General and Comparative Endocrinology 284 (2019) 113129

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

A conceptual framework for understanding sexual differentiation of the teleost brain

Kataaki Okubo*, Daichi Miyazoe, Yuji Nishiike

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan

ARTICLE INFO

Keywords:
Brain
Sexual differentiation
Sexual lability
Sex steroid
Sex steroid receptor
Teleost

ABSTRACT

Vertebrate brains are sexually differentiated, giving rise to differences in various physiological and behavioral phenotypes between the sexes. In developing mammals and birds, the neural substrate underlying sex-dependent physiology and behavior undergoes an irreversible process of sexual differentiation due to the effects of perinatal gonadal steroids and sex chromosome complement. The differentiated neural substrate is then activated in the adult by the sex-specific steroid milieu to facilitate the expression of sex-typical phenotypes. However, this well-established concept does not hold for teleost fish, whose sexual phenotypes (behavioral or otherwise) are highly labile throughout life and can be reversed even in adulthood. Indeed, the available evidence suggests that, in teleosts, neither gonadal steroids early in development nor the sex chromosome complement contribute much to brain sexual differentiation; instead, steroids in adulthood serve to both differentiate the neural substrate and activate it to elicit sex-typical phenotypes in a transient and reversible manner. Evidence further suggests that marked sexual dimorphisms and adult steroid-dependent lability in the neural expression of sex steroid receptors constitute the primary molecular basis for sexual differentiation and lability of the teleost brain. The consequent sexually dimorphic but reversible steroid sensitivity in response to the adult steroid milieu may enable the teleost brain to maintain lifelong sexual lability and to undergo phenotypic sex reversal.

1. Introduction

In vertebrates, once the gonads differentiate into testes in males or ovaries in females, they establish a sex-specific circulating sex steroid milieu that directs sexual differentiation of the rest of the body. In males, the androgen-dominated steroid milieu produced by the testes masculinizes (induces male-typical phenotypes of) and defeminizes (prevents female-typical phenotypes of) the structure and function of various extra-gonadal organs; in females, by contrast, the estrogen-dominated steroid milieu produced by the ovaries feminizes (induces female-typical phenotypes of) and demasculinizes (prevents male-typical phenotypes of) those organs (McCarthy and Arnold, 2011; McCarthy et al., 2017). A notable example of such organs is the brain, which, although similar in appearance in males and females, is clearly sexually differentiated, giving rise to sex differences in various physiological and behavioral traits. For example, males of a given species generally prefer to mate with females, whereas females generally prefer to mate with males. The pattern of mating behaviors also differs between the sexes: males commonly perform elaborate courtship displays to attract females for mating, while females evaluate male courtship displays to decide whether to mate. In addition, males and females have distinct patterns of pituitary gonadotropic hormone secretion to regulate gametogenesis in a sex-specific manner. These sex differences clearly result from sexual differentiation of the underlying neural circuitry in the brain (Yang and Shah, 2014, 2016). Sexually differentiated neural circuits can also serve to prevent sex differences in some physiological and behavioral traits, by compensating for differences in the sex steroid milieu that may otherwise cause undesirable sex differences in these traits (De Vries, 2004).

Among teleost fish, males and females also show differences in various physiological and behavioral traits, indicating that their brains are sexually differentiated. In contrast to mammals and birds, however, many teleost species undergo spontaneous sex reversal in adulthood and their physiological and behavioral sexual phenotypes are highly labile. In this review, we summarize the process of sexual differentiation of the mammalian and avian brain, and describe the contrasting high sexual lability of the teleost brain. We summarize recent findings on the mechanisms governing this sexual lability, enabling us to present a likely molecular basis for sexual differentiation of the teleost brain.

* Corresponding author.
E-mail address: okubo@marine.fs.a.u-tokyo.ac.jp (K. Okubo).

https://doi.org/10.1016/j.ygcen.2019.02.020
Received 14 December 2018; Received in revised form 8 February 2019; Accepted 26 February 2019
Available online 27 February 2019

2. Sexual differentiation of the mammalian and avian brain

How do gonadally-derived sex steroids direct sexual differentiation of the brain? Accumulating evidence has shown that, in addition to genetic factors, environmental events early in life such as infection, altered nutrition, and exposure to toxins and hormones are important determinants of physiological and behavioral phenotypes later in life (Gluckman et al., 2008). The effects of such events are irreversible (or not easily reversible) and can persist into adulthood, often leading to adult-onset phenotypes; therefore, this phenomenon is termed "early-life programming". Studies in mammals (mainly rodents) and birds spanning more than half a century have demonstrated that sexual differentiation of their brains begins with early-life programming (McCarthy et al., 2017). In mammals and birds, sex steroids secreted from the fetal gonads in a sex-specific manner act on the developing brain to induce permanent and irreversible sex differences in the neural substrate underlying sex-dependent physiology and behavior. These early steroid effects have traditionally been referred to as "organizational effects" in the field of sexual differentiation of the brain.

When looking at the details of this process, however, certain variations among species become apparent (Adkins-Regan, 2009; Maekawa et al., 2014). In rodents, the fetal male testis secretes large quantities of testosterone, whereas the fetal female ovary remains hormonally quiescent. Upon reaching the developing male brain, a large proportion of the testicular testosterone is converted by the enzyme aromatase to estradiol-17β (E2), which serves to masculinize/defeminize the neural substrate. In the absence of circulating testosterone, as occurs in females, the developing neural substrate of the brain is feminized/demasculinized. On the other hand, in the developing male primate brain, testosterone acts directly without conversion to E2. In contrast to these mammals, in quail, E2 secreted from the fetal female ovary acts on the developing brain to feminize/demasculinize the neural substrate, whereas zebra finches seem to undergo the same process as in rodents. Despite these variations, early-life programming represents a common mechanism for initiating sexual differentiation of the mammalian and avian brain.

Importantly, most of the early steroid effects emerge only after onset of puberty, when increasing amounts of steroids are secreted from the gonads in a sex-specific manner and activate the sexually differentiated neural substrate to facilitate the expression of sex-typical physiological and behavioral phenotypes (McCarthy and Arnold, 2011; McCarthy et al., 2017). More specifically, the high and steady levels of circulating testosterone typical of adult males activate the masculinized neural substrate to actuate male-typical phenotypes, whereas an adult female-typical cyclic pattern of circulating E2 associated with ovulation activates the feminized neural substrate to induce female-typical phenotypes. These effects of steroids in the adult are referred to as "activational effects". In contrast to the early steroid effects, which are typically permanent and irreversible, thereby persisting even after the hormonal stimulus is removed, the adult steroid effects are transient and reversible in nature, and are maintained only in the continued presence of hormones.

Although the majority of sex differences in the brain are a consequence of the effects of gonadal steroids, strong evidence has emerged over the past few decades for the contribution of the sex chromosome complement to some sex differences in the brain: namely, male and female brain cells are intrinsically different in their sex chromosome complement (XY versus XX in mammals, or ZZ versus ZW in birds), which directly and constitutively affects the sexual differentiation of some neural and behavioral phenotypes, independent of gonadal steroids (McCarthy and Arnold, 2011; Arnold, 2017). In zebra finches, for example, sex differences in the development of specific nuclei within the song system (i.e., the neural substrate underlying the learning and production of song, a male-biased behavioral trait in song birds) have been shown to result from the sex chromosome complement (Agate et al., 2003). These effects, referred to as "sex chromosome effects", also contribute to sex differences in several traits, including aggressive and parental behaviors, nociception, and social interactions (McCarthy and Arnold, 2011), although the genes and pathways underlying the sex differences in these traits remain largely unknown.

Collectively, sexual differentiation of the mammalian and avian brains involves (i) the permanent and irreversible effects of gonadal steroids in the early-life programming of the neural substrate during development; (ii) the transient and reversible effects of gonadal steroids in adulthood to elicit sex-typical physiological and behavioral phenotypes; and (iii) constitutive sex chromosome effects probably due to direct actions of sex chromosome genes within the brain, independent of the steroid effects. The effects of adult gonadal steroids are sometimes not considered to be sexual differentiation because they do not differentiate the neural substrate, but rather transiently activate the already masculinized/feminized substrate. Arnold (2017), by contrast, argues that all factors that cause sex differences, including the transient effects of adult steroids, need to be incorporated into a theory of sexual differentiation, and indeed these effects may be the most potent proximate factors that make males and females different. We agree with Arnold's idea and use it as a basis for our review.

3. The brain of teleost fish is highly sexually labile throughout life

In general, sex differences in neural and behavioral traits (particularly those associated with reproduction including sexual preference, courtship display, copulatory behavior, intrasexual competition, and neural control of gonadotropic hormone secretion patterns) are robust and irreversible in mammals and birds, most probably because the neural substrate for these traits undergoes an irreversible process of sexual differentiation early in development. Interestingly, however, this irreversibility is not the case in teleost fish. Male and female fish display differences in a wide range of physiological and behavioral traits, thus signifying that their brains are also sexually differentiated; unlike mammals and birds, however, many teleost species (from at least 27 families across 9 teleost orders) undergo spontaneous sex reversal, involving both behavioral and morphological changes, in adulthood in response to social and physical stimuli (Munakata and Kobayashi, 2010; Todd et al., 2016; Liu et al., 2017; Capel, 2017). Even in teleost species that do not normally switch sex as adults, such as medaka (*Oryzias latipes*), tilapia (*Oreochromis niloticus*), zebrafish (*Danio rerio*), and an African cichlid (*Astatotilapia burtoni*), functional (often including behavioral) sex reversal can occur when adult genetic females are chronically treated with an aromatase inhibitor, which prevents androgen conversion to estrogen (Paul-Prasanth et al., 2013; Takatsu et al., 2013; Sun et al., 2014; Göppert et al., 2016). These phenomena indicate that, unlike mammalian and avian brains, the teleost brain is highly sexually labile across the lifespan and can be reversed even in adulthood.

This concept is further supported by behavioral observations of adult fish receiving acute hormone treatments. Treating female stickleback (*Gasterosteus aculeatus*) with androgen as adults induces a nest-building behavior that is typical of males (Wai and Hoar, 1963). Furthermore, treating adult female and male goldfish (*Carassius auratus*) with androgen and prostaglandin F2α (an ovulatory hormone), respectively, can lead to the reversal of sex-typical mating behaviors, even though the gonadal phenotype remains unchanged (Stacey and Kyle, 1983; Stacey and Kobayashi, 1996; Ghosal and Sorensen, 2016). Of note, these species do not spontaneously undergo sex reversal, suggesting that the brains of teleosts, irrespective of whether the species normally switch sex or not, preserve a considerable degree of sexual lability throughout their lifetime.

4. Sexual differentiation of the teleost brain does not rely on early-life programming or sex chromosome complement

Considering that the sexual phenotype, behavioral or otherwise, of

teleosts can be reversed in adults, the teleost brain must have distinctive mechanisms of sexual differentiation that enable it to maintain high levels of sexual lability throughout life. It seems reasonable to assume that sex steroids early in development induce few (if any) irreversible sex differences in the neural substrate, or that such differences can easily be reversed by the prevailing steroid milieu in adults. In support of the former assumption, studies in medaka have shown that there is no significant increase in testosterone or E2 levels in developing embryos (Iwamatsu et al., 2005, 2006). Another study in medaka has shown that aromatase is expressed at much lower levels in the developing brain than in the adult brain, and exhibits neither a transient elevation nor a sex difference (Okubo et al., 2011). This observation is in stark contrast to the situation in rodents, where a transient, male-biased induction of high levels of aromatase activity in the developing brain is involved in masculinization/defeminization of the neural substrate (George and Ojeda, 1982; Weisz et al., 1982; Tobet et al., 1985; Erskine et al., 1988).

The marked sexual lability of the adult teleost brain also suggests that the sex chromosome complement, which should be invariably effective, does not contribute much, if anything at all, to sexual differentiation of the teleost brain. This idea is consistent with the general observation that sex-reversed XX/ZW male and XY/ZZ female teleosts seem to be as fertile and behaviorally active in mating as normal XY/ZZ males and XX/ZW females (Volff et al., 2007; Paul-Prasanth et al., 2013; Chen et al., 2014), although no quantitative studies have been performed on behavior or neural function in sex-reversed fish. Teleosts differ from mammals and birds in that their sex chromosomes arose fairly recently and independently in each genus or even species (in fact many lack sex chromosomes entirely) (Marshall Graves and Peichel, 2010; Kikuchi and Hamaguchi, 2013; Capel, 2017). Because the sex chromosomes of teleosts are still in the early stages of differentiation, the sex chromosome pairs (i.e., X and Y, or Z and W chromosomes) are morphologically indistinguishable and likely to be almost identical, differing at only one or a few loci. In medaka, for example, the Y chromosome is genetically the same as the X chromosome except for the addition of a 258-kb sequence including the sex-determining gene *dmy* (Kondo et al., 2006). Thus, the absence of extensive genetic differences between the sexes may limit the potential impact of the sex chromosome complement on brain sexual differentiation in teleosts.

Despite these arguments, a recent study in medaka has shown that two gametologous gene pairs (homologous genes on opposite sex chromosomes) (García-Moreno and Mindell, 2000), namely, *cntfa* (encoding a neurotrophic factor belonging to the interleukin-6 family) and *pdlim3a* (encoding a member of the PDZ-LIM protein family), are differentially expressed between adult male and female brains owing to greater expression from the Y allele (Maehiro et al., 2014). The significance of their differential expression remains to be determined; however, these genes have been implicated in the self-renewal and differentiation of neural stem cells, the survival of their progeny, and cytoskeletal assembly (Bauer et al., 2007; Krcmery et al., 2010), thus potentially playing a role in sexual differentiation of the neural substrate. Notably, each genus or species of teleosts has its own particular sex chromosomes and thereby its own gametologous genes, some of which may be differentially expressed between male and female brains, as is the case for *cntfa* and *pdlim3a* in medaka. Teleosts display genus- or species-specific sex differences in various physiological and behavioral traits; some of these differences are possibly a result of the sex-differential expression and/or function of their gametologous genes (Maehiro et al., 2014).

5. Sexual differentiation of the teleost brain is highly dependent on the sex-specific adult steroid milieu

Assuming that early-life programming and the sex chromosome complement do not contribute much to sexual differentiation of the teleost brain, what mechanism is primarily responsible for this process? The most likely candidate for this mechanism is, of course, the effects of sex steroids in adulthood. The adult steroid effects are transient and reversible, which is consistent with the high levels of sexual lability of the teleost brain. The reversal of sex-typical behaviors in response to experimental manipulations of the adult steroid milieu described above is indeed reflective of the effects of adult steroids, and suggests that these effects have an essential role in sexual differentiation of the teleost brain.

In further support of this suggestion, several studies have shown that sexually differentiated patterns of gene expression in the medaka brain can be reversed between males and females by altering the sex steroid milieu in adulthood. The genes showing this reversal encode, for example, brain-type aromatase (*cyp19a1b*), androgen receptors (*ara* and *arb*), estrogen receptors (*esr1*, *esr2a*, and *esr2b*), a novel heme-binding protein (*hebp3*), and neuropeptide B (*npb*) (Okubo et al., 2011; Hiraki et al., 2012, 2014; Nakasone et al., 2013). Differential expression of these genes in the male and female brains becomes apparent only after onset of puberty and is dependent on gonadal phenotype (testis or ovary), but entirely independent of sex chromosome complement (XX or XY). These findings indicate that the sexually differentiated expression of these genes does not involve early gonadal steroids or sex chromosome complement, but instead relies mainly on adult gonadal steroids.

Taken together, these lines of evidence suggest that adult gonadal steroids are largely, if not solely, responsible for sexual differentiation of the teleost brain. It seems plausible that, in teleosts, adult gonadal steroids serve to both differentiate the neural substrate and activate it to actuate sex-typical phenotypes in a transient and reversible manner. This less rigid process of sexual differentiation may enable the teleost brain to maintain lifelong sexual lability and to undergo phenotypic sex reversal.

6. Sexual dimorphism and lability of sex steroid receptor expression in the teleost brain

Crucially, then, what is the molecular basis of the probable uniqueness of sexual differentiation in the teleost brain? Recent evidence suggests that sexual dimorphism (i.e., a large sex difference with little or no overlap between male and female values; McCarthy et al., 2012; Ball et al., 2014) and lability of the expression of sex steroid receptors in the brain may play a key role in this process.

Sex steroids exert their effects primarily by binding to specific nuclear receptors, including androgen receptor (AR) and estrogen receptor (ER), which act as ligand-gated transcription factors to alter gene expression. Hiraki et al. (2012) have shown that, in adult medaka, ER and AR are expressed almost exclusively in the female brain in neurons of several nuclei, including the ventral telencephalic and magnocellular preoptic nuclei, which have been implicated in mating behavior (Demski et al., 1975; Kyle and Peter, 1982; Koyama et al., 1984; Satou et al., 1984). Because the expression pattern of sex steroid receptors determines the sensitivity of cells and/or organs to sex steroids, these behaviorally relevant brain nuclei are directly sensitive to androgen and estrogen only in females. This is an interesting finding in light of the fact that, in many animal species including medaka, mature males are always sexually active, but females readily engage in mating only at or around the time of ovulation. Thus, the female-specific influence of sex steroids on behaviorally relevant brain nuclei may underlie this phenomenon. This finding also leads to the assumption that sex steroid signaling elicits female-specific gene expression in these nuclei that is decisive for female-typical mating behavior. Indeed, follow-up studies have identified a neuropeptide gene (*npb*) as a direct transcriptional target of estrogen in these nuclei that mediates female receptivity to male courtship (Hiraki et al., 2014; Hiraki-Kajiyama et al., unpublished data). As expected, the expression of *npb* therein is essentially confined to females and dependent on the sex steroid milieu. These studies suggest that the sexually dimorphic expression of sex steroid receptors

in behaviorally relevant brain nuclei constitutes a molecular basis for sexual differentiation of mating behavior in teleosts.

Importantly, the sexually dimorphic pattern of AR and ER expression in the ventral telencephalic and magnocellular preoptic nuclei is highly labile and can be even reversed, solely depending on the sex steroid milieu in adulthood (Hiraki et al., 2012). More specifically, the estrogen-dominated steroid milieu stimulates, whereas the androgen-dominated steroid milieu inhibits, the expression of these receptors, regardless of genetic or gonadal sex. The labile nature of the sexual dimorphism in sex steroid receptor expression means that the sexually differentiated sensitivity of relevant brain nuclei to sex steroids can be reversed even in adulthood. Sex differences in, and effects of the adult steroid milieu on, the expression of steroid receptors have also been documented in the brains of several mammalian and avian species (e.g., Lauber et al., 1991; Shughrue et al., 1992; Zhou et al., 1995; McGinnis and Katz, 1996; Lu et al., 1998; Scott et al., 2000, 2004; Fernández-Guasti et al., 2000; Voigt et al., 2009); however, the degree of both sex differences and steroid effects is modest and much less pronounced as compared with medaka. Therefore, the all-or-none sex differences and marked adult steroid-dependent lability in sex steroid receptor expression seem to be unique properties of the teleost brain. Although there are discrepancies among studies, the available evidence in rodents suggests that the effects of perinatal sex steroids in early-life programming are mediated, in part, by epigenetic modifications (DNA methylation) of steroid receptor genes; these sex-dependent modifications limit the flexibility of receptor gene expression in relevant brain nuclei, and consequently their sensitivity to sex steroids, later in life (Westberry et al., 2010; Kurian et al., 2010; Schwarz et al., 2010). It seem unlikely that the teleost brain undergoes such a developmental process, given the lability of steroid receptor expression in adults.

7. A molecular basis for sexual differentiation and lability of the teleost brain

Based on the above considerations, we propose that marked sexual dimorphisms and significant adult steroid-dependent lability in the neural expression of steroid receptors constitute the primary molecular basis for sexual differentiation and lability of the teleost brain. The consequent sexually dimorphic but reversible steroid sensitivity of relevant brain nuclei presumably enables the teleost brain to possess lifelong sexual lability and to undergo phenotypic sex reversal.

As described above, sexual differentiation of the teleost brain is not likely to involve the long-lasting effects of early sex steroids or sex chromosome complement observed in mammals and birds, but instead relies largely or solely on the transient effects of sex steroids in adulthood. Given that circulating steroid levels fluctuate considerably with reproductive status in adults, one may argue that sole reliance on the adult steroid milieu renders brain sexual phenotypes highly unstable and might often lead to unforeseen phenotypic reversals. The teleost brain most probably avoids this difficulty by producing marked sexual dimorphisms in the neural expression of steroid receptors, which ensure stable persistence of the sex-specific responsiveness of the brain to sex steroids even though steroid levels are transiently altered. Simultaneously, the adult steroid-dependent lability of steroid receptor expression would facilitate the reversal of sexual phenotypes if the steroid milieu is massively altered by certain life events, including sex change. Hence, the sexually dimorphic but reversible neural steroid receptor expression seems to represent a simple yet efficient mechanism, whereby the brain is sexually differentiated but retains the potential for sex reversal. In mammals and birds, perinatal sex steroids differentiate the neural substrate to promote the expression of phenotypes typical of one sex (masculinize/feminize) and suppress that of the other sex (demasculinize/defeminize) later in life; in teleosts, the sexually dimorphic expression of neural steroid receptors in adulthood, which results from the sex-specific adult steroid milieu, may serve this role.

It may be of interest to note here that, in teleosts, circulating testosterone levels in females are substantial and are sometimes equal to the levels found in males (although 11-ketotestosterone, the more potent androgen in teleosts similar to 5α-dihydrotestosterone in mammals, occurs in higher levels in males than females) (Katz and Eckstein, 1974; Borg, 1994). In addition, brain aromatase activity in teleosts is 100–1000 times higher than in mammals and birds, resulting in a high amount of estrogen even in the male brain (Pasmanik and Callard, 1985). These facts suggest that sex differences in the sex steroid milieu are smaller in the teleost brain than in the mammalian and avian brain. Sex differences in the action of sex steroids in the teleost brain may be primarily controlled at the receptor level rather than at the ligand level.

The importance of sex steroids in sexual differentiation of the brain is conserved across vertebrate taxa. In mammals and birds, the effects of sex steroids early in life are rendered persistent and more or less irreversible, thereby limiting the impact of steroids later in life. This has the consequence that the sexual phenotypes of their brains are rather robust and irreversible in adulthood. In teleosts, by contrast, sex steroids have transient and reversible effects throughout the lifespan to ensure that brain sexual phenotype always correlates with gonadal phenotype even when gonadal sex is reversed.

Unfortunately, no information is yet available on the mechanisms by which sex steroids regulate the neural expression of steroid receptors and the signaling pathways initiated by steroid receptors in the brain to mediate sex-typical phenotypes. These issues clearly warrant future investigations. Future research is also needed to investigate if the findings and concepts described above can be generalized across species in teleosts. Teleosts are the most diversified group of vertebrates, but sexual differentiation of the teleost brain has been studied only in a few species. Caution must be exercised in assuming that findings in these species are applicable to other species.

8. Is sexual lability of the adult brain really a characteristic peculiar to teleosts?

Among vertebrates, teleosts have exceptionally high levels of adult sexual lability in neural and behavioral phenotypes. While this is certainly true, it is worth noting that several studies have reported that mammals and birds also have some degree of sexual lability in these phenotypes as adults, notwithstanding the widely held view that their brains undergo irreversible sexual differentiation early in life. In quail, for example, the volume of the medial preoptic nucleus, which is implicated in male-typical behaviors, is approximately 40% larger in males, and this sex difference is largely dependent on adult sex steroids (Adkins-Regan, 2009; Balthazart et al., 2010). Consistent with this observation, female quail that are given testosterone as adults exhibit some male-typical courtship behaviors, such as crowing and strutting (Adkins-Regan, 2009; Ball et al., 2014). Similarly, female rodents display male-like mating behaviors either when treated with testosterone or E2 as adults (Edwards and Burge, 1971; Södersten, 1972), or when deprived of pheromonal signaling by genetic disabling of the vomeronasal organ (Kimchi et al., 2007).

Although the mechanisms underlying these findings remain to be determined, it seems likely that the reversal of brain sexual phenotypes in response to changes in the adult steroid milieu is not a phenomenon peculiar to teleosts, but more or less common across vertebrate taxa, including mammals and birds. A better understanding of sexual differentiation of the teleost brain will provide valuable insight into the molecular basis for determining the robustness or lability of sex differences in vertebrate brains and its evolutionary aspects — insight that might otherwise be difficult to attain.

Because the focus of this review is on the conceptual understanding of sexual differentiation and lability of the teleost brain, a detailed description of sex differences so far observed in the teleost brain is beyond the scope of this review. However, sex differences in the

expression of several genes, which are often steroid-sensitive, have been reported in the teleost brain, and their possible involvement in the development and reversal of sex-typical phenotypes has been suggested. These genes include those for brain-type aromatase (Goto-Kazeto et al., 2004; Forlano and Bass, 2005; Strobl-Mazzulla et al., 2005; Patil and Gunasekera, 2008; Okubo et al., 2011), vasotocin (Grober and Sunobe, 1996; Foran and Bass, 1998; Godwin et al., 2000; Grober et al., 2002; Maruska et al., 2007; Maruska, 2009; Kawabata et al., 2012), isotocin (Black et al., 2004; Kawabata et al., 2012; Yamashita et al., 2017), gonadotropin-releasing hormones (Grober et al., 1994; Elofsson et al., 1997, 1999; Ishizaki et al., 2004; Kuramochi et al., 2011; Kawabata et al., 2012), kisspeptin (Kanda et al., 2008), tyrosine and tryptophan hydroxylases (Sudhakumari et al., 2010; Raghuveer et al., 2011; Kawabata et al., 2012; Mamta et al., 2014; Goebrecht et al., 2014; Saha et al., 2015), and glutamate decarboxylases (Trudeau et al., 2000; Bosma et al., 2001; Larivière et al., 2005). Although there is no direct evidence, even from gene knockout studies in zebrafish (Yin et al., 2017; Tang et al., 2017), it has been repeatedly proposed that, among these genes, brain-type aromatase plays a particularly important role by regulating neurogenesis, which is highly active even during adulthood in teleosts (Chapouton et al., 2007; Ganz and Brand, 2016). The reader is referred to several excellent reviews on this topic for further information (Blázquez and Somoza, 2010; Le Page et al., 2010; Diotel et al., 2010, 2018).

Declaration of interest

The authors have nothing to declare.

Acknowledgements

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) in Japan (Grant number 17H06429) and the Japan Society for the Promotion of Science (JSPS) (Grant number 16H04979).

References

Adkins-Regan, E., 2009. Hormones and sexual differentiation of avian social behavior. Dev. Neurosci. 31, 342–350.
Agate, R.J., Grisham, W., Wade, J., Mann, S., Wingfield, J., Schanen, C., Palotie, A., Arnold, A.P., 2003. Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. Proc. Natl. Acad. Sci. U.S.A. 100, 4873–4878.
Arnold, A.P., 2017. A general theory of sexual differentiation. J. Neurosci. Res. 95, 291–300.
Ball, G.F., Balthazart, J., McCarthy, M.M., 2014. Is it useful to view the brain as a secondary sexual characteristic? Neurosci. Biobehav. Rev. 46, 628–638.
Balthazart, J., Charlier, T.D., Barker, J.M., Yamamura, T., Ball, G.F., 2010. Sex steroid-induced neuroplasticity and behavioral activation in birds. Eur. J. Neurosci. 32, 2116–2132.
Bauer, S., Kerr, B.J., Patterson, P.H., 2007. The neuropoietic cytokine family in development, plasticity, disease and injury. Nat. Rev. Neurosci. 8, 221–232.
Black, M.P., Reavis, R.H., Grober, M.S., 2004. Socially induced sex change regulates forebrain isotocin in *Lythrypnus dalli*. Neuroreport 15, 185–189.
Blázquez, M., Somoza, G.M., 2010. Fish with thermolabile sex determination (TSD) as models to study brain sex differentiation. Gen. Comp. Endocrinol. 166, 470–477.
Borg, B., 1994. Androgens in teleost fish. Comp. Biochem. C Pharmacol. Toxicol. Endocrinol. 109, 219–245.
Bosma, P.T., Blázquez, M., Fraser, E.J., Schulz, R.W., Docherty, K., Trudeau, V.L., 2001. Sex steroid regulation of glutamate decarboxylase mRNA expression in goldfish brain is sexually dimorphic. J. Neurochem. 76, 945–956.
Capel, B., 2017. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. Nat. Rev. Genet. 18, 675–689.
Chapouton, P., Jagasia, R., Bally-Cuif, L., 2007. Adult neurogenesis in non-mammalian vertebrates. Bioessays 29, 745–757.
Chen, S., Zhang, G., Shao, C., Huang, Q., Liu, G., Zhang, P., Song, W., An, N., Chalopin, D., Volff, J.N., Hong, Y., Li, Q., Sha, Z., Zhou, H., Xie, M., Yu, Q., Liu, Y., Xiang, H., Wang, N., Wu, K., Yang, C., Zhou, Q., Liao, X., Yang, L., Hu, Q., Zhang, J., Meng, L., Jin, L., Tian, Y., Lian, J., Yang, J., Miao, G., Liu, S., Liang, Z., Yan, F., Li, Y., Sun, B., Zhang, H., Zhang, J., Zhu, Y., Du, M., Zhao, Y., Schartl, M., Tang, Q., Wang, J., 2014. Whole-genome sequence of a flatfish provides insights into ZW sex chromosome evolution and adaptation to a benthic lifestyle. Nat. Genet. 46, 253–260.
De Vries, G.J., 2004. Sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology 145, 1063–1068.
Demski, L.S., Bauer, D.H., Gerald, J.W., 1975. Sperm release evoked by electrical stimulation of the fish brain: a functional-anatomical study. J. Exp. Zool. 191, 215–232.
Diotel, N., Charlier, T.D., Lefebvre d'Hellencourt, C., Couret, D., Trudeau, V.L., Nicolau, J.C., Meilhac, O., Kah, O., Pellegrini, E., 2018. Steroid transport, local synthesis, and signaling within the brain: roles in neurogenesis, neuroprotection, and sexual behaviors. Front. Neurosci. 12, 84.
Diotel, N., Le Page, Y., Mouriec, K., Tong, S.K., Pellegrini, E., Vaillant, C., Anglade, I., Brion, F., Pakdel, F., Chung, B.C., Kah, O., 2010. Aromatase in the brain of teleost fish: expression, regulation and putative functions. Front. Neuroendocrinol. 31, 172–192.
Edwards, D.A., Burge, K.G., 1971. Early androgen treatment and male and female sexual behavior in mice. Horm. Behav. 2, 49–58.
Elofsson, U., Winberg, S., Francis, R.C., 1997. Number of preoptic GnRH-immunoreactive cells correlates with sexual phase in a protandrously hermaphroditic fish, the dusky anemonefish (*Amphiprion melanopus*). J. Comp. Physiol. A 181, 484–492.
Elofsson, U., Winberg, S., Nilsson, G.E., 1999. Relationships between sex and the size and number of forebrain gonadotropin-releasing hormone–immunoreactive neurones in the ballan wrasse (*Labrus berggylta*), a protogynous hermaphrodite. J. Comp. Neurol. 410, 158–170.
Erskine, M.S., Tobet, S.A., Baum, M.J., 1988. Effect of birth on plasma testosterone, brain aromatase activity, and hypothalamic estradiol in male and female ferrets. Endocrinology 122, 524–530.
Fernández-Guasti, A., Kruijver, F.P., Fodor, M., Swaab, D.F., 2000. Sex differences in the distribution of androgen receptors in the human hypothalamus. J. Comp. Neurol. 425, 422–435.
Foran, C.M., Bass, A.H., 1998. Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. Gen. Comp. Endocrinol. 111, 271–282.
Forlano, P.M., Bass, A.H., 2005. Steroid regulation of brain aromatase expression in glia: female preoptic and vocal motor nuclei. J. Neurobiol. 65, 50–58.
Ganz, J., Brand, M., 2016. Adult neurogenesis in fish. Cold Spring Harb. Perspect. Biol. 8, a019018.
García-Moreno, J., Mindell, D.P., 2000. Rooting a phylogeny with homologous genes on opposite sex chromosomes (gametologs): a case study using avian CHD. Mol. Biol. Evol. 17, 1826–1832.
George, F.W., Ojeda, S.R., 1982. Changes in aromatase activity in the rat brain during embryonic, neonatal, and infantile development. Endocrinology 111, 522–529.
Ghosal, R., Sorensen, P.W., 2016. Male-typical courtship, spawning behavior, and olfactory sensitivity are induced to different extents by androgens in the goldfish suggesting they are controlled by different neuroendocrine mechanisms. Gen. Comp. Endocrinol. 232, 160–173.
Gluckman, P.D., Hanson, M.A., Cooper, C., Thornburg, K.L., 2008. Effect of in utero and early-life conditions on adult health and disease. N. Engl. J. Med. 359, 61–73.
Godwin, J., Sawby, R., Warner, R.R., Crews, D., Grober, M.S., 2000. Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. Brain Behav. Evol. 55, 77–84.
Goebrecht, G.K., Kowtoniuk, R.A., Kelly, B.G., Kittelberger, J.M., 2014. Sexually-dimorphic expression of tyrosine hydroxylase immunoreactivity in the brain of a vocal teleost fish (*Porichthys notatus*). J. Chem. Neuroanat. 56, 13–34.
Göppert, C., Harris, R.M., Theis, A., Boila, A., Hohl, S., Rüegg, A., Hofmann, H.A., Salzburger, W., Böhne, A., 2016. Inhibition of aromatase induces partial sex change in a cichlid fish: distinct functions for sex steroids in brains and gonads. Sex. Dev. 10, 97–110.
Grober, M.S., Fox, S.H., Laughlin, C., Bass, A.H., 1994. GnRH cell size and number in a teleost fish with two male reproductive morphs: sexual maturation, final sexual status and body size allometry. Brain Behav. Evol. 43, 61–78.
Grober, M.S., George, A.A., Watkins, K.K., Carneiro, L.A., Oliveira, R.F., 2002. Forebrain AVT and courtship in a fish with male alternative reproductive tactics. Brain Res. Bull. 57, 423–425.
Grober, M.S., Sunobe, T., 1996. Serial adult sex change involves rapid and reversible changes in forebrain neurochemistry. Neuroreport 7, 2945–2949.
Goto-Kazeto, R., Kight, K.E., Zohar, Y., Place, A.R., Trant, J.M., 2004. Localization and expression of aromatase mRNA in adult zebrafish. Gen. Comp. Endocrinol. 139, 72–84.
Hiraki, T., Nakasone, K., Hosono, K., Kawabata, Y., Nagahama, Y., Okubo, K., 2014. Neuropeptide B is female-specifically expressed in the telencephalic and preoptic nuclei of the medaka brain. Endocrinology 155, 1021–1032.
Hiraki, T., Takeuchi, A., Tsumaki, T., Zempo, B., Kanda, S., Oka, Y., Nagahama, Y., Okubo, K., 2012. Female-specific target sites for both oestrogen and androgen in the teleost brain. Proc. Biol. Sci. 279, 5014–5023.
Ishizaki, M., Iigo, M., Yamamoto, N., Oka, Y., 2004. Different modes of gonadotropin-releasing hormone (GnRH) release from multiple GnRH systems as revealed by radioimmunoassay using brain slices of a teleost, the dwarf gourami (*Colisa lalia*). Endocrinology 145, 2092–2103.
Iwamatsu, T., Kobayashi, H., Hamaguchi, S., Sagegami, R., Shuo, T., 2005. Estradiol-17 β content in developing eggs and induced sex reversal of the medaka (*Oryzias latipes*). J. Exp. Zool. A Comp. Exp. Biol. 303, 161–167.

Iwamatsu, T., Kobayashi, H., Sagegami, R., Shuo, T., 2006. Testosterone content of developing eggs and sex reversal in the medaka (*Oryzias latipes*). Gen. Comp. Endocrinol. 145, 67–74.
Kanda, S., Akazome, Y., Matsunaga, T., Yamamoto, N., Yamada, S., Tsukamura, H., Maeda, K., Oka, Y., 2008. Identification of KiSS-1 product kisspeptin and steroid-sensitive sexually dimorphic kisspeptin neurons in medaka (*oryzias latipes*). Endocrinology 149, 2467–2476.
Katz, Y., Eckstein, B., 1974. Changes in steroid concentration in blood of female *Tilapia aurea* (teleostei, cichlidae) during initiation of spawning. Endocrinology 95, 963–967.
Kawabata, Y., Hiraki, T., Takeuchi, A., Okubo, K., 2012. Sex differences in the expression of vasotocin/isotocin, gonadotropin-releasing hormone, and tyrosine and tryptophan hydroxylase family genes in the medaka brain. Neuroscience 218, 65–77.
Kikuchi, K., Hamaguchi, S., 2013. Novel sex-determining genes in fish and sex chromosome evolution. Dev. Dyn. 242, 339–353.
Kimchi, T., Xu, J., Dulac, C., 2007. A functional circuit underlying male sexual behaviour in the female mouse brain. Nature 448, 1009–1014.
Kondo, M., Hornung, U., Nanda, I., Imai, S., Sasaki, T., Shimizu, A., Asakawa, S., Hori, H., Schmid, M., Shimizu, N., Schartl, M., 2006. Genomic organization of the sex-determining and adjacent regions of the sex chromosomes of medaka. Genome Res. 16, 815–826.
Koyama, Y., Satou, M., Oka, Y., Ueda, K., 1984. Involvement of the telencephalic hemispheres and the preoptic area in sexual behavior of the male goldfish, *Carassius auratus*: a brain-lesion study. Behav. Neural. Biol. 40, 70–86.
Krcmery, J., Camarata, T., Kulisz, A., Simon, H.G., 2010. Nucleocytoplasmic functions of the PDZ-LIM protein family: new insights into organ development. Bioessays 32, 100–108.
Kuramochi, A., Tsutiya, A., Kaneko, T., Ohtani-Kaneko, R., 2011. Sexual dimorphism of gonadotropin-releasing hormone type-III (GnRH3) neurons and hormonal sex reversal of male reproductive behavior in Mozambique tilapia. Zool. Sci. 28, 733–739.
Kurian, J.R., Olesen, K.M., Auger, A.P., 2010. Sex differences in epigenetic regulation of the estrogen receptor-α promoter within the developing preoptic area. Endocrinology 151, 2297–2305.
Kyle, A.L., Peter, R.E., 1982. Effects of forebrain lesions on spawning behaviour in the male goldfish. Physiol. Behav. 28, 1103–1109.
Larivière, K., Samia, M., Lister, A., Van Der Kraak, G., Trudeau, V.L., 2005. Sex steroid regulation of brain glutamic acid decarboxylase (GAD) mRNA is season-dependent and sexually dimorphic in the goldfish *Carassius auratus*. Brain Res. Mol. Brain Res. 141, 1–9.
Lauber, A.H., Mobbs, C.V., Muramatsu, M., Pfaff, D.W., 1991. Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. Endocrinology 129, 3180–3186.
Le Page, Y., Diotel, N., Vaillant, C., Pellegrini, E., Anglade, I., Mérot, Y., Kah, O., 2010. Aromatase, brain sexualization and plasticity: the fish paradigm. Eur. J. Neurosci. 32, 2105–2115.
Liu, H., Todd, E.V., Lokman, P.M., Lamm, M.S., Godwin, J.R., Gemmell, N.J., 2017. Sexual plasticity: a fishy tale. Mol. Reprod. Dev. 84, 171–194.
Lu, S.F., McKenna, S.E., Cologer-Clifford, A., Nau, E.A., Simon, N.G., 1998. Androgen receptor in mouse brain: sex differences and similarities in autoregulation. Endocrinology 139, 1594–1601.
Maehiro, S., Takeuchi, A., Yamashita, J., Hiraki, T., Kawabata, Y., Nakasone, K., Hosono, K., Usami, T., Paul-Prasanth, B., Nagahama, Y., Oka, Y., Okubo, K., 2014. Sexually dimorphic expression of the sex chromosome-linked genes *cntfa* and *pdlim3a* in the medaka brain. Biochem. Biophys. Res. Commun. 445, 113–119.
Maekawa, F., Tsukahara, S., Kawashima, T., Nohara, K., Ohki-Hamazaki, H., 2014. The mechanisms underlying sexual differentiation of behavior and physiology in mammals and birds: relative contributions of sex steroids and sex chromosomes. Front. Neurosci. 8, 242.
Mamta, S.K., Raghuveer, K., Sudhakumari, C.C., Rajakumar, A., Basavaraju, Y., Senthilkumaran, B., 2014. Cloning and expression analysis of tyrosine hydroxylase and changes in catecholamine levels in brain during ontogeny and after sex steroid analogues exposure in the catfish, *Clarias batrachus*. Gen. Comp. Endocrinol. 197, 18–25.
Marshall Graves, J.A., Peichel, C.L., 2010. Are homologies in vertebrate sex determination due to shared ancestry or to limited options? Genome Biol. 11, 205.
Maruska, K.P., 2009. Sex and temporal variations of the vasotocin neuronal system in the damselfish brain. Gen. Comp. Endocrinol. 160, 194–204.
Maruska, K.P., Mizobe, M.H., Tricas, T.C., 2007. Sex and seasonal co-variation of arginine vasotocin (AVT) and gonadotropin-releasing hormone (GnRH) neurons in the brain of the halfspotted goby. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147, 129–144.
McCarthy, M.M., Arnold, A.P., 2011. Reframing sexual differentiation of the brain. Nat. Neurosci. 14, 677–683.
McCarthy, M.M., Arnold, A.P., Ball, G.F., Blaustein, J.D., De Vries, G.J., 2012. Sex differences in the brain: the not so inconvenient truth. J. Neurosci. 32, 2241–2247.
McCarthy, M.M., Nugent, B.M., Lenz, K.M., 2017. Neuroimmunology and neuroepigenetics in the establishment of sex differences in the brain. Nat. Rev. Neurosci. 18, 471–484.
McGinnis, M.Y., Katz, S.E., 1996. Sex differences in cytosolic androgen receptors in gonadectomized male and female rats. J. Neuroendocrinol. 8, 193–197.
Munakata, A., Kobayashi, M., 2010. Endocrine control of sexual behavior in teleost fish. Gen. Comp. Endocrinol. 165, 456–468.
Nakasone, K., Nagahama, Y., Okubo, K., 2013. *hebp3*, a novel member of the heme-binding protein gene family, is expressed in the medaka meninges with higher abundance in females due to a direct stimulating action of ovarian estrogens. Endocrinology 154, 920–930.
Okubo, K., Takeuchi, A., Chaube, R., Paul-Prasanth, B., Kanda, S., Oka, Y., Nagahama, Y., 2011. Sex differences in aromatase gene expression in the medaka brain. J. Neuroendocrinol. 23, 412–423.
Pasmanik, M., Callard, G.V., 1985. Aromatase and 5α-reductase in the teleost brain, spinal cord, and pituitary gland. Gen. Comp. Endocrinol. 60, 244–251.
Patil, J.G., Gunasekera, R.M., 2008. Tissue and sexually dimorphic expression of ovarian and brain aromatase mRNA in the Japanese medaka (*Oryzias latipes*): implications for their preferential roles in ovarian and neural differentiation and development. Gen. Comp. Endocrinol. 158, 131–137.
Paul-Prasanth, B., Bhandari, R.K., Kobayashi, T., Horiguchi, R., Kobayashi, Y., Nakamoto, M., Shibata, Y., Sakai, F., Nakamura, M., Nagahama, Y., 2013. Estrogen oversees the maintenance of the female genetic program in terminally differentiated gonochorists. Sci. Rep. 3, 2862.
Raghuveer, K., Sudhakumari, C.C., Senthilkumaran, B., Kagawa, H., Dutta-Gupta, A., Nagahama, Y., 2011. Gender differences in tryptophan hydroxylase-2 mRNA, serotonin, and 5-hydroxytryptophan levels in the brain of catfish, *Clarias gariepinus*, during sex differentiation. Gen. Comp. Endocrinol. 171, 94–104.
Saha, S., Patil, S., Singh, U., Singh, O., Singru, P.S., 2015. Sexual dimorphism in the hypophysiotropic tyrosine hydroxylase-positive neurons in the preoptic area of the teleost *Clarias batrachus*. Biol. Sex Differ. 6, 23.
Satou, M., Oka, Y., Kusunoki, M., Matsushima, T., Kato, M., Fujita, I., Ueda, K., 1984. Telencephalic and preoptic areas integrate sexual behavior in hime salmon (landlocked red salmon, *Oncorhynchus nerka*): results of electrical brain stimulation experiments. Physiol. Behav. 33, 441–447.
Schwarz, J.M., Nugent, B.M., McCarthy, M.M., 2010. Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. Endocrinology 151, 4871–4881.
Scott, C.J., Clarke, I.J., Rao, A., Tilbrook, A.J., 2004. Sex differences in the distribution and abundance of androgen receptor mRNA-containing cells in the preoptic area and hypothalamus of the ram and ewe. J. Neuroendocrinol. 16, 956–963.
Scott, C.J., Tilbrook, A.J., Simmons, D.M., Rawson, J.A., Chu, S., Fuller, P.J., Ing, N.H., Clarke, I.J., 2000. The distribution of cells containing estrogen receptor-α (ERα) and ERβ messenger ribonucleic acid in the preoptic area and hypothalamus of the sheep: comparison of males and females. Endocrinology 141, 2951–2962.
Shughrue, P.J., Bushnell, C.D., Dorsa, D.M., 1992. Estrogen receptor messenger ribonucleic acid in female rat brain during the estrous cycle: a comparison with ovariectomized females and intact males. Endocrinology 131, 381–388.
Södersten, P., 1972. Mounting behavior in the female rat during the estrous cycle, after ovariectomy, and after estrogen or testosterone administration. Horm. Behav. 3, 307–320.
Stacey, N.E., Kobayashi, M., 1996. Androgen induction of male sexual behaviors in female goldfish. Horm. Behav. 30, 434–445.
Stacey, N.E., Kyle, A.L., 1983. Effects of olfactory tract lesions on sexual and feeding behavior in the goldfish. Physiol. Behav. 30, 621–628.
Strobl-Mazzulla, P.H., Moncaut, N.P., López, G.C., Miranda, L.A., Canario, A.V., Somoza, G.M., 2005. Brain aromatase from pejerrey fish (*Odontesthes bonariensis*): cDNA cloning, tissue expression, and immunohistochemical localization. Gen. Comp. Endocrinol. 143, 21–32.
Sudhakumari, C.C., Senthilkumaran, B., Raghuveer, K., Wang, D.S., Kobayashi, T., Kagawa, H., Krishnaiah, Ch., Dutta-Gupta, A., Nagahama, Y., 2010. Dimorphic expression of tryptophan hydroxylase in the brain of XX and XY Nile tilapia during early development. Gen. Comp. Endocrinol. 166, 320–329.
Sun, L.N., Jiang, X.L., Xie, Q.P., Yuan, J., Huang, B.F., Tao, W.J., Zhou, L.Y., Nagahama, Y., Wang, D.S., 2014. Transdifferentiation of differentiated ovary into functional testis by long-term treatment of aromatase inhibitor in Nile tilapia. Endocrinology 155, 1476–1488.
Takatsu, K., Miyaoku, K., Roy, S.R., Murono, Y., Sago, T., Itagaki, H., Nakamura, M., Tokumoto, T., 2013. Induction of female-to-male sex change in adult zebrafish by aromatase inhibitor treatment. Sci. Rep. 3, 3400.
Tang, H., Chen, Y., Liu, Y., Yin, Y., Li, G., Guo, Y., Liu, X., Lin, H., 2017. New insights into the role of estrogens in male fertility based on findings in aromatase-deficient zebrafish. Endocrinology 158, 3042–3054.
Tobet, S.A., Shim, J.H., Osiecki, S.T., Baum, M.J., Canick, J.A., 1985. Androgen aromatization and 5α-reduction in ferret brain during perinatal development: effects of sex and testosterone manipulation. Endocrinology 116, 1869–1877.
Todd, E.V., Liu, H., Muncaster, S., Gemmell, N.J., 2016. Bending genders: the biology of natural sex change in fish. Sex. Dev. 10, 223–241.
Trudeau, V.L., Bosma, P.T., Collins, M., Priede, I.G., Docherty, K., 2000. Sexually dimorphic expression of glutamate decarboxylase mRNA in the hypothalamus of the deep sea armed grenadier, *Coryphaenoides (Nematonurus) armatus*. Brain Behav. Evol. 56, 269–275.
Yamashita, J., Kawabata, Y., Okubo, K., 2017. Expression of isotocin is male-specifically up-regulated by gonadal androgen in the medaka brain. J. Neuroendocrinol. 29, e12545.
Yang, C.F., Shah, N.M., 2014. Representing sex in the brain, one module at a time. Neuron 82, 261–278.
Yang, T., Shah, N.M., 2016. Molecular and neural control of sexually dimorphic social

behaviors. Curr. Opin. Neurobiol. 38, 89–95.
Yin, Y., Tang, H., Liu, Y., Chen, Y., Li, G., Liu, X., Lin, H., 2017. Targeted disruption of aromatase reveals dual functions of *cyp19a1a* during sex differentiation in zebrafish. Endocrinology 158, 3030–3041.
Voigt, C., Ball, G.F., Balthazart, J., 2009. Sex differences in the expression of sex steroid receptor mRNA in the quail brain. J. Neuroendocrinol. 21, 1045–1062.
Volff, J.N., Nanda, I., Schmid, M., Schartl, M., 2007. Governing sex determination in fish: regulatory putsches and ephemeral dictators. Sex. Dev. 1, 85–99.
Wai, E.H., Hoar, W.S., 1963. The secondary sex characters and reproductive behavior of gonadectomized sticklebacks treated with methyl testosterone. Can. J. Zool. 41, 611–628.
Weisz, J., Brown, B.L., Ward, I.L., 1982. Maternal stress decreases steroid aromatase activity in brains of male and female rat fetuses. Neuroendocrinology 35, 374–379.
Westberry, J.M., Trout, A.L., Wilson, M.E., 2010. Epigenetic regulation of estrogen receptor gene expression in the mouse cortex during early postnatal development. Endocrinology 151, 731–740.
Zhou, Y., Shughrue, P.J., Dorsa, D.M., 1995. Estrogen receptor protein is differentially regulated in the preoptic area of the brain and in the uterus during the rat estrous cycle. Neuroendocrinology 61, 276–283.