

# Neuronal diversity, dendro-spinous characterization in the hippocampal complex of *Coracias benghalensis*

Kirti Ojha, Krishna Pal Singh\* 

Neurobiology Lab, Department of Zoology, Science Faculty, University of Allahabad, Prayagraj, India

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## ABSTRACT

*Coracias benghalensis*, a seasonally breeding sub-tropical bird, displays a peculiar rolling behavior to attract the female for courtship and territoriality. The avian hippocampal complex (HCC) is a dorsomedial component of the forebrain and is involved in cognitive functions and sexual behavior. The present study was aimed to identify and characterize the neuronal classes, dendritic arborization, and spines density in the HCC of *C. benghalensis* during the quiescent phase of the breeding cycle. Adult male birds were collected during the winter season from a wild zone of Prayagraj (25° 28' N, 81° 54' E), U.P., India. These birds were transcardially perfused, and whole brains were stained with Cresyl-violet and Golgi-Colonnier staining method. We observed unipolar, bipolar, pyramidal, and multipolar types of projection neurons and local circuit (aspinous) neurons with a qualitative and quantitative characterization of soma size, dendritic arborization, and spine density. The multipolar neurons with the highest spine density were predominantly present in the HCC of *C. benghalensis*. This study concludes that the bird's neuronal diversity and neuronal characteristics suggest a unique character that helps the bird perform overt rolling behavior as functional (learning and memory) intervention of the HCC.

## 1. INTRODUCTION

The hippocampal complex (HCC), a prominent component of the brain network and is considered to be involved in learning, memory, spatial navigation, and sexual behavior [1,2] in birds. Topologically, avian HCC is divided into three main components, dorsolateral hippocampus (DLH), dorsomedial hippocampus (DMH), and ventral hippocampus (VH) [3,4]. The avian HCC is homologous to the mammalian hippocampus for structural and functional entities [5,6]. The neuronal diversity in the HCC of the avian brain is also similar to the mammalian hippocampus [1,7].

In avian HCC, two main neuronal classes, spinous projection neurons and several types of aspinous local circuit neurons, were reported in *Taeniopygia guttata* [8], *Gallus domesticus* [1],

*Columba livia* [5], *Estrilda amandava* [9], and *Corvus splendens* [10]. Tömböl *et al.* [1] classified three types of spinous projection neurons, pyramidal, pyramidal-like and multipolar neurons, and aspinous multipolar local circuit neurons. There are several types of projection neurons in *E. amandava* [9] and *C. splendens* [10]: monotufted, bitufted, pyramidal, pyramidal type, and multipolar. Apart from these types of HCC neurons, another neuron, stellate neurons, were also identified [9,10]. A characteristic feature of dendrites is that the spine and spine are divided into head and neck regions. Excitatory synapse, learning, and memory development may be related to an increase in the hippocampus's spine density [11]. Spine morphology was described in the HCC of the *E. amandava* [9], *C. splendens* [10].

However, neuronal classes in the HCC of *Coracias benghalensis* (an Indian Roller bird displaying a characteristic ornate rolling behavior by a male bird during the breeding season to attract a female for courtship) have not been identified and characterized so far. Therefore, the present study has been undertaken to reveal the neuronal classes and their characterization in the HCC of *C. benghalensis* using Cresyl-violet and Golgi impregnation staining techniques.

### \*Corresponding Author

Krishna Pal Singh, Neurobiology Lab, Department of Zoology,  
Science Faculty, University of Allahabad, Prayagraj, India.  
E-mail: [kpsald29@gmail.com](mailto:kpsald29@gmail.com)

## 2. MATERIALS AND METHODS

As per our study design, four adult male birds, *C. benghalensis* (weight,  $90 \pm 10$  g; size,  $26 \pm 27$  cm), were collected during the November–January from the subtropical wild zone of Prayagraj ( $25^{\circ} 28' N$ ,  $81^{\circ} 54' E$ ), U.P., India.

### 2.1. Ethical Clearance

Ethical permission was taken from the Principal Chief Conservator of Forest and Wildlife, U.P., India, vide letter no. 23-2-12(G), 26-04-2016.

### 2.2. Cresyl-Violet Staining

For the cytoarchitectonic study of the HCC, one adult male bird was perfused transcardially with 10% formalin solution, brain dissected out, dehydrated in ascending grades of alcohol series, and finally embedded in paraffin wax. Furthermore,  $10 \mu\text{m}$  thick serial sections were cut and stained with Cresyl-violet [12] solution for studying the cytoarchitecture of the HCC, as previously reported by this laboratory [13].

### 2.3. Golgi-Colonnier Staining

In our study, Golgi-Colonnier staining method [14] was used to assess neuronal morphological characteristics in three brains of adult male birds. This staining procedure was earlier reported in detail [15,16]. In brief, all birds were transcardially perfused with saline, followed by 2% paraformaldehyde (PFA) (0.1 mol/l phosphate buffer at  $4^{\circ}\text{C}$ , pH 7.4 for 40 minutes). The dissected-out brain was submerged in the same fixative for 24 hours, then transferred into 2.5%  $\text{K}_2\text{Cr}_2\text{O}_7$  for 1 hours with two changes as a pre-chroming process. Finally, it was transferred for 72 hours for chroming to the 5% glutaraldehyde and 2% potassium dichromate solution. For impregnation, the brain was transmitted for 48 hours into a 0.62% solution of the  $\text{AgNO}_3$ . Both the chroming and impregnation steps were repeated twice. Furthermore, the brain was dehydrated into increasing alcohol series, cleared in xylene, and fixed in paraffin wax. The  $100 \mu\text{m}$  thick serial sections of the whole brain were cut (rostral to caudal) by a rotary microtome. Sections were deparaffinized in xylene, dehydrated in graded alcohol followed by xylene treatment, and mounted with DPX. After thorough observation, selected Golgi-Colonnier stained sections were photomicrographed under Leica DM 2500. The camera lucida drawings of each neuronal class were sketched using a light microscope.

### 2.4. Measurement of Spine Density

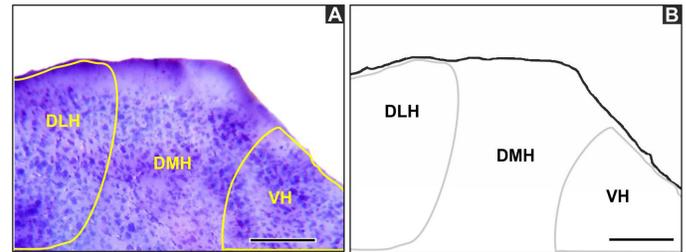
To predict “true” total spine density ( $N$ ), the following equation given by Feldman and Peters (1979) was used:

$$N = \frac{n\pi \left[ (Dr + SpL)^2 - (Dr + SpD)^2 \right]}{\left[ \frac{\theta}{90\pi} (Dr + SpL)^2 \right] - 2 \left[ (Dr + SpL) \sin \theta (Dr + SpD) \right]}$$

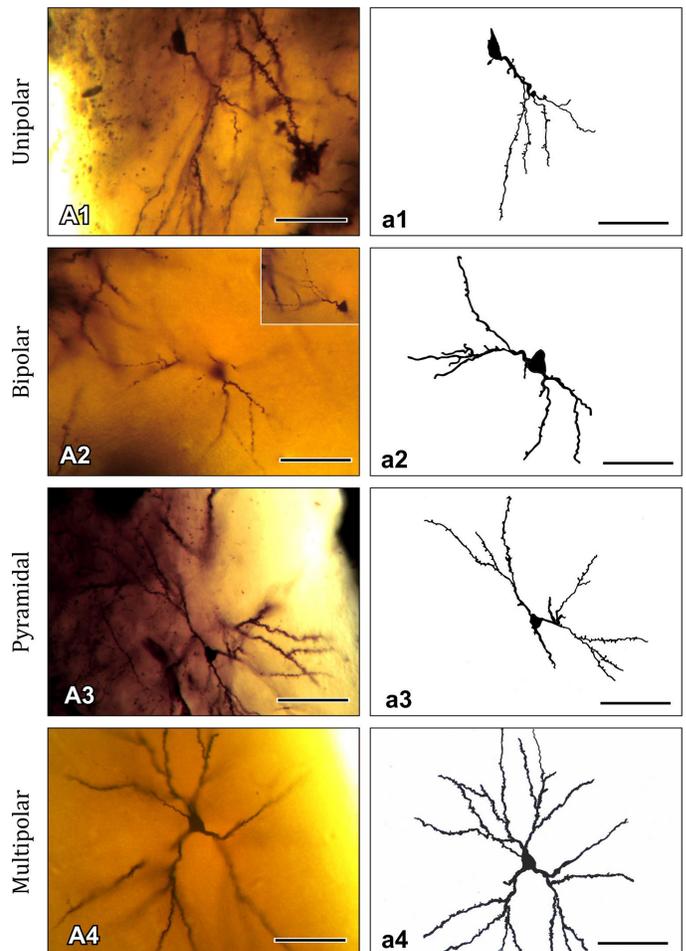
where  $N$  = true spine density,  $n$  = number of visible spines,  $Dr$  = radius of a dendrite,  $SpL$  = spine length,  $SpD$  = spine head diameter, and  $\theta$  = central angle.

## 3. RESULTS

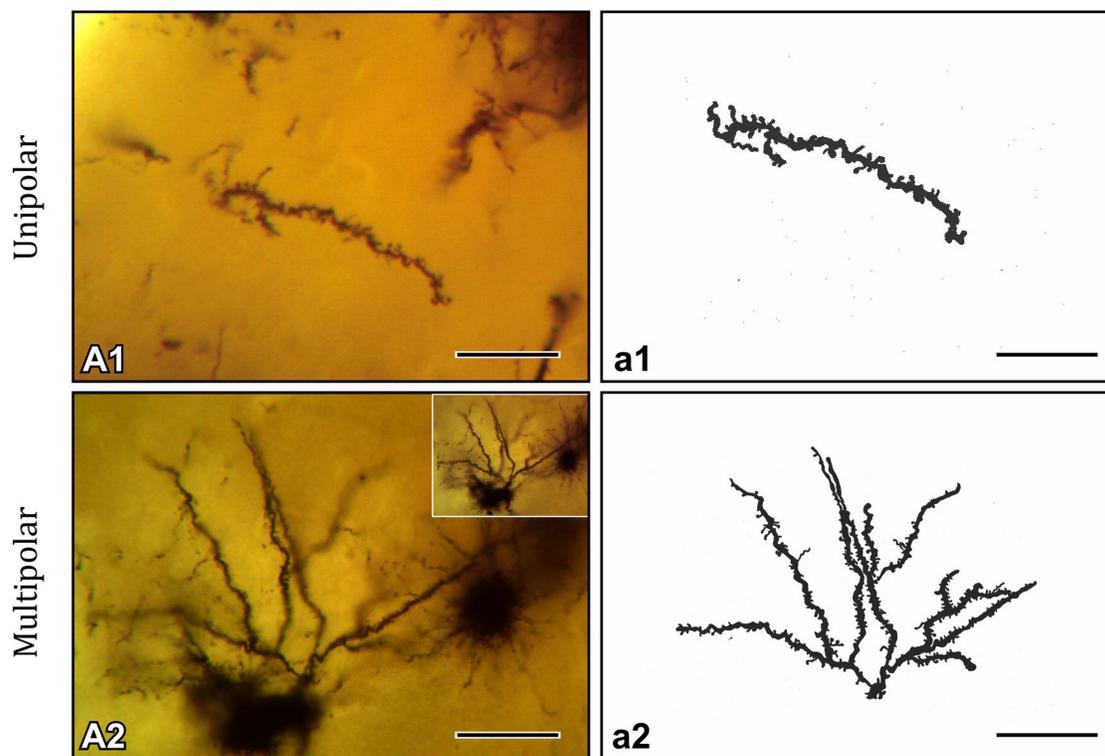
The present study revealed the cytoarchitectural pattern of HCC (Fig. 1) characterized with two major classes of neurons, projection neurons (Figs. 2, 3 and Table 1) and local circuit neurons (Fig. 4 and Table 1). These neurons were ubiquitously distributed in the entire regions (DLH, DMH, and VH) of HCC; however, most neurons were present in the DMH region.



**Figure 1:** (A) Cresyl-violet stained sections showed major sub-fields of hippocampus complex (HCC) of a male *C. benghalensis*. Abbreviations: DMH, dorsomedial hippocampus; DLH, dorsolateral hippocampus; VH, ventral hippocampus. (B) Camera lucida drawing of (A). Scale bar=  $50 \mu\text{m}$ .



**Figure 2:** A1 to A4 represents the photomicrographs of different types of projection neurons (unipolar, bipolar, pyramidal, and multipolar neurons) in the hippocampal complex (HCC) *C. benghalensis*, whereas a1 to a4 represent camera lucida drawings of the photomicrographs of A1 to A4. Scale bar =  $50 \mu\text{m}$ .



**Figure 3:** A1 and A2 represent photomicrographs of the dendritic segment(s) of projection neuron showing spines on the unipolar (minimum spines) and the multipolar (maximum spines) neurons of the hippocampal complex (HCC) of *C. benghalensis*, whereas a1 & a2 represent camera lucida drawings of the photomicrographs of A1 and A2. Scale bar = 50  $\mu\text{m}$ .

**Table 1:** Measures of neuronal entities of projection and local circuit neurons in the HCC of *C. benghalensis*.

Type of neurons	Soma diameter ( $\mu\text{m}$ )	Dendritic length ( $\mu\text{m}$ )	Spine density ( $\mu\text{m}$ )
Unipolar	6–10	70–105	8.93–15.44
Bipolar	7–11	98–220	10.81–18.03
Pyramidal	10–15	120–290	12.76–24.96
Multipolar	11–16	135–320	14.62–31.98
Local circuit neurons	Aspinous or sparsely spinous multipolar	110–265	5.02–8.92

### 3.1. Projection Neurons (Spinous Neurons)

We have identified and characterized four types of projection neurons in the present study, namely, unipolar, bipolar, pyramidal, and multipolar (Fig. 2 and Table 1). Among these neuronal types, multipolar neurons were found predominantly throughout the HCC region. The multipolar neurons were endowed with the soma of oval, spherical, rectangular, multiangular shaped with a diameter of 11–16  $\mu\text{m}$ . Two or more (4–7) dendritic branches, apical (thick) and basal (thin), were emanated in all possible directions from the soma or cell body of neurons. The dendritic length, either apical or basal direction, was ranged from 135–320  $\mu\text{m}$  from the soma, whereas spine density was found between 14.62 and 31.98  $\mu\text{m}$ .

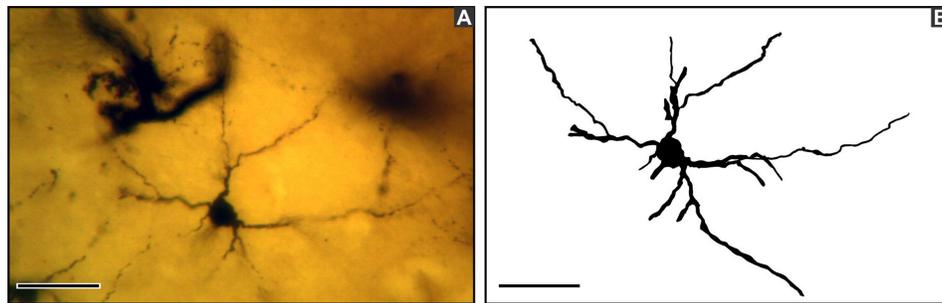
The pyramidal neurons possessed with medium-sized soma of triangular or pyramidal shaped with diameter 10–15  $\mu\text{m}$  give rise thick apical dendrite that runs toward the pia and dividing into a few side branches fashioned as a tree-shaped structure, and two to four thin basal dendrites directing toward ventricle.

Dendritic length (120–290  $\mu\text{m}$ ) and spine density (12.76–24.96  $\mu\text{m}$ ) of pyramidal neurons were also measured (Fig. 2 and Table 1).

The bipolar neurons with a bean-shaped soma of 7–11  $\mu\text{m}$  diameter give rise to dendritic branches in two directions: the pia and the other toward the ventricle. The bipolar neurons were observed to be the least in number out of four types of neurons present in the HCC. The dendritic length and spine density of bipolar neurons were ranged from 98 and 220  $\mu\text{m}$  and 10.81–18.03  $\mu\text{m}$ , respectively (Fig. 2 and Table 1).

The unipolar neurons have oval or rounded soma (6–10  $\mu\text{m}$  diameter), and dendrites were intended for one direction, either toward the pia or ventricle. The dendritic length (70–105  $\mu\text{m}$ ) and spine density (8.93–15.44  $\mu\text{m}$ ) of unipolar neurons were also recorded (Fig. 2 and Table-1).

Overall, soma size, dendritic length, and spine density of unipolar, bipolar, pyramidal, and multipolar neurons of HCC were found



**Figure 4:** (A) The photomicrograph of local circuit neuron and (B) respective camera lucida drawing of the hippocampal complex (HCC) of *C. benghalensis*. Scale bar = 50  $\mu$ m.

in ascending order (minimum in unipolar and maximum in multipolar), respectively (Fig. 3).

### 3.2. Local Circuit Neurons (Aspinous Neurons)

Local circuit or aspinous neurons were endowed with a medium-sized soma (11–15  $\mu$ m diameter) and dendritic length (110–265  $\mu$ m), whereas spine density was found to be the lowest (5.02–8.92  $\mu$ m). Local circuit neurons dendritic trees were mostly multipolar, giving four to six smooth or sparsely spinous branches (Fig. 4 and Table 1).

## 4. DISCUSSION

In the present study, the cytoarchitectonic subdivisions (DLH, DMH, and VH) of the HCC of *C. benghalensis* observed demonstrates neuroarchitectural similarities with the HCC of the *C. livia* [5], *Gallus gallus* [17], and *C. splendens* [10] based on the Cresyl-violet analysis. We have identified four major types of projection (spinous) neurons (unipolar, bipolar, pyramidal, and multipolar) and local circuit (aspinous) neurons in the HCC of *C. benghalensis* by the Golgi-impregnation stain. Our findings corroborate with those investigators who had reported various types of projection neurons in the HCC of birds such as *G. domesticus* [1], *C. livia* [5,17], *T. guttata* [8], *E. amandava* [9], *G. domesticus* [1], and *C. splendens* [10].

The larger soma diameter of multipolar and pyramidal neurons that lead to the biosynthesis support and a cell metabolic requisite governs soma functionality alongside the dendritic length [18].

Dendrites bear a small structure in the form of a knob emerging from the dendritic shaft. These extensions include post-synaptic densities [19]. Spines are suggested to be the critical sites of synaptic excitatory transmission within the central nervous system [20]. It has been anticipated that spine density represents overall mental alertness [21]. In the HCC of *C. benghalensis*, the spine density of multipolar neurons has been observed to be greater than other neurons indicating a functional level or high efficiency of the region, as it has been suggested that spine density in the sensory area is correlated with activation of the related sense organ [22].

There is minimal variation in local circuit neuron forms than the various investigator's studies in other bird species. The aspinous neurons in the HCC of *C. benghalensis*, with its local dendritic

arborization pattern, are similar to the various subtypes of the *T. guttata* [8], *E. amandava* [9], and *C. splendens* [10] aspinous local circuit neuron. These neurons also show similarity to the local circuit neurons present in the *G. domesticus* [1] and *C. livia* [5,17].

In the avian HCC, several researchers documented stellate type of neurons, but no such types of neurons were observed in the *C. benghalensis*.

## 5. CONCLUSION

In conclusion, the HCC of *C. benghalensis* was endowed with four types of neurons, unipolar, bipolar, pyramidal, and multipolar as projection neurons and local circuit (aspinous) neurons with characteristic features soma size, dendritic arborization, and spine density. The multipolar neurons with the highest spine density were predominantly present in the HCC of *C. benghalensis* during the reproductive cycle's quiescent phase.

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## 7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## 9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 10. ETHICAL APPROVALS

Ethical permission was taken from the Principal Chief Conservator of Forest and Wildlife, U.P., India, vide letter no. 23-2-12(G), 26-04-2016.

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