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***Research Article** (Open Access)*

EFFECT OF ACUTE STRESS ON NEURONAL CHARACTERISTICS OF THE DORSOLATERAL FOREBRAIN OF 30 DAYS OLD CHICK

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ABSTRACT
The available literature on stress studies in birds have shown that stress exposure may improve or impair both learning as well as memory processes. The present study aims to evaluate the role of acute stress (AS) exposure in inducing the plasticity in neuronal characteristics of the dorsolateral forebrain of 30 days old chick, *Gallus domesticus*, by using Cresyl Violet, and Golgi Cox staining techniques. The cytoarchitectonic analysis shows that the chick corticoid complex (CC) is formed at the dorsolateral surface of the telencephalic hemisphere and is further differentiated into two subfields: an intermediate corticoid subfield (CI) (consisting of dorsolaterally arranged three layers) and a dorsolateral corticoid subfield (CDL) (without layer patterns). The corticoid complex shows three main spinous neuronal groups: multipolar projection neurons, pyramidal projection neurons, and stellate neurons. The Golgi study revealed that the spinous projection neurons along with stellate neurons observed in the dorsolateral forebrain show remarkable significant variations in their architecture in terms of spine density, dendritic field, soma diameter, spine length, etc. due to acute stress exposure. These plastic changes in the form of either decrease or increase in various neuronal features due to acute-stress effect have been found very significant in the intermediate corticoid subfield as compared to the dorsolateral corticoid subfield which could be linked to more effect of acute stress in this area. The present study will establish that slight modifications in natural stimuli or environmental changes have effect on the neurons of dorsolateral forebrain which may help the animal to adapt stressful environmental conditions.

Keywords: *Acute Stress, Corticoid Complex, Multipolar Neurons, Dorsolateral forebrain, Projection Neurons, Spine Density*

INTRODUCTION
The dorsolateral forebrain or corticoid complex (CC) of birds is present at the dorsolateral surface of the telencephalic pallium and consists of two subfields: an intermediate corticoid area (CI) and a dorsolateral corticoid (CDL) area (Montagnese *et al.*, 1996; Atoji and Wild, 2005; Srivastava *et al.*, 2009, 2014). Several studies, have proposed the functional aspect of the CC in different birds like strawberry finch (Srivastava *et al.*, 2009), Indian house crow (Srivastava *et al.*, 2014), the zebra finch (Montagnese *et al.*, 1996; Colombo *et al.*, 2001; Atoji & Wild, 2005; Srivastava *et al.*, 2009, 2014; Singh *et al.*, 2019) and recommended that the avian CC has participated in spatial memory, based on neuronal types, neuronal density, neuronal morphology etc. The main basic feature of neuronal cells is the presence of dendritic spines as primary postsynaptic structures (Feldman & Peters, 1979; Ballesteros *et al.*, 2006) over their dendritic shafts and are the primary site of structural plasticity (Holtmaat & Svoboda, 2009) due to dynamic nature (Aguayo *et al.*, 2018). Various stimuli such as stress prompted structural plasticity of neuronal cells by strengthening the synaptic transmission for the stressor adaptation in changing environment (Krugers *et al.*, 2010), and failing to do so may leads to anxiety and depression-like mood disorders (McEwen, 2007; Southwick & Charney, 2012).
Stress may produce fluctuations both due to acute (short term) as well as chronic (long term) exposure at the level of structural plasticity such as dendritic branching pattern, dendritic field, and spine density (McEwen, 1998; Buwalda *et al.*, 2005; Chattarji *et al.*, 2015; Hammels *et al.*, 2015). Acute

stress exposure exerts beneficial effects on memory acquisition (Joëls *et al.*, 2006) as well as impairs memory retrieval that is important for animal survival (de Quervain, Roozendaal & McGaugh, 1998) and also modifies memory processes. In Pigeon, based on afferent as well as efferent CDL networks, it is recommended that the CDL belongs to the limbic system, and in some aspects, it may be comparable with the hippocampal parts of the mammalian entorhinal cortex (Atoji & Wild, 2005).
The results of various stress studies concerning the structural plasticity of neurons due to acute stress observed in birds like chicken (Ellethey *et al.*, 2001; Shini *et al.*, 2009; Goerlich *et al.*, 2012), show little/no consistency and needs further evaluation. The domestic chicks are the primary model for studying the development and neurobiology of learning and memory (Nakamori *et al.*, 2013). The present study was designed to investigate the anatomical aspect of acute stress effects on three-dimensional neuronal architecture, in the dorsolateral forebrain of 30 days old chick, *Gallus domesticus*.

MATERIALS AND METHODS

Animal model: Total ten chicks, *Gallus domesticus* of 40 cm mean length (from beak tip to the tail feather endpoint) and having an average weight of 210 gram, were used in the present study. They were purchased from the nearby PAHARI Poultry House (A government department) Hawalbag, Almora, Uttarakhand India. The chicks were brought to the laboratory on the 27thday of their hatching and maintained in the laboratory condition for 24 hours to release stress due to their transport. After 24 hours chicks were divided into two groups of six (Group 1, Non-Stress) and four (Group 2, Acute-Stress). The chicks of Group 1 (NS, Non-Stress) were maintained in the laboratory under normal environmental conditions with free access to food and water, while the chicks of Group 2 (AS, Acute Stress) were maintained in similar environmental conditions without food and water for next 24 hours. At the end of this period, all the chicks were sacrificed by administering a lethal dose of ketamine; their brain was immediately taken out from the skull and processed according to different staining protocols. The present study was carried out according to the animal care guidelines of the Animal Ethics Committee of Kumaun University, Nainital; Uttarakhand, India.
***Cresyl Violet Staining Method*:** For cytoarchitectonic analysis two brains of non-stressed chicks were fixed in 10% formalin at 4°C for 24 hours. Then the brains were washed in water, dehydrated, cleared in xylene and embedded in paraffin wax (56°C- 58°C). Thereafter, 10 µm thick serial sections were cut by using a rotary microtome and stained with Cresyl-Violet Staining solution (Srivastava et al., 2009).
Golgi Cox Staining Method: For neuronal architecture examination, eight brains (4 from group-I & 4 from group-II) were immersed in the filtered Golgi-Cox staining solution for 24 hours at room temperature and followed by 14 days impregnation in fresh solution at room temperature. Thereafter, each brain was washed, dipped in 1% potassium dichromate solution for 24 hours, washed and dehydrated, cleared in xylene and embedded in paraffin wax for section cutting. After block formation, 120 µm thick brain sections were cut with the help of microtome. The sections were deparaffinized in xylene, and rehydrated. Sections were then placed in 1% potassium dichromate, 28% ammonia solution, and 1% sodium thiosulfate for 5 minutes in each (Levine et al., 2013). Thereafter, the sections were dehydrated; cleared in xylene; mounted in D.P.X and studied under the microscope.
Microscopic analysis: The microphotographs of Cresyl Violet stained sections and Golgi impregnated neurons were taken from the original well labeled permanent slides with the help of a computer-aided microscope (Leica) at 40X & 400X magnifications. Camera lucida drawings of all the selected neurons were drawn from the original permanent slides with the help of a camera lucida attached to a light microscope at various focal planes at 400X primary magnification. All the drawings were scanned by using the scanner and corrected with the help of Adobe Photoshop 7.0 computer software. The drawings of the synaptic distributions represent the actual observations.
Neurohistological data analysis: Various morphological characteristics of neurons were calculated with the help of a computer-aided microscope (Leica) at 400X magnification. The numbers of

dendritic branches were counted directly from camera lucida drawings of the neurons. To calculate spine density (N), the total numbers of dendritic spines (n) were counted per 25 µm of dendritic segment in ten fragments from each type of neuron. For analysis, only those dendrites were used which are lying in a plane, parallel to the section. The corrected spine numbers (Srivastava *et al.*, 2014) calculated as per the mathematical formula given by Feldman and Peters (Feldman & Peters, 1979) as the following equation:

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

Where, (n), number of visible spines; (Dr), radius of the dendrite; (Sd), spine head diameter; (Sl), spine length; & (θ), central angle.

Statistical analysis: In the present study student's unpaired t-test (with Welch's correction) has been applied to find out the differences in corrected dendritic spine number between non-stress and acute stressed chicks. A basic minimum criterion of probability level $P < 0.05$ was accepted as indicative of a significant difference. All the results were presented as the Mean ± SEM. All the statistical analysis of neuronal classes was performed by using Microsoft Excel, Graph Pad Prism, and Adobe Photoshop 7.0 software.

RESULTS AND DISCUSSION

Table 1: Showing the total number and percentage (%) of neurons; mean (Mean ± SEM) values of dendritic field, soma diameter, dendritic diameter, spine head diameter, and spine length of different neuronal classes observed in the intermediate corticoid area (CI) and dorsolateral corticoid area (CDL) of corticoid complex of 30 days old non-stress (NS) and acute stress (AS) chick, *Gallus domesticus*.

Field	Neuronal Classes	Stress Type	Total Neuron (x) N	Neuronal %age	Dendritic Field (µm) (Mean ± SEM)	Soma Diameter (µm) (Mean ± SEM)	Dendritic Diameter (µm) (Mean ± SEM)	Spine Head Diameter (µm) (Mean ± SEM)	Spine Length (µm) (Mean ± SEM)
CI	Multipolar	NS	240	47.06	287.70±3.46	20.65±0.38	1.46 ± 0.03	1.22±0.02	1.77±0.02
		AS	480	52.63	268.00±3.12	17.86±0.34	1.52±0.02	1.20±0.02	1.69±0.02
	Pyramidal	NS	120	23.53	305.40±1.91	20.48±0.27	1.51±0.03	1.26±0.01	1.78±0.02
		AS	192	21.05	254.70±3.00	17.24±0.36	1.52±0.03	1.18±0.02	1.66±0.02
	Stellate	NS	150	29.41	183.40±3.35	19.22±0.80	1.34±0.02	1.21±0.01	1.73±0.03
		AS	240	26.32	134.20±3.12	17.10±0.55	1.51±0.03	1.24±0.01	1.72±0.02
CDL	Multipolar	NS	420	58.33	276.20±10.93	18.73±0.46	1.44±0.02	1.21±0.01	1.73±0.02
		AS	240	44.44	237.70±3.80	16.24±0.29	1.37±0.02	1.21±0.01	1.67±0.03
	Pyramidal	NS	120	16.67	289.40±3.19	17.70±0.36	1.44±0.02	1.23±0.02	1.75±0.02
		AS	180	33.33	224.80±2.05	16.31±0.69	1.48±0.03	1.24±0.02	1.72±0.02
	Stellate	NS	180	25.00	182.30±2.43	19.36±0.31	1.46±0.02	1.22±0.02	1.76±0.02
		AS	120	22.22	134.30±2.69	16.92±0.30	1.49±0.03	1.23±0.01	1.65±0.02

Results

Cytoarchitechtonic analysis of the dorsolateral forebrain: The present study revealed that the dorsolateral forebrain of the chick is formed after the progressive replacement of laterally situated superficial layer of visual wulst (VW) that is hyperpallium accessorium (HA) at the dorsolateral surface of the telencephalic hemisphere. The dorsolateral forebrain consists of two subfields: an intermediate corticoid subfield (CI) and a dorsolateral corticoid subfield (CDL). The CI subfield constitutes an intermediate region between the CDL of CC and the parahippocampalis (APH) area of the hippocampal complex (HCC) (Fig. 1.1). In CI, based on the neuronal soma size and density three

dorsolaterally arranged layers can be recognized: Layer-1; Layer-2, and Layer-3 (Fig. 1.2). At the caudal most level of the telencephalic hemisphere, the CI along with the different subfields of HCC progressively disappeared. Thus, caudally at the level of the cerebellum, only the APH of HCC was observed limited laterally by the CDL of the CC (Fig. 1.3). The CDL of the chick telencephalon is a thin, narrow, superficial strip-like structure adjoining the medially situated hippocampal formation (HF) without any layered pattern as observed in the CI subfield (Fig. 1.4).

Table 2: Showing the average number of dendritic branches at 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre; range of axonal length and axonal projections in different neuronal classes observed in the intermediate (CI) and dorsolateral corticoid (CDL) areas of corticoid complex (CC) of 30 days old non-stress (NS) and acute stress (AS) chick, *Gallus domesticus*. L, local; D, dorsal surface; V, ventral surface; P-V, parallel to ventricle; APH, parahippocampalis; CI, intermediate corticoid area; CDL, dorsolateral corticoid area.

Field	Neuronal Classes	Stress Type	Number of dendritic branches at different radius circles from soma centre (Mean ± SEM)				Axonal Length Range (µm) (min-max)	Axonal Projection
			25µm	50µm	75µm	100µm		
CI	Multipolar	NS	13.80±0.42	14.20±0.68	12.70±0.88	8.90±0.53	66.99-108.69	D, V, CDL, APH
		AS	14.10±0.53	13.70±0.76	11.70±0.52	6.30±0.45	55.06-88.87	D,L,V,P-V,CDL,APH
	Pyramidal	NS	12.90±0.62	14.00±0.70	11.40±0.67	8.40±0.45	72.27-98.71	D, V, P-V,CDL,APH
		AS	13.50±0.40	15.20±0.98	13.10±0.64	8.50±0.60	45,48-96.01	L,P-V,D,CDL
	Stellate	NS	10.00±0.42	11.50±0.62	9.30±0.54	4.80±0.55	51.07-78.30	CDL,V,D,P-V
		AS	13.40±0.79	11.20±0.85	7.70±0.37	0.60±0.16	40.39-84.15	L,P-V,CDL,D
CDL	Multipolar	NS	17.30±0.47	18.90±1.29	14.00±0.95	8.80±0.55	53.08-95.45	D,L,V,P-V, CI
		AS	13.50±0.50	17.70±0.75	15.70±0.56	9.20±0.39	30.68-78.99	D,V,L,CI
	Pyramidal	NS	12.80±0.76	16.60±0.85	13.40±0.85	7.20±0.42	55.75-83.43	D,V,L
		AS	12.90±0.59	15.20±1.08	12.70±1.11	8.40±0.56	41.97-80.69	CI,D,V,
	Stellate	NS	10.20±0.81	9.00±0.86	6.00±0.76	0.60±0.27	60.26-83.19	V,D, P-V, CI
		AS	9.90±0.38	10.20±0.79	6.80±0.59	1.30±0.37	38.33-82.10	D, L, V, P-V

Table 3: Showing the results of unpaired t-test with Welch's correction to determine the significant and insignificant dissimilarity between the mean (Mean ± SEM) values obtained from the intermediate (CI) & dorsolateral corticoid (CDL) areas of two independent groups of chicks for corrected spine density (calculated from mean values of spine density) per 25 µm, the long spinous dendritic segment of multipolar, pyramidal, and stellate neurons observed in the corticoid complex (CC) of 30 days old non-stress (NS) and acute stress (AS) chick, *Gallus domesticus*. Data with variance is significantly different at level *P < 0.05, **P < 0.01.

Field	Neuronal Classes	Spine Density		Corrected Spine Number / 25 µm (Mean ± SEM)		t test (with Welch's correction) at P < 0.05			
		NS	AS	NS	AS	Df Value	$t_{calculated}$ values	t_{table} values	Significant/ Insignificant
CI	Multipolar	21.00±0.26	17.20±0.39	68.60±1.416	59.92±2.261	15	3.254	2.131	Significant**
	Pyramidal	21.30±0.84	17.20±0.02	72.40±3.146	60.48±1.959	15	3.217	2.131	Significant**
	Stellate	19.40±0.48	16.60±0.45	64.95±3.256	58.60±1.966	14	1.669	2.145	Insignificant
CDL	Multipolar	20.90±0.48	17.30±0.50	70.86±2.120	61.54±2.762	16	2.676	2.120	Significant*
	Pyramidal	21.80±0.79	17.50±0.40	73.30±2.704	61.96±2.203	17	3.250	2.110	Significant**
	Stellate	19.50±0.40	16.50±045	65.38±2.266	61.99±2.056	17	1.106	2.110	Insignificant

Neuronal classes observed in the dorsolateral forebrain: The complete analysis of the results obtained from Golgi stained sections showed that, based on criteria such as dendritic field, perikaryon diameter, dendritic diameter and branching pattern, spine length & head diameter, axonal length & projection, spine density (Table 1, 2, & 3), three spinous neuronal classes (multipolar projection neurons; pyramidal projection neurons, and stellate neurons) can be distinguished in the CI (Fig. 2 , 3) and CDL subfield (Fig. 2 , 3) of the dorsolateral forebrain of 30 days old chick, *Gallus domesticus.*
The presence of dense spinous sheath over the dendritic branches is the main characteristic feature of the spinous neurons (Fig. 1.5, 1.6). The spinous neurons observed in present study bear four types of dendritic spines namely: filopodia, stubby, thin, mushroom-shaped over their primary, secondary, and tertiary dendritic branches (Fig. 1.5, 1.6). All the neurons give rise to primary dendritic shafts which usually divide into spinous secondary branches which further divide and give rise to tertiary branches that gives a tree-shaped appearance to the neuron (Fig. 2, 3). The axons of these neurons generally originate from the perikaryon, further bifurcates into collaterals (side branches) which make contact with their dendritic branches or the branches of the dendrites of the neighboring neurons and run in all possible directions (Table 2) (Fig. 2, 3).

Acute stress effects on neuronal characteristics in the intermediate corticoid subfield:

Multipolar projection neurons: The most dominant type of neuronal cells which show equal distribution throughout the intermediate corticoid subfield are the spinous multipolar projection neurons (x = 240 & 47.06% in non-stressed & x = 480 & 52.63% in acute-stressed chick) (Table 1). They possess spherical, oval, elongated, angular or irregular shaped soma (Fig. 2.3; Fig. 3.3) having mean soma diameter of 20.65 ± 0.38 µm, in non-stressed &17.86 ± 0.34 µm in acute-stressed chick (Table 1).The extension of dendritic field (287.70 ± 3.46 µm in NS & 268.00 ± 3.12 µm in AS) shows mean number of dendritic branches 13.80 ± 0.42, 14.20 ± 0.68, 12.70 ± 0.88, 8.90 ± 0.53 in NS & 14.10 ± 0.53, 13.70 ± 0.76, 11.70 ± 0.52, 6.30 ± 0.45 in AS at 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre respectively (Fig. 2.3; Fig. 3.3) (Table 2). On the basis of different dendritic characters (Table 1) the corrected spine number observed 68.60±1.416 in non-stressed & 59.92±2.261 in acute stressed chick (Table 3). The axons of the multipolar neurons have an average length range 66.99 - 108.69 µm in NS & 55.06 - 88.87 µm in AS chick (Table 2).

Pyramidal projection neurons: The uniformly distributed spinous pyramidal projection neurons of intermediate corticoid subfield accounts for x = 120 & 23.53% in non-stressed and x = 192 & 21.05% in acute-stressed chick (Table 1). They possess pyramidal like triangular or cone-shaped soma (Fig. 2.5; Fig. 3.5) having mean soma diameter of 20.48 ± 0.27 µm, in non-stressed &17.24 ± 0.36 µm in acute-stressed chick and mean dendritic field 305.40 ± 1.91 µm in NS & 254.70 ± 3.00 µm in AS (Table 1). The mean dendritic branches observed in these neurons at 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre are 12.90 ± 0.62, 14.00 ± 0.70, 11.40 ± 0.67, 8.40 ± 0.45 in NS & 13.50 ± 0.40, 15.20 ± 0.98, 13.10 ± 0.64, 8.50 ± 0.60 in AS respectively (Table 2). The corrected spine number observed 72.40±3.146 in non-stressed & 60.48±1.959 in acute stressed chick (Table 3) on the basis of dendritic characteristics such as dendritic diameter, spine length, spine head diameter (Table 1). The axons have an average length range 72.27 - 98.71 µm in NS & 45.48 - 96.01 µm in AS chick (Table 2).

Acute stress effects on neuronal characteristics in the dorsolateral corticoid subfield:

Multipolar projection neurons: The most dominant type of neuronal cells distributed throughout the dorsolateral corticoid subfield are the spinous multipolar projection neurons (x = 420, 58.33% in non-stressed & x = 240, 44.44 % in acute-stressed chick) (Table 1). They possess spherical, oval, elongated, angular or irregular shaped soma (Fig. 2.4; Fig. 3.6) with mean soma diameter of 18.73 ± 0.46 µm, in non-stressed & 16.24 ± 0.29 µm in acute-stressed chick (Table 1). At 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre these neurons show mean number of dendritic branches 17.30 ± 0.47, 18.90 ± 1.29, 14.00 ± 0.95, 8.80 ± 0.55 in NS & 13.50 ± 0.50, 17.70 ± 0.75, 15.70 ± 0.56, 9.20 ± 0.39 in AS chick (Table 2). The extension of dendritic field observed in these neurons is 276.20 ± 10.93µm in NS & 237.70 ± 3.80µm in AS (Table 2). On the basis of different dendritic characters (Table 1) the corrected spine number observed 70.86±2.120 in non-stressed &

61.54±2.762 in acute stressed chick (Table 3). The axons of the multipolar neurons have an average length range 53.08 - 95.45µm in NS & 30.68 - 78.99 µm in AS chick (Table 2).

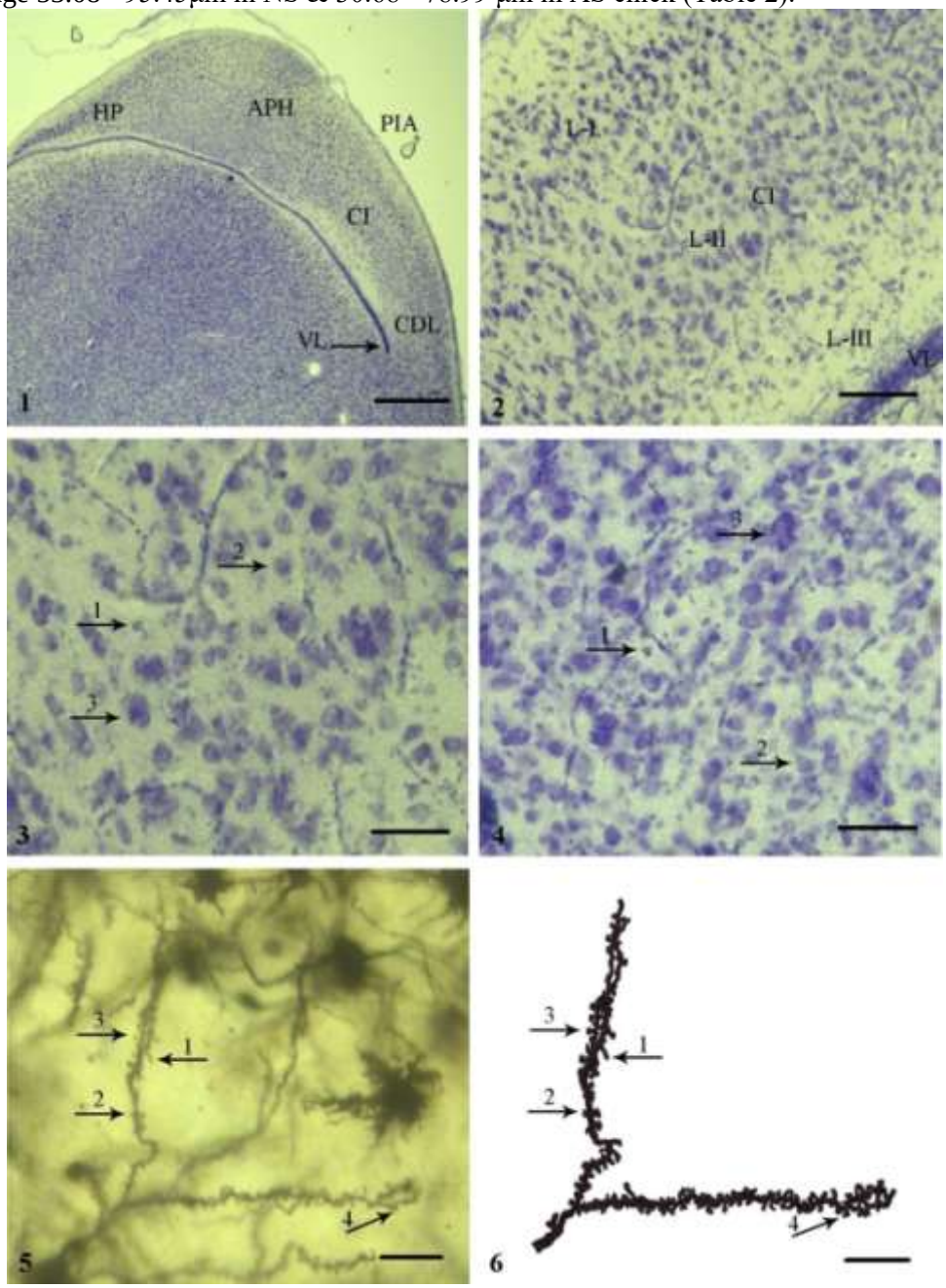

Figure 1: Microphotographs of the dorsolateral forebrain of 30 days old chick, *Gallus domesticus*: (1) Cresyl Violet stained section showing the position of different regions; (2) Cresyl Violet stained section showing the Layer-I (L-I), Layer-II (L-II), & Layer-III (L-III) of the intermediate corticoid; (3-4) showing Cresyl Violet stained blue-purple colored neuronal perikaryon present in the CI and CDL respectively; Arrows: 1, 2, & 3 represents the small, medium, and large-sized soma; (5-6) showing Golgi stained dendritic segment with camera lucida drawing; Arrows 1, 2, 3 & 4 represent the filopodia, stubby, thin, mushroom shaped spines. CI-intermediate corticoid, CDL-dorsolateral corticoid, HP-hippocampus proper, APH-parahippocampalis area, PIA-pia matter, VL-lateral ventricle. Scale bars: (1) 500 µm; (2) 200 µm; (3-4) 50 µm; (5-6) 20 µm.

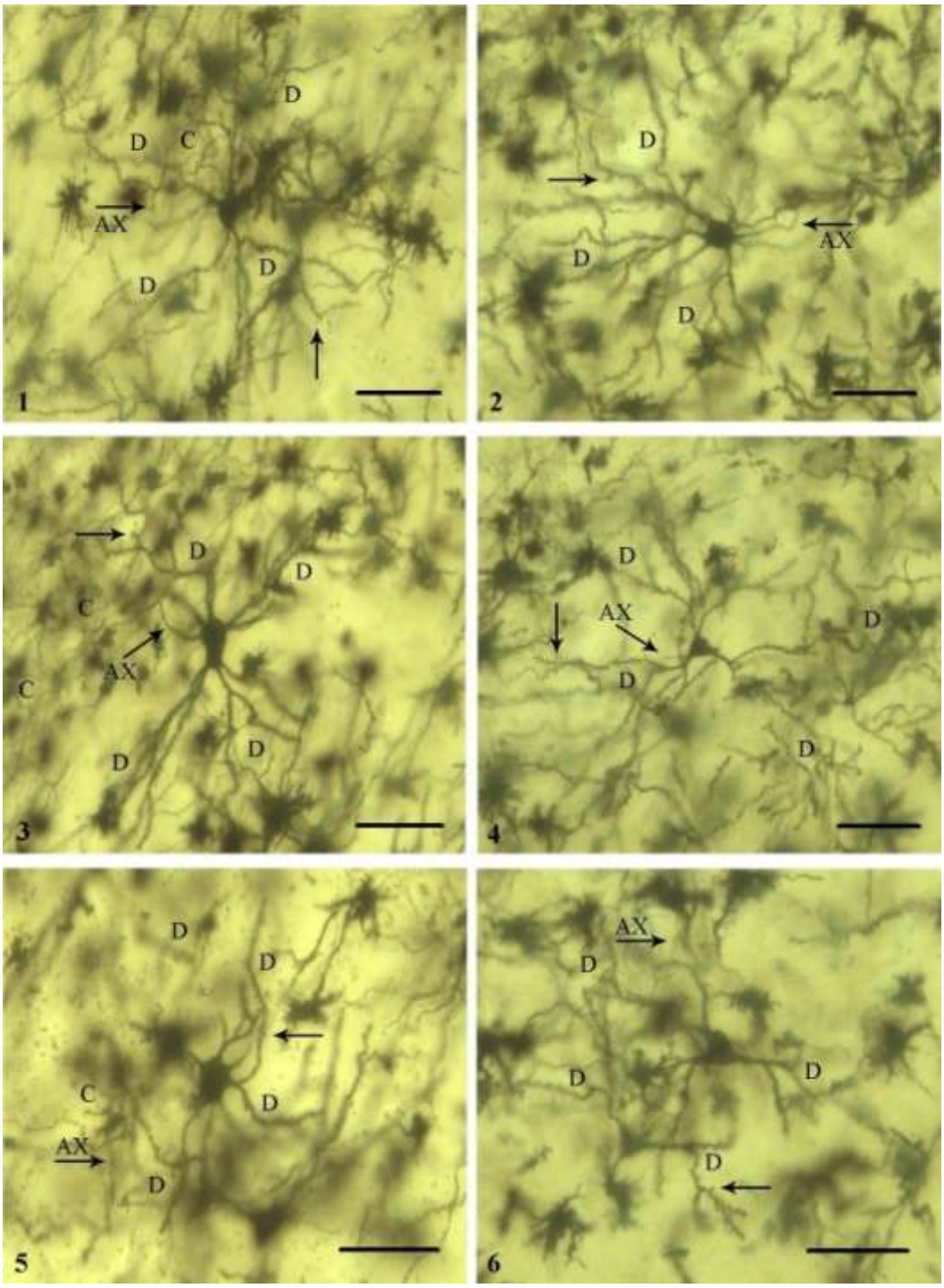

Figure 2: Microphotographs of Golgi-Cox stained sections of the corticoid complex (CC) of 30 days old chick, *Gallus domesticus*: (1, 3, & 5) showing the Golgi stained multipolar, pyramidal, and stellate neurons observed in the intermediate corticoid (CI) subfield of corticoid complex (CC); (2, 4, & 6) showing the Golgi stained dorsolateral corticoidal (CDL) neurons viz., multipolar, pyramidal, and stellate neurons, respectively. D-dendrites, AX-axon, C-axon collaterals, Arrow-spines. Scale bar: 50 µm.

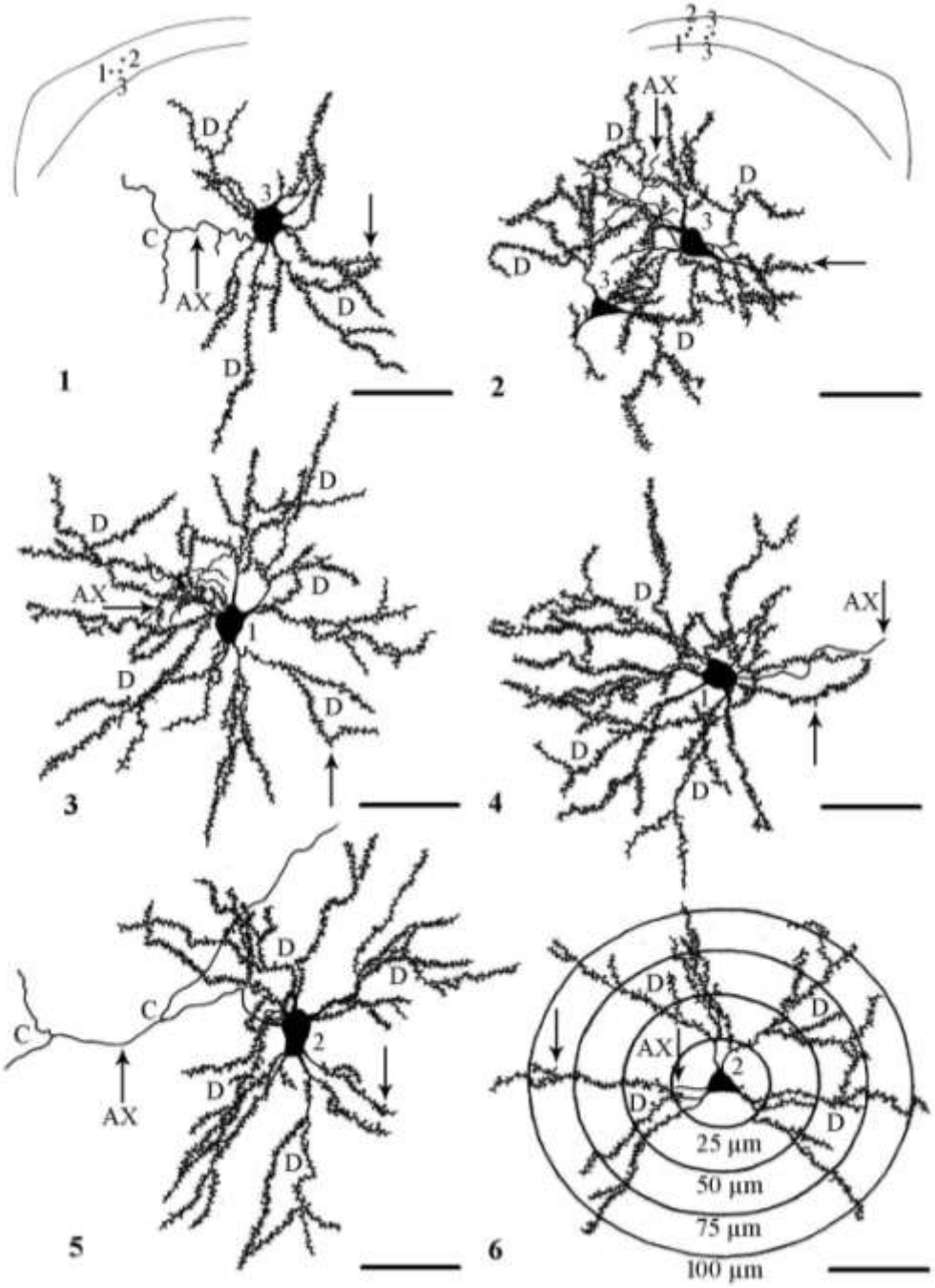

Figure 3: Camera lucida drawings of different spinous neuronal cells observed in intermediate (CI) and dorsolateral corticoidal subfields of the dorsolateral forebrain of 30 days old chick, *Gallus domesticus*: (1, 3, & 5) showing the stellate, multipolar, and pyramidal neurons of the intermediate corticoidal subfield; (2, 4, & 6) showing dorsolateral corticoidal stellate, multipolar, and pyramidal neurons. Cells-1, 2, & 3 represent the multipolar, pyramidal, and stellate neurons observed in both CI and CDL areas of the dorsolateral forebrain. Inset shows the position of different cells in section (6) Cell-2 showing the circles of 25 µm, 50 µm, 75 µm, and 100 µm, radius from the soma centre for dendritic branches counting. D-dendrites, AX-axon, C-axon collaterals, Arrow-spines. Scale bar: 50 µm.

Pyramidal projection neurons: The spinous pyramidal projection neurons of dorsolateral corticoid subfield accounts for x = 120 & 16.67% in non-stressed and x = 180 & 33.33% in acute-stressed chick (Table 1). They possess pyramidal like triangular or cone-shaped soma (Fig. 2.6; Fig. 3.6) having mean soma diameter of 17.70 ± 0.36 µm in non-stressed & 16.31 ± 0.69 µm in acute-stressed chick and mean dendritic field 289.40 ± 3.19 µm in NS & 224.80 ± 2.05 µm in AS (Table 1). The mean dendritic branches observed in these neurons at 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre are 12.80±0.76, 16.60±0.85, 13.40±0.85, 7.20±0.42 in NS & 12.90±0.59, 15.20±1.08, 12.70±1.11, 8.40±0.56 in AS (Table 2). The corrected spine number observed 73.30±2.704 in non-stressed & 61.96±2.203 in acute stressed chick (Table 3) on the basis of dendritic characteristics such as dendritic diameter, spine length, spine head diameter (Table 1). The axons have an average length range 55.75±83.43 µm in NS & 41.97±80.69 µm in AS chick (Table 2).
Stellate neurons: The spinous stellate neurons of the dorsolateral corticoid subfield show uniform distribution and accounts for x = 180 & 25.00% in non-stressed and x = 120 & 22.22% in acute-stressed chick (Table 1). They are characterized by spherical, round, or oval-shaped soma (Fig. 2.2; Fig. 3.2) with mean soma diameter of 182.30 ± 2.43 µm in non-stressed & 134.30 ± 2.69 µm in acute-stressed chick (Table 1). The extension of dendritic field is 182.30 ± 2.43 µm in NS & 134.30 ± 2.69 µm in AS, which shows mean number of dendritic branches 10.20 ± 0.81, 9.00 ± 0.86, 6.00 ± 0.76, 0.60 ± 0.27 in NS & 9.90 ± 0.38, 10.20 ± 0.79, 6.80 ± 0.59, 1.30 ± 0.37 in AS at 25 µm, 50 µm, 75 µm, and 100 µm radius circle from soma centre. Different dendritic characteristics of the stellate neurons (Table 1) show the corrected mean spine number 65.38±2.266 in non-stressed & 61.99±2.056 in acute stressed chick (Table 3). The axons of the stellate neurons with an average length 60.26-83.19 µm in NS & 38.33 ± 82.10 µm in AS (Table 2), originate either from the cell body or from a dendrite (Fig. 2.2; Fig. 3.2).
Statistical data analysis: Statistical analysis of the data revealed that the acute stress (AS) of 24 hours food and water deprivation induces remarkable plasticity in neuronal architecture (most especially in spine density) in both the intermediate and dorsolateral corticoid subfields of the dorsolateral forebrain of 30 days old chick, *Gallus domesticus*. The results of student's unpaired t-test (with Welch's correction) for corrected spine number (N) of the different neurons observed in dorsolateral forebrain of non-stressed and acute stress chick shows that in both intermediate corticoid area the projection neurons show a significant decrease (**) in their spine density within 25µm dendritic segment while the stellate neurons show an insignificant decrease. In the dorsolateral corticoid subfield of corticoid complex due to acute stress effect, the multipolar neurons show significant (*) decrease; pyramidal neurons also show significant (**) decrease while the stellate neurons display an insignificant decrease in their spine density due to stress effect (Table 3)

Discussion

The present study examines various fluctuations observed in the neuronal architecture of dorsomedial forebrain of 30 days old chick, *Gallus domesticus*, as a result of 24 hours of acute stress (AS) exposure. The Nissl study reveals that the dorsolateral forebrain of chick is present at the dorsolateral surface of the cerebral hemisphere and consists of two subfields: an intermediate and dorsolateral corticoid subfield which is also shown in other studies conducted in birds (Montagnese *et al.*, 1996; Tömböl *et al.*, 2000; Srivastava *et al.*, 2007, 2009; Srivastava, Singh & Singh, 2012; Srivastava *et al.*, 2014; Chand *et al.*, 2013). The CI subfield is found to be further composed of three dorsolaterally arranged layers: the superficial Layer-I, the intermediate Layer-II, and the innermost layer-III, as reported in Zebra finch (Montagnese *et al.*, 1996), Chick and pigeon (Tömböl *et al.*, 2000), Strawberry finch (Srivastava *et al.*, 2009), Indian house crow (Srivastava *et al.*, 2014). At the caudal most level, only the parahippocampal area of hippocampal complex and dorsolateral corticoid subfield of corticoid complex is observed as the CI subfield along with the various subfields of the dorsomedial forebrain progressively disappeared. The dorsolateral corticoid subfield is devoid of layering pattern and was observed to be a narrower thin strip-like structure that shares homology with other studies in birds (Székely & Krebs, 1996; Srivastava *et al.*, 2009).

The multipolar and pyramidal projection neurons of the dorsolateral forebrain of the chick are homologous with the projection neurons present in the ventral telencephalon of the chick (Tömböl *et al.*, 1988), corticoid complex of Zebra finch (Montagnese *et al.*, 1996), Strawberry finch (Srivastava *et al.*, 2009), *Corvus splendens*, (Srivastava *et al.*, 2014), & *P. krameri* (Srivastava *et al.*, 2012) and several hippocampal parts of the mammalian entorhinal cortex (Hamam *et al.*, 2002). In the present study, the multipolar neurons are found to be the most dominant neuronal type which is also reported in other studies conducted in birds (Tömböl *et al.*, 2000; Srivastava et al., 2009, 2014) and their morphology may play an essential role in their adaptations to varied ecological niches for survivals under stressful conditions as the dorsolateral forebrain has been suggested to contribute in spatial memory (Colombo *et al.*, 2001; Atoji and Wild 2005). The presence of spinous pyramidal neurons in the dorsolateral corticoid subfield of chick may be associated with memory and cognitive ability as found in house crow (Srivastava *et al.*, 2014). The axonal collaterals of the multipolar and pyramidal projection neurons act as the key afferent source of stellate neurons it may have some essential role in local circuitry (Tömböl *et al.*, 2000; Srivastava *et al.*, 2014). Spinous dendritic shafts of the stellate neurons of chick links to the stellate neurons presented in the visual wulst of the chick (Tömböl *et al.*, 1988) and the corticoid stellate neurons of the *Corvus splendens* (Srivastava *et al.*, 2014).

The present study determines that the single acute stress may induce plasticity in neuronal architecture like reduction in spine density that shows variability between two subfields of corticoid region. Previous studies (Montagnese *et al.*, 1996; Tömböl *et al.*, 2000; Srivastava *et al.*, 2007, 2009, 2012, 2014; Chand *et al.*, 2013) are in agreement with our outcome that the spine density more or less depends on neuronal activity. The stress response may be improved by environmental effects as found in *Rissa tridactyla* and *R. brevirostris* chicks due to food restriction led to heightened stress response (Kitaysky *et al.*, 1999, Brewer *et al.*, 2008). The present findings are in line with the earlier studies of birds that delivered indications about the functional aspects of the corticoid complex in learning and smemory (Colombo *et al.*,2001; Atoji and Wild 2005; Goerlich *et al.*, 2012).

Conclusion

The significant reduction in dendritic spine density in projection neurons of both CI and CDL, due to stress effects supports that the spine density is more or less dependent on neuronal activity. Acute stress modulates the synaptic plasticity, by influencing the particular brain circuits which finally affect the animal's behavior and is essential for the adequate functioning of an individual under continuously changing environmental conditions. Furthermore, the findings of the present study open new avenues of research to understand how a stress exposure may trigger a fast-adaptive response in individuals to overcome the stressful environmental conditions. Further studies will develop our understanding of these plastic changes and their reciprocal interactions in other telencephalic regions.

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