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Gonadotropin-releasing hormone-independent effects of recombinant vertebrate ancient long opsin in the goldfish *Carassius auratus* reveal alternative reproduction pathways

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Abstract Vertebrate ancient long (VAL)-opsin is a green-sensitive photoreceptor that shows high sequence similarity to vertebrate ancient opsin, which is considered to play a role in sexual maturation via gonadotropin-releasing hormone (GnRH); however, the role of VAL-opsin in vertebrate sexual maturity remains unclear. Therefore, we investigated the possible role of VAL-opsin in reproduction in the goldfish *Carassius auratus* under a state of GnRH inhibition. Goldfish were injected with recombinant VAL-opsin protein (0.5 μg/g body mass) and/or the GnRH antagonist cetrorelix (0.5 μg/fish), and changes in the mRNA expression levels of genes associated with goldfish reproduction were measured by quantitative polymerase chain reaction, including those involved in the hypothalamus-pituitary-gonad (HPG) axis, VAL-opsin, GnRH, the gonadotropins (GTHs) luteinizing hormone and follicle-stimulating hormone, and estrogen receptor (ER). Moreover, the fish were irradiated with a green light-emitting diode (520 nm) to observe the synergistic effect on the HPG axis with VAL-opsin. Green LED exposure significantly and slightly increased the VAL-opsin and GnRH levels, respectively; however, these effects were blocked in groups injected with cetrorelix at all time points. Cetrorelix significantly decreased the mRNA levels of GTHs and ER, whereas these hormones recovered by co-treatment with VAL-opsin. These results indicate that green LED is an effective light source to promote the expression of sex hormones in fish. Moreover, VAL-opsin not only affects activity of the HPG axis but also appears to act on the pituitary gland directly to stimulate a new sexual maturation pathway that promotes the secretion of GTHs independent of GnRH.

Keywords GnRH · Goldfish · Green light · Photoreceptor · Reproduction · VAL-opsin

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Introduction

The sexual development and maturation of fish are controlled by various sex hormones such as gonadotropin-releasing hormone (GnRH), gonadotropins (GTHs) and steroid hormones, neuroendocrine substances, and gonadal hormones (Lee et al. 2001). In particular, GTH has been reported to play a major role in gonadal development and maturation and is known to stimulate reproduction and steroid hormone regulation in vertebrates, including fish (Kim et al. 1995; Grandi et al. 2003). Of the main GTHs, follicle-stimulating hormone (FSH) in fish is involved in early oocyte formation, vitellogenin synthesis, and spermatogenesis, whereas luteinizing hormone (LH) is involved in the final maturation, ovulation, and localization of sex steroid hormones (Nagahama et al. 1995; Kobayashi et al. 2006). Moreover, estrogen is a typical sex steroid

hormone that binds to the estrogen receptor (ER) and affects the vitellogenesis and sexual maturation of vertebrates, including fish (Bowman et al. 2002; Davis et al. 2009).

Sexual maturity of fish is affected by various environmental factors such as water temperature, salinity, and photoperiod (Pankhurst and Thomas 1998; Duncan et al. 2000). Among them, light is known to regulate not only sexual maturity but also the hormone secretion that affects biorhythm, growth, and behavioral changes (Migaud et al. 2006; Pierce et al. 2008). Information from the light signal is transmitted to the central nervous system by photoreceptors, which are distributed in the pineal gland, deep brain, retina, and skin tissue (Karakatsouli et al. 2010; Nakane et al. 2010; Owen et al. 2010). A study in birds showed that the deep brain photoreceptors in the hypothalamus regulate the activity of the hypothalamus-pituitary-gonad (HPG) axis and play a role in sexual maturation Foster and Soni 1998; Halford et al. 2009; Nakane et al. 2010), in addition to regulating physiological changes such as skin color change and growth (Blanco-Vives et al. 2011; Blaser and Rosemberg 2012; Villamizar et al. 2014).

The deep brain photoreceptors identified to date include rhodopsin, cone-like opsin, melanopsin, vertebrate ancient (VA) opsin, and VA long (VAL)–opsin (Minamoto and Shimizu 2002; Drivenes et al. 2003). In particular, VA opsin is considered to directly or indirectly regulate sexual maturation through its interaction with GnRH since it is was detected in neurons that synthesize algae GnRH (García-Fernández et al. 2015). VAL-opsin is a variant of VA opsin that has the same core sequence but contains a cytoplasmic tail at the C-terminal region (Soni and Foster 1997; Davies et al. 2012). VAL-opsin is present in the hypothalamus in the diencephalic ventricle and retinal horizontal cells and transmits light information to the central nervous system through binding with the light-sensitive compound 11-cis-retinal (Kojima et al. 2008). In particular, VAL-opsin is known to be a green-sensitive pigment that reacts sensitively to light in the green-wavelength region (Kojima et al. 2000). However, information on the detailed role and function of VAL-opsin in the regulation of sexual maturity is limited.

Therefore, in this study, we aimed to gain insight into the association between VAL-opsin and the HPG axis in the goldfish *Carassius auratus*.

Materials and methods

Experimental fish and conditions

Female goldfish *Carassius auratus* ($n = 408$; length, 9.3 ± 0.5 cm; mass, 10.5 ± 0.8 g; gonadosomatic index = gonad weight × 100/mass (Crim and Glebe 1990), 4.5 ± 10) were purchased from a commercial aquarium (Busan, Korea) and maintained in five 300-L circulation filter tanks to acclimate to the laboratory conditions for 24 h prior to conducting the experiments. A total of eight experimental conditions were established, including the control (no injection) and injection with the GnRH inhibitor or recombinant VAL-opsin alone or in combination with either white or green light exposure. The fish were randomly allocated to each of the eight groups in duplicate with 34 fish per tank. The goldfish were reared under an automatic temperature regulation system (JS-WBP-170RP; Johnsam Co., Buchoen, Korea) with the water temperature maintained at 22 °C.

The fish were exposed to light from a green-wavelength (520 nm) LED (Daesin LED Co., Kyunggi, Korea) providing light intensity at the water surface of approximately 0.5 W/m^2, or with a white fluorescent bulb (27 W, wavelength range 350–650 nm) providing light intensity at the water surface of approximately 0.96 W/m^2 (Behrens et al. 2000; Choi et al. 2015). The fish were exposed to a 12-h light:12-h dark photoperiod (lights on at 07:00 h and lights off at 19:00 h). The fish were reared under these conditions with daily feeding of a commercial feed until the day prior to sampling. Spectral analysis of the lights was performed using a spectroradiometer (FieldSpec®, ASD, CO, USA).

Injections of VAL-opsin and GnRH antagonist (cetrorelix)

To investigate the effects of VAL-opsin on sexual maturation, the fish were anesthetized with 2-phenoxyethanol and then injected (intramuscular) with recombinant VAL-opsin (0.5 μg/g BM; CSB-EP517517SWI, Cusabio, MD, USA) and/or a GnRH antagonist (cetrorelix, 0.5 μg/fish; C5249, Sigma, MO, USA; cetrorelix was used as an inhibitor of GnRH because it is well established to play a role in inhibiting the synthesis and release of FSH and LH by binding to the GnRH receptor of the pituitary gland, thereby preventing binding of GnRH (Reissmann et al. 2000;

Gábor and Marilena 2009)) dissolved in 0.9% physiological saline; the concentration of injection was determined to be 10 μL/g BM considering the daily dose (3 mg) of humans and the weight of the experimental fish (Diedrich et al. 1994; Choi and Choi 2018). The control group (no treatment of either substance) comprised the control fish for the light experiment. Each tank included 34 fish.

One week after the injection, the fish were anesthetized with 200 μL/L of 2-phenoxyethanol (Daejung Co., Kyunggi, Korea) to minimize stress prior to total RNA extraction. The fish were euthanized by spinal transection (sampling at 6, 12, and 24 h after injections and at 0 h before injection; first sampling at 07:00 h) to collect the brain, pituitary, and gonads under dim light using an attenuated white fluorescent bulb. Tissues were separated by centrifugation (4 °C, 1000×*g*, 10 min) and stored at − 80 °C until analysis.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from each sample using TRI reagent® (Molecular Research Center, Inc., OH, USA) according to the manufacturer's instructions. After DNase treatment, the integrity of total RNA was confirmed by agarose gel electrophoresis, and the purity was measured on a NanoDrop spectrophotometer (BioDrop, England) for determination of the optical density at 260 nm (OD_{260}), OD_{280}, and OD_{230} ($OD_{260/280} \geq 1.8$, $OD_{260/230} \geq 1.5$). Total RNA (2 μg) was reverse-transcribed in a total volume of 20 μL using an oligo-d$(T)_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol. The resulting cDNA was diluted and stored at 4 °C for use in quantitative polymerase chain reaction (QPCR).

QPCR

QPCR was performed according to the recommendations of the Minimum Information for publication of Quantitative real-time PCR Experiments guidelines (Bustin et al. 2009). QPCR was conducted to determine the relative expression levels of VAL-opsin, GnRH, FSH, LH, ER, and β-actin mRNA using the cDNA reverse-transcribed from the total RNA extracted from the hypothalamus, pituitary, and gonads. The primers (Table 1) were designed for each gene using Beacon Designer software (Bio-Rad, Hercules, CA, USA). Primer alignments were performed with the BLAST database to ensure their specificity. PCR amplification was conducted using a Bio-Rad CFX96™ Real-time PCR Detection System (Bio-Rad) with a 20-μL reaction volume containing 10 μL iQ™ SYBR Green Supermix (Bio-Rad), 5 μL of diluted cDNA (50-fold), 0.5 μL of 300 nM each of forward and reverse primers, and 4 μL H_2O. The QPCR program was as follows: 95 °C for 5 min, followed by 50 cycles of 95 °C for 20 s and 55 °C for 20 s. Amplification of a single product from PCR was confirmed by melting curve analysis, and representative samples were electrophoresed to verify that only a single product was present. As internal controls, the experiments were duplicated with β-actin, and all data are expressed relative to the corresponding β-actin threshold cycle (ΔCt) levels. The calibrated ΔCt value (ΔΔCt) for each sample and internal controls (β-actin) was calculated using the $2^{-\Delta\Delta Ct}$ method: ($\Delta\Delta Ct = 2\text{^}-(\Delta Ct_{sample} - \Delta Ct_{internal\ control})$).

Statistical analysis

All data were analyzed using the SPSS statistical package (version 19.0; SPSS Inc., USA). A two-way analysis of variance followed by Tukey's post hoc test was used to compare differences among groups ($P < 0.05$). The values are expressed as the means ± standard deviation (SD).

Results

Changes in VAL-opsin mRNA expression in the brain

The expression of VAL-opsin mRNA using cDNA extracted from the goldfish brain was not significantly different between the cetrorelix-injected groups and non-injected control groups at any time point, indicating that GnRH inhibition did not affect VAL-opsin expression on its own. However, as expected, VAL-opsin injection significantly increased the VAL-opsin mRNA levels compared with those of the control groups (non-injection). In particular, the VAL-opsin expression levels in the groups exposed to green-wavelength LED light were higher than those in the groups exposed to white fluorescent light. Val-opsin mRNA expression levels in G in the Val-opsin treatment group at 6 h were higher than in the other treatment groups ($P < 0.05$) and were maintained after 6 h (Fig. 1).

Table 1 Primers used for QPCR amplification

Genes (accession no.)	Primer	DNA sequences
VAL-opsin (AB383149)	Forward	5′-CAC CAC CTG CTT CAT CTT-3′
	Reverse	5′-TCA TCA CAA CCA CCA TAC G-3′
sGnRH (U30301)	Forward	5′-CCA ACA GAC GAG GAA GAG-3′
	Reverse	5′-CGA TTC AGG ACG CAA ACT-3′
FSH (D88023)	Forward	5′-CCT GGA AAG TGA GGA ATG-3′
	Reverse	5′-GTT CTG GTA AGA CAG CAT CA-3′
LH (D88024)	Forward	5′-TGT CCT ATT CTC TGT AAT TGT CC-3′
	Reverse	5′-GTC TCA TTA ACT GGC TCA CA-3′
ER (AY055725)	Forward	5′-AGC GGT AAT GAA GAT CGT G-3′
	Reverse	5′-TGT GTT TCT GTC GTG AGC-3′
β-actin (AB039726)	Forward	5′-ATT TGG CAT CAC ACC TTC T-3′
	Reverse	5′-TTC TCC CTG TTG GCT TTG-3′

Changes in GnRH mRNA expression in the brain

The expression level of GnRH mRNA using cDNA extracted from the goldfish brain was not significantly different between the cetrorelix-injected groups and non-injected groups at all time points. Thus, GnRH mRNA expression was not affected by cetrorelix injection. However, the expression levels of GnRH were higher in the VAL-opsin injection groups than those in the control groups (non-injection), with a higher increase observed in the groups that were also exposed to green-wavelength light compared with those exposed to white fluorescent light. GnRH mRNA expression levels in the Val-opsin treatment group at 6 h were higher than in the other treatment groups ($P < 0.05$) and were maintained after 6 h. In addition, for GnRH mRNA expression at 12 h, Val-opsin in the green-wavelength light–exposed groups was higher than in the groups exposed to white fluorescent light ($P < 0.05$), other treatments, and control conditions (Fig. 2).

Changes in GTH mRNA expression levels in the pituitary

The expression levels of FSH and LH mRNA using cDNA extracted from the goldfish pituitary were decreased in the cetrorelix-injected group compared with those in the non-injected groups. By contrast, the expression levels in the VAL-opsin injection group were significantly higher than those in the control groups (non-injection) with time. FSH and LH mRNA expression levels in G in Val at 6 h were higher than those in the control group ($P < 0.05$) and were high at 0 h (Fig. 3).

Changes in ER mRNA expression levels in the gonads

Similar to the results for FSH and LH mRNA expression, the expression level of ER mRNA using cDNA extracted from the goldfish gonads was decreased in the cetrorelix-injected group compared with that in the non-injected group. However, the ER expression levels in the VAL-opsin injection groups were significantly higher than those in the control groups (non-injection); ER mRNA expression levels in the Val-opsin treatment groups at 6 h were higher than in the other treatment groups ($P < 0.05$) and were increased after 6 h. However, in the cetrorelix treatment groups, ER mRNA expression levels were decreased (Fig. 4).

Discussion

In this study, we investigated the effect of VAL-opsin on the HPG axis and the possibility of its involvement in sexual maturation by participating in visual pathways other than the HPG axis. To investigate this possibility, we irradiated female fish with light in the green wavelength and then injected cetrorelix as a GnRH inhibitor and/or recombinant VAL-opsin and observed the expression changes of sex-related genes.

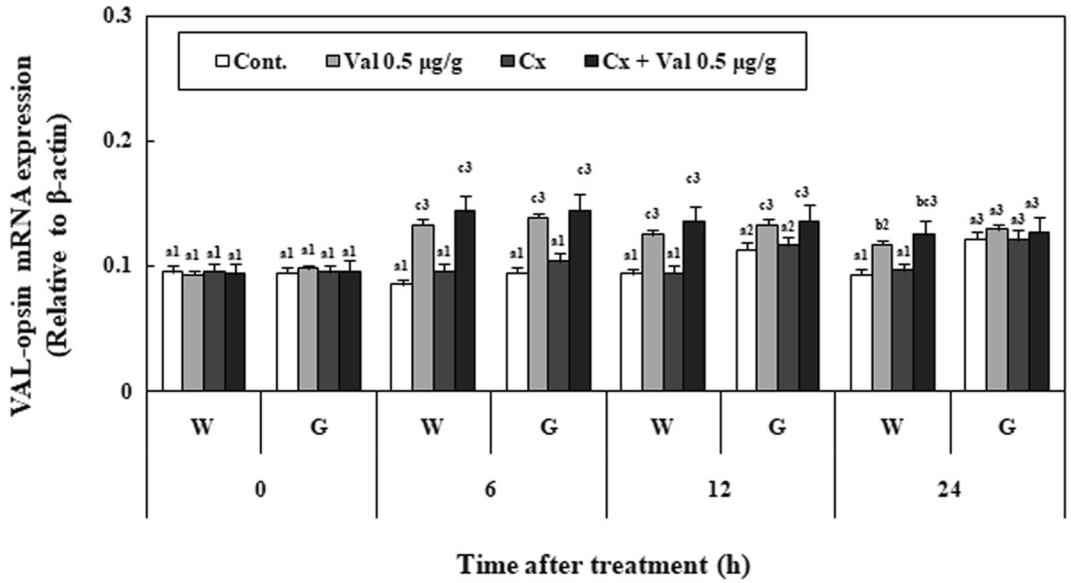

Fig. 1 Changes in vertebrate ancient long opsin (VAL-opsin) mRNA expression levels upon light treatment (green LED (520 nm) (G) and a white fluorescent bulb (W)) in the hypothalamus after VAL-opsin (0.5 μg/g BM), cetrorelix (Cx) (0.5 μg/fish), or cetrorelix (Cx) (0.5 μg/fish) + VAL-opsin (0.5 μg/g BM) injections, and no injection as a control group (Cont.). Different letters indicate significant differences between groups with respect to simultaneous VAL-opsin injection ($P < 0.05$). Different numbers indicate significant differences between groups with respect to simultaneous cetrorelix injection ($P < 0.05$). All values are presented as the means ± SD ($n = 5$)

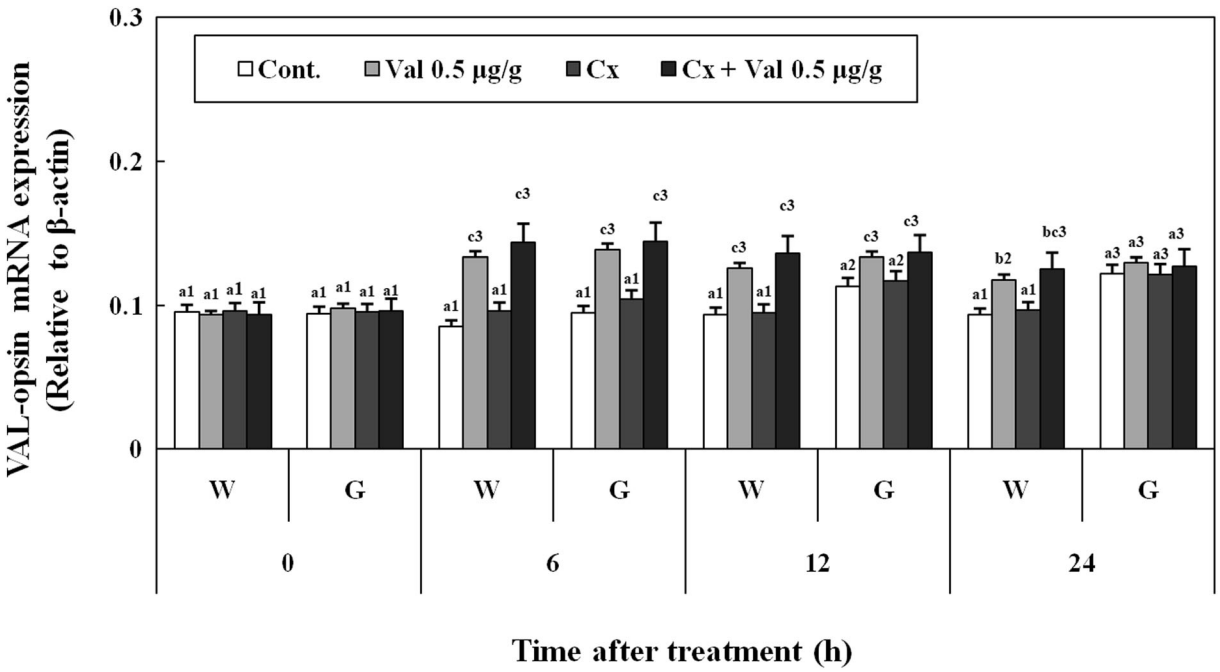

Fig. 2 Changes in GnRH mRNA expression levels upon light treatment (green LED (520 nm) (G) and a white fluorescent bulb (W)) in the hypothalamus after VAL-opsin (0.5 μg/g BM), cetrorelix (Cx) (0.5 μg/fish), or cetrorelix (Cx) (0.5 μg/fish) + VAL-opsin (0.5 μg/g BM) injections, and no injection as a control group (Cont.). Different letters indicate significant differences between groups with respect to simultaneous VAL-opsin injection ($P < 0.05$). Different numbers indicate significant differences between groups with respect to simultaneous cetrorelix injection ($P < 0.05$). All values are presented as the means ± SD ($n = 5$)

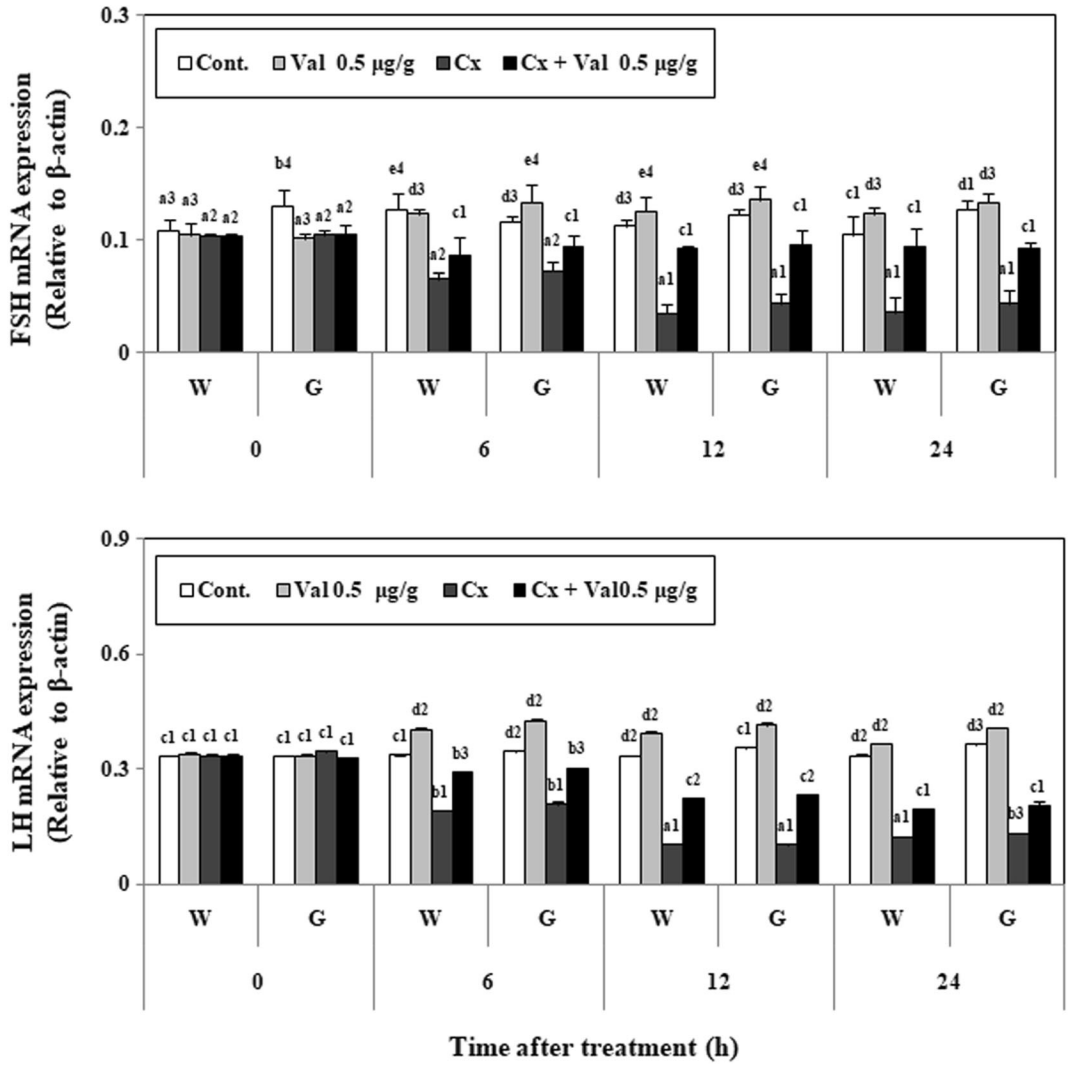

Fig. 3 Changes in FSH (A) and LH (B) mRNA expression levels upon light treatment (green LED (520 nm) (G) and a white fluorescent bulb (W)) in the pituitary after VAL-opsin (0.5 μg/g BM), cetrorelix (Cx) (0.5 μg/fish), or cetrorelix (Cx) (0.5 μg/fish) + VAL-opsin (0.5 μg/g BM) injections, and no injection as a control group (Cont.). Different letters indicate significant differences between groups with respect to simultaneous VAL-opsin injection ($P < 0.05$). Different numbers indicate significant differences between groups with respect to simultaneous cetrorelix injection ($P < 0.05$). All values are presented as the means ± SD ($n = 5$)

First, we confirmed that the expression level of VAL-opsin mRNA increased over time after recombinant VAL-opsin injection, with significantly higher expression levels observed in the fish also exposed to green light, and this increase was not affected by injection with the GnRH antagonist cetrorelix.

In a similar study, Kojima et al. (2008) reported that the expression level of the VAL-opsin gene was significantly increased in the brain after zebrafish (*Danio rerio*) were irradiated with green-wavelength light. In addition, Song et al. (2016) reported that the expression level of the VAL-opsin gene was significantly increased in the brain after administration of GTH to goldfish. In line with these studies, we found that green-wavelength light was directly responsible for the increase in the expression of the VAL-opsin gene. This further suggested that cetrorelix treatment was the main cause of inhibition of HPG axis activity.

The hypothalamic GnRH gene is expressed in the center of the HPG axis and regulates the maturation of goldfish. VAL-opsin injection significantly increased the expression level of GnRH mRNA in a time-

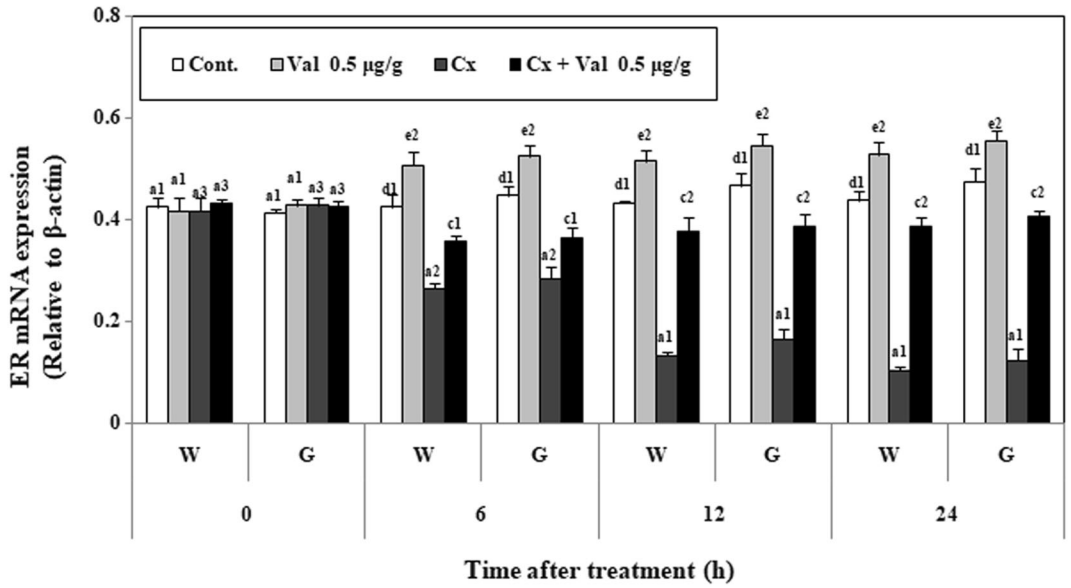

Fig. 4 Changes in ER mRNA expression levels upon light treatment (green LED (520 nm) (G) and a white fluorescent bulb (W)) in the gonads after VAL-opsin (0.5 μg/g BM), cetrorelix (Cx) (0.5 μg/fish), or cetrorelix (Cx) (0.5 μg/fish) + VAL-opsin (0.5 μg/g BM) injections, and no injection as a control group (Cont.). Different letters indicate significant differences between groups with respect to simultaneous VAL-opsin injection ($P < 0.05$). Different numbers indicate significant differences between groups with respect to simultaneous cetrorelix injection ($P < 0.05$). All values are presented as the means ± SD ($n = 5$)

dependent manner, and exposure to green-wavelength light further increased these levels. However, the group treated with recombinant VAL-opsin and cetrorelix did not show a significant difference in the expression of GnRH mRNA. Halford et al. (2009) reported that chicken VAL-opsin neurons are located at the same position in the hypothalamus as GnRH. In addition, Song et al. (2016) reported that exposure of goldfish to green light increased the expression levels of both VAL-opsin and GnRH. Thus, our results suggest that inhibition of GnRH by cetrorelix could still not increase the GnRH mRNA expression even under green light.

Both green light and recombinant VAL-opsin injection increased the levels of FSH, LH, and ER, which are regulated by GnRH. Moreover, in contrast to the results for hypothalamic GnRH, the FSH, LH, and ER mRNA expression levels in the cetrorelix-injected group were significantly (approximately 70%) decreased over time, and these levels were reduced with combined cetrorelix and recombinant VAL-opsin treatment compared with those of the group injected with only recombinant VAL-opsin. These results are supported by a previous study in which Fischer et al. (2013) suggested that VAL-opsin would coexist with teleost multiple tissue opsin (TMT opsin), one of the deep brain "photoreceptors", to play a role in the sexual maturation of fish. Although the effect of cetrorelix on the secretion of GTHs and steroid hormones remains unknown, Ganesh (2017) reported that injection of cetrorelix significantly delayed the ovarian development of the cichlid *Oreochromis mossambicus*. Similar findings have been reported in humans. Berardelli et al. (2011) showed that plasma FSH, LH, and estradiol concentrations decreased at 24 h after cetrorelix injection (3.0 mg) to healthy women (body mass index 21.5 ± 1.7 kg/m) and then returned to their initial levels after 96 h. Pechstein et al. (2000) also reported that FSH, LH, and testosterone levels decreased by 80%, 45%, and 95%, respectively, after injection of 3.0 mg cetrorelix in healthy human subjects.

Therefore, considering the present results and previous findings, we conclude that VAL-opsin regulates the overall activity of the HPG axis. In addition, although the expression level of GnRH mRNA was increased in all experimental groups injected with recombinant VAL-opsin, co-injection with cetrorelix decreased the expression levels of FSH, LH, and ER, even below control levels, suggesting that recombinant VAL-opsin is involved in different maturation pathways in the HPG axis. In other words, it seems that VAL-opsin directly acts on the pituitary gland of HPG axis to directly increase the levels of GTHs.

Overall, our results indicate that VAL-opsin increases the expression of sex hormones associated with the HPG axis in goldfish. Moreover, green LED seems to be an effective light source to increase the expression of green-sensitive VAL-opsin and consequently promote the expression of sex hormones. Ultimately, these results suggest that VAL-opsin affects GnRH secretion and gonadal maturation through the normal HPG axis. Moreover, the significant increase in the expression of GTHs under GnRH inhibition by cetrorelix combined with VAL-opsin injection compared with their marked reduction under cetrorelix treatment alone indicates that opsin directly acts on the pituitary gland in the HPG axis to promote the secretion of GTHs. In other words, this result suggests the possibility of a distinct sexual maturity path outside the normal HPG axis that is dependent on GnRH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Human and animal rights All handling and experimental protocols with fish complied with ethical guidelines in accordance with the Animal Protection Act and were approved by the Institutional Animal Care Use Committee of Korea.

References

Behrens UD, Douglas RH, Sugden D, Davies DJ, Wagner HJ (2000) Effect of melatonin agonists and antagonists on horizontal cell spinule formation and dopamine release in a fish retina. Cell Tissue Res 299:299–306. https://doi.org/10.1007/s004419900161

Berardelli R, Gianotti L, Karamouzis I, Picu A, Giordano R, Angelo V, Zinnà D, Lanfranco F, Ghigo E, Arvat E (2011) Effects of cetrorelix, a GnRH-receptor antagonist, on gonadal axis in women with functional hypothalamic amenorrhea. Gynecol Endocrinol 27:753–758. https://doi.org/10.3109/09513590.2010.526661

Blanco-Vives B, Aliaga-Guerrero M, Canavate JP, Munoz-Cueto JA, Sanchez-Vazquez FJ (2011) Does lighting manipulation during incubation affect hatching rhythms and early development of sole? Chronobiol Int 28:300–306. https://doi.org/10.3109/07420528.2011.560316

Blaser RE, Rosemberg DB (2012) Measures of anxiety in zebrafish (*Danio rerio*): dissociation of black/white preference and novel tank test. PLoS One 7:e36931. https://doi.org/10.1371/journal.pone.0036931

Bowman CJ, Kroll KJ, Gross TG, Denslow ND (2002) Estradiol-induced gene expression in largemouth bass (*Micropterus salmoides*). Mol Cell Endocrinol 196:67–77. https://doi.org/10.1016/S0303-7207(02)00224-1

Bustin SA, Benes V, Garson JA (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622. https://doi.org/10.1373/clinchem.2008.112797

Choi JY, Choi CY (2018) Effects of recombinant vertebrate ancient long opsin on reproduction in goldfish, *Carassius auratus*: profiling green-wavelength light. Fish Physiol Biochem 44:1027–1036. https://doi.org/10.1007/s10695-018-0490-4

Choi CY, Shin HS, Kim NN, Yang SG, Kim BS, Yu YM (2015) Time-related effects of various LED light spectra on reproductive hormones in the brain of the goldfish *Carassius auratus*. Biol Rhythm Res 46:671–682. https://doi.org/10.1080/09291016.2015.1046247

Crim LW, Glebe BD (1990) Reproduction. In: Schreck CB, Moyle PB (eds) Methods for fish biology. Amer Fish Soc Bethesda, p 529–547

Davies WI, Turton M, Peirson SN, Follett BK, Halford S, Garcia-Fernandez JM, Sharp PJ, Hankins MW, Foster RG (2012) Vertebrate ancient opsin photopigment spectra and the avian photoperiodic response. Biol Lett 8:291–294. https://doi.org/10.1098/rsbl.2011.0864

Davis LK, Visitacion N, Riley L, Hiramatsu N, Sullivan C, Hirano T, Gordon Grau E (2009) Effects of o,p'-DDE, heptachlor, and 17β-estradiol on vitellogenin gene expression and the growth hormone/insulin-like growth factor-I axis in the tilapia, *Oreochromis mossambicus*. Comp Biochem Physiol C 149:507–514. https://doi.org/10.1016/j.cbpc.2008.11.007

Diedrich K, Diedrich C, Santos E, Zoll C, Al-Hasani S, Reissmann T, Klingmüller D (1994) Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist cetrorelix during ovarian stimulation. Hum Reprod 9:788–791. https://doi.org/10.1093/oxfordjournals.humrep.a138597

Drivenes O, Soviknes AM, Ebbesson LO, Fjose A, Seo HC, Helvik JV (2003) Isolation and characterization of two teleost melanopsin genes and their differential expression within the inner retina and brain. J Comp Neurol 456:84–93. https://doi.org/10.1002/cne.10523

Duncan NJ, Selkirk C, Porter M, Hunter D, Magwood S, Bromage N (2000) The effect of altered photoperiods on maturation of male and female Atlantic salmon (*Salmo salar*), observations of different responses and mechanisms. In: Norberg B, Kjesbu OS, Taranger GL, Andersson E, Stefansson SO (eds) Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish. Bergen, Norway, p 344

Fischer RM, Fontinha BM, Kirchmaier S, Steger J, Bloch S, Inoue D, Panda S, Rumpel S, Tessmar-Raible K (2013) Co-expression of VAL- and TMT-opsins uncovers ancient photosensory interneurons and motorneurons in the vertebrate brain. PLoS Biol 11:e1001585. https://doi.org/10.1371/journal.pbio.1001585

Foster RG, Soni BG (1998) Extraretinal photoreceptors and their regulation of temporal physiology. Rev Reprod 3:145–150. https://doi.org/10.1530/revreprod/3.3.145

Gábor M, Marilena M (2009) Luteinizing hormone-releasing hormone antagonists. Expert Opin Ther Pat 19:1771–1785. https://doi.org/10.1016/S0090-4295(01)01238-9

Ganesh CB (2017) Influence of leucine-enkephalin on pituitary-ovary axis of the cichlid fish *Oreochromis mossambicus*. Fish Physiol Biochem 43:1253–1264. https://doi.org/10.1007/s10695-017-0369-9

García-Fernández JM, Cernuda-Cernuda R, Davies WI, Rodgers J, Turton M, Peirson SN, Follett BK, Halford S, Hughes S, Hankins MW, Foster RG (2015) The hypothalamic photoreceptors regulating seasonal reproduction in birds: a prime role for VA opsin. Front Neuroendocrinol 37:13–28. https://doi.org/10.1016/j.yfrne.2014.11.001

Grandi G, Colombo G, Chicca M (2003) Immunocytochemical studies on the pituitary gland of *Anguilla anguilla* L., in relation to early growth stages and diet-induced sex differentiation. Gen Comp Endocrinol 131:66–76. https://doi.org/10.1016/S0016-6480(02)00646-9

Halford S, Pires SS, Turton M, Zheng L, González-Menéndez I, Davies WL, Perison SN, García-Fernández JM, Hankins MW, Foster RG (2009) VA opsin-based photoreceptors in the hypothalamus of birds. Curr Biol 19:396–1402. https://doi.org/10.1016/j.cub.2009.06.066

Karakatsouli N, Papoutsoglou ES, Sotiropoulosa N, Mourtikasa D, Stigen-Martinsena T, Papoutsoglou SE (2010) Effects of light spectrum, rearing density and light intensity on growth performance of scaled and mirror common carp *Cyprinus carpio* reared under recirculating system conditions. Aqauculture 42:121–127. https://doi.org/10.1016/j.aquaeng.2010.01.001

Kim MH, Oka Y, Amano M, Kobayashi M, Okuzawa K, Hasegawa Y, Kawashima S, Suzuki Y, Aida K (1995) Immunocytochemical localization of sGnRH and cGnRH-II in the brain of goldfish, *Carassius auratus*. J Comp Neurol 356:72–82. https://doi.org/10.1016/j.cbpb.2012.04.006

Kobayashi T, Nishizawa K,Mitsumori, K (2006) Individual variation of hormonal recovery after cessation of luteinizing hormone-releasing hormone agonist therapy in men receiving long-term medical castration therapy for prostate cancer. Scand J Urol Nephrol 40:98–203. https://doi.org/10.1080/00365590600641533

Kojima D, Mano H, Fukada Y (2000) Vertebrate ancient-long opsin: a green-sensitive photoreceptive molecule present in zebrafish deep brain and retinal horizontal cells. J Neurosci 20:2845–2851. https://doi.org/10.1523/JNEUROSCI.20-08-02845.2000

Kojima D, Torii M, Fukada Y, Dowling JE (2008) Differential expression of duplicated VAL-opsin genes in the developing zebrafish. J Neurochem 104:1364–1371. https://doi.org/10.1111/j.1471-4159.2007.05093.x

Lee YH, Du JL, Yen FP, Lee CY, Dufour S, Huang JD, Sun LT, Chang CF (2001) Regulation of plasma gonadotropin II secretion by sex steroids, aromatase inhibitors, and antiestrogens in the protandrous black porgy, *Acanthopagrus schlegeli* Bleeker. Comp Biochem Physiol B 129:399–406. https://doi.org/10.1016/S1096-4959(01)00337-2

Migaud H, Taylor JF, Taranger GL, Davie A, Cerda-Reverter JM, Carrillo M, Hansen T, Bromage NB (2006) A comparative ex vivo and in vivo study of day and night perception in teleosts species using the melatonin rhythm. J Pineal Res 41:42–52. https://doi.org/10.1111/j.1600-079X.2006.00330.x

Minamoto T, Shimizu I (2002) A novel isoform of vertebrate ancient opsin in a smelt fish, *Plecoglossus altivelis*. Biochem Biophys Res Commun 290:280–286. https://doi.org/10.1006/bbrc.2001.6186

Nagahama Y, Yoshikuni M, Yamashita M, Tokumoto T, Katsu Y (1995) Regulation of oocyte growth and maturation in fish. Curr Top Dev Biol 30:103–45. https://doi.org/10.1016/s0070-2153(08)60565-7

Nakane Y, Ikegami K, Ono H, Yamamoto N, Yoshida S, Hirunagi K, Ebihara S, Kubo Y (2010) A mammalian neural tissues opsin (Opsin 5) is a deep brain photoreceptor in birds. Proc Natl Acad Sci 107:15264–15268. https://doi.org/10.1073/pnas.1006393107

Owen MAG, Davies SJ, Sloman KA (2010) Light colour influences the behaviour and stress physiology of captive tench (*Tinca tinca*). Rev Fish Biol Fisher 20:375–380. https://doi.org/10.1007/s11160-009-9150-1

Pankhurst NW, Thomas PM (1998) Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquaculture 166:163–177. https://doi.org/10.1080/00365590600641533

Pechstein B, Nagaraja NV, Hermann R, Romeis P, Locher M, Derendorf H (2000) Pharmacokinetic-pharmacodynamic modeling of testosterone and luteinizing hormone suppression by cetrorelix in healthy volunteers. J Clin Pharmacol 40:266–274. https://doi.org/10.1177/00912700022008937

Pierce LX, Noche RR, Ponomareva O, Chang C, Liang JO (2008) Novel function for period 3 and Exo-rhodopsin in rhythmic transcription and melatonin biosynthesis within the zebrafish pineal organ. Brain Res 1223:11–24. https://doi.org/10.1016/j.brainres.2008.05.020

Reissmann T, Schally AV, Bouchard P, Riethmuller H, Engel J (2000) The LHRH antagonist cetrorelix: a review. Hum Reprod Update 6:322–331. https://doi.org/10.1093/humupd/6.4.322

Song JA, Kim NN, Choi YJ, Choi JY, Kim B-S, Choi CY (2016) Effects of light-emitting diode spectra on the vertebrate ancient long opsin and gonadotropin hormone in the goldfish *Carassius auratus*. Biochem Biophys Res Commun 476:541–545. https://doi.org/10.1016/j.bbrc.2016.05.159

Soni BG, Foster RG (1997) A novel and ancient vertebrate opsin. FEBS Lett 406:279–283. https://doi.org/10.1016/S0014-5793(97)00287-1

Villamizar N, Vera LM, Foulkes NS, Sanchez-Vazquez FJ (2014) Effect of lighting conditions on zebrafish growth and development. Zebrafish 11:173–181. https://doi.org/10.1089/zeb.2013.0926