Neuropeptide FF indirectly affects testicular morphogenesis and functions in medaka

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Testicular morphogenesis and functions are considered to be under the control of neural and endocrine systems. However, the available literature is mainly limited to mammals, and it remains unclear how they are regulated in teleost species. Here, we demonstrated that neuropeptide FF (NPFF) in the brain is responsible for the follicle-stimulating hormone expression in the pituitary, which facilitates the testicular morphogenesis and androgen synthesis, and subsequently contributes to successful spermatogenesis. The present findings give us important insights into the neuroendocrine regulatory mechanisms underlying the testicular morphogenesis and functions in teleosts.

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The authors declare no competing interest.

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expression in WT and npff−/− pituitaries. We detected significantly lower fshb expression in npff−/− pituitaries (Fig. 2C). In addition, 5 to 6 mpf FSH-KO (fshb+/−) males showed lower fertilization rates (Fig. 2D) and smaller testis (Fig. 1B) compared with WT (Fig. 2B). Furthermore, retrograde labeling of hypophysiotropic neurons demonstrated that a part of gpr147.74-2-expressing neurons in the POm (the area preopticus pars magnocellularis) projected to the pituitary (Fig. 2F).

**Discussion**

The present study demonstrates that NPFF plays a crucial role in testicular morphogenesis and functions by increasing the expression of fshb in the pituitary, because npff−/− males showed a low fertilization rate (Fig. 1A), small testes (Fig. 1B and C), and low fshb expression in the pituitary (Fig. 2C). We have previously shown that NPFF is specifically expressed in the terminal nerve in the teleost brain and the NPFF neurons project their axons broadly in the brain but not the pituitary (13, 14), and here we showed that NPFF receptors (gpr147.74-2) expressing neurons in the POm projected to the pituitary (Fig. 2F). Taken together, it is conceivable that NPFF modulates fshb expression via neurons expressing NPFF receptors in the POm (Fig. 2G). It should be noted that FSH- or FSH receptor–KO males have been reported to demonstrate normal testes (3–5). In the present study, experiments using older fish (at 5 to 6 mpf) than those in the previous studies (at ~3 mpf) enabled us to find that FSH is involved in the testicular functions, at least in medaka.

Interestingly, 11-KT levels of WT males showed the difference between morning and evening (Fig. 1K), suggesting that there is a diurnal fluctuation of 11-KT levels in WT males. Also, our results showed that npff−/− males showed lower 11-KT levels in the evening (Fig. 1A), lower expression of odf3 (Fig. 1D), and a lower fertilization rate (Fig. 1A), which suggests that sufficient levels and/or diurnal fluctuation of testosterone play a role in appropriate spermatogenesis in teleost species. It should be noted that npff−/− showed higher expression of 3bhsd1/cyp17 (Fig. 1H and I), which contradicts the results of lower 11-KT levels. It is conceivable that these steroidogenic genes are up-regulated to compensate for the deficiency of FSH signaling caused by npff KO, but it is insufficient to reinstate 11-KT level due to the small size of the KO’s testis.

In summary, our present study suggests that NPFF affects testicular morphogenesis and functions, as illustrated in Fig. 2G. Since we demonstrated NPFF function in males, further analysis of NPFF in females will also help understanding teleost reproduction. In teleosts, the neuroendocrine mechanism
regulating testicular function has been relatively unraveled compared to that of the ovary. Our study may advance the understanding of brain functions for successful reproduction in teleost males.

### Materials and Methods

We kept and used d-i:RT WT, npff<sup>−/−</sup> and fshb<sup>−/−</sup> medaka (Oryzias latipes) as described by Umatani et al. (14) and Takahashi et al. (5). All of the experiments were conducted in accordance with the protocols approved by the animal care and use committee of the Graduate School of Science, University of Tokyo (permission no. 20-6). Detailed procedures for other analyses are described in SI Appendix.

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**Data, Materials, and Software Availability.** All of the study data are included in the article and/or supporting information.

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