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A comparative histological and immunohistochemical study of the brain of three birds (myna, racing pigeon, and Cochin chicken)

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Abstract

Twelve birds (mynas, racing pigeons, and Cochin chickens) suffering from fractures in various parts of their bodies were used in this study from local veterinary clinics in Diyala and Baghdad. The current study was conducted between November 2024 and February 2025. The birds were weighed and euthanized using an overdose of ketamine and xylazine. The current study revealed that the brains of the three birds exhibited the presence of certain types of glial cells. The myna brain contained a distinct arrangement of glial cells and neurons, as well as a special arrangement of nerve fibers in a circular and annular pattern, which was not observed in the other two birds. Mynas had a higher number of glial cells and neurons per micrometer than the other two birds in this study. NSE protein showed significantly higher expression in the hippocampus of mynas compared to the other two birds

Keywords: Brain, glial cells, myna, hippocampus

I. Introduction

The vertebrates nervous system is studied according to functional or structural features. Functionally consider as the somatic and autonomic nervous system, and these are according to conscious and involuntary influences, respectively [Catala, M., Kubis, N., 2013: Gross anatomy and development of the peripheral nervous system. Handb. Clin. Neurol., 115, 29–41. .]. structurally, the nervous system is compsed of : the peripheral nervous system (PNS) comprising the cranial, spinal and autonomic nerves, and the enteric nervous system; and the central nervous system (CNS), instituted by the brain and spinal cord [Esteves, M., Almeida, A., Leite-Almeida, H., 2020: Insights on nervous system biology and anatomy. Handbook of Innovations in Central Nervous System Regenerative Medicine. Elsevier, pp 28]. The brain regulates and controls many vital functions and is the most important structure of the body [Usende, I. L., Attah, O. R., Oyelowo, F. O., Shokoye, I., Rassaq, A. A., Madubuike, S. A. 2022: Morphology and morphometry of the brain of African side necked turtle (Pelusios castaneus): A preliminary investigation. Sahel J. Vet. Sci., 19, 3, 20–27.]. all vertebrates have the same structural parts of the brain, but the complexity and organization are differ [Shimizu, T., Patton, T. B., Husband, S. A., 2010: Avian visual behaviour and the organization of the telencephalon. Brain Behav. Evol., 75, 3, 204–217.].mammals have different brain from birds , alot of variations existing in their structural shape and situation [Medina, L., Reiner, A., 2000: Do birds possess homologues of mammalian primary visual, somatosensory and motor cortices ? Trends Neurosci., 23, 1, 1–12]. Functionally, the avian nervous system obtains information through sensory receptors are responsible for carry information to the brain that about environment surrounded , the brain filtrated this information and respond and, also it coordinates the motor impulses to all body [Kubke, M. F., Wild, J. M., 2009: Evolution of avian brains. In Binder, M. D., Hirokawa, N., Windhorst. U. (Eds.), Butler, A. (Section Ed.): Encyclopedia of Neuroscience. Springer-Verlag GmbH, Berlin, Heidelberg, 1312–1318]. cerebrum is the most important part of the brain composed of two regions, the first called pallium which includes the external corticoid area which involve the hyperpallium and the dorsolateral corticoid area, it consists of piriform cortex and hippocampal complex. The last region is the internal corticoid areas dorsal ventricular ridge which comprises the mesopallium, nidopallium and archopallium. the large size pyramidal neurons appeared in the nidopallium surrounded the crescent shaped lateral ventricles. The subpallium region consists of two regions represented by striatum which contains neuronal fibers and pallium which is appeared small and

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pale colour and represented the deepest part of cerebrum. (Abid, A. B., & Al-Bakri, N. A. (2017). Morphological and Histological Study of The Fore Brain (Cerebrum) In Quail Coturnix coturnix (Linnaeus, 1758). *Ibn AL-Haitham Journal For Pure and Applied Science*, *29*(1).) Birds communicate using visual signals as well as through the use of calls and song. The testing of intelligence in birds is therefore usually based on studying responses to sensory stimuli.

The corvids (ravens, crows, jays, magpies, etc.) and parrots and myna are often considered the most intelligent birds, .(Emery, N. J. (2006). Cognitive ornithology: the evolution of avian intelligence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1465), 23-43.) The current study aimed to compare the morphological and fine structural differences of the brain parts that associated with intelligence among three different types of bird (Myna birds are very ingenious, and are able to familiarize to a range of habitats. it have an ability to sing, speak , pigeon which able to remember locations and finally chicken which consider as have no high intelligence.

II. Materials and methods

twelve birds(myna, racing pigeon, and cochin chicken) suffering from broken in different parts of their bodies from local veterinary clinics in Diyala and Baghdad between November 2024 to February 2025. The birds weighted and euthanized by using over dose of ketamine and xylazine. morphometric analysis will determine the length, weight of the bird body and brain as well as the relative weight and relative length calculated. The specimen was fixated in 10%neutral buffered formalin for 3days,then washed by tap water to remove the fixative and dehydration by different alcoholic concentration (ethanol) 70%,80%,90%,100%, clearing by the xylene then paraffin infiltrated and embedded in paraffin block. The sections were cut a 5-6 um thick by rotary microtome and then stained with (H&E) stain. (15)

Immunohistochemistry.

1.The Sections after mounted on positive charge slides immediately, Deparaffinized with Xylene 3 times for 3 minutes, then Washed with Ethanol three times for 3 minutes each.

2. Wash the slides 3 times in PBS for 3 minutes each ,after that the slides removed from PBS and cover each section with 3% H2O2 for 10 minutes at room .

3.Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.

4.Tip off the blocking buffer, wipe gently around each section and cover tissues with anti-NSE monoclonal antibody (ready for use).

5. Incubate the sections for 1 hour at room temperature then washed the slides twice in PBS for 5 minutes each.

6. Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) Incubate for 30 minutes at room temperature, Wash as in step 10. Visualize by reacting for 5 minutes with Histostar™™ DAB Substrate Solution (MBL; code no. 8469). ,Wash the slides in water for five minutes.

7. Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for five minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each and examined.

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III. The results

The cerebrum of the three birds show some kinds of glial cells which are chiefly in white matter of the brain are called **oligodenderocytes** which are small round and have a little clear zone around them, these are the most predominant glial cell of white matter and located between neurons and blood vessels and act as the blood brain barrier and some of them have a nucleus that looks like oligodenderocytes but their location next to the blood vessel where there's neurons present these small round nuclei perhaps cigar shape called **microglia** and they can be visible in routine stains to identify microglia. the third type of the glial cells found in all portions of the central nervous system and their function in being the **macrophage** like cells of the central nervous system the last two type of glial cells are not routinely seen in the substance of the brain. By examining the tissue sections of the cerebrum in the three birds, it was found that the Mina bird cerebrum contains a distinct arrangement of glial cells and neurons, in addition to the presence of a special arrangement of nerve fibers in a circular and ring-like manner, which was not observed in the other two birds mynas bird have number per micrometer of glial cells as a compared to the other two birds in this study fig.(1). The current study The neuron cells number per micrometer very high in mynas as a compared to the other two birds in our study. (Olkowicz, S., Kocourek, M., Lučan, R. K., Porteš, M., Fitch, W. T., Herculano-Houzel, S., & Němec, P. (2016).mentioned that the Birds have primate-like numbers of neurons in the forebrain.brains of songbirds and parrots contain very large numbers of neurons, at neuronal densities considerably exceeding those found in mammals. Avian brains thus have the potential to provide much higher "cognitive power" per unit mass than do mammalian brains.) the neuron density differed among species and when increase indicate that bird tend to be more developed than others. Emery, N. J. (2006). referred that the bird brains have two-to-four times the neuron packing density of mammal brains, for higher overall efficiency. Emery, N. J. (2006). Cognitive ornithology: the evolution of avian intelligence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1465), 23-43.)

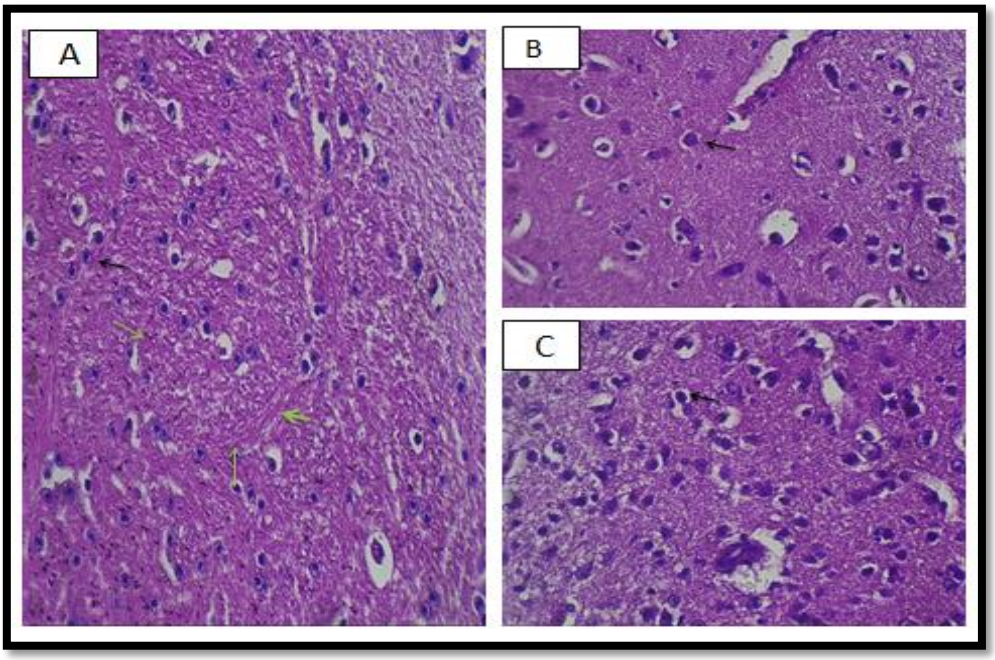

fig.1 Histological section through cerebrum(gray matter) in Mynas(A),pigeon(B) and cochin bird (C), show glial cells (black arrow),nervous fibers as special arrangement(green arrows).H&E stain.40X.

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The histometric Findings indicated that presence of a significance differences in the number of neuron and glial cells of cerebrum at level 0.005 in the Mynas bird compared to the cochin bird and pigeon, also there was significance differences in the glial cells of cerebrum at level 0.005 between pigeon and cochin as shown below(fig. 2,3) Many kinds of glial cells present in white matter of the brain . Myna bird cerebrum contains a distinct arrangement of glial cells and neurons(Kálmán, M., Szekely, A. D., & Csillag, A. (1998). Distribution of glial fibrillary acidic protein and vimentin-immunopositive elements in the developing chicken brain from hatch to adulthood. *Anatomy and embryology*, *198*(3), 213-235. Referred that Four classes of glial cells can be recognized in the central nervous system of turtles and birds on the basis of nuclear characteristics), Nissal granules Bunyamin ., et al (2001)observed that "the glial cells in the white matter were characterized by small bodies and small dark nuclei".

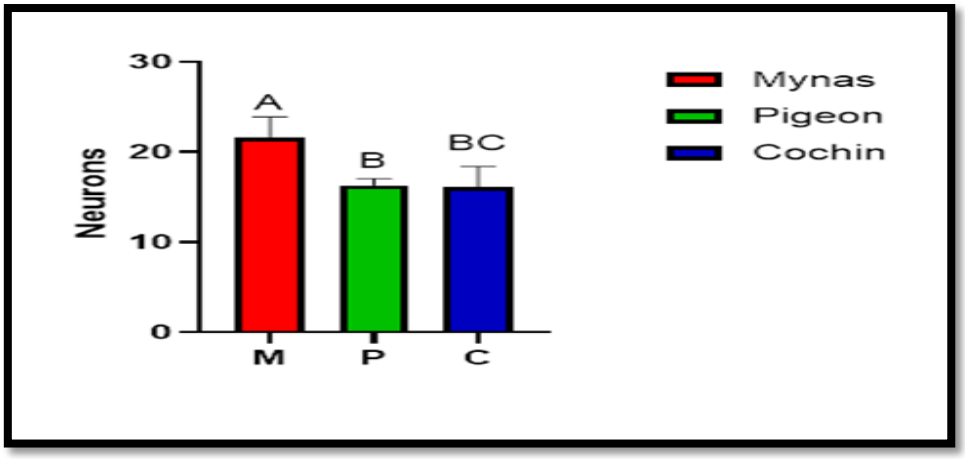

Fig.2 **Showed the comparison in the number of neurons among three groups Mynas , pigeon and cochin presence of different letters indicate presence of significant differences at level 0.005**

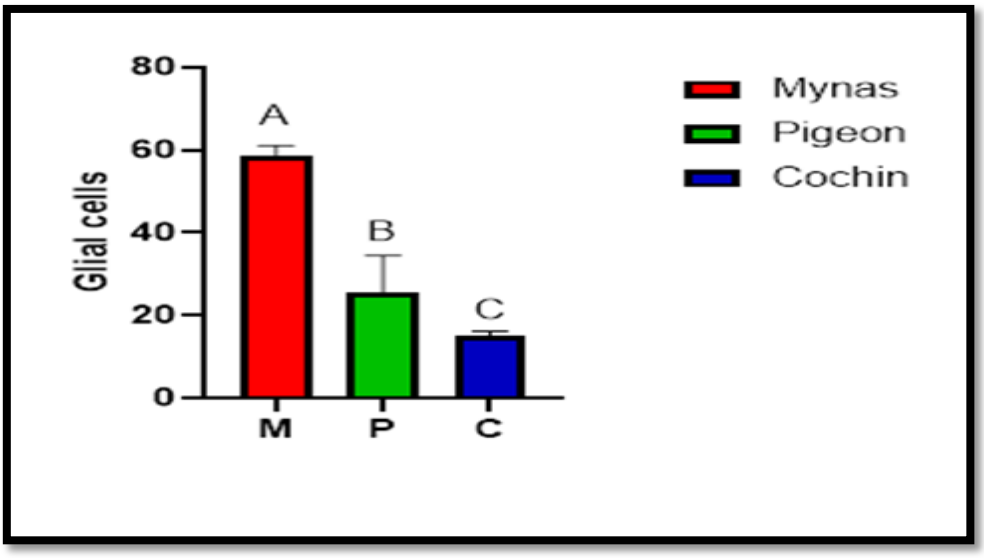

Fig.3 **Showed the comparison in number of glial cells among three groups Mynas , pigeon and cochin presence of different letters indicate presence of significant differences at level 0.005**

Neuron-specific enolase (NSE) is an acidic soluble protein, which functions as a glycolytic isoenzyme and is predominantly present in cytoplasm of neurons and involved with regulating intraneuronal

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chloride levels during neural activity.in this study NSE marker express the positive expression in the cytoplasm of neuron and glial cells.(Schmechel, D., Marangos, P. J., Zis, A. P., Brightman, M., & Goodwin, F. K. (1978). Brain enolases as specific markers of neuronal and glial cells. *Science*, *199*(4326), 313-315.), proposed that a specific biomarker for neuronal cells). Neuron-specific enolase (NSE) is one of five isomers of the glycolytic enzyme enolase. ( ) It is localized within central and peripheral neurons as well as in neuroendocrin cells, but not in glial cells.(Maxwell, G. D., Whitehead, M. C., Connolly, S. M., & Marangos, P. J. (1982). Development of neuron-specific Enolase Immunoreactivity in avian nervous tissue in vivo and in vitro. *Developmental Brain Research*, *3*(3), 401-418.)

The current study expressed that NSE protein have shown highly significant expression in the hippocampus in the brain of mynas bird.(fig.4)

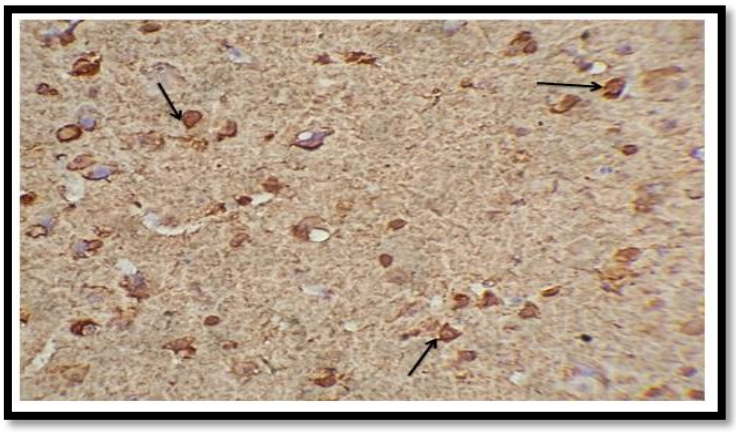

Figure 4. Immunoreactivity stain of mynas bird cerebrum showing the highly expressional pattern of NSE receptor in the hippocampus .

The current study showed a significant difference in the expression of the NSI protein, as the myna bird showed high expression, while the racing pigeon showed moderate expression(fig.5, while the cochin chicken showed mild expression(fig.6).

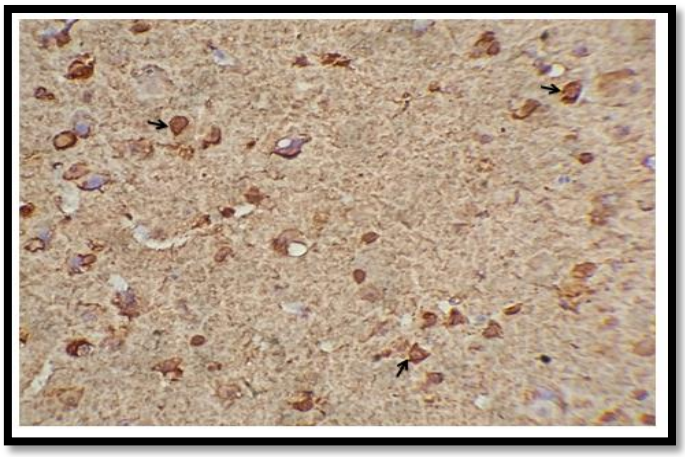

Figure 5. Immunoreactivity stain of racing pigeon cerebrum showing the moderate expressional pattern of NSE receptor in the hippocampus .

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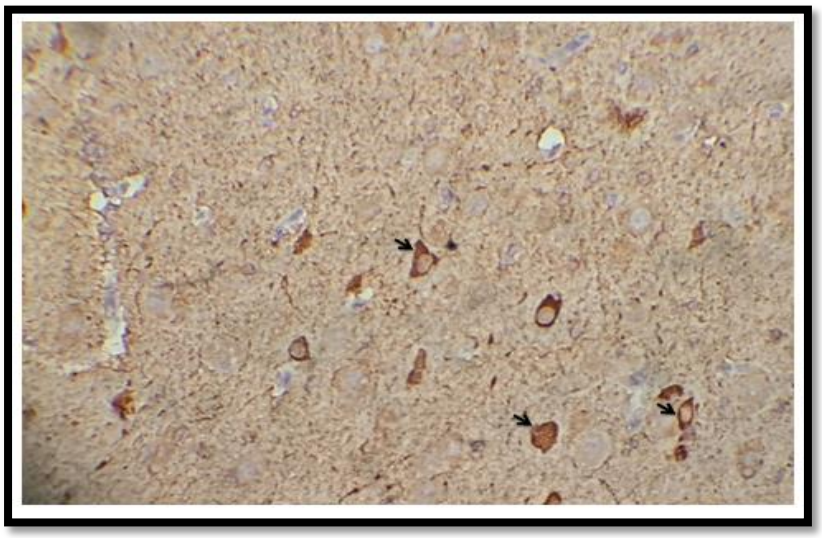

Figure 6 . Iimmunoreactivity stain of cochin chicken cerebrum showing the highly expressional pattern of NSE receptor in the hippocampus .

IV. Conclusion

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.

Recommendation

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.

3. The comparative variations must be studied with the aid of electron microscopy to identify the cytological changes .

V. Refrences

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