was 1.5 ± 0.10 cm, while the body length was 21.5 ± 0.02 cm, resulting in a relative length of 0.06% (Table 1).""Azeez *et al*. (2023) mentioned that the relative weight and relative length reflect the evolution of the brain." "Body weight was strongly positively correlated with brain weight and brain length Jarvis *et al.* (2005) stated, 'The avian cerebrum has a large pallial territory that performs functions similar to those of the mammalian cortex.'" "Although the avian pallium is nuclear and the mammalian cortex is laminar in organization, the avian pallium supports cognitive abilities similar to, and in some species more advanced than, those of many mammals"The high relative brain weight in birds is due to natural selection, driven by ecological and social challenges." "Species with higher brain-to-body ratios have evolved sophisticated behaviors and cognitive abilities, enabling them to thrive in diverse and changing environments." "This relationship illustrates how brain size is a critical factor in the evolutionary success of many avian species." "Comparing the relative weight of avian brains across species typically highlights variations that correspond to differences in behavior, ecology, and evolutionary adaptations." "Their brains emphasize spatial memory, hunting strategies, and precision." "Acute vision, precise predatory strategies, and exceptional flight control.

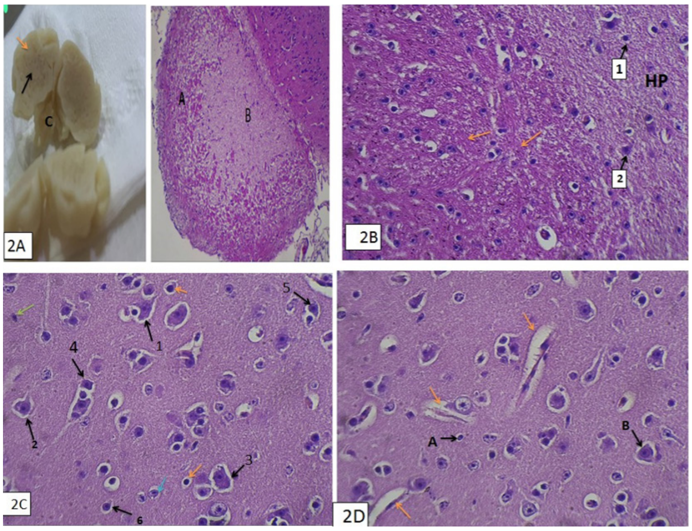

Figure 2A: Cerebrum section showing white and gray matter; **B:** Hippocampal region showing glial cells and neurons; **C:** Cerebrum white matter showing different types of neurons and glial cells; **D:** Hippocampal region showing pyramidal neurons and blood vessels.

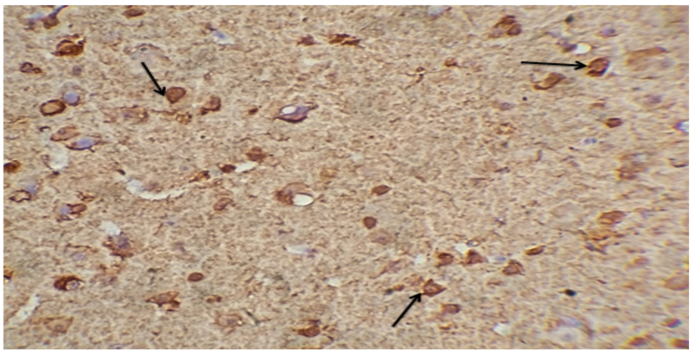

Figure 3: Immunoreactivity stain of myna bird cerebrum showing the highly "expression." pattern of NSE receptor in the hippocampus.

The hippocampus of the myna bird, like that of other birds, is more specialized because this part of the brain has an abundance of neurons, called gray matter, while the parts where neurons are largely absent are called white matter. The white color is due to the presence of myelinated cells, primarily from a certain type of glial cell (Herold *et al*., 2019). It was noted that the large cells with cytoplasmic processes extending as either axons or dendrites are neural cells or neurons (Figure 2)." There are two types of cells in the central nervous system: the large cells, called neurons, and the small cells, called glial cells (Figure 3)." "Neurons are larger than glial cells, with a large cell body containing a nucleus and sometimes extensions of cytoplasm called axons or dendrites." "The myna's cerebrum shows some types of glial cells, primarily in the white matter of the brain, called oligodendrocytes. These are small, round cells with a clear zone around them and are the most predominant glial cells in the white matter. They are located between neurons and blood vessels and act as part of the blood-brain barrier. Some of these cells have nuclei that resemble oligodendrocytes but are located next to blood vessels where neurons are present. These small, round nuclei, possibly cigar-shaped, are called microglia and can be identified in routine stains.". "The third type of glial cells is found throughout the central nervous system and functions as the macrophage-like cells of the central nervous system. The last two types of glial cells are not routinely seen in the brain's substance "The number of neurons and glial cells per micrometer is very high in mynas compared to other birds in previous studies. Myna birds also show a high density of blood vessels per micrometer." "Neuron-specific enolase (NSE)." (NSE) is an acidic soluble protein that functions as a glycolytic isoenzyme and is predominantly present in the cytoplasm of neurons, where it is involved in regulating intraneuronal chloride levels during neural activity. In this study, a marker was used to express the positive expression of NSE in the cytoplasm of neurons and glial cells." "Neuron-specific enolase (NSE)." (NSE) is one of five isomers of the glycolytic enzyme enolase.". "Haque *et al*. (2018) noted that NSE is localized within central and peripheral neurons as well as in neuroendocrine cells, but not in glial cells. This finding was not accepted by Schmechel *et al*. (1978). proposed that NSE is a specific biomarker for neuronal cells Our study suggests that the activity of the cytoplasmic NSE protein expression plays a role in the viability of the birds, particularly in the processes of navigation and memory." "The current study found that NSE protein showed highly significant expression in the hippocampus of the myna bird's brain. The high expression density of NSE protein in the brain suggests its role in cellular functions related to cognition and the bird's tendency to exhibit special traits such as speech and signs of mental development"In this study, NSE was predominantly and strongly expressed in the cytoplasm of cortical pyramidal neurons" - Lee *et al*. (2021) reported similar findings in their study.

CONCLUSION AND RECOMMENDATIONS

The results of the current study indicate that the evolution that maintains the behavior of the myna bird is important due to the fact that this bird has a high weight and relative weight of the brain compared to other birds. The histological side, it is clear that the myna bird excels over the rest of the birds in the experiment, as it has a large number of nerve cells, supported by the presence of similar numbers of cells and the rest of the thinkers with those present in a large number of blood supply that magnify and what confirms the validity of the conclusions is the presence of a large number of individuals who need blood nutrition. The myna bird has a unique arrangement of nerve fibers in the circular form which enhances its ability to process information quickly.

1. The current findings could be useful for other veterinary and medical investigations related to the cerebrum in birds. It may give many supports to the physiological investigations of cerebrum for the postnatal period starting from hatching to the puberty.
2. The comparative variations must be studied with the aid of electron microscopy to identify the cytological changes.

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NOVELTY STATEMENT

The novelty of this study lies in understanding the levels of intelligence among birds, including myna, racing pigeons, and cougar chickens, and identifying the differences from an anatomical and histological perspective." This study can be used to understand diseases that affect the nervous system, particularly the brain.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to this study.

Conflict of Interest

The authors declare no conflict of interest.

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