CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2023 Vol.12, pp.176-189/Kavita et al.
***Research Article** (Open Access)*

CHRONIC MILD STRESS SHOWS NEURONAL REMODELING IN PYRAMIDAL PROJECTION NEURONS OF HIPPOCAMPAL COMPLEX IN POSTNATAL CHICKS

Kavita Tamta[1,2], Hemlata Arya[1,2], Adarsh Kumar[3], Shweta Arya[1], Ram Chandra Maurya[1,2]*
[1]Department of Zoology (DST-FIST Sponsored), Soban Singh Jeena, University, Almora
[2]Kumaun University, Nainital, Uttarakhand, India
[3]Department of Zoology, The Maharaja Sayajirao University of Baroda, Gujrat, India
**Author for Correspondence: mauryarc@gmail.com*

ABSTRACT
Various studies reported that the hippocampal complex (dorsomedial forebrain) shows homology with the mammalian HCC and is more prone to stress. The hippocampal complex plays an important role in spatial navigation, social behavior, learning, and memory. The study was based on Golgi Cox method to evaluate neuronal remodeling such as dendritic field, secondary branching, corrected spine number, axonal length, and the number of branching patterns at 25, 50, 75, and 100µm due to chronic mild stress in various age group (3-, 5-, 7-, and 9-week-old) of chicks (*Gallus gallus domesticus*). The main finding of the present study shows pyramidal projection neurons with dendritic spines i.e., filopodia, stubby, thin and mushroom-shaped spines. The dendritic field of pyramidal neurons in 3-, 5-, 7-, and 9-week-old chicks shows a significant decrease due to chronic mild stress. But secondary branches show significant decrease only in 9-week-old chicks. A significant decrease in the spine density of 3-, 5-, and 9-week-old chicks was observed under stressed conditions. The dendritic branches show significant decrease at 50, 100µm in 3 weeks old chicks and at 75µm in 5 weeks old chicks, while at 25µm significant increase was observed in 9 weeks old chicks, due to stress. Thus, the present study reveals that the neurons of HCC show continuous modification in postnatal chick in response to stress due to changing environmental conditions.

Keywords: *Hippocampal Complex; Neuronal Remodeling; Chronic Mild Stress; Pyramidal Neuron; Golgi Cox*

INTRODUCTION
The avian hippocampal complex (HCC) is located in the posterior part of the dorsomedial telencephalon, it comprises two major divisions, the hippocampus and the area parahippocampalis (Atoji & Wild, 2006; Montagnese *et al.,* 1996; Tömböl, Davies, Németh, Sebestény, *et al.,* 2000). The HCC of aves and mammals are homologous in terms of function but differs anatomically (Atoji & Wild, 2006; Herold *et al.,* 2014; Székely, 1999). The avian HCC is not differentiated clearly from adjacent structures, it comprises of a layered arrangement of densely occupied neurons, while the mammalian HCC contains of two areas of three-layered structures such as Ammon's horn and dentate gyrus (Atoji & Wild, 2006; Atoji *et al.,* 2002; Montagnese *et al.,* 1996; Tömböl, Davies, Németh, Sebestény, *et al.,* 2000). The avian HCC was reported to be involved in memory and food-storing behavior (Hampton & Shettleworth, 1996; Volman *et al.,* 1997), spatial navigation (Tömböl, Davies, Németh, Sebestény, *et al.,* 2000), learning (Bingman & Able, 2002; Vargas *et al.,* 2004), spatial cognition (Bingman. *et al.,* 2003; Bingman *et al.,* 2005; Jacobs, 2003), and sexual behavior (Atoji & Wild, 2006).
In the HCC of the avian brain various types of neurons are present such as projection neurons (multipolar, pyramidal) and stellate neurons, which share some common features with the neurons present in the reptiles and mammals brain (Atoji & Wild, 2004; Montagnese *et al.,* 1996; Srivastava & Maurya, 2010). The pyramidal and pyramidal-like neurons are present in the pyramidal layer II, while multipolar neurons are localized in all layers of avian HCC (Montagnese *et al.,* 1996; Srivastava, Chand, *et al.,* 2007). There are major differences in the distribution pattern of the pyramidal neurons in the HCC of birds (chick, homing pigeons, strawberry finch, zebra finch, etc.) and the reptilian cerebral cortex. These are found restricted to the medial hippocampus in the

zebra finch (Montagnese *et al.,* 1996), Strawberry finch (Srivastava, Chand, *et al.,* 2007), chicks and pigeon (Tömböl, Davies, Németh, Sebestény, *et al.,* 2000), while they were also present in all the cortical areas of the reptilian cerebral cortex (Maurya & Srivastava, 2006; Srivastava & Maurya, 2009). In the food storing birds, the HCC pyramidal neurons are involved in cognition, memory, and learning as compared to non-food-storing birds (D. Singh *et al.,* 2014; Srivastava, Chand, *et al.,* 2007). Similarly, the pyramidal projection neurons in cerebral cortex and prefrontal cortex of mammalian brain play an important role in neuronal circuitry, cognition as well as object recognition (Elston, 2003; S. Singh *et al.,* 2018).

A common characteristic feature of neuron is dendritic spines, originating as small protrusions from the dendritic shaft (A. Kumar *et al.,* 2019, 2023). Dendritic spines are mainly four types (filopodia, stubby, thin and mushroom- shaped spines) which were described in previous studies in the HCC of birds (A. Kumar *et al.,* 2023; Srivastava *et al.,* 2016). The spine density, dendritic arbor, branching pattern as well as synaptic connections vary due to internal and external circumstances (or stimuli) that influences the structure and function of the brain is known as neuronal remodeling (Aguayo *et al.,* 2018; Mcewen & Gianaros, 2010). The stimuli like stress and ageing are known to cause negative effects in the neuronal characteristics (McEwen, 2000).

Stress is an aversive condition based on the daily life experiences, behavior, and well-being of an animal (E. J. Kim *et al.,* 2015; J. J. Kim & Diamond, 2002). Stress may be short-term (acute) and long-term (chronic), in the long term they cause atrophy in HCC. Chronic stress also alters neuronal morphology, cognitive functions viz., learning memory, and normal behavioral patterns (McEwen, 2000; McEwen *et al.,* 2016; McEwen & Sapolsky, 1995). Various human and animal research has shown that the HCC is highly susceptible to both acute and chronic stress (McEwen, 1999, 2000). Various studies reported regarding susceptibility to stress-related destruction and negative feedback regulation of the stress through the hypothalamic-pituitary-adrenal axis (HPA) in HCC (Herman & Cullinan, 1997; Jacobson & Sapolsky, 1991; McEwen *et al.,* 2016; Sapolsky *et al.,* 1991). Several studies reported that in extreme situations it causes various neuropathological diseases such as PTSD (post-traumatic stress disorder), schizophrenia, anxiety, and depression in animals (E. J. Kim *et al.,* 2015; E. J. Kim & Kim, 2023; Widiger & Clark, 2000). The main purpose of the present study was to evaluate the effect of chronic mild stress (CMS) such as food deprivation, social isolation, dark, and cold temperature on neuronal remodeling in HCC of various age group (3-, 5-, 7-, and 9-week-old) of chicks (*Gallus gallus domesticus*).

MATERIAL AND METHODS

Experimental animals

The 5-day old male chicks having an average length of 10-15 cm and weight 35-45 g were purchased from the Pahari Poultry farm (a government department), Hawalbag, Almora. The sex of chicks was determined by feather sexing method which was previously described (Abbas *et al.,* 2019). The chicks were kept in the laboratory conditions, i.e., in an animal house ($0.90\times0.60\times0.60$ m^3) with food and water ad libitum. The animal house was maintained with a 12/12 light/dark cycle with a moderate temperature of 22-26°C. In this study, 32 male chicks were divided into five groups: Group 1 (16 chicks) non-stressed (NS), Group 2 (4 chicks), Group 3 (4 chicks), Group 4 (4 chicks, and Group 5 (4 chicks) were the stressed groups. Group 1 was left undisturbed, but the chicks of groups 2, 3, 4, and 5 were exposed to chronic mild stress (CMS) from one of the following stresses such as food deprivation, social isolation, dark, and cold temperature every day for 4 hours, until 2, 4, 6 and 8 weeks at the same time. All the experimental procedures were carried out according to the Institutional Animal Ethical Committee (IAEC) guidelines of Kumaun University, Nainital, Protocol no. KUDOPS/181).

Golgi Cox methods

According to the experimental design, after 2, 4, 6, and 8 weeks of CMS exposure, 4 chicks of each group (NS and CMS) were sacrificed with a lethal dose of ketamine (Thermo Fisher Scientific, India). The brain was rapidly removed from the skull and kept in 4% paraformaldehyde for 30 minutes at room temperature (RT). After 30 minutes, the brain was shifted to a freshly prepared Golgi Cox solution for 24 hours, and the next day, for 14 days in the same solution in dark at RT (Cox, 1891; Levine *et al.,* 2013). Thereafter, the brain was

washed with distilled water (3×5 minutes), and each brain sample was kept in 1% potassium dichromate (Thermo Fisher Scientific, Mumbai India) solution for 24 hours. The next day, brain was washed (3×5 minutes), dehydrated with 30%, 50%, 70%, 90%, and 100% alcohol (Thermo Fisher Scientific, Mumbai India) for 30 minutes in each, cleared with xylene for 15 minutes (Thermo Fisher Scientific, Mumbai India). The brain was then transferred to paraffin wax (Molychem, Mumbai, India) at 56℃ for 6 hours (3 changes 1, 2, and 3 hours respectively). Later on, the brain was embedded in paraffin wax for section cutting. The brain was sectioned at a thickness of 120µm on a rotary microtome (Spencer), and deparaffinized in xylene for 10 minutes. After the deparaffinization, the sections were rehydrated with alcohol viz., 100%, 90%, 70%, 50%, and 30% for 5 minutes in each. Sections were stained with 1% potassium dichromate, 28% ammonia solution (Thermo Fisher Scientific, India), and 1% sodium thiosulfate (Thermo Fisher Scientific, India), for 5 minutes in each. After this, the sections were dehydrated with 30%, 50%, 70%, 90%, and 100% alcohol (5 minutes in each), cleared in xylene (5 minutes) and mounted in D.P.X. (Molychem, Mumbai, India) (A. Kumar *et al.*, 2021, 2023).

Microscopic Analysis

The microphotographs of pyramidal projection neurons in the Golgi Cox sections were taken under the light microscope (Leica-DMIL) at magnification 400X (40X × 10X). The drawing of all selected neurons was drawn with the help of camera lucida attached to the light microscope at primary magnification of 400X. Additionally, neuron drawing was scanned, all the drawings and microphotographs were labelled and photoplates were made by Adobe Photoshop 7.0.

Data Analysis

Several morphological features of neurons such as dendritic field, the distance of secondary branches from the soma, corrected spine number, and axonal length were calculated with the help of a computer-aided microscope (Leica-DMIL) at 400X magnification. From Golgi-impregnated slides, the total number of pyramidal neurons was counted through a light microscope at 400X, and their percentage was calculated.

At 25, 50, 75, and 100µm radius circles from the soma center, the number of dendritic branches of neurons was counted with the help of camera lucida drawings. The spine density (N) was calculated per 25µm of a dendritic shaft of each neuron. The dendritic diameter was taken three times, their mean was calculated, and half of the dendritic diameter was measured as dendritic radius (Dr). The spine length (Sl) was measured to the extended spines of the dendritic segment, and also the spine head diameters (Sd) were taken three times and their mean was calculated. The following equation was used to calculate the corrected spine number (Feldman & Peters, 1979):

$$N = \frac{n\pi[(Dr + Sl)^2 - (Dr + Sd)^2]}{[\frac{\theta}{90}\pi(Dr + Sl)^2] - 2[(Dr + S1)\sin\theta(Dr + Sd)]}$$

Here, N, corrected spine number, n, number of visible spines, Dr, radius of the dendrite, Sd, spine head diameter, Sl, spine length, and ɵ, central angle.

$$\text{Cos}\ \theta = \frac{Dr + Sd}{Dr + Sl}$$

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 9.0. All data were represented as Mean±Standard error mean (SEM). The various morphological characters of pyramidal projection neurons were analyzed by using an unpaired t-test with Welch's correction in various age groups i.e., 3-, 5-, 7-, and 9-week-old of NS and CMS chicks. Data is significantly different at level: *P < 0.05; **P < 0.01; ***P < 0.001.

RESULTS AND DISCUSSION

The main finding of the present study was pyramidal projection neuron with several neuronal characteristic features such as dendritic field, the distance of secondary branches from the soma, corrected spine number, axonal length, its projection, and branching pattern of the radius circles at 25, 50, 75, and 100µm from the soma center represents variations in different age groups.

Morphological analysis of pyramidal neuron

The study revealed the effect of CMS in various age groups viz., 3-, 5-, 7-, and 9-week-old chicks on neuronal remodeling in HCC of chicks. The pyramidal neurons composed of spinous dendrites 10-12 originated from the soma, containing fusiform to rectangular, pyramidal or cone-shaped, and medium-sized triangular soma, with basal and apical and dendritic extension (Figure 1&2). The dendrites that are initiated from the soma possess small protrusions originating directly from the dendritic shaft of the neurons are called spines, that are of four types- filopodia, stubby, thin, and mushroom-shaped spines observed in pyramidal neurons (Figure 3 & 4). The axon initiates directly from the dendrites or soma, it sometimes splits in all probable directions later a short interval to make axon collaterals (c). The axonal projection was observed in Hp, APH, dorsal, ventral, and local in the HCC of all group chicks (Figure 1& 2). In the HCC of 3-week-old chick pyramidal neurons number and percentage were calculated as 139 and 21.89% in NS, 166 and 26.14% in CMS chicks, whereas in 5-week-old chick 200 and 25.00% in NS, 172 and 22.84% in CMS. Additionally, the neuronal number and percentage were 115 and 16.38% in NS whereas 145 and 22.48% in CMS of 7-week-old chicks. In 9-week-old chicks, 270 and 33.29% in NS while 170 and 26.23% was observed in CMS chick's HCC.

The outcome of CMS in neuronal characters of different age groups (3, 5, 7, and 9 weeks) chicks

Dendritic field

The statistical analysis revealed that the dendritic field of pyramidal neurons shows a significant decrease due to CMS in 3 (*), 5 (**), 7 (**), and 9 (***) week old chicks (Figure1,2 & 4, Table 1).

Distance of secondary branches from the soma

Under stress circumstances the secondary branches of only 9-week-old chicks show significant (*) decrease while 3-, 5-, and 7- weeks-old chicks does not differ significantly (Figure 1,2 & 4, Table 1).

Spine density

The statistical analysis revealed that the pyramidal projection neurons depict significant decrease in the 3 (*), 5 (***), and 9 (***) weeks old chicks, whereas 7 weeks does not differ significantly due to CMS (Figure 1,2, 3 & 4, Table 1).

Axonal Length

The axonal length in various age groups of chicks (3-, 5-, 7-, and 9-week-old) does not differ significantly under stress conditions (Figure 1,2 & 4, Table 1).

Dendritic branches at 25, 50, 75 and 100µm

In HCC pyramidal projection neurons, the dendritic branches at 50µm (*) and 100µm (*) show significant decrease, while at 25 and 75µm does not differ significantly in 3-week-old chicks due to CMS (Figure 1,2 & 4, Table 2).

In 5-week-old chicks only at 75µm shows significant (*) decrease, whereas 25, 50, and 100µm does not differ significantly due to CMS (Figure 1,2 and 4, Table 2).

The 7-week-old chicks under stressed condition does not differ significantly at 25, 50, 75, and 100µm (Figure 1,2 & 4, Table 2).

At 25µm the dendritic branches show significant (*) increase, while at 50, 75, and 100µm does not differ significantly due to CMS in 9-week-old chicks (Figure 1,2 & 4, Table 2).

Table 1: Depicts the mean values of dendritic field, distance of secondary branches from soma, axonal length and corrected spine number, Unpaired two tailed t-test (with Welch's correction), degree of freedom, $t_{calculated}$ value and p value in the HCC pyramidal projection neurons of NS and CMS various age groups (3, 5, 7, and 9 weeks old) of chicks (*Gallus gallus domesticus).* Data is significantly different at level: *P < 0.05; **P < 0.01; ***P < 0.001. HCC- Hippocampal complex, NS- Non-stress, CMS- Chronic mild stress.

Neuronal Characters	Chick's age	NS	CMS	Unpaired t-test (with Welch's correction) at P < 0.05		
				Degree of Freedom	$t_{calculated}$ value	P value
Dendritic Field	3 weeks	192.5 ± 8.34	163.5 ± 6.92	27	2.679	0.0124*
	5 weeks	183.3 ± 7.05	155.6 ± 6.59	27	2.874	0.0078**
	7 weeks	180.8 ± 6.93	151.1 ± 6.32	27	3.165	0.0038**
	9 weeks	231.8 ± 11.74	157.5 ± 5.7	20	5.694	P<0.0001***
Secondary Branches	3 weeks	13.66 ± 0.84	13.25 ± 1.26	24	0.2707	0.7889
	5 weeks	12.67 ± 1.11	11.95 ± 1.04	27	0.4704	0.6418
	7 weeks	14.88 ± 1.39	12.9 ± 0.98	25	1.165	0.2549
	9 weeks	15.78 ± 1.46	11.49 ± 0.78	21	2.588	0.0172*
Corrected Spine Number	3 weeks	49.05 ± 2.6	41.69 ± 0.87	17	2.678	0.0159*
	5 weeks	49.11 ± 1.65	38.67 ± 1.6	27	4.536	0.0001***
	7 weeks	42.81 ± 1.84	39.05 ± 0.93	20	1.826	0.0829
	9 weeks	66.27 ± 3.33	43.5 ± 1.6	20	6.161	P<0.0001***
Axonal length	3 weeks	73.16 ± 6.75	70.75 ± 4.81	21	1.225	0.2343
	5 weeks	70.73 ± 5.8	56.14 ± 5.43	27	1.836	0.0775
	7 weeks	61.82 ± 6.45	66.52 ± 3.86	22	0.6261	0.5377
	9 weeks	84.95 ± 9.53	62.16 ± 5.96	23	2.027	0.0544

Table 2: Depicts the mean values of dendritic branches at 25, 50, 75 and 100µm radius circles from soma centre, with Unpaired two tailed t-test (with Welch's correction), degree of freedom, $t_{calculated}$ value and p value in the HCC pyramidal projection neurons of NS and CMS various age groups (3, 5, 7, and 9 weeks old) of chicks (*Gallus gallus domesticus).* Data is significantly different at level: *P < 0.05; **P < 0.01; ***P < 0.001. HCC- Hippocampal complex, NS- Non-stress, CMS- Chronic mild stress.

Mean dendritic branches at different radius circles from soma center (Mean ± SEM) of pyramidal neurons				Unpaired t-test (with Welch's correction) at P < 0.05		
Radius Circle	Chick's age	NS	CMS	Degree of Freedom	$t_{calculated}$ value	P value
25µm	3 weeks	12.4 ± 0.41	11.6 ± 0.41	27	1.374	0.1808
	5 weeks	8.133 ± 0.57	7.067 ± 0.64	27	1.251	0.2217
	7 weeks	7.867 ± 0.4	7.733 ± 0.51	26	0.2052	0.8390
	9 weeks	6.2 ± 0.42	7.533 ± 0.4	27	2.307	0.0289*
50µm	3 weeks	15.2 ± 0.7	12.93 ± 0.52	25	2.602	0.0153*
	5 weeks	12.8 ± 0.98	11.4 ± 0.77	26	1.124	0.2714
	7 weeks	10.4 ± 0.43	10.93 ± 0.66	24	0.6765	0.5052
	9 weeks	9.933 ± 0.49	10.93 ± 0.66	25	1.217	0.2351
75µm	3 weeks	12.2 ± 0.68	10.73 ± 0.96	25	1.241	0.2261
	5 weeks	12.73 ± 0.99	9.067 ± 0.88	27	2.770	0.0100*
	7 weeks	8.4 ± 0.59	8.2 ± 0.61	27	0.2351	0.8159
	9 weeks	10.53 ± 0.57	8.933 ± 0.65	27	1.852	0.0749
100µm	3 weeks	8 ± 0.59	5.933 ± 0.57	27	2.505	0.0186*
	5 weeks	9 ± 0.84	6.733 ± 0.75	27	2.010	0.0545
	7 weeks	5.8 ± 0.59	5.4 ± 0.56	27	0.4934	0.6257
	9 weeks	7 ± 0.8	4.867 ± 0.75	27	1.941	0.0628

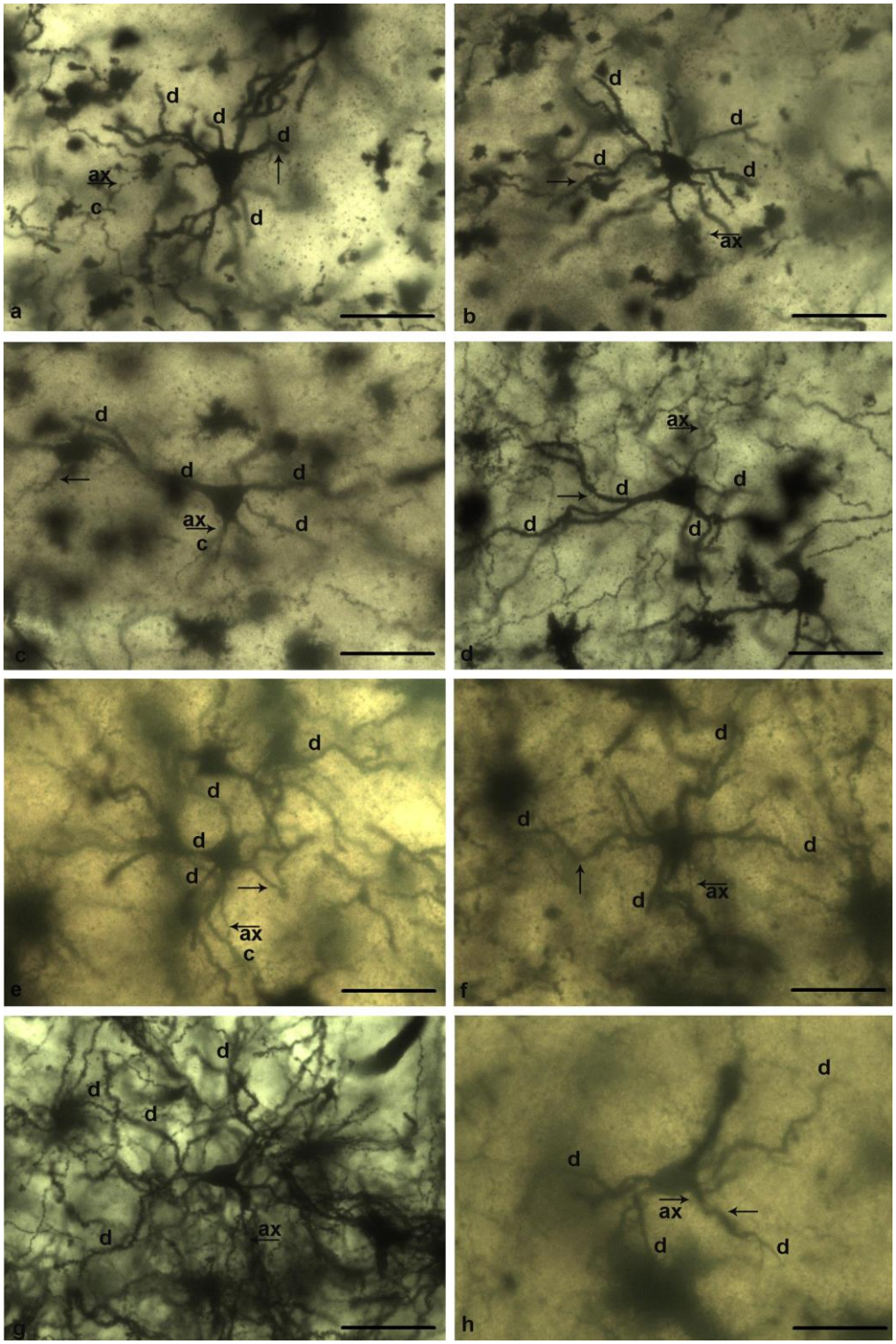

Figure 1: Microphotographs showing the Golgi Cox impregnated pyramidal projection neurons with spinous dendrites, axon and axon collaterals in the hippocampal complex (HCC) of various age groups viz., 3 (A- NS; B- CMS), 5 (C- NS; D- CMS), 7 (E- NS; F- CMS) and 9 (G- NS; H- CMS) weeks old chicks (*Gallus gallus domesticus*). d- dendrites, ax- axon, c- axon collaterals, arrow- spines. Scale bar- 50 µm

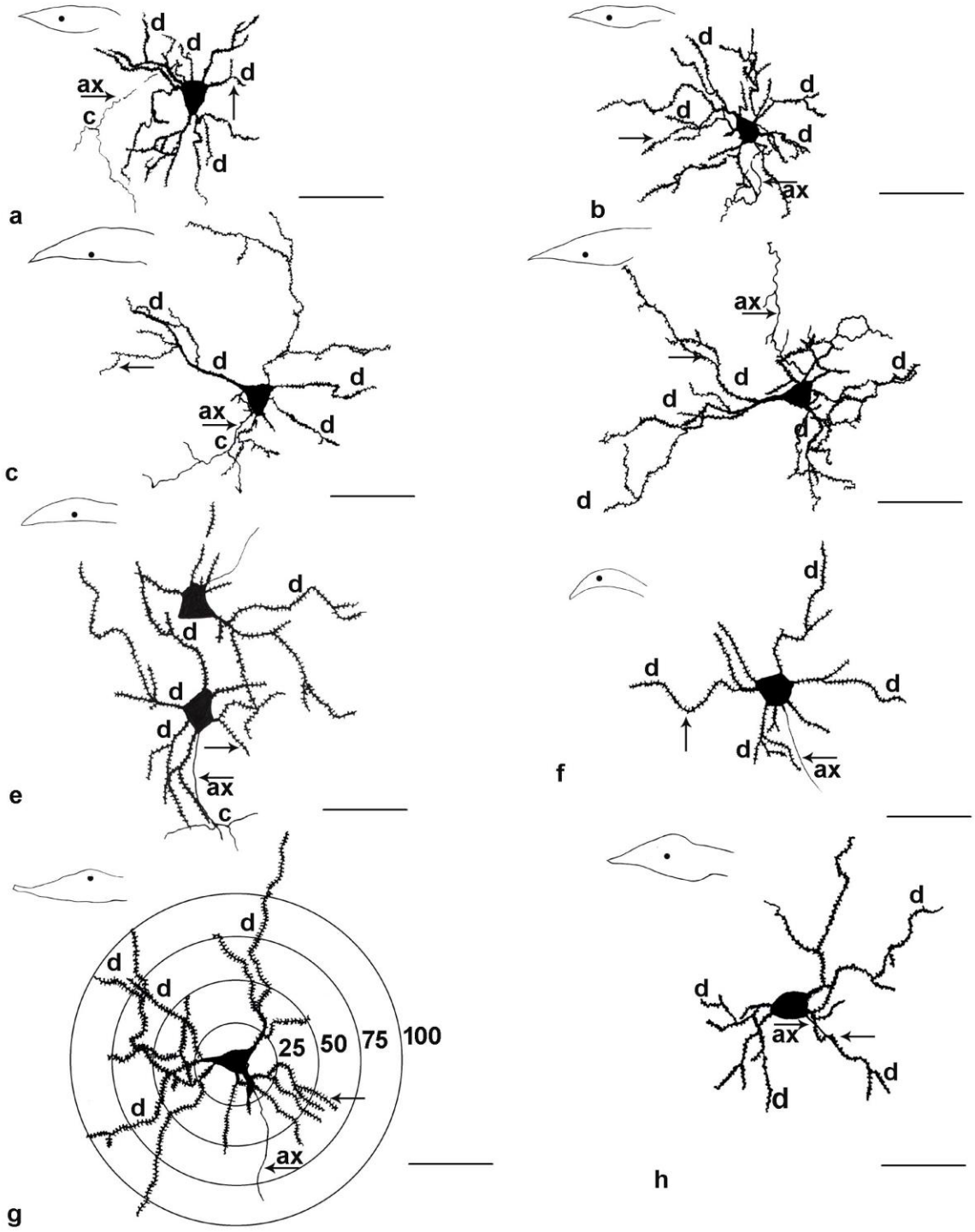

Figure 2: Camera lucida drawings of the pyramidal projection neurons with spinous dendrites, axon and axon collaterals (c) in the hippocampal complex (HCC) of various age groups viz., 3 (A- NS; B- CMS), 5 (C- NS; D- CMS), 7 (E- NS; F- CMS) and 9 (G- NS; H- CMS) weeks old chicks (*Gallus gallus domesticus*). Insets depicts the position of pyramidal neurons. d- dendrites, ax- axon, c- axon collaterals, arrow- spines. Scale bar- 50 µm.

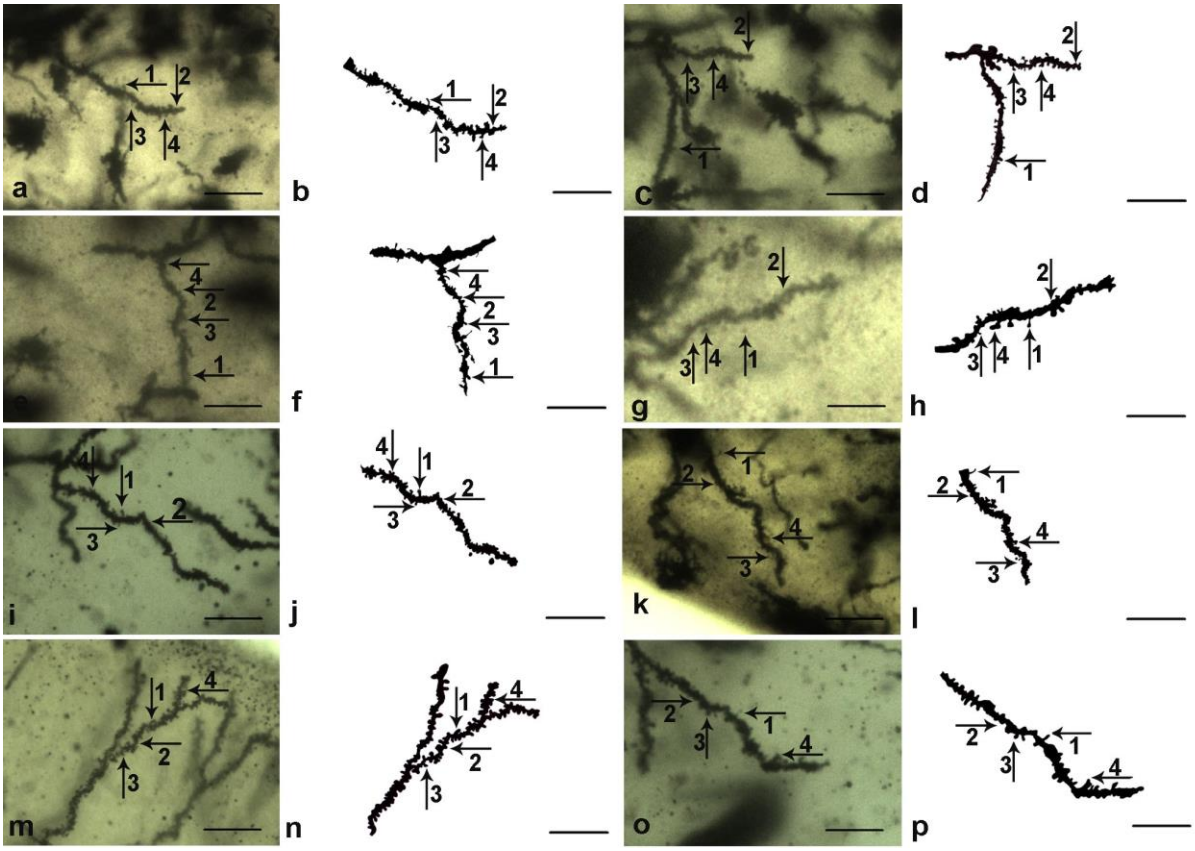

Figure 3: Microphotographs and camera lucida drawings depicts the pyramidal projection neurons dendritic segments with four types of spines of various age groups viz., 3 weeks (A, B), 5 weeks (E, F), 7 weeks (I, J) and 9 weeks (M, N) of NS chicks and 3 weeks (C, D), 5 weeks (G, H), 7 weeks K, L) and 9 weeks (O, P) CMS chicks. Arrows- 1, 2, 3 and 4 depicts the filopodia, stubby, thin and mushroom-shaped spines respectively, scale bars = 20µm

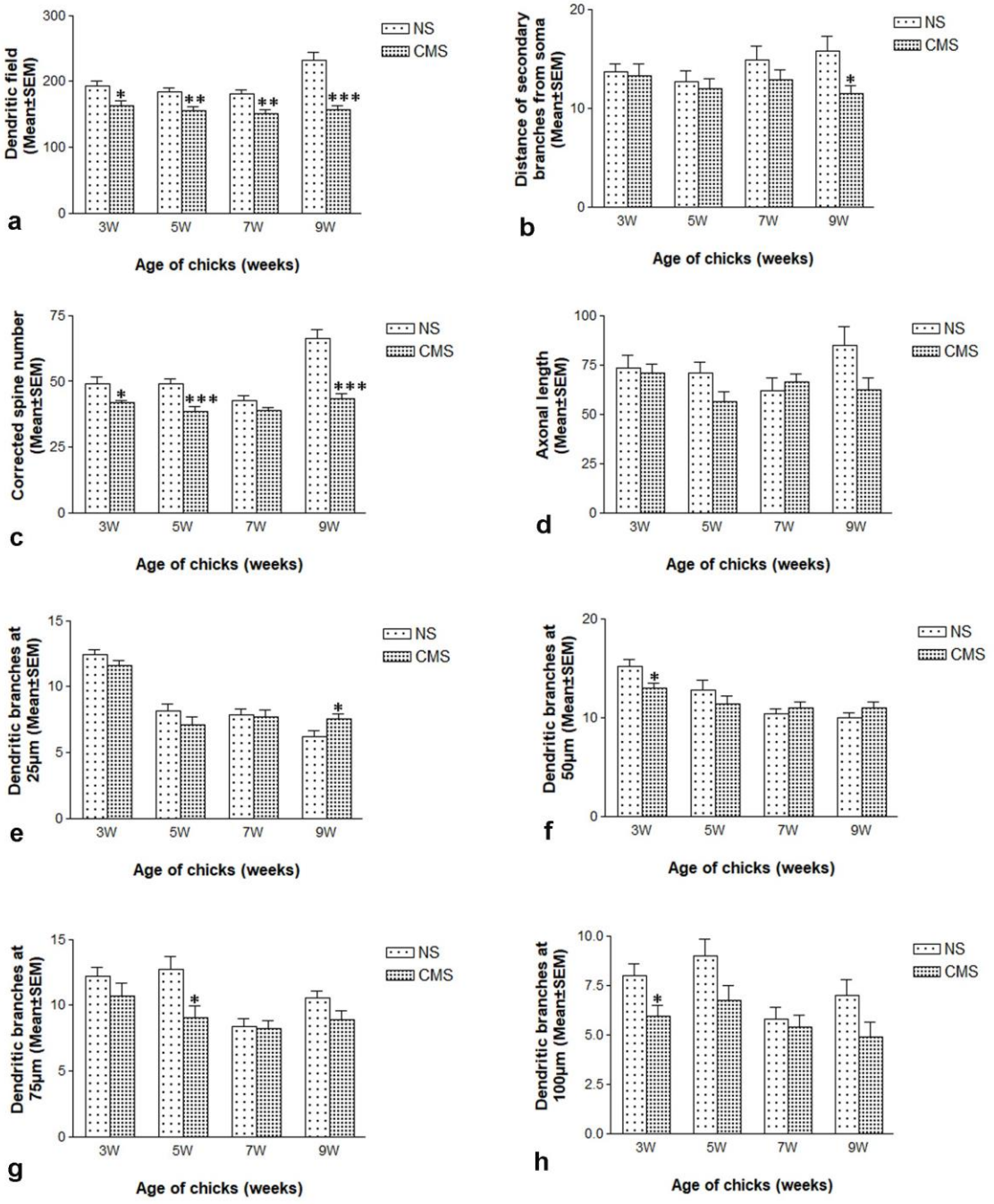

Figure 4: Graphs represents the different neuronal characteristics of pyramidal projection neurons: Dendritic field (A), Distance of secondary branches from soma (B), Corrected spine number (C), Axonal length (D), and Dendritic branches at 25 µm (E),50 µm (F),75 µm (G) and 100µm (H) of various age groups (3, 5, 7, and 9 weeks old) of chicks.

DISCUSSION

The present study revealed the consequences of CMS (food deprivation, social isolation, dark, and cold temperature) on neuronal remodeling of HCC in various age groups of chicks. The avian HCC is a brain region, a curved cortical strip presents in the dorsomedial telencephalon. The HCC is essential for the process of

learning, memory, behavioral regulation, navigation, and cognition (Bingman *et al.,* 2005; Emery, 2006; Sherry, 2005; Shettleworth, 2003; D. Singh *et al.,* 2014; Smulders, 2017; Vargas *et al.,* 2004). The present study found that pyramidal projection neurons with various neuronal characteristics such as dendritic field, secondary branches, corrected spine number, axonal length, and dendritic branches at 25, 50, 75, and 100µm by using the Golgi-Cox method. This type of similar study has also been found in many avian species such as zebra finch (Montagnese *et al.,* 1996), chick, *Columba livia* (Tömböl, Davies, Németh, Sebestény, *et al.,* 2000), pigeon (Atoji & Wild, 2006), strawberry finch, (Srivastava, Chand, *et al.,* 2007), *Psittacula krameri* (Srivastava & Singh, 2012), *Corvus splendens* (Srivastava *et al.,* 2016); male and female vanraja birds (P. K. Kumar *et al.,* 2016), broiler chicken (Kumaravel *et al.,* 2019) and *Coracias benghalensis* (Ojha & Singh, 2021a, 2021b). Previous studies described that the various type of neuronal cells was found in reptiles, avian, and mammal's brain. Likewise, in the cerebral cortex of reptiles multipolar, pyramidal, inverted pyramidal, bipolar, monotufted and bitufted neurons were reported from which bitufted neurons is the dominant type (Maurya & Srivastava, 2006; Srivastava *et al.,* 2009a; Srivastava, Maurya, *et al.,* 2007; Srivastava & Maurya, 2010), but in mammals the pyramidal neurons are dominant (El-Falougy & Benuska, 2006; Hassiotis & Ashwell, 2003; Keuker *et al.,* 2003). However, in the HCC of birds, the multipolar projection neuron was reported as dominant type (A. Kumar *et al.,* 2023; Montagnese *et al.,* 1996; Tömböl, Davies, Németh, Alpár, *et al.,* 2000), whereas in intermediate corticoid (CI), the pyramidal projection neuron is dominant (Srivastava *et al.,* 2009b). Additionally, in the guinea fowl, pyramidal and polymorphic multiangular neurons were observed in the hippocampus (Showers MJC, 1982; Tömböl, Davies, Németh, Alpár, *et al.,* 2000).

Moreover, pyramidal neurons possess spines on their dendrites. The dendritic spines are the small bulbous flanges present all over the dendritic shaft in neurons (A. Kumar *et al.,* 2019). Based on the size and shape, spines are generally classified as filopodia, stubby, thin and mushroom-shaped spines (A. Kumar *et al.,* 2019; Peters & Kaiserman-Abramof, 1970; Rochefort & Konnerth, 2012; Runge *et al.,* 2020). Various studies described that the spine density increases during breeding season, and enriched environment (D. Singh *et al.,* 2014), while stress or negative environmental interactions decrease the spine density (Aguayo *et al.,* 2018; A. Kumar *et al.,* 2023).

Stress can be threatening or valuable for an organism, mild stressors can improve learning and memory processes, while severe stressors can cause impairments in memory function (McEwen & Gianaros, 2011). The HCC comprises a remarkable ability to adapt, change with experience and displays continuous neuronal remodeling in their dendritic extension, spine density and branching pattern under stress conditions (McEwen, 2010; McEwen *et al.,* 2016).Various studies demonstrate that the chronic stress exposure for 21 days causes dendritic retraction in the HCC CA3 region of rat (Conrad *et al.,* 1999; Watanabe *et al.,* 1992). In the CA3 region of hippocampus in male tree shrews, daily psychosocial stress depicts apical dendritic atrophy in pyramidal neurons (Magariños *et al.,* 1996; Watanabe *et al.,* 1992). The stress and high-fat diet caused a significant decrease in CA3 neurons of both apical dendrites and length of dendrites in male adult rat (Baran *et al.,* 2005). Similar type of study was observed in present study, due to stress the dendritic field of 3-, 5-, 7-, and 9-week-old chicks shows significant decrease. Stress is reported to induce structural changes (Banasr & Duman, 2008; Czéh *et al.,* 2006), neuronal remodeling such as a reduction in the synapses number of pyramidal neurons in CA3 regions (Fuchs *et al.,* 2006) and alterations in dendritic spine density (Conrad, 2008). In the avian hippocampus seasonal fluctuations affects the dendritic thickness, spine density, and spine morphology (S. Singh *et al.,* 2015; Srivastava *et al.,* 2012; Srivastava & Singh, 2012). The same was observed after stress exposure in the spine density which shows significant decrease in 3-, 5- and 9-week-old chicks. Stress causes dendritic atrophy as indicated by decrease in the number and length of branch points of the CA3 apical dendrites (Yau & So, 2014). The dendritic branches were also affected by CMS in the various age groups of chicks, in 3-week-old chicks at 50µm and 100µm, while in 5 weeks only at 75µm significant decrease was observed. Moreover, at 25µm due to CMS, significant increase was observed in 9-week-old chicks.

Therefore, the finding of present study delivers a valuable information regarding effect of CMS of the pyramidal projection neurons in HCC of various age groups of chicks. This study also provides a new research avenue in the scientific field and discovery of new targets for treating many neurobiological disorders of HCC derived from maladaptive responses to stress.

CONCLUSION

The present study concludes that the pyramidal neurons are also found in the HCC of chicks. Additionally, in various groups (3-, 5-, 7-, and 9-week-old) of chicks, CMS affects the neuronal morphology such as the dendritic extension, branching pattern, and spine density in various age groups of chicks differently. Although sometimes the chicks also overcome the stress and try to adapt in adverse conditions.

ABBREVIATIONS

HCC- Hippocampal complex, NS- Non stress, CMS- Chronic mild stress, ax- axon c- axon collateral, ɵ- Theta, d- dendrites, D.P.X.- Dibutylphthalate Polystyrene xylene, Dr- Dendritic radius, HPA- Hypothalamic–pituitary–adrenal axis, IAEC- Institutional Animal Ethical Committee, n- Number of dendritic spines N- Corrected spine number, RT- Room temperature, Sd- spine head diameter, SEM- Standard error mean, Sl- Spine length, CA- Cornu Ammonis, CI-Intermediate corticoid, CDL-Dorsolateral corticoid

ACKNOWLEDGEMENT

The authors thank the Head, Department of Zoology (DST FIST Sponsored), Soban Singh Jeena Campus Almora, Kumaun University Nainital for providing essential infrastructural support for the present research work. The present research study has received funding from UGC Fellowship No. F. 82-1/2018 (UGC ref. No.2856).

Ethical Guidelines

All the experimental procedures were carried out according to the Institutional Animal Ethical Committee (IAEC) guidelines of Kumaun University, Nainital, Protocol no. KUDOPS/181).

Conflict of interest: *none*

REFERENCES

Abbas FI, Abbas AH, & Hussain L (2019). Identification sex of newly hatched chicks through images: A survey. *International Journal of Civil Engineering and Technology*, **10**(1), 595–606.

Aguayo FI, Tejos-Bravo M, Díaz-Véliz G, Pacheco A, García-Rojo G, Corrales W, Olave FA, Aliaga E, Ulloa JL, Avalos AM, Román-Albasini L, Rojas PS, & Fiedler JL (2018). Hippocampal Memory Recovery After Acute Stress: A Behavioral, Morphological and Molecular Study. *Frontiers in Molecular Neuroscience*, **11**(August), 1–14.

Atoji & Wild JM (2006). Anatomy of the Avian Hippocampal Formation. *Reviews in the Neurosciences*, **17**(1–2), 3–15.

Atoji Y & Wild JM (2004). Fiber connections of the hippocampal formation and septum and subdivisions of the hippocampal formation in the pigeon as revealed by tract tracing and kainic acid lesions. *Journal of Comparative Neurology*, **475**(3), 426–461.

Atoji Y, Wild JM, Yamamoto Y & Suzuki Y (2002). Intratelencephalic connections of the hippocampus in pigeons (*Columba livia*). *The Journal of Comparative Neurology*, **447**(2), 177–199.

Banasr M & Duman R (2008). Regulation of Neurogenesis and Gliogenesis by Stress and Antidepressant Treatment. *CNS & Neurological Disorders - Drug Targets*, ***6***(5), 311–320.

Baran SE, Campbell AM, Kleen JK, Foltz CH, Wright RL, Diamond DM & Conrad CD (2005). Combination of high fat diet and chronic stress retracts hippocampal dendrites. *NeuroReport*, **16**(1), 39–43.

Bingman Hough GE, Kahn MC & Siegel JJ (2003). The homing pigeon hippocampus and space: In search of adaptive specialization. *Brain, Behavior and Evolution*, ***62***(2), 117–127.

Bingman, V. P., & Able, K. P. (2002). Maps in birds: representational mechanisms and neural bases. *Current Opinion in Neurobiology*, **12**(6), 745–750.

Bingman, V. P., Gagliardo, A., Hough, G. E., Ioalé, P., Kahn, M. C., & Siegel, J. J. (2005). The avian hippocampus, homing in pigeons and the memory representation of large-scale space. *Integrative and Comparative Biology*, **45**(3), 555–564.

Conrad, C. D. (2008). Chronic Stress-induced Hippocampal Vulnerability: The Glucocorticoid Vulnerability Hypothesis. *Reviews in the Neurosciences*, **19**(6), 395–411.
Conrad, C. D., LeDoux, J. E., Magariños, A. M., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioral Neuroscience*, **113**(5), 902–913.
Cox, W. H. (1891). Imprägnation des centralen Nervensystems mit Quecksilbersalzen. *Archiv Für Mikroskopische Anatomie*, **37**(1), 16–21.
Czéh, B., Simon, M., Schmelting, B., Hiemke, C., & Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology*, **31**(8), 1616–1626.
El-Falougy, H., & Benuska, J. (2006). History, anatomical nomenclature, comparative anatomy and functions of the hippocampal formation. *Bratislavské Lekárske Listy*, *107*(4), 103–106.
Elston, G. N. (2003). Cortex, Cognition and the Cell: New Insights into the Pyramidal Neuron and Prefrontal Function. *Cerebral Cortex*, **13**(11), 1124–1138.
Emery, N. J. (2006). Cognitive ornithology: The evolution of avian intelligence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **361**(1465), 23–43.
Feldman, M. L., & Peters, A. (1979). A technique for estimating total spine numbers on golgi-impregnated dendrites. *Journal of Comparative Neurology*, **188**(4), 527–542.
Fuchs, E., Flugge, G., & Czeh, B. (2006). Remodeling of neuronal networks by stress. *Frontiers in Bioscience*, **11**(SUPPL. 2), 2746–2758.
Hampton, R. R., & Shettleworth, S. J. (1996). Hippocampal lesions impair memory for location but not color in passerine birds. *Behavioral Neuroscience*, **110**(4), 831–835.
Hassiotis, M., & Ashwell, K. W. S. (2003). Neuronal classes in the isocortex of a monotreme, the Australian echidna (*Tachyglossus aculeatus*). *Brain, Behavior and Evolution*, **61**(1), 6–27.
Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neurosciences*, **20**(2), 78–84.
Herold, C., Bingman, V. P., Ströckens, F., Letzner, S., Sauvage, M., Palomero-Gallagher, N., Zilles, K., & Güntürkün, O. (2014). Distribution of neurotransmitter receptors and zinc in the pigeon (*Columba livia*) hippocampal formation: A basis for further comparison with the mammalian hippocampus. *Journal of Comparative Neurology*, **522**(11), 2553–2575.
Jacobs, L. F. (2003). The evolution of the cognitive map. *Brain, Behavior and Evolution*, **62**(2), 128–139.
Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews*, **12**(2), 118–134.
Keuker, J. I. H., Rochford, C. D. P., Witter, M. P., & Fuchs, E. (2003). A cytoarchitectonic study of the hippocampal formation of the tree shrew (*Tupaia belangeri*). *Journal of Chemical Neuroanatomy*, **26**(1), 1–15.
Kim, E. J., & Kim, J. J. (2023). Neurocognitive effects of stress: a metaparadigm perspective. *Molecular Psychiatry*, *January*.
Kim, E. J., Pellman, B., & Kim, J. J. (2015). Stress effects on the hippocampus: A critical review. *Learning and Memory*, **22**(9), 411–416.
Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Reviews Neuroscience*, **3**(6), 453–462.
Kumar, A., Arya, H., & Maurya, R. C. (2019). *Neuronal Remodeling and Dendritic Spines: A Review*. *January*.
Kumar, A., Arya, H., Tamta, K., & Maurya, R. C. (2021). Acute stress-induced neuronal plasticity in the corticoid complex of 15-day-old chick, *Gallus domesticus*. *Journal of Anatomy*, **239**(4), 869–891.
Kumar, A., Tamta, K., Arya, H., & Maurya, R. C. (2023). Acute-stress induces the structural plasticity in hippocampal neurons of 15 and 30-day-old chick, *Gallus gallus domesticus*. *Annals of Anatomy*, **245**, 151996.
Kumar, P. K., Behera, K., Mohapatra, L. M., Mandal, A. K., Sethy, K., Gupta, S. K., Panda, S., Behera, D., & Sahu, S. K. (2016). HISTOLOGICAL STUDY OF HIPPOCAMPUS IN MALE AND FEMALE

VANARAJA BIRDS. *International Journal of Agriculture Sciences*, **8**(53), 2711–2714.
Kumaravel, A., Ramesh, G., Paramasivan, S., & Sivagnanam, S. (2019). MICROANATOMICAL OBSERVATIONS ON THE HIPPOCAMPUS (CORNU AMMONIS) IN BROILER CHICKEN. *International Journal of Science, Environment and Technology*, **8**(1), 95–101.
Levine, N. D., Rademacher, D. J., Collier, T. J., O'Malley, J. A., Kells, A. P., San Sebastian, W., Bankiewicz, K. S., & Steece-Collier, K. (2013). Advances in thin tissue Golgi-Cox impregnation: Fast, reliable methods for multi-assay analyses in rodent and non-human primate brain', Journal of Neuroscience Methods, **213**(2), pp. 214–227.
Magariños, A. M., McEwen, B. S., Flügge, G., & Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *Journal of Neuroscience*, **16**(10), 3534–3540.
Maurya, R. C., & Srivastava, U. C. (2006). Morphological diversity of the medial cortex neurons in the common Indian wall lizard, *Hemidactylus flaviviridis*. *National Academy Science Letters*, **29**(9–10), 375–383.
McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annual Review of Neuroscience*, **22**(1), 105–122.
McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance. *Brain Research*, **886**(1–2), 172–189.
McEwen, B. S. (2010). Stress, sex, and neural adaptation to a changing environment: Mechanisms of neuronal remodeling. *Annals of the New York Academy of Sciences*, **1204**(SUPPL.1), 38–59.
Mcewen, B. S., & Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. *Annals of the New York Academy of Sciences*, **1186**, 190–222.
McEwen, B. S., & Gianaros, P. J. (2011). Stress- and allostasis-induced brain plasticity. *Annual Review of Medicine*, **62**, 431–445.
McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*, **41**(1), 3–23.
McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology*, **5**(2), 205–216.
Montagnese, C. M., Krebs, J. R., & Meyer, G. (1996). The dorsomedial and dorsolateral forebrain of the zebra finch, *Taeniopygia guttata*: A Golgi study. *Cell and Tissue Research*, **283**(2), 263–282.
Ojha, K., & Singh, K. P. (2021a). Neuronal diversity, dendro-spinous characterization in the hippocampal complex of *Coracias benghalensis*. *Journal of Applied Biology and Biotechnology*, **9**(4), 112–116.
Ojha, K., & Singh, K. P. (2021b). Seasonal plasticity in the morphology and cytoarchitecture of the hippocampal complex of the Indian Roller, *Coracias benghalensis*. *Zoology*, *147*.
Peters, A., & Kaiserman-Abramof, I. R. (1970). The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *American Journal of Anatomy*, *127*(4), 321–355.
Rochefort, N. L., & Konnerth, A. (2012). Dendritic spines: From structure to in vivo function. *EMBO Reports*, **13**(8), 699–708.
Runge, K., Cardoso, C., & de Chevigny, A. (2020). Dendritic Spine Plasticity: Function and Mechanisms. *Frontiers in Synaptic Neuroscience*, **12**(August).
Sapolsky, R. M., Zola-Morgan, S., & Squire, L. R. (1991). Inhibition of glucocorticoid secretion by the hippocampal formation in the primate. *Journal of Neuroscience*, **11**(12), 3695–3704.
Sherry, D. F. (2005). Do ideas about function help in the study of causation? *Animal Biology*, **55**(4), 441–456.
Shettleworth, S. J. (2003). Memory and hippocampal specialization in food-storing birds: Challenges for research on comparative cognition. *Brain, Behavior and Evolution*, **62**(2), 108–116.
Showers MJC. (1982). Telencephalon of birds. Macmillan, New York,. *In: Crosby EC, Schnitzleds N (Eds) Comparative Correlative Neuroanatomy of the Vertebrate Telencephalon.*, 218– 246.
Singh, D., Kumar, P., & Srivastava, U. C. (2014). Neuronal specialization in hippocampal complex of food storing indian bird *corvus splendens*. *National Academy Science Letters*, **37**(3), 233–235.
Singh, S., Chauhan, P., Singh, D., & Srivastava, U. C. (2018). Distribution of Non-pyramidal Neurons in the Frontal Lobe of Indian Gray Mongoose (*Herpestes edwardsii*). *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, **88**(1), 209–217.

Singh, S., Singh, D., & Srivastava, U. C. (2015). Seasonal dynamics within the neurons of the hippocampus in adult female Indian Ring neck Parrots (*Psittacula krameri*) and Asian Koels (*Eudynamys scolopaceus*). *Canadian Journal of Zoology*, **93**(3), 157–175.
Smulders, T. V. (2017). The Avian Hippocampal Formation and the Stress Response. *Brain, Behavior and Evolution*, **90**(1), 81–91.
Srivastava et al. (2009a). Neuronal morphology of lateral cerebral cortex of the indian wall lizard, H. *flaviviridis*. *National Academy Science Letters*, **32**(9–10), 291–295.
Srivastava, U. C., Chand, P., & Maurya, R. C. (2007). Cytoarchitectonic organization and morphology of the cells of hippocampal complex in strawberry finch, *Estrilda amandava*. *Cellular and Molecular Biology*, **53**(5), 103–120.
Srivastava, U. C., Chand, P., & Maurya, R. C. (2009b). Neuronal classes in the corticoid complex of the telencephalon of the strawberry finch, *Estrilda amandava*. *Cell and Tissue Research*, **336**(3), 393–409.
Srivastava, U. C., & Maurya, R. C. (2009). Neuronal morphology of lateral cerebral cortex of the indian wall lizard, *H. flaviviridis*. *National Academy Science Letters*, **32**(9–10), 291–295.
Srivastava, U. C., & Maurya, R. C. (2010). Evolution of the cerebral cortex in amniotes: Anatomical consideration of neuronal types. In *Nature at Work: Ongoing Saga of Evolution* (Issue January, pp. 329–354).
Srivastava, U. C., Maurya, R. C., & Shishodiya, U. (2007). Cyto-architecture and morphology of the different neuronal types of the cerebral cortex of an Indian lizard, *Mabouia carinata*. *Proc. Nat. Acad. Sci., India*, **77**(IV), 331–347.
Srivastava, U. C., Singh, D., Kumar, P., & Singh, S. (2016). Neuronal diversity and their spine density in the hippocampal complex of the house crow (*Corvus splendens*), a food-storing bird. *Canadian Journal of Zoology*, **94**(8), 541–553.
Srivastava, U. C., & Singh, S. (2012). Seasonal plasticity in neurons of APH in female Indian ringneck parrot (*Psittacula krameri*). *National Academy Science Letters*, **35**(4), 259–262.
Srivastava, U. C., Singh, S., & Singh, D. (2012). Seasonal fluctuation in the neuronal classes of parahippocampal area of *P. krameri* (Scopoli, 1769) and *E. scolopaceus* (Linnaeus, 1758). *Cellular and Molecular Biology (Noisy-Le-Grand, France)*, *Suppl.58*, OL1768-79.
Székely, A. D. (1999). The avian hippocampal formation: Subdivisions and connectivity. *Behavioural Brain Research*, **98**(2), 219–225.
Tömböl T, Davies DC, Németh A, Alpár A & Sebestény T (2000). A Golgi and a combined Golgi/GABA immunogold study of local circuit neurons in the homing pigeon hippocampus. *Anatomy and Embryology*, **201**(3), 181–196.
Tömböl T, Davies DC, Németh A, Sebestény T & Alpár A (2000). A comparative Golgi study of chicken (*Gallus domesticus*) and homing pigeon (*Columba livia*) hippocampus. *Anatomy and Embryology*, **201**(2), 85–101.
Vargas JP, Petruso EJ & Bingman VP (2004). Hippocampal formation is required for geometric navigation in pigeons. *European Journal of Neuroscience*, ***20***(7), 1937–1944.
Volman SF, Thomas C. Grubb J & Schuett KC (1997). Relative Hippocampal Volume in Relation to Food-Storing Behavior in Four Species of Woodpeckers. *Brain, Behavior and Evolution*, **49**(2), 110–120.
Watanabe Y, Gould E, Daniels DC, Cameron H & McEwen BS (1992). Tianeptine attenuates stress-induced morphological changes in the hippocampus. *European Journal of Pharmacology*, **222**(1), 157–162.
Widiger TA & Clark LA (2000). Toward DSM-V and the classification of psychopathology. *Psychological Bulletin*, **126**(6), 946–963.
Yau SY & So KF (2014). Adult neurogenesis and dendritic remodeling in hippocampal plasticity: Which one is more important? *Cell Transplantation*, **23**(4–5), 471–479.