

CYTOARCHITECTONIC PATTERN OF THE OLFACTORY SYSTEM, FOREBRAIN AND PITUITARY GLAND OF THE INDIAN MAJOR CARP, LABEO ROHITA

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Abstract

To study the organization and cytoarchitecture of the entire olfactory organ, forebrain and pituitary gland in the Indian major carp *Labeo rohita*, Kluver and Barrera and Aldehyde fuschsin staining techniques are used. In the olfactory system, important cell groups and nuclei identified are mitral cells, smaller sized granular cells and giant cells of nucleus terminalis. In the forebrain, important nucleus of larger sized neurons forming a cluster is located adjacent to the lateral forebrain bundle which is nucleus entopeduncularis in the telencephalon. Diencephalon is divided into preoptic area, hypothalamus, thalamus and epithalamus. In the preoptic area, both AF-positive and AF-negative neuronal groups are identified. Nucleus preopticus which is divided into preopticus pars paraventricularis and pars magnocellularis are AF-positive. In the preoptic area, nucleus of anterior commissure and nucleus preopticus periventricularis are AF-negative. On the ventrolateral side of the horizontal commissure (HC) in the preoptic area, nucleus of HC is localized. In the hypothalamus, behind the HC, tuberal area starts. In this area, nucleus hypothalamicus is found on lateral side of the third ventricle. In the tuberal area near the infundibulum, nucleus lateralis tuberis is identified. In the caudal tuberal area, lateral side of the third ventricle shows nucleus recesses lateralis. Dorsal to the tuberal area, paraventricular organ is identified and nucleus inferior lobi is located on lateral side. Dorsal to the hypothalamus, thalamus shows dorsal and ventral thalamic nuclei. In the epithalamus, paired habenular ganglia are present dorsal to the thalamus. Pituitary is platybasic with short stalk and is forward in position.

Key words: Olfactory organ, Forebrain, Pituitary, Teleost, Indian major carp.

Introduction

Though teleostean brain conforms to the general vertebrate pattern, the unique feature is the absence of median eminence in teleosts and hypothalamic neurons directly innervating the pituitary cells. Teleostean hypothalamo-hypophysial system, therefore, offers a simple model to determine the anatomical relationship

between the specific neurons, the bioactive substances present in them and the pituitary cell types on which they act. This type of brain structure has attracted the researchers over last few years. In several teleosts, olfactory bulb extends away from the telencephalon, thus increasing the length of olfactory peduncles or tracts. Such type of olfactory bulbs are known as pedunculated bulbs. They lie closely opposed to the peripheral olfactory epithelium (1, 2). The cytoarchitectonic pattern of the forebrain of teleosts is studied in a number of species (*Ictalurus punctatus*;, *Carassius auratus*;, *Clarias batrachus*;, *Astyanax hubbsi*;, *Solea senegalensis*;, *Dicentrarchus labrax*) (1, 3-7). Stereotaxic atlases for the forebrain nuclei of the goldfish *Carassius auratus* and the killifish: *Fundulus heteroclitus* are also available (3,8). Pituitary gland in teleosts is unique amongst the vertebrates in that it is directly innervated by the neurosecretory fibers (9).

Teleosts represent the most abundant group of bony fishes covering about 25,000 species, but the organization of olfactory system, forebrain and pituitary gland is studied in only a limited number of species (10). Furthermore, no information is available pertaining to the organization of the pituitary of any of the Indian major carps. *Labeo rohita* offers a good model to study cytoarchitecture of the olfactory system, forebrain and pituitary gland.

Materials and Methods

Adult males of *Labeo rohita* (n = 12) weighing 1.5 kg to 2 kg and ranging in length between 30 to 40 cms were collected from the natural habitat all around the City of Nagpur. They were brought to the laboratory in tin containers and acclimatized in small ponds. They were anaesthetized with 0.2% 2-phenoxy ethanol. Fishes were perfused transcardially with 750-850 ml ice cold phosphate buffere saline (PBS, pH 7.45) followed by the same volume of ice-cold bouin's fixative. Olfactory organ with bulb and brain with pituitary gland were dissected out and post-fixed in the same fixative overnight. Olfactory organ and brain with pituitary gland were cut in transverse and sagittal planes at 10µm thickness. Alternate sections were stained with Nissl's stain and Aldehyde fuschsin to localize the neurosecretory material (11,12).

Kluver and Barrera technique

Sections of olfactory organ with bulb and brain with pituitary were deparaffinised in xylene and passed through different grades of alcohol bringing upto 95%. They were stained in 0.1% solution of Luxol Fast Blue in 95% alcohol for 6 to 8 hours at 57°C. Sections were rinsed in 95% alcohol to wash off the excess stain and brought down to distilled water through descending grades of alcohol, differentiated quickly in 0.1% Lithium carbonate solution and passed through several changes of 70% alcohol till there was a sharp contrast between the greenish blue white matter and colourless gray matter. Sections were washed thoroughly in distilled water and stained in 0.1% cresyl violet solution for 2 hours, then differentiated in several changes of 95% alcohol, dehydrated, cleared in xylene and mounted in DPX and coverslipped.

Aldehyde Fuschin (AF)

The deparaffinized sections of brain and pituitary were passed through descending grades of alcohol, brought to water and oxidized for 1 minute in 0.3% potassium permagnate solution acidified with sulphuric acid. They were decolorized quickly in 0.1% sodium metabisulphite. The sections were rinsed in distilled water and 70% alcohol, and then stained with AF, dehydrated in ascending grades of alcohol and mounted in DPX after clearing in xylene. Brain charts were prepared for the forebrain by plotting the sections from the series of transverse and sagittal sections. Each nuclear group in the forebrain was explored using cytoarchitectonic criteria.

Results

In the Indian major carp *Labeo rohita*, paired olfactory organs with bulb are located in the snout region. This organ is connected to the forebrain by the medial olfactory tract (MOT) and lateral olfactory tract (LOT).

Olfactory system

The olfactory organ has a central raphae, from which radiate lamellae on both the sides. These lamellae receive fascicles or fibers from its proximal end and extend into olfactory nerve (Fig-1). Olfactory nerve is short and caudally connected to the olfactory bulb. The olfactory bulb is divisible into four layers. Outer layer is olfactory nerve layer (ONL) which has axons of olfactory receptor neurons (ORN's). Below ONL, axons group together forming glomeruli called as Glomerular layer (GL). The glomeruli innervate the bigger sized neurons called mitral cells which form the Mitral cell layer (MCL). In the centre, densely packed small cells are present forming Granular cell layer (GCL) (Fig. 1, 4A, 4B).

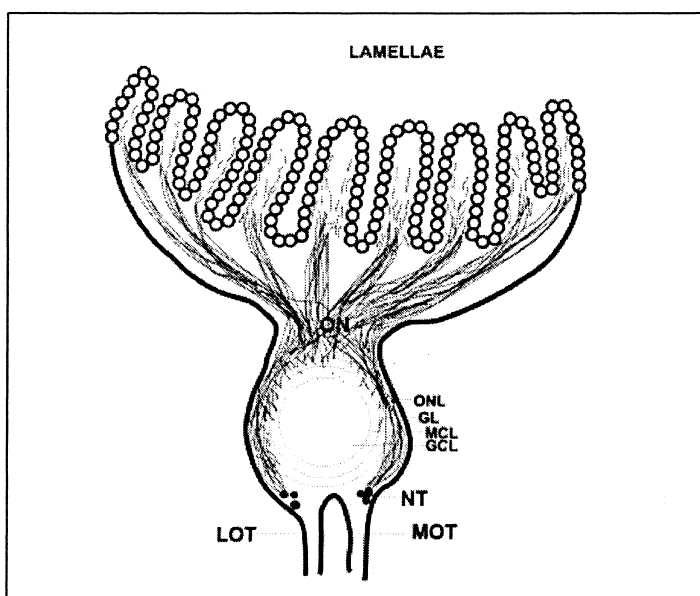


Fig. 1. Diagrammatic representation of the sagittal section of the olfactory system of *L. rohita* showing, GCL, granular cell layer; GL, glomerular layer; MCL, mitral cell layer; MOT, medial olfactory tract; ON, olfactory nerve; ONL, olfactory nerve layer.

The fibers from the olfactory nerve penetrate the olfactory bulb peripherally and innervate profusely forming glomerular structure over the larger sized mitral cells which show intense Nissl staining (Fig.4A). Some smaller cells found in GCL also show intense Nissl staining. The cells which are dorsal to olfactory bulb situated at the base of olfactory bulb are intensely stained and these are giant cells of nervus terminalis (NT) (Fig.4B).

Forebrain

Forebrain in *Labeo rohita* is divided into telencephalon and diencephalon.

Telencephalon

Telencephalon is divisible into two main regions i.e. area dorsalis telencephali and area ventralis telencephali. Its organization is shown in figure-2 ,3. Area dorsalis telencephali is subdivided into pars medialis (Dm), pars centralis (Dc), pars lateralis dorsal (Dld) and pars lateralis ventral (Dlv). Small sized cells located near the medial region of dorsal telencephalon is Dm which show moderate Nissl staining. The neurons in the area Dc are few in number and show intense Nissl staining. Cells in the area Dld are rounded in shape and are moderately stained, while neurons in the area Dlv are spindle shaped, small in size and moderately stained. Area ventralis telencephali is broad, divided into pars ventralis (Vv), pars dorsalis (Vd) and pars lateralis (Vl). Neurons in the Vv are sparsely arranged, smaller in size and exhibit moderate Nissl staining. Cells of the Vd are bigger as compared to the cells of Vv, compactly distributed and show intense Nissl staining. In the area Vl, few larger sized cells showing intense staining are observed. The medial and lateral forebrain bundles are present in ventrolateral position of the telencephalon. Telencephalic lobes are connected with each other by Anterior commissure and Commissure of Goldstein which are located dorsal to the preoptic recess. These two commissures are parallel to each other (Fig.4C).

Adjacent to the forebrain bundle, few bigger sized neurons are seen forming a cluster which is nucleus entopeduncularis (NE) (Fig.4D). It shows intense Nissl staining (Fig.4D). However these neurons are AF negative.

Diencephalon

In *Labeo rohita*, diencephalon includes four regions- preoptic area, hypothalamus, thalamus and epithalamus.

In the preoptic area, hypothalamus forms a major part of ventral diencephalon. Preoptic area is the portion of prosencephalic wall that covers the preoptic recess of third ventricle. It is covered anteriorly by Anterior commissure and commissure of Goldstein, dorsally by telencephalon and ventrally by the optic chiasma. Preoptic area contains both AF positive and AF negative neuronal groups. The neurons of the Nucleus preopticus (NPO) are AF positive and other nuclei in the preoptic area are AF negative.

Neuronal complex of the preoptic area is paired in paraventricular region of the preoptic recess which is AF negative. Nucleus of anterior commissure (NAC) is one of the nuclei of preoptic area

which is AF-negative. It is located near the ventral margin of anterior commissure and dorsal margin of the commissure of Goldstein on lateral side of the preoptic recess. Neurons of NAC are spindle shaped and show weak Nissl staining. Another AF negative nucleus is nucleus preopticus periventricularis (NPP). It is present close to the preoptic recess on both the sides. NPP is divided into nucleus preopticus periventricularis dorsalis (NPPd) and ventralis (NPPv) (Fig.2). Cells of NPPv are small, aggregated and show intense Nissl staining. Neurons of NPPd area are comparatively less in number and show moderate Nissl staining.

In *Labeo rohita*, neurons of NPO are present on either side of the preoptic recess. On the basis of size of these neurons, NPO is divisible into NPO pars paraventricularis (NPOp) and NPO pars magnocellularis (NPOm). Neurons of NPOp are located above the optic chiasma and around the third ventricle, NPOm neurons are larger in size as compared to the NPOp and are located dorsally around the third ventricle (Fig.2, 3). Neurons of NPOp and NPOm show intense Nissl staining (Fig.4E). These are AF positive and show accumulation of neurosecretory material in the cytoplasm of NPOp and NPOm. They have long axons which collectively form the hypothalamo-hypophysial tract which innervates the pituitary gland.

Suprachiasmatic nucleus (SCN)

On either side of the third ventricle is located the SCN (Fig.2). It has small spindle shaped neurons which show moderate Nissl staining.

Nucleus of horizontal commissure (NHC)

Horizontal commissure is present in the post optic area. It has paired nuclei that cover the horizontal commissure on ventrolateral side (Fig.2). The nuclei are rounded and show intense Nissl staining.

Nuclear groups of tuberal area

Behind the Horizontal commissure, tuberal area starts. It consists of neurons located on either side of the third ventricle and its lateral recess. In the tuberal area, lateral side of the third ventricle shows three prominent nuclei i.e nucleus hypothalamicus ventralis (Nhv), nucleus hypothalamicus dorsalis (Nhd) and nucleus hypothalamicus medialis (Nhm) (Fig.2, 3). Nhv consists of comparatively small cells and shows moderate Nissl staining. Above Nhv, round shaped cells of Nhm show weak Nissl staining. Dorsal to the Nhm, spindle or round shaped cells of Nhd show moderate staining. Nucleus lateralis tuberis (NLT) is an important nuclear group of the tuberal area located near the infundibulum (Fig.2, 3). It is present ventral to the horizontal commissure and extends along the base of hypothalamus. NLT is divisible on the basis of position and size of the neurons into NLT pars lateralis (NLTl) and NLT pars medialis (NLTm). NLTl neurons are located laterally, large in size and rounded in shape showing intense Nissl staining (Fig.4F). NLTm neurons are located near the third ventricle and comparatively smaller in size than NLTl neurons. NLTm cells are few in number and show moderate Nissl staining.

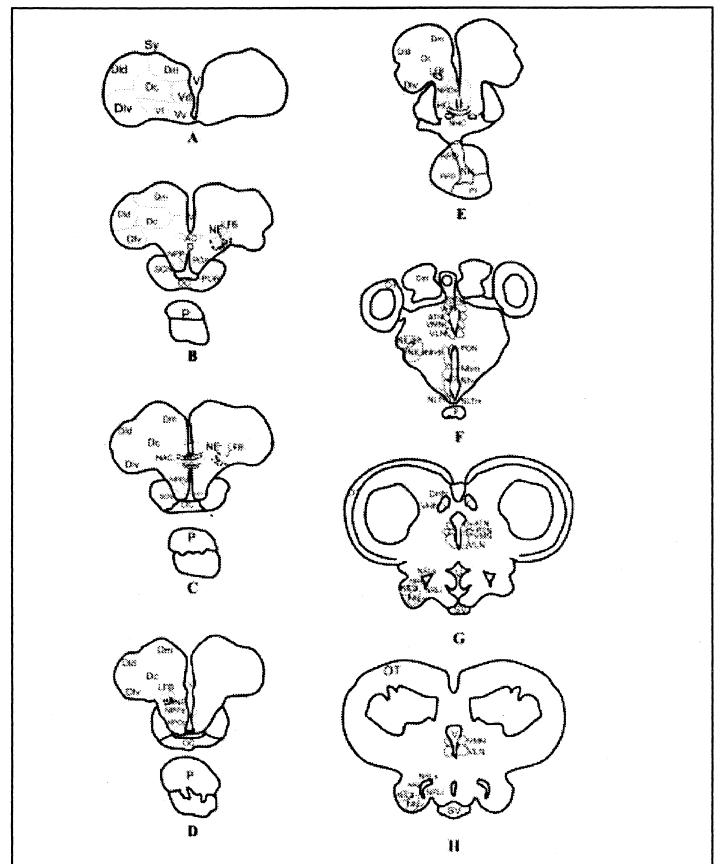


Fig. 2. Diagrammatic representation of the transverse section of the forebrain of *L. rohita* showing, AC, anterior commissure; ATN, anterior thalamic nucleus; CG, commissure of Goldstein; Dc, area dorsalis telencephali pars centralis; DHN, dorsal habenular nucleus; Dld, area dorsalis telencephali pars lateralis dorsal; Dlv, pars lateralis ventral; Dm, pars medialis; HC, Horizontal commissure; LFB, Lateral forebrain bundle; NE, Nucleus entopeduncularis; NH, Neurohypophysis; NHC, Nucleus horizontal commissure; Nhd, nucleus hypothalamicus dorsalis; Nhm, nucleus hypothalamicus medialis; Nhv, nucleus hypothalamicus ventralis; NILd, nucleus inferior lobi pars dorsalis; NILv, nucleus inferior lobi pars ventralis; NPOm, Nucleus preopticus pars magnocellularis; NPOp, nucleus preopticus pars parvocellularis; NPPd, nucleus preopticus periventricularis dorsalis; NPPv, nucleus preopticus periventricularis ventral; NRL, nucleus recesses lateralis; NRLi, nucleus recesses lateralis inferior; NRLs, nucleus recesses lateralis superior; OC, optic chiasma; OT, optic tectum; P, pituitary gland; PI, pars intermedia; POA, preoptic area; POR, preoptic recesses; PPD, proximal pars distalis; PTN, posterior thalamic nucleus; PVO, paraventricular organ; RPD, rostral pars distalis; SCN, suprachiasmatic nucleus; SV, succus vasculosus; V, ventricle; VHN, ventral hypothalamic nucleus; VLH, ventrolateral thalamic nucleus; VMN, ventromedial thalamic nucleus.

Caudal to the tuberal area, lateral side of the third ventricle give rise to the lateral recesses. Compactly arranged cells on the periphery of the lateral recesses are nucleus recesses lateralis (NRL) (Fig.2, 3). This nucleus is again sub grouped into superior and inferior groups on the basis of their position i.e. nucleus recesses lateralis superior (NRLs) located on the dorsal side of the lateral recess and nucleus recesses lateralis inferior (NRLi) present on the ventral side of the lateral recess. The NRLs consists of large number of cells and show moderate Nissl staining. Cells of NRLi are smaller in size and compactly arranged as compared to the NRLs cells. They show intense Nissl staining.

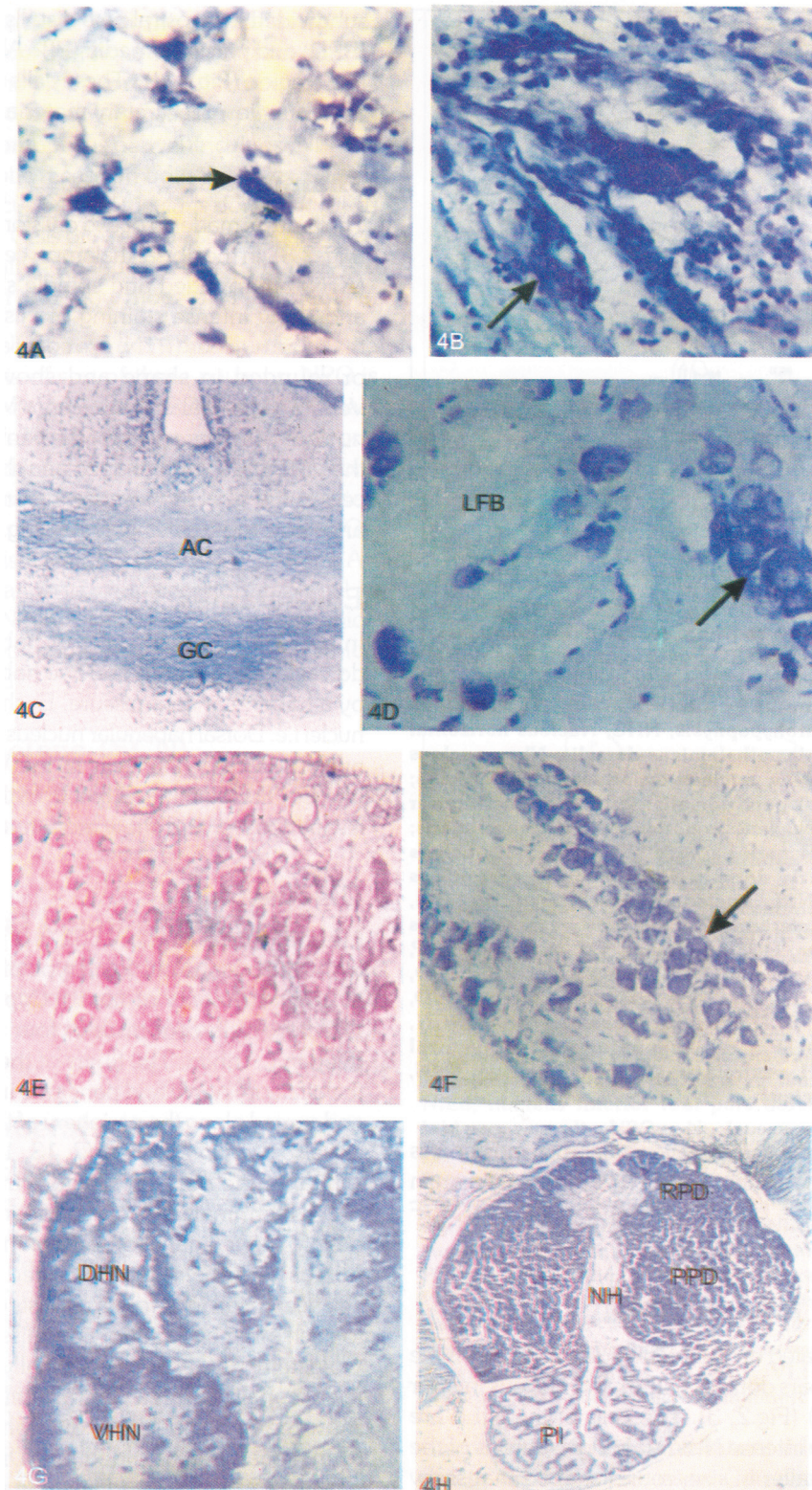


Fig.4. Sagittal and transverse sections of olfactory bulb and brain of *L. rohita*. A) Olfactory bulb showing larger sized mitral cells with intense Nissl staining (arrow) and B) bigger sized neurons of nervus terminalis (NT) (arrow). C) Telencephalon showing anterior commissure (AC) and Commissure of Goldstein (CG) and D) bigger size neurons adjacent to the lateral forebrain bundle (LFB) in nucleus entopeduncularis (NE) (arrow) with intense Nissl staining. E) Diencephalon showing intense staining of Alcian blue in nucleus preopticus pars magnocellularis (NPOm) and F) tuberal area showing bigger sized neurons located laterally showing intense Nissl staining in nucleus lateralis tuberis pars lateralis (NLTl) (arrow). G) Epithalamus showing habenular ganglion with dorsal habenular nucleus (DHN) and ventral habenular nucleus (VHN). H) Transverse section of pituitary gland showing Neurohypophysis (NH), rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Magnification: (A,B), 400X; (C, E, F, G), 160X; (D), 500X; (H), 25X.

thalamus, hypothalamus and preoptic area are included as the parts of the diencephalon in *Dicentrarchus labrax* (17). This type of division of diencephalon is also used in the cytoarchitectural analysis to study the organization of the neuronal groups in the brain of teleosts (3, 5, 6, 7, 8, 17). In *L. rohita*, same pattern of division is observed. Preoptic area is the part of rostralmost diencephalic subdivision (16). A dense aggregation of nuclei around the third ventricle is an important landmark to localize the preoptic area. This area has been subdivided into magnocellular, parvocellular, preoptic nucleus and suprachiasmatic nucleus (*Carassius auratus*; *Haplochromis*;) (17,18). In the present study, AF positive small NPO cells referred as *pars parvocellularis* and large cells called as *pars magnocellularis* are observed as shown in some of the teleosts, situated at the level of third ventricle (19, 20). Other workers employed the term *magnocellularis* for AF positive and *parvocellularis* for AF negative 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 *L. rohita* is important because of its association with the anterior commissure. This nucleus is also described in other fish and reptiles (4, 21). SCN nucleus in *L. rohita* is an aggregation of small cells which is also reported in other fish, amphibians, reptiles and mammals (22, 23, 24). This nucleus receives retinal projections (25). NLT has been extensively studied in several teleosts (4, 19). In some teleosts, NLT neurons are reported to be AF positive (26, 27). In *L. rohita* also, they are AF positive. Functionally, the role of NLT in control of gonadotropic cells is well documented in number of teleosts (28, 29).

In *L. rohita*, 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, 18, 30).

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 *L. rohita*. In salmonids, catecholamine producing cells of nucleus *recesses lateralis* (NRL) and NRL in *Clarias batrachus* seem to be the similar ones. The nucleus *paraventricular organ* (NPVO) associated with *paraventricular organ* (PVO), shown in *Clarias batrachus* is comparable with the one found in *L. rohita* (4).

In *L. rohita*, inferior lobe in hypothalamus has two prominent nuclei, nucleus *inferior lobi pars dorsalis* (NILd) and nucleus *inferior lobi pars ventralis* (NILv) but earlier workers have shown only one nucleus i.e. nucleus *diffuse lobi inferioris* (3, 31). Same findings are noted in *Clarias batrachus* (4). In *L. rohita*, 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 *ementia*, the *ventrolateral thalamic nucleus*, *ventromedial thalamic nucleus* and the *intermediate thalamic nucleus* (7, 17, 18). Such type of division however, is not clearly marked in *L. rohita*.

Epithalamus region in *L. rohita* consist of habenular ganglion and the habenular commissure. Habenular ganglion consist of the dorsal and ventral nuclei, as reported in other teleosts (7, 16, 17).

Pituitary gland in *L. rohita* 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 (9, 32).

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 there is direct innervation of hypothalamic nuclei in *adenohypophysis*. This study will thus be helpful to find out the correlation of brain and pituitary gland in the Indian major carps.

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