

ORGANIZATION OF OLFACTORY SYSTEM, FOREBRAIN AND PITUITARY GLAND OF A TELEOST, *NOTOPTERUS NOTOPTERUS*

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Abstract

Nuclear identification of olfactory system, forebrain and pituitary gland in *Notopterus notopterus* (order - Osteoglossiformes) is studied using Klüver and Barrera and Aldehyde fuschin staining techniques. Olfactory system has a short olfactory nerve which connects the olfactory epithelium with the bulb. From the bulb, two tracts run posteriorly and enter the telencephalon. In the olfactory bulb, mitral cells and giant cells of nervus terminalis (NT) are observed. In the forebrain, near the lateral forebrain bundle (LFB), multipolar cells of nucleus entopeduncularis (NE) are located in the telencephalon. Diencephalon is divided into preoptic area, hypothalamus, thalamus and epithalamus. In the diencephalon, Suprachiasmatic nucleus (SCN), Nucleus preopticus (NPO) and nucleus anterior commissure (NAC) are also identified. In the preoptic area, nucleus of subventricular gray (NSG) and organasum vasculosum lamellae terminalis (OVLT) is observed. Nucleus preopticus magnocellularis (NPOm) is the only AF-positive nucleus in the neuronal complex. Caudal to preoptic region, the tuberal area starts which has nucleus hypothalamicus (Nh) and nucleus lateralis tuberis (NLT). Above the hypothalamus, thalamic area shows thalamic nuclei. In the epithalamus, paired habenular ganglia are connected to each other by habenular commissure. Pituitary is platybasic which has flat neurohypophysis and disc shaped adenohypophysis.

Key words : Cytoarchitecture, Osteoglossiformes, Olfactory organ, Forebrain.

Introduction

Teleosts represent the most abundant group of bony fishes covering about 28,400 species (1). Pisces is phylogenetically the oldest vertebrate group holding large number of species with distinct morphological variations. These morphological variations have arisen as result of the diverse environments that they inhabit. They occupy a variety of freshwater and marine habitats and display a wide range of structural and functional responses to these environments.

In the early periods of brain research, the only neurosecretory neurons known in the brain were those of the nucleus preopticus; later, other neurons in the hypothalamus and recently other areas of the brain were also found to have this quality and such neurons. For studying the neurobiology of any brain area, it is a basic requisite to understand the morphological organization of the area by various neuroanatomical techniques.

Cytoarchitectural analysis appears to be the widely accepted criteria to study the organization of the neuronal groups in the brain of teleosts (2-10).

Although, information about the importance of olfactory organ, forebrain and pituitary gland in the regulation of reproduction is available, no reports are available on the cytoarchitectonic pattern of osteoglossiformes though it is a important and major order in the teleosts among the indigenous fishes. The fish *N. notopterus* selected for the present study is common in lakes throughout the greater parts of India. Its unique feature related to reproduction is the presence of single gonad in the body. The cytoarchitectonic pattern of the olfactory organ, forebrain and pituitary of *N. notopterus* is explored using standard techniques at light microscopic level.

Materials and Methods

Notopterus notopterus is found in clear streams and enters the brackish waters. It inhabits standing and sluggish waters of lakes, floodplains, canals and ponds, feeds on insects, fish, crustaceans and some young roots of aquatic plants. It is active during twilight and night, colonizes and breeds seasonally during rainy days and migrates back to permanent waters in dry season.

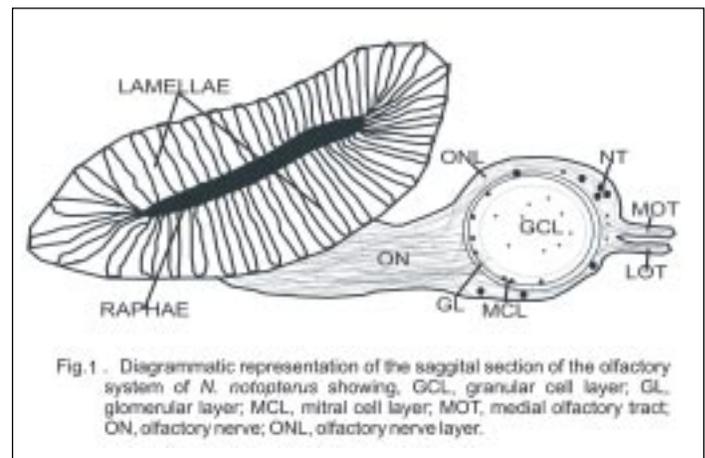


Fig.1 . Diagrammatic representation of the saggital section of the olfactory system of *N. notopterus* showing, GCL, granular cell layer; GL, glomerular layer; MCL, mitral cell layer; MOT, medial olfactory tract; ON, olfactory nerve; ONL, olfactory nerve layer.

Collection and maintenance of fish: Fishes were collected from natural habitat all around the Nagpur City. They were brought to the laboratory and acclimatized in small ponds. Sexually matured fishes were selected with body weight ranging between 75 gm to 125 gm and length 15 to 20 cm.

Histological Methods: Fishes were anaesthetized with 0.2% 2-phenoxy ethanol. They were perfused transcardially with 150-200 ml ice cold phosphate buffered saline (PBS, pH 7.45),

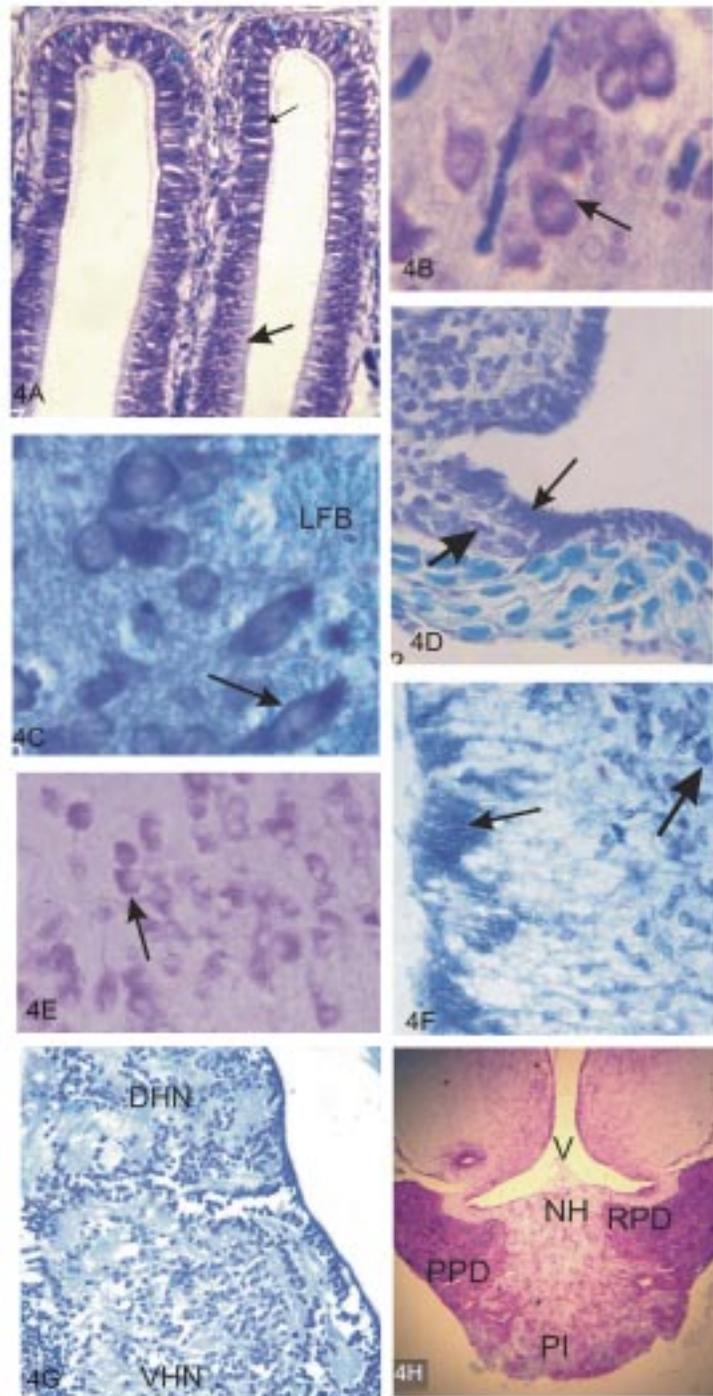


Fig. 4 A. Transverse section of the olfactory organ showing the sensory (arrow) and nonsensory (thick arrow) regions in the lamellae. Kluver and Barrera 450X.
 Fig. 4 B. Sagittal section of olfactory bulb showing bigger sized intense Nissl's stained neurons of nervus terminalis (NT) (arrow). Kluver and Barrera. 400X.
 Fig. 4 C. Transverse section of the brain through telencephalon showing bigger sized neurons adjacent to the lateral forebrain bundle (LFB) in the nucleus entopeduncularis (NE) (arrow) with intense Nissl's staining. Kluver and Barrera. 1000X.
 Fig. 4 D. Transverse section of the brain through diencephalon showing nucleus of subventricular gray (thick arrow) and organum vasculosum laminae terminalis (arrow) located at the base of the preoptic recess. Kluver and Barrera. 400X.
 Fig. 4 E. Transverse section of the brain through diencephalon showing moderate staining of Aldehyde Fuschin in nucleus preopticus pars magnocellularis (arrow) (NPOm). 400X.
 Fig. 4 F. Transverse section of the brain through tuberal area showing paraventricular organ (PVO) (arrow), cells of nucleus of PVO (NPVO) (thick arrow) showing intense staining of Nissl's. Kluver and Berrera. 400 X.
 Fig. 4 G. Transverse section of brain through epithalamus showing habenular ganglion with dorsal habenular nucleus (DHN) and ventral habenular nucleus (VHN). Kluver and Berrera. 160 X.
 Fig. 4 H. Transverse section of brain with pituitary gland. Pituitary attached to the brain below third ventricle (V). Pituitary gland showing neurohypophysis (NH), rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Kluver and Berrera. 50 X.

which is nucleus entopeduncularis (NE) (Fig. 4C). It shows intense Nissl's staining (Fig. 4C). These neurons are AF- negative. Telencephalic lobes are connected with each other by anterior commissure which is located dorsal to the preoptic recess.

Diencephalon: In *N. notopterus*, diencephalon includes four regions i.e. preoptic area, hypothalamus, thalamus and epithalamus.

Preoptic area: Preoptic area of *N. notopterus* is an important region which constitutes major part of anterior diencephalon. It is the portion of prosencephalic wall covering preoptic recess of the third ventricle. Anteriorly it is covered by anterior commissure, dorsally by the telencephalon and ventrally by the optic chiasma. The preoptic area contains AF-positive and AF- negative neuronal groups. The neurons of the nucleus preoptics magnocellularis (NPOm) are AF-positive and other nuclei in the preoptic area are AF- negative. Nucleus of subventricular gray (NSG) is an unpaired group of cells situated ventral to the preoptic recess. It begins at the anterior limit of preoptic recess, and comprises of neurons of oval shape showing moderate Nissl's staining (Fig. 4D). Organum vasculosum laminae terminalis (OVL) is located in ventral wall of preoptic recess and is organized by epidymal and parenchymal layers. OVL shows many parenchymal cells sending apical processes into the third ventricle and shows intense Nissl's staining (Fig. 4D).

In the preoptic area, there is paired paraventricular neuronal complex of the preoptic recess which is AF-negative. Nucleus of anterior commissure (NAC) is one of the important nuclei of preoptic area which is AF-negative. It is located near the ventral margin of the anterior commissure and lateral side of the preoptic recess. The neurons of NAC are spindle or oval in shape and show moderate Nissl's staining. Suprachiasmatic nucleus (SCN) is an important AF-negative nucleus in the preoptic area. SCN shows rounded, compactly arranged neurons and shows moderate Nissl's staining. Nucleus preopticus periventricularis (NPP) is also AF-negative. It is located close to the preoptic recess on both the sides. NPP is divided into nucleus preopticus periventricularis dorsal (NPPd) and ventral (NPPv). Neurons of NPPd area are comparatively bigger in size, round in shape and show moderate Nissl's staining. NPPv cells are sparsely distributed, rounded and show moderate Nissl's staining.

In *N. notopterus*, neurons of NPO are present on either side of the preoptic recess. On the basis of their size, NPO neurons have been categorized into NPO pars paraventricularis (NPOp) and NPO pars magnocellularis (NPOm) on the basis of their size. The neurons of NPOp are located above the optic chiasma and around the third ventricle, NPOm neurons are larger in size as compared to those of NPOp and located dorsally around the third ventricle. Neurons of NPOp and NPOm show intense Nissl's staining. NPOp neurons are AF-negative and NPOm neurons are AF-positive and show accumulation of neurosecretory material in the cytoplasm (Fig. 4E).

Horizontal commissure (HC): HC is present in the post optic area.

Hypothalamus: Hypothalamus is made up of tuberal area and

inferior lobe.

Nuclei of tuberal area: Tuberal area starts behind the horizontal commissure. These neuronal groups are located on either side of the third ventricle and its lateral recess. In this area, three important nuclei are seen adjacent to the third ventricle which are nucleus hypothalamicus ventralis (Nhv), nucleus hypothalamicus medialis (Nhm) and nucleus hypothalamicus dorsalis (Nhd). Nhv consists of sparsely distributed, rounded neurons showing moderate Nissl's staining. In Nhm, neurons present in the paraventricular position also are round in shape but show intense Nissl's staining. Above Nhv, rounded cells of Nhd are compactly arranged and show intense Nissl's staining. The nucleus lateralis tuberis (NLT) is an important nuclear group of the tuberal area located near the infundibulum. It is present on the ventral side of the horizontal commissure and extends continuously along the base of hypothalamus. On the basis of their position NLT is divided into NLT pars anterior (NLTa) and NLT pars posterior (NLTp). NLTa neurons are rounded or spindle shaped and compactly arranged showing intense Nissl's staining. NLTp neurons are rounded in shape, few in number and exhibit moderate Nissl's staining.

Paraventricular organ (PVO) is one of the important circumventricular organs, situated in the dorsal wall of the third ventricle. It begins with the pituitary gland and extends upto the region where the lateral recess joins the third ventricle. This organ consists of loosely arranged columnar cells perpendicular to the third ventricle. Two types of cells encountered in the PVO are (i) cells embedded within the ependyma called as intraependymal cells which are directly in the contact with the CSF and (ii) cells located slightly away from the ependyma called as subependymal cells that communicate by way of short processes. Both the types of cells are elongated and there are no morphological differences. PVO cells show intense Nissl's staining (Fig. 4F). Adjacent to the PVO, spindle shaped neurons are present i.e. nucleus paraventricular organ which show moderate Nissl's staining (Fig. 4F).

Caudal to the tuberal area, lateral side of the third ventricle shows lateral recesses. Around the lateral recess, compactly arranged cells of nucleus recesses lateralis (NRL) are observed. This nucleus is again sub divided on the basis of their position into nucleus recesses lateralis superior (NRLs) and nucleus recesses lateralis inferior (NRLi). NRLs consists of compactly arranged spindle shaped cells and shows intense Nissl's staining. Distribution of neurons in NRLi is sparse compared to those in NRLs. These are spindle shaped and show intense Nissl's staining.

At the level where pituitary gland terminates, two pouches of infundibular recess appear. These pouches are called mamillary recesses (MR). They have densely aggregated round shaped cells on the periphery called as nucleus mamillaris, which show moderate Nissl's staining.

Nuclear groups of inferior lobe: The nucleus inferior lobi located on the lateral side is divided into nucleus inferior lobi pars dorsalis (NILd), nucleus inferior lobi pars ventralis (NILv)

and nucleus inferior lobi pars medialis (NILm). The cells of NILd are round and show intense Nissl's staining. The neurons of the NILv are spindle shaped and show moderate Nissl's staining. The neurons of NILm are rounded or spindle shaped and show weak Nissl's staining.

Thalamus: Thalamus is made up of two divisions: dorsal and ventral thalamus. In the dorsal thalamus, cells of anterior thalamic nucleus (ATN) are compactly arranged, oval and/or spindle shaped and show intense staining of Nissl's. The neurons in the posterior thalamic nucleus (PTN) are comparably less in number, sparsely distributed, spindle or rounded in shape and show moderate Nissl's staining. Ventromedial thalamic nucleus (VMN) and ventrolateral thalamic nucleus (VLN) are the parts of ventral thalamus. The neurons of the VMN are oval shaped and show intense Nissl's staining. The cells in the VLN are spindle shaped, bigger in size and show intense Nissl's staining.

Epithalamus: In the epithalamus, a pair of habenular ganglia (HG) are present dorsal to the thalamus. Both the HG are connected by habenular commissure (HbC). Each HG has two nuclei i.e. dorsal habenular nuclei (DHN), and ventral habenular nuclei (VHN). In the DHN, cells are rounded in shape, comparatively bigger in size and show intense Nissl's staining (Fig. 4G). The cells in the VHN are smaller in size than DHN, spindle shaped and show intense Nissl's staining (Fig. 4G).

Pituitary gland: Pituitary of *N. notopterus* is of platybasic type. It is attached to the hypothalamus and is forward in position. The pituitary gland is divided into adenohipophys and neurohipophys. Axons of the hypothalamic nuclei penetrate the neurohipophys. Adenohipophys is subdivided into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). The AF-positive cells are seen in PPD region (Fig. 4H), these cells are large in size and show accumulation of secretory material.

Discussion

In *N. notopterus*, olfactory epithelium has lamellae radiating from the raphae which show the sensory and nonsensory regions. Location of these regions varies in different species. The sensory region is at the proximal end and basal region of the lamellae in *N. notopterus*. However, in the Cyprinid *L. rohita*, sensory region is at the middle of the lamellae and nonsensory region is at the proximal and basal regions of lamellae (10), in *Rhodeus amarus*, sensory region is at base and middle of the lamellae and nonsensory region is at the proximal end (12). This suggests that even within the groups of Cyprinids such variation is observed (Undocumented data). In *N. notopterus*, olfactory bulb is of pedunculated type, connected to the telencephalon. Such type of olfactory organ is also found in other members of family Cyprinidae and Acanthidae (13). Olfactory bulb comprises of four layers from superficial to the deep in *N. notopterus*. The fibers of the olfactory receptor neurons (ORNs) extend caudally over the olfactory nerve and penetrate the bulb from anterior side and spread along the periphery of the bulb in some teleosts (10, 12, 14) which are also seen in *N. notopterus*. In the olfactory

bulb of *N. notopterus*, on the ventromedial and dorsomedial side, giant cells of the nervus terminalis have been identified. Smaller cluster of the deeply stained ganglion cells along ventromedial surface of the nervus terminalis is also noted in *Ictalurus punctatus* (13). Telencephalon in *N. notopterus* consists of two hemispheres connected to each other by anterior commissure (AC) and is basically divided into area dorsalis telencephali and area ventralis telencephali. Other teleosts also exhibit the same pattern of division (3, 6, 8, 10). Area dorsalis telencephali is subdivided into Dm, Dc, Dld, Dlv according to reviews by Meek and Nieuwenhuys and Rodriguez-Gomez et al. (8, 15). In number of other teleosts, area ventralis telencephali is subdivided into Vv, Vd, Vl and NE (2, 8, 10, 15, 16) depending on the area which they occupy and same is observed in *N. notopterus*.

Diencephalon is considered to be the most complex region in the brain of the teleosts (9). It is the neuronal integration centre in the goldfish for gustatory information, reproduction and vision (17). Recently, using cytoarchitectonic criteria, epithalamus, thalamus, hypothalamus and preoptic area are included as the parts of the diencephalon in *Dicentrarchus labrax* (9, 10). This type of division of diencephalon is used in the cytoarchitectural analysis to study the organization of the neuronal groups in the brain of teleosts (2, 6, 8-10, 16, 17). In *N. notopterus*, same pattern of division is observed. Preoptic area is the part of rostralmost diencephalic subdivision (15). A dense aggregation of nuclei around the third ventricle is an important landmark to localize the preoptic area. Nucleus of subventricular gray is unpaired group of cells at the preoptic recess in *N. notopterus* which appears to be homologous to the thin subventricular strand of gray which interconnects the nuclei of two sides in sunfish, perch, darter and goldfish (18). This nucleus is also reported in reptiles (19). The organum vasculosum laminae terminalis (OVLt) is located in the ventral wall of preoptic recess and is well developed and highly vascularised in *N. notopterus*. OVLt has been reported in almost all the vertebrate classes (20) and is considered as neurohaemal organ. NPO of teleosts is known to be homologous to the supraoptic and paraventricular nuclei of the higher vertebrates; the aspect has been extensively studied and/or reviewed (21-23). NPO area has been subdivided into magnocellular, parvocellular, preoptic nucleus and suprachiasmatic nucleus (17, 23). In the present study, AF-negative small NPO cells, referred as pars parvocellularis and AF-positive large cells called as pars magnocellularis are observed as shown in some of the other teleosts (22, 24), situated at the level of third ventricle. Other workers employed AF-positive term for magnocellularis and parvocellularis cell groups. In the preoptic area, AF-negative smaller cell groups are divided into Nucleus preopticus periventricularis dorsalis (NPPd) and ventralis (NPPv) which are comparable to that of *Clarias batrachus* (4). The nucleus of anterior commissure in *N. notopterus* is important because of its association with the anterior commissure. This nucleus is also described in other fish and reptiles (4). Suprachiasmatic nucleus (SCN) in *N. notopterus* is an aggregation of small cells which is also reported in other fish, amphibians, reptiles and mammals (25). This nucleus receives retinal projections (26).

Another nuclear group, nucleus lateralis tuberis (NLT) has been extensively studied in several teleosts (4, 24). In some teleosts, NLT neurons are reported to be AF-positive (27), but in *N. notopterus* also, they are AF-negative. Functionally, the role of NLT in the control of gonadotropic cells is well documented in number of teleosts(28).

In *N. notopterus*, on the lateral side of the third ventricle, the anterior tuberal area consists of Nhm, Nhd and Nhv. The Nhm and Nhd are comparable with the nucleus periventricularis posterior of *Corydora palitus* but in other vertebrates it is described as a dorsal hypothalamic nucleus in a similar position (17, 23, 29).

In caudal part of the tuberal area, on the lateral side of the third ventricle, hypothalamus gives extensions called as the lateral recesses: lateralis superior and inferior in *N. notopterus*. In salmonids, catecholamine producing cells of nucleus recesses lateralis (NRL) and NRL in *Clarias batrachus* seem to be the similar ones (4). The nucleus paraventricular organ (NPVO) associated with paraventricular organ (PVO), shown in *Clarias batrachus* by Ramakrishna and Subhedar is comparable with the present study(4). At the caudal end of the hypothalamus, mamillary recess appears and around the recess, mamillary nucleus is observed in *N. notopterus*. Such, studies are also reported in other teleosts (2, 17, 23).

In *N. notopterus*, inferior lobe in the hypothalamus has two prominent nuclei, nucleus inferior lobi pars dorsalis (NILd), nucleus inferior lobi pars ventralis (NILv) and nucleus inferior lobi pars medialis (NILm) but earlier workers have shown only one nucleus i.e. nucleus diffuse lobi inferioris (2). Same findings are noted in *Clarias batrachus* by Ramakrishna and Subhedar (4). In *N. notopterus*, thalamus is divided into dorsal and ventral portions. In the dorsal division, there is anterior thalamic nucleus (ATN) and posterior thalamic nucleus (PTN), while in the ventral thalamus, two nuclei are identified i.e. the ventrolateral thalamic nucleus (VLN) and ventromedial thalamic nucleus (VMN), but in *Dicentrarchus labrax*, dorsal thalamus is formed by three nuclei: the ATN, PTN and the central posterior thalamic nucleus. Ventral thalamus is made up of nucleus of thalamic eminentia, the VLN, VMN and the intermediate thalamic nucleus (9, 17, 23). Such type of division however, is not clearly marked in *N. notopterus*.

Epithalamus region in *N. notopterus* consists of habenular ganglia and the habenular commissure. Habenular ganglia consist of the dorsal and ventral nuclei, as reported in other teleosts (9, 15, 17).

Pituitary gland in *N. notopterus* is platybasic and is divided into adenohypophysis and neurohypophysis. Adenohypophysis is subdivided into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Similar pattern is also reported in other teleosts (30).

As teleost is a big group of fishes showing enormous diversity, it is important to uncover the underlying pattern of brain to determine the anatomical and functional correlation of its various parts. Teleosts are further unique in vertebrates, in that they show

direct innervations of hypothalamic nuclei in adenohypophysis. This study will thus be helpful to find out the correlation of brain and pituitary gland in the order Osteoglossiformes.

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ERRATUM

Article “Emerging Role of Astrocytes in Cerebral Ischemia/Reperfusion Injury” published in *Ann Neurosci* 2008; 15: 17-24 authored by Dr. Ram Raghubir, Head Division of Pharmacology, CDRI. The names of co authors for this article RK Verma, Vikas Mishra were inadvertently not included. The citation of this article should now read as Raghubir R, Verma R K, Mishra V. Emerging Role of Astrocytes in Cerebral Ischemia/Reperfusion Injury. *Ann Neurosci* 2008; 15: 17-24