Journal of Applied and Natural Science
13(2): 686 - 699 (2021)
Published online: June 12, 2021
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Multifactorial control of gonadotropin release for induction of oocyte maturation: Influence of gonadotropin-releasing hormone, gonadotropin release-inhibiting factor and dopamine receptors in the catfish, *Heteropneustes fossilis*

Pooja Kumari
Department of Zoology, University of Delhi, Delhi-110007, India
Neeta Sehgal
Department of Zoology, University of Delhi, Delhi-110007, India
S. V. Goswami
Department of Zoology, University of Delhi, Delhi-110007, India
Neerja Aggarwal*
Department of Zoology, University of Delhi, Delhi-110007, India

*Corresponding author. E mail: neerja.agg@gmail.com

Article Info
https://doi.org/10.31018/jans.v13i2.2695
Received: April 24, 2021
Revised: June 3, 2021
Accepted: June 6, 2021

How to Cite
Kumari, P. *et al.* (2021). Multifactorial control of gonadotropin release for induction of oocyte maturation: Influence of gonadotropin-releasing hormone, gonadotropin release-inhibiting factor and dopamine receptors in the catfish, *Heteropneustes fossilis. Journal of Applied and Natural Science*, 13(2), 686 - 699. https://doi.org/10.31018/jans.v13i2.2695

Abstract
Several external and internal factors contribute to the reproductive success of teleosts, which makes the reproductive process complex and unique. In the Indian freshwater catfish, *Heteropneustes fossilis*, monsoon plays a crucial role as it fine tunes the neuroendocrine axis, culminating in oocyte maturation. Therefore, induction of oocyte maturation requires the coordinated interaction among hypothalamic, hypophyseal, and peripheral hormones. In the present investigation, dual neuroendocrine control of oocyte maturation has been demonstrated in the catfish, *H. fossilis*. The maturational response in gravid catfish is inhibited in the presence of dopamine but GnRH evokes the oocyte maturation and ovulation. GnRH upregulates the expression of *lhb* gene as well as increases plasma levels of LH significantly within 30 minutes of its administration. Destruction of the preoptic region in gravid catfish by electrolytic or chemical lesions also causes oocyte maturation and ovulation. But this response is inhibited if dopamine is injected into the nucleus preopticus periventricularis-lesioned fishes. These observations support the role of dopamine as an inhibitory factor, therefore specific receptors of dopamine have been characterized in catfish and their expression in the brain has been quantified. Dopamine receptors are upregulated in dopamine-treated fishes and downregulated if a dopamine antagonist (pimozide) is injected. The present study suggests the presence of inhibitory mechanism for LH secretion in gravid catfish. Abolition of this inhibition is necessary to release LH surge, which in turn stimulates resumption of meiosis and ovulation. Thus peptidergic as well as aminergic systems regulate oocyte maturation in *H. fossilis*. Neuroendocrine regulation of oocyte maturation and ovulation has major implications for inducing spawning in aquaculture.

Keywords: Dopamine, GnRH, Lesioning, Monosodium L-glutamate, Pimozide

INTRODUCTION

In teleost fishes, like the other vertebrates, the neuroendocrine control of reproduction is based on the activity of the hypothalamus-pituitary-gonad (HPG) axis (Dufour *et al*., 2010). The hypothalamus produces the gonadotropin-releasing factor (GnRH) which stimulates gonadotrophs of the pituitary gland to control the synthesis and release of the gonadotropic hormones (GtHs). The gonadotropic hormones in turn regulate the gametogenesis in females as well as in males. (Blázquez *et al*., 1998, Dufour *et al*., 2010). The hypothalamus also produces dopamine, which, on the contrary, shows inhibitory effects on the HPG axis (Rainis and Ballestrazzi, 2005, Zohar *et al*., 2010).

At the brain level, GnRH and also GRIF (gonadotropin release-inhibiting factor, reported in few teleost) secreting neurons originate in the preoptic area (nucleus preopticus, NPO) of the hypothalamus and innervate the pituitary gland (Peter and Paulencu, 1980, Yamamoto

et al., 1998, Zohar *et al*., 2010, Bryant *et al*., 2016). Interestingly, dopaminergic neurons have also been shown to originate from the NPO and innervate directly pituitary gonadotrophs (Kah *et al*., 1987, Corio *et al*., 1991), and regulate the GRIF activity (Peter and Paulencu, 1980, Dufour *et al*., 2005). Dopamine strongly inhibits gonadotropin release in *Carassius auratus* (Peter and Paulencu, 1980); *Clarias gariepinus* (Corio *et al*., 1991); *Danio rerio* (Fontaine *et al*., 2013); *Astatiotilapia burtoni* (Bryant *et al*., 2016) and has no inhibitory role in perciformes fishes like *Sparus aurata*, *Morone saxatilis*, *Dicentrarchus labrax, Pagarus major* (Paullada-Salmeron *et al*., 2016). This dual hypothalamic control (stimulation by GnRH and inhibition by dopamine) of gametogenesis is achieved by the activation of specific receptor subtypes (Levavi-Sivan *et al*., 2005, Fontaine *et al*., 2013). Both, GnRH and dopamine effects on pituitary gland are mediated by distinct receptor subtypes, which belong to the G-protein-coupled-receptor (GPCR) type (Zohar *et al*., 2010, Fontaine *et al*., 2013). GnRH exert their biological response via GnRH receptors (type I and type II) by interacting through two types of protein, $G_{q/11}$- leads to activation of protein kinase C (PKC), and G_s- leads to activation of protein kinase A (PKA) (Ciani *et al*., 2020). Similarly, the dopamine receptors, particularly the D1-like (*D1* and *D5*) or D2-like (*D2, D3* and *D4*), are involved in the regulation of the activity of HPG axis via modulating the adenylyl cyclase activity (Dufour *et al*., 2010, Fontaine *et al*., 2013). D1-like receptors stimulate adenylyl cyclase activity, whereas D2-like receptors inhibit the adenylyl cyclase activity (Dufour *et al*., 2010, Yamamoto *et al*., 2015).

The annual reproductive cycle in fish is most distinctly defined in females by the maturation of oocytes and ovulation (Goswami and Sundararaj, 1971). This is influenced at the level of gonads by luteinizing hormone (LH) surge, under influence of hypothalamic activation. Peter *et al*. (1978) reported that a lesion at the nucleus lateral tuberis (NLT) of caudal hypothalamus led to the release of a large amount of LH which in turn induced ovulation in gravid (mature) female goldfish (*Carassius auratus*) even when they were maintained under conditions that prevented the ovulation. An inhibitory control over LH release and ovulation was mimicked and blocked by dopamine agonist and antagonist (Mylonas and Zohar, 2007, Bryant *et al*., 2016, El-Hawarry *et al*., 2016). Furthermore, dopaminergic inhibitory tone intensity varies at the time of spawning among teleosts (Saligaut *et al*., 1999, Aizen *et al*., 2005). In captive conditions, few fishes do not spawn due to lack of release of gonadotropins (FSH or LH) in the absence of environmental stimuli or unavoidable stress by captivity. *Heteropneustes fossilis*, is a seasonally breeding catfish, with its spawning season extending from July to August. It is a medicinal fish with high aquaculture value. Understanding of reproductive physiology of the catfish will help in its increased production. Previous studies have shown that this species responds to the annual cycle of photoperiod and temperature (Sundararaj and Vasal, 1976). Goswami and Sundararaj (1971) have shown that steroid hormones like deoxycorticosterone (DOC) and 17α, 20β dihydroxy-4-pregnen-3-one (DHP) induce oocyte maturation *in vitro*. Further studies explain that DHP acts as a terminal enzyme for final meiotic oocyte maturation whose production is regulated by levels of LH in the fish (Nagahama and Yamashita, 2008). LH release is under the control of dual neurohormonal system that may be stimulatory or inhibitory. So, this study was conducted to understand whether LH release in this catfish is regulated by stimulatory hormone or inhibitory hormone or combination of the two. This study was done using different experimental approaches (i) disruption of brain nuclei using electrolytic lesioning, chemical lesioning or mechanical disruption (ii) treatment of the catfish with different hormones and drugs like GnRH, Dopamine, Pimozide and Monosodium L-glutamate (iii) gene expression studies of D2-like receptors.

MATERIALS AND METHODS

Animal maintenance and Ethics statement

This study was done on adult catfish, *H. fossilis*, as per approval of the Institutional Animal Ethics Committee (IAEC) of the University of Delhi (DU/ZOOLD/IAEC-R/2017/25A). The experiment used adult females (70-80 gm) which were collected during the month of June to August from backwaters of the river Yamuna, Delhi (Lat. 28°35´ N, Long. 77°12´E). At this time, fishes were in the late pre-spawning/ spawning (gravid) phase of their annual reproductive cycle (Lamba *et al*., 1983) and experiencing natural photoperiod (13.9 hrs-13.1 hrs, sunrise to sunset) and temperature (39°C – 33.6° C). They were brought to the laboratory and kept in aquaria (size: 90x45x45 cm, n = 30 per aquarium), and acclimated to captive conditions under the artificial photoperiod (12 h light: 12 h darkness) and temperature (25 ±1°C) conditions for a week before an experiment. Such change in the photoperiod and temperature does not impact reproductive physiology in the short term (Lamba *et al*., 1983). Fishes were provided *ad libitum* minced meat as food suspended in the aquarium water, which was renewed every day during the light-on period (Lamba *et al*., 1983).

Experiments

Experiment 1. Involvement of specific hypothalamic areas in meiotic oocyte maturation

Involvement of hypothalamic NPP, NPO and NLT in meiotic oocyte maturation by using three different experimental approaches was performed.

Experiment 1A: Mechanical disruption of the neuronal communication in the NPP and NPO

This experiment investigated if the transduction of the information to and/ or from the hypothalamic NPP and NPO regions was important for meiotic maturation. These regions are known to be involved in meiotic maturation (Peter and Paulencu, 1980). To examine this, mechanical disruption of the transduction of 'information' through axonal pathway was performed by the insertion of a hollow cannula. For this, in fishes (n = 18) anaesthetized with 2-phenoxyethanol at 1:1000 dilution, a circular hole measuring 5 mm was drilled into the skull of the fish on the stereotaxic apparatus. A stainless-steel cannula fixed onto the electrode holder of the stereotaxic apparatus was gradually lowered at coordinates (+1.0, midline, D1.5) for the NPP and NPO regions of the fish brain (Aggarwal *et al*., 2012). Then, the cannula was withdrawn immediately, and the hole in the skull sealed and the fish was returned to its aquarium. The same procedure was repeated for the control group except that the cannula was inserted in a region anterior or right or left of the coordinates for NPP and NPO regions. After 15 hours of the procedure, fish was evaluated for maturational response in both experiment and control groups. A positive response was considered positive if ovulated eggs had been spawned or a slight pressure on the abdomen yielded egg release; conversely the negative response was considered when fish neither spawned nor yielded ripe eggs on stripping (Goswami and Sundararaj, 1971). Thereafter, fish were sacrificed by decapitation and brains were processed for bulk-staining (Sundararaj and Viswanathan, 1971) to show the axonal connection between hypothalamic nuclei (NPP and NPO) and the pituitary. Briefly, the brains were fixed in 10% formaldehyde-saline. After 72 h, the thoroughly washed brains with water were oxidized with performic acid, and stained with Victoria blue-4R for 18 to 21 hours and cleared in methyl salicylate.

Experiment 1B: Electrolytic lesioning of NPP, NPO and NLT regions

Here, electrolytic lesioning of the NPP, NPO and NLT was performed to examine if these regions play crucial roles in the meiotic maturation response. This was done by placing electrolytic lesions in gravid female catfish (n = 10, per region) in the hypothalamic region containing NPP (coordinates = +1.4, midline, 1.5), NPO (coordinates = +0.6, midline, 1.2) and NLT (coordinates = -0.2, 0.5, 2.4) as per the stereotaxic atlas (Aggarwal *et al*., 2012). Specifically, insulated unipolar electrodes (0 number stainless steel pins) leaving a tip (0.5mm) were used for lesioning. For this, in anesthetized fishes, a circular hole was drilled into the skull at the junction of the frontal and parietal bones, and using stereotaxic coordinates, the insulated unipolar electrode was placed at the desired location, and a current of 1 mA at 20 volts was passed for 10 sec. The electrode was then withdrawn, and the circular bone was sealed with the dental cement and fish was returned to its aquarium. The sham-lesioned catfish served as controls, wherein all the procedures were identically adopted except the passing of the electrical current. After 15 hours, fish were evaluated for the oocyte maturational response, as described earlier. Thereafter, fish were sacrificed by decapitation, and the brain was fixed in Bouin's fluid and sectioned at 8 µm followed by staining with Bargmann's chrome alum hematoxylin phloxine to ascertain if the specific hypothalamic nucleus was completely lesioned.

In a sub-experiment, it was further tested if dopamine elicited a response in fishes lesioned for NPP. It is known that hypophysiotropic DA neurons of the NPP play an inhibitory role in the LH release. For this, electrolytic lesioning of NPP region (coordinates: +1.4, midline, 1.5) of gravid fish (n = 10) was performed, followed by administration of DA (100 µg/ g body weight). The NPP-lesioned fishes (n = 10 each), and those NPP -intact but administered with DA served as controls. After 15 hours, fishes were evaluated for the oocyte maturational response, as described earlier.

Experiment 1C: Chemically induced brain lesions

Monosodium L-glutamate (MSG) was used to induce brain lesions (albeit non-specific) to assess the effects of neurotransmitters, in particular glutamate, in the oocyte maturation. Two groups of gravid fishes were injected intraperitoneally the monosodium L-glutamate, which was dissolved in 0.3% NaCl at 2.5 mg and 5 mg/ g body weight; simultaneously, fishes injected with 0.3% NaCl served as the control. At 2.5 mg/g body weight dose, the MSG could not elicit the oocyte maturational response, whereas, at 5 mg/g body weight dose, 18 out of 31 fishes showed a positive response. These 18 fishes with positive oocyte maturational response were killed by decapitation. Their brains were fixed in the Bouin's fluid and processed for the histological examination to ascertain necrotic cells in the NPP region with pycnotic nuclei.

Experiment 2. Role of GnRH and dopamine on oocyte maturation

Experiment 2A. Effect of GnRH-analogue on oocyte maturation, plasma LH, and gene expressions

In an initial experiment, the effective dose of GnRH-analogue (Busereline acetate, Hoechst, AG, West Germany) on oocyte maturation was determined. Groups of gravid female fishes were injected intraperitoneally GnRH-analogue dissolved in the distilled water at doses of 0.05, 0.10, 0.15 and 0.20 µg/g body weight; a group injected with distilled water (vehicle) served as

the control. After 15 hours, fishes were checked for the maturational response as described earlier.

GnRH-analogue at the dose of 0.20 µg/g body weight elicited the maximum maturational response in catfish, and hence selected for the further experiment to determine it's physiological (rise in plasma LH) and molecular (genes coding for *D2* and *D4* receptors, and *lhb* gene) functions. The experiment used four groups of fishes: 2 experimental and 2 control groups of 6 fishes each. To 2 experimental groups of gravid fishes, GnRH at the dose of 0.20 µg/g body weight was administered intraperitoneally, while the two control groups of fishes were injected with distilled water and served as controls. Of two groups of fishes, one was sampled after 0.5 h and the other was sampled after 8 h of the GnRH administration; the control groups were sampled simultaneously. Using the hypodermic needle fitted to heparinized syringe, blood was collected from the caudal artery and centrifuged at 1200 g for 15 min. Plasma was harvested and processed for the measurement of LH. Then, fishes were decapitated, and the pituitary was collected and stored at -80 °C until processed for gene expression assays.

Measurement of plasma LH

To measure LH in plasma, samples were collected at two time points from both control and experimental fishes by ELISA. For this, MBS283097 ELISA KIT (Mybiosource, San Diego, CA, USA) was used, which was checked for its specificity for catfish, cross reactivity or interference between fish LH and analogue. Intra-assay and inter-assay coefficient of variations were < 8 and 10 %, respectively. ELISA was performed in 96-well plate, as per the manufacturer's protocol. Briefly, 50 µl of plasma and 50 µl of HRP-conjugate were added to each well, except those for the blank. The mixture was incubated for 1.5 h at 37°C. The samples were thoroughly washed with wash buffer and decanted completely, and 100 µl of substrate was added to each well. The plate was re-incubated at 37°C for 15 min in the dark, and the reaction was stopped by adding stop solution (50 µl). The optical density (OD) was read at 450 nm using the ELISA plate reader (Epoch, BioTek, USA).

Gene expression assays

The mRNA expression of gene coding for the *D2* and *D4* receptors, and of *lhb* gene was measured by qPCR using the gene-specific primers. The partial gene sequence of *D2*, *D4* receptors was cloned, and an already available sequence of *lhb* gene was used (Accession no. KF573628). Degenerate primers were used to amplify partial stretch of both *D2* and *D4* receptor cDNA. A single-step method employing guanidinium thiocyanate-phenol-chloroform was used for the extraction of total RNA from pituitary tissue using TRIZOL (Sigma) (Chomczynski and Sacchi, 1987). Diluted RNA samples were used to assess the quantity and quality of extracted RNA. Thereafter, PCR, cDNA cloning and sequencing of *D2* and *D4* receptor were performed (Kumari *et al*., 2020). The *D2* and *D4* receptor transcripts were analyzed by PRALINE online server and MEGA software version 6 (Kumar *et al*., 2018).

The specific primers were designed, and mRNA expression of *D2* and *D4* and *lhb* was quantified by using Power SYBR™ Green PCR master mix (Thermo Fisher scientific, USA) in 10 µl reaction volume (for details, see Kumari *et al*.,2020) and the reaction was run on 7500 Applied Biosystem Fast DxReal-time PCR. Both sample and reference (*β*-actin) were run in duplicates. The fold change of mRNA expression level of the target gene relative to the reference gene was calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Additionally, a small piece of the ovarian tissue containing approximately 50-60 oocytes was maintained in modified Wolf and Quimby medium for 15 h for *in-vitro* culture and evaluated for GVBD, which is an indicator of the maturational response (Goswami and Sundararaj, 1971).

Experiment 2B. Effect of dopamine and GnRH on oocyte maturation response

This experiment contained three subsets of experiment and examined the roles of dopamine and GnRH on oocyte maturation in the catfish. The first subset of the experiment used four groups of fishes (n = 10 each) and compared the oocyte maturation response in fishes injected intraperitoneally with *monosodium L-glutamate* (group 1: dose = 2.5 mg/g body weight; to induce chemical lesioning of hypothalamic nuclei involved in oocyte maturation;), or with pimozide (10 µg/g body weight, a dopamine antagonist hence removes the dopamine blockade; group 2), or with pimozide followed by MSG (group 3: doses as in groups 1 and 2) after an interval of 6 hours or vehicle alone (control; group 4). After 15 h of the injection, fishes were sampled and evaluated for the oocyte maturational response, as described in experiment 1A.

The second subset of the experiment contained six groups of 10 fishes each and tested the effects of both GnRH and dopamine together. The first three groups of fishes were injected intraperitoneally with dopamine (DA; 100 µg/g body weight) or GnRH (0.20 µg/g body weight) or pimozide (10 µg/g body weight) alone. The fishes of the fourth group were administered with both DA (100 µg/g body weight) and GnRH (0.2 µg/g body weight). To the fifth group of fishes, pimozide (10 µg/g body weight) followed by DA (100 µg/g body weight) and GnRH (0.20 µg/g body weight) was injected. Sixth group was injected with vehicle and served as control. After 15 h of the treatment, fishes were sampled and assessed for the oocyte maturational response as described above.

In the third subset of the experiment gravid fishes were administered intraperitoneally with GnRH alone, or with GnRH and DA together, or with pimozide followed by DA and GnRH sequentially. The dosages were the same as described above. The control fish were injected with vehicle alone. Fishes were sampled after 0.5 h and 8 h after the injection. Blood was collected, and plasma was harvested for the measurement of plasma LH, as described above. From fishes collected at both time points, the pituitary was removed and processed to isolate total RNA. As described above, the mRNA expression of *D2, D4* receptor and *lhb* genes was measured. In addition, the ovarian samples were collected and transferred to a culture medium. Cultured oocytes from the short term *in vitro* cultures were evaluated for GVBD after 15 hours.

Statistics

Statistical analysis was done by using by IBM® SPSS 23.0 software. Two-way Analysis of Variance (ANOVA) tested the effects, if ANOVA indicated a significant difference, Duncan post hoc test was used for group comparisons. p <0.05 was considered a significant difference.

RESULTS

Experiment 1. Involvement of specific hypothalamic areas in meiotic oocyte maturation

Experiment 1A: Mechanical disruption of the neuronal communication in the NPP and NPO

Figure 1 shows bulk-stained preparation of the brain. Clearly, the axonal tract connects the NPP and NPO hypothalamic nuclei, and the pituitary gland. The insertion of a hollow cannula mechanically disrupted the neuronal connection between these two hypothalamic nuclei and pituitary gland, and all these fishes showed the oocyte maturation, ovulation and spawning. By contrast, fishes in which a similar cannula was inserted anterior or lateral to these hypothalamic nuclei did not exhibit the maturational response (Table 1).

Experiment 1B: Electrolytic lesioning of NPP, NPO and NLT regions

Electrolytic lesioning of NPP, NPO and NLT regions in gravid female catfish was performed to ascertain if these three hypothalamic nuclei indeed played a role in the oocyte maturation. The success of lesioning was confirmed by histological examination. For example, the histological examination showed complete destruction of cells in the NPP region of lesioned, compared to normal catfish; cells were with shrunken nuclei and depleted neurosecretory material (Fig. 2a-d). Data summarized in Table 1 reveal that lesion placed in the NPP region (+1.4, midline, 1.5) evoked oocyte maturation, ovulation and spawning in 100% of NPP-lesioned fishes (10 out of 10). On the other hand, a similar lesion in the NPO (+0.6, midline, 1.2) and NLT (0.2, 0.5, 2.4) region did not evoke such oocyte maturational response. The sham-lesioned fishes also did not show oocyte maturation. Further, when administered at a dose of 100 µg/g body weight, DA suppressed the oocyte maturation in 80 % of the NPP-lesioned catfish (only 2 out of 10 fishes showed a maturational response; Table 1).

Experiment 1C: Chemically induced brain lesions

The involvement of hypothalamic regions was confirmed, in particular the NPP, in oocyte maturation by chemical-induced lesions with intraperitoneal injections of MSG, which elicited a dose-dependent effect. At a dose of 2.5 mg/g body weight MSG was ineffective, whereas at 5.0 mg/g body weight, it induced maturational response (oocyte maturation, ovulation, and spawning) among 58% of fishes (Table 1). Histological examination further supported this; fishes treated with MSG at 5.0 mg/ g body weight showed complete necrosis in the NPP region (Fig. 3c). The affected brain regions showed complete depletion of the neurosecretory material, shrunken nuclei and the overall hypertrophied cells (Fig. 3d).

Experiment 2. Role of GnRH and dopamine on oocyte maturation

Experiment 2A. Effect of GnRH-analogue on oocyte maturation, plasma LH, and gene expressions

Analysis of cloned, D2 receptor and D4 receptor transcripts in catfish: Cloned PCR amplicons of 309 bp for *D2 receptor* and 641 bp for *D4* receptor (GenBank Accession no.: MN706261 and MK682512) using primer pairs (Table 3) were obtained. Open reading frame (ORF) of 309 bp for *D2* receptor encoding a protein of 85 aa and ORF of 597 bp encoding a protein of 198 aa was reported. Multiple sequences of deduced amino acids aligned by PRALINE online server showed conserved sequences available in D2 and D4 receptor with other catfish (Fig. 4). Amino acid sequence alignment and phylogenetic analysis revealed that D2 receptor is in close relation with *Ictalurus punctatus* and D4 receptor of *H. fossilis* is very closely related to the *Clarias gariepinus* (Fig. 5). Primers used for expression studies by real-time PCR are given in Table 3.

This experiment was conducted to examine if a single injection of GnRH would affect the oocyte maturation and associated changes. A dose-dependent effect of GnRH was observed, the effect at the dose of 0.05 µg was significantly lower than 0.10 µg, 0.20 µg of GnRH (Table 2). Whereas, nearly half (45%) of the fishes tested showed positive oocyte maturation in response to 0.05 µg, the maturation response was increased to 85

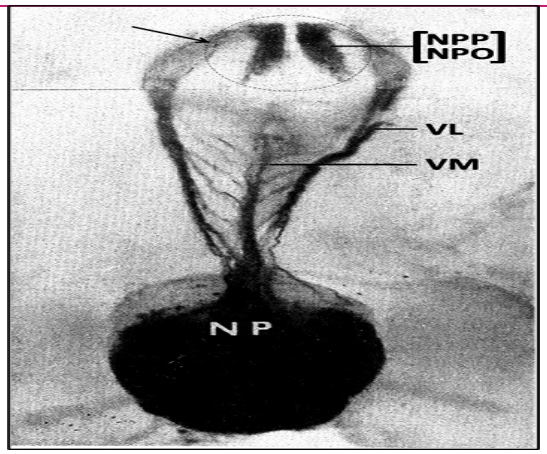

Fig. 1. *In situ preparation of the brain of the catfish stained with Victoria blue (Ventral View). Photograph illustrates Hypothalamic nuclei {Nucleus preopticus periventricularis (NPP) and Nucleus preopticus (NPO)}, Axonal Tracts {Ventrolateral (VL) and Ventromedial (VM)} and Neurohypophysis (NP). Arrow indicates the site of mechanical disruption of axonal tract by insertion of cannula in preoptic region.*

and 90 % in fishes administered with GnRH at 0.10 µg and 0.20 µg/g body weight (Table 2).

GnRH effects was further tested *in vitro* when administered at a dose of 0.20 µg/ g body weight to gravid catfish. In particular, the effects on GVBD by the culture of ovaries (oocytes) was assessed, and plasma LH was measured to show concurrent hormonal changes associated with oocyte maturation. There was a time lag in the effect of GnRH on oocyte maturation: whereas only 23% oocytes indicated GVBD if collected within 0.5 h, 82 % oocytes showed GVBD if cultured after 8 h of the GnRH treatment (Fig. 6A). The meiotic maturation in *in vitro* culture was thus in agreement with the observed *in vivo* maturational response (Table 2).

Interestingly, compared to controls, LH levels were elevated in plasma collected within 0.5 h, and were decreased significantly in plasma collected 8 h after the GnRH treatment (Fig. 6B). Corresponding to plasma LH levels, there was 8-fold increase in the *Ihb* mRNA expression transcript in the pituitary harvested within 0.5 h of the GnRH treatment, whereas no significant

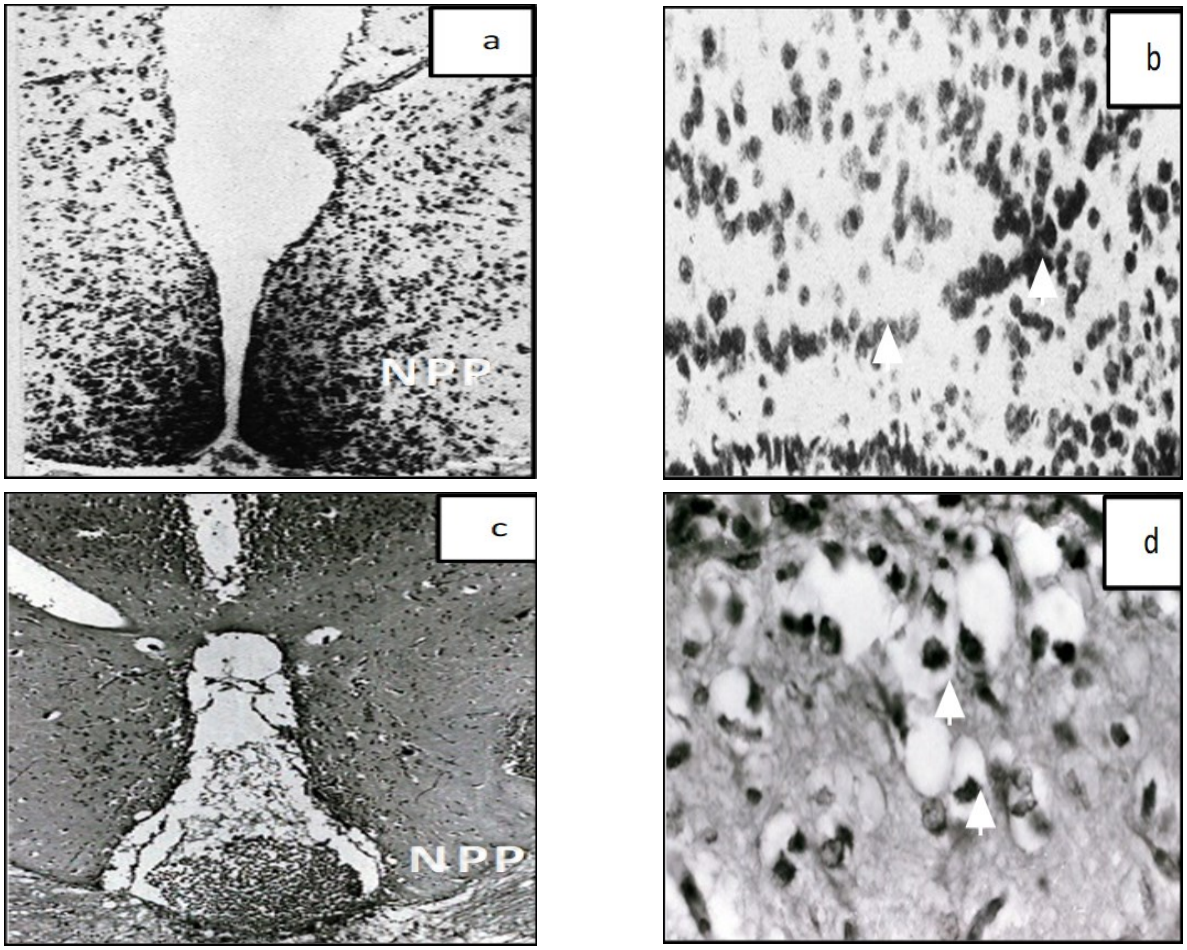

Fig. 2. *Photomicrographs of Nucleus preopticus periventricularis (NPP) region of the brain of female catfish H. fossilis. Bargmann's chrome alum hematoxylin phloxine. {x 80 (**a** and **c**); x 900 (**b** and **d**)}*

*T.S. of the brain of sham operated catfish through NPP region (**a** and **b**) Note Cells with prominent nuclei (arrow-heads in **b**). T.S. of the brain of NPP-lesioned catfish (**c** and **d**). Wide-intercellular spaces in the NPP region of lesioned catfish (**c**) and shrunken cells (arrow-heads in **d**) with damaged nuclei and little neurosecretory material.*

difference was observed in transcript level of *lhb* after 8 hrs of GnRH treatment in comparison to control group (Fig. 6C). However, the expression of genes coding for D2-like receptors (*D2* and *D4 receptor genes*) in the pituitary in response to exogenous GnRH was not observed (Fig. 6D and E).

Experiment 2B. Effects of dopamine and GnRH on oocyte maturation

It was observed that both PIM (10 µg/ g body weight) and MSG (2.5 mg/g body weight) when given alone did not elicit the oocyte maturation in gravid female catfish (Table 2). However, when fishes were pretreated with PIM (10 µg/g body weight) and six hours later injected with MSG (2.5 mg/g body weight), 4 out of 7 catfish showed positive maturational response including the ovulation and spawning (Table 2). Similarly, the maturational response was not elicited in catfish when they were injected with DA (100 µg/g body weight) or the PIM (10 µg/g body weight). However, as expected from experiment 2A, GnRH at a dose of 0.20 µg/g body weight induced oocyte maturation in 9 out of 10 catfish. This maturational response was reduced to 20% (2 out of 10 catfish) when DA (100 µg/g body weight) was injected simultaneously with GnRH (0.20 µg/g body weight). Interestingly, however, the PIM ameliorated the suppressive effect of DA, as evidence by 100% maturational response in catfish administered with PIM (10 µg/g body weight) along with DA and GnRH (Table 2).

When further tested *in vitro* for GVBD with oocytes those were collected at 0.5 h and 8 h of administration of GnRH or GnRH and DA or PIM followed by DA and GnRH, 23% GVBD among oocytes collected within 0.5 h opposed to 82% GVBD among oocytes cultured after 8 h of the GnRH treatment was observed. The meiotic maturation was found significantly inhibited among oocytes collected both within 0.5 h and after 8 h if DA was administered along with GnRH. However, the oocytes showed 94% GVBD if they were collected after 8 h, compared to 27% GVBD if collected within 0.5 h from catfish injected with PIM followed by DA and GnRH (Fig. 6A).

Plasma levels of LH in treated catfish of above-mentioned three groups were compared (Fig. 6B). An

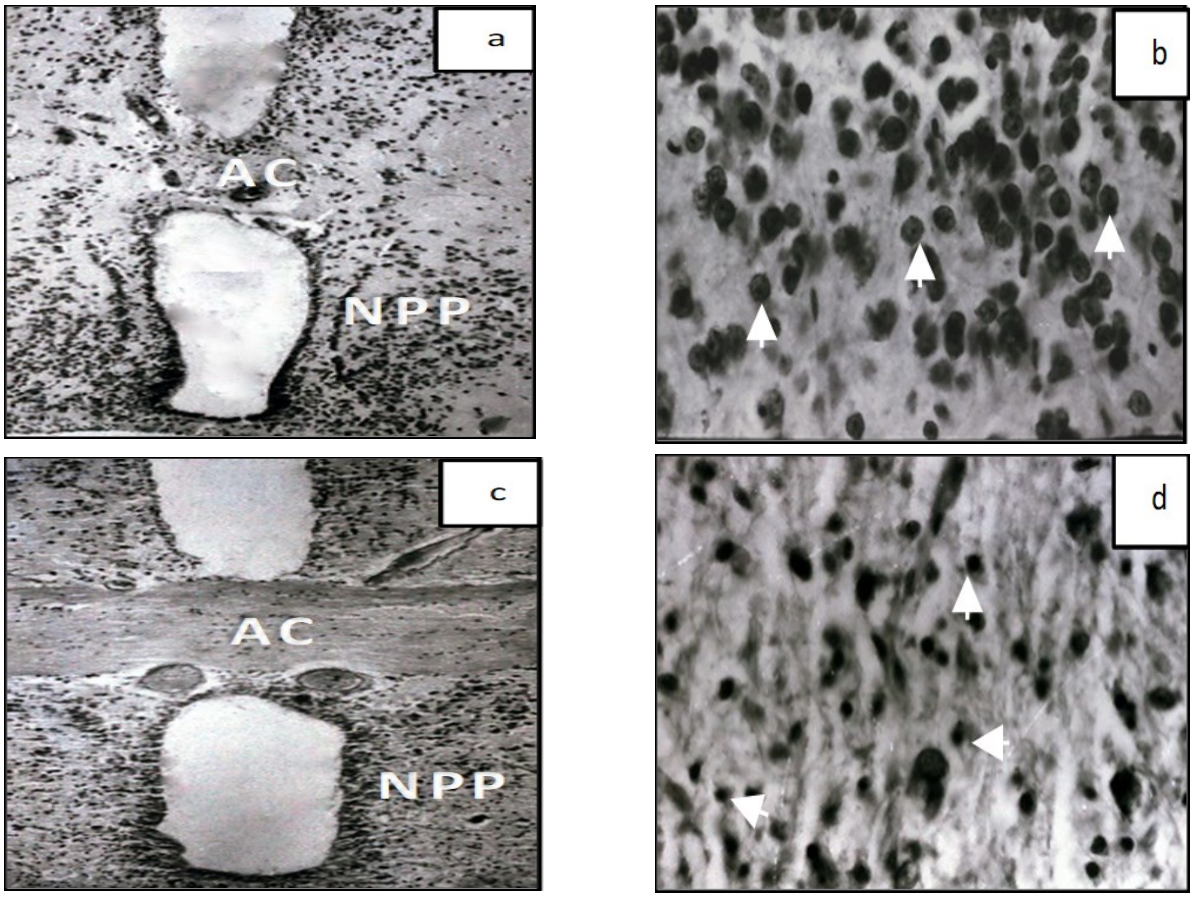

Fig. 3. *Photomicrographs of Nucleus preopticus periventricularis (NPP) region of the brain of female catfish H. fossilis. Bargmann's chrome alum hematoxylin phloxine {x 80 (a and c); x 900 (b and d)}. T.S. of the brain of control catfish showing NPP region situated ventral to anterior commissure (AC) (a and b). Note cells with prominent nuclei (arrow-heads in b). T.S. of the brain of MSG (5 mg/g body weight) - injected catfish through NPP region (c and d). Note lesion in the NPP region (c) and necrotic cells with pyknotic nuclei (arrow-heads in d).*

Table 1. Effect of mechanical disruption/ Lesioning (electrolytic or chemical) of specific hypothalamic nuclei on oocyte maturational response in the gravid catfish.

Sr. no	Treatment (*in vivo*)	Total no. of fish	Positive maturational response		
			No. of fish	Percentage	Graphical representation
	Mechanical disruption of axonal tracts				% positive maturational response (0, 50, 100)
a	1. Cannula inserted in the NPP and NPO regions	18	18	100	
	2. Sham Operated {Control}	12	0	0	
	Electrolytic lesioning of the NPP region				
b	1. NPP-lesioned	10	10	100	
	2. NPO-lesioned	10	0	0	
	3. NLT-lesioned	10	0	0	
	4. NPP-lesioned + DA (100 µg)	10	2	20	
	5. Sham operated {Control}	10	0	0	
	Chemical Lesioning of NPP region				
c	1. MSG (2.5 mg)	10	0	0	
	2. MSG (5 mg)	31	18	58	
	3. 0.3% NaCl (1 µL) {Control}	10	0	0	

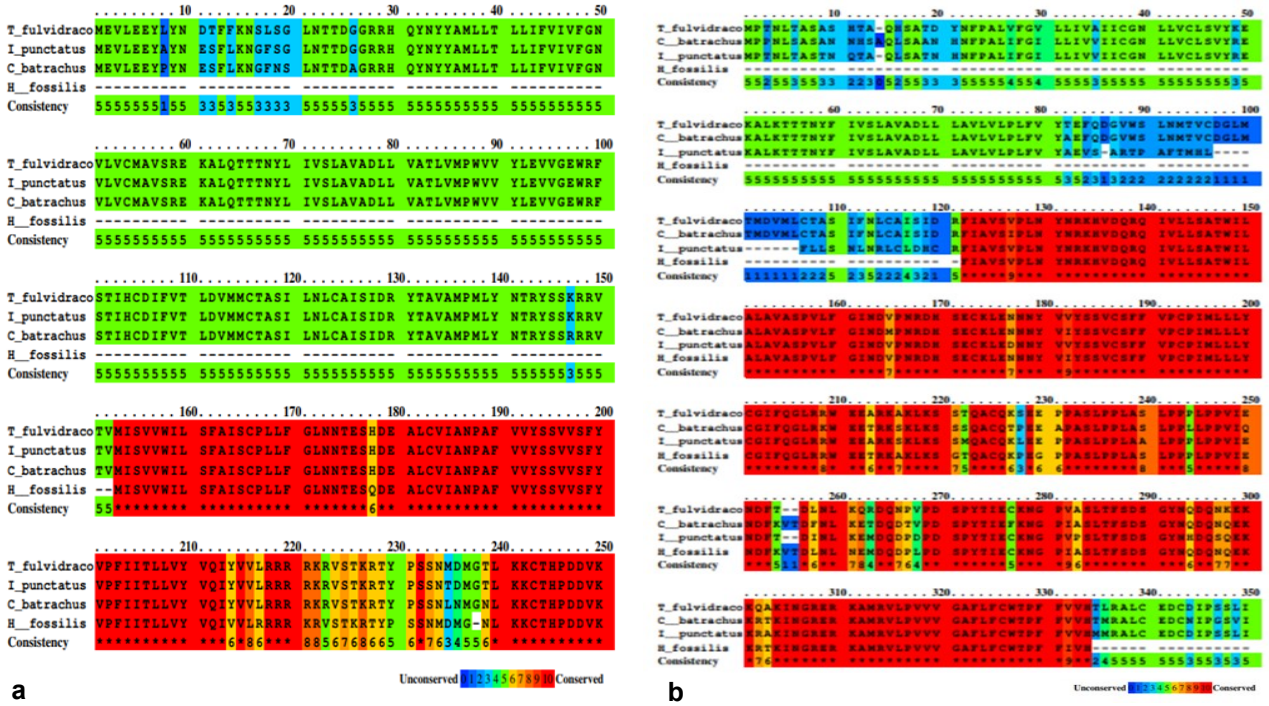

Fig. 4. *(a) Multiple sequence alignment of D2 receptor (D2-like) using PRALINE software. GenBank accession numbers of sequences used for analysis of D2 receptor are as follows: Tachysurus fulvidraco (XP_027029730), Ictalurus punctatus (XP_017347172), Clarias batrachus (QIX02979) and Heteropneustes fossilis (MN706261). (b) Multiple sequence alignment of D4 receptor (D2-like) using PRALINE software. GenBank accession numbers of sequences used for analysis of D4 receptor are as follows: Tachysurus fulvidraco (XP027019615), Clarias batrachus (AWK21972), Ictalurus punctatus (XP017340258) and Heteropneustes fossilis (QEE04228). The least conserved alignment position has been denoted by blue color with value 0 and most conserved alignment are shown in red color with maximum value of 10.*

Table 2. Effect of various treatments on oocyte maturational response in the gravid catfish.

Sr. no	Treatment (*in vivo*)	Total no. of fish	Positive maturational response		
			No. of fish	Percent-age	Graphical representation
	Administration of GnRH (per gm body weight)				% positive maturational response 0 50 100
a	1. GnRH (0.05 µg)	11	5	45	
	2. GnRH (0.1 µg)	20	17	85	
	3. GnRH (0.15 µg)	9	8	89	
	4. GnRH (0.2 µg)	22	20	90	
	5. Distilled Water (1 µL) {Control}	10	0	0	
b	**Administration of pimozide (PIM) or monosodium L-glutamate (MSG) alone or in combination (per gm body weight)**				
	1. PIM (10 µg)	5	0	0	
	2. MSG (2.5 mg)	9	0	0	
	3. PIM (10 µg) + MSG (2.5 mg)	7	4	57	
	4. DMSO + 0.3% NaCl (1:1) (1 µL) {Control}	10	0	0	
c	**Administration of DA, GnRH and PIM alone or in combination (per gm body weight)**				
	1. DA (100 µg)	10	0	0	
	2. GnRH (0.20 µg)	10	9	90	
	3. PIM (10 µg)	10	0	0	
	4. DA (100 µg) + GnRH (0.20 µg)	10	2	20	
	5. PIM (10 µg) + DA (100 µg) + GnRH (0.20 µg)	10	10	100	
	6. DMSO + Distilled Water (1:1) (1 µL) {Control}	10	0	0	

elevated titre of LH within 0.5 h was also noted, which was decreased significantly after 8 h of the GnRH treatment (Fig. 6B). Corresponding to elevated plasma LH levels, 8-fold increase in the *lhb* mRNA levels in pituitary samples harvested within 0.5 h of the GnRH treatment was also observed (Fig. 6C). However, both plasma levels of LH and *lhb* mRNA levels were not elevated if fish injected with dopamine and GnRH together (Fig. 6B, C). Notably, a significant surge in the plasma levels of LH was found within 0.5 h in fishes if administered with pimozide (a potent inhibitor of dopamine) followed by GnRH and DA (Fig. 6B). Subsequently (i.e. in samples collected 8 h after the treatment), plasma LH levels were reduced in treated groups and were similar to the control group; a similar expression pattern was also noticed for *lhb* gene (Fig. 6C).

Further, the mRNA expression of genes coding for D2-like receptors (*D2* and *D4 receptors*) in the pituitary was compared among the afore-mentioned treatments (Fig. 6D, E). Both receptor genes were found not expressed in the pituitary of catfish treated with GnRH alone. However, mRNA expression of *D2 receptor* gene was increased by 14-fold and of *D4 receptor* gene by 10-fold in the pituitary of catfish collected within 0.5 h of treatment with DA and GnRH. Both receptors were found expressed even 8 h after the treatment, albeit in lower levels. Furthermore, D2-like receptor genes were found not expressed within 0.5 h and were found down regulated in pituitary samples collected 8 h after the administration of PIM followed by DA and GnRH (Fig. 6D, E).

DISCUSSION

In fishes of the Indian subcontinent, the onset of the maturational phase remains indefinitely blocked if envi-

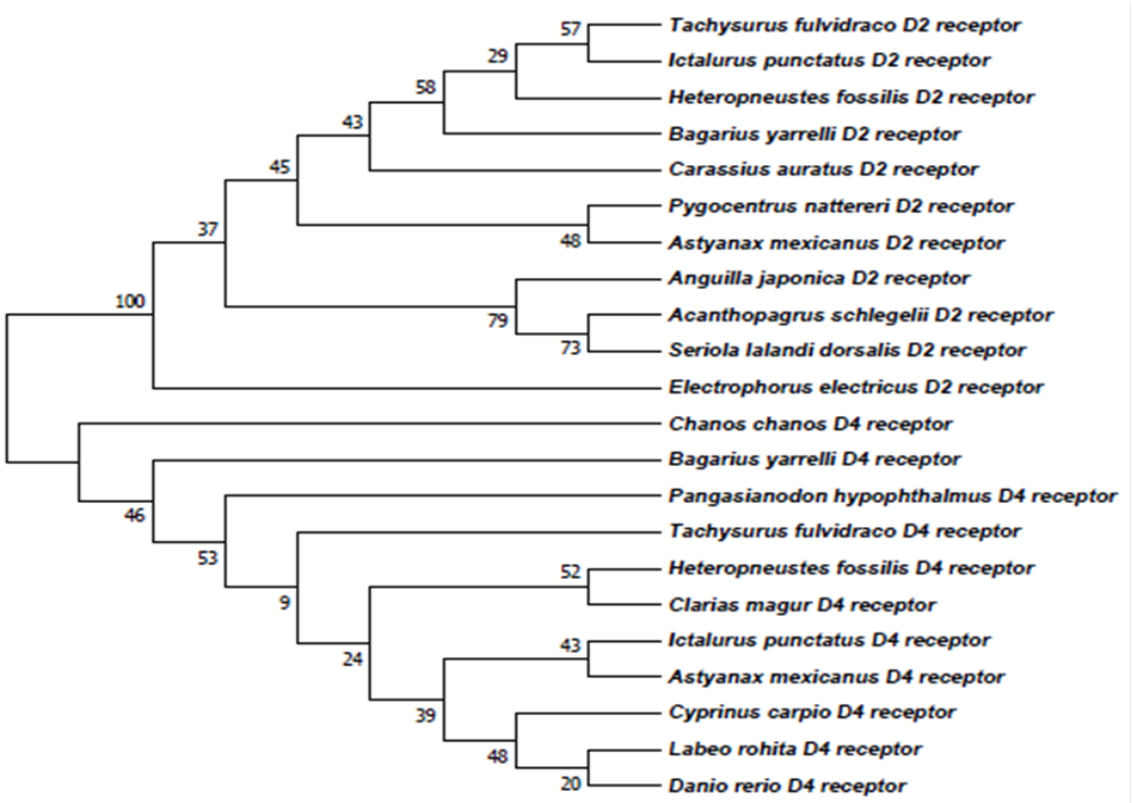

Fig. 5. *Phylogenetic relationship of deduced amino acid sequence of D2 and D4 receptors of H. fossilis with other teleost species using Neighbor-joining algorithm to infer the consensus tree and 1000 bootstrap replications for validation of branch point.*

The GenBank accession numbers of sequences used for analysis of D2 receptor are as follows: Tachysurus fulvidraco (XP_027029730), Ictalurus punctatus (XP_017347172), Heteropneustes fossilis (MN706261), Bagarius yarrelli (TSQ58042), Carassius auratus (XP_026082110), Pygocentrus nattereri (XP_017568544), Astyanax mexicanus (XP_022530824), Anguilla japonica (AFS30510), Acanthopagrus schlegelii (ABV21761), Seriola lalandi (XP_023275664), Electrophorus electricus (XP_026869479).

The GenBank accession numbers of sequences used for analysis of D4 receptor are as follows: Chanos chanos (XP_030630776), Bagarius yarrelli (TSK13470), Pangasianodon hypophthalmus (XP026775444), Tachysurus fulvidraco (XP027019615), Heteropneustes fossilis (QEE04228), Clarias magur (AWK21972), Ictalurus punctatus (XP017340258), Astyanax mexicanus (XP007235199), Cyprinus carpio (CAA74977), Labeo rohita (RXN10811), Danio rerio (NP001012634).

ronmental conditions are not conducive to spawning. But, a single injection of a superactive analogue of GnRH induces the postvitellogenic oocytes to undergo maturation, ovulation and spawning in a dose-related manner which is similar to that evoked by exogenous administration of mammalian LH or partially purified piscine gonadotropin (Sloley *et al*., 1992, Yaron *et al*., 2003, Podhorec and Kouril, 2009, Azuadi *et al*., 2011; Spicer *et al*., 2017; Fallah and Habibi, 2020). It suggests that in catfish GnRH evokes maturational response by stimulating endogenous LH release from the pituitary. Further, when environmental or physiological conditions are not favorable, the release of the LH is inhibited. It is likely that there exist some inhibitory factors in the brain. Therefore, this perimaturational gonadotropin release is under rigorous hypothalamic control (Peter *et al*., 1978, Dufour *et al*., 2010, Zohar *et al*., 2010, Bryant *et al*., 2016; Beriotto *et al*., 2020).

We have demonstrated that postvitellogenic oocytes underwent the meiotic division leading to the maturation, ovulation and spawning in those catfish in which the function of preoptic area was manipulated either by the mechanical disruption or by the electrolytic/ chemical lesioning. Even a transitory disruption of the neuronal communication between the NPO and NPP cell bodies by the insertion of a hollow cannula altered the oocyte response i.e. disruption led to the oocyte maturation. Similarly, a lesion placed in the preoptic area (NPP), but not in the other regions interfered with oocyte response, as NPP-lesioned fishes showed oocyte maturation response. This suggested that NPP of the catfish brain released GRIF, which when reduced/ abolished by lesioning, resulted in oocyte maturation and ovulation. This also substantiates the existence of a GRIF in the NPO region of catfish that possibly serves to inhibit a spontaneous or unseasonal gonadotropin

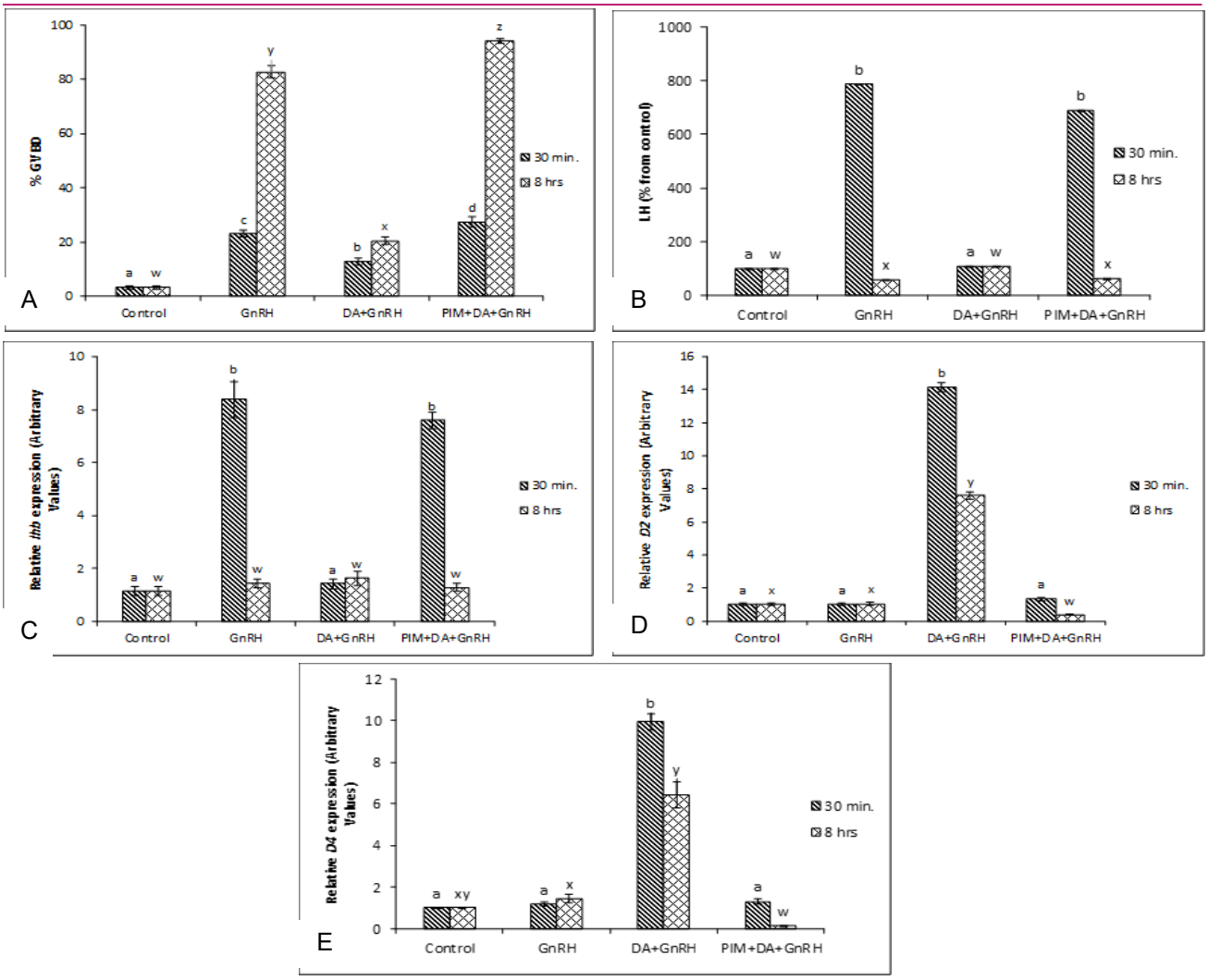

Fig. 6. *Effect of administration of GnRH alone or in combination with dopamine (DA) and pimozide (PIM) on GVBD in oocytes (A); plasma levels of LH (B); expression patterns of Ihb gene (C), D2 receptor gene (D), D4 receptor gene (E) in pituitary. Samples collected at two time points (30 minutes and 8 hrs) after treatment were analyzed.*
Values are expressed as mean ± SEM. n=12 for (A) and n=6 for (B, C, D and E). Bars with different superscripts (a-d) for 30 min. and (w-z) for 8 hrs. indicate statistically significant differences between groups. Two-way ANOVA followed by Duncan as Post hoc test, p <0.05.

release by the pituitary gland during unsuitable environmental conditions. These results are in the overall agreement with (Peter *et al*., 1978, Peter and Yu, 1997; Beriotto *et al*., 2020) reporting that GRIF possibly originated from antero-ventral NPP of the preoptic recess in goldfish. The results of chemical-induced lesion by systemic administration of MSG induce extensive brain lesions (Peter and Paulencu, 1980, Sloley *et al*., 1992; Li *et al*., 2020), further corroborate this.

It was found that MSG at 5 mg/g body weight caused specific cell necrosis in a portion of both NPP and NLT. We suggest that necrotic changes in NPP resulted in the release of gonadotropin from the pituitary gland, as evidenced by oocyte maturation, ovulation and spawning in MSG-injected catfish. These results are in accordance to that was found in goldfish (Peter and Paulencu, 1980, Kah *et al*., 1987, Sloley *et al*., 1992). Although MSG at a lower dose (2.5 mg/g body weight) failed to induce a maturational response, the pretreatment with pimozide (dopamine receptor antagonist) potentiated MSG-effect even at the lower dose to oocyte maturation; this implied the presence of an inhibitory factor in the catfish.

Even GnRH administered with DA failed to initiate meiotic division in the primary vitellogenic oocytes that is in agreement with the studies in goldfish (Peter and Yu, 1997). On the contrary, pimozide, along with GnRH and DA, led to a positive maturational response in all catfish oocytes, although pimozide given alone failed to induce the oocyte maturation, as evident in the study on *Cyprinus carpio* (Drori *et al*., 1994). It can be therefore concluded that only blocking the dopamine receptors may not evoke a maturational response. We suggest that dopamine inhibits the release of gonadotropin, which in

turn blocks the oocyte maturation in catfish, and DA-induced inhibitory effect is removed by pimozide and leads to LH release and in turn the oocyte maturation. Although this cannot be confirmed from the present study but we speculate that dopamine binds to D2-like receptors in *H. fossilis* and acts as the gonadotropin release inhibitory factor. This is consistent with the evidence that D2-like receptors (*D2, D3* and *D4*) are involved in the neuroendocrine control of pituitary function in fishes like zebrafish (Fontaine *et al*., 2013), grey mullet (Nocillado *et al*., 2007) and gambusia (Bhat and Ganesh, 2019).

Dopamine also plays a pivotal role in the neuroendocrine control of LH release in the catfish, similar to evidence from many other fishes, viz. rainbow trout (Linard *et al*., 1995, Vacher *et al*., 2002), salmon (Saligaut *et al*., 1999), grey mullet (Aizen *et al*., 2005), tilapia (Levavi-Sivan *et al*., 2005), zebrafish (Fontaine *et al*., 2013, Golan *et al*., 2015; Spicer *et al*., 2017) and eel (Maugars *et al*., 2020). We propose that GnRH and dopamine interact at the brain level and control oocyte maturation. Although this is achieved mechanistically cannot be elucidated by the present study, but we can provide a tenable explanation(s) based on the overall results on changes in LH, gene expressions and oocyte maturation. First, the temporal expressions of *lhb*, *D2* and *D4* receptor genes were correlated with plasma LH levels and, consequently, with oocytes' meiotic maturation. We suggest that GnRH upregulated the expression of genes, as was shown by an enhanced mRNA expression of *lhb* gene within half an hour of its administration. It is speculated that *lhb* gene gets translated and secreted, therefore a significant increase in plasma titre of LH has been observed.

Consequently, *lhb* expression is downregulated and this lowers the plasma LH levels. A LH titre initiates maturational changes in oocytes that finally culminate into GVBD. Second, in the presence of DA, *lhb* expression was suppressed, leading to the reduction in the synthesis of LH and finally its low titre in the plasma. Thus, dopamine suppresses GnRH regulated transcription and translation of LH, and in turns led to the failure of oocytes to mature. This is substantiated by the results from DA antagonist (pimozide) treatment, which countered the DA-induced suppression, and hence potentiates LH secretion, leading to oocyte maturation in gravid catfish. The suppression of *D2* and *D4* receptor expression by pimozide supported the involvement of D2-like receptors in the neuroendocrine regulation of oocyte maturation in catfish, as has been suggested in the reproductive regulation of white sturgeon (Pavlick and Moberg, 1997), tilapia (Levavi-Sivan *et al*., 2005), grey mullet (Nocillado *et al*., 2007), rainbow trout (Vacher *et al*., 2002) and zebrafish (Fontaine *et al*., 2013; Fallah *et al*., 2020).

Fish oocyte maturation and ovulation are very critical reproductive events that involves various morphological changes which is under the control of HPG axis. In our study also, GnRH analogue along with dopamine inhibitor has been shown to be very effective for inducing oocyte maturation. In drug treated group (GnRH alone or GnRH along with dopamine and pimozide) the nuclear membrane was dissolved, GVBD takes place and the yolk globules coalesced and became homogenous suggesting a role of these neurohormones in progression of oocyte maturation in the catfish.

Conclusion

In summary, the NPP is an important hypothalamic site in the catfish because interference with its function alters LH release from the pituitary gland. Dopamine inhibits GnRH-induced secretion of the pituitary LH via D2-like receptors. Therefore, it is suggested that secretions of NPP, presumably dopamine, inhibit spontaneous LH release from the pituitary gland in the *H. fossilis*. Hence, in all likelihood, the perimaturational gonadotropin surge requires the abolition of the GRIF and GnRH availability in the catfish.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Science and Technology (DST) for providing the research grant (SR/WOS-A/LS-358/2016) and the University of Delhi for providing the infrastructural facilities. We thank Prof. Vinod Kumar from the Department of Zoology, the University of Delhi, for his insightful and valuable inputs in the manuscript. Pooja Kumari (PK) greatly acknowledges the University Grant Commission (UGC) for providing the stipend.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Aggarwal, N., Goswami, S. & Sehgal, N. (2012). A stereotaxic atlas and technique for nuclei of the diencephalon of catfish, *Heteropneustes fossilis* (Bloch). *J Env Bio-Sci,* 26**,** 15.
2. Aizen, J., Meiri, I., Tzchori, I., Levavi-Sivan, B. & Rosenfeld, H. (2005). Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *General and Comparative Endocrinology,* 142**,** 212-221. https://doi.org/10.1016/j.ygcen.2005.01.002
3. Azuadi, N., Siraj, S., Daud, S., Christianus, A., Harmin, S., Sungan, S. & Britin, R.(2011). Enhancing ovulation of Malaysian mahseer (*Tor tambroides*) in captivity by removal of dopaminergic inhibition. *Journal of Fisheries and Aquatic Science,* 6**,** 740.

4. Beriotto, A. C., Di Yorio, M. P., Sirkin, D. I. P., Toledo-Solis, F. J., Peña-Marín, E. S., Álvarez-González, C. A., & Vissio, P. G. (2020). Gonadotropin-inhibitory hormone (GnIH) distribution in the brain of the ancient fish *Atractosteus tropicus* (Holostei, Lepisosteiformes). *General and Comparative Endocrinology*, *299*, 113623. https://doi.org/10.1016/j.ygcen.2020.113623
5. Bhat, S. K. & Ganesh, C. B. (2019). Dopamine receptor agonist bromocriptine restrains the follicular development, hatchling success and puberty in Gambusia affinis. *Journal of Applied Ichthyology*, *35*(2), 501-511. https://doi.org/10.1111/jai.13875
6. Blázquez, M., Bosma, P., Fraser, E., Van Look, K. & Trudeau, V. (1998). Fish as models for the neuroendocrine regulation of reproduction and growth. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* 119**,** 345-364. https://doi.org/10.1016/S0742-8413(98)00023-1
7. Bryant, A. S., Greenwood, A. K., Juntti, S. A., Byrne, A. E. & Fernald, R. D. (2016). Dopaminergic inhibition of gonadotropin-releasing hormone neurons in the cichlid fish *Astatotilapia burtoni*. *J. Exp. Biol.,* 219**,** 3861-3865. https://doi.org/10.1242/jeb.147637
8. Chomczynski, P. & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem,* 162**,** 156-9. https://doi.org/10.1016/0003-2697(87)90021-2
9. Ciani, E., Fontaine, R., Maugars, G., Nourizadeh-Lillabadi, R., Andersson, E., Bogerd, J., Von Krogh, K. & Weltzien, F.-A. (2020). Gnrh receptor gnrhr2bbα is expressed exclusively in *lhb*-expressing cells in Atlantic salmon male parr. *General and Comparative Endocrinology,* 285**,** 113293. https://doi.org/10.1016/j.ygcen.2019.113293
10. Corio, M., Peute, J. & Steinbusch, H. W. (1991). Distribution of serotonin- and dopamine-immunoreactivity in the brain of the teleost *Clarias gariepinus*. *J Chem Neuroanat,* 4**,** 79-95. https://doi.org/10.1016/0891-0618(91)90033-9
11. Drori, S., Ofir, M., Levavi-Sivan, B. & Yaron, Z. (1994). Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture,* 119**,** 393-407. https://doi.org/10.1016/0044-8486(94)90303-4
12. Dufour, S., Sebert, M. E., Weltzien, F. A., Rousseau, K. & Pasqualini, C. (2010). Neuroendocrine control by dopamine of teleost reproduction. *J Fish Biol,* 76**,** 129-60. https://doi.org/10.1111/j.1095-8649.2009.02499.x
13. Dufour, S., Weltzien, F. A., Sebert, M. E., Le Belle, N., Vidal, B., Vernier, P. & Pasqualini, C. (2005). Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary implications. *Annals of the New York Academy of Sciences,* 1040**,** 9-21. doi: 10.1196/annals.1327.002
14. El-Hawarry, W. N., Abd El-Rahman, S. H. & Shourbela, R. M. (2016). Breeding response and larval quality of African catfish (*Clarias gariepinus*, Burchell 1822) using different hormones/hormonal analogues with dopamine antagonist. *The Egyptian Journal of Aquatic Research,* 42**,** 231-239. https://doi.org/10.1016/j.ejar.2016.06.003
15. Fallah, H. P. & Habibi, H. R. (2020). Role of GnRH and GnIH in paracrine/autocrine control of final oocyte maturation. *General and Comparative Endocrinology*, *299*, 113619. https://doi.org/10.1016/j.ygcen.2020.113619
16. Fallah, H. P., Rodrigues, M. S., Corchuelo, S., Nóbrega, R. H. & Habibi, H. R. (2020). Role of GnRH isoforms in paracrine/autocrine control of zebrafish (Danio rerio) spermatogenesis. *Endocrinology*, *161*(2), bqaa004. https://doi.org/10.1210/endocr/bqaa004
17. Fontaine, R., Affaticati, P., Yamamoto, K., Jolly, C., Bureau, C., Baloche, S., Gonnet, F., Vernier, P., Dufour, S. & Pasqualini, C. (2013). Dopamine inhibits reproduction in female zebrafish (*Danio rerio*) via three pituitary D2 receptor subtypes. *Endocrinology,* 154**,** 807-18. https://doi.org/10.1210/en.2012-1759
18. Golan, M., Zelinger, E., Zohar, Y. & Levavi-Sivan, B. (2015). Architecture of GnRH-Gonadotrope-Vasculature Reveals a Dual Mode of Gonadotropin Regulation in Fish. *Endocrinology,* 156**,** 4163-73. https://doi.org/10.1210/en.2015-1150
19. Goswami, S. V. & Sundararaj, B. I. (1971). In vitro maturation and ovulation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch): effects of mammalian hypophyseal hormones, catfish pituitary homogenate, steroid precursors and metabolites, and gonadal and adrenocortical steroids. *J. Exp. Zool,* 178**,** 467-77. https://doi.org/10.1002/jez.1401780405
20. Kah, O., Dulka, J. G., Dubourg, P., Thibault, J. & Peter, R. E. (1987). Neuroanatomical substrate for the inhibition of gonadotrophin secretion in goldfish: existence of a dopaminergic preoptico-hypophyseal pathway. *Neuroendocrinology,* 45**,** 451-8. https://doi.org/10.1159/000124774
21. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.,* 35**,** 1547-1549. 10.1093/molbev/msy096
22. Kumari, P., Kumar, M., Sehgal, N. & Aggarwal, N. (2020). In silico analysis of kiss2, expression studies and protein–protein interaction with gonadotropin-releasing hormone 2 (GnRH2) and luteinizing hormone beta (LHβ) in *Heteropneustes fossilis*. *Journal of Biomolecular Structure and Dynamics***,** 1-15. https://doi.org/10.1080/0739 1102.20 20.1860820
23. Lamba, V. J., Goswami, S. V. & Sundararaj, B. I. (1983). Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17 beta, estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *Gen Comp Endocrinol,* 50**,** 205-25. https://doi.org/10.1016/0016-6480(83)90221-6
24. Levavi-Sivan, B., Aizen, J. & Avitan, A. (2005). Cloning, characterization and expression of the D2 dopamine receptor from the tilapia pituitary. *Mol Cell Endocrinol,* 236**,** 17-30. https://doi.org/10.1016/j.mce.2005.03.010
25. Li, X., Zheng, S., & Wu, G. (2020). Nutrition and metabolism of glutamate and glutamine in fish. *Amino acids*, *52*, 671-691. https://doi.org/10.1007/s00726-020-02851-2
26. Linard, B., Bennani, S. & Saligaut, C. (1995). Involvement of estradiol in a catecholamine inhibitory tone of gonadotropin release in the rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol,* 99**,** 192-6. https://doi.org/10.1006/gcen.1995.1101
27. Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR

and the 2− ΔΔCT method. *methods,* 25**,** 402-408. https://doi.org/10.1006/meth.2001.1262

28. Maugars, G., Pasquier, J., Atkinson, C., Lafont, A. G., Campo, A., Kamech, N. & Rousseau, K. (2020). Gonadotropin-inhibitory hormone in teleosts: New insights from a basal representative, the eel. *General and comparative endocrinology*, *287*, 113350. https://doi.org/10.1016/j.ygcen.2019.113350
29. Mylonas, C. C. & Zohar, Y. (2007). Promoting oocyte maturation, ovulation and spawning in farmed fish. *The Fish Oocyte.* Springer. https://doi.org/10.1007/978-1-4020-6235-3_15
30. Nagahama, Y. & Yamashita, M. (2008). Regulation of oocyte maturation in fish. *Development, growth* and *differentiation,* 50**,** S195-S219. https://doi.org/10.1111/j.1440-169X.2008.01019.x
31. Nocillado, J. N., Levavi-Sivan, B., Carrick, F. & Elizur, A. (2007). Temporal expression of G-protein-coupled receptor 54 (GPR54), gonadotropin-releasing hormones (GnRH), and dopamine receptor D2 (drd2) in pubertal female grey mullet, *Mugil cephalus*. *Gen Comp Endocrinol,* 150**,** 278-87. https://doi.org/10.1016/j.ygcen.2006.09.008
32. Paullada-Salmeron, J. A., Cowan, M., Aliaga-Guerrero, M., Morano, F., Zanuy, S. & Munoz-Cueto, J. A. (2016). Gonadotropin Inhibitory Hormone Down-Regulates the Brain-Pituitary Reproductive Axis of Male European Sea Bass (*Dicentrarchus labrax*). *Biol Reprod,* 94**,** 121. https://doi.org/10.1095/biolreprod.116.139022
33. Pavlick, R. J. & Moberg, G. P. (1997). Dopaminergic influence on gonadotropin secretion in white sturgeon (*Acipenser transmontanus*). *Fish Physiology and Biochemistry,* 16**,** 35-43.
34. Peter, R. & Yu, K. (1997). Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Reviews in Fish Biology and Fisheries,* 7**,** 173-197. https://doi.org/10.1023/A:1018431610220
35. Peter, R. E., Crim, L. W., Goos, H. J. & Crim, J. W. (1978). Lesioning studies on the gravid female goldfish: neuroendocrine regulation of ovulation. *Gen Comp Endocrinol,* 35**,** 391-401. https://doi.org/10.1016/0016-6480(78)90133-8
36. Peter, R. E. & Paulencu, C. R. (1980). Involvement of the preoptic region in gonadotropin release-inhibition in goldfish, *Carassius auratus*. *Neuroendocrinology,* 31**,** 133-41. https://doi.org/10.1159/000123064
37. Podhorec, P. & Kouril, J. (2009). Induction of final oocyte maturation in Cyprinidae fish by hypothalamic factors: a review. *Veterinarni Medicina,* 54**,** 97-110.
38. Rainis, S. & Ballestrazzi, R. (2005). The control of reproduction in finfish species through GnRH treatments. *Italian Journal of Animal Science,* 4**,** 345-353. https://doi.org/10.4081/ijas.2005.345
39. Saligaut, C., Linard, B., Breton, B., Anglade, I., Bailhache, T., Kah, O. & Jego, P. (1999). Brain aminergic systems in salmonids and other teleosts in relation to steroid feedback and gonadotropin release. *Aquaculture,* 177**,** 13-20. https://doi.org/10.1016/S0044-8486(99)00065-4
40. Sloley, B. D., Kah, O., Trudeau, V. L., Dulka, J. G. & Peter, R. E. (1992). Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish: involvement in the regulation of gonadotropin secretion. *J Neurochem,* 58**,** 2254-62. https://doi.org/10.1111/j.1471-4159.1992.tb10971.x
41. Spicer, O. S., Zmora, N., Wong, T. T., Golan, M., Levavi-Sivan, B., Gothilf, Y. & Zohar, Y. (2017). The gonadotropin-inhibitory hormone (Lpxrfa) system's regulation of reproduction in the brain–pituitary axis of the zebrafish (Danio rerio). *Biology of reproduction*, *96*(5), 1031-1042. https://doi.org/10.1093/biolre/iox032
42. Sundararaj, B. I. & Vasal, S. (1976). Photoperiod and temperature control in the regulation of reproduction in the female catfish *Heteropneustes fossilis*. *Journal of the Fisheries Board of Canada,* 33**,** 959-973. https://doi.org/10.1139/f76-123
43. Sundararaj, B. I. & Viswanathan, N. (1971). Hypothalamo-hypophyseal neurosecretory and vascular systems in the catfish, *Heteropneustes fossilis* (Bloch). *J Comp Neurol,* 141**,** 95-106. https://doi.org/10.1002/cne.901410108
44. Vacher, C., Ferrière, F., Marmignon, M.-H., Pellegrini, E. & Saligaut, C. (2002). Dopamine D2 receptors and secretion of FSH and LH: role of sexual steroids on the pituitary of the female rainbow trout. *General and comparative endocrinology,* 127**,** 198-206. https://doi.org/10.1016/S0016-6480(02)00046-1
45. Yamamoto, K., Fontaine, R., Pasqualini, C. & Vernier, P. (2015). Classification of Dopamine Receptor Genes in Vertebrates: Nine Subtypes in Osteichthyes. *Brain Behav Evol,* 86**,** 164-75. https://doi.org/10.1159/000441550
46. Yamamoto, N., Parhar, I. S., Sawai, N., Oka, Y. & Ito, H. (1998). Preoptic gonadotropin-releasing hormone (GnRH) neurons innervate the pituitary in teleosts. *Neuroscience research,* 31**,** 31-38. https://doi.org/10.1016/S0168-0102(98)00022-4
47. Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A. & Levavi-Sivan, B. (2003). Regulation of fish gonadotropins. *Int Rev Cytol,* 225**,** 131-85. https://doi.org/10.1016/S0074-7696(05)25004-0
48. Zohar, Y., Munoz-Cueto, J. A., Elizur, A. & Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *Gen Comp Endocrinol,* 165**,** 438-55. https://doi.org/10.1016/j.ygcen.2009.04.017