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Seasonal changes in hypothalamo-hypophysial complex in relation to reproduction in murrel fish *Channa punctatus* (Bloch)

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Abstract

Seasonal changes in the secretory activities of hypothalamic nuclei, the nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) and pituitary gonadotropic cells were studies in relation to reproductive cycle of a fresh water murrel fish Channa punctatus. Hypothalamo-neurosecretory complex consisted mainly of NPO, NLT and their axonal tracts. NPO is a paired structure situated on either side of third ventricle anterodorsal to the optic chiasma. NPO consists of dorsal pars magnocellularis (PMC) of larger and ventral pars parvocellularis (PPC) of smaller neurosecretory cells. The NLT is situated at infundibular floor region adjacent to pituitary gland. NLT cells are of two types, the larger NLT-I cells generally, placed on the anterolateral side and the smaller NLT-II cells posteroventral region in the infundibular floor adjacent to the pituitary gland. NPO and NLT cells of hypothalamus and pituitary gonadotropic cells exhibit conspicuous seasonal changes in relation to reproductive cycle. During resting phase these cells remain inactive and show poor concentration of neurosecretory granules and glycoproteinous contents in the pituitary gonadotropic cells. Progressive increased synthesis of granules occur during preparatory phase. During prespawning phase these cells exhibit maximum synthetic activities as they were laden with the neurosecretory / hormonal materials whereas they exhibit degranulation and partial vacuolisation (releasing activities) during spawning phase. These cells sharply release their contents during postspawning phase showing depleted appearance with increased vacuolisation.

Key Words- Hypothalamic nuclei, nucleus preopticus, nucleus lateralis tuberis, gonadotropic cells, seasonal changes, reproductive cycle, *Channa punctatus* .

Introduction

Hypothalamus is a strategic point and a seat of neuroendocrine regulation in the vertebrate's brain that mediates the organismic endocrine responses and adjustment to the environmental changes (Ball, 1981). It comprises groups of neurosecretory cells that secrete neurohormones known as releasing (-RH) and inhibiting (-IH) hormones which modulate the secretion of various trophic hormones of the pituitary gland (Makshimovich, 1987; Peter *et al*., 1991; Bhattacharya *et al.,* 1994; Peter and Yu 1997; Prasada Rao, 1999; Goos *et al*., 1999). Hypothalamus also possesses hormone specific receptors which coordinates its activity through feedback mechanism (Ball, 1981; Maksimovich, 1987; Sherwood and Hew, 1994; Evans 1998; Melamed and Sherwood , 2005). Fish pituitary (hypophysis) plays a central role in the control of growth, development, adaptation to the environment and reproduction. Unlike mammals, teleost fish lack a hypothalamo-hypophysial portal system for the transport of neurohormonal regulators. Instead, a direct axonal transport exists between hypothalamic neurons and pituitary endocrine cells via the hypophysial stalk and the neurohypophysis (Weltzien *et al., 2004).* The persual of literature revealed that in fishes too, the hypophysial functions are modulated by the secretion of hypothalamic neurohormones but its regulatory mechanism is yet obscure (Goos *et al.,* 1999 ; Subhedar *et al.,* 1999; Melamed and Sherwood, 2005). The role of hypothalamic nuclei, the nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) were studied in many teleosts in relation to reproduction (Viswanathan and Sundararaj, 1974; Saxena, 1976; Zolotnitskiy, 1980; Moitra and Medya, 1980; Prakash *et al.,* 1984, Rai and Pandey, 1986; Maksimovich, 1987, Das and Sinha, 1988; Mandal, 1990; Peter *et al.,* 1991; Bhattacharya *et al.,* 1994; Okuzawa & Kobayashi 1999; Lal and Pandey, 2007; Sinhababu *et al.,* 1999, 2001; Pandey & Mani, 2006, 2009; Pandey *et al.,* 2000; Pandey, 2008). In the present work, an attempt has been made to observe the changes in the hypothalamo-neurosecretory cells and pituitary gonadotropic cells in relation to the gonadal maturation of a fresh water murrel fish *Channa punctatus.*

Materials and Methods

Adult specimens of *Channa punctatus* were collected locally throughout the year. The intact brain alongwith pituitary gland and gonads were carefully dissected out and fixed in aqueous Bouin's solution and were subjected to routine procedures for histological studies and for identification of its reproductive status. Serial paraffin sections of the tissues were obtained at 6 µm thickness. Hypothalamic neurosecretory system alongwith pituitary were stained with Heidenhain's azan, PAS and AF and gonads with hematoxylin /eosin. Size of NPO cells , NLT cells and pituitary gonadotropic cells were recorded with the help of ocular micrometer. 100 nuclei were measured from each type of cells. The Student's't' test was used for statistical calculations. The activity of neurosecretory cells and gonadotrops were determined by the changes observed in the cytoplasmic granulation and nuclear size.

Results

The salient features of ovaries and testes of *Channa punctatus* corresponding to various phases of reproductive cycle are described elsewhere (Srivastava and Singh, 1990, 1994). In the reproductive cycle of *Channa punctatus* five distinct phases of ovary and testes have been recognized i.e. i) resting, ii) preparatory, iii) prespawning iv) spawning, and v) postspawning phases. Seven types of germ cells were encountered in the maturation process of the testes viz. i) resting germ cells (diameter 16.5 µm); ii) primary spermatogonia (diameter 12.6 µm); iii) secondary spermatogonia (diameter 8.7 µm); iv) Primary spermatocytes (diameter 8.1 µm); v) secondary spermatocytes (diameter 6.4 µm); vi) spermatids (diameter 2.7 µm); vii) spermatozoa (diameter 1.0 µm) (Srivastava and Singh, 1994).

Hypothalamo-hypophysial complex- The hypothalamo-hypophysial complex of *C.punctatus* consists of a paired nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) neurosecretory tracts, neurohypophysis and the hypophysis (pituitary) (fig. 1). The general organization of neuro-secretory cells is given in the Table-1.

Table 1: General organization of neurosecretory cells in hypothalamus of *C.punctatus*

Cell type	Position	Shape	Size Cell (µm) ± SEM	Cytoplasm	Staining affinity
1- NPO	On either side of III ventricle, anterodorsal to optic chiasma				
i). PMC	Dorsal to optic chiasma	Rounded oval or polygonal	8.87±0.042	Dense	AF-positive^{+++}
ii). PPC	Ventral to optic chiasma	Rounded	5.55±0.06	Scanty	AF-positive^{++}
2- NLT	Infundibular floor region, adjacent to Pituitary				
i). Cell type I (NLT I)	Anterodorsal	Rounded	5.71±0.035	Dense	Acid fuchsin-positive
ii). Cell type II (NLT II)	Posteroventral	Rounded	3.85±0.15	Scanty	Acid fuchsin-positive

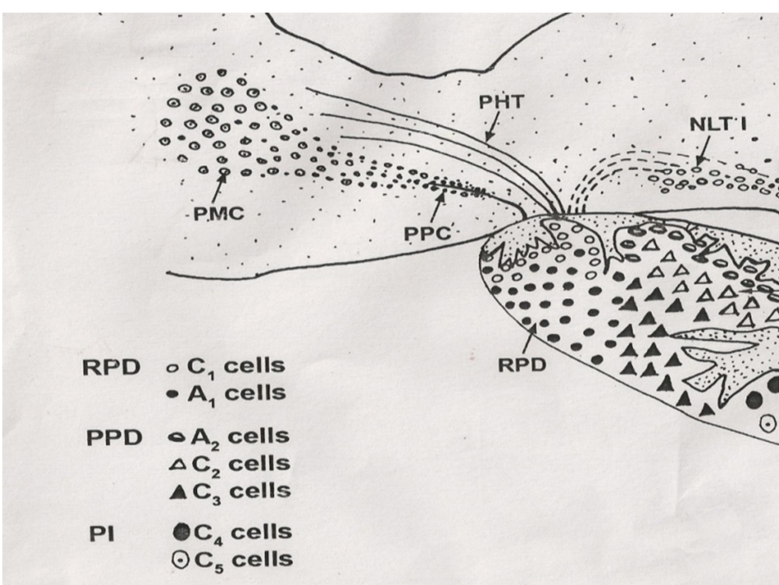
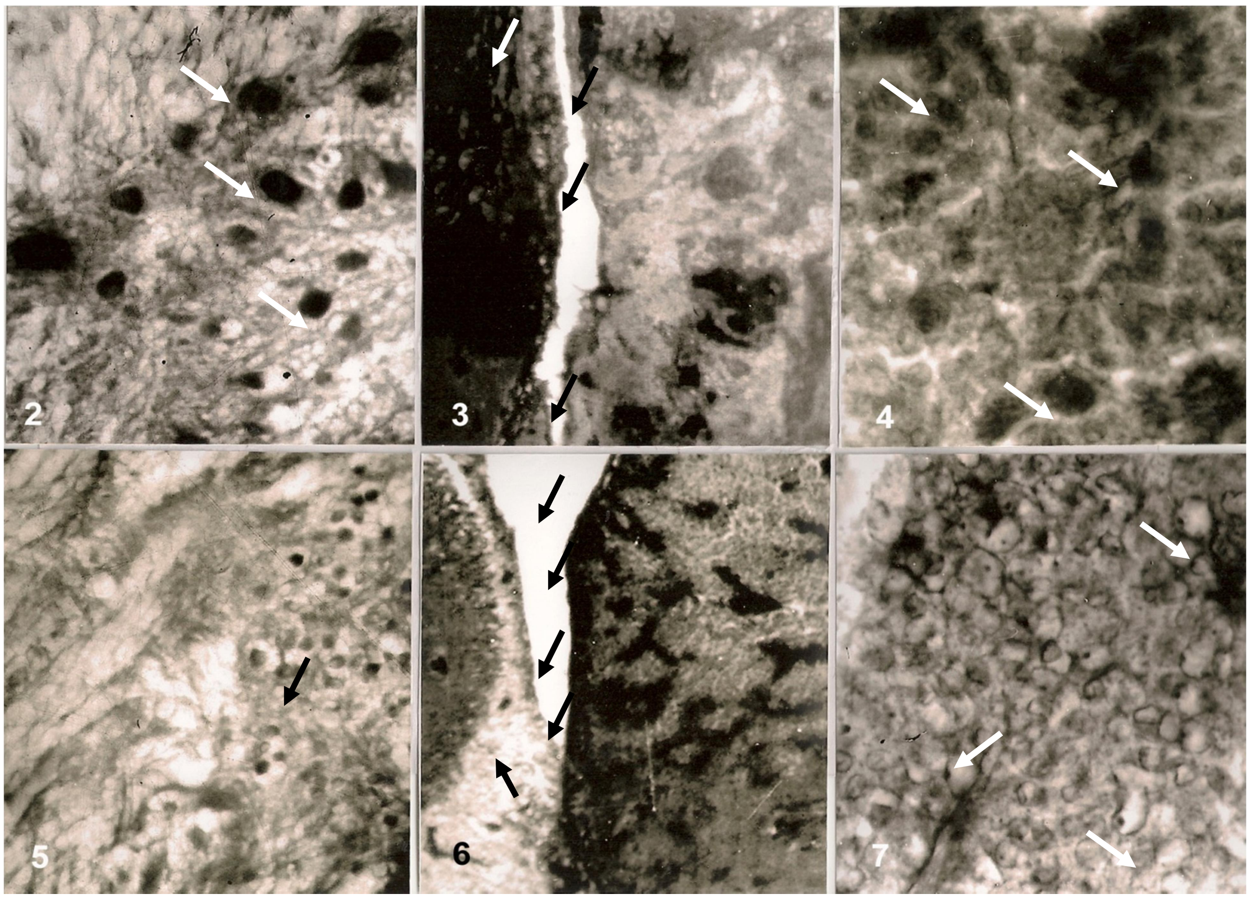


Fig. 1



Legends

Fig.1 Diagrammatic representation of sagittal section of the hypothalamus- neurohypophysial system of ***C.punctatus*** showing the neurosecretory centers. **Abbreviations :** NPO-Nucleus preopticus, PMC-Pars magnocellularis, PPC- Pars parvocellularis, PHT-Preoptic hypophysial tract, NLT I- Nucleus lateralis tuberis I, NLT II- Nucleus lateralis tuberis II, NH-Neurohypophysis, RPD-Rostral pars distalis, PPD-Proximal pars distalis, PI-Pars intermedia

Fig.2 NPO cells (larger PMC & smaller PPC)showing increased granulation (arrows) during prespawning phase AF x 1000.

Fig.3 NLT cells (larger NLT I & smaller NLT II)showing granulation (arrows) during prespawning phase. Triple mallory x 125.

Fig.4 Gonadotrop cells of pituitary showing heavy cytoplasmic granulation (arrows) during prespawning phase PAS x 1000.

Fig.5 NPO cells showing degranulation and vacuolisation (arrows) during spawning phase AF x 500.

Fig.6 NLT cells showing degranulation and partial vacuolisation (arrows) during spawning phase. Triple mallory x 125.
Fig.7 Gonadotrop cells of pituitary showing vacuolisation (arrows) during spawning phase PAS x 1000.

Nucleus preopticus- The cells of the nucleus preopticus are located in the walls of the hypothalamus on either side of the III ventricle and antero-dorsal to optic chiasma. Morphologically, they may be divided into two groups: the dorsal half of the nucleus preopticus made up of larger cells forming the pars magnocellularis (PMC), while the ventral part of the nucleus preopticus is constituted by smaller cells termed as pars parvocellularis (PPC) (Figs. 1 & 2). NPO was highly vascularised structure. Its neurosecretory cells were positive to aldehyde fuchsin (AF), chrome-alum-hematoxylin-phloxine (CAHP) and acid fuchsin. Most of the neurons of PMC and PPC were bipolar and contributed beaded axons to form left and right neurohypophysial main tracts. NPO cells laden with neurosecretions during breeding season (Fig. 2).

Nucleus lateralis tuberis- The cells of the nucleus lateralis tuberis are located in the infundibular region at the base of the hypothalamus dorsolateral to the pituitary gland. Morphologically they are distinguished into two types with reference to difference in their size and position. The cells of anterolateral components are larger, forming the cell type NLT I, while the smaller cells of posteroventral region constitute the cell type NLT II (Figs. 1 & 3). The cells of the nucleus lateralis tuberis are AF negative but positive to triple mallory. The neurohypophysial tracts (NHT) enter the pituitary through infundibulum. The NLT cells also exhibit enhanced activity during breeding season (Fig. 3).

Hypophysis(Pituitary)-The pituitary of *C.punctatus* is oval and platybasic and composed of a glandular adenohypophysis and a neurohypophysis. The adenohypophysis is distinguishable into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). The neurohypophysis is formed of loosely arranged fibres which ramify extensively in the pars intermedia, few smaller branches penetrate into both proximal pars distalis and rostral pars distalis. (Fig. 1). On the basis of tinctorial and histochemical properties, seven cell types have been identified in the adenohypophysis (Srivastava and Singh, 1992b). The rostral pars distalis contains two cell types: PbH-positive C_1 cells and an acidophil A_1 cells. The proximal pars distalis comprises of acidophils A_2 cells and two types of cyanophils, C_2 and C_3 (gonadotropic) cells. The cyanophils are PAS, AF and aniline blue positive. The pars intermedia possesses two cells types: PbH-positive C_4 cells and PAS-positive C_5 cells. (Fig. 1).

Correlative seasonal changes in hypothalamo-hypophysial-gonadal system

Seasonal changes in the neurosecretory cells (NPO and NLT) and gonadotropic cells (C_3 cells) of the proximal pars distalis of pituitary glands are conspicuous and they closely follow the reproductive cycle (Table-2). There is poor concentration of neurosecretory materials in the neurosecretory cells and glycoproteinaceous contents in the gonadotropic cells (C_3 cells) during the resting phase, progressive increased synthesis occur during preparatory phase whereas maximum synthetic activity occur during prespawning phase exhibiting intense staining affinity (Table 2 & Figs. 2,3,4). The neurosecretory cells (NPO and NLT) and gonadotropic cells (C_3 cells) undergo a process of hypertrophy, granulation, degranulation and vacuolisation during the spawning phase (Table 2 & Fig. 5, 6, 7). They almost empty their contents during the postspawning phase.

Discussion

Hypothalamus is a major integrating centre of regulatory mechanism concerned with neuro-endocrine control of gonadotropins secretions, hence the reproductive activities (Goos *et al.,* 1999). Hypothalamo-neurosecretory complex of *C.punctatus* comprised of NPO, NLT and their axonal tracts. The hypothalamo-neurosecretory-hypophysial complex of *C.punctatus* is comparable to those reported in other teleosts (Sathyanesan and Kulkarni, 1983; Maksimovich, 1987; Subhedar *et al*., 1999; Prasada Rao, 1999; Pandey and Mani, 2006; Pandey, 2008). The neurosecretions of the nucleus preopticus (NPO) alongwith nucleus lateralis tuberis (NLT) directly influence the pituitary gonadotropic cells activities (Maksimovich, 1987; Mandal, 1990; Peter *et al*., 1991; Okuzawa and Kobayashi, 1999; Sinhababu *et al*., 1999, 2001; Lal and Pandey, 2007, Jadhao, 2007; Pandey, 2008; Pandey and Mani, 2009; Singh and Abhinav, 2009). These neurosecretions pass through neurohypophysial tracts (NHT) into the pituitary as also reported in other teleosts (Peter *et al*., 1991; Subhedar *et al*., 1999; Prasada Rao, 1999; Sinhababu *et al*., 1999, 2001; Lal and Pandey, 2007; Pandey and Mani, 2006, 2009). In *C.punctatus* little neurosecretory materials were observed in NPO and NLT cells during resting and postspawning phases (indicate inactivity) whereas enhanced neurosecretions were noticed during prespawning and spawning phases indicate that these cells are possibly involved in regulation of gonadal activities through its neurosecretions. The concomitant changes in the activity of NPO and NLT cells (granulation, degranulation and vacuolisation) with gonadal maturation and spawning have also been reported in a number of teleosts (Vishwanathan and Sundararaj 1974; Saxena 1976; Zolotnitskiy, 1980; Moitra and Medya, 1980; Prakash *et al*., 1984; Rai and Pandey, 1986; Das and Sinha, 1988; Mandal, 1990; Sinhababu *et al*., 1999, 2001; Weltzien *et al*., 2004; Pandey and Mani, 2006, 2009; Lal and Pandey, 2007; Pandey, 2008).In the present study low profile of activity or inactivity with scanty granulation were noticed in gonadotrophic cells of pituitary during resting and postspawning phases whereas intense granulation and more activity were recorded during breeding season in *C.punctatus*. Similar seasonal changes in the activity of gonadotrophs in relation to gonadal cycle have also been reported in other teleosts (Saksena, 1976; Srivastava, 1983; Prakash *et al*., 1984; Peute *et al*., 1986; Borg *et al*., 1988; Peter *et al*., 1991; Bhattacharya *et al.,* 1994; Srivastava and Singh, 1992a; Peter and Yu, 1997; Khanna, 2006; Lal and Pandey, 2007; Pandey and Mani, 2006, 2009).

It may be inferred that NPO and NLT neurosecretory cells of hypothalamus in *C.punctatus* exhibit seasonal morphohistological changes with regards to synthesis, storage and release of neurosecretory materials on demand. These neurosecretions probably regulate the synthesis and release of gonadotropins from pituitary gonadotrophs. Gonadotropins ultimately regulate the gonadal activities. Thus, gonadotropin hormones are a central component of brain-pituitary-gonadal axis that initiate various aspects of gonadal activities like gonadal recrudescence, maturation and spawning. This is supported by immuno-cytochemical studies which revealed that NPO and NLT neurosecretory cells contain several hypophysial related releasing factors or neurohormones out of which gonadotropin releasing hormone (GnRH) regulate gonadal functions (See reviews Peter *et al.*, 1991; Bhattacharya *et al.,* 1994). Further, reverse transcription polymerase chain reaction (RT-PCR) studies support that gonadotrophs are directly innervated by neurosecretory fibres (Parhar *et al*., 2003). However, Goos *et al*., (1999) stated that the mechanism of integration of neuroendocrine signals for GnRH system and hypophysial gonadotropin release for gonadal function at every stage of reproductive cycle is yet obscure and incomplete and needs further research at organism and environmental level.

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