

Chapter 7

Sex Steroid Regulation of Male- and Female-Typical Mating Behaviors in Teleost Fish



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Abstract Males and females exhibit innate sex-specific mating behaviors, which are established developmentally. In mammals and birds, phenotypic sex differences in mating behaviors are stable and essentially irreversible, because the underlying neural substrates are irreversibly sex-differentiated prior to puberty due to the effects of gonadal steroids and the sex chromosome complement. In contrast, experimental manipulation of the hormonal milieu of teleost fish in adulthood effectively reverses male and female mating behaviors, illustrating the lifelong sexual lability in their underlying neural substrates. Consistent with this, evidence is accumulating that, in teleosts, both early gonadal steroids and the sex chromosome complement have little effect on the development of sex-typical mating behaviors; instead, recent work in medaka (*Oryzias latipes*) demonstrates that mutual antagonism between estradiol-17 β signaling through an estrogen receptor subtype Esr2b and 11-ketotestosterone signaling through androgen receptor in adulthood, rather than during development, mediates sex-typical mating behaviors in a reversible and transient manner. Further evidence is provided that the pronounced sexual dimorphism and adult steroid-dependent lability in the expression of Esr2b and downstream effectors, including neuropeptide B, in the telencephalic and preoptic nuclei underlie the neural basis of induction, maintenance, and reversal of male and female mating behaviors in teleosts.

Keywords Brain · Mating behavior · Sex steroid · Sexual spectrum · Teleost

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7.1 Introduction

From invertebrates to humans, males and females of a given species display differences in a wide range of physiological and behavioral traits. Perhaps the most obvious differences are found in traits directly relevant to reproduction, including mating behaviors. Males commonly perform elaborate courtship displays to attract females for mating, while female behavior is primarily an evaluation of male suitability. These differences result from differential development and activation of the underlying neural substrates in males versus females (Yang and Shah 2014, 2016; Chen and Hong 2018).

In vertebrates, sex-specific mating behaviors and the underlying neural substrates are highly dependent on the milieu of gonadal hormones, particularly sex steroids, which is typical of each sex (McCarthy et al. 2017; Jennings and de Lecea 2020). In adult males, high and persistent levels of circulating androgens activate the neural substrates to facilitate the expression of male-typical mating behaviors, whereas in adult females, cyclic elevations in circulating estrogens, progestins, and prostaglandins activate the neural substrates to achieve female-typical mating behaviors. While this mechanism seems to be fundamental to the expression of sex-typical mating behaviors in all vertebrate taxa, large variations in the impact and mode of action of gonadal hormones are apparent across taxa.

This review briefly summarizes current knowledge on how gonadal hormones regulate male and female mating behaviors in teleost fish, which are unique among vertebrates in that their sex-specific phenotypes, including behaviors, are quite labile and can even be reversed as adults (Okubo et al. 2019; Nagahama et al. 2021). Considering the framework of this book, which regards sex as a spectrum rather than a binary, teleosts can transition from one end of the spectrum to the other throughout their lifetime. This, combined with knowledge from other vertebrates, highlights the mechanistic underpinnings of the permanence/labability of sex-specific mating behaviors, or in other words, the settlement and transition of these behaviors on the sexual spectrum.

7.2 Sex Differences in Mating Behaviors Are Stable and Essentially Irreversible in Mammals and Birds

While the pattern of gonadal hormone secretion in adulthood is certainly a proximate factor that influences sex-typical mating behaviors, the overall picture of the mechanism underlying these behaviors is not that simple. Reversal of sex-typical mating behaviors in adult mammals and birds does not generally occur. Even after ovariectomy and androgen administration to alter their sex steroid milieu to that typical of males, females do not exhibit male-typical mating behaviors to the same extent as males. Similarly, adult males of these taxa, if castrated and treated with estrogens and progestins, fail to show female-typical behaviors. These observations indicate

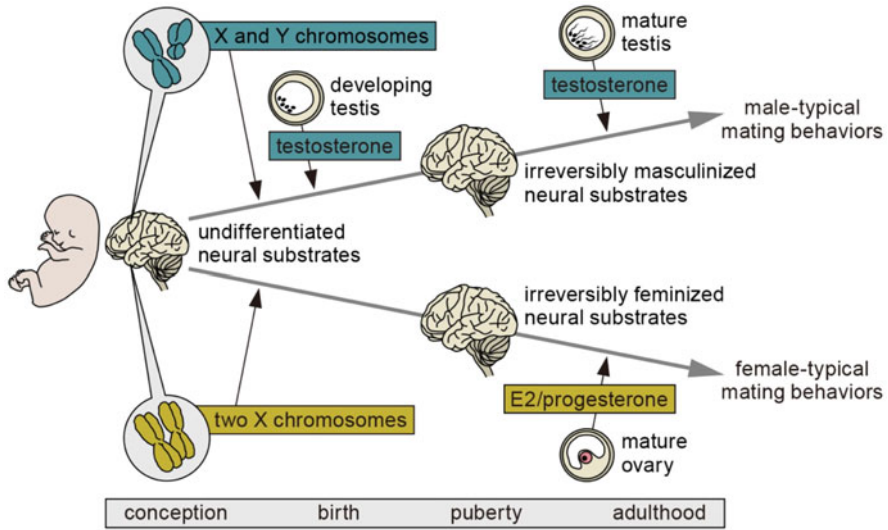


Fig. 7.1 Mechanisms underlying the development of sex-typical mating behaviors in mammals. The neural substrates that mediate mating behaviors are irreversibly and constitutively sex-differentiated prior to puberty, depending on early developmental exposure to gonadal steroids (testicular testosterone) and the sex chromosome complement of brain cells (XX vs. XY chromosomes). The sex-differentiated neural substrates are then activated in the adult by increased steroid secretion from mature gonads in a sex-specific manner (testosterone from the testes and estradiol-17 β (E2) and progesterone from the ovaries) for manifestation of sex-typical behaviors

that differential expression of mating behaviors by males and females is essentially permanent and irreversible in mammals and birds.

More than half a century of research on these taxa (mostly in rodents) has revealed the mechanisms underlying the permanent differentiation of the male and female brain (McCarthy and Arnold 2011; McCarthy et al. 2017; McCarthy 2020; Balthazart 2019) (Fig. 7.1). In rodents, the fetal testes of males secrete large amounts of testosterone, whereas the fetal ovaries of females remain hormonally quiescent. Once in the developing male brain, testicular testosterone is largely converted by cytochrome p450 aromatase into estradiol-17 β (E2), which then acts to masculinize (induce male-typical phenotypes of, or direct toward the male end of the sexual spectrum) and defeminize (prevent female-typical phenotypes of, or direct away from the female end of the spectrum) the neural substrates that later mediate sex-specific behaviors. In the absence of testosterone during development, as in the female brain, the neural substrates are feminized (female-typical phenotypes are induced or directed toward the female end of the sexual spectrum) and demasculinized (male-typical phenotypes are prevented or directed away from the male end of the spectrum), although exposure to E2 during the prepubertal period is required for full feminization (McCarthy et al. 2017; Balthazart 2019). A similar mechanism is present in primates, although testosterone acts directly to masculinize the brain, without prior conversion to E2 (Bao and Swaab 2011). In quail, in contrast

to rodents, the fetal female ovaries secrete E2, which acts on the developing brain to feminize and demasculinize the neural substrates, while the absence of fetal E2 secretion masculinizes and defeminizes them as occurs in males (Adkins-Regan 2009; Maekawa et al. 2014; Balthazart 2019). In spite of such variations among species, these steroidal effects early in development, traditionally referred to as “organizational effects”, represent a common mechanism for sexual differentiation of brain and behavior in mammals and birds. Importantly, the early steroid effects are basically irreversible and permanently differentiate the neural substrates. This allows for the manifestation of behaviors typical of one sex later in life, while concurrently preventing that of the other sex.

The sex-differentiated neural substrates are then activated in adulthood by increasing sex steroid secretion from mature gonads (McCarthy and Arnold 2011; McCarthy et al. 2017). In adult males, testicular testosterone activates the masculinized neural substrates to allow male-typical mating behaviors, whereas in adult females, E2 and progesterone arising from the ovaries activate the feminized neural substrates to allow female-typical behaviors (in male rodents, the bulk of adult testicular testosterone acts after being aromatized to E2 in the brain, as fetal testosterone does, and thus E2 is primarily responsible for mating behaviors, as in females). These effects of sex steroids in adulthood are traditionally referred to as “activational effects” and differ from early steroid effects in that they are reversible and transient in nature, thus existing only when steroids are continuously present.

Although gonadal sex steroids are the dominant drivers of most sexually differentiated behaviors, studies over the past few decades have established the contribution of the sex chromosome complement (XX vs. XY in mammals, or ZZ vs. ZW in birds) which directly and constitutively affects the sexual differentiation of some behaviors, independent of steroidal effects (Arnold 2017, 2020). A notable example comes from zebra finches, where the sex chromosome complement contributes to the masculine development of the song system, a series of interconnected nuclei that are essential for learning and producing courtship songs, a male-biased behavioral trait (Agate et al. 2003). These effects, referred to as “sex chromosome effects”, have also been shown to contribute to parental and aggressive behaviors and social interactions (McCarthy and Arnold 2011), although the proximate mechanisms of these effects remain elusive.

Collectively, sex-specific behaviors and the underlying neural substrates are shaped by (1) the effects of gonadal sex steroids early in life, which cause irreversible and enduring sex differences in the developing neural substrates; (2) the effects of gonadal sex steroids in adulthood, which activate the differentiated neural substrates to drive sex-specific behaviors in a reversible and transient manner; and (3) the effects of sex chromosome complement, where direct sex-specific actions of sex chromosome genes within the brain induce sex differences in some behavioral phenotypes (Fig. 7.1). Mammals and birds exhibit stable sex differences in mating behaviors, most probably because their underlying neural substrates are irreversibly and constitutively sex-differentiated prior to puberty under the influence of early gonadal steroids and sex chromosome complement.

7.3 Mating Behaviors in Teleosts Are Highly Sexually Labile Across Their Lifetime

Although differential expression of mating behaviors by males and females is essentially fixed in mammals and birds, some studies, albeit few, have challenged the permanence of these behaviors and suggest that a certain degree of sexual lability is retained into adulthood. For example, female rodents can exhibit mating behaviors typical of males, though less frequently, either in response to testosterone or E2 treatment in adulthood (Edwards and Burge 1971; Södersten 1972). In addition, the medial preoptic nucleus of the quail brain, which reportedly is involved in male mating behaviors, is about 1.4 times larger in males than in females, and this sex difference relies to some extent on the adult steroid milieu (Adkins-Regan 2009; Balthazart et al. 2010; Balthazart 2019). In agreement with these findings, female quail treated with testosterone as adults display some male-specific courtship behaviors, such as crowing and strutting (Ball et al. 2014).

Notably, and interestingly, sexual lability in behavioral phenotypes is seen much more frequently and thoroughly in teleost fish, in which experimental manipulation of the hormonal milieu of adult males and females effectively reverses sex-typical behaviors. For example, adult male and female goldfish (*Carassius auratus*) receiving acute treatment with prostaglandin F₂ α (PGF₂ α) or androgens, respectively, display mating behaviors typical of the opposite sex (Stacey and Kyle 1983; Stacey and Kobayashi 1996; Ghosal and Sorensen 2016). Similarly, female stickleback (*Gasterosteus aculeatus*) and medaka (*Oryzias latipes*) respectively exhibit nest-building behavior or courtship displays that are typical of males, when treated with androgens as adults (Wai and Hoar 1963; Nishiike et al. 2021). Moreover, the frequency of courtship displays induced in androgen-treated female medaka can be as high as in males (Nishiike et al. 2021).

Teleosts are even more unique in that many species spontaneously undergo phenotypic sex reversal, involving both anatomical and behavioral changes, depending on physiological, environmental, and social cues (Godwin 2010; Liu et al. 2017; Capel 2017). Even in teleost species that do not normally sex-reverse in adult life, such as tilapia (*Oreochromis niloticus*), medaka, African cichlid (*Astatotilapia burtoni*), and zebrafish (*Danio rerio*), morphological, and often behavioral, sex reversal can be induced by chronically exposing adult females to an aromatase inhibitor to block the conversion of androgens to estrogens (Paul-Prasanth et al. 2013; Takatsu et al. 2013; Sun et al. 2014; Göppert et al. 2016). These facts strongly imply that brain and behavior of teleosts, regardless of whether the species normally switch sex or not, are sexually labile throughout their lifetime and can even be reversed as adults (Okubo et al. 2019).

7.4 Sex-Typical Mating Behaviors in Teleosts Are Largely Dependent on the Adult Steroid Milieu

The enduring adult sexual lability in mating behaviors in teleost fish suggests that their neural substrates undergo a unique process of sexual differentiation from those of mammals and birds. It is plausible to assume that gonadal sex steroids early in life cause only minimal, if any, irreversible sex differences in the neural substrates, or that these differences can readily be reversed by the prevailing sex steroid milieu in adulthood. The former supposition is supported by the observation that neither testosterone nor E2 levels are appreciably elevated in medaka embryos (Iwamatsu et al. 2005, 2006). In addition, the levels of aromatase expression in the developing medaka brain are much lower than those in adult fish, with no transient increase or sex difference (Okubo et al. 2011). This is in sharp contrast to what occurs in developing rodent brain, where high levels of aromatase activity are transiently induced in a male-biased manner to help masculinize and defeminize the neural substrates (Okubo et al. 2019).

Presumably, the sex chromosome complement also plays a minor role, if any, in sexual differentiation of the neural substrates in teleosts, because the permanent and invariable effects that the constitutive presence of sex chromosomes should produce are inconsistent with the sexual lability of teleosts. In line with this notion, in medaka and tongue sole (*Cynoglossus semilaevis*), sex-reversed males and females (i.e., males with the sex chromosome constitution XX or ZW and females with XY or ZZ chromosomes) appear to be as fertile and behaviorally active in mating as normal males and females (Voff et al. 2007; Paul-Prasanth et al. 2013; Chen et al. 2014), although there has been no quantitative examination of behavior or neural function of sex-reversed fish. The sex chromosomes of teleosts have arisen only recently and independently many times in different families, genera, or species (many teleost species do not have sex chromosomes, and sex is instead determined by environmental signals) (Marshall Graves and Peichel 2010; Kikuchi and Hamaguchi 2013; Gammerdinger and Kocher 2018). Since their sex chromosomes are still in the early stages of differentiation, the chromosome pairs are not morphologically distinguishable and are almost identical with only one or a few different loci. As an extreme example, in some fish species including pufferfish (*Takifugu rubripes*) and amberjacks (*Seriola dumerili*), the sex chromosome pairs essentially differ by only a single nucleotide (Kamiya et al. 2012; Koyama et al. 2019). The lack of extensive genetic differences between males and females probably limits the potential impact of the sex chromosome complement on sex-typical behaviors and the underlying neural substrates in teleosts (Okubo et al. 2019).

Assuming that neither early gonadal steroids nor the sex chromosome complement has much effect on sexual differentiation of the neural substrates underlying sex-typical mating behaviors, it is highly likely that gonadal steroids in adulthood play a decisive role in teleosts. The reversible and transient nature of the adult steroidal effects agrees with the marked sexual lability of behavioral phenotypes in teleosts. The reversal of sex-typical mating behaviors upon manipulation of the adult

hormonal milieu described above exactly reflects such a nature, and suggests that these effects are largely, if not solely, responsible for sex-typical mating behaviors. In teleosts, gonadal steroids in adult life may serve to both differentiate the neural substrates and activate them to actuate sex-typical mating behaviors in a reversible and transient manner. It appears that the neural substrates for sex-typical mating behaviors in teleosts remain undifferentiated until the onset of puberty, after which they are differentiated and simultaneously activated depending on the sex-specific steroid milieu in the adult.

7.5 Teleosts Have a Unique Adult Steroid Milieu Compared to Mammals and Birds

The adult steroid milieu of teleost fish differs from that of mammals and birds in several important respects (Figs. 7.2 and 7.3). The mature teleost testes secrete copious quantities of 11-ketotestosterone (11KT) in addition to testosterone into the circulation, which eventually gain access to the brain. 11KT is generally more effective than testosterone in stimulating the development of secondary sexual characteristics, spermatogenesis, and mating behaviors in teleosts (Borg 1994). Because of this and the fact that the circulating levels of 11KT, unlike testosterone (see below), are much higher in males than in females, 11KT is regarded as the primary testicular androgen in teleosts (Devlin and Nagahama 2002). Importantly, 11KT also differs from testosterone in that it cannot be aromatized into estrogens, and all of its actions are mediated by the androgen receptor (AR) (Borg 1994). This mode of action is also distinct from that of 5 α -dihydrotestosterone (DHT), a potent


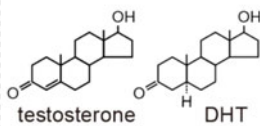
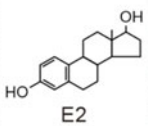
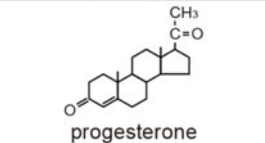

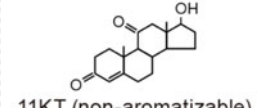
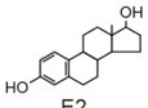
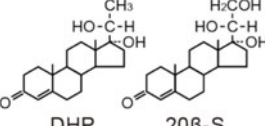
	major androgen	major estrogen	major progestin
 mammals	 testosterone DHT	 E2	 progesterone
 teleosts	 11KT (non-aromatizable)	 E2	 DHP 20 β -S

Fig. 7.2 Comparison of major sex steroids in mammals and teleosts. The major androgen in teleosts is 11-ketotestosterone (11KT; which cannot be aromatizable), and not testosterone or 5 α -dihydrotestosterone (DHT) as in mammals. In teleosts, testosterone serves as a precursor to 11KT and estradiol-17 β (E2), rather than as an androgenic steroid. In addition, the major progestin in teleosts is not progesterone as in mammals, but 17,20 β -dihydroxy-4-pregnen-3-one (DHP), or in some species, 17,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S). The major estrogen in both mammals and teleosts is E2

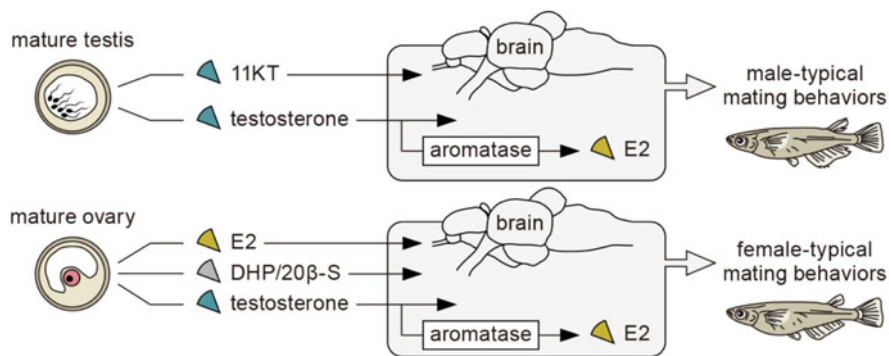


Fig. 7.3 The adult sex steroid milieu in teleosts. The mature testis primarily secretes 11-ketotestosterone (11KT) and testosterone, whereas the mature ovary mainly secretes estradiol- 17β (E2), 17,20 β -dihydroxy-4-pregnen-3-one (DHP) (or 17,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S) in some species), and testosterone. Sex-typical mating behaviors in teleosts largely depend on the sex-specific adult steroid milieu. Of note, teleosts have much higher brain aromatase activity than any other vertebrates. Hence, circulating testosterone, once in the brain, is converted to E2, resulting in a significant accumulation of E2 even in the male brain

androgen in mammals and birds, which cannot be aromatized into estrogens, but whose metabolite, 3β -androstenediol, binds to the estrogen receptor (ESR, also known as ER) and exerts estrogenic effects (Handa et al. 2009).

The mature ovaries of teleosts secrete large amounts of E2 and progestin into the circulation, as do those of mammals and birds; however, the major progestin in teleosts is not progesterone as in mammals and birds, but 17,20 β -dihydroxy-4-pregnen-3-one (DHP), or in some species, 17,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S) (Scott et al. 2010). Strikingly, teleost ovaries also produce substantial amounts of testosterone, and its circulating levels in adult females are comparable to and sometimes higher than the levels found in adult males (Katz and Eckstein 1974; Borg 1994). Testosterone therefore likely functions as a precursor to both the major androgen 11KT and the major estrogen E2, rather than as an androgenic steroid, in teleosts. DHT is also produced in both the testes and ovaries of teleosts; however, its circulating levels are typically lower than those of testosterone and 11KT and are not significantly male-biased, leaving the role of DHT in teleosts largely unexplored (Margiotta-Casaluci et al. 2013; Martyniuk et al. 2013; Nishiike et al. 2021; Yazawa et al. 2021).

Another salient feature of the sex steroid milieu in teleosts is that their adult brain, irrespective of sex, has more than 100-fold greater aromatase activity than the adult mammalian and avian brains, and is thus a major site of E2 production along with the ovary (Pasmanik and Callard 1985). In rodents, brain aromatase activity reaches its peak during fetal life and declines gradually thereafter to adult levels, representing only a small percentage of the fetal peak; the remaining activity in adulthood is, however, still important for mating behaviors (Tobet et al. 1985; Beyer et al. 1993; Lephart 1996) (Fig. 7.4). In contrast, brain aromatase expression and activity in

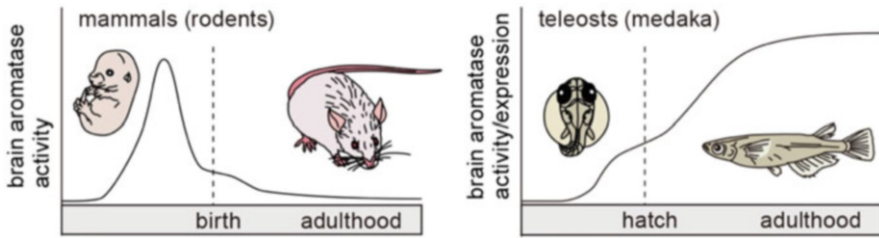


Fig. 7.4 Lifetime changes in brain aromatase activity/expression in mammals and teleosts. In mammals (rodents), brain aromatase activity peaks during fetal development and gradually decreases toward adulthood. In contrast, in teleosts (medaka), brain aromatase activity/expression increases with age and sexual maturation, reaching levels in adulthood comparable to or higher than the fetal peak in rodents

teleosts increases with age and sexual maturation and reaches levels comparable to or higher than the fetal peak in rodents during adult life (Le Page et al. 2010; Okubo et al. 2011) (Fig. 7.4). Due to this very high level of aromatase activity, a substantial fraction of the circulating testosterone that reaches the adult brain is converted to E2, resulting in high levels of E2 even in the male brain (though not as much as in the female).

7.6 Duplicated Steroid Receptor Subtypes in Teleosts Have Largely Nonredundant Roles in Mediating Sex-Typical Mating Behaviors

What then is the mechanism of action of adult sex steroids in establishing and reversing sex-typical mating behaviors in teleosts? The action of sex steroids is primarily mediated via binding to specific intracellular receptors, including AR, ESR, and progesterin receptor (PGR, also known as PR), which function as ligand-dependent transcription factors to regulate the expression of downstream genes. While tetrapods have a single AR subtype and two ESR subtypes (ESR1 and ESR2), most teleosts have two AR subtypes (Ara and Arb) and three ESR subtypes (Esr1, Esr2a, and Esr2b), except for some species, including zebrafish, which have only a single AR (Ikeuchi et al. 1999; Hawkins et al. 2000; Douard et al. 2008) (Fig. 7.5). Ara and Arb are duplicates that arose because of a whole-genome duplication event in the teleost lineage and are co-orthologous to tetrapod AR (Ogino et al. 2018). Similarly, Esr2a and Esr2b are co-orthologs of tetrapod ESR2, arising from the teleost-specific genome duplication (Ogino et al. 2018). In contrast to AR and ESR, both tetrapods and teleosts have only a single subtype of PGR.

Duplicated genes often diverge in function, partitioning the multiple functions of their single ancestral gene (Force et al. 1999; Lynch and Force 2000; Postlethwait et al. 2004). Studies of mice and several teleost species rendered deficient in AR and

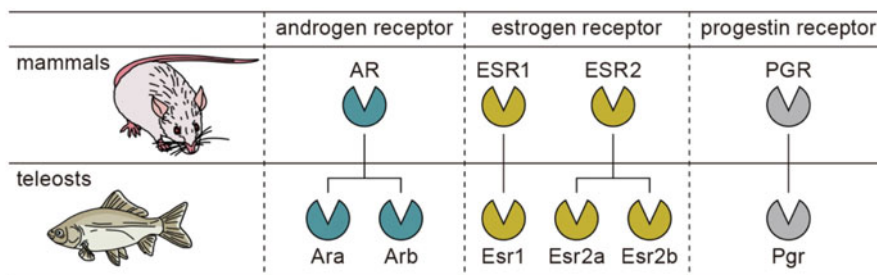


Fig. 7.5 Comparison of intracellular steroid receptor subtypes in mammals and teleosts. While mammals have a single androgen receptor (AR) subtype and two ESR subtypes (ESR1 and ESR2), most teleosts have two AR subtypes (Ara and Arb) and three ESR subtypes (Esr1, Esr2a, and Esr2b). Orthologous subtypes are indicated by connecting lines. Teleosts have more subtypes of these receptors as a consequence of a whole-genome duplication event early in their evolution. In contrast to AR and ESR, teleosts have only a single progesterin receptor (PGR) subtype, as do mammals

ESR subtypes have revealed that they indeed have largely nonredundant roles in mediating mating behaviors. In male rodents, testicular testosterone, once in the brain, is aromatized to E2, which then acts through ESR1 to masculinize and activate the neural substrates to actuate male-typical mating behaviors and, in addition, through ESR2 to defeminize the neural substrates (McCarthy and Arnold 2011; McCarthy et al. 2017). The most compelling evidence for this is the finding that male mice lacking *Esr1* show greatly reduced male-typical mating behaviors (Ogawa et al. 1997, 1999), whereas those lacking *Esr2* show no overt defect in these behaviors, but display lordosis, a characteristic mating posture of female rodents, when castrated and given estrogens and progesterone as adults (Kudwa et al. 2005). Testosterone also acts directly on AR in the male rodent brain without being aromatized to E2. In male mice, however, dysfunction of AR in the brain leads to only a modest reduction in mating behaviors (Juntti et al. 2010), while loss of both ESR subtypes completely abolishes them (Ogawa et al. 2000). It thus seems that AR-mediated signaling is not essential for the masculinization of mating behaviors, but rather regulates the extent of behavioral displays in males (Juntti et al. 2010).

In contrast, in teleosts, where nonaromatizable 11KT is the primary androgen, studies with different agonists and antagonists have repeatedly shown that AR-mediated 11KT signaling is critical for male-typical mating behaviors (e.g., Stacey and Kobayashi 1996; van Breukelen 2013; Alward et al. 2019). This is confirmed by recent findings in zebrafish that *ar*-deficient males (zebrafish have only a single AR) are fertile, but court females less vigorously (Yong et al. 2017) and that males deficient in a steroidogenic enzyme *cyp17a1* show reduced androgen levels and impaired mating behaviors, which can be rescued by administration of 11KT (Zhai et al. 2018; Shu et al. 2020). In addition, in African cichlid, loss of *ara*, but not *arb*, results in males with diminished mating behaviors, indicating that *ara* mediates the effects of 11KT on male mating behaviors (Alward et al. 2020). In contrast, the involvement of ESR in male mating behaviors remains unclear in

teleosts. Male medaka lacking *esr2b* show no defects in mating behaviors, suggesting that this ESR subtype does not play a role in eliciting male-typical mating behaviors (Nishiike et al. 2021); however, no attempt has been made to determine whether *esr1* and *esr2a* have any such role. Considering that large amounts of E2 are produced locally by aromatase in the male brain of teleosts, ESR may play some role, albeit minor, in male mating behaviors in teleosts as it does in rodents. In fact, it has been reported that treating adult male guppies (*Poecilia reticulata*) with an aromatase inhibitor attenuates their courtship activities (Hallgren et al. 2006). However, the aromatase inhibitor has no such effect in African cichlid, and further testing with other species would be necessary to draw any conclusions (Huffman et al. 2013).

Then, what about female-typical mating behaviors? In female rodents, E2 activates the feminized neural substrates to induce female-typical mating behaviors mainly through *Esr1*. This is evidenced by the finding that female mice lacking *Esr1* are not receptive to male courtship, while those lacking *Esr2* mate normally (Ogawa et al. 1996, 1998, 1999; Rissman et al. 1997). Recent work in medaka has shown that female-typical mating behaviors in teleosts also require ESR-mediated E2 signaling (Nishiike et al. 2021). However, unlike in rodents, the effect of E2 in teleosts appears to be mediated primarily by *Esr2b* (a teleost ortholog of ESR2), not *Esr1*; female medaka deficient in *esr2b* are not sexually receptive to courting males, despite retaining normal ovarian function with an unaltered sex steroid milieu (Nishiike et al. 2021), whereas *esr1* and *esr2a* deficiency do not prevent normal mating behaviors in females (Tohyama et al. 2017; Kayo et al. 2019). Even more strikingly, *esr2b*-deficient female medaka are not only unreceptive to male courtship, but often court other females. This finding indicates that *Esr2b* is the major determinant of sex-typical mating behaviors, playing a decisive role in demasculinization as well as feminization of these behaviors. This is in marked contrast to rodents, in which neither E2/ESR1 nor E2/ESR2 signaling is apparently involved in demasculinization of mating behaviors (rather they serve critical roles in the masculinization and defeminization, respectively, as mentioned above). It should be noted, however, that recent evidence suggests that, in quail, fetal E2 demasculinizes the neural substrates of mating behaviors primarily through *Esr2* (Court et al. 2020). The extent to which the demasculinizing effect of E2/ESR2 signaling is prevalent in vertebrates is an important question that warrants further investigation.

7.7 E2/*Esr2b* and 11KT/AR Signaling Act Antagonistically to Regulate Mating Behaviors in Teleosts

The study of *esr2b*-deficient female medaka also revealed that they develop male-typical mating behaviors due to the AR-mediated action of small amounts of 11KT (or, less likely, DHT) secreted by the ovary (Nishiike et al. 2021). Although the ovary of normal females also secretes small amounts of 11KT, they do not show

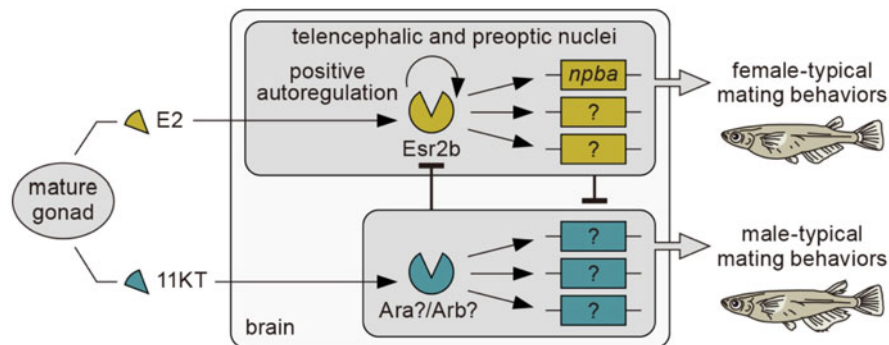


Fig. 7.6 Regulation of teleost mating behaviors by gonadal steroid signaling in adulthood, as revealed by studies in medaka. Estradiol-17 β (E2) signaling through an estrogen receptor subtype Esr2b is essential for the induction of female-typical, and the suppression of male-typical, mating behaviors, acting antagonistically to androgen receptor (Ara and/or Arb)-mediated 11-ketotestosterone (11KT) signaling. Presumably, the positive autoregulation of Esr2b expression in the telencephalic and preoptic nuclei by E2, followed by the activation of downstream effector genes such as *npba*, feminizes and demasculinizes mating behaviors, whereas repression of Esr2b expression therein by 11KT masculinizes and defeminizes these behaviors

male-typical behaviors, probably because the masculinizing effect of 11KT/AR signaling is suppressed by the demasculinizing effect of E2/Esr2b signaling. It seems that mutual antagonism between Esr2b-mediated E2 signaling and AR-mediated 11KT signaling in adult life serves to establish and actively maintain sex-typical mating behaviors (Fig. 7.6).

Another study in medaka unveiled that *esr2b* is expressed exclusively in females due to positive autoregulation by E2 in several brain regions, including the ventral telencephalic (Vs/Vp) and magnocellular preoptic (PMm/PMg) nuclei (Hiraki et al. 2012), which have been implicated, by classic lesion and stimulation studies, in mating behaviors (Demski et al. 1975; Kyle and Peter 1982; Koyama et al. 1984; Satou et al. 1984). Vs/Vp and PMm/PMg are part of the evolutionarily conserved “social behavior network” and considered homologous to the bed nucleus of the stria terminalis/subpallial amygdala and the paraventricular nucleus in the rodent brain, respectively (Newman 1999; Goodson 2005; O’Connell and Hofmann 2011, 2012; Goodson and Kingsbury 2013; Herget et al. 2014), which are also relevant to mating behaviors (Veenema and Neumann 2008; Yao et al. 2017; Bayless et al. 2019). It is reasonable to assume that activation of E2/Esr2b signaling in Vs/Vp and PMm/PMg is responsible for the expression of female-typical mating behaviors, and that males do not display female-typical behaviors because E2/Esr2b signaling is not activated in these brain nuclei. Crucially, 11KT has an inhibitory effect on *esr2b* expression in Vs/Vp and PMm/PMg; high levels of 11KT secretion from the male testis may contribute to the defeminization of male mating behaviors by inhibiting E2/Esr2b signaling in these brain nuclei (Hiraki et al. 2012; Nishiike et al. 2021). This defeminizing effect of 11KT, along with its ability to promote male-typical

(masculinize) behaviors, presumably induces and maintains the male pattern of mating behaviors.

It is noteworthy that the sexually dimorphic *esr2b* expression in Vs/Vp and PMm/PMg of medaka can be reversed between the sexes by altering the adult sex steroid milieu; the E2-dominated adult steroid milieu stimulates, whereas the 11KT-dominated adult steroid milieu inhibits, *esr2b* expression therein, regardless of sex (Hiraki et al. 2012; Nishiike et al. 2021). Sex-differentiated expression of steroid receptors and effects of the adult steroid milieu on it have also been reported in the brain of mammals and birds (Okubo et al. 2019). However, the degree of both sex differences and steroid effects is modest and much less extensive than *esr2b* expression in medaka. These differences between medaka and mammals/birds highlight the profound sexual dimorphism and adult steroid-dependent lability in the activation of E2/Esr2b signaling in behaviorally relevant nuclei of the teleost brain. These particular modes of E2/Esr2b signaling are most likely responsible for the adult sexual lability in teleost mating behaviors.

Evidence has accumulated in rodents that the effects of gonadal sex steroids early in life on brain and behavior are mediated, in part, by DNA methylation of steroid receptor genes, although there are some discrepancies between studies (Westberry et al. 2010; Kurian et al. 2010; Schwarz et al. 2010). This epigenetic modification limits the flexibility of receptor gene expression in relevant brain nuclei later in life in a sex-specific manner. Considering the lability of steroid receptor expression in adults, the teleost brain is unlikely to undergo such a developmental process. This suggests again that steroids early in life have little effect on sexual differentiation of brain and behavior in teleosts.

7.8 Neuropeptide B Is a Direct Mediator of E2/Esr2b Signaling and Required for Female Sexual Receptivity

Recent studies, particularly in mice employing optogenetic and chemogenetic manipulations, have identified sex steroid-responsive neural circuits that underlie sex-specific mating behaviors, as well as the effector genes downstream of sex steroids that mediate these behaviors (Bayless and Shah 2016; Chen and Hong 2018; Jennings and de Lecea 2020). Nonetheless, very few genes have been identified in any species that are sex-specific direct transcriptional targets of sex steroids for mediating sex-specific mating behaviors (Yang and Shah 2014). Given that the social behavior network comprises subsets of peptidergic neurons, neuropeptides and their receptors seem to be good candidates for direct targets of sex steroids. Work in the past few years in medaka has revealed that the gene encoding a neuropeptide, neuropeptide B (NPB), is an immediate downstream effector of E2/Esr2b signaling that mediates female receptivity to male courtship, as detailed below.

NPB, together with its close relative, neuropeptide W (NPW), was originally identified as a ligand for the orphan receptors GPR7 and GPR8 (now designated NPBWR1 and NPBWR2) (Fujii et al. 2002; Brezillon et al. 2003; Tanaka et al. 2003). In mammals, NPB and NPW have been implicated in diverse physiological processes, including energy homeostasis, food intake, inflammatory pain response, social interaction, and pituitary hormone secretion (Sakurai 2013; Watanabe and Yamamoto 2015). Teleost fish have two NPB genes (*npba* and *npbb*) and one NPBWR2 gene, while lacking the NPW and NPBWR1 genes. A search for genes differentially expressed between the sexes in the medaka brain identified *npba* as being much more highly expressed in females (Hiraki et al. 2014). It was subsequently found that *npba* is expressed exclusively in females in Vs/Vp and PMm/PMg, specifically in *esr2b*-positive neurons, as a result of direct transcriptional activation by E2/Esr2b signaling. Similarly, expression of *npbb* in these brain nuclei is nearly confined to females and is E2-dependent (Hiraki et al. 2014; Hiraki-Kajiyama et al. 2019; Nishiike et al. 2021). Behavioral testing revealed that both *npba*- and *npbwr2*-deficient female medaka require more time to accept males after receiving courtship stimulation. In addition, *npbwr2*-deficient females and females that are simultaneously deficient in both *npba* and *npbb* tend to accept males without being courted (Hiraki-Kajiyama et al. 2019). These findings suggest that NPB signaling in teleosts plays a significant role in female mate choice, possibly by facilitating the acceptance of males performing courtship display and the refusal of males exhibiting no courtship. Notably, the sexually dimorphic expression of *npba* and *npbb* in Vs/Vp and PMm/PMg can be reversed, to some extent, between female and male patterns in response to changes in the adult steroid milieu, as with *esr2b*. Furthermore, the morphological, transcriptional, and electrophysiological phenotypes of *npba/npbb*-expressing neurons that indicate cellular activation (e.g., large euchromatic nuclei with abundant cytoplasm, high levels of RNA polymerase II activity and histone marks of active transcription, and relatively high spontaneous firing rates) are all critically dependent on the adult E2 milieu (Kikuchi et al. 2019). Altogether, E2-dependent, and thus female-specific, NPB signaling is likely to be a crucial element of the neural circuitry that underlies sexual dimorphism and lability of teleost mating behaviors.

However, it is of note that the behavioral defects in females deficient in NPB signaling are much less severe than those in *esr2b*-deficient females. A possible explanation for this is that E2/Esr2b signaling regulates most, if not all, aspects of female mating behaviors by simultaneously affecting multiple behavior-related genes, including *npba/npbb*, and that each of these genes handles one or a few aspects separately (Fig. 7.6). This speculation is consistent with the view that sexually dimorphic social behaviors are regulated in a modular fashion by multiple sexually dimorphic genes that function downstream of sex steroid signaling (Xu et al. 2012).

7.9 Conclusions and Future Directions

Considering the evidence presented above, it is highly probable that, in teleost fish, neither early gonadal steroids nor the sex chromosome complement contributes much to the development of sex-typical mating behaviors; instead, steroids in adulthood serve to both differentiate the neural substrates and activate them to facilitate the expression of these behaviors in a reversible and transient manner. Recent evidence from studies in medaka further suggests that mutual antagonism between *Esr2b*-mediated E2 signaling and AR-mediated 11KT signaling in adult life underlies the induction, maintenance, and reversal of sex-typical mating behaviors. Individual adult fish can display a spectrum of sex-typical behaviors ranging from exclusively masculine to exclusively feminine, probably as a result of this mutual antagonism. These studies also suggest that the striking sexual dimorphism and steroid-dependent lability in *Esr2b* expression in the telencephalic and preoptic nuclei are the primary molecular basis for sexual differentiation and lability of teleost mating behaviors. The consequent sex-differentiated but reversible activation of downstream effectors, including *Npba/Npbb*, in response to the adult steroid milieu may allow for the transition of mating behaviors from one end of the sexual spectrum to the other.

Besides *Npba/Npbb*, several other neuropeptides, including gonadotropin-releasing hormones (*Gnrh2* and *Gnrh3*) (Yamamoto et al. 1997; Ogawa et al. 2006; Okuyama et al. 2014; Marvel et al. 2021), nonapeptides (vasotocin, the teleost ortholog of mammalian vasopressin, and isotocin, the teleost ortholog of mammalian oxytocin) (Yokoi et al. 2015, 2020), and secretogranins (*Scg2a* and *Scg2b*) (Mitchell et al. 2020), have been shown to be involved in mating behaviors in teleosts. Sex-differential expression of these neuropeptides has been reported in the brains of many teleost species (e.g., Grober et al. 1994, 2002; Elofsson et al. 1997, 1999; Foran and Bass 1998; Ishizaki et al. 2004; Black et al. 2004; Maruska et al. 2007; Maruska 2009; Kuramochi et al. 2011; Kawabata et al. 2012). The expression of these peptides has also been reported to be significantly altered with sex change in gobies (*Trimma okinawae*) and wrasses (*Thalassoma bifasciatum*) (Grober and Sunobe 1996; Godwin et al. 2000; Todd et al. 2019). However, only limited information is available on whether the behaviorally relevant expression of these neuropeptides is under the control of sex steroids, and if so, through what signaling pathways (Yamashita et al. 2017; Narita et al. 2018). This issue certainly warrants future research to determine the signaling pathways initiated by sex steroids in the brain to mediate sex-typical behaviors.

Another important issue to be addressed in the future is the functional connection between E2/*Esr2b* signaling and *PGF2 α* in regulating female mating behaviors. Evidence from several teleost species suggests that gonadal steroids, including E2, are not required for the execution of female mating behaviors, provided that sufficient *PGF2 α* is available (Munakata and Kobayashi 2010). In goldfish, for example, ovariectomized and immature females, and even males, are sexually receptive to courting males when administered with *PGF2 α* but not when administered with E2

(Stacey and Kyle 1983; Kobayashi and Stacey 1993). Similarly, in African cichlid, administration of PGF2 α to females in a nonreproductive state elicits female-typical mating behaviors (Kidd et al. 2013) and, moreover, genetic disruption of the PGF2 α receptor abolishes female receptivity (Juntti et al. 2016). These observations seem to be incompatible with the findings in medaka that defined the relevance of E2/Esr2b signaling to female mating behaviors (Nishiike et al. 2021). The reason for this incompatibility is currently unknown, but it could be due to species differences in the impact and mode of action of E2 and PGF2 α . Alternatively, and more likely, E2/Esr2b signaling for female mating behaviors may involve E2 produced locally in the brain, rather than, or in addition to, circulating E2 derived from the ovary, and acts upstream of PGF2 α signaling. This is in accordance with the fact stated above that the adult teleost brain has more than 100-fold greater aromatase activity than the adult mammalian and avian brains and thus produces greater amounts of E2 (Pasmanik and Callard 1985). Future work is needed to clarify the behavioral role of E2 produced in large amounts in the teleost brain.

Finally, future work is also necessary to ascertain whether the findings and implications presented in this review can be generalized across species in teleost fish. As the most diverse group of vertebrates (comprising half of all vertebrate species), teleosts exhibit a remarkable diversity in reproductive strategies. However, sex steroid regulation of sex-typical behaviors has been studied only in a few species. One should be cautious in assuming that findings from these species will apply to other species.

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