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Effect of sublethal exposure of Cartap on hypothalamo-neurosecretory system of the freshwater spotted murrel, *Channa punctatus* (Bloch)

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Abstract: *In order to record the effect of carbamate pesticide on hypothalamus of Channa punctatus, fish were exposed to sublethal concentration (0.18 mg l^{-1}, 30% LC_{50} for 96 hr) of Cartap for 24, 48, 72 and 96 hr under static bioassay condition. Hypothalamo-neurosecretory complex of the murrel consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO is a paired structure situtated on either side of the third ventricle anterodorsal to the optic chiasma and looked inverted L-shape in the sagittal section. NPO is morphologically divisible into a dorsal pars magnocellularis (PMC) consisting of large neurons and ventral pars parvocellularis (PPC) formed of smaller neurosecretory cells. NLT cells are distributed in the infundibular floor adjacent to the pituitary stalk. Sublethal Cartap treatment induced an initial hypertrophy of the neurosecretory cells of NPO and NLT followed by loss of staining affinity as well as varying degrees of cytoplasmic vacuolization and necrosis. Herring bodies (HB) were also encountered in the neurohypophysis of the treated fishes.*

Key words: *Cartap, Hypothalamo-neurosecretory complex, Hypophysial vesicle, Channa punctatus*
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Introduction

Hypothalamus in vertebrates comprises groups of neurosecretory cells which control secretion of the various trophic hormones of the pituitary by elaborating releasing (-RH) and /or inhibiting (-IH) hormones (Ball, 1981; Maksimovich, 1987; Evans, 1998). Recent studies in mammals have demonstrated it to be a strategic point in the vertebrate brain where several exteroceptive neural stimuli converge. Hypothalamus also contains receptors specifically sensitive to the hormones which, in turn, regulate its activity through feedback mechanism (Peter, 1986; Maksimovich, 1987; Peter *et al*., 1991; Peter and Yu, 1997; Evans, 1998; Rokade *et al*., 2006). There are increasing evidences that hypophysial functions in fishes mediated by hypothalamic neurohormones, but the regulatory mechanisms are not properly defined (Peter, 1986; Peter *et al*., 1991; Goos *et al*., 1999; Subhedar *et al*., 1999). Since information pertaining to the effects of pesticides on hypothalamus of the teleosts is scanty (Shukla and Pandey, 1986; Ram *et al*., 2001), an attempt was made to record the effects of sublethal exposure of Cartap on hypothalamo-neurosecretory system of the freshwater spotted murrel, *Channa punctatus*.

Materials and Methods

Healthy *Channa punctatus* (measuring 14.28+1.03 cm; weighing 41.9+8.75 g) were collected from the natural ponds (wild) near Bhubaneswar (Orissa). They were acclimatized to the laboratory conditions for two weeks before initiation of the experiment. Fish were fed with minced goat liver and earthworm *ad libitum* during entire period of the experiment to avoid the effect of starvation. Cartap (S,S'-[2-(dimethylamino)-1, 3-propanediyl] dicarbamethioate) is the widely used carbamate pesticide in agriculture on a variety of crops for pest control (Anon, 2005). Technical grade Cartap 50 SP (Caldan; Dhanuka Pesticides Ltd., Gurgaon: Batch No. DCS/09087) was procured and dissolved in water. Static bioassays were conducted according to the method recommended by APHA (1991). From their mortality, the LC_{50} values for 24, 48, 72 and 96 hr were determined (Litchfield and Wilcoxon, 1949) which were 2.4, 1.8, 1.6 and 0.6 mg l^{-1} for Cartap (Mishra and Bohidar, 2005). 30% LC_{50} value for Cartap (0.18 mg l^{-1}) was taken as sublethal concentration for the present study.

Forty fish were divided into two equal groups. In group 1, fish were maintained in tap water (control) whereas in group 2, they were exposed to sublethal concentration of Cartap (experimental). Fish of all the groups were maintained in glass aquaria containing 200 litres of water. The test solution was changed everyday to give constant effect of the pesticide. Fish from all the groups were killed after 0, 24, 48, 72 and 96 hr. Brains (along with pituitary) were sugrically removed and fixed immediatily in freshly prepared aqueous Bouin's solution. After 24 hr of fixation, the tissues were washed thoroughly in a running tap water, dehyderated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Serial sections (sagittal, frontal, and horizontal) of the brain were cut at 6 μm, stained in Mallory's triple, aldehyde fuchsin (AF) and chrome-alum-hematoxlyin-phloxine (CAHP) and viewed in microscope (Leitz, Germany).

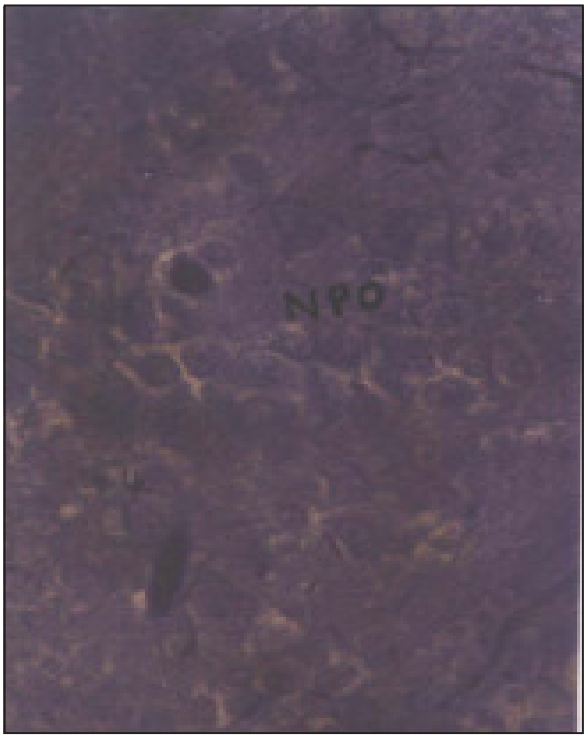

Fig. 1: Brain of *Channa punctatus* showing nucleus preopticus (NPO) of hypothalamus. Mark dorsal pars magnocellularis with large neurons and ventral pars parvocellularis with smaller neurosecretory cells. Mallory's triple. x 500

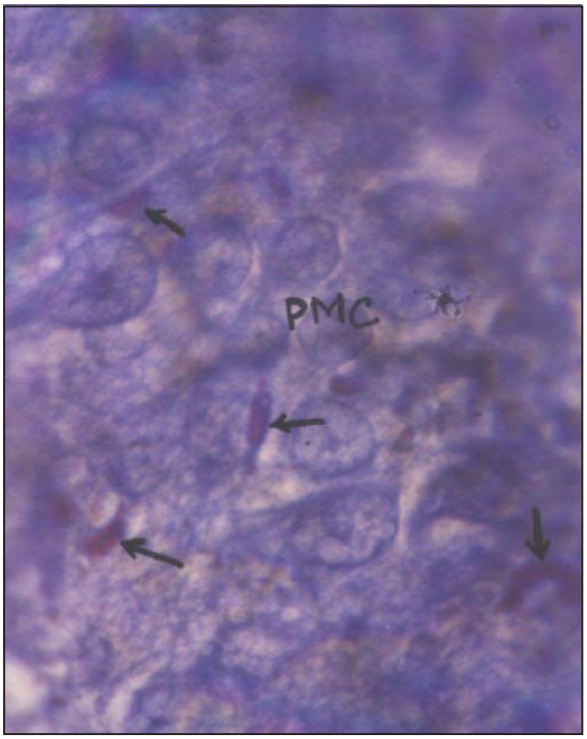

Fig. 2: NPO pars magnocellularis (PMC) of experimental *C. punctatus* at 24 hr of the treatment depicting enlarged neurosecretory cells and vascularization (arrow). Mallory's triple. x 1,000

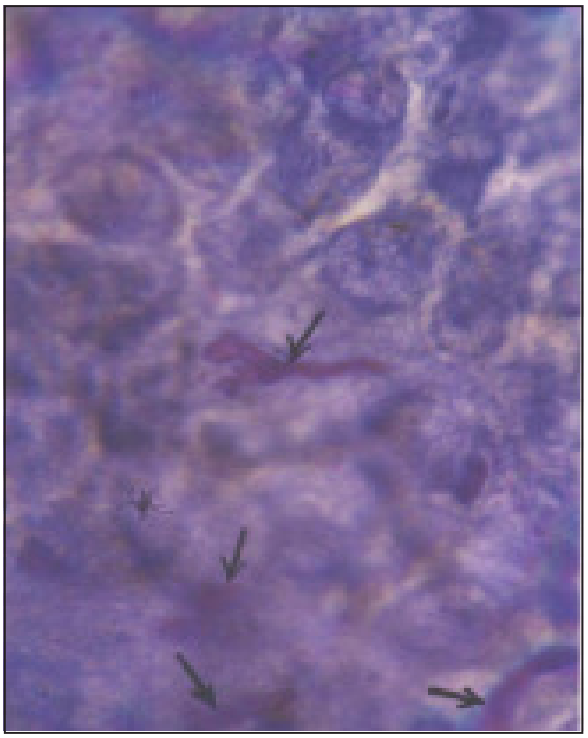

Fig. 3: PMC of *C. punctatus* at 48 hr of the treatment to Cartap exhibiting hypertrophied neurosecretory cells, partial loss of staining affinity and enhanced vascularization (arrow). Mallory's triple. x 1,000

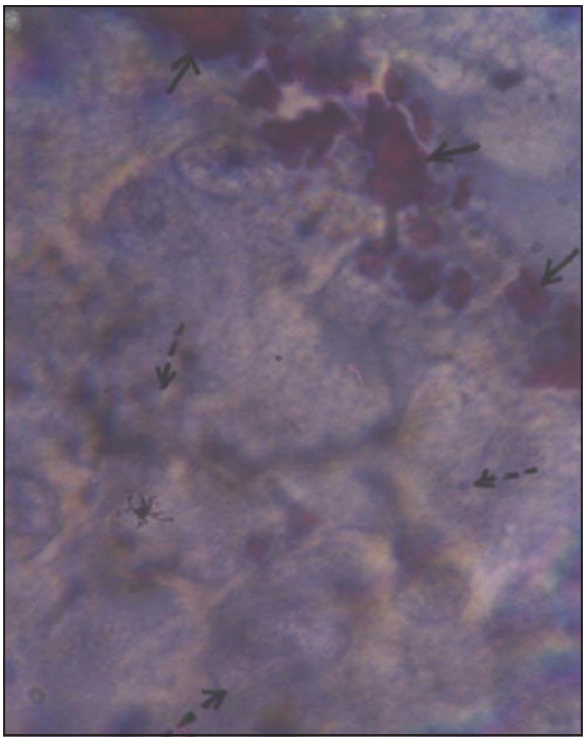

Fig. 4: PMC of *C. punctatus* at 72 hr of exposure to Cartap showing vacuolization and pyknosis (broken arrow) in the neurosecretory cells. Mallory's triple. x 1,000

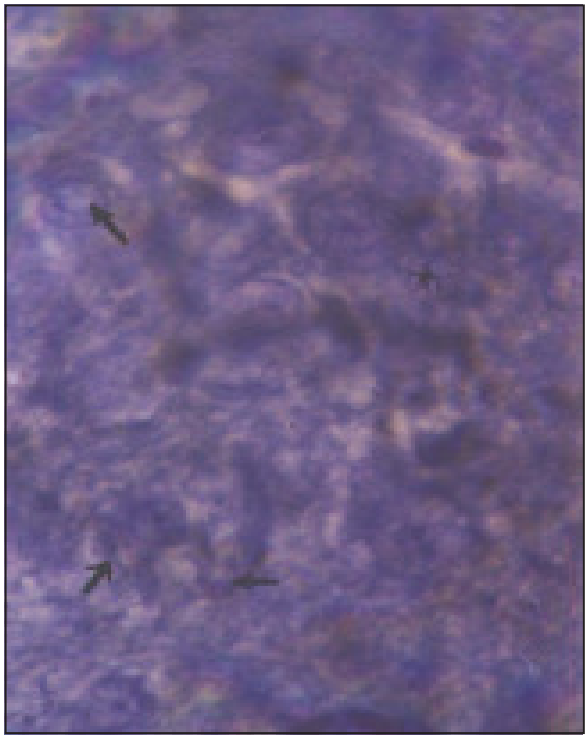

Fig. 5: PMC of *C. punctatus* at 96 hr of exposure to Cartap depicting degenerative changes in the neurosecretory cells. Mark a few neurosecretory cells with pyknotic nuclei (arrow). Mallory's triple. x 1,000

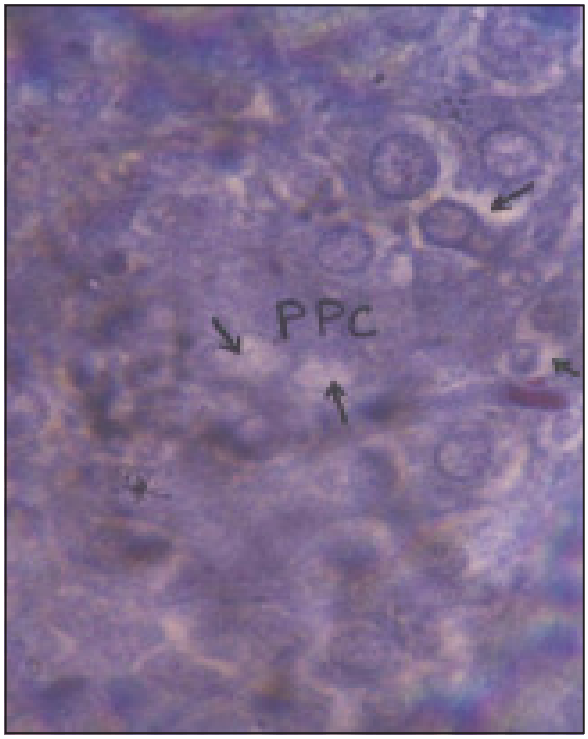

Fig. 6: PPC of *C. punctatus* at 96 hr of treatment to Cartap exhibiting loss in the staining affinity and vacuolization (arrow) in the neurosecretory cells. Mallory's triple. x 1,000

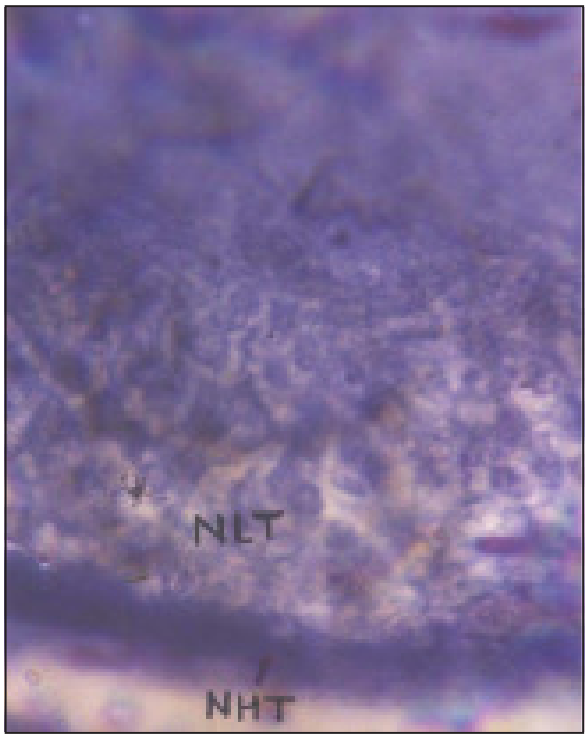

Fig. 7: NLT of *C. punctatus* at 96 hr of exposure to Cartap showing excessive vacuolization and pyknosis in the neurosecretory cells. (NHT, neurohypophysial tract). Mallory's triple. x 1,000

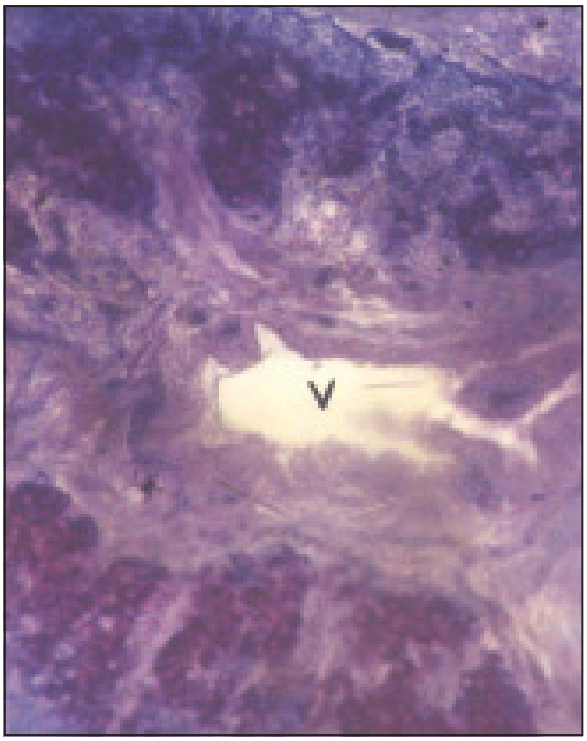

Fig. 8: Proximal pars distalis of *C. punctatus* showing large and well-demarcated vesicle (V) in the hypophysis. Mallory's triple. x 600

Results and Discussion

Hypothalamo-neurosecretory complex of *Channa punctatus* consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO was a paired structure situated topographically on either side of the third ventricle dorsal to the optic chiasma and looked inverted L-shape in sagittal section (Fig. 1). The horizontal limb of NPO comprised sparsely distributed neurons whereas the neurosecretory cells were closely packed in the vertical limb. NPO was morphologically divisible into dorsal pars magnocellularis (PMC) formed of larger neuronal cells (Fig. 2) and a ventral pars parvocellularis (PPC) comprising smaller cells (Fig. 6). Thus, a progessive reduction in the size of neurons from dorsal to ventral aspect of NPO was discernible (Fig. 1). NPO was highly vascularized structure and its neurosecretory cells were positive to aldehyde fuchsin (AF), chrome-alum-hematoxylin-phloxine (CAHP) and acid fuchsin (in Mallory's triple stain). Generally, neurons of PMC and PPC were bipolar and contributed beaded axons to form left and right neurohypophysial main tracts. NLT cells of *Channa punctatus* were distributed in the infundibular floor adjacent to the pituitary stalk. They were negative to AF and CAHP but stained readily with acid fuchsin (Fig. 7). This structure was also highly vascularized and several neurons were seen in close association with the blood vessels. Neurohypophysial tracts entered the pituitary through the infundibulum (Fig. 7).

Sublethal exposure of Cartap induced an increase in size of neurosecretory cells of PMC and PPC of *Channa punctatus* at 24 hr (Fig. 2). Though hypertrophy persisted by 48 hr, increased vascularity was also observed in NPO (Fig. 3). By 72 hr, there was increased vacuolization in the neurosecretory cells. However, accumulation of acid fuchsin-positive materials were also noticed in PMC cells of the treated fish (Fig. 4). At 96 hr of exposure, there was increased vacuolization as well as necrosis in both NPO (PMC and PPC) (Fig. 5-6) and NLT cells (Fig. 7). Varying sizes of Herring bodies (HB) were encountered in the neurohypophysis of the treated murrels at 96 hr of exposure. Interestingly, a large vesicle was noticed in proximal pars distalis (PPD) of the pituitary gland of control *Channa punctatus* (Fig. 8).

The basic structural pattern of hypothalmo-neurosecretory system of *Channa punctatus* resembles to those described for the other teleosts (Sundararaj and Viswanathan, 1971; Terlou and Ekengren, 1979; Thomas and Sathyanesan, 1982; Peter, 1986; Rama Krishna and Subhedar, 1991). Generally, the cells of NPO stain with AF and CAHP (Maksimovich, 1987) but in *Channa punctatus* they are stainable with acid fuchsin in Mallory's triple preparation. A similar staining response of NPO cells has also been observed in *Notopterus chitala* (Prakash *et al*., 1984), *Rastrelliger kanagurta* (Pandey, 1993a), *Megalaspis cordyla* (Pandey, 1993b), *Decapterus tabl* (Pandey and Mohamed, 1993), *Sphyraena obtusata* (Pandey and Mohamed, 1997), *Lates calcarifer* (Lal and Pandey, 1998), *Ariomma indica* (Pandey and Mohamed, 1999) and *Tor putitora* (Pandey *et al*., 2000). Since there are instances of accumulation of the neurosecretory material during prespawning phase as well as cyclical changes in activity of the neurosecretory cells in relation to reproduction (Belsare, 1967; Viswanathan and Sundararaj, 1974; Prakash *et al*., 1984; Pandey, 1993b; Pandey and Mohamed, 1993, 1997, 1999; Lal and Pandey, 1998), NPO has been implicated in controlling reproduction in fish (Peter, 1986; Subhedar *et al*., 1987, 1999; Peter *et al*., 1991; Peter and Yu, 1997; Goos *et al*., 1999). Immunoreactive gonadotropin-releasing hormone (GnRH) has also been localized in the NPO of a few teleostean species (Goos *et al*., 1985; Peter, 1986; Subhedar and Rama Krishna, 1988).

Nucleus lateralis tuberis (NLT) is the second important neurosecretory centre in the hypothalamus of fish (Dixit, 1967; Sathyanesan, 1973; Viswanathan and Sundararaj, 1974; Maksimovich, 1987; Pandey, 1993a,b; Pandey and Mohamed, 1993, 1997, 1999; Lal and Pandey, 1998; Pandey *et al*., 2000). The neurosecretory cells of NLT exhibit correlative cyclic activity with ovarian maturation in teleosts suggesting its important role in controlling reproduction (Dixit, 1967; Viswanathan and Sundararaj, 1974; Pandey, 1993b; Pandey and Mohamed, 1993, 1997, 1999; Lal and Pandey, 1998). The recent localization of immunoreactive gonadotropin-releasing hormone (GnRH) in pericarya of the NLT cells of a few teleosts has also strengthened this assumption (Goos *et al*., 1985; Peter, 1986; Subhedar and Rama Krishna, 1988).

In the present study, sublethal exposure of *Channa punctatus* to Cartap induced an initial hypertrophy in the NPO and NLT neurosecretory cells followed by varying degrees of cytoplasmic vaccuolation and pyknosis. Shukla and Pandey (1986) reported almost similar responses in both NPO and NLT cells of *Sarotherodon mossambicus* exposed to sublethal concentration (0.01 ppm) of Endosulphan for 20 days. Loss of neurosecretory material, inactivity in neurosecretory cells and darkly stained necrotic neurons were observed in the NPO of *Channa punctatus* in response to chronic (4.5 ppm) exposure to Carbofuran for 6 months (Ram *et al.,* 2001). Haider and Sathyanesan (1975) noticed depletion of neurosecretory materials from the NPO of *Clarias batrachus* due to injection of 0.2 ml of 2% formalin (three times a day) for 2 days. Katti and Sathyanesan (1986-*Clarias barachus*), Ram and Joy (1988-*Channa punctatus*), Pandey (1994-*Liza parsia*) and Hontella *et al.* (1997-*Esox lucius* and *Perca flavescens*) have also recorded varying degrees of inactivity and degenerative changes in the neurosecretory cells of hypothalamus of fishes exposed to sublethal concentrations of lead, mecuric chloride and bleached craft mill effluent. The observed vacuolization and necrotic changes in hypothalamic neurosecretory cells of *Channa punctatus* at 96 hr of exposure may probably depict the signs of exhaution due to perpetual pesticidal stress. Varying sizes of Herring bodies (HB) were observed in the neurohypophysis of the murrels at 96 hr of exposure to Cartap. These structures are assumed to be accumulated neurosecretory material that help increase the surface area for release of biologically active principles in the blood circulation (Zolotnitskiy, 1980).

Generally, teleostean pituitary is a solid structure comprising adenohypophysis and neurohypophysis, however, there are rare instances of the presence of vesicles of varying sizes in the gland (Schreibman, 1986). These vesicles are normally located in proximal pars distalis (PPD) of the hypophysis (Sathyanesan, 1963; Olsson, 1974; Swarup and Srivastava, 1976; Dar and Sathyanesan, 1973; Das and Sinha 1988). The large and well-demarcated vesicle observed in hypophysis of *Channa punctatus* (Fig. 8) has not yet been recorded in any other teleostean species. These vesicles are assumed to be a primitive character that might be having some significance in the evolution of pituitary gland in teleosts (Holmes and Ball, 1974; Schreibman, 1986).

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