Biochem. Cell. Arch. Vol. 11, No. 2, pp. 341-347, 2011 | *ISSN 0972-5075*

NEUROARCHITECTURE OF VOCAL CONTROL AREAS IN NIDOPALLIAL REGION OF PARROT BRAIN

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(Accepted 23 September 2011)

ABSTRACT –Vocal learning, cognition and emotions are arguably the most important human cognitive capacity. In the search for the neural mechanisms of cognition and memory, in mammals and humans, birds are usually preferred as model systems, because of their closer evolutionary relatedness to humans. The morphological characteristics of the neurons found in nidopallium vocal control areas in the brain of Indian ring neck parrot (*Psittacula krameri*) were examined by Nissl and Golgi-Cox method. Cytoarchitecture elucidated that, in Nidopallium region of the telencephalon various types of neurons found in different vocal control nuclei of this region. Neurons present in these areas have been identified on the basis of their architecture. We have observed multipolar neurons, spindle shaped bipolar neurons, pyramidal neurons, chandelier neurons, Basket cells and fusiform cells. Present work aims to study the complete architecture of vocal control area and to understand which type of neurons are involved in vocal learning and high level of cognition, learning and emotions in parrot family.

Key words : Nidopallium, vocal learning, consciousness, cognition, spindle cells, prefrontal cortex, parrot, ***Psittacula krameri*** **.**

INTRODUCTION

Parrots are popular as pets due to their sociable and affectionate nature, high intelligence, and ability to imitate human voices. Parrots can count, remember specific past events, can learn vocal sequences (Pepperberg 1999a, Butler *et al* 2006 Jarvis 2004), have insight and mental time travel (Clayton *et al* 2003, Clayton and Dickson 1998, 1999) bears no physical resemblance to a human; but has ability requiring a high level of intelligence and social behavior. Language and social cognition have also not found in primates and other mammalian taxa except hominids. while a few taxa of birds display behavior reminiscent of the sophisticated cognition and higher level of consciousness, for example new Caledonian crows can make and use tools, (Kirsch *et al* 2008, Hunt and Gray2004), parrots can count, remember specific past events, can learn vocal sequences (Weir *et al* 2002, Pepperberg 1999a, Butler *et al* 2006 Jarvis 2004), parrots and crow have insight and mental time travel (Clayton *et al* 2003, Clayton and Dickson, 1998, 1999). Pigeons are able to memorize up to 725 different visual patterns (von Fersen and Delius, 1989). Birds also have working memory (Honing 1978) which has been proposed to require higher level of consciousness. Perception, memory and attention are the cognitive abilities which are well recorded in birds (Fuster, 2003) contribute to consciousness. This is, as yet, unclear, how do birds achieve such complex cognition with relatively small brains? While the high brain body ratio, and many aspect of brain circuitry and connectivity of birds and mammals are remarkably similar, the neural substrate for complex cognitive function could not been fully determined in birds.

Kirsch *et al* (2008) demonstrated, functionally avian and mammalian pallia resemble each other even though they do not share the architectural feature of lamination. The purpose of the present investigation is to determine whether a morphologically similar neuroarchitecture that found in humans and other vocal learner mammals, is also present in parrots which display complex intelligent behavior similar to mammals.

MATERIALS AND METHODS

One pair of Indian green neck parrot approximately ten years old were our pet and were caged for behavioral studies from last two years , suddenly attacked by a cat. The pair was seriously injured and died. Brain of both animals were dissected out and immediately fixed in 4% Para formaldehyde in 0.1m phosphate buffer (4ºC, pH 7.4) for four days. Both brains were cut sagittally into two halves. Paraffin blocks were prepared of one hemisphere of each specimen. 20 µm serial sections were cut by sliding microtome and processed for the procedure of Nissl staining by Cresyl Violet (Sigma Aldrich, USA).

Other hemisphere o feach specimen was processed for the procedure of Golgi-Colonnie method. 2 mm coronal pieces were cut of both half hemisphere and were prechromed twice in 2.5% potassium dichromate for (60 min, each treatment). These tissues were left in 5% gluteral dehyde and 2% potassium dichromate solution at 4ºC for 3 days for chroming. This was followed by customary procedure for impregnation by 0.75% solution of silver nitrate at 4ºC for two days. Both chroming and impregnation steps were repeated twice. In all cases blocks were washed in distilled water between changes in solutions and fixative was replaced with a freshly prepared solution. After the completion of third and final impregnation blocks were dehydrated for two to five minutes immersions in 100% alcohol, and were immersed in xylene for 5 min, coated with paraffin wax (56º C M.P). Sections of 110 µm thicknesses were cut on a sliding microtome. The sections were than dewax treating with different grade of alcohol. Each section then mounted and cover slipped using DPX mounting medium.

Series sections from both hemispheres of the brain were studied for detailed morphological analysis of Golgi impregnated and Nissl stained neurons, Sections were studied and photographed, by using digital photo automat camera (Nikon). The vocal control nuclei in parrot, has been identified with the help of budgerigar brain atlas. Morphometric analysis and scales shown in photomicrographs and drawings were determined by an ocular scale calibrated for each objective using a stage micrometer. Terminology and abbreviations are used according to revised nomenclature for telencephalon and some related brainstem nuclei. (Reiner *et al* 2004).

OBSERVATION AND RESULTS

Current evidence showed that avian taxa as do mammals possess a complex forebrain that contains a well developed upper part called the pallium and a smaller ventral part called the subpallium. The term pallium refers to the upper part of the developing telencephalon and its adult derivatives (Reiner, Puelles, *et al* 2000). It has been revealed that, unlike mammalian pallium, the avian pallium does not have a cortical organization, but rather is organized into a largely continuous field of nuclei (Karten, H. 1969, Karten, 1991 and Reiner, 1984).

According to new nomenclature avian pallium have been sub-divided into(Fig I)

Hyperpallium (hypertrophied pallium): The upper part of pallium including wulst, an upper bulging part of upper pallium.

Mesopallium (middle pallium): Region located between hyperpallium and the subdivision below it (nidopallium).

Nidopallium: (nest pallium): The prefix **"Nido"** was derived from Latin word **"nidus" (nest),** since it is pallial structure in which the overlying pallial structures are nested.

Arcopallium (arched pallium): a region of pallium inferior to the posterior striatum.

Three of the vocal control nuclei are located in anterior medial telencephalon, contributes to form anterior vocal pathway and the other four forms posterior vocal pathway (two nuclei located anterior- laterally overlying Bas and two other located laterally but more posterior to the other regions). In addition to this one vocal counterpart was also found in dorsal thalamus and one in mid brain.

Neuroarchitecture of Nidopallium nuclei

Out of nine vocal counterparts three are found to be located in nidopallium

1. Oval Nucleus of Anterior Nidopallium (NAOc)

Previously this nucleus was designated as NAOC, (NAO complex, Vocal anterior neostriatal field including and surrounding NAO.)

A round nucleus, comprising of small tightly packed cells, can be distinguished laterally within the comparative larger area of nidopallium (Fig IA). It has been clearly observed that small densely packed multipolar and fusiform cells (Fig I D) were present in medial portions of nucleus, However, the presence of comparatively larger cells have been noticed on the caudolaterale side of the nucleus which also contributes to form NCL (Nidopallium caudolaterale). This caudolaterale area exhibits the presence of exclusively large spindle shaped bipolar cells and comparatively smaller pyramidal cells. (Fig. I E). Occasional presence of basket cell and Chandelier cells have also been observed in this area (Fig. II D). The soma of the chandelier neuron was located in the center of a large networked structure of axon filaments.

In Golgi stained sectons two neuronal classes, viz: typical pyramidal neurons, large spindle shaped bipolar neurons, were found to arrange in a characteristic manner Pyramidal cells are arranged dominated in medial position and are further surrounded by large bipolar cells. Bipolar spindle cells were characterized by an apical dendrite extending upwards and a basal dendrite extending downwards and possess large, elongated spindle soma (Fig: II B). The shape of these cells is distinct from the pyramidal cells found in this region (II A). Their axon exits at a right angle from the cell body. Sparse dendritic arborization was detected after traveling long distance from soma. Apical and basal dendrites show similar branchiness in spindle cells and spread out to a distance of 200-250 µm from opposite sides of soma and are vertically oriented. Soma size was approximately 85 to 100µm in length and 20-25µm in width. Total dendritic length was measured approximately 400-500µm. total neuronal size varies from 550-600 µm.

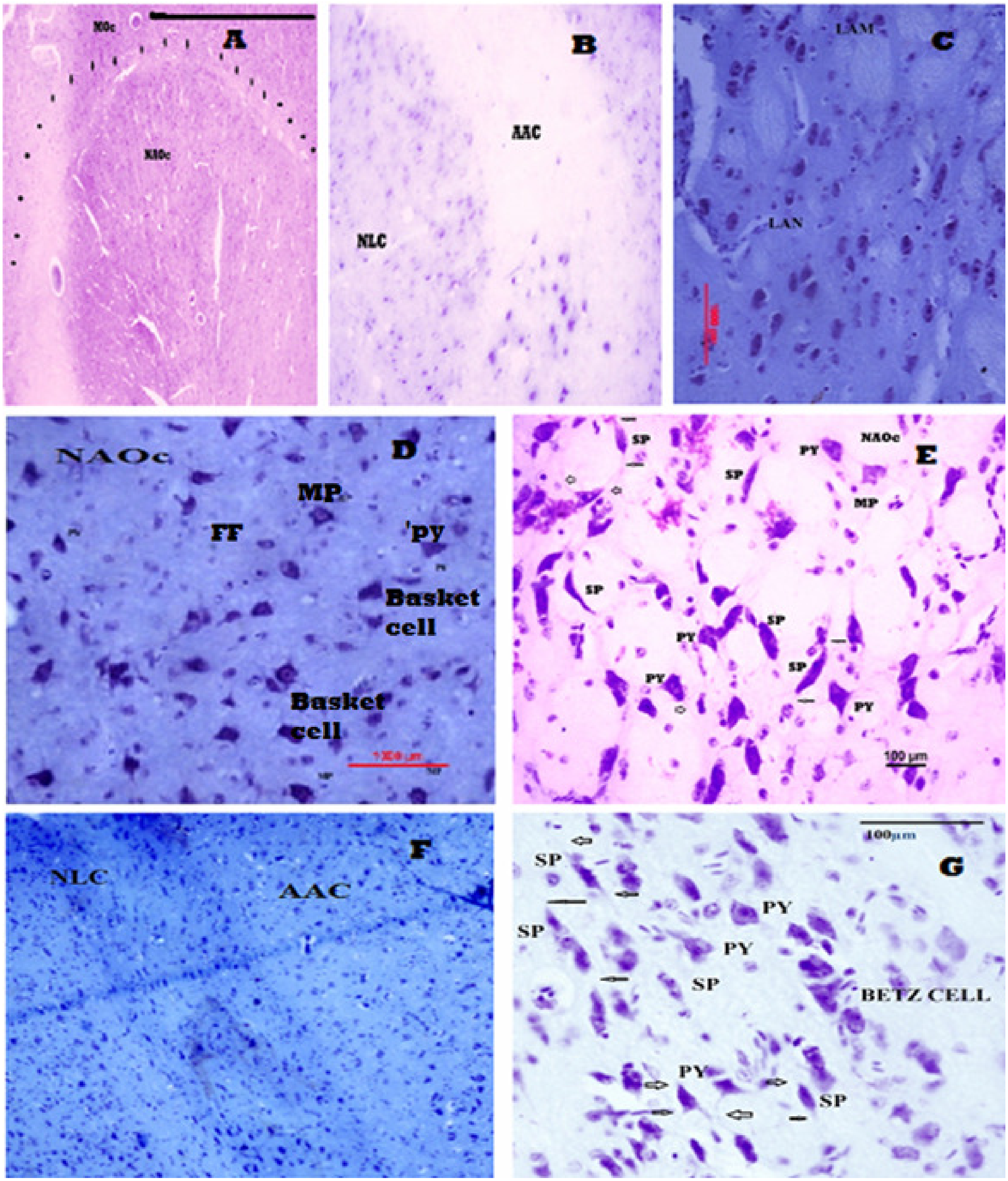


FIG : I A, B, C; respectively represents the photomicrograph of Nissl defined boundaries of NAOc, NLC & LAN vocal nucleus of nidopallium at low power, scale bar = 100ìm. I **D;** shows NAOc at higher magnification and represents mixed population of distinct cell types in middle of the nucleus. PY indicates (Pyramidal cell), SP (spindle cell), MP (multipolar cell), FF (fusiform cell) &basket cells. I **E;** exhibits the SP and PY cells in lateral part of NAOC at higher magnifications. Dendrites are indicated by arrows. I **F;** Illustrates the photomicrograph of NLC at low power in which both vertical and radial arrangement of cells is clearly seen. I **G;** indicates SP&PY cells along with occasional presence of Betz cells(very large pyramidal cells).

Pyramidal neurons had a typical morphology with triangular shaped Soma; the long axis measured approximately 25 to 30 µm and the short axis varied between 20 to 25 µm. Whip like long apical dendrites, 120-125 µm long and had branched basal skirt having 2-4 primary basal dendrites which further bifurcates into secondary dendrites. (Fig. II A) All primary dendrites radiates from two opposite angles of soma base and extends up to 70-100µm towards basal region. At higher magnification (40×10X) the dendritic surface appears to be spinous and was occasionally wrinkled. Spines are observed in dendrites of both pyramidal and bipolar cells at higher magnification.

Chandelier neuron :

These neuronal types were found to be arranged near the boundaries of the nucleus some of the basket cells are also present in close proximity to this cell type. The cell body of the chandelier neuron is located in the center of a large networked structure of axon filaments. The axon arbors form an array that is called a cartridge. Each cartridge establishes large number of links with pyramidal neuron. Single chandelier cell represented in (Fig. IID).

Basket neuron :

Basket cells have sparsely branched axons giving off small pericellular, basket-shaped elaborations at several intervals along their length. Fig. II C indicates single basket cell. Basket cells along with the Chandelier neuron contributed to form about 7% of total neuronal population in the nucleus.

2. Central nucleus of the lateral Nidopallium (NLC)

This nucleus was located posterio- laterally in nidopallium towards lateral side of arcopallium and found to be vertically oriented, oval shaped region which forms the border of lateral surface of brain (Fig. I B). Nissl stained cells of these area are on average smaller in comparison to other adjacent areas of nidopallium. This area was confined with fusiform cells and large spindle shaped bipolar cells along with pyramidal cells of comparatively smaller size. (Fig: I F)

Detailed study of Golgi impregnation have revealed that, the neuroarchitecture of this nidopallium vocal counterpart was similar to NAOc up to some extent having some similar type of neuronal classes viz ; spindle shaped bipolar cells and pyramidal cells with almost similar morphology including same dendritic arborization branching. However, a minor variation has also been noticed in pyramidal cells morphology. The soma shape of NAOc PY cells was triangular and in NLC PY cells soma shape varies between fusiform to pyramidal.

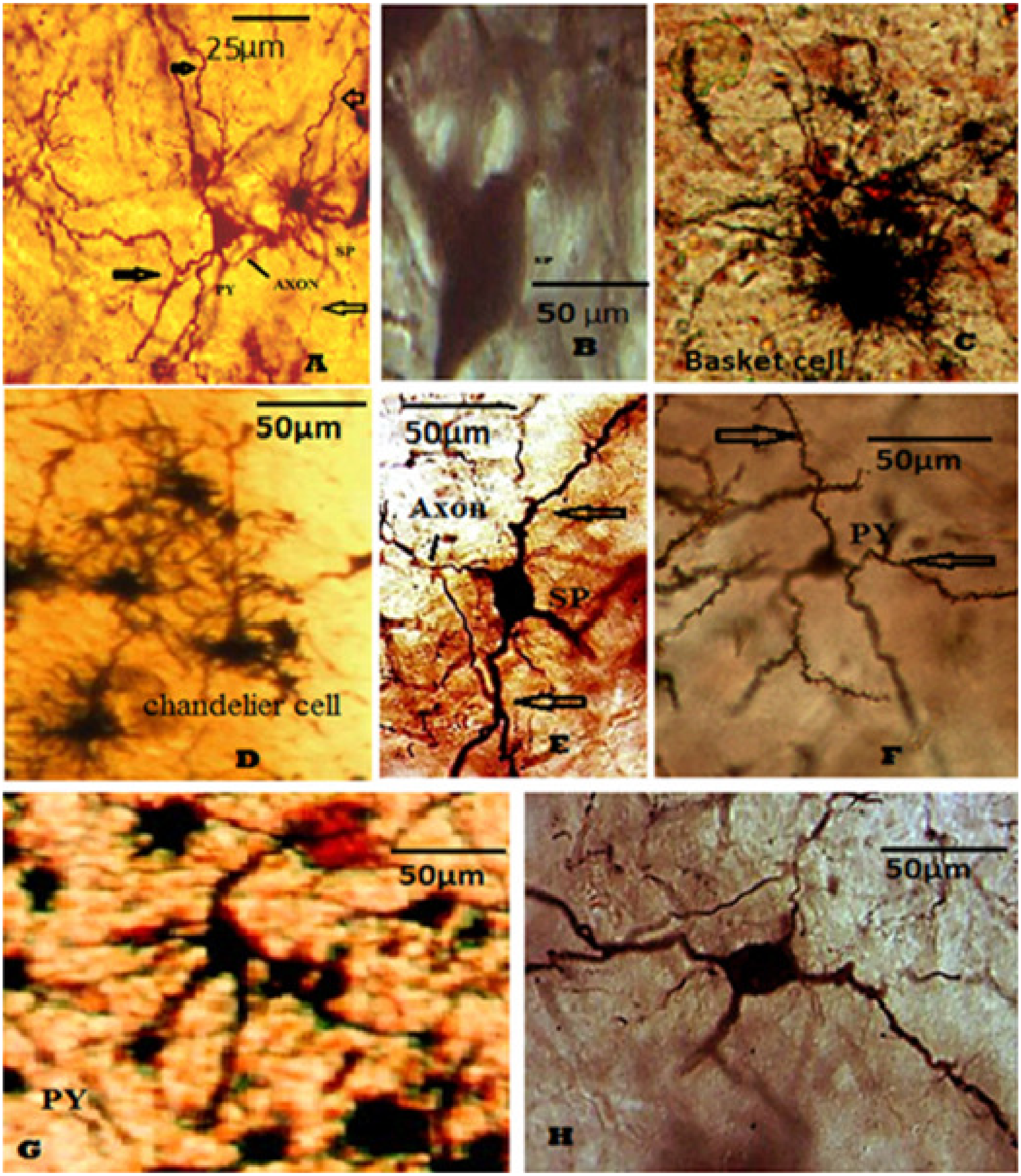
3. Lateral nucleus of the anterior Nidopallium (LAN)

It is located at the anterior lateral surface of the brain and acquires circular shape cap over the underlined cone shaped mesopallium vocal counterpart LAM. LAN exhibits the presence of small pyramidal cells and other type neuron (Fig. II G & H) which were scattered between fusiform cells. Golgi impregnated neurons of this area exhibits same morphological features as do represented by PY and FF cells of other regions which has been previously described in this section for NAOc region. However, a small population of other type of neuron has also been observed along with pyramidal and fusiform cells having simple but different morphological features. Soma shape was oval measuring about 35 to 40ìm in long axis and 25 to 30 ìm in short axis. Single thick dendrite radiates out from all four poles of soma and extends up to varying length. Axon was observed at the base of the soma.

DISCUSSION

In the present study, the presence of spindle cells has been observed, in two of the major nidopallium vocal nucleus including NAOc and NLC. Lateral part of NAOc and NLC being the part of lateral nidopallium also contributes to NCL region, the nidopallium caudolaterale, a callopallial anterior region, proposed as homologous to mammalian prefrontal cortex 'PFC' (Waldmann and Güntürkün, 1993; Hartmann and Güntürkün, 1998; Diekamp *et al*, 2001; Lissek *et al* 2002; Rose and Colombo 2005, Kirsch *et al* 2008). Recent evidence suggests that avian NCL is functionally equivalent to the mammalian PFC and is involved in higher cognitive functions like consciousness (Kirsch *et al* 2008). It is believed till date that large bipolar spindle neurons are uniquely shaped neurons located in the layer V of the anterior cingulated cortex (ACC), fronto- insula cortex (FI) and prefrontal cortex (PFC) of the brain of only species of large brains, such as humans, (Watson *et al*, 2006) great apes, (Nimchinsky et.al 1999) cetaceans (Hof and Vander Gucht, 2007) orangutan, African great apes, and elephants, (Hakeem *et al* 2009). The emergence and localization of spindle neurons suggests their role in the development of intelligent behavior and in many cognitive abilities (Watson *et al* 2006) like social interaction (Seeley *et al* 2006), language and emotion (Allman *et al* 2005). Presence of spindle cells in homologous area of parrot suggests that complex cognitive functions of birds are due to similar neuronal components of homologous regions of brain.

Watson *et al* (2006) also proposed that because of force of natural selection these cells have had a relatively



FigII A; photomicrograph of Golgi impregnated NAOc pyramidal neurons (PY) surrounded by spindle bipolar (Sp) cells, Left small black arrow indicates apical dendrite of PY cell and long arrow indicates basal dendritic skirt, right small light arrow indicates single apical dendrite of SP cell and long arrow indicates single basal dendrite, **B;** represents photomicrograph of Single spindle cell with single apical & basal dendrites, **C;** Large spindle shaped soma & D chandelier cell, **E & F;** indicates NLC SP and PY cell respectively, **G & H;** shows PY and other type of cell.

short time to shape their functioning and integration with other cell population and may be vulnerable to frontotemporal dementia, FTD (social behavior disorder), autism (speech disorder) and Alzheimer's disease memory loss). These spindle cells were not observed in any other primate species or any other mammalian taxa, which do not have historical backgrounds in social behavior, interaction, vocal learning, explained, that this unique Cytoarchitecture is responsible for social interaction and vocal learning.

A rare ability of vocal learning the substrate for human language (social construct) found to date in only distantly related groups of mammals humans, bats, cetaceans (Janik and Slater,1997), and elephants (Sanvito, *et al*, 2007, Poole *et al* 2005) and three related groups of birds parrots, hummingbirds and songbirds (Nottebohm 1972). Presence of spindle large bipolar neurons in three vocal learner groups of mammals- human, elephants and cetaceans is reported. (Watson *et al* 2006, Hakeem *et al* 2009, Hof and Vander Gucht, 2007) present study noticed for the first time presence of these cells in a bird's taxa which display behavioral correlates of higher level consciousness, like vocal learning, episodic memory, emotions etc.

In present study, the presence of few chandelier neuron and basket cells along with spindle and pyramidal neurons in nidopallial regions is also significant. It is believed that the main function of the chandelier neuron is an inhibitory action on pyramidal neurons. Outputs from Chandelier cells connect to Basket cells and Pyramidal neurons through complex axon arbors labeled cartridges. The complexity of cartridges seems to increase in higher functioning mammals, especially in the frontal and pre-frontal cortex. Increased complexity of Chandelier cells might be directly correlated to increased intelligence in mammals. Appearance of such combination of highly specialized neurons in the present investigation represents higher level of consciousness and intelligence of parrot.

ACKNOWLEDGEMENT

We thank Prof. U.C. Srivastava (Professor in Neurobiology, Dept of Zoology, University of Allahabad U.P.(India) for their critical and invaluable suggestions and allowing us to use laboratory space to perform photography by using digital photo automat camera (Nikon eclipse- Boi). We thank Dr. K. P. Singh (Asst. Professor in Neurobiology, Dept of zoology, University of Allahabad U.P. India.) for providing his laboratory facilities to perform some experiments during this work. We are also thankful to Dr. R. K. Tiwari and Dr. V. M. S. Srivastava for their valuable suggestions.

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