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NEUROPEPTIDE-Y LIKE IMMUNOREACTIVITY IN THE OLFACTORY SYSTEM, FOREBRAIN AND PITUITARY GLAND OF THE MALE TELEOST, *LABEO ROHITA* (HAMILTON)

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ABSTRACT:

Neuropeptide-Y (NPY), a 36 amino peptide was examined within the olfactory area, forebrain and pituitary within the grown-up Indian major carp *Labeo rohita*, using immunocytochemical technique. Within the olfactory bulb, NPY positive fibers were dispersed within the olfactory nerve layer, mitral cell layer, granular cell layer and NPY positive response was observed within the mitral cells as well as within the nervous terminalis (NT) which extended within the medial olfactory tract (MOT). NPY positive cells were localized within the MOT. Within the telencephalon, multipolar NPY positive cells were found within the nucleus entopeduncularis (NE). Within the optic chiasma, both cells and filaments were disseminated. Nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) both were innervated by NPY positive fibers. CSF reaching NPY positive cells were too oserved within the nucleus hypothalamicus medialis (Nhm). Within the epithalamic area, habenular ganglion had NPY positive cells. Within the pituitary organ, NPY positive fibers innervated the neurohypophysis and proximal pars distalis (PPD). These discoveries in *L. rohita* proposed that NPY might play a part of neurotransmitter within the olfactory receptor neurons that transmit the data to the olfactory bulb. NPY positive response found within the MOT proposes that it might carry signals to the telencephalon and other ranges within the brain. Localizationof NPY positive fibers within the neurohypophysis and PPD which has gonadotropin discharging cells show coordinate association of NPY in the process of reproduction, probably regulating spawning activity in the males of *L. rohita*.

Keywords:- labeo rohita, Neuropeptide, olfactory nerve

INTRODUCTION :

Peptides and neurohormones in olfactory system participate a major role in controlling the reproductive behavior through fore brain is known. Role of neuropeptides in reproduction has been studied in most of the vertebrates including fish. Olfactory system is one of the most important sites that processes the signaling related to reproduction (Schreibeman et al., 1984). Gonadotropin releasing hormone (GnRH), a leading peptide that is localized in olfactory organ (Biju et al., 2003) and forebrain (Chiba et al., 1996) known to control fish reproductive physiology. Other peptides and neurohormones in the olfactory system of teleosts that regulate reproduction include neuropeptide-Y (NPY), -endorphin, vasoactive intestinal polypeptide and nitric oxide synthase (Alanso et al., 1989, Batten et al., 1990; D'Aniello et al., 1994; Khan et al., 1999; Baby et al., 2000; Singru et al., 2003).

NPY family of peptides, shows notable sequence homology since fishes to mammals (Larhammar, 1996). Fishes are the most spread vertebrates more than 34,000 species has been reported (Froese and Pauly, 2019). NPY play important role in the food intake in fishes (Assan et al., 2021). NPY immunopositive fibers have been reported in close association with gonadotrophs in the goldfish, suggesting its neuroendocrine function (Kah et al., 1989). Goldfish NPY excites the discharge of GTH-II in a dose requirement mode (Peng et al., 1994). It has 86% homology with human NPY (Blomquist et al., 1992).

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Further, porcine NPY (Kah et al., 1989) and human NPY (Peng et al., 1990) stimulate GTH release from goldfish pituitary. Neuropeptide Y distribution also reported in the brain of the chicken (Sartsoongnoen et al., 2020; Boonyarit and Yupaporn, 2022).

In spite of the fact that data around the significance of olfactory organ and forebrain within the control of reproduction is accessible, exceptionally less information is available on the NPY distribution and its role in any of the Indian Major carps. Indian major carps contribute major aquaculture system in Indian subcontinent and almost 75 % of fishery comes from them. To elucidate NPY localization in the olfactory organ, forebrain and pituitary, we used immunocytochemical approach in the males of, *Labeo rohita.*

MATERIAL AND METHODS

Adult males of *L. rohita* were collected water bodies from Nagpur, Central India. They were acclimatized in ponds. Sexually mature males with body weight ranging from 1.5- 2 kg and 30 to 40 cm in length were selected for the present investigation.

Immunocytochemistry

The fishes were anesthetized using 2-phenoxyethanol and perfused transcardially with 550 ml to 750 ml ice cold phosphate buffer saline (PBS) having pH 7.45 taken after the same quantity of ice cold Bouin's fixative. Olfactory organ along with olfactory bulb and brain with pituitary were dissect out, post fixed in the identical fixative for 20 - 24 hrs, cryoprotected in sucrose solution prepared in PBS and sections were cut on Leica cryostat at 15$^{\mu}$ thickness in sagittal and transverse planes and processed for immunocytochemical labeling.

NPY immunocytochemistry

The tissue sections were brought to the room temperature and washed in PBS for 15 min taken after by the treatment of 2% bovine serum egg whites (BSA) in PBS containing 0.4% triton X-100 for 10 min. Sections were at that point incubated with antibodies against NPY (Sigma,USA) at 1:2000 dilution for 2hrs at room temperature. The sections were washed in PBS for 11 min and incubated with biotinylated secondary antibody (Bangalore Genei, India) for 45 min. The sections were at that point washed in PBS and incubated in streptavidin peroxidase conjugate (Bangalore Genei, India) for 45 min. Sections were again rinsed in PBS for 11 min. For visualization of the antigen antibody complex, the sections were incubated for 5-7 min in solution containing hydrogen peroxide that served as a substrate and 3-amino-9-ethylcarbazole as chromogen. Reddish-brown precipitate demonstrated the presence of antigen within the areas.

Specificity of the antibodies

The specificity of the NPY antibodies used in the present study was confirmed by the following direct procedures:

1. Exclusion of the primary antibody from the reaction.
2. Substitute of antiserum against NPY with BSA which produced no immunoreaction.
3. Western blot study of unfinished homogenates of olfactory organ with bulb, forebrain and pituitary of *L. rohita* was carried out according to the details given below.

Polyacrylamide gel electrophoresis and Western blotting

The fish were killed by decapitation. Olfactory organ and bulb, brain and pituitary were taken out and homogenized in lysis buffer discretely containing 50 mM $MgCl_2$, 50 mM tris-HCl (pH 7.5), 1% Triton X-100, 1mM EDTA, and 1mM PMSF (Phenyl methyl sulphonyl fluoride) as protease inhibitor. The homogenized solution were centrifuged and supernatants were in use as a protein sample. Protein sample was added to an equal volume of sample buffer (0.1 M Tris-

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HCl, pH 6.8; 10% glycerol; 1% sodium dodecyl sulphate, 0.02% bromophenol blue), treated with 1% β -mercaptoethanol and denatured at 98°C for 4 min. olecular weight marker (Bangalore Genei, India) was included in each gel and 100 μ g protein was used in 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on a vertical gel (16 × 14 cm) system (Bangalore Genei, India). The gel was then stained with Coomassie Brilliant Blue R-250 (SRL, India) and distained.

Proteins isolated by SDS-PAGE were exchanged to nitrocellulose layer by electro-blotting within the blotting buffer (192 mM glycine, 25 mM Tris, 0.1% SDS, 20% methanol) overnight at 10V utilizing electro transfer system (Bangalore Genei, India). The nitrocellulose sheet was soaked in PBS for 1 hr some time recently beginning the immunoblotting try; this was taken after by blocking for 1 hr at room temperature in presoaked solution in PBS (0.1 m, pH 7.45) containing 0.1% triton X-100 and 1% BSA. Blots were at that point incubated overnight at 4 °C with anti-NPY antibodies at 1:5000 dilution in presoaked solution. Film was washed three times for 5 min each in PBS and was incubated with biotylated secondary anibody (Bangalore Genei, India) at 1:500 dilution for 2 hrs taken after by incubation with streptavidin-peroxidase conjugate (Bangalore Genei, India) at 1:500 dilution for 2 hrs at room temperature. 3-amino-9-ethylcarbazole was utilized as chromogen to imagine the response product.

RESULTS AND DISCUSSION:

Organization of NPY immunoreactivity in the olfactory system, forebrain and pituitary gland is diagrammatically illustrated in figure 1, 2 and 3. Immunoreactivity is observed in the olfactory receptor neurons (ORN's) (Fig. 4A) but such NPY positive ORN's are limited in number throughout the sensory region. NPY-negative cells are however large in number but no specific pattern of arrangement is seen. Few NPY positive fibers extend from ORN's in the vicinity of basal lamina (Fig. 1). NPY positive fiber density is also observed in the olfactory nerve which entered the olfactory bulb in the olfactory nerve layer (ONL) (Fig. 1). In the olfactory bulb, NPY immunoreactivity is seen in the mitral cells and fibers of mitral cell layer (MCL) (Fig. 1, 4B). In the granular cell layer (GCL), NPY positive beaded fibers are also located (Fig. 1).

The medial olfactory tract (MOT) and lateral olfactory tract (LOT) extend caudally from the olfactory bulb towards telencephalon. NPY positive fibers enter MOT from the olfactory bulb. (Fig. 1, 4C) while in MOT, few NPY positive neurons are also located (Fig. 4C). In the telencephalon, area dorsalis telencephali pars lateralis dorsalis (Dld) shows densely distributed beaded NPY positive fibers (Fig. 3). Area dorsalis telencephali pars medialis (Dm) shows NPY immunoreactive neurons and beaded fibers (Fig. 2, 3).

NPY immunostained fibers are easily distinguished in the area dorsalis telencephali pars centralis (Dc) (Fig. 3), where they are beaded but sparsely distributed. In the area dorsalis telencephali pars lateralis ventralis (Dlv), few positive fibers (Fig. 3) are seen. NPY immunoreactive fibers were also located in the area ventralis telencephali pars ventralis (Vv), area ventralis telencephali pars lateralis (Vl) and area ventralis telencephali pars dorsalis (Vd) (Fig. 3) . Unipolar, bipolar and/or multipolar NPY positive perikarya were distributed in the nucleus entopeduncularis (NE) (Fig. 2, 3, 4D). Ventrolaterally in the optic chiasma, NPY immunoreactive neurons and fibers were observed (Fig. 4E).

As far as nucleus preopticus is concerned, immunoreactive fibers are observed around

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nucleus preopticus pars parvocellularis (NPOp) and they also innervate the NPOp region and nucleus preopticus pars magnocellularis (NPOm) region (Fig. 3).

In the hypothalamus, maximum area shows network of NPY immunoreactive fibers (Fig. 2, 3). In the wall of third ventricle, few NPY immunoreactive neurons are observed nuceus hypothalamicus pars medialis (Nhm) (Fig. 4F). Some NPY positive fibers are seen innervating NLT but no NPY immnoreactivity is observed in the NLT perikarya (Fig. 2, 3). NPY positive beaded immunoreactive fibers are distributed in the inferior lobe (Fig. 2, 3). The habenular ganglia show NPY immunoreactive neurons and fibers in the dorsal habenular nucleus (DHN) and ventral habenular nucleus (VHN) (Fig. 2, 3, 4G). NPY positive immunoreactivity is also observed in the pituitary gland (Fig. 2, 3, 4H). NPY positive beaded fibers are localized in the neurohypophysis and they innervate pars intermedia (PI), rostral pars distalis (RPD) and proximal pars distalis (PPD) while NPY positive cells are observed in the PPD (Fig. 2, 3, 4H).

Immunoblot analysis

The homogenates from the olfactory system, forebrain and pituitary gland show a single band of approximately 4.3 kDa (Fig. 5).

Discussion

Present investigation is the first report on the understanding of pattern of NPY immunoreactivity in the olfactory organ, forebrain and pituitary of commercially important, Labeo rohita. Western blotting analysis of this peptide was also confirmed in the reactivity in these organs. We have for the initial time documented NPY immunoreactivity in the olfactory receptor neurons (ORN's) in carp which suggests NPY possibly will play a significant role as a neurotransmitter in ORN's with the purpose to send the information to the olfactory bulb. Among the teleosts, such type of distribution pattern is reported in Clarias batrachus (Gaikwad et al., 2004) however immunoreaction was not noticed in the ORN's of bichir, Polypterus senegahus (Chiba, 1997). Reactivity of NPY in the ORN's and their axon penetrating in the ONL suggest possible role in signaling. In the ORN's, other neurotransmitters such as GnRH in Clarias batrachus and Rhodeus amarus (Subhedar and Ramakrishna, 1988; Baby et al., 2000), β-endorphin in C. batrachus (Khan et al., 1999) and Nitric oxide synthase for Nitric oxide in Oreochromis mossambicus (Singru et al., 2003) are also reported.

In the olfactory bulb towards the periphery, NPY positive fibers enter the ONL from the axons of the ORN's. These fibers do not form fascicles because the number of ORN's present in the olfactory organ is limited, rather they innervate the mitral cells and these cells show NPY immunoreactions. Innervations of fibers of ORN's enter into the dendrites of mitral cells was reported in the teleosts (Satou, 1990). In the granular cell layer (GCL), NPY positive fibers were located and such fibers were also reported in the olfactory bulb of some teleosts (Pontet et al., 1989; Chiba et al., 1996; Subhedar et al., 1996; Gaikwad et al., 2004). The granular cells were reported to extend their dendrites, penetrate glomeruler layer and establish reciprocal synapse with the mitral cell dendrites in the teleosts (Satou, 1990). Other vertebrate such as bichir (Chiba, 1997), amphibians (Lazar et al., 1993), birds (Kuenzel and McMurtry, 1988) and mammals (Gall et al., 1986; Matsutani et al., 1989) also exhibit such NPY immunoreactive fibers. Along with all these cell layers , NPY positive reaction is also noted in the MOT that sends signals to the telencephalon and other areas in the brain. MOT was known to be involved in the regulation of spawning activity in the goldfish (Kyle et al., 1987). Hence we propose that NPY may play the same role in this pathway in L. rohita.

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In the forebrain, telencephalon and diencephalon were the two divisions. In the telencephalon, extensive distribution of NPY immunoreactivity was observed in L. rohita. Dorsal and lateral parts of telencephalon receive maximum NPY fibers in the Goldfish (Kah et al., 1989; Pontet et al., 1989), Atlantic salmon and gambusia (Garcia-Fernandez et al., 1992), platyfish (Cepriano and Schreibman, 1993) and killifish (Subhedar et al., 1996). In carp, higher density of fibers was noticed in the dorsal telencephalon than in the lateral and ventral regions (Marchetti et al., 2000). Dense innervation of NPY positive fibers in the rostral and lateral telencephalon and much lesser in other areas in C. gariepinus and C. batrachus was reported (Zangdbrgen et al., 1994; Gaikwad et al., 2004). In L. rohita, though NPY immunoreactive fibers are observed throughout the telencephalon, particularly dense network is found in the dorsal and lateral regions but in Dc NPY positive immunoreactivity was observed in neurons as well as in fibers.

NPY positive fibers were distributed in ventral telencephalic region. NPY immunoreactive cells and fibers were seen in the Vd of goldfish (Pontet et al., 1989) and carp (Marchetti et al., 2000). In C. batrachus, weak NPY positive reaction was reported (Gaikwad et al., 2004) but no immunoreactivity was detected in C. gariepinus (Zandbergen et al., 1994), trout (Danger et al., 1991) and killifish (Subhedar et al., 1996). In Vl region, NPY positive cells and fibers were observed in several teleosts such as the goldfish (Pontet et al., 1989), Xiphophorus maculates (Cepriano and Schreibman, 1993), Salmo salar (Garacia-Fernandez et al., 1992) and Pejerry (Traverso et al., 2003) but in others such as Onchorynchus mykiss (Danger et al., 1991), C. gariepinus (Zandbergen et al., 1994) and C. batrachus (Gaikwad et al., 2004), immunoreactive cells were not observed in Vl.

NPY immunoreactive neurons of nucleus entopeduncularis (NE) were the important cells of the neuronal system of L. rohita. In NE, unipolar, bipolar or multipolar types of NPY positive neurons were located. Similarly, NPY immunoreactive neurons were also found in other teleosts such as goldfish (Kah et al., 1989; Pontet et al., 1989), rainbow trout (Danger et al., 1991), brown trout (Castro et al., 1999), catfish (Zandbergen et al., 1994; Gaikwad et al., 2004) and carp (Marchetti et al., 2000). The neurons of NE also have fiber connections with the pituitary gland (Anglade et al., 1993; Kah et al., 1993). These were reported to play an important role in the regulation of pituitary hormone secretion (Peng et al., 1993). NPY positive cells were observed in the optic chiasma of L. rohita however Gaikwad et al., (2004) have reported only NPY immunoreactive fibers in C. batrachus. We, therefore for the first time report that NPY positive cells were present in the optic chiasma of any teleosts including Indian major carp. Neuropeptide Y possesses a widespread distribution in the amphibian brain in the anterior pre-optic area of telencephalon, ventromedial, central and posterior thalamic nuclei, suprachiasmatic nuclei (Ali et al., 2016).

In the preoptic area of L. rohita NPY immunoreactive fibers innervate both the divisions of the nucleus preopticus (NPO). This region has already shown NPY positive perikarya in the goldfish (Pickavance et al., 1992) and rainbow trout (Danger et al., 1991) and NPY positive fibers in goldfish (Pontet et al., 1989), catfish (Zandbergen et al., 1994) and Cyprinus carpio (Marchetti et al., 2000) like that of L. rohita. NPO is also known to contain GnRH and dopamine cells that regulate the activity of the gonadotrophs of the pituitary gland (Rodrigeuz-Gomez et al., 2000). Therefore, there is a possibility that NPY positive fibers from NE or MOT innervate the NPO neurons which may be responsible for the synthesis of GnRH and

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dopamine that are involved in the control of gonadotrophs of the pituitary gland. However, this hypothesis requires further investigation.

NPY Immunoreactive fibers enter the nucleus lateralis tuberis (NLT) in L. rohita. Dense NPY fibers were reported in the tuberal hypothalamus (Pontet et al., 1989; Danger et al., 1991; Chiba and Honma, 1994, Chiba, 1997). Presence of the GnRH in NLT neurons is extensively worked out in the teleosts such as X. maculeatus (Schreibman et al., 1979) Carassius auratus (Kah et al., 1984a) and C. batrachus (Subhedar and Ramakrishna, 1988) and NLT neurons were known to innervate the pituitary gland and play an important role in the regulation of reproduction (Peter and Fryer, 1983). In L. rohita interaction of NPY and GnRH in the NLT may be responsible for the control of GTH release from pituitary gland.

In the inferior lobe, NPY positive beaded fibers were observed in L. rohita similar to those reported in brown trout (Castro et al., 1999).

The neurons of nucleus hypothalamicus medialis (Nhm) show NPY positive reaction in L. rohita. No report was available for the presence NPY positive neuronal cells in Nhm other than this except dense NPY fibers were seen in several bony fishes (Pontet et al., 1989; Danger et al., 1991; Chiba and Honma, 1994; Subhedar et al., 1996; Gaikwad et al., 2004).

Both NPY positive neurons and fibers were demonstrated in the habenular ganglion of L. rohita. Such findings were also reported in the killifish (Subhedar et al., 1996), but in Clarias batrachus, only fibers are reported (Gaikwad et al., 2004).

In the neurohypophysis, NPY positive fibers were distributed and NPY positive cells are seen in the PPD in L. rohita. Similar findings were reported in killifish (Subhedar et al., 1996), Ayu (Chiba et al., 1996), Pejerrey (Traverso et al., 2003) and C. batrachus (Gaikwad et al., 2004). Presence of NPY fibers in the neurohypophysis and its innervation in the proximal pars distalis (PPD) area which has gonadotropin secreting cells, suggests that it may be involved in GTH release from the pituitary.

Hence we propose that the results of present investigation will prove a landmark hypothesis in the understanding of role of NPY in commercial production of Indian major carps.

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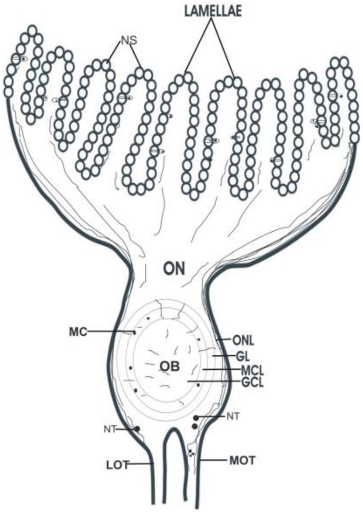

Fig. 1.Diagrammatic representation of distribution of NPY immunoreaction in the olfactory system of *L. rohita* showing, GCL, granular cell layer; GL, glomerular layer; LOT, lateral olfactory tract; MC, mitral cell; MCL, mitral cell layer; MOT, medial olfactory tract; NT, nervus terminalis; OB, Olfactory bulb; ON, olfactory nerve; ONL, olfactory nerve layer.

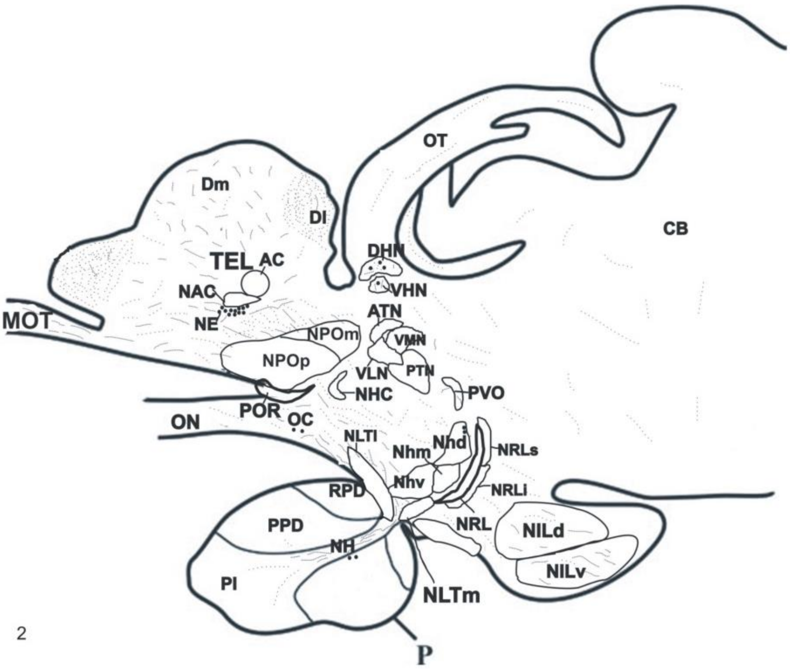

Fig. 2. Diagrammatic representation of the sagittal section of the forebrain and pituitary gland of *L. rohita* showing the distribution of NPY immunoreactivity in the cells and fibers. AC, anterior commissure; ATN, anterior thalamic nucleus; CB, cerebellum; Dm, area dorsalis telencephali pars medialis; DHN, dorsal habenular nucleus; Dl, area dorsalis telencephali pars lateralis ; MOT, medial olfactory tract; NAC, nucleus anterior commissure; 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; NLTl, nucleus lateralis tuberis pars latralis; NLTm, nucleus lateralis tuberis pars medialis; NPOm, Nucleus preopticus pars magnocelullaris; NPOp, nucleus preopticus pars parvocelullaris; NRL, nucleus recesses lateralis; NRLi, nucleus recesses lateralis inferior; NRLs, nucleus recesses superior; OC, optic chiasma; ON, optic nerve ;OT, optic tectum; P, pituitary gland; PI, pars intermedia; POR, preoptic recesses; PPD, proximal pars distalis; PTN, posterior thalamic nucleus; PVO, paraventricular organ; RPD, rostral pars distalis; SCN, suprachiasmiatic nucleus; TEL, telencephalon; VHN, ventral habelnuar nucleus; VLN, ventrolateral thalamic nucleus; VMN, ventromedial thalamic nucleus.

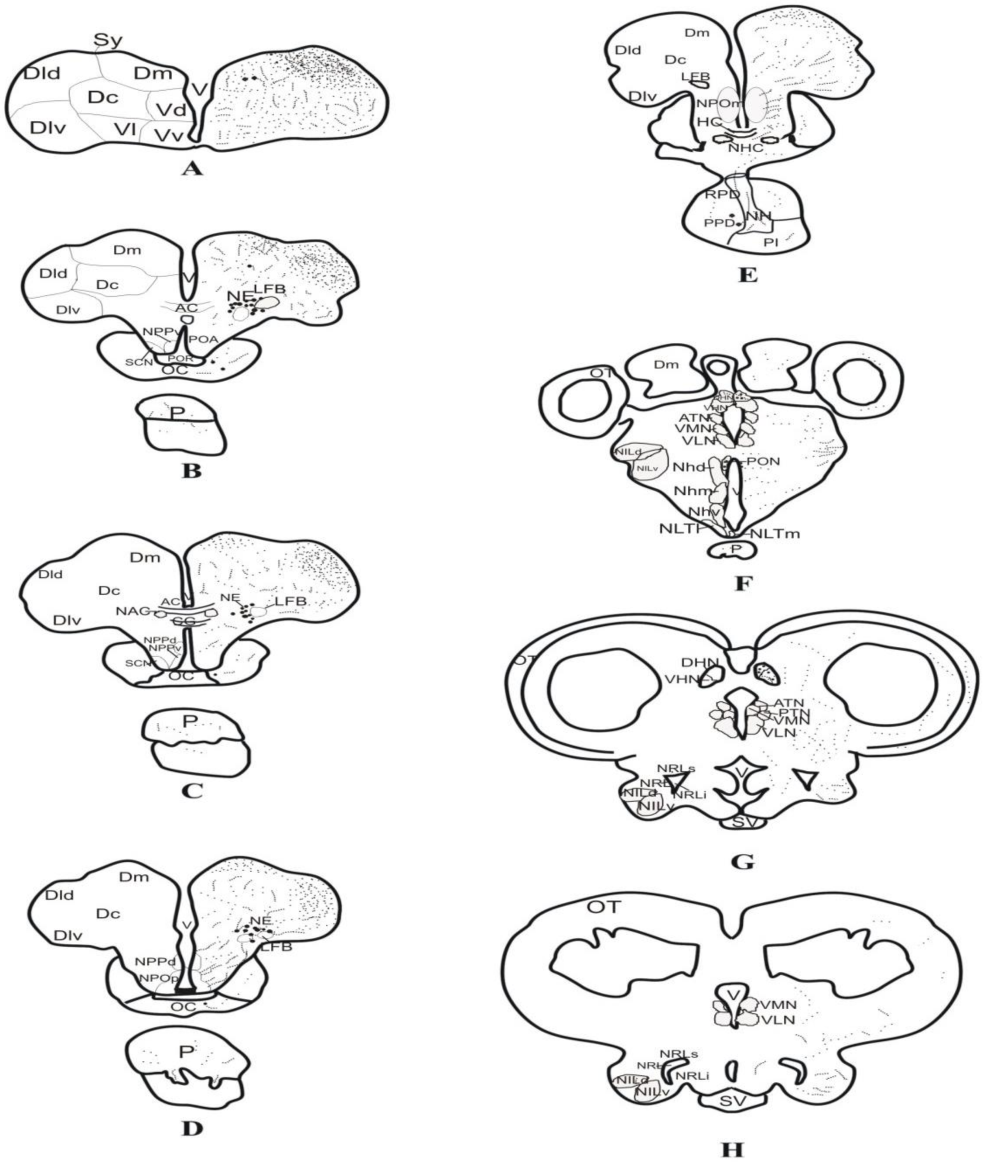

Fig. 3. Diagrammatic representation of transverse section through forebrain and pituitary gland showing the distribution of NPY immunoreactivity in the cells and fibers. AC, anterior commissure; ATN, anterior thalamic nucleus; CG, commissure of goldstein; Dc, area dorsalis telencephali pars centralis; DHN, dorsal habenular nucleus; Dld, area dorsalis telencephali pars lateralis dorsal; Dlv, area dorsalis telencephali pars lateralis ventral; Dm, area dorsalis telencephali pars medialis; HC, Horizontal commissure; LFB, lateral forebrain bundle; NAC, nucleus anterior commissure; 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 magnocelullaris; NPOp, nucleus preopticus pars parvocelullaris; NPPd, nucleus preopticus periventricularis dorsal; 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; PON, nucleus paraventricular organ; POR, preoptic recesses; PPD, proximal pars distalis; PTN, posterior thalamic nucleus; PVO, paraventricular organ; RPD, rostral pars distalis; SCN, suprachaismatic nucleus; SV, saccus vasculossus; V, ventricle; VHN, ventral habelnuar nucleus; Vd, area ventralis telencephali pars dorsalis; Vl, area ventralis telencephali pars latralis; VLN, ventrolateral thalamic nucleus; VMN, ventromedial thalamic nucleus. Vv, area ventralis telencephali pars ventralis.

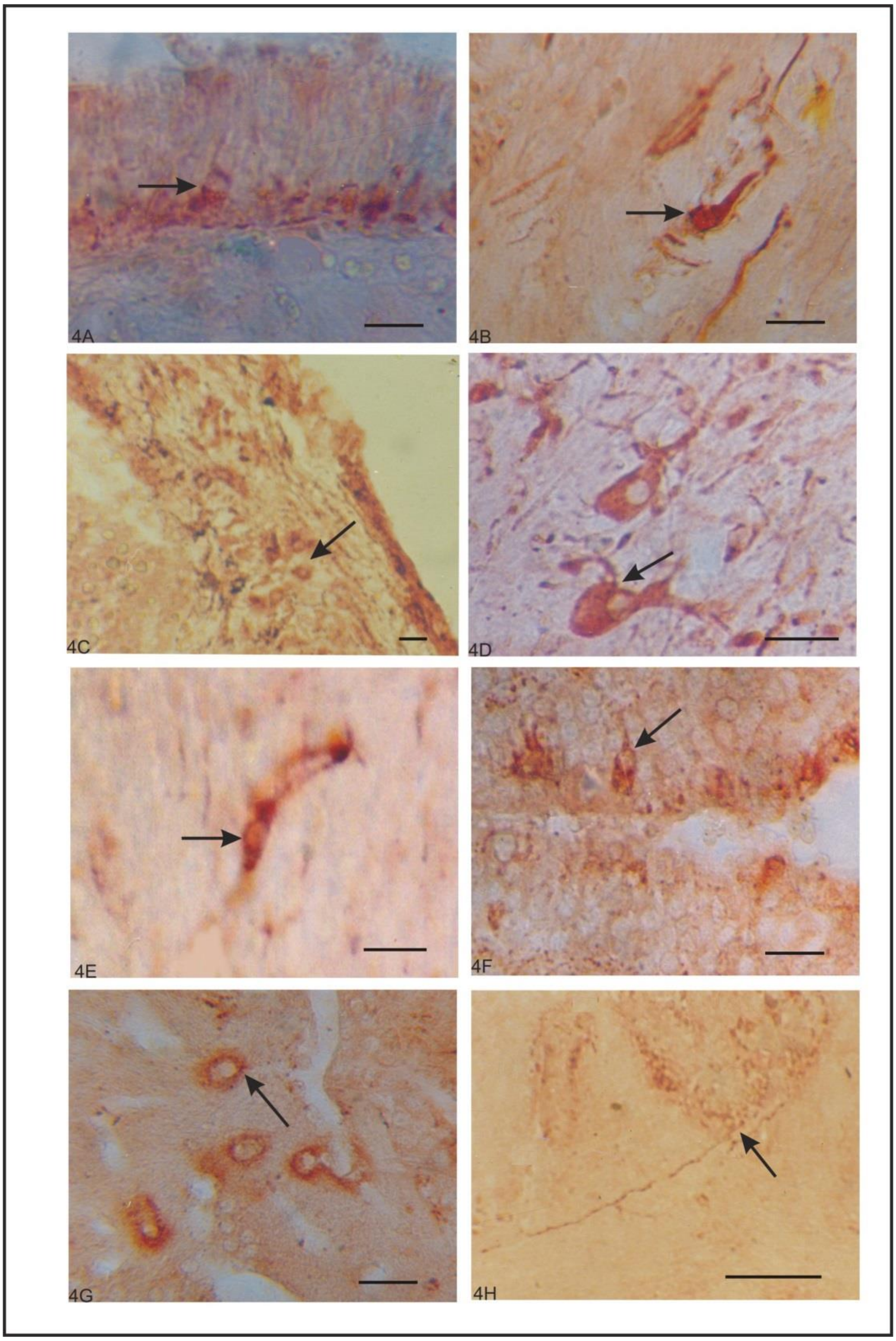

Fig. 4 **A.** Sagittal section through the olfactory epithelium showing presence of NPY immunoreactivity in the olfactory receptor neurons (ORN's) (arrow) and their apical dendrites. Figs. 4A-4H. Bar, 20µm. **B.** Sagittal section through olfactory bulb showing NPY immunoreactive mitral cell (arrow). **C.** Sagittal section through olfactory bulb showing NPY immunoreactivity in cells (arrow) and fibers of the medial olfactory tract (MOT). **D.** Transverse section of the brain through telencephalon showing positive multipolar neurons of the nucleus entopeduncularis (arrow) (NE). **E.** Transverse section of the brain through hypothalamus showing presence of NPY positive neurons (arrow) and fibers in the optic chiasma. **F.** Transverse section of the brain through nucleus hypothalamicus medialis showing NPY positive cell (arrow) and fibers adjacent to the ventricle. **G.** Transverse section of the brain through epithalamus showing NPY positive cells (arrow) and fibers in the habenular ganglion. **H.** Sagittal section of pituitary gland showing NPY positive fibers in the neurohypophysis innervating proximal pars distalis (arrow).

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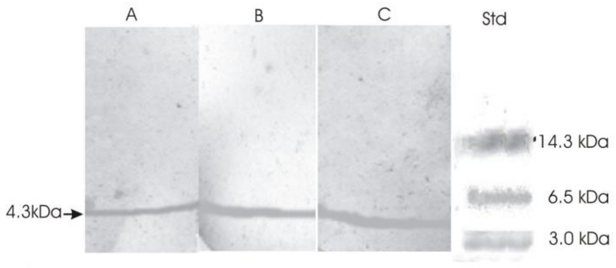

Fig. 5. Western blot analysis of extract of the olfactory organ, hypothalamus and pituitary gland showing the single band of 4.3 kDa (arrow) (A) Olfactory organ (B) Hypothalamus (C) Pituitary, Std.- Molecular marker.

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