



Identification of the Different Types of Neurons in the Cerebral Cortex of Post Hatch Broiler Chicken

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10.18805/IJAR.B-5503

ABSTRACT

Background: Broiler chickens are most widely utilized among all poultry species across the world. As brain of birds is capable of sophisticated cognitive, social and motor behaviours, it is not surprising that they possess large and complex brains. The cognitive ability of a species might be due to its total number of brain neurons.

Methods: A total of seventy (70) day-old broiler chicks were reared up to 42 days. The whole experimental period of study was divided into seven (07) at weekly interval. Brain tissues preservation and slide preparation were made as per standard protocol. The histological slides were stained with Cresyl violet and Haematoxylin and Eosin stains.

Result: The microstructure of cerebral cortex in almost all age groups of broiler chicken showed two regions *i.e.* pallium and subpallium. The former one was identified as hyperpallium, dorsolateral corticoid area, mesopallium, nidopallium, archipallium, piriform cortex and the hippocampal complex. In all age groups of broiler chicken the hyperpallium was differentiated into fibro molecular layer or reticular layer, hyperpallium accessorium (HA), intercalated hyperpallium accessorium (IHA), hyperpallium intercalates suprema (HISM) and hyperpallium densocellulare (HD). Based on the shape, different types of neurons were identified in different layers of the cerebral cortex.

Key words: Cortical area, Histological, Neurons, Poultry.

INTRODUCTION

Poultry industry has witnessed rapid expansion in the last few decades due to the increasing demand for poultry meat (Anurag, 2025). Broiler chickens are one of the primary sources of meat worldwide. They are bred specifically for rapid growth and efficient meat production, providing a reliable and affordable protein source for millions of people. Fast-growing chicken strains are favored for industrial and consumer use, while slow-growing strains offer healthier meat with less fat, better with consumer health preferences (Anita, 2019). The development of the brain in broiler chickens is a critical aspect of their overall growth and welfare, influencing behavior, learning and feeding efficiency. As brain of birds is capable of sophisticated cognitive, social and motor behaviors, it is not surprising that they possess large and complex brain. The cognitive ability of a species might be due to its total number of brain neurons (Herculano-Houzel, 2011). The avian cerebral hemispheres are pear-shaped structure situated on the dorsal view of brain (Balkaya, 2016). The cerebral hemispheres of birds consist of two regions, the dorsal pallium and ventral subpallium. The pallium comprised extensive outer and larger inner cortical regions. The outer area is the hyperpallium (wulst) and the inner cortical area is dorsal ventricular ridge (Alrahman, 2012). The dorsal ventricular ridge has three sub divisions, the mesopallium, nidopallium and archipallium. The subpallium occupying the inner parts is further divided into the striatum and the pallidum. The well-developed cerebrum in birds helps in body movements during flight and also provides a great

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How to cite this article: Gautam, A.K., Ray, S., Das, P., Mandal, A.K., Sinha, M.K., Bharti, S.K. and Azad, C.S. (2025). Identification of the Different Types of Neurons in the Cerebral Cortex of Post Hatch Broiler Chicken. Indian Journal of Animal Research. 1-9. doi:10.18805/IJAR.B-5503.

Submitted: 10-10-2024 **Accepted:** 27-02-2025 **Online:** 19-05-2025

capacity for learning, memory, attention, integration and consciousness (Alrahman, 2012). The cerebral cortex is composed of six histological layers containing an outer molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer and multiform layer, while the medulla contains dense bundle of fibers and glial nerve cells (Banks, 1993, Eurell and Frappier, 2006 and Batah *et al.*, 2012). In all layers of the cerebral cortex showed the presence of large number of pyramidal cell, satellites cell and blood vessels (Karkoura *et al.*, 2015). The use of brain morphometry can greatly enhance our understanding of various aspects related to physiology, ecology and developmental changes (Panigrahy *et al.*, 2018). The importance of the cerebral cortex in various motor and cognitive functions has drawn

scientist's attention to the study of its age-related modifications in the last few decades. Decreases in the functional capacity of the central nervous system with age occur universally in all living organisms. For instance, significant alteration in the gait control, sleeping cycle and learning and memory with age are the three commonest neural impairments in aged humans. In conclusion, understanding the development of the brain in broiler chickens is essential for improving welfare and productivity in poultry farming. By fostering optimal developmental conditions, producers can ensure healthier birds that are better equipped to thrive in their environments.

MATERIALS AND METHODS

Ethical approval

The current retrospective study received approval from the institutional animal ethics committee (IAEC) of the faculty of veterinary and animal sciences at the West Bengal university of animal and fishery sciences, Kolkata-700037 (Reference No. IAEC/67/III, B, dated 19/08/2019).

Rearing of birds

A total of seventy (70) day-old broiler chicks were reared for 42 days (market age) in the experimental pens (cage system) at the Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata-700037, India. Standard management practices, including housing, feeding and vaccination, were uniformly applied to all birds. The chicks had access to feed (starter followed by grower feed) and water *ad libitum* throughout the experimental period. The study was divided into seven (7) intervals at weekly increments (days 3, 7, 14, 21, 28, 35 and 42). The birds were randomly assigned to seven (07) groups (Groups I to VII), each consisting of ten (10) birds, regardless of sex.

Collection of specimens

The birds were euthanized by administering an overdose of sodium pentobarbital (IP) at a dose of 120 mg/kg at weekly intervals and brain samples were carefully collected (Gourdan, 2016; Gautam *et al.*, 2020).

Histological observation

For histological studies, tissue samples were fixed in 10% neutral buffered formalin (NBF) for 48 hours at room temperature after recording biometrical parameters. Following fixation, the tissues were rinsed in running tap water overnight, dehydrated through ascending grades of ethyl alcohol (70%, 80%, 90%, 95% and absolute alcohol) and then cleared in acetone and benzene before undergoing paraffin impregnation in a thermostatically controlled oven to create paraffin blocks (Luna, 1968). The trimmed paraffin blocks were sectioned using a semi-motorized rotary microtome (Leica, 2125 DM) to produce serial sections measuring 5-7 microns thick. The sections were stained with Hematoxylin and Eosin (Luna, 1968) for general histoarchitecture, Cresyl violet (Bancroft and

Stevens, 1977) and Toluidine blue stain (Sivaraman *et al.* 2015) to identify neurons and glial cells and Silver staining for neurons and glial cells/ astrocytes (Bancroft and Stevens, 1977). Stained slides were examined under a light microscope to assess the histoarchitecture of the cerebrum. Photomicrography and histomorphometry were performed using the Leica Qwin Image Analyzer software on a DM 2000 microscope, under both lower and higher magnifications.

Data analysis

For statistical analysis, data was entered into a Microsoft excel spreadsheet and then analyzed by IBM SPSS (version 20.0). Data had been summarized as mean and standard error. Analysis of variation (ANOVA) is used With 'F' statistics to find the group variation. Tukey's HSD test is used for multiple comparison of mean.

RESULTS AND DISCUSSION

Cerebrum

In the current study, the cerebral cortex of brain showed two region i.e Pallium and Subpallium in broiler chicken of all age group. Former identified as hyperpallium, dorsolateral corticoid area, mesopallium, nidopallium, archipallium and the hippocampal complex as earlier identified by Alrahman (2012) in the brain of Barn Owl, Abid and Al-Bakri, (2016) in quail and Nakeeb and Jasim (2018) in the *Columba livia domestica*.

Pallium

Hyperpallium

In the present study the hyperpallium was observed as multilaminated cellular structures at the dorsal side of each cerebral hemisphere in broiler chicken. From day 7 onwards, it was identified as fibro molecular layer or reticular layer, Hyperpallium accessorium (HA), Intercalated hyperpallium accessorium (IHA), Hyperpallium intercalates suprema (HISM) and Hyperpallium densocellulare (HD) in broiler birds (Fig 1). Similar laminated structures of hyperpallium were observed by Alrahman (2012) in the brain of Barn Owl, Abid and Al-Bakri, (2016) in quail and Nakeeb and Jasim (2018) in the *Columba livia domestica* in support of present findings. Iwaniuk and Wylie (2006) documented that the hyperpallium neurons were selective for orientation, movement direction, spatial frequency and binocular disparity in potoos (Nyctibidae) and nightjars (Caprimulgidae) birds.

Fibromolecular or reticular layer

In group I of broiler chicken, the fibromolecular layer was differentiated from rest of the hyperpallium layer which had few small scattered neurons and dendritic extensions with horizontally oriented axons. From Group II onwards, the stellate neurons, spherical neurons, glial cells and few horizontal cells were identified (Fig 2).

The mean diameter of stellate neurons were recorded as $5.55 \pm 0.45 \mu$ in group I, $7.02 \pm 0.39 \mu$ in group II, $7.55 \pm 0.31 \mu$ in group III, $11.09 \pm 0.86 \mu$ in group IV, $10.32 \pm 0.51 \mu$ in group

V, $10.59 \pm 0.63 \mu$ in group VI and in group VII it was $11.05 \pm 0.56 \mu$. The mean diameter of spherical neurons and glial cells were recorded as $6.24 \pm 0.41 \mu$ and $2.58 \pm 0.16 \mu$ in group I, $7.55 \pm 0.30 \mu$ and $2.70 \pm 0.20 \mu$ in group II, $9.04 \pm 0.38 \mu$ and $3.53 \pm 0.19 \mu$ in group III, $10.24 \pm 0.67 \mu$ and $4.07 \pm 0.34 \mu$ in group IV, $9.50 \pm 0.46 \mu$ and $3.71 \pm 0.16 \mu$ in group V, $9.74 \pm 0.54 \mu$ and $4.01 \pm 0.25 \mu$ in group VI and $10.20 \pm 0.55 \mu$ and $4.22 \pm 0.19 \mu$ (Table 1). Present findings were in accordance with Abd-Alrahman (2012) in Barn owl, Abid and Al-Bakri (2016) in quail and Nakeeb and Jasim (2018) in the *Columba livia domestica*. Abid and Al-Bakri (2016) found that the mean diameter of stellate neuron, spherical neurons and glial cells were $10 \mu\text{m}$, $7 \mu\text{m}$ and $2 \mu\text{m}$ respectively in adult quail which is slightly less than the present findings in broiler chicken; it might be due to species variation.

Reticular layer of different group under study statistically analysed and it was revealed that the mean diameter of stellate, spherical neurons and glial cells were increased from group I onwards. The maximum diameter of stellate neurons was in group IV, but it was not significant from group I to III. However, the highly significant values were observed from group I to III with that of group IV to VII in broiler chicken (Table 1). The maximum diameter of spherical neurons and glial cells were in group IV and group VII respectively.

The spherical neurons were significantly less in group I from the rest of groups. The glial cells were not significant in group I and group II. However, first three groups (I to III) were highly significant with rest groups (IV to VII). The maximum diameter of stellate and spherical neurons was in group VII.

Hyperpallium accessorium (HA)

In present observation, at day 3 broiler chicks had different types of neurons in hyperpallium accessorium. From day 7 onwards (group II) chiefly the stellate and spherical neurons were dominated (Fig 2). Similar findings was made by Abid and Al-Bakri (2016) in quail and Nakeeb and Jasim (2018) in the *Columba livia domestica*. The mean diameter of stellate and spherical neurons were recorded as $7.54 \pm 0.57 \mu$ and $5.53 \pm 0.41 \mu$ in group I, $8.24 \pm 0.50 \mu$ and $6.09 \pm 0.41 \mu$ in group II, $9.03 \pm 0.57 \mu$ and $8.52 \pm 0.45 \mu$ in group III, $10.65 \pm 0.59 \mu$ and $9.44 \pm 0.50 \mu$ in group IV, $10.59 \pm 0.53 \mu$ and $9.24 \pm 0.51 \mu$ in group V, $10.66 \pm 0.84 \mu$ and $9.53 \pm 0.44 \mu$ in group VI and in group VII it was $10.76 \pm 0.53 \mu$ and $9.77 \pm 0.58 \mu$ (Table 1).

Hyperpallium accessorium of different group under study statistically analysed and it was revealed that the mean diameter of stellate and spherical neurons was increased from group I onwards. The mean diameter of stellate neurons of group I was highly significant with group IV onwards. However, no significant difference was observed from group I to III and group IV to VII. The mean diameter of spherical neurons was highly significant in first two group (I and II) with rest of the groups (group III to VII). However, no significant difference was observed from

group I to II and group III to VII. Abid and Al-Bakri (2016) recorded the mean diameter of stellate and spherical neurons were $9 \mu\text{m}$ and $5 \mu\text{m}$ respectively in quail.

Intercalated hyperpallium accessorium (IHA)

From the beginning of the experiment this layer was dominated with stellate neurons and few glial cells. By group III onwards few small pyramidal cells were well differentiated as earlier reported by Nakeeb and Jasim (2018) in the *Columba livia domestica* (Fig 2 and 3). The mean diameter of stellate neurons were recorded as in group I $8.58 \pm 0.47 \mu$, $10.08 \pm 0.57 \mu$ in group II, $12.03 \pm 0.75 \mu$ in group III, $12.56 \pm 0.58 \mu$ in group IV, $12.10 \pm 0.78 \mu$ in group V, $12.20 \pm 0.85 \mu$ in group VI and $12.50 \pm 0.83 \mu$ in group VII

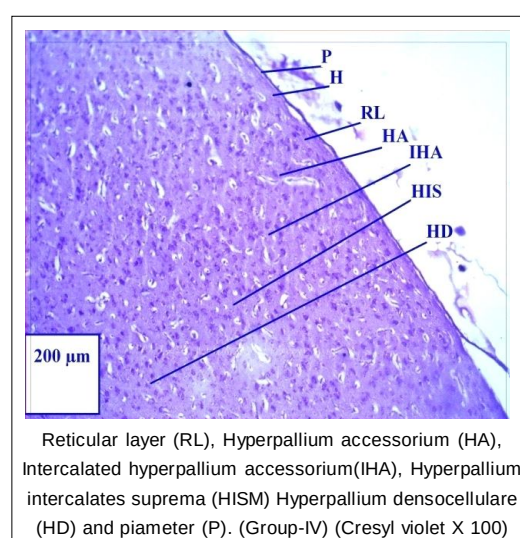


Fig 1: Photomicrograph showing different layer of hyperpallium (H).

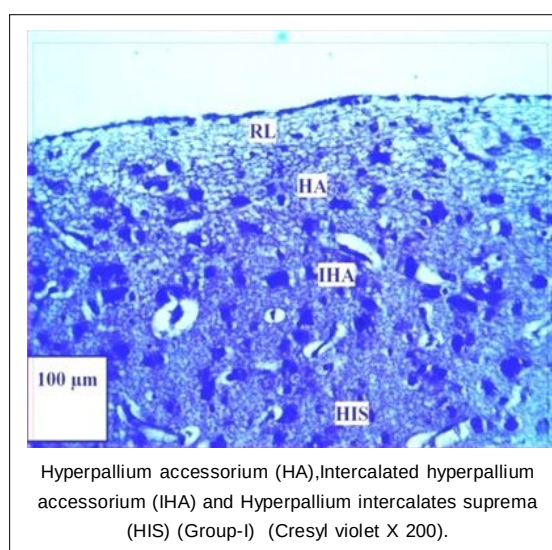


Fig 2: Photomicrograph of hyperpallium showing the neurons in Reticular layer (RL),

Table 1: Micrometrical observations showing mean diameter of neurons in different layers of hyperpallium of cerebral cortex from group I to VII.

Group	Fibro molecular layer			Hyperpallium accessorium (HA)		Intercalated hyperpallium accessorium (HA layer)	Hyperpallium intercalates suprema (HISM)	Hyperpallium densocellulare (HD)			
	Stellate neurons	Spherical neurons	Stellate neurons	Fusiform neurons	Spherical neurons	Stellate neurons	Fusiform neurons	Pyramidal neurons	Stellate neurons	Fusiform neurons	Spherical neurons
I	5.55±.45 ^b	6.24±.41 ^c	8.58±.47 ^b	11.15±.56	5.53±.41 ^b	8.58±.47 ^b	11.15±.56	14.49±.70	6.63±.45 ^b	10.73±.50 ^a	8.04±.51
II	7.02±.39 ^b	7.55±.30 ^{bc}	10.08±.57 ^a	13.04±.60	6.09±.41 ^b	10.08±.57 ^a	13.04±.60	14.57±.63	6.97±.39 ^{ab}	8.93±.44 ^b	9.02±.37
III	7.55±.31 ^b	9.04±.38 ^{ab}	12.03±.75 ^a	14.64±.94	8.52±.45 ^a	12.03±.75 ^a	14.64±.94	15.42±.86	7.73±.48 ^{ab}	10.64±.58 ^a	9.45±.54
IV	11.09±.86 ^a	10.24±.67 ^a	12.56±.58 ^a	14.01±.1.44	9.44±.50 ^a	12.56±.58 ^a	14.01±.1.44	15.17±.89	9.12±.56 ^a	11.38±.91 ^a	10.13±.57
V	10.32±.51 ^a	9.50±.46 ^{ab}	12.10±.78 ^a	14.05±.83	9.24±.51 ^{ab}	12.10±.78 ^a	14.05±.83	15.03±.1.29	8.94±.71 ^a	10.75±.83 ^a	10.15±.64
VI	10.59±.63 ^a	9.74±.54 ^a	12.20±.85 ^a	14.10±.93	9.53±.44 ^a	12.20±.85 ^a	14.10±.93	15.18±.1.27	9.07±.48 ^a	10.78±.69 ^a	10.13±.65
VII	11.05±.56 ^a	10.20±.55 ^a	12.50±.83 ^a	14.40±.84	9.77±.58 ^a	12.50±.83 ^a	14.40±.84	15.26±.1.11	9.24±.57 ^a	8.58±.48 ^b	10.18±.87
F-value	16.47	9.64	4.62	1.71	13.56	4.62	1.71	.125	4.40	2.61	1.77
P-value											

(Table 1). These observations of the present study showed that mean diameter of stellate neurons in group I was highly significant with that of rest of groups. However, no significant difference was observed from group II to group VII. Abid and Al-Bakri (2016) reported the mean diameter of stellate neuron was 8 μ m in intercalated hyperpallium accessorium in one month age of quail birds in support of present findings of group I of broiler chickens.

Hyperpallium intercalates suprema (HISM)

By day 3 post hatch broiler chicks HISM layer was differentiated into fusiform neuron, few stellate cells and glial cell. From group II onwards fusiform neurons and glial cells were dominated (Fig 2,3 and 4). The mean diameter of fusiform neurons were recorded as 11.15±0.56 μ m in group I, 13.04±0.60 μ m in group II, 14.64±0.94 μ m in group III, 14.01±1.44 μ m in group IV, 14.05±0.83 μ m in group V, 14.10±0.93 μ m in group VII and it was 14.40±0.84 μ m in group VII. Present observations showed that mean diameter of fusiform neurons were increased from group I onwards and maximum was in group III. However, statistically there was no significant differences were observed from group I to group VII (Table 1). In support of present findings, Abd-Alrahman (2012) in Barn owl and Abid and Al-Bakri (2016) reported the presence of fusiform neurons in this layer and its mean diameter was 7 μ m quail birds.

Hyperpallium densocellulare (HD)

Hyperpallium densocellulare (HD) was last and deepest layer of hyperpallium in all group of broiler chicken. From group I onwards mainly four types of neurons were differentiated i.e. pyramidal cells, stellate cells, fusiform cells and spherical cells. Beside these, glial cells were also present (Fig 5). The mean diameter of pyramidal neurons was varied from 14.49±0.70 μ m in group I to 15.26±1.11 μ m in group VII, whereas the maximum was 15.42±0.86 μ m in group III. Statistically there was no significant differences were observed from group I to group VII (Table 1). The mean diameter of stellate neurons were varied in group I 6.63±0.45 μ m to 9.24±0.57 μ m in group VII.

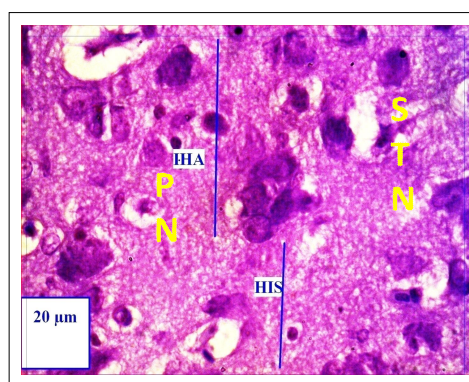


Fig 3: Photomicrograph showing stellate neurons (STN) and pyramidal cells (PN) in IHA layer of hyperpallium. (Group-III). (Haematoxylin and Eosin X 1000).

The mean diameter of stellate neurons were statistically analysed and observed that group I was highly significant with group IV and remaining all were non significant (Table 1). The mean diameter of fusiform neurons were varied from 10.73 ± 0.50 in group I and $8.58 \pm 0.48 \mu$ in group VII, whereas the maximum was $11.38 \pm 0.91 \mu$ in group IV. Statistically analysis showed that group II and group VII was significant with all other groups. The mean diameters of spherical neurons were varied from $8.04 \pm 0.51 \mu$ in group I and $10.18 \pm 0.87 \mu$ in group VII. Statistically there was no significant differences were observed from group I to group VII (Table 1). Present findings were in accordance with Nakeeb and Jasim (2018) in the *Columba livia domestica* and Abid and Al-Bakri (2016) who recorded the pyramidal shaped neurons with mean diameter of 18μ , stellate neurons with 10μ diameter and fusiform neurons of 10μ mean diameter in quail. Abd-Alrahman (2012) had not reported pyramidal shaped neurons in in Barn owl.

Dorsolateral corticoid area

The dorsolateral corticoid area (DLC) of cerebral cortex was superficial and had horizontally placed fusiform neurons, stellate and spherical neurons in group I, however, it was well differentiated from group II onwards. (Fig 6, 7 and 8). The mean diameter of fusiform neurons were varied from $14.96 \pm 1.09 \mu$ in group I to $19.92 \pm 1.21 \mu$ in group VII, where as the maximum was in $20.18 \pm 1.51 \mu$ in group IV. A significant difference was observed in mean diameter of fusiform neurons in group I and group II, however from group II onwards no significant difference was observed with advancement of age. The mean diameter of spherical neurons were varied from $7.02 \pm 0.54 \mu$ in group I to $10.64 \pm 0.72 \mu$ in group VII. It was gradually increased with advancement of age but no significant difference was observed (Table 2). In group I, the mean diameter of stellate neurons was recorded $7.53 \pm 0.43 \mu$ and $9.66 \pm 0.73 \mu$ in group VII (Table 2). A significant difference was observed in

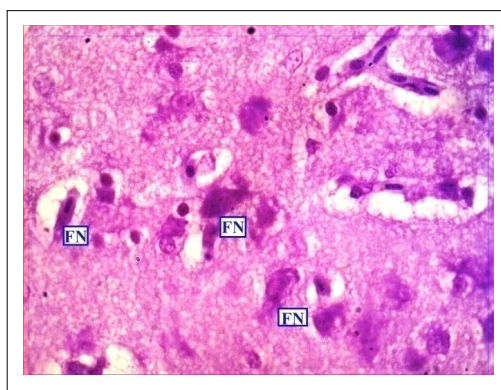


Fig 4: Photomicrograph showing fusiform neurons (FN), stellate neurons and glial cell in HISM layer of hyperpallium. (Group-II). (Haematoxylin and Eosin X 1000).

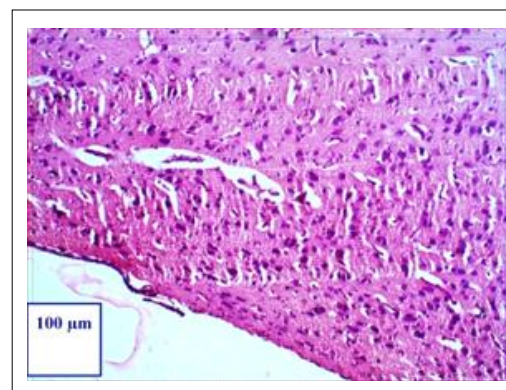


Fig 6: Photomicrograph showing the dorsolateral corticoid area (DLC) of cerebral cortex. (Group-I). (Haematoxylin and Eosin X 200).

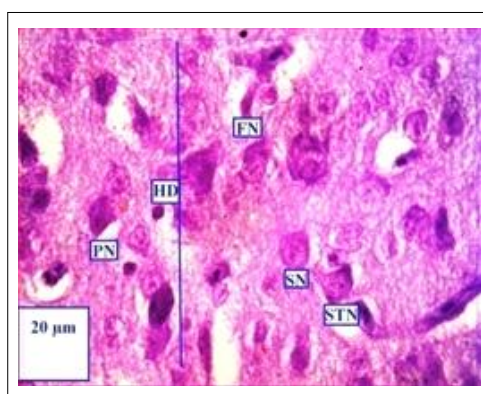


Fig 5: Photomicrograph showing fusiform neurons (FN), stellate neurons (STN), Pyramidal neuron (PN) and spherical neuron (SN) in HD layer of hyperpallium. (Group-VII). (Haematoxylin and Eosin X 1000).

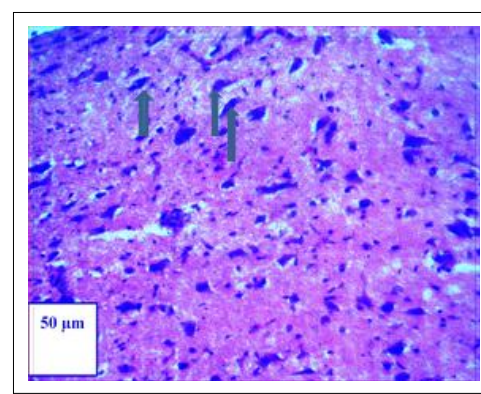


Fig 7: Photomicrograph showing fusiform neurons (arrow) in dorsolateral corticoid area (DLC) of cerebral cortex. (Group-III). (Cresyl violet X 400).

mean diameter of stellate neurons between group I and II. However, from group II onwards no significant difference was observed with advancement of age. Present findings regarding the presence of different types of neurons were well supported by Shepherd (1994) in adult birds, Srivastava *et al.* (2009) in Estrilda amandava birds, Abd-Alrahman (2012) in Barn owl and Abid and Al-Bakri (2016) in fore brain of quail who recorded the mean diameter of stellate neurons (10 μ m), spherical neurons (9 μ m) and horizontal neurons of fusiform (20 μ m).

Mesopallium

It was situated just deep to the dorsolateral cortical area in cerebral cortex of broiler chicken. In group I, the cells of mesopallium was identified as different types of cells like pyramidal, stellate, horizontal neurons and glial cells. These cells were well differentiated into group II onwards (Fig 9 and 10). The mean diameter of pyramidal neurons were varied from $7.11 \pm 0.57 \mu$ in group I to $11.08 \pm 0.59 \mu$ in group VII. The mean diameter of pyramidal neurons were statistically analysed and observed that group I and II was significant with group VII and remaining all were non significant (Table 2). The mean diameter of spherical neurons, horizontal neurons and glial cells were varied from $6.65 \pm 0.42 \mu$, $11.06 \pm 0.68 \mu$ and $3.26 \pm 0.27 \mu$ in group I to $9.13 \pm 0.77 \mu$, $13.04 \pm 0.65 \mu$ and $4.89 \pm 0.21 \mu$ in group VII respectively. The statistical analysis of spherical neurons and glial cells showed significant differences in group I with group V, VI and VIII. No significant differences were observed in horizontal neurons with advancement of age. (Table 2). In accordance with the findings of the present study, Abd-Alrahman (2012) reported the presence of a heterogeneous neuron groups and pyramidal cells in Barn owl and Abid and Al-Bakri (2016) observed the pyramidal neurons with mean diameter of (7.5 μ m), spherical neurons (7.5 μ m) and horizontal neurons (18 μ m) in mesopallium of quail.

In present study the nidopallium was situated around the lateral ventricle in all age groups of broiler chicken. By day 3 of post hatch, it had pyramidal neurons and glial cells (Fig 11). From group III onwards large pyramidal neurons contained apical single thick dendrite extended to the upper layers of the cortical area, besides these cells few spindle shaped fusiform and multipolar neurons were observed (Fig 11 and 12). The mean diameter of pyramidal neurons and glial cells were varied from $11.86 \pm 0.74 \mu$ and $3.35 \pm 0.19 \mu$ in group I to 12.96 ± 0.92 and $5.08 \pm 0.25 \mu$ in group VII respectively. The maximum mean diameter of pyramidal neurons and glial cells were observed in group V. The statistical analysis of mean diameter of pyramidal neurons showed that there was no significant difference was observed with advancement of age. However, glial cells of group I was highly significant with group III to VII (Table 2). Weathers (1992) documented that nidopallium in birds represented as centre for neuronal input and output of the brain. Abd-Alrahman (2012) reported that the higher density of large, pyramidal cells was present in the

Table 2: Micrometrical observations showing mean diameter of neurons in different layers of pallium of cerebral cortex from group I to VII.

Group	Dorsolateral corticoid area				Mesopallium			Nidopallium			Archipallium			Piriform cortex		
	Fusiform neurons	Spherical neurons	Stellate neurons	Pyramidal neurons	Spherical neurons	Pyramidal neurons	Glial cells	Pyramidal neurons	Spherical neurons	Fusiform neurons	Stellate neurons	Multipolar neurons	Pyramidal neurons	Glial cells		
I																
II	14.96 ± 1.09^a	$7.02 \pm .54^b$	$7.53 \pm .43^a$	$7.11 \pm .57^b$	$6.65 \pm .42^b$	$11.06 \pm .68$	$3.26 \pm .27^c$	$11.86 \pm .74$	$3.35 \pm .19^c$	$12.80 \pm .64^b$	$6.89 \pm .31$	14.11 ± 1.02	$10.10 \pm .91^b$	$12.64 \pm .65$	$7.50 \pm .51^b$	$4.73 \pm .34$
III	16.55 ± 1.20^b	$7.88 \pm .56^{ab}$	$8.11 \pm .44^b$	$7.38 \pm .61^b$	$7.12 \pm .44^{ab}$	$12.12 \pm .1.13$	$3.54 \pm .30^{bc}$	$12.17 \pm .53$	$3.92 \pm .23^{bc}$	$13.93 \pm .77^b$	$7.78 \pm .48$	$14.88 \pm .94$	$11.58 \pm .55^b$	$13.19 \pm .63$	$8.70 \pm .48^{ab}$	$5.50 \pm .38$
IV	19.12 ± 1.19^b	$9.04 \pm .47^{ab}$	$8.65 \pm .36^b$	$9.04 \pm .62^{ab}$	$7.76 \pm .50^{ab}$	$12.72 \pm .77$	$4.12 \pm .31^{abc}$	$13.66 \pm .77$	$4.59 \pm .18^a$	$19.41 \pm .91^a$	$7.80 \pm .50$	15.62 ± 1.04	$12.96 \pm .75^a$	14.12 ± 1.07	$10.75 \pm .64^a$	$5.73 \pm .31$
V	20.18 ± 1.51^b	$9.70 \pm .67^{ab}$	$9.56 \pm .39^b$	9.81 ± 1.12^{ab}	$8.27 \pm .44^{ab}$	$13.08 \pm .76$	$4.67 \pm .31^{ab}$	$13.91 \pm .83$	$5.01 \pm .25^{ab}$	21.05 ± 1.51^a	$7.93 \pm .60$	$15.75 \pm .86$	$14.04 \pm .87^a$	15.58 ± 1.178	$10.79 \pm .54^a$	$5.90 \pm .30$
VI	20.11 ± 1.34^b	$9.76 \pm .78^{ab}$	$9.63 \pm .67^b$	$10.05 \pm .93^{ab}$	$8.97 \pm .64^a$	$13.12 \pm .76$	$4.65 \pm .29^{ab}$	$13.95 \pm .99$	$5.32 \pm .19^a$	20.07 ± 1.48^a	$7.81 \pm .36$	15.73 ± 1.12	14.28 ± 1.26^a	15.48 ± 1.50	$10.53 \pm .93^{ab}$	$5.94 \pm .27$
VII	19.82 ± 1.22^b	$9.86 \pm .72^a$	$9.48 \pm .47^b$	$10.33 \pm .79^{ab}$	$9.13 \pm .77^a$	$13.04 \pm .65$	$4.89 \pm .21^a$	$13.20 \pm .96$	$5.22 \pm .15^a$	19.50 ± 1.15^a	$7.89 \pm .53$	$15.95 \pm .94$	14.37 ± 1.08^a	15.54 ± 1.12	$10.76 \pm .97^a$	$6.06 \pm .34$
F-value	19.92 ± 1.21^b	$10.64 \pm .72^a$	$9.66 \pm .73^b$	$11.08 \pm .59^a$	$9.32 \pm .39^a$	$13.07 \pm .90481$	$5.00 \pm .19^a$	$12.96 \pm .92$	$5.08 \pm .25^a$	19.35 ± 1.19^a	$7.87 \pm .40$	15.63 ± 1.44	14.33 ± 1.30^a	$15.43 \pm .44$	$10.60 \pm .73^a$	$5.95 \pm .50$
P-value	2.61	3.81	2.83	3.78	3.81	.87	6.04	.990	12.69	8.09	.61	.37	2.81	1.22	3.41	1.67

nidopallium in support of present findings. Abid and Al-Bakri (2016) documented that the pyramidal neurons with mean diameter of 51 μm which was larger than present findings and the glial cells were found with mean diameter of 5 μm in support of present observation.

Archipallium

It represented the cerebral cortex which was situated at caudal arch like area in cerebrum of broiler chicken. By day 3 of post hatch, it had heterogeneous neuron groups in archipallium of broiler chicken (Fig 13). Different classes of neurons were identified from group I onwards as pyramidal, spherical, fusiform, stellate and multipolar neurons beside the glial cells. (Fig 13). The mean diameter of pyramidal, spherical, fusiform, stellate and multipolar neurons were varied from $12.80 \pm 0.64 \mu$, $6.89 \pm 0.31 \mu$, $14.11 \pm 1.02 \mu$, $10.10 \pm 0.91 \mu$ and $12.64 \pm 0.65 \mu$ in group I and $19.35 \pm 1.19 \mu$, $7.87 \pm 0.40 \mu$, $15.63 \pm 1.44 \mu$, $14.33 \pm 1.30 \mu$ and $15.43 \pm 0.44 \mu$ respectively in group VII (Table 2). The statistical analysis of mean diameter of pyramidal neurons showed that there highly significant difference was

observed between group II and III, but no significant difference was observed from group III onwards with advancement of age. In mean diameter of spherical, fusiform and multipolar neurons did not showed significant difference with advancement of age. However, significant difference was observed from group II to group III in mean diameter of stellate neurons. Similar findings were made by Abid and Al-Bakri (2016) regarding the pyramidal neurons, spherical neurons and stellate neurons in quail. However, they did not observe the fusiform and multipolar neurons in archipallium of quail.

Piriform cortex

It represents the ventrolateral part of cerebral cortex in frontal transverse section of brain. In all age groups of birds, it had pyramidal neurons and glial cells (Fig 14). The mean diameter of pyramidal and glial cells were ranged from $7.50 \pm 0.51 \mu$ and $4.73 \pm 0.34 \mu$ respectively in group I to $10.60 \pm 0.73 \mu$ and $5.95 \pm 0.50 \mu$ respectively in group VII. (Table 2). The statistical analysis of mean diameter of pyramidal neurons showed that the significant difference

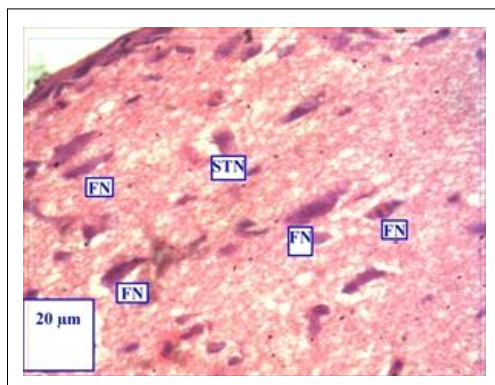


Fig 8: Photomicrograph showing fusiform neurons (FN) and stellate neurons (STN) in dorsolateral corticoid area (DLC) of cerebral cortex. (Group-VII). (Haematoxylin and Eosin X 1000).

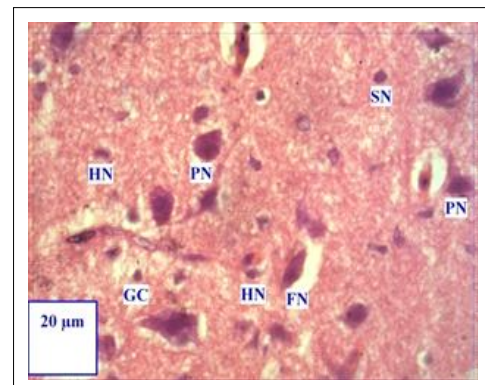


Fig 10: Photomicrograph showing the pyramidal (PN), spherical (SN), Fusiform (FN), horizontal neurons (HN) and glial cells (GC) in mesopallium (Group-VI). (Haematoxylin and Eosin X 1000)

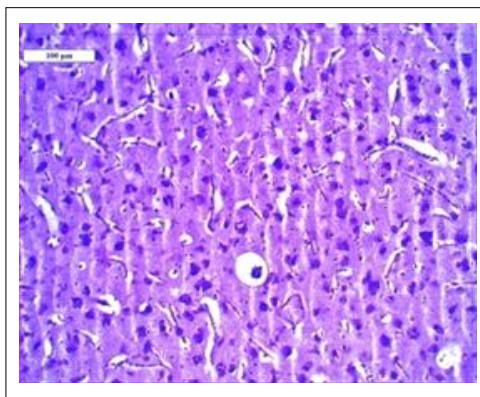


Fig 9: Photomicrograph showing the neurons of mesopallium in cerebral cortex (Group-III). (Cresyl violet X 200).

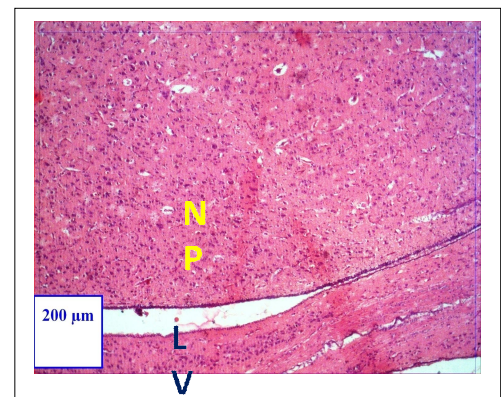


Fig 11: Photomicrograph showing the nidopallium (NP) in cerebral cortex. (Group-I). (Haematoxylin and Eosin X 100).

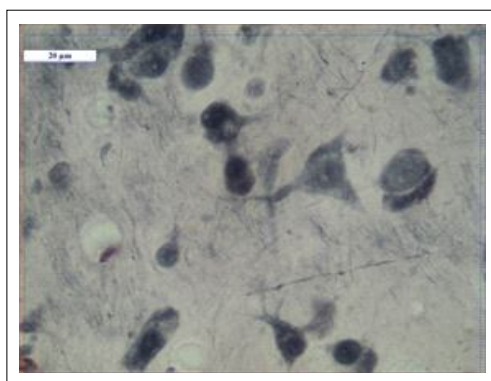


Fig 12: Photomicrograph showing the neurons in nidopallium in cerebral cortex. (Group-VII). (Silver Impregnation X 1000).

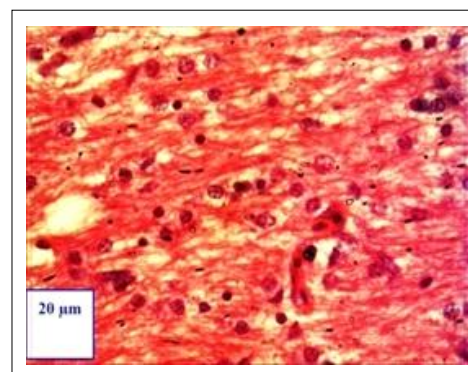


Fig 15: Photomicrograph showing the neurons of striatum in sub pallium of cerebrum hemisphere. (Group-VI). (Haematoxylin and Eosin X 1000).

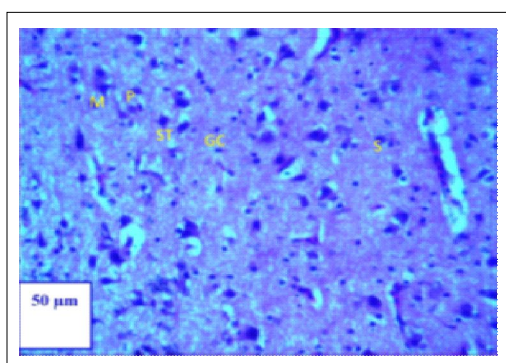


Fig 13: Photomicrograph showing pyramidal (P), spherical (S), stellate (ST), multipolar neurons (M) and glial cells (GC) in archipallium of cerebral cortex. (Group-IV). (Cresyl violet X 400).

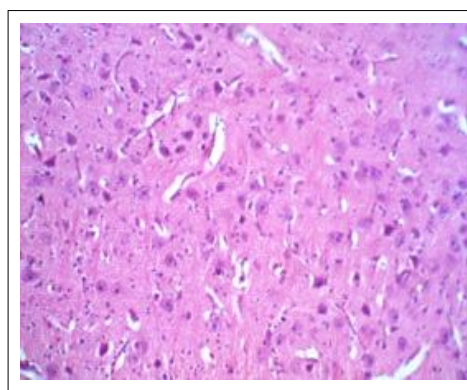


Fig 16: Photomicrograph showing the pallidum of sub pallium of cerebrum hemisphere. (Group-I). (Haematoxylin and Eosin X 200)

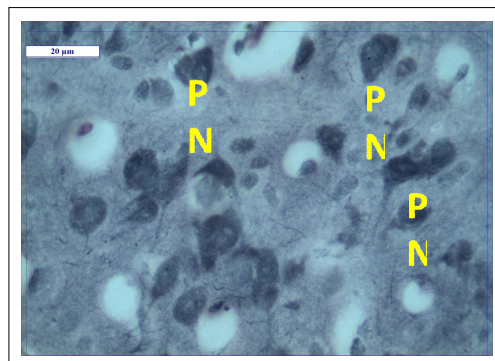


Fig 14: Photomicrograph showing pyramidal neurons (PN) in pyriform cortex. (Group-VI). (Silver Impregnation X 1000).

observed in group VI and VII with that of group I. However, no significant difference was observed in mean diameter of glial cells with advancement of age. Present observation in accordance with findings of Abd-Alrahman (2012) in Barn owl and Abid and Al-Bakri (2016) in quail birds.

Sub pallium

It was inner layer of cerebrum hemisphere situated deep to the nidopallium in all age groups of broiler chicken.

It consisted two parts *i.e.* Striatum and palladium as earlier reported by Nakeeb and Jasim (2018) in the *Columba livia domestica*. The striatum containing neuronal fibers and spherical neurons which was distinguished from group II onwards. Between spherical neurons a large bundles of myelinated axons and many spiny dendrites were present which gave striated appearance (Fig 15). The palladium was situated in deepest part of cerebrum containing few pyramidal neurons of small size (Fig 16). Similar findings were made by Abd-Alrahman (2012) in Barn owl, Abid and Al-Bakri (2016) in quail birds and Nakeeb and Jasim (2018) in the *Columba livia domestica*.

CONCLUSION

The microstructure of cerebral cortex in almost all age groups of broiler chicken showed two regions *i.e.* pallium and subpallium. In present study neurons were identified on the basis of its morphological appearance *viz.* Stellate, spherical, fusiform, pyramidal and multipolar neurons. The mean diameter of neurons in all layers of cerebral cortex increases with age, *i.e.* from group I to group VII. The cellular organization of cerebral cortex doesn't show distinct six

layered arrangement like in mammals. In present study the microscopic structures of the cerebral cortex was different from that of mammalians cerebral cortex.

ACKNOWLEDGEMENT

The authors want to thank and acknowledge the West Bengal University of Animal and Fisheries Sciences, Kolkata for funding this research and providing supports.

Conflict of interest

There is no conflict of interest for authors to declare.

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