General and Comparative Endocrinology 206 (2014) 8–15

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Neuroendocrine gene expression reveals a decrease in dopamine D2B receptor with no changes in GnRH system during prepubertal metamorphosis of silvering in wild Japanese eel

Shan-Ru Jeng [a,*], Wen-Shiun Yueh [a], Yi-Ting Pen [a], Yan-Horn Lee [b], Guan-Ru Chen [c], Sylvie Dufour [d], Ching-Fong Chang [e,f,*]

[a] Department of Aquaculture, National Kaohsiung Marine University, Kaohsiung 811, Taiwan
[b] Tungkang Biotechnology Research Center, Fisheries Research Institute, Council of Agriculture, Tungkang 928, Taiwan
[c] Freshwater Aquaculture Research Center, Fisheries Research Institute, Council of Agriculture, Lukang 505, Taiwan
[d] Muséum National d'Histoire Naturelle, Sorbonne Universités, Research Unit BOREA, Biology of Aquatic Organisms and Ecosystems, CNRS 7208 – IRD207 – UPMC – UCBN, 75231 Paris Cedex 05, France
[e] Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan
[f] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 202, Taiwan

ARTICLE INFO

Article history:
Received 28 May 2014
Revised 20 July 2014
Accepted 5 August 2014
Available online 12 August 2014

Keywords:
Gonadotropins
GnRH
Dopamine
Dopamine D2 receptor
Silvering

ABSTRACT

Silvering is a prepubertal metamorphosis preparing the eel to the oceanic reproductive migration. A moderate gonad development occurs during this metamorphosis from the sedentary yellow stage to the migratory silver stage. The aim of this study was to elucidate the molecular aspects of various endocrine parameters of BPG axis at different ovarian developmental stages in wild yellow and silver female Japanese eels. The GSI of the sampled female eels ranged between 0.18 and 2.3%, corresponding to yellow, pre-silver and silver stages. Gonad histology showed changes from previtellogenic oocytes in yellow eels to early vitellogenic oocytes in silver eels. Both serum E_2 and T concentrations significantly increased with ovarian development indicating a significant activation of steroidogenesis during silvering. In agreement with previous studies, significant increases in pituitary gonadotropin beta subunits *FSH-β* and LH-*β* transcripts were also measured by qPCR, supporting that the activation of pituitary gonadotropin expression is likely responsible for the significant ovarian development observed during silvering. We investigated for the first time the possible brain neuroendocrine mechanisms involved in the activation of the pituitary gonadotropic function during silvering. By analyzing the expression of genes representative of the stimulatory GnRH control and the inhibitory dopaminergic control. The transcript levels of mGnRH and the three GnRH receptors did not change in the brain and pituitary between yellow and silver stages, suggesting that gene expression of the GnRH system is not significantly activated during silvering. The brain transcript levels of tyrosine hydroxylase, limiting enzyme of DA synthesis did not change during silvering, indicating that the DA synthesis activity was maintained. In contrast, a significant decrease in *DA-D2B receptor* expression in the forebrain and pituitary was observed, with no changes in *DA-D2A receptor*. The decrease in the pituitary expression of *DA-D2BR* during silvering would allow a reduced inhibitory effect of DA. We may raise the hypothesis that this regulation of *D2BR* gene expression is one of the neuroendocrine mechanisms involved in the slight activation of the pituitary gonadotropin and gonadal activity that occur at silvering.

1. Introduction

The eel is a catadromous basal teleost, which exhibits a complex life cycle with spawning in the ocean and growing up in continental waters. Leptocephali larvae drift towards the coast and metamorphose into glass eels, which grow in continental habitat where they develop as yellow eels. After several years of growth phase, the yellow eels undergo a prepubertal secondary

* Corresponding authors. Addresses: Department of Aquaculture, National Kaohsiung Marine University, Kaohsiung 811, Taiwan. Fax: +886 7 366 3570 (S.-R. Jeng), Department of Aquaculture, National Taiwan Ocean University, Keelung 202, Taiwan. Fax: +886 2 2462 1579 (C.-F. Chang).
E-mail addresses: srjeng@webmail.nkmu.edu.tw (S.-R. Jeng), b0044@mail.ntou.edu.tw (C.-F. Chang).

http://dx.doi.org/10.1016/j.ygcen.2014.08.001
0016-6480/

metamorphosis called silvering and transform into silver eels (Rousseau et al., 2013). The silver eels start their downstream migration toward their sea spawning ground (Tesch, 1977; Tsukamoto, 1992; Thillart et al., 2009). During silvering, eels undergo significant morphological, physiological and behavioral changes, including some changes related to reproductive function. The GSI (gonadosomatic index) and the plasma levels of sex steroids increase significantly during silvering (Han et al., 2003c; Aroua et al., 2005). The gonads of yellow eels show small primary, non-vitellogenic oocytes, while the oocytes of silver eels correspond to the early vitellogenic stage (Han et al., 2003a; Aroua et al., 2005).

Gonad development remains limited in silver eels. Naturally mature eels are never been found in captivity and only a few matured eels have been caught in the open ocean (Chow et al., 2009). Japanese eel (*Anguilla japonica*) is an economically important species for aquaculture in Asia, but eel farming completely relies on wild elvers caught during their migration to the coasts. The catches of glass eels are drastically reduced, mainly because of overfishing, pollution, and destruction of habitats (Stone, 2003), and causing a serious crisis in eel aquaculture industry. The artificial induction of sexual maturation on eels is only based on gonadal stimulation with exogenous gonadotropic treatments and leading to extra-physiological activation of gonadal steroidogenesis, inadequate kinetic of vitellogenesis, inappropriate oocyte stores and poor quality eggs. Therefore, fundamental information on regulatory mechanisms of the neuroendocrine control at various ovarian development stages of eels is necessary to further comprehend eel reproduction.

In teleosts, as in other vertebrates, the reproductive functions are governed by the brain–pituitary–gonadal (BPG) axis (Weltzien et al., 2004). Brain gonadotropin-releasing hormone (GnRH) acts on the pituitary to stimulate the production of gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which act on the gonads to induce gametogenesis and steroidogenesis (Nagahama et al., 1995; Simoni et al., 1997; Dufau, 1998). Previous studies in Japanese and European eels (*Anguilla Anguilla*) have shown that the expression of both pituitary *FSH-β* and *LH-β* significantly increased during silvering (Han et al., 2003b; Aroua et al., 2005). It has been proposed that this moderate activation of pituitary gonadotropin expression may account for the slight but significant gonadal development at silvering.

Multiple variants of GnRH have been found in non-mammalian vertebrates. In the eel, previous studies reported that the mammalian form of GnRH (mGnRH) would be involved in the neuroendocrine control of reproduction (Dufour et al., 1993). Recently, three GnRH receptor genes (*GnRHR-1a*, *-1b*, and *-2*) were characterized in the European eel. The three *GnRHRs* are expressed in the brain and pituitary, as well in several peripheral tissues (Peñaranda et al., 2013).

In various teleosts, including the eel, a dopaminergic inhibitory control may counter-act the GnRH-stimulatory control of gonadotropin production (for review: Dufour et al., 2005, 2010).Tyrosine hydroxylase (TH) is the rate-limiting enzyme in dopamine biosynthesis.TH has been characterized in the European eel (Boularand et al., 1998; Weltzien et al., 2005). Dopamine acts through the dopamine D2 receptor (D2R) to inhibit basal and GnRH-induced GtH release (Yu and Peter, 1992; Yaron et al., 2003), and may modulate pituitary sensitivity to GnRH by down regulating the synthesis of GnRH receptors (De Leeuw et al., 1988; Omeljaniuk et al., 1989; Levavi-Sivan et al., 2004). Two dopamine D2 receptors have been identified in the European eel (Pasqualini et al., 2009).

It has been proven that the deficit in the pituitary gonadotropic function in the silver eel resulted from both a lack of stimulatory input from GnRH and low pituitary sensitivity to GnRH, as well as from a strong inhibition by dopamine (Dufour et al., 2003, 2005; Vidal et al., 2004). This revealed that a dual brain control was responsible for the arrest of eel sexual maturation at a prepubertal stage. Activation of GnRH system and reduction of the inhibition effects of DA should be able to trigger the expression of eel endogenous GtHs, but limited information is available about the neuroendocrine regulatory systems in the wild yellow and silver eels. Therefore, the aim of this study was to elucidate the molecular aspects of some endocrine parameters of BPG axis at different ovarian developmental stages in wild yellow and silver Japanese eels.

2. Materials and methods

2.1. Animals

Forty-two wild female Japanese eels, were collected by eel traps in the lower reach of Kaoping River in Pingtung County in the South of Taiwan from December 2008 to March 2009, and transported to the Tungkang Biotechnology Research Center, Fisheries Research Institute, Tungkang. The eels were placed in outdoor 2.5 ton-tanks in brackish water (salinity of 5 ppt), under natural light and temperature. The eels were sacrificed within a week. All procedures and investigations were approved by the College of Life Science of the National Taiwan Ocean University (Affidavit of Approval of Animal Use Protocol: No. 98029) and were performed in accordance with standard guiding principles.

2.2. Sampling procedure

Eels were anesthetized with 800 ppm 2-phenoxyethanol before being sacrificed. Body weight (BW) and ovarian weight were measured for the calculation of the gonadosomatic index (GSI = gonad weight/BW × 100%). The forebrain (olfactory bulbs and telencephalon), midbrain (optic tectum, mesencephalon and diencephalon), pituitary and ovaries were collected and stored at −80 °C for quantitative real-time PCR analysis. A piece of ovarian tissue was stored in Bouin's solution, and the ovarian sections were stained with hematoxylin and eosin for histological observation. Blood samples were allowed to clot at 4 °C, and serum was collected and stored at −20 °C for steroid immunoenzymatic assays.

2.3. Quantification of gene transcripts by real-time PCR analysis

Quantitative real-time PCR (qPCR) analyses for gene transcripts of *mGnRH GnRHRs, TH, D2AR, D2BR, FSH-β* and *LH-β* were conducted according to previously described methods (Jeng et al., 2007, 2012). Partial sequences of Japanese eel *mGnRH* (264 bp), *TH* (1140 bp), *GnRHR-1a* (1107 bp), *GnRHR-1b* (597 bp), *GnRHR-2* (1000 bp), *D2AR* (692 bp, GenBank Accession No. JX305466) and *D2BR* (1088 bp, GenBank Accession JX305467) cDNAs were cloned and used as standards for qPCR. These cDNAs were cloned from the total RNA of Japanese eel brain by RT-PCR using degenerated primers or specific primers designed from the published sequences. The sequences obtained were 100% identical to the published Japanese eel sequences (*mGnRH*: GenBank Accession No. BAA82608; *TH*: GenBank Accession No. BAJ83551) and 98–99% identical to the published European eel sequences (*GnRHR-1a*: GenBank Accession No. JX567769.1; *GnRHR-1b*: GenBank Accession No. JX567770.1; *GnRHR-2*: GenBank Accession No. JX567771.1; *D2AR*: GenBank Accession No. DQ789976; *D2BR*: GenBank Accession No. ABH06894).

Specific primers for the genes analyzed in this study and reference gene glyceraldehyde -3-phosphate dehydrogenase (*GAPDH*) (GenBank Accession No. AB049458.1) were designed for qPCR (Table 1). Gene quantification of standards (plasmids with cDNA

Table 1
Specific primers used for quantitative real-time PCR analyses (S, sense strand; AS, antisense strand).

Gene		Sequences	Amplicon size
FSH-β	S	5′-GCG GTG GTG TTG AAG GTG AT-3′	69 bp
FSH-β	AS	5′-CAG TTG TGG TCT CGC CAA CAT-3′	
LH-β	S	5′-GCG TGG ATC CCC ATG TGA-3′	88 bp
LH-β	AS	5′-ACT CTG GAT GGC GCA GTC A-3′	
mGnRH	S	5′-TGG CTG GGG CTG GCT GTG-3′	88 bp
mGnRH	AS	5′-GCT GGG CAA ACT GGA GGT GTC-3′	
GnRH-1a	S	5′-TGG TCA TGA GTT GCT GCT ACA-3′	81 bp
GnRH-1a	AS	5′-AGA CAC ACC TCT CCG TCT TT-3′	
GnRH-1b	S	5′-GGT CAC GCA CTG GGT GAA GT-3′	66 bp
GnRH-1b	AS	5′-TCC CCG CAG CTC TTC ATC T-3′	
GnRH-2	S	5′-TCA CCT TCT CCT GCC TCT TTC-3′	108 bp
GnRH-2	AS	5′-TTG GAA GAT GCC TTC CCT TT-3′	
TH	S	5′-GCC CAG TTT TCT CAG AAC ATT G-3′	170 bp
TH	AS	5′-TGC ACC AGC TCT CCA TAG G-3′	
D2AR	S	5′-CGA CGG TGA TGC TAA CGC TAC-3′	93 bp
D2AR	AS	5′-TGC CAT TGG ACT TGA CAA TCA GC-3′	
D2BR	S	5′-CAC ACG CTA CAG CTC CAA AA-3′	99 bp
D2BR	AS	5′-GTC TTC ACG GGT GGC TGT AT-3′	
GAPDH	S	5′ GCC AGC CAG AAC ATC ATC 3′	110 bp
GAPDH	AS	5′ GAC ACG GAA AGC CAT ACC 3′	

sequence) and samples were conducted simultaneously by qPCR (Applied Biosystems 7300 Real-Time. PCR System.; Applied Biosystems, Foster City, CA) with SYBR green I as a dsDNA minor-groove binding dye. Melting curves indicated the amplification of a single amplicon for each gene. The slopes of the respective standard and sample curves of the log (cDNA concentrations) *vs.* Ct (the calculated fractional cycle number at which the PCR-fluorescence product is detectable above a threshold) were −3.3 to −3.5, indicating an amplification efficiency of 100–90%. The transcript values of each gene were calibrated with the internal reference gene (*GAPDH*). No significant changes were observed in *GAPDH* transcript levels in any tissue with silvering (Supplemental Fig. S1).

2.4. Immunoenzymatic assays of serum E_2 and T

The concentrations of E_2 and T in serum were measured by Cayman Chemical's ACE™ enzyme immunoassay kit (EIA) Kits (Estradiol EIA Kit and Testosterone EIA Kit, Cayman Chemical Company, Ann Arbor, MI).

2.5. Data analysis

Linear regression analyses were conducted on the relationships between individual serum steroid or gene expression levels and GSI.

3. Results

3.1. Ovarian development and serum steroid levels in wild female Japanese eels

Forty-two wild female Japanese eels of various stages of ovarian development were sampled. The GSIs ranged between 0.18% and 2.3%. According to the histological observation and the report of Han et al. (2003a), the maturities of the wild female Japanese eels in this study were divided into yellow (the gonad showed small, primary non-vitellogenic oocytes and a dense nucleus with a large nucleolus, for example Fig. 1(A): GSI = 0.25%), pre-silver (oocytes at peri-nucleolar stage and a few lipidic vesicles were observed in the cytoplasma, for example Fig. 1(B): GSI = 0.56%) and silver (oocytes at oil-droplet stage, with numerous lipidic vesicles and a few yolk vesicles, for example Fig. 1(C): GSI = 1.65%) stages. Thus, the wild eels used in this study represented various stages of the initiation of the ovarian vitellogenesis process that occurs during silvering.

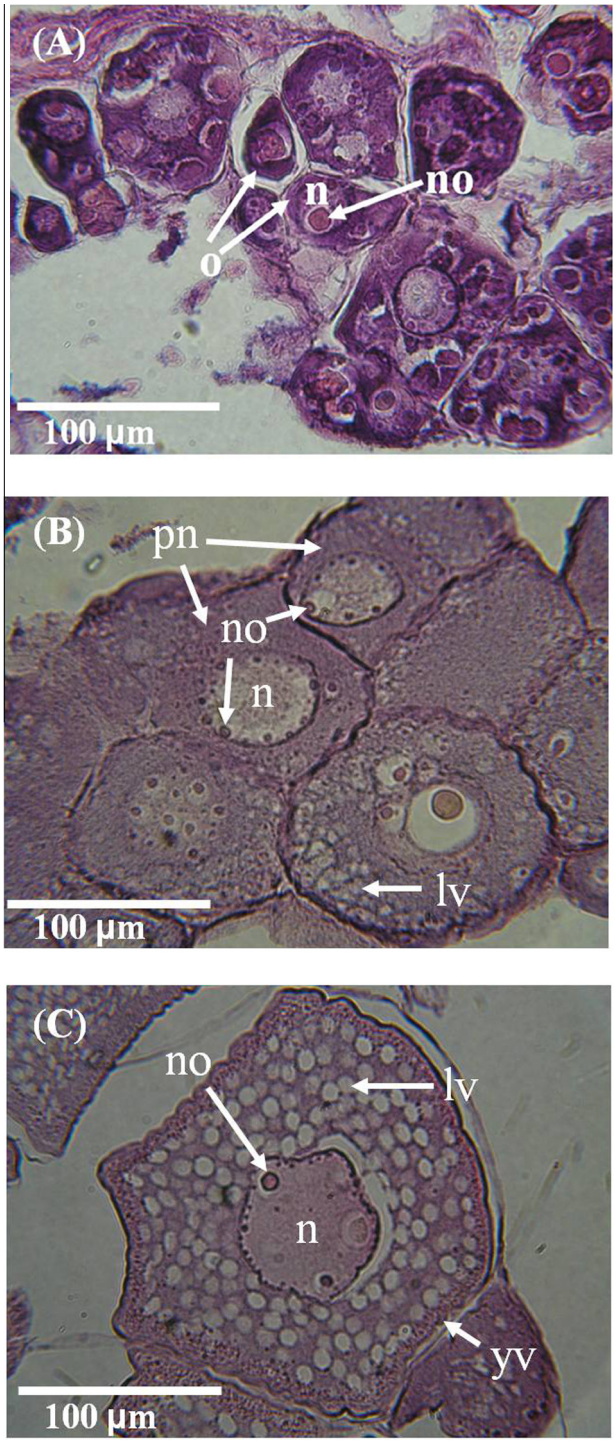

Fig. 1. (A–C) Transverse sections of ovarian tissues of wild female Japanese eels stained with hematoxylin and eosin. (A) Yellow eel (GSI = 0.25%): previtellogenic stage (non-vitellogenic oocytes with a dense cytoplasma and a large nucleolus in the nucleus). (B) Pre-silver eel (GSI = 0.56%): peri-nucleolar stage (oocytes with small nucleolus at the periphery of the nucleus; presence of a few lipidic vesicles in the cytoplasma). (C) Silver eel (GSI = 1.65%): early vitellogenic stage (oocytes at oil-droplet stage, with numerous lipidic vesicles and a few yolk vesicles). lv, lipid vesicles; n, nucleus; no, nucleoli; o, oocyte; pn, perinucleolar stage; yv, yolk vescles. GSI = gonadosomatic index.

Immunoassays of E_2 and T serum levels showed a significant increase of both steroids with ovarian development of wild female eels (Fig. 2).

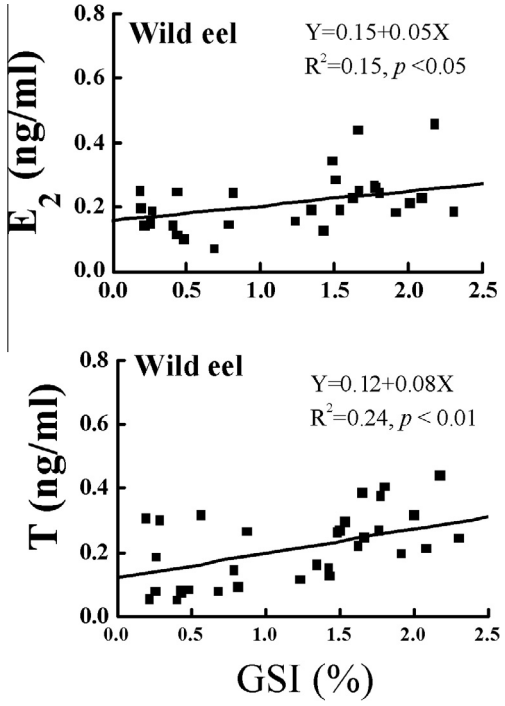

Fig. 2. Concentrations of E_2 and T in serum in individual wild female Japanese eels during silvering as measured by immunoenzymatic assays. GSI = gonadosomatic index.

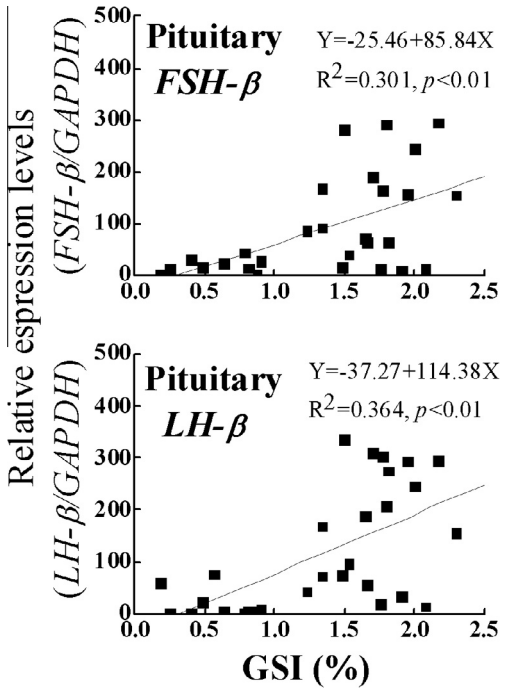

Fig. 3. Expression profiles of pituitary *FSH-β* and *LH-β* in individual wild female Japanese eels during silvering as measured by qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is used as reference gene. GSI = gonadosomatic index.

3.2. Gonadotropin gene expression

Transcript levels for gonadotropin-specific beta subunits were measured in the pituitary. Both *FSH-β* transcript ($p < 0.01$) and *LH-β* ($p < 0.01$) transcript levels significantly increased with ovarian development of wild female eels (Fig. 3).

3.3. GnRH and GnRH receptor gene expression

Transcripts for *mGnRH* were mainly expressed in the forebrain and also at a lower level in the midbrain. In both parts of the brain, mGnRH transcript levels did not significantly change with the ovarian development (Fig. 4). Transcripts of mGnRH were also expressed in the pituitary and ovary, and showed no significant change with ovarian development (Fig. 4).

The three *GnRHRs* transcripts were expressed in the brain and showed no significant change with ovarian development (Fig. 5(A)–(C)). In the pituitary, *GnRHR-1b* transcript was the most abundant as compared to *GnRHR-1a* and *GnRHR-2*, which were even undetectable in some individuals. On the opposite, in the ovary, *GnRHR-1a* and *GnRHR-2* transcripts were more abundant then *GnRHR-1b* which was undetectable in some individuals. No significant change in transcript levels in the pituitary nor in the ovary was observed for the three receptors with ovarian development (Fig. 5(A)–(C)).

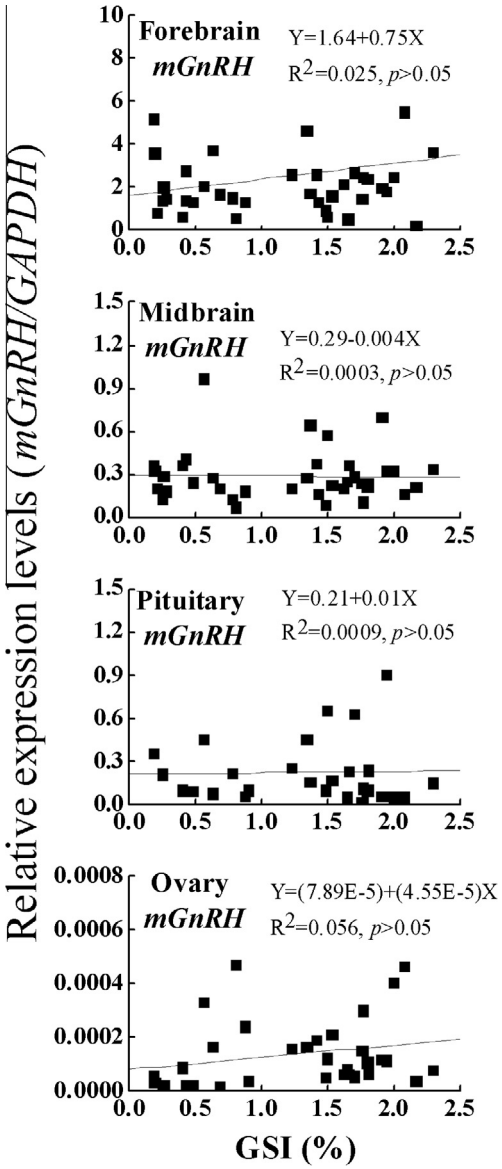

Fig. 4. Expression profiles of *mGnRH* in forebrain, midbrain, pituitary and ovary in individual wild female Japanese eels during silvering as measured by qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is used as reference gene. GSI = gonadosomatic index.

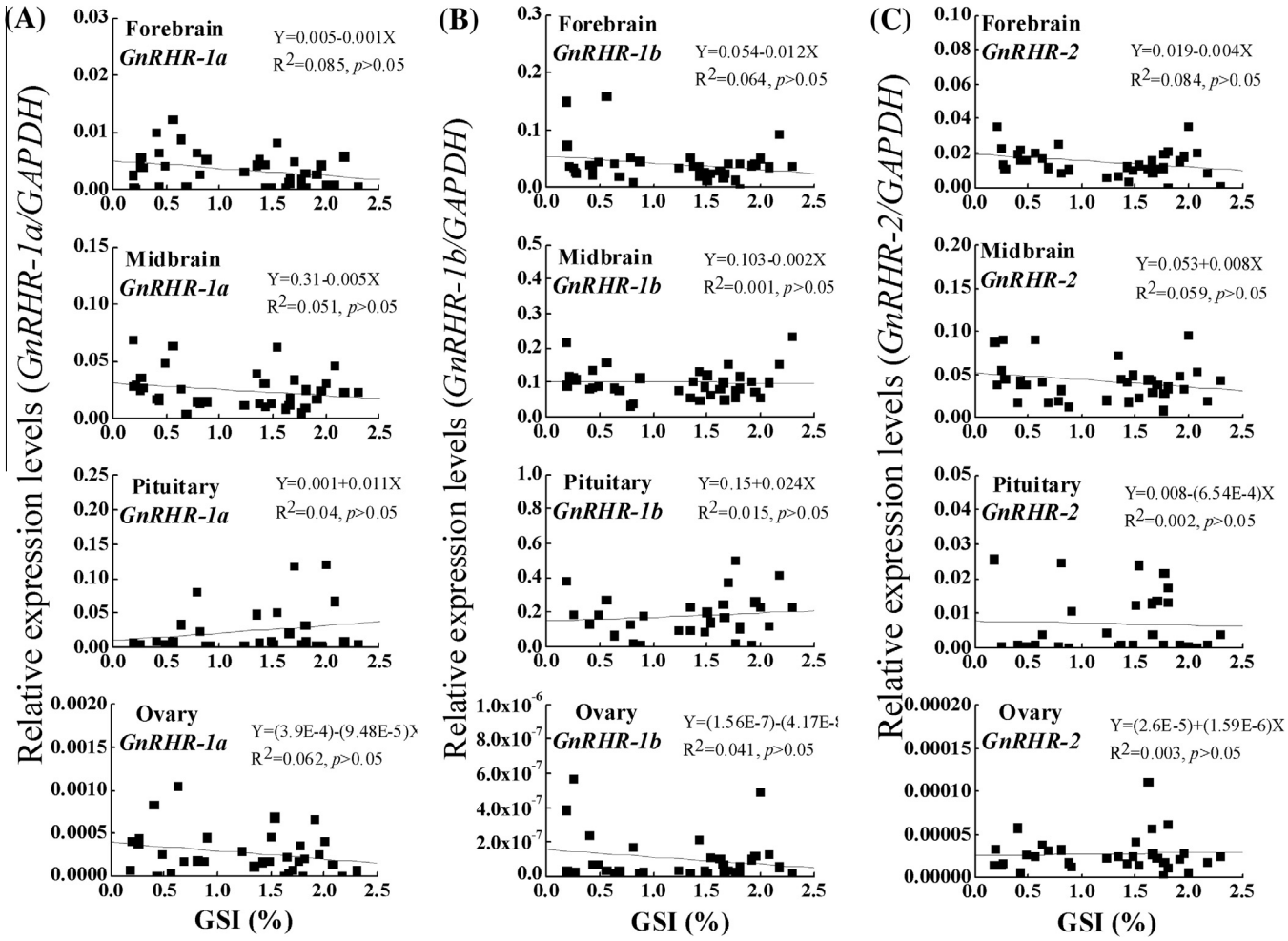

Fig. 5. Expression profiles of GnRH receptors in forebrain, midbrain, pituitary and ovary in individual wild female Japanese eels during silvering as measured by qRT-PCR. (A) *GnRHR-1a*, (B) *GnRHR-1b* and (C) *GnRHR-2*. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is used as reference gene. GSI = gonadosomatic index.

3.4. Tyrosine hydroxylase gene expression

TH transcripts were expressed in both parts of the brain and their levels did not significantly change with ovarian development in wild female eels (Fig. 6). TH transcripts were undetectable in the pituitary and ovary.

3.5. Dopamine D2 receptor gene expression

Both *D2AR* and *D2BR* transcripts were expressed in the brain and pituitary but were undetectable in the ovary of Japanese eel. In the brain, the expression of *D2AR* transcripts did not change significantly in the forebrain nor in the midbrain with ovarian development (Fig. 7(A)). In contrast, *D2BR* transcript levels significantly decreased in the forebrain ($p < 0.05$) (Fig. 7(B)).

In the pituitary, *D2AR* transcript levels did not change, while *D2BR* transcript levels significantly decreased ($p < 0.05$) with the ovarian development in wild female eels (Fig. 7(A) and (B)).

4. Discussion

4.1. Ovarian development during silvering in wild female Japanese eels

In this study, the GSI of the wild female eels ranged between 0.18% and 2.3%, corresponding to yellow, pre-silver and silver stages, as assessed by the histological observation of the gonads. Yellow eels showed previtellogenic primary oocytes, while silver eels showed early vitellogenic oocytes containing abondant lipid vesicles and a few yolk granules. The increase in GSI and the initiation of vitellogenesis occurring during silvering are in agreement with previous studies in the European and Japanese eels (Dufour et al., 2003; Nagae et al., 1996). The silver stage, referred to as prepubertal stage (Dufour et al., 2003), is the last stage of the eel life cycle in continental waters, preceding the reproductive oceanic migration. The ovarian development observed between yellow and silver stage, remains however limited as compared to experimentally mature eels presenting a GSI of more than 20% (Ohta et al., 1997; Jeng et al., 2007).

Both serum E_2 and T concentrations significantly increased with ovarian development between yellow and silver stages in agreement with previous data by Han et al. (2003c) for the Japanese eel. Similar results were reported in New Zealand eels (*Anguilla australis* and *Anguilla dieffenbachiai*) (Lokman and Young, 1998), American eel, *Anguilla rostrata*, (Cottrill et al., 2001) and European eel (Sbaihi et al., 2001; Aroua et al., 2005).

4.2. Increase in pituitary gonadotropin gene expression during silvering

The transcript levels of both pituitary gonadotropin beta subunits *FSH-β* and LH-*β* were significantly increased between yellow

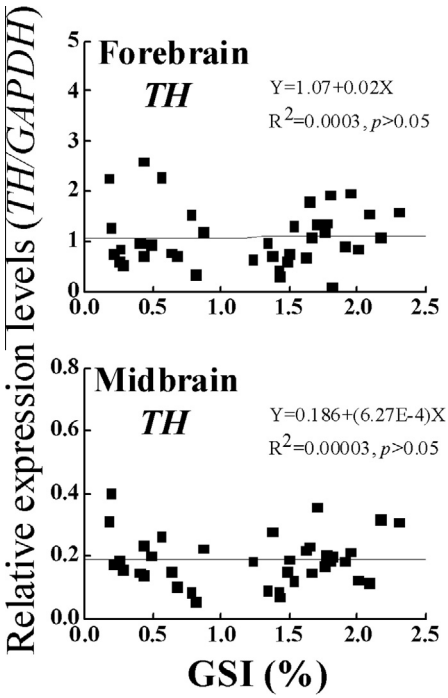

Fig. 6. Expression profiles of tyrosine hydroxylase (*TH*) in forebrain and midbrain in individual wild female Japanese eels during silvering as measured by qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is used as reference gene. GSI = gonadosomatic index.

and silver stages. The activation of pituitary gonadotropin expression during silvering is in agreement with previous results reported by Han et al. (2003a) in the Japanese eel, and Aroua et al. (2005) in the European eel. All these data suggest that the activation of pituitary gonadotropin expression is likely responsible for the significant ovarian development observed during silvering.

In the present study, we investigated for the first time the possible brain neuroendocrine mechanisms involved in the activation of the pituitary gonadotropic function during silvering. We analyzed the expression of genes representative of the stimulatory GnRH control and the inhibitory dopaminergic control.

4.3. No change in the expression of genes of the GnRH stimulatory control of gonadotropins

We did not observe any change in the transcript levels of mGnRH nor of the three GnRH receptors in the brain and pituitary between yellow and silver stage. This suggests that gene expression of the GnRH system is not significantly activated during silvering. A significant increase in mGnRH expression was showed in experimentally matured female European eels (Pasquier et al., 2012). Furthermore, Peñaranda et al. (2013) showed that pituitary expression of *GnRHR-1b* and *GnRHR-2* were increased in experimentally matured female European eels, and inferred that GnRHR-1b and GnRHR-2 are likely hypophysiotropic GnRH receptors in eels. Interestingly, the pituitary *GnRHR-2* transcripts show a very low expression, and are even undetectable in some individuals in the present study, further suggesting that the GnRH

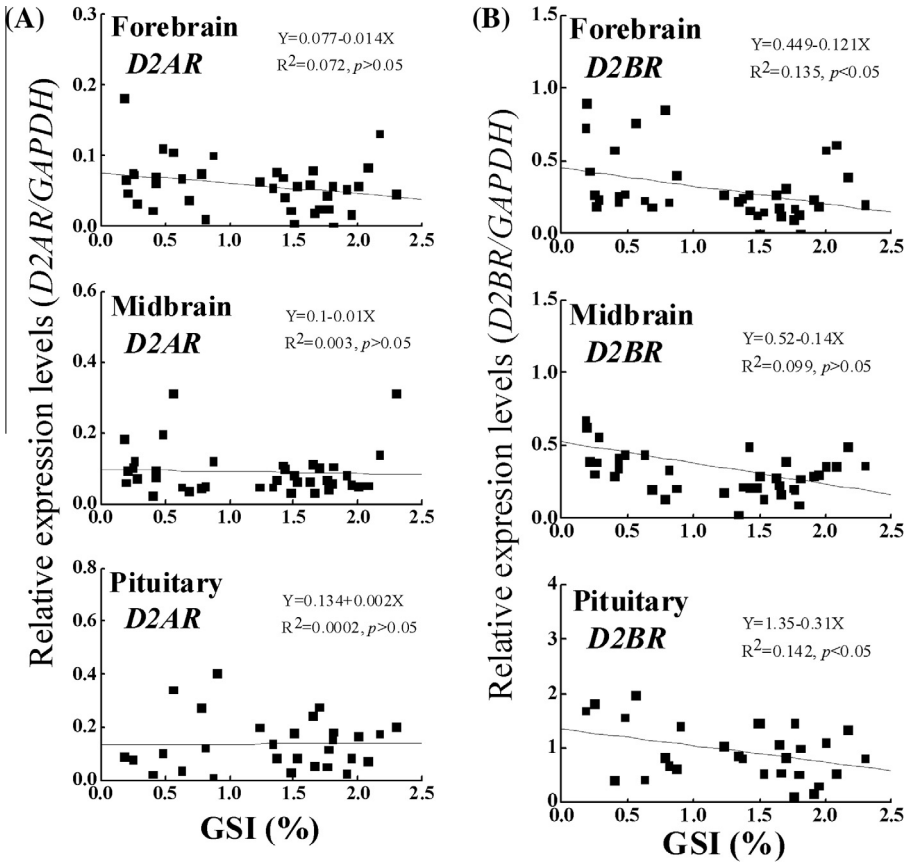

Fig. 7. Expression profiles of dopamine D2 receptors in forebrain, midbrain and pituitary in individual wild female Japanese eels during silvering as measured by qRT-PCR. (A) *D2AR* and (B) *D2BR*. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is used as reference gene. GSI = gonadosomatic index.

hypophysiotropic control is not yet significantly activated at silvering. A significant activation of the GnRH system may take place later in the sexual maturation process, during the oceanic reproductive migration and at the spawning ground. In other teleosts, increases in GnRH (for review: Dufour et al., 2010; Zohar et al., 2010) and GnRHR gene expression have been reported during sexual maturation. For example, *GnRHR-2a,* the expression of which increases in the pituitary in mature fish, is considered the main hypophysiotropic receptor in the Atlantic cod (Hildahl et al., 2013); pituitary GnRH receptor expression was also showed to increase during gonad maturation in pejerrey and European sea bass (Guilgur et al., 2009; Alvarado et al., 2013).

4.4. Changes in the expression of genes of the dopaminergic inhibitory control of gonadotropins

We found that the *TH* transcripts in the brain did not change between yellow and silver stages, suggesting that DA synthesis remained constant during silvering in the Japanese eels. It enhances the idea that DA plays an inhibitory role on pituitary gonadotropin synthesis and release, and eels remain blocked at a prepubetal stage if their reproductive migration does not occur (Sebert et al., 2008). Together with the lack of stimulation by GnRH, it also explains why eels need chronic treatment with exogenous hormones to stimulate the gonadal development.

We also analyzed the expression of the two dopamine D2 receptors present in the eel, *D2AR* and *D2BR*. We observed no change in *D2AR* transcript levels in the brain and pituitary between yellow and silver stage. In contrast, we evidenced a significant decrease in *D2BR* expression in the forebrain and pituitary. Remarkably, ongoing studies from our group revealed the expression of *D2BR* in pituitary gonadotropic cells in the European eel (Jolly, Dufour et al., unpublished data). The decrease in the pituitary expression of *D2BR* during silvering, as shown by the present study, would thus allow a reduced inhibitory effect of DA. We may raise the hypothesis that this regulation of *D2BR* gene expression is one of the neuroendocrine mechanisms involved in the slight activation of the pituitary gonadotropin expression and gonadal activity that occur at silvering.

In conclusion, this study investigated the differential expressions of various neuroendocrine parameters possibly involved in the regulation of the reproductive function during silvering in wild female Japanese eels. The expression of pituitary gonadotropins, LH and FSH, increased during silvering, which may account for the significant stimulation of ovarian activity. Concerning the stimulatory GnRH control of gonadotropic function, our results indicated no sign of activation of gene expression during silvering. Concerning the dopaminergic inhibitory control, the DA synthesis activity, as reflected by tyrosine hydroxylase expression, was maintained, but a significant decrease in *D2BR* transcripts in the pituitary occurred during silvering. This reveals that a reduction in the DA inhibitory control, via the decrease in pituitary *D2BR* expression, may constitute a regulatory mechanism for the moderate but significant activation of the pituitary–gonadal axis during silvering. Activation of GnRH system, and further reduction of the DA inhibition, may occur during the reproductive oceanic migration and at the spawning ground, leading to further activation of pituitary gonadotropins and complete gonadal development.

Acknowledgments

This study was supported by the bilateral France ANR/Taiwan NSC project PUBERTEEL: NSC98-2923-B-019-001-MY3/ANR-08-BLAN-0173.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ygcen.2014.08.001.

References

Alvarado, M.V., Carrillo, M., Felip, A., 2013. Expression of kisspeptins and their Receptors, gnrh-1/gnrhr-II-1a and gonadotropin genes in the brain of adult male and female European sea bass during different gonadal stages. Gen. Comp. Endocrinol. 187, 104–116.

Aroua, S., Schmitz, M., Baloche, S., Vidal, B., Rousseau, K., Dufour, S., 2005. Endocrine evidence that silvering, a secondary metamorphosis in the eel, is a pubertal rather than a metamorphic event. Neuroendocrinology 82, 221–232.

Boularand, S., Biguet, N.F., Vidal, B., Veron, M., Mallet, J., Vincent, J.D., Dufour, S., Vernier, P., 1998. Tyrosine hydroxylase in the european eel (*Anguilla anguilla*): cDNA cloning, brain distribution, and phylogenetic analysis. J. Neurochem. 71, 460–470.

Chow, S., Kurogi, H., Mochioka, N., Kaji, S., Okazaki, M., Tsukamoto, K., 2009. Discovery of mature freshwater eels in the open ocean. Fish Sci. 75, 257–259.

Cottrill, R.A., McKinley, R.S., van der Kraak, G., Dutil, J.D., Reid, K.B., McGrath, K.J., 2001. Plasma non-esterified fatty acid profiles and 17β-oestradiol levels of juvenile immature and maturing adult American eels in the St Lawrence River. J. Fish Biol. 59, 364–379.

De Leeuw, R., Van 't Veer, C., Goos, H.J., Van Oordt, P.G., 1988. The dopaminergic regulation of gonadotropin-releasing hormone receptor binding in the pituitary of the African catfish, *Clarias gariepinus*. Gen. Comp. Endocrinol. 72, 408–415.

Dufau, M.L., 1998. The luteinizing hormone receptor. Ann. Rev. Physiol. 60, 461–496.

Dufour, S., Burzawa-Gerard, E., Le Belle, N., Sbaihi, M., Vidal, B., 2003. Reproductive endocrinology of the European eel, *Anguilla anguilla*. In: Aida, K., Tsukamoto, K., Yamauchi, K. (Eds.), Eel Biology. Springer Verlag, Tokyo, pp. 373–383.

Dufour, S., Montero, M., Le Belle, N., Bassompierre, M., King, J.A., Millar, R.P., Peter, R.E., Fontaine, Y.A., 1993. Differential distribution and response to experimental sexual maturation of two forms of brain gonadotropin-releasing hormone (GnRH) in the European eel *Anguilla anguilla*. Fish Physiol. Biochem. 11, 99–106.

Dufour, S., Sébert, M.E., Weltzien, F.A., Rousseau, K., Pasqualini, C., 2010. Neuroendocrine control by dopamine of teleost reproduction (Invited review). J. Fish Biol. 76, 129–160.

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. Ann. N. Y. Acad. Sci. 1040, 9–21.

Guilgur, L.G., Strüssmann, C.A., Somoza, G.M., 2009. MRNA expression of GnRH variants and receptors in the brain, pituitary and ovaries of pejerrey (*Odontesthes bonariensis*) in relation to the reproductive status. Fish Physiol. Biochem. 35, 157–166.

Han, Y.S., Liao, I.C., Huang, Y.S., He, J.T., Chang, C.W., Tzeng, W.N., 2003a. Synchronous changes of morphology and gonadal development of silvering Japanese eel *Anguilla japonica*. Aquaculture 219, 783–796.

Han, Y.S., Liao, I.C., Huang, Y.S., Tzeng, W.N., Yu, J.Y., 2003b. Profiles of PGH-alpha, GTH I-beta, and GTH II-beta mRNA transcript levels at different ovarian stages in the wild female Japanese eel *Anguilla japonica*. Gen. Comp. Endocrinol. 133, 8–16.

Han, Y.S., Liao, I.C., Tzeng, W.N., Huang, Y.S., Yu, J.Y., 2003c. Serum estradiol-17beta and testosterone levels during silvering in wild Japanese eel *Anguilla japonica*. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 136, 913–920.

Hildahl, J., Taranger, G.L., Norberg, B., Haug, T.M., Weltzien, F.A., 2013. Differential regulation of GnRH ligand and receptor genes in the brain and pituitary of Atlantic cod exposed to different photoperiod. Gen. Comp. Endocrinol. 180, 7–14.

Jeng, S.R., Pasquier, J., Yueh, W.S., Chen, G.R., Lee, Y.H., Dufour, S., Chang, C.F., 2012. Differential regulation of the expression of cytochrome P450 aromatase, estrogen and androgen receptor subtypes in the brain-pituitary-ovarian axis of the Japanese eel (*Anguilla japonica*) reveals steroid dependent and independent mechanisms. Gen. Comp. Endocrinol. 175, 163–172.

Jeng, S.R., Yueh, W.S., Chen, G.R., Lee, Y.H., Dufour, S., Chang, C.F., 2007. Differential expression and regulation of gonadotropins and their receptors in the Japanese eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 154, 161–173.

Levavi-Sivan, B., Safarian, H., Rosenfeld, H., Elizur, A., Avitan, A., 2004. Regulation of gonadotropin-releasing hormone (GnRH)-receptor gene expression in tilapia: effect of GnRH and dopamine. Biol. Reprod. 70, 1545–1551.

Lokman, P.M., Young, G., 1998. An in tersexual migratory (silver) longfinned New Zealand eel and its gonadal response to treatment with salmon pituitary homogenate. J. Fish Biol. 52, 547–555.

Nagae, M., Todo, T., Gen, K., Kato, Y., Young, G., Adachi, S., Yamauchi, K., 1996. Molecular cloning of the cDNAs encoding pituitary glycoprotein hormone alpha- and gonadotropin II beta-subunits of the Japanese eel, *Anguilla japonica*, and increase in their mRNAs during ovarian development induced by injection of chum salmon pituitary homogenate. J. Mol. Endocrinol. 16, 171–181.

Nagahama, Y., Yoshikuni, M., Yamashita, M., Tokumoto, T., Katsu, Y., 1995. Regulation of oocyte growth and maturation in fish. Curr. Top. Dev. Biol. 30, 103–145.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Iinuma, N., Hirose, K., 1997. Artificial induction of maturation and fertilization in the Japanese eel *Anguilla japonica*. Fish Physiol. Biochem. 17, 163–169.

Omeljaniuk, R.J., Habibi, H.R., Peter, R.E., 1989. Alterations in pituitary GnRH and dopamine receptors associated with the seasonal variation and regulation of gonadotropin release in the goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 74, 392–399.

Pasqualini, C., Weltzien, F.A., Vidal, B., Baloche, S., Rouget, C., Gilles, N., Servent, D., Vernier, P., Dufour, S., 2009. Two distinct dopamine D2 receptor genes in the European eel: molecular characterization, tissue-specific transcription, and regulation by sex steroids. Endocrinology 150, 1377–1392.

Pasquier, J., Lafont, A.G., Jeng, S.R., Morini, M., Dirks, R., van den Thillart, G., Tomkiewicz, J., Tostivint, H., Chang, C.F., Rousseau, K., Dufour, S., 2012. Multiple kisspeptin receptors in early osteichthyans provide new insights into the evolution of this receptor family. PLoS One 7, e48931.

Peñaranda, D.S., Mazzeo, I., Hildahl, J., Gallego, V., Nourizadeh-Lillabadi, R., Perez, L., Asturiano, J.F., Weltzien, F.A., 2013. Molecular characterization of three GnRH receptor paralogs in the European eel, *Anguilla anguilla*: tissue-distribution and changes in transcript abundance during artificially induced sexual development. Mol. Cell. Endocrinol. 369, 1–14.

Rousseau, K., Lafont, A.G., Pasquier, J., Maugars, G., Jolly, C., Sébert, M.E., Aroua, S., Pasqualini, C., Dufour, S., 2013. Advances in eel reproductive physiology and endocrinology. In: Trischitta, F., Takei, Y., Sébert, P. (Eds.), Eel Physiology. CRC Press, London, pp. 1–43.

Sbaihi, M., Fouchereau-Pero, M., Meunier, F., Elie, P., Mayer, I., Burzawa-Gerard, E., Vidal, B., Dufour, S., 2001. Reproductive biology of the conger eel from the south coast of Brittany, France and comparison with the Europe eel. J. Fish Biol. 59, 302–318.

Sebert, M.E., Legros, C., Weltzien, F.A., Malpaux, B., Chemineau, P., Dufour, S., 2008. Melatonin activates brain dopaminergic systems in the eel with an inhibitory impact on reproductive function. J. Neuroendocrinol. 20, 917–929.

Simoni, M., Gromoll, J., Hoppner, W., Nieschlag, E., 1997. Molecular pathophysiology of the pituitary-gonadal axis. Adv. Exp. Med. Biol. 424, 89–97.

Stone, R., 2003. Freshwater eels are slip-sliding away. Science 302, 221–222.

Tesch, F.W., 1977. The Eel, Biology and Management of Anguillid Eels. Chapman & Hall, London, p. 434.

van den Thillart, G., Dufour, S., Rankin, J.C., 2009. Spawning migration of the European eel, in: Fish & Fisheries Series, vol. 30, Springer Verlag, Tokyo.

Tsukamoto, K., 1992. Discovery of the spawning area for Japanese eel. Nature 356, 789–791.

Vidal, B., Pasqualini, C., Le Belle, N., Holland, M.C., Sbaihi, M., Vernier, P., Zohar, Y., Dufour, S., 2004. Dopamine inhibits luteinizing hormone synthesis and release in the juvenile European eel: a neuroendocrine lock for the onset of puberty. Biol. Reprod. 71, 1491–1500.

Weltzien, F.A., Andersson, E., Andersen, O., Shalchian-Tabrizi, K., Norberg, B., 2004. The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*Pleuronectiformes*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 137, 447–477.

Weltzien, F.A., Pasqualini, C., Le Belle, N., Vidal, B., Vernier, P., Dufour, S., 2005. Brain expression of tyrosine hydroxylase and its regulation by steroid hormones in the European eel quantified by real-time PCR. Ann. N. Y. Acad. Sci. 1040, 518–520.

Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A., Levavi-Sivan, B., 2003. Regulation of fish gonadotropins. Int. Rev. Cytol. 225, 131–185.

Yu, K.L., Peter, R.E., 1992. Adrenergic and dopaminergic regulation of gonadotropin-releasing hormone release from goldfish preoptic-anterior hypothalamus and pituitary in vitro. Gen. Comp. Endocrinol. 85, 138–146.

Zohar, Y., Munoz-Cueto, J.A., Elizur, A., Kah, O., 2010. Neuroendocrinology of reproduction in teleost fish. Gen. Comp. Endocrinol. 165, 438–455.