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Diversity and Characteristics of Neurons Observed in Medial Hippocampus (HCm) of Blue Jay (*Coracias benghalensis*)

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Abstract: Hippocampus, a dorsomedial part of forebrain present at the periphery of telencephalon as a narrow strip is included in the limbic system and is known to perform diverse functions such as food storing behavior, memorizing ability and sexual discrimination. Hippocampal region in birds comprises of five regions: Lateral hippocampus (HCl), Parahippocampal area (APH), Central field of Parahippocampus (PHc), medial hippocampus (HCm) and crescent field (CF). HCm is the outer peripheral region close to pia divided in three layer- suprapyramidal, pyramidal and infrapyramidal. Multipolar is the main subtype of neuron of HCm region. Various studies have been performed on Jay species of birds but on Indian jay species the findings remain limited therefore, it has been attempted in the present investigation to study the diversity of neurons in HCm region of Indian Blue jay. The neuronal characteristics and spine density have also been taken into account. During the study it was observed that Pyramidal neurons had better dendritic field and axonal length. Multipolar neurons had greater soma size (diameter) and spine density in comparison to other neuronal types. Multipolar and pyramidal neurons were observed to be prominent in distribution while bitufted neurons were observed to be in least count during the study.

Keywords: *Coracias benghalensis*, Multipolar neurons, Soma diameter, Dendritic field

Introduction

Avian hippocampus, a homologue to mammalian hippocampus exhibits morphological as well as functional similarities (Montagnese *et al.,* 1996). Avian dorsomedial forebrain has been suggested to participate in memory formation, storing of food and spatial navigation (El Falougy and Benuska, 2006). Hippocampal lesion experiment showed that the lesions in hippocampus impaired the navigation skills in pigeons (Bingman *et al.,* 1990). Hippocampal damage in food storing bird unable them to retrieve their food (Sherry *et al.,* 1989). Several workers have worked out to draw parallels among the mammalian and avian

hippocampus on the basis of laminar organisation and cell type (Molla *et al.,* 1986; Pisana, 1986) or immunocytochemistry (Krebs *et al.,* 1991; Erichsen *et al.,* 1991). These efforts focused the interest of researchers towards the avian hippocampus, its importance in functions like formation of memory, cache retrieval and spatial navigation. At the intermediate level hippocampal complex size increases dorsally and laterally, a dense packed cells appears in the two-third of ventral portion of hippocampal complex, this hippocampus proper can be differentiated into lateral (HCl) and medial hippocampus (HCm), outer peripheral part of this hippocampus proper close to pia is known as medial hippocampus (HCm). Generally the HCm region is present in serial sections in rostral and medial region but not in caudal sections (Srivastava *et al.,* 2007).

Cort and Clayton (2006) studied different jay species (including blue jay – *Cyanocita cristata*) and categorized them as food storing bird in caching category hierarchy. Brodin and Lundborg (2003) investigated several birds of paridae family (tits, titmice and chickadees) and corvidae family (crow, nutcrackers, jays etc.) and pointed out that food storing jay species have larger hippocampus than non-storers. These studies included food storing jay species, describing their food caching behavior and its relation with hippocampus but lacked the information regarding neuronal distribution within the hippocampus. To the best of authors' knowledge, there is no literature on neuronal classes and its parameter of jay bird and hence the present study was undertaken to investigate the neuronal classes and its characteristic within the HCm region of blue jay (*Coracias benghalensis*).

Materials and Methods

Three adult *Coracias benghalensis* used in the present study were collected from Allahabad ($25^0 28' N$, $81^0 54' E$), India. Golgi Colonnier Method (Blaesing *et al*., 2001) and Nissl (cresyl violet) method were employed for neuronal study. All the protocols were in accordance to institutional animal care guideline. A total of six hemispheres were used for study, four for golgi colonnier and two for cresyl violet method. During golgi experiment, the brain of transcardially perfused birds (by solution of physiological saline and 2% paraformaldehyde dissolved in 0.1 M phosphate buffer (pH 7.4) fixative) were withdrawn from skull and kept in 2% paraformaldehyde fixative dissolved in 0.2 M phosphate buffer (pH 7.4) for 24 h. Further the brains were treated with 2.5% $K_2Cr_2O_7$ solution for 1 h. The prechromation step was repeated. Brains were dipped into solution of gluteraldehyde (5% w/v) and $K_2Cr_2O_7$ (2% w/v) for a span of 3 days at 4 C followed by impregnation in 0.65% $AgNO_3$ (w/v) solution for 2 days at 4 C. Chromation and impregnation steps were repeated for better results. Brains were dehydrated and blocked in paraffin wax and sectioned at 100 µm thickness. Sections were treated with xylene and finally mounted in DPX. In cresyl method, birds were perfused transcardially, the brains were dehydrated in increasing alcohol grades, dipped in xylene and finally moulded in paraffin. Brains were sectioned at 10 µm, deparafinized in xylene, treated with decreasing grades of alcohol and stained with cresyl violet. Finally sections were dehydrated through different alcohol

grades, dipped in xylene and mounted with DPX. Sections containing neurons were photomicrographed with Nikon eclipse 80i (Software, ACT-1) computer-aided microscope at magnification of 40x. Camera lucida of selected neurons were drawn. Microsoft paint, Adobe photoshop version 7.0 and graphpad prism 5.0 software were used for statistical calculation. For spine density calculations, number of spine was calculated per 10 µm of dendritic segment. Number denoted as observable spine does not reflect the correct number of spine as a few spines that are present on the opaque portion of dendrite are obscured and not taken into consideration. For the calculation of true spine density, formula given by Feldman and Peters (1979) was used. All the protocols were in accordance to institutional animal care guideline.

Results

Uneven distribution of small to large sized cell was found in the HCm region of hippocampal complex of Blue jay *Coracias benghalensis*. Nissl (cresyl violet) study (Fig. 1) showed that the region is divided into three subparts namely Suprapyramidal (layer I), Pyramidal (layer II) and Infrapyramidal (layer III). During the investigation, four types of projection neurons were observed namely-- Multipolar, Pyramidal, Bipolar and Bitufted. Multipolar neurons (Figs. 2a, b) had small to large sized soma; distantly projecting axon which extended to other subfields; large dendritic field; oval or multiangular soma shape with 3-6 dendrites emerging out from soma. Pyramidal neurons (Figs. 2c, d) had pyramidal shaped or triangular soma of medium size with dendrites emerging from its entire corner. Bipolar neurons (Figs. 3a, b) had two dendritic branch extending towards the opposite direction while bitufted neurons (Figs. 3c, d) sent dendrites in both the direction mostly in form of tuft. Multipolar and pyramidal neurons were the dominant neuronal class in HCm region of presently studied bird C*oracias benghalensis* having higher soma size, dendritic field (Table 1) and spine density while bipolar and bitufted had lesser dendritic field and spine density. Spine density was greater in multipolar and lesser in bipolar, dendritic field was higher in pyramidal and lesser in bitufted, soma diameter was highest in multipolar and lesser in bipolar (Table 2) while axonal length had greater value for pyramidal and lesser value for bipolar neurons (Table 1).

Discussion

Laminar organization of HCm region in *Coracias benghalensis* is similar to the earlier studies on non-food storing bird species viz *Estrilda amandava* (Srivastava *et al.,* 2007); *Taeniopygia guttata* (Montagnese *et al.,* 1996); *Psittacula krameri* and *Eudynamys scolopaceus* (Singh *et al.,* 2015) as well as food storing *Corvus splendens* (Srivastava *et al.,* 2016). Multipolar neurons were observed to be the dominant type of neuron in this region which is in consonance with the previous findings in *Taeniopygia guttata* (Montagnese *et al.,* 1996), *Estrilda amandava* (Srivastava *et al.,* 2007), *Eudynamys scolopaceus* and *Psittacula krameri* (Srivastava *et al.,* 2016).

Study by Srivastava *et al.* (2007) on *Estrilda amandava* (non-storing bird) highlighted many of the neuronal characteristics in HCm region. These observations when compared with the presently studied bird reveals that soma size, dentritic field, axonal projection and spine

Table 1: Different neuronal characteristic of HCm region in *Coracias benghalensis*

Field	Type of neuron	Soma diameter (µm±SD)	Dendritic Field (µm±SD)	Axonal length (µm±SD)	Neuronal Population (%)
HCm	Multipolar	16.20±3.24	236±38.09	176±22.33	48%
	Pyramidal	14.30±4.80	276±34.80	181±19.46	38%
	Bipolar	11.45±2.40	219±21.08	141±21.05	11%
	Bitufted	13.10±4.60	180±27.50	166±10.88	3%

(SD= Standard Deviation)

density of food storing *Coracias benghalensis* is greater than the non-food storing *Estrilda amandava*. Such result indicates the preeminence of food storing bird over the non-food storers in terms of neuronal parameters which in turn can be correlated with better functioning of concerned region of brain in food storing birds (Srivastava *et al.,* 2016). Comparison with *Taeniopygia guttata* (Montagnese *et al.,* 1996) another non-storing bird also leads to similar conclusion. Studies by Srivastava *et al.* (2016) on neuronal classes and characteristics in food storing *Corvus splendens* showed larger soma size, dendritic field, axonal length and spine density in comparison to non-food storers. All the neuronal parameters in the presently studied bird were very close to *Corvus splendens,* suggesting comparable results in both the food storing bird species. Adequate amount of pyramidal neurons within the HCm region in *Coracias benghalensis* possessing larger dendritic field and dendritic thickness than the other neuronal types indicates better memory and cognitive ability of the bird (Elston, 2003).

Soma size of neurons observed in HCm region of *Coracias benghalensis* were found to be greater as compared to the non-food storing *Estrilda amandava* (Srivastava *et al*., 2007) and *Taeniopygia guttata* (Montagnese *et al.*, 1996), whereas comparable to those observed in HCm of food storing *Corvus splendens*. Larger soma size in *Coracias benghalensis* might correspond to support longer axon and dendritic arborisation as soma serves as metabolic depot that supports conducting dendrites and axon (Lieberman, 1976). Greater soma size in

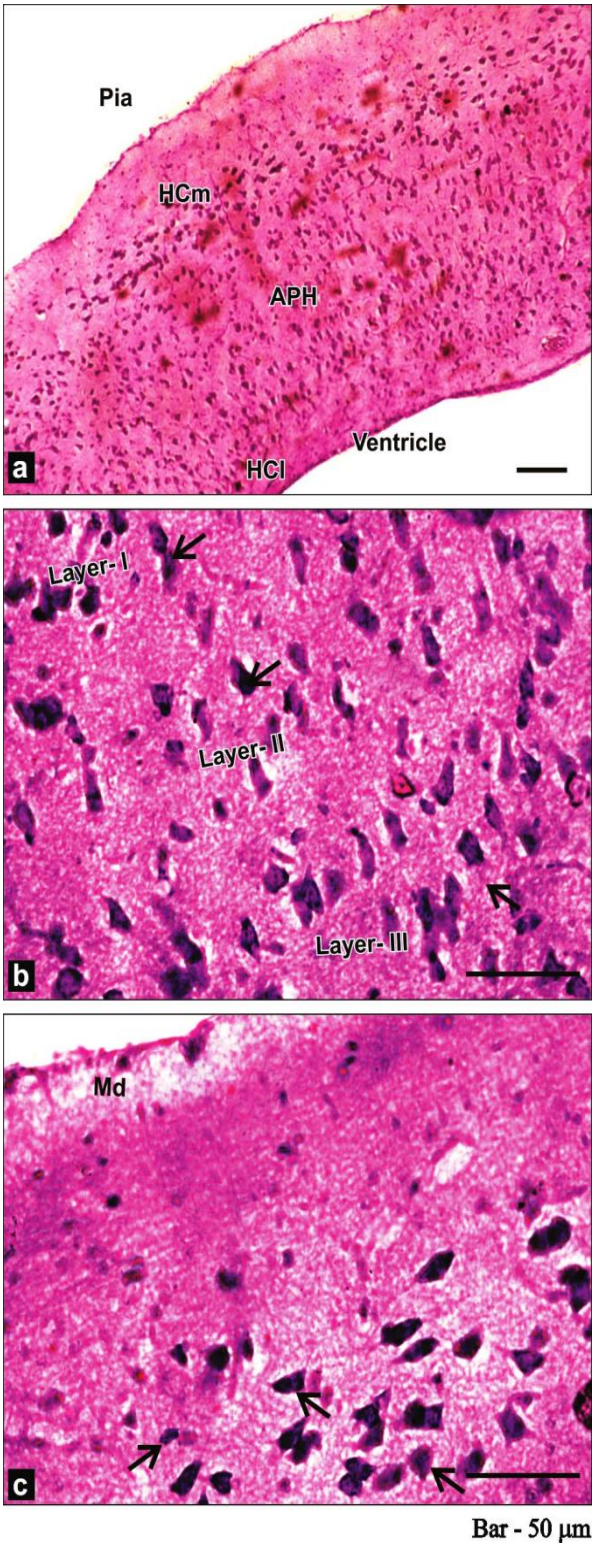

Fig.1 : **a-** Photomicrograph of Hippocampal complex showing medial hippocampus (HCm) **b-** Photomicrograph showing three cell layer's in HCm i.e. suprapyramidal (I), pyramidal (II) and infrapyramidal (III). pyramidal cells (↑) are present in layer with other cells.**c-**HCm at Pial surface showing cell free region at mid-dorsal position (Md).

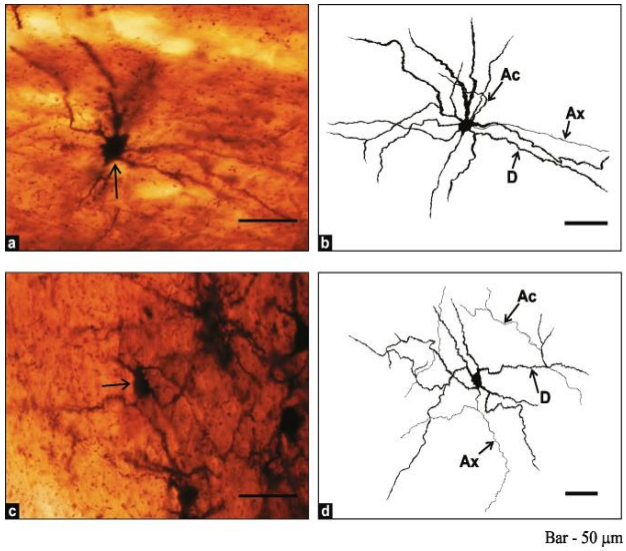

Fig. 2 : **a -** Photomicrograph of multipolar neuron of HCm region showing soma(↑)and dendritic arborization **b-**Camera lucida drawing of respective neuron showing orientation of Dendrite (D), Axon (Ax) and Axon Collaterals (Ac) **c-**Photomicrograph of Pyramidal neuron of HCm region showing soma (↑)and dendritic arborization **d-**Camera lucida drawing of Pyramidal neuron of HCm region showing orientation of Dendrite (D), Axon (Ax) and Axon Collateral (Ac).

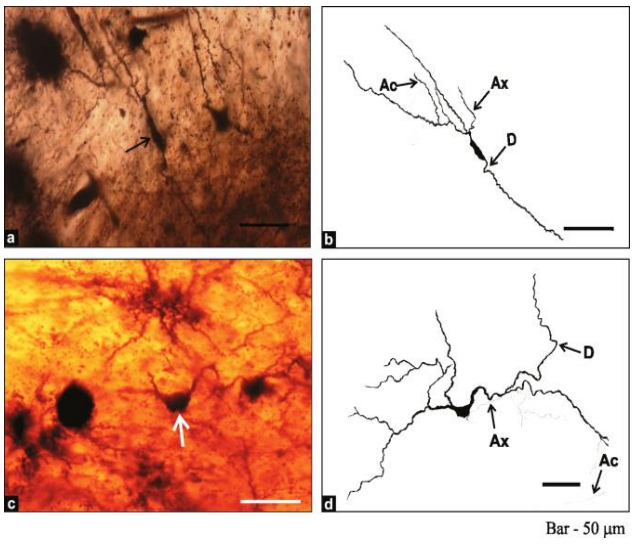

Fig. 3: **a-** Photomicrograph of Bipolar neuron of medial hippocampus (HCm) region showing soma (↑)and dendritic arborization. **b-** Camera lucida drawing of respective neuron showing orientation of dendrite (D),Axon (Ax) and Axon collaterals (Ac). **c-** Photomicrograph of Bitufted neuron of HCm region showing soma (↑)and dendritic arborization **d-** Camera lucida drawing of respective neuron showing orientation of Axon (Ax), Axon Collaterals (Ac) and Dendrite (D).

Table 2: Different neuronal parameter viz. spine length, spine head diameter, dendritic thickness, number of visible and true number of estimated spines of different neuronal types of HCm region of hippocampal complex of Blue jay (*Coracias benghalensis*)

Hippocampal subfield	Type of Neuron	Spine length (µm±SD)	Spine head diameter (µm±SD)	Dentritic thickness (µm±SD)	No. of visible spine (n)	True no. of Estimated spine (N)
HCm	Multipolar	2.10±0.62	1.02±0.16	1.34±0.34	12.80±2.23	23.02±10.12
	Pyramidal	2.21±0.31	0.84±0.07	1.56±0.41	13.50±2.10	19.84±8.75
	Bipolar	2.04±0.25	0.72±0.06	1.26±0.51	7.25±0.98	14.02±3.42
	Bitufted	1.68±0.21	0.82±0.21	1.23±0.23	8.32±1.08	14.43±6.85

(SD= Standard Deviation)

presently studied bird can therefore be correlated with food storing habit of the bird allowing more conducting dendrites and longer axon length. Similarly, the comparison of dendritic field and axon length of *Coracias benghalensis* with that of non-food storing *Estrilda amandava* (Srivastava *et al*., 2007) shows marked difference. Such differences indicate ascendance of food storing *Coracias benghalensis* over non-storing birds.

As far as spine density is considered it also shows similar trend i.e. spine density of neurons within HCm region in *Coracias benghalensis* was comparatively more in comparison to non- food storing *Estrilda amandava* (Srivastava *et al*., 2007) and *Taeniopygia guttata* (Montagnese *et al.,* 1996), but very close to food storing *Corvus splendens* (Srivastava *et al.,* 2016). Greater spine density and other parameters (spine head and neck length) adds to the food storing behavior in blue jay (*Coracias benghalensis*) by enabling better memorizing and cognitive abilities (Burgos *et al.,* 2012).

Conclusion

This study provides the details of neuronal diversity in HCm region of *C. benghalensis.* Neuronal diversity in *C. benghalensis* alongwith the neuronal parameters indicate specialized characters which help the bird in food storing behavior. The present finding shows relationship between neuronal specialization and food storing behavior. However, to draw any broad conclusion or to draw any phylogenic relationship of hippocampus neuron size between blue jay and crow warrants further study.

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