

## MICROANATOMICAL OBSERVATIONS ON THE HIPPOCAMPUS (CORNU AMMONIS) IN BROILER CHICKEN

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**Abstract:** The brain samples were collected from broiler birds aged from 2 weeks to 8 weeks at an interval of 2 weeks. Small tissue pieces were collected from dorsomedial part of each cerebral hemisphere and processed for histological studies. The hippocampus appeared as a separate structure demarcated from the rest of the hemisphere by a narrow slit-like lateral ventricle and composed of 4 regions namely, Anterior continuation, Dorsomedial part, Dorsal part and Dorsolateral parts. Anterior continuation constituted of full of small, round and spindle neurons with their processes forming a rich fibrous network. The dorsomedial part showed few granular neurons in 2 weeks old birds and the polymorphous layer had oval and stellate neurons, in all the age groups studied. Small pyramidal neurons were present in 2 and 4 week old birds and they were of medium sized in 6 and 8 week old birds. The dorsal part of hippocampus showed more of arborizing fibres of the spherical neurons and pyramidal cell layer with medium sized pyramidal and polymorphous neurons in 2 and 4 weeks old birds while in 6 and 8 weeks old birds, large pyramidal neurons with dendritic spines were also noticed. In the current study, arrangement of different layers of neurons and neuroglia were compared with various species.

**Keywords:** Microanatomy, Histology, Hippocampus, Chicken, Broiler.

### Introduction

Brain morphology reflects the behavioural repertoire, cognitive capacity and evolutionary history of a species (Kawabe *et al.*, 2013). Role of avian hippocampus in spatial learning, memory, cognitive and navigation is well established (Srivastava *et al.*, 2007). The hippocampus share many pathway connections with septum, hypothalamus, monoaminergic nuclei and telencephalic sensory processing areas in brain (Hampson *et al.*, 1999). Both anatomical and physiological observation recorded proves that the hippocampus is organized in a lamellar fashion and the orientation of the lamella differs among species (Warwick and Willams, 1989). The avian hippocampus shoulders an important role in learning and memory (Sherry *et al.*, 1992). Hence, the hippocampus is larger in food storing birds among the passerine birds. Hippocampus is better developed in birds living water and on the ground though small in some birds such as Parakeet and Sparrow (Sherry and Vaccarino, 1989).

Available literatures suggest that there has not been a continuing thread of neurological research on the hippocampus of chicken. Hence, this study was under taken to explore the histomorphological aspects in the hippocampus of various age groups of broiler chicken reared commercially in organized poultry farms.

### **Materials and Methods**

The brain samples of the broiler birds were collected from the commercial broiler farms in and around the Veterinary College and Research Institute, Namakkal and Poultry Research Station, Chennai. The brain samples obtained were from broiler birds (2 weeks to 8 weeks at an interval of 2 weeks). In broiler, samples were collected from both sexes at the rate of 6 samples per sex for every age group. The heads of birds were separated at the level of 2<sup>nd</sup> cervical vertebrae and the cranial cavity was cut open carefully. The meninges of the brain were dissected out from the bones of attachment. The olfactory lobes and optic nerves at the level of optic chiasma on the ventral surface of the brain were carefully dissected out to remove the whole brain from the cranial cavity. The cerebellum was removed and the cerebral hemispheres were collected by dissection for gross morphological and morphometrical observations. Small tissue pieces were collected from dorsomedial (DM) part of each cerebral hemisphere through the transverse section and were immediately fixed in 10% buffered neutral formalin for 2-3 days. For histological studies the tissues were inducted to further tissue processing viz. dehydration, clearing and embedding procedures were followed as indicated for the brain tissue (Luna, 1968).

Paraffin impregnation was performed in a thermostatically controlled oven to prepare the paraffin blocks. The trimmed paraffin blocks were cut with the help of a semi-motorized rotary microtome (Leica RM make) to obtain 5-20  $\mu\text{m}$  thick paraffin section as warranted for different staining procedures. The tissue sections were mounted on clean, grease free, albumenized glass slides. The tissues sections were stained with the routine and special staining procedures viz. 1. Ehrlich's haematoxylin and Eosin staining for routine histological observations, 2. Golgi Cox method for nerve cells, 3. Vogt's method for nuclei and Nissl substance, 4. Holme's method for nerve cells and fibres, 5. Bielschowsky's method for axis cylinders and dendrites, 6. Ramon Y Cajal's method for staining astrocytes.

### **Results and Discussion**

The histological observations were recorded from the hippocampus and adjacent associated structure i.e. hippocampal formation along with lateral ventricle. The hippocampus appeared as a separate structure demarcated from the rest of the hemisphere by a narrow slit-like lateral

ventricle. The hippocampus of the chicken, a non food storer, was smaller than the hippocampus of the Homsing Pigeons, Owl and European Magpie and these are food storers. In these birds, the hippocampus was said to occupy the entire dorsomedial wall of the cerebral hemisphere (Rehkamper *et al.*, 1988). Four continuous regions were discernible in the hippocampus of the chicken based on the histomorphological differences. However, the sexual dimorphism did not exist. The 4 histologically demarcated regions were termed as 1. Anterior continuation, 2. Dorsomedial part, 3. Dorsal part and 4. Dorsolateral part.

### **1. Anterior continuation**

This part was constituted of full of small, round and spindle neurons with their processes forming a rich fibrous network (Fig.1). In 2 weeks old birds, the terminal part of the anterior continuation just in front of the dorsomedial part, had smaller spherical neurons. In 4 and 6 week old birds, the neurons were medium sized and oval and in 8 weeks old birds, they were larger and stellate. Ariens Kappers *et al.*, (1960) found that in Dog fish Shark, the anterior continuation has a band of neurons in between the fibres. This is in consonant with the present study but unlike in Dog fish Shark, the dorsal part of the hippocampus in chicken showed clear lamination. Neurons of several contours such as spindle and large multipolar pyramidal types recorded in the hippocampus of Dog fish shark, were also observed in the corresponding part of that of chicken.

The multi laminar pattern recorded in the anterior continuation as plexiform, granular and polymorphous layer in the hippocampus of Edentate (Humphrey, 1966) is not agreeable with the present study as the anterior continuation in chicken did not have three layers. Similarly, variation in the configuration of the anterior continuation of the hippocampus in chicken, when compared to Rodents (Siegal *et al.*, 1974) was also observed. Because, in that part in chicken, medium sized pyramidal cell column at four cell thickness was not recorded as present in rodents.

### **2. Dorsomedial part**

In 2 week old birds, the fibrous layer had a few granular neurons. The polymorphous layer had oval and stellate neurons, in all the age groups studied. Small pyramidal neurons were present in 2 and 4 week old birds (Fig.2) and they were of medium sized in 6 and 8 week old birds. The vascularization in the form of capillaries were seen throughout the hippocampus and ependymal lining of lateral ventricle. In the Eel, a small zona limitans of fibres is observed between the subiculum and cornu Ammonis (Pearson and Pearson, 1976). This fact is acceptable with the present study, where a ventricular fissure was found in between the

dorsolateral part and dorsal part of the hippocampus in chicken in all the age groups of broiler chicken.

### **3. Dorsal part**

The first layer was composed of more of arborizing fibres of the spherical neurons. The second, pyramidal cell layer had medium sized pyramidal and polymorphous neurons in 2 and 4 weeks old birds while in 6 and 8 weeks old birds, large pyramidal neurons with dendritic spines were also noticed. In 2 to 6 weeks old birds the polymorphous layer revealed clusters of spherical, round, oval and stellate neurons whereas small pyramidal neurons were seen in 8 week old birds. In 2 to 4 week old birds, the last lateral most layer possessed a rich network of fibres with a few smaller round neurons and it was medium sized in 6 and 8 week old birds. Occasional double pyramidal cells and a separate small pyramidal cells layer formed out of the polymorphous layer and a small polymorphous layer formed out of the large celled layer were the characteristic features in the 8 weeks old birds. Like in Owl and unlike in Tinamou bird, the anterior continuation and the dorsomedial part of the hippocampus of chicken was distinct. However, the dorsal and dorsolateral part in the owl and Tinamous bird is well developed, as observed in the present study (Abd-Alrahman, 2012).

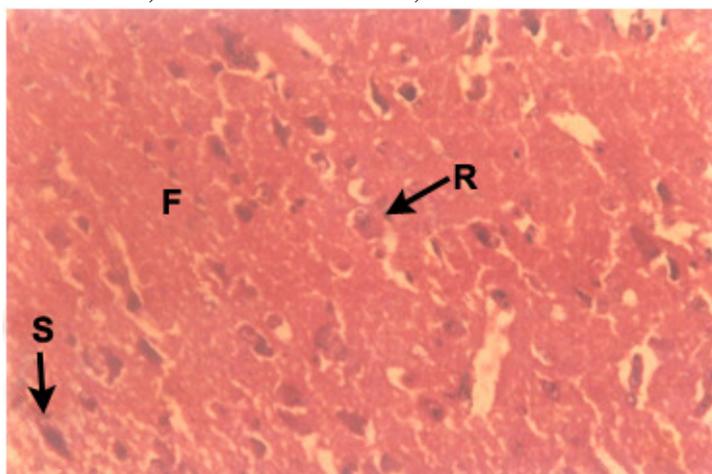
In the hippocampus of chicken, the dorsal and dorsolateral part differed in their laminar pattern by revealing 4 to 6 layers and only 4 layers respectively. But in Rhyncotous bird, both these parts are identical with respect to number of layers. The dorsal part of the hippocampus in chicken had at the most of six layers whereas the corresponding part in mammal has seven layers (Lopes Da Silva *et al.*, 1990).

### **4. Dorsolateral part**

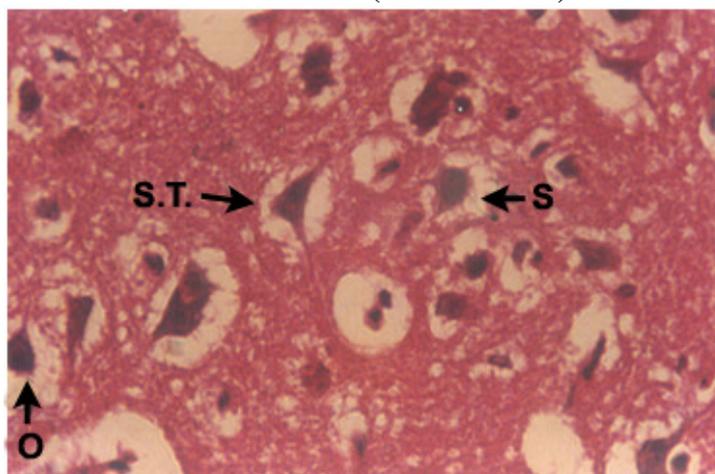
The dorsolateral part of the hippocampus in chicken had four layers which was different from the finding of Warwick and Williams (1989) as the corresponding part, termed subiculum in mammalian hippocampus had only two layers. The small round neurons with their processes formed the fibrous meshwork in the first layer. The pyramidal cell layer was of lesser density than the dorsal part and had small to medium sized neurons. Few large pyramidal neurons were seen in the 6 and 8 week old birds. The mixed cell layer had only small, round or oval neurons in 2 and 4 weeks old birds. In 6 and 8 weeks old birds it was interspersed with some small pyramidal neurons (Fig.3). The fourth layer was an admixture of smaller oval neurons and fibres whose density increased with the age.

The type of neuronal population such as double pyramidal cells and glial cells recorded in the hippocampus of rat fish (Faucette, 1969) was also recorded in the various components of the hippocampus in chicken in 8 weeks old broiler birds. Aggregation of neurons of various sizes in the cornu Ammonis in the paddle fish is in concurrence with the histological structure of the cornu Ammonis in chicken. In all the age groups studied, the shape of the nucleus of the neurons was constantly spherical, large and central in position with clear nucleolus. The thin cytoplasm of the neurons had irregular aggregations of the Nissl bodies (Fig.4).

**Figure 1.** Photmicrograph showing anterior continuation of hippocampus in 2 weeks old broilers. S - Spindle neurons, R - Round neurons, F - Rich fibrous network (H&E X 100)



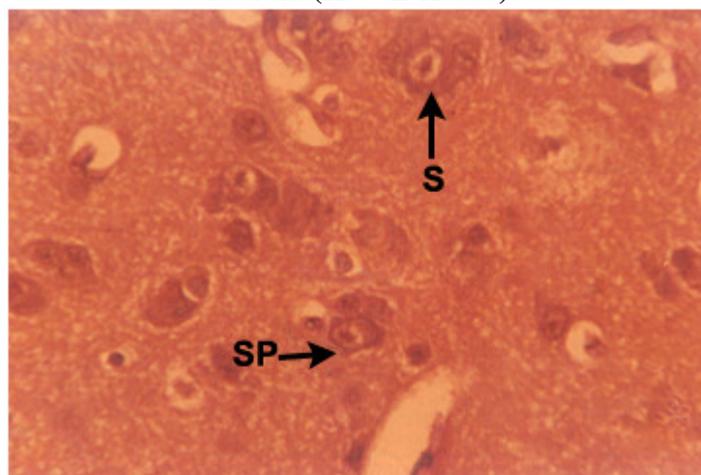
**Figure 2.** Photomicrograph showing the polymorphous layer in the dorsal medial part of the hippocampus in 4 weeks old broilers. S - Small pyramidal neuron, O - Oval neuron, S.T. - Stellate neuron. (H & E X 500)



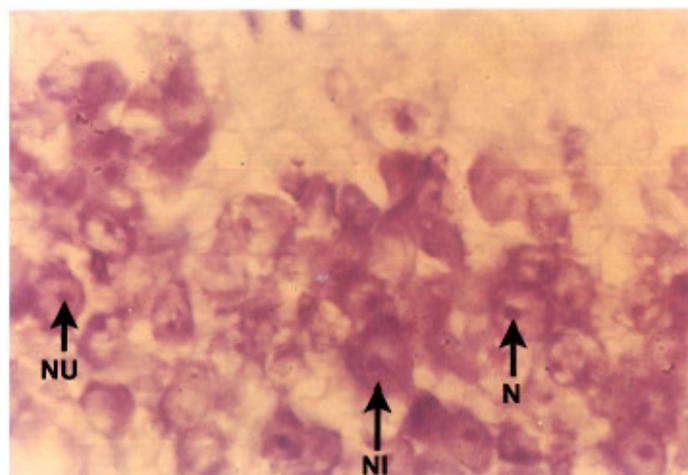
The glial cell population was more in dorsal part, less in the anterior continuation and moderate in the rest of the regions. The astrocytes and oligodendroglia were clustered with the

neurons, forming a thick feltwork by their short, branching processes. Some of the processes of the astrocytes had close association with the blood vessels and formed the vascular pedicles. Small microglia showed short processes with spines as reported by Potier *et al.* (1985) in primates and Machado and Alessi (1997) in the hippocampus of adult cattle.

**Figure 3.** Photomicrograph showing the mixed cell layer in the dorso-lateral part of the hippocampus in 8 weeks old broilers. S – Spherical neurons in clusters, SP – Small pyramidal neuron. (H & E X 300)



**Figure 4.** Photomicrograph showing the dorsal part of the Hippocampus in 8 weeks old broiler birds. N – Nucleus, NU – Nucleolus, NI – Nissl substance. (Cresyl Violet X 500)



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