SHORT COMMUNICATION

# Age Specific Anatomical Studies on the Medulla Oblongata in Post-Hatch Broiler Chicken

Avnish Kumar Gautam*, Sanjay Ray, Partha Das, Arun Kumar Mandal, Nirmal Kumar Tudu, Manoj Kumar Sinha

## Abstract

Present study was conducted on 70 day-old broiler chicks which were reared up to 42 days (market age) of post-hatch period. The birds were divided into 7 groups, each containing 10 birds, irrespective of sex. The whole experimental period was divided into seven weekly intervals (days 0, 7, 14, 21, 28, 35 & 42). The birds of age-group I to VII were sequentially euthanized at weekly interval and intact brains were harvested carefully. Grossly, the medulla oblongata was situated behind the optic lobes just ventral to the cerebellum and it became gradually narrowed posterior. Histologically, the medulla oblongata at all the age groups was made up of chiefly the broad bands of nerve fibres, which were longitudinally placed. The nuclei of the medulla oblongata were formed by few multipolar neurons, spindle shape neurons and very few pyramidal neurons supported by few neuroglia cells. On PAS staining a weak to mild lipofuscin pigmentation in neurons of medulla oblongata was observed in day old chicks, while in 42 days old chicks (group VII) moderate pigmentation was recorded.

**Key words:** Broiler chicken, Gross anatomy, Histology, Medulla oblongata.

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## Introduction

Avian brain research has evolved into a major biological field in recent years. The study of the brain in birds is very important because it controls physiology, gesturing, maintenance, regulation of muscle tension, and function of the body (Gupta *et al.,* 2016). Brain research is important for a better understanding of neuro-physiological coordination *vis-à-vis the* growth and production of birds. The medulla oblongata is responsible for maintaining vital body functions, namely regulation of breathing, heart and blood vessel function, digestion, and swallowing. Moreover, it is home to all ascending and descending nerve tracts that communicate between the brain and spinal cord. It is composed of three zones in gray matter: the first zone contains medium-sized nerve cells; the second zone is situated on the medial side, which has large nerve cells; and the last zone is situated on the lateral side of the medulla oblongata, which has small nerve cells (Goodmon and Schein, 1974). Several studies have focused on the brains of adult avian species (Barkan *et al.,* 2017). Age-wise information on the gross and histological changes in medulla oblongata is limited; therefore, this investigation was made to explore the same in broiler chickens.

## Materials and Methods

The present study was duly approved by the Institutional Animal Ethics Committee (IAEC), Faculty of Veterinary and Animal sciences, West Bengal University of Animal & Fishery Sciences, Kolkata-3, India (References no. IAEC/67/III, B, dated 19/08/2019).

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### Grouping of Chicken and Specimen Collection

A total of seventy (70) day-old broiler chicks were reared up to 42 days (market age) in the experimental pens (cage system) at Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata, India. The birds were randomly allocated to 7 equal groups (Groups I to VII), each containing ten birds, irrespective of sex. The whole experimental period was divided into seven weekly intervals (days 0, 7, 14, 21, 28, 35 & 42).

The birds were euthanized by injecting overdose of Sodium pentobarbital IP at the dose rate of 120 mg/kg at weekly interval and brain samples were harvested carefully (Gourdon, 2016; Gautam *et al.,* 2020). The head of the birds was carefully separated at the level of second cervical vertebrae. The cranial cavity was exposed very carefully with the help of forceps, scissors, and scalpel. The meninges

covering the brain and its attachment with cranial bones was cut followed by severing of rostral attachment of olfactory lobes and optic nerves at the level of optic chiasma on the ventral surface of the brain; the intact brain was removed from the cranial cavity.

### Histological and Histochemical Study

For histological studies, brain tissue samples were fixed in 10% neutral buffered formalin (NBF) for 48 h at room temperature after recording the biometrical parameters. After fixation, the tissues were washed in running tap water for overnight, dehydrated in ascending grades of ethyl alcohol (70%, 80%, 90%, 95% and absolute alcohol), cleared in acetone and benzene followed by paraffin impregnation in a thermostatically controlled oven to prepare paraffin blocks (Luna,1968). The trimmed paraffin blocks were cut with the help of semi motorised rotary microtome (Leica, 2125 DM) to obtain 5-7 µ thick serial paraffin sections. Sections were stained with Haematoxylin and Eosin staining (Luna, 1968) for general histo-architecture.

Cresyl violet and silver staining for neurons and glial cells/astrocytes and Periodic Acid Schiff's (PAS) method for glycogen or neutral muco-polysaccharide as well as for glycolipids (cerebrosides and gangliosides of nervous tissue) were done by method of Bancroft and Stevens (1977) using NBF fixed tissue. The stained slides were observed under light microscope to study the histoarchitecture of the optic tectum. Photomicrography was done with the help of Leica Qwin Image Analyser software in DM 2000 microscope under lower as well as higher magnification.

## Results and Discussion

The medulla oblongata was situated posterior to the optic lobes and ventral to the cerebellum. The medulla oblongata gradually narrowed caudally and continued as the spinal cord towards the foramen magnum and vertebral canal. A ventro-median fissure was prominent along the ventral midline of the pons and medulla oblongata, and it continued caudally in all age groups of broiler chickens. There was no clear-cut demarcation observed between pons and medulla oblongata in any group of chickens (Fig. 1). Similar findings were observed by Nickel *et al.* (1977) in fowl, Peng *et al.*(2010) in African ostrich, and Balkaya and Toprak (2018) in sparrow hawk.

Histologically, the medulla oblongata of broiler chickens was covered by pia matter, which was made up of loose connective tissue with blood vessels in all age groups of birds (Fig. 2). From day 3 onwards, it was chiefly made up of the nucleus and the broad bands of nerve fibres, which were longitudinally placed and had some glial cells scattered among them. In between these longitudinally arranged nerve fibres, spindle-shaped neurons and stellate-type neurons were observed (Fig. 3). Although neurons of different sizes were found in the superficial layer, only medium- to large-sized neuronal cells were observed in the deeper layer of the medulla oblongata in all age groups of broiler chickens (Fig. 4). The present observation was well supported by the findings of Batah *et al.* (2012) in chickens and Karkoura *et al.* (2015) in African ostriches.

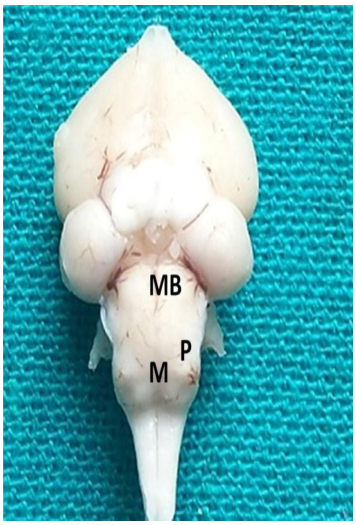


**Fig. 1:** Photograph of ventral view of brain in group IV broiler chicken showing Pons (P) and Medulla oblongata (M) and mid brain (MB).

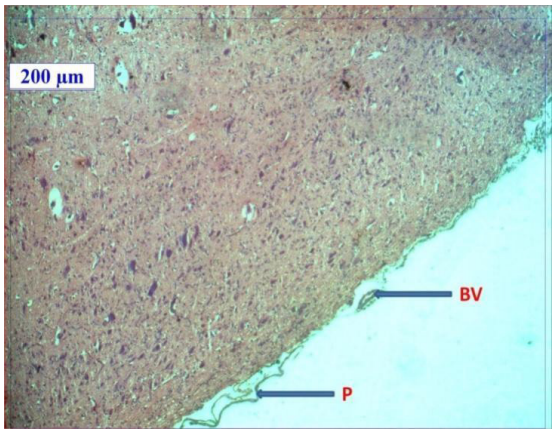


**Fig. 2:** Photomicrograph showing medulla oblongata covered with pia matter (P) (Group-I) (Haematoxylin and Eosin X 100)

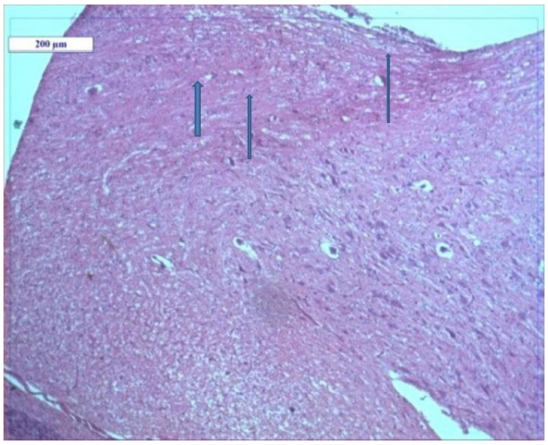


**Fig. 3:** Photomicrograph showing the longitudinally arranged nerve fibres (arrow head) in medulla oblongata (Group-IV) (Haematoxylin and Eosin X 100)

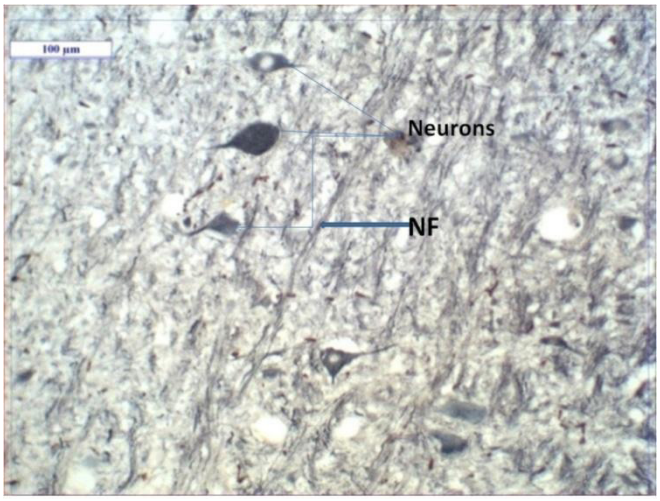


**Fig. 4:** Photomicrograph showing the nerve fibres (arrow head) and different types of neurons in medulla oblongata (Group-V) (Silver Impregnation X 200)

From the beginning the nuclei of the medulla oblongata were formed by a few multipolar neurons, a spindle-shaped neuron, and very few pyramidal neurons supported by a few neuroglia cells (Fig. 5). A similar observation was made by Karkoura *et al.* (2015) in African ostrich. During the present study, the rostral transverse section of the medulla oblongata on either side of the 4$^{th}$ ventricle dorsally had a nucleus that was chiefly made up of multipolar neurons in all age age-groups of broiler chickens (Fig. 6). The fourth ventricle was lined by a single layer of ependymal cells, as earlier reported by Karkoura *et al.* (2015) in an African ostrich.

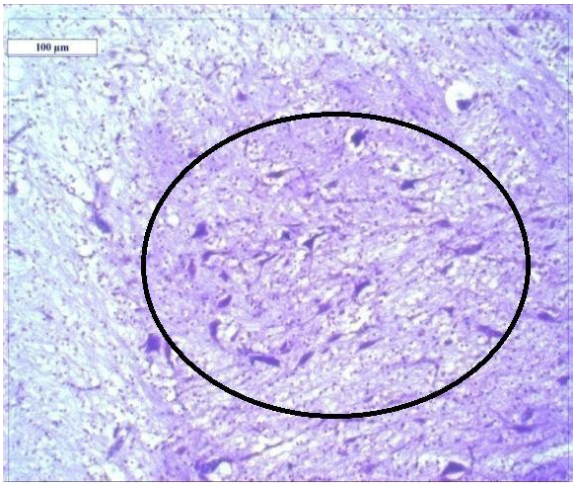


**Fig. 5:** Photomicrograph showing the different types of neurons in nucleus of medulla oblongata (Group-I) (Cresyl violet X 200)

In the present study, weak to mild PAS reactivity was observed in the cellular component of the medulla oblongata in group I (1 day old), whereas it was mild to moderate in the medulla oblongata in group VII (42 days old), the PAS reactivity increased with the advancement of age (Fig. 7). Similar observations were reported by Gerhauser *et al.* (2013) in hamsters and Salankar (2017) in goats.

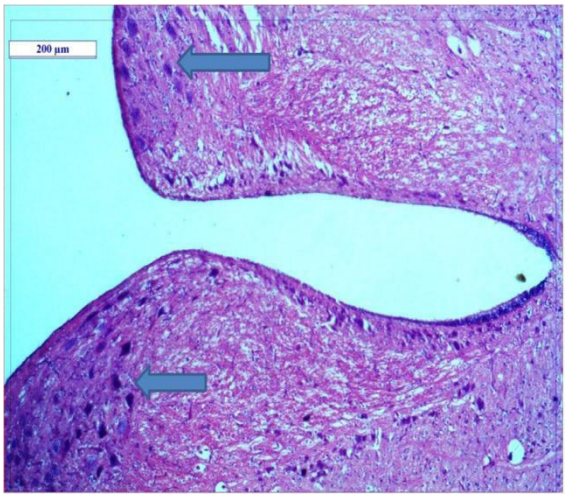


**Fig. 6:** Photomicrograph showing the 4th ventricle in rostral transverse section of medulla oblongata. (Group-IV) (Haematoxylin and Eosin X 100)

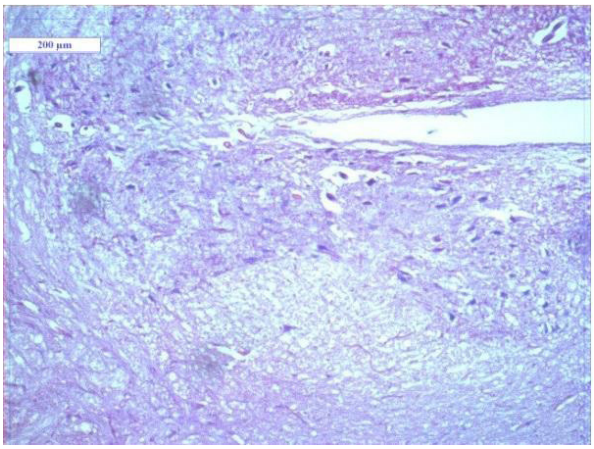


**Fig. 7:** Photomicrograph showing the mild to moderate PAS positive activity in cellular component of medulla oblongata (Group-I) PAS-AB X200

## Conclusion

In brief, medulla oblongata was chiefly made up of the nuclei, longitudinally arranged nerve fibres, spindle shaped neurons and stellate neurons in all age-groups of broiler chicken.

## Acknowledgement

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