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Social Regulation of Gene Expression in the Hypothalamic-Pituitary-Gonadal Axis

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Reproduction is a critically important event in every animals' life and in all vertebrates is controlled by the brain via the hypothalamic-pituitary-gonadal (HPG) axis. In many species, this axis, and hence reproductive fitness, can be profoundly influenced by the social environment. Here, we review how the reception of information in a social context causes genomic changes at each level of the HPG axis.

Reproduction is arguably the most important event in any animals' life. Thus understanding how reproduction is regulated offers important insights into the evolution of a species. In particular, learning how social and physiological factors collaborate to control reproductive activity is essential for understanding selective pressures that have shaped reproductive control. For example, how does reception of social information reach brain regions responsible for initiating reproductive behaviors, how is gamete (sperm, oocyte) production and steroid hormone release controlled, and, ultimately, how do social interactions influence gene expression to control reproduction? Although there are many studies on regulation of social behaviors from molecular or genomic perspectives (120, 121), much less is known about how social information directly influences the reproductive genome from the brain to the gonads. Here, we review how social information can influence plasticity in gene expression of the highly conserved vertebrate hypothalamic-pituitary-gonadal (HPG) reproductive control system.

For the purpose of this review, we define social behaviors as interactions among members of the same species that influence immediate or future behaviors (120). Importantly, these interactions include the production, reception, and interpretation of communicative signals that influence individual behaviors in a context-dependent manner. Specifically, we concentrate on how communication signals in a social context produce genomic changes to alter the function of the HPG axis. We focus on key elements in the signaling pathway initiated by gonadotropin-releasing hormone (GnRH) neurons in the brain, their impact on gonadotrope-producing cells [luteinizing hormone (LH); follicle-stimulating hormone (FSH)] of the anterior pituitary gland, and the activation of LH and FSH receptors in the testes that leads to stimulation of steroid biosynthesis and sperm production. In light of the recent evidence showing the RFamides, including kisspeptin and gonadotropin inhibitory hormone, also play critical roles in HPG axis function, we discuss the social influence of these upstream regulators of GnRH neurons as well. We use examples from the social African cichlid fish *Astatotilapia* (formerly *Haplochromis*) *burtoni* as a model to illustrate how rapidly the social environment can influence the genome at every level of the HPG axis. Finally, we discuss how little is actually known about the neural pathways and mechanisms that translate social information into a genomic response that ultimately changes the activity of the reproductive axis. This sociogenomic frontier awaits future comparative research in diverse taxa.

GnRH and the HPG Axis in Fishes and Mammals

Reproduction in all vertebrates is controlled by the highly conserved HPG axis (FIGURE 1). At its apex are the GnRH neurons in the hypothalamic-preoptic area of the brain that ultimately control reproduction by integrating information from social and environmental signals with internal information such as nutritional and hormonal state. The output of this integration controls GnRH production in the brain and its release to the pituitary gland. GnRH, a decapeptide, is conserved across all vertebrates and was recently also found in several invertebrates, emphasizing its evolutionary importance (91, 140). There are multiple GnRH forms found across taxa, all of which have close phylogenetic relationships and putative common origin (20, 150). Teleost fishes have two or three different forms located in distinct brain regions, whereas most mammals express only two forms (20, 131). GnRH3 ({Trp^{7}Leu^8}-GnRH) has only been found in fishes, where it is localized in the terminal nerve ganglia, olfactory bulb, and ventral forebrain, and some evidence suggests it may be a neuromodulator (1, 57, 58, 85, 100). GnRH2 ({His^{5}Trp^{7}Tyr^8}-GnRH) is identical in all species examined to date, including humans, and is produced by neurons in the midbrain tegmentum. Limited data are

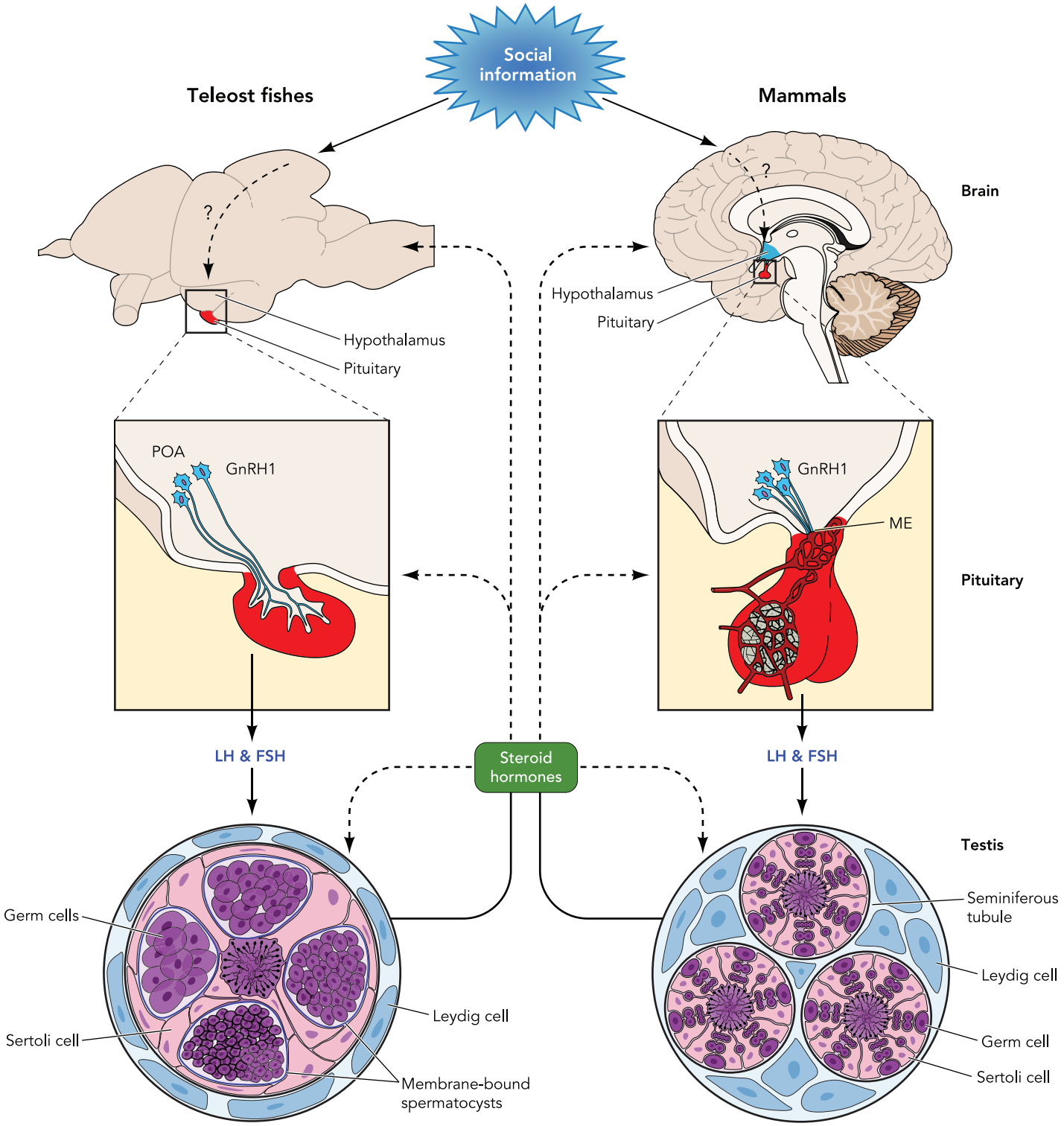

FIGURE 1. **Schematic representation of the hypothalamic-pituitary-gonadal axis in teleost fishes and mammals**
Information from social contexts is ultimately integrated by gonadotropin-releasing hormone (GnRH1) neurons in the preoptic-hypothalamic region of the brain to regulate the output of the reproductive axis. In fishes, GnRH1 neurons project directly to the pituitary gland (shown in red), whereas in mammals GnRH1 neurons release peptide into the median eminence (ME) where it is then transported via the hypothalamic-hypophyseal portal system to the anterior pituitary. In both taxa, LH and FSH are released from the pituitary and travel through the bloodstream to their target receptors in the testes to stimulate sperm production and steroid hormone synthesis and release. The organization of the testes also differs between fishes and mammals, as well as the cellular localization of LH and FSH receptors. In fishes, the testes is organized into membrane-bound spermatocysts that contain a group of synchronously developing germ cells derived from the same spermatogonial stem cell, followed by release of the mature spermatozoa into a central lumen. In mammals, spermatogenesis takes place in the seminiferous tubules where germ cells develop from the tubule wall in a centripetal direction toward the central lumen. POA, preoptic area.

consistent with a role for GnRH2 in coordinating reproduction with metabolic state (56, 86), modulating pineal gland activity (127), modulating sensory system function (85), and possibly influencing motor reproductive pathways (18, 77). GnRH1 is the hypophysiotrophic form produced by neurons in the hypothalamic-preoptic area that stimulates the pituitary gland. Despite its conserved function, the amino acid sequence of GnRH1 differs slightly among species (see Refs. 20, 60, 91 for reviews).

Although the functional actions of the HPG axis are conserved across all vertebrates, the basic anatomical organization of the hypothalamic-pituitary connection differs fundamentally between fishes and mammals (FIGURE 1). In teleost fishes, GnRH1 neurons in the preoptic area of the brain send axonal projections directly to the anterior pituitary gland where GnRH1 is released from nerve terminals in the vicinity of the gonadotrope cells, binds to membrane-bound G-protein-coupled GnRH receptors, and causes synthesis and release of the two gonadotropins, LH and FSH (72). This direct neural innervation of the pituitary is functionally equivalent but differs from mammals where the GnRH1 neurons release peptide into the hypothalamic median eminence for delivery via a hypothalamo-hypophyseal blood portal system to the anterior pituitary. Furthermore, whereas the different hormone-producing cell types (e.g., gonadotropes, somatotropes, thyrotropes, etc.) are arranged in a mosaic pattern in the mammalian pituitary, the fish pituitary maintains the embryonic compartmental organization where each specific cell type is localized in a different pituitary compartment.

The gonadotropin hormones LH and FSH released from the pituitary gland then travel via the general circulation to the testes where they bind to LH and FSH receptors, a family of rhodopsin-like G-protein-coupled receptors. Here again, there are noteworthy differences in cellular localization of the receptors and how LH and FSH act in the testes between fishes and mammals. In mammals, LHR is expressed in Leydig cells and primarily functions to stimulate steroid biosynthesis and release, whereas FSHR is found in Sertoli cells and plays a major role in sperm production (72, 97, 146). In teleosts, however, LHR is expressed in both Leydig and Sertoli cells, and FSHR is found in Leydig, Sertoli, and early germ cells (41, 42, 72). Thus the duality of gonadotropin function found in mammals is not apparent in most fishes because both LH and FSH are often equipotent in stimulating steroid production and can regulate spermatogenesis at different stages (42, 72).

The African Cichlid Fish *Astatotilapia Burtoni* as a Model for Socially Induced Genomic Plasticity of the Reproductive Axis

The African cichlid fish *Astatotilapia burtoni* lives in shallow shore pools and river estuaries of Lake Tanganyika in the rift valley system in Eastern Africa (38), and has proven to be an ideal model to examine how social information regulates genomic changes along the reproductive axis (FIGURE 2). The value of this system lies in its phenotypic plasticity: Males exist as two distinct reversible phenotypes, and their reproductive capacity and HPG axis activity are tightly coupled to social status (37). Dominant (also called territorial) males are brightly colored (blue or yellow) with a black stripe through the eye (eye bar), an opercular black spot

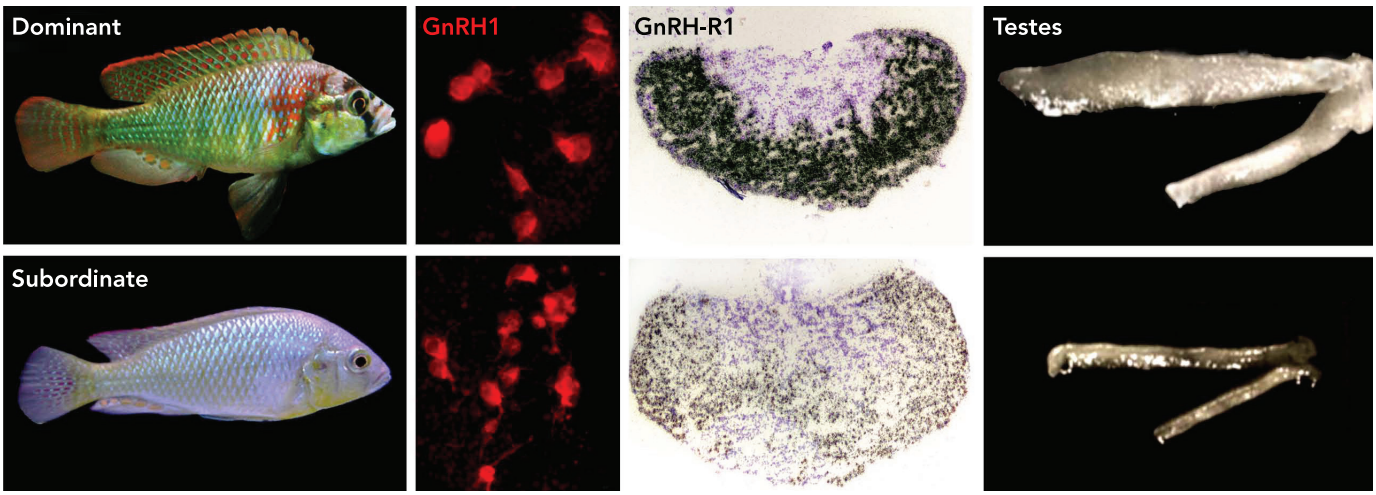

FIGURE 2. **The African cichlid fish *Astatotilapia burtoni* as a model for social regulation of the hypothalamic-pituitary-gonadal axis**
Phenotypic characters of reproductively active dominant males (*top*) and socially suppressed subordinate males (*bottom*). Dominant males have larger GnRH1 neurons (red; immunohistochemical staining) in the preoptic area of the brain (32, 151), higher GnRH-R1 levels (black, GnRH-R1 in situ hybridization; purple, cresyl violet counterstain) in the pituitary gland (4, 39, 83), and larger testes (32, 40, 81) compared with subordinate males.

at the caudal tip of the gill cover, prominent egg spots on the anal fin, and a red humeral patch on the body. Dominant males vigorously defend territories against rival males where they actively court and spawn with females (36, 38). In contrast, subordinate (also called nonterritorial) males are dull in coloration, do not hold territories or typically reproduce, school with females and other subordinate males, and flee from the aggressive attacks of dominant males. This disparity in behavior and appearance between males is also associated with important reproductive physiological differences such that dominant males have an active and upregulated HPG axis compared with subordinate males. For example, along the HPG axis, dominant males have larger GnRH1 neurons (32) with distinct membrane properties (e.g., higher membrane capacitance, lower input resistance, shorter action potential duration) (48), higher GnRH1, kisspeptin receptor (*kiss1r*), and steroid receptor mRNA levels in the brain (12, 49, 151), higher GnRH receptor type I, LHβ, and FSHβ mRNA levels in the pituitary (4, 83), higher circulating levels of androgens (testosterone and 11-ketotestosterone), estradiol, LH, and FSH (79, 82, 83, 103), higher levels of LH receptor, FSH receptor, and multiple steroid receptor subtypes in the testes (81), and larger testes with higher density of luminal sperm and spermatogenic potential (40, 68, 81) compared with subordinate males (FIGURE 3).

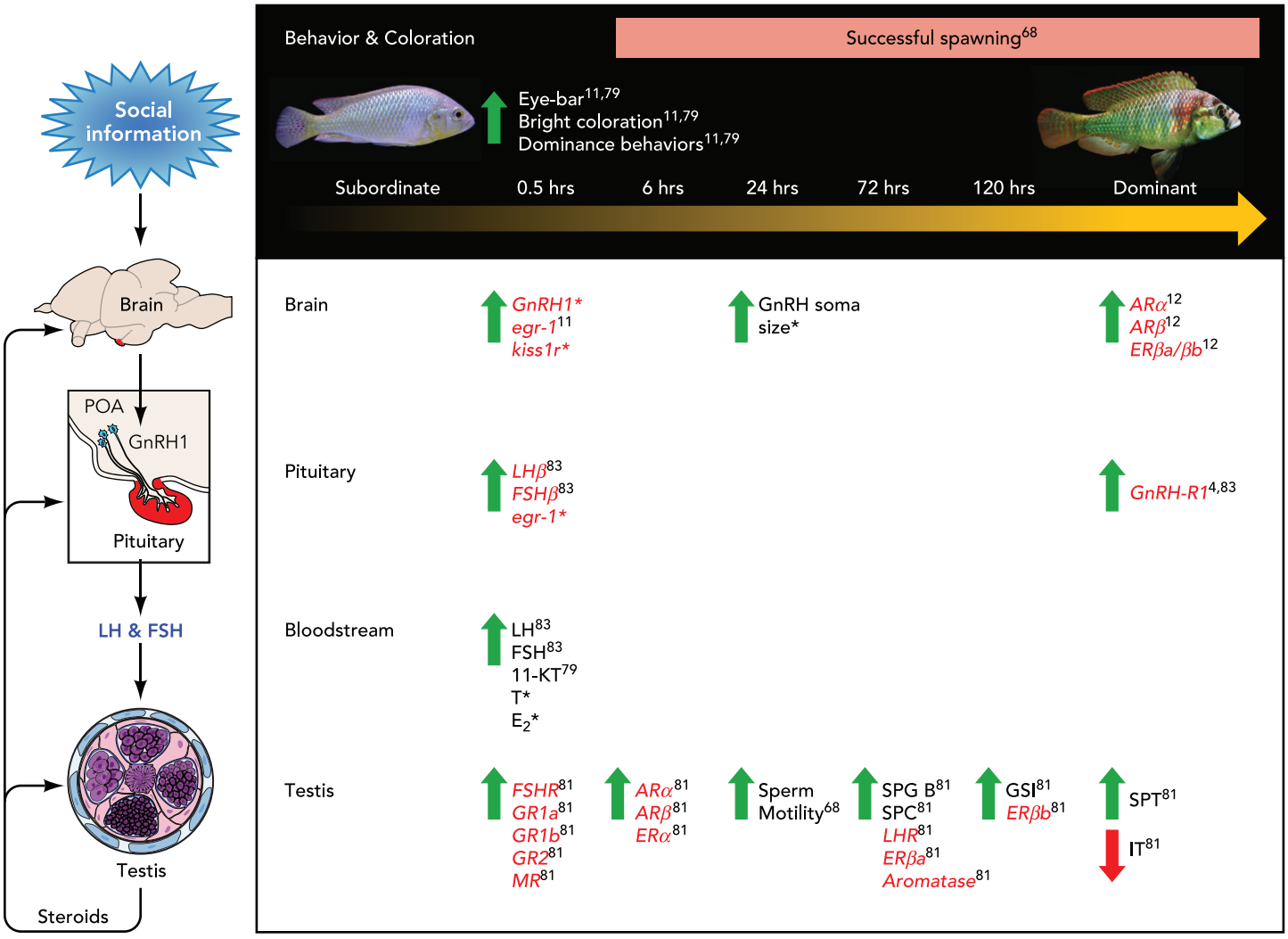

FIGURE 3. **Temporal summary of behavioral, morphological, and physiological changes in the HPG axis during social ascent in the male cichlid fish *Astatotilapia burtoni***

Arrows indicate the time point after social opportunity at which the first significant increase (up arrows) or decrease (down arrows) from stable subordinate male values was observed. Genomic changes (e.g., as determined by in situ hybridization or qRT-PCR) are indicated in red. Data were compiled from the following Refs. 4, 11, 12, 68, 79, 81, 83, as well as some unpublished data from our laboratory (*). Note also that not all measures were quantified at each time point, and several are only known for subordinate and dominant states. ARα/β, androgen receptor subtypes α and β; E_2, 17β-estradiol; egr-1, early growth response factor-1; ERα/βa/βb, estrogen receptor subtypes α, βa, and βb; FSH, follicle stimulating hormone; FSHβ, β-subunit of FSH; FSR, FSH receptor; GnRH1, gonadotropin-releasing hormone 1; GR1a/1b/2, glucocorticoid receptor subtypes 1a, 1b, and 2; GSI, gonadosomatic index; IT, interstitial tissue; kiss1r, kisspeptin receptor 1; LH, luteinizing hormone; LHβ, β-subunit of LH; LHR, LH receptor; MR, mineralocorticoid receptor; POA, preoptic area; SPG B, B-type spermatogonia; SPC, spermatocytes; SPT, spermatids; T, testosterone; 11-KT, 11-ketotestosterone.

Importantly, these behavioral, morphological, and physiological features of each male phenotype are reversible and under social control such that when a dominant male disappears, vacating a territory, a subordinate male will quickly take it over and rise in social rank. Furthermore, this transition between subordinate and dominant states can be experimentally controlled in the laboratory by manipulating the composition of the social environment (11, 79), which allows us to examine the precise timing of genomic changes along the HPG axis induced by social interactions. The recently sequenced genome of *A. burtoni* also makes it a powerful model to address specific questions on how the social environment is translated into genomic change on multiple levels and temporal scales. We use examples from *A. burtoni* to illustrate how tightly and rapidly the social environment can be linked to genomic changes of the HPG axis from the brain to the testes.

Social Regulation of Gene Expression in the Brain: GnRH1, Kisspeptin, and GnIH

GnRH1 neurons in the hypothalamic-preoptic area of the brain sit at the apex of the HPG axis and thus are the master regulators of reproduction across vertebrates. However, recent studies also highlight the critical role that kisspeptins (a group of RFamide peptides encoded by the *Kiss1*, and in some species, the *Kiss2* gene) and gonadotropin inhibitory hormone (GnIH; another RFamide peptide) play in controlling both GnRH1 neuron activity and the reproductive axis across taxa (see Refs. 2, 24, 30, 50, 90, 98, 142, 143 for reviews). Here, we examine current evidence for the link between reproductively relevant social signals and genomic activation of GnRH1, kisspeptin, and GnIH systems among vertebrates.

In vertebrates, numerous studies demonstrate activation of the reproductive axis caused by olfactory (47, 94, 111, 155), auditory (7, 13, 22, 76), tactile (104, 148), and visual (17) social signals that are often measured as changes in the number, size, or axonal densities of GnRH1-immunoreactive neurons, alterations in neuronal firing patterns, surges in circulating LH or steroid levels, increased testicular activity, or increases in sexual arousal and behavior. For example, plasma LH levels are elevated in female ring doves after hearing species-specific coo vocalizations compared with white noise or altered sounds, which is thought to mediate ovarian growth in this species (22). Exposure to female urinary pheromones causes rapid LH release in male mice (26), and reception of mating calls in the green treefrog is associated with increased numbers of GnRH-immunoreactive neurons and higher plasma androgen levels (13). In addition to sensory channel-specific signals, contextual social interactions with multimodal sensory information such as courtship, mating, exposure to the opposite sex, parental care, and opportunities to rise in social rank are also known to influence GnRH neurons and the HPG axis in many vertebrates (5, 11, 15, 33, 69, 78, 116, 125, 126, 134, 149, 152). There is also evidence in mammals that social signals reach the GnRH1 neurons via the kisspeptin neuronal pathway (24, 98, 101, 107) and that activation of the reproductive axis by sensory signals causes GnRH release to the pituitary, but it is often not associated with genomic changes in GnRH mRNA levels (47, 115). However, despite numerous studies that demonstrate links between socially salient signals and activation of the reproductive axis, there are comparatively fewer studies that examine how these social sensory signals cause changes, either directly or indirectly, within GnRH1 or kisspeptin neurons at the genomic level.

Immediate early genes (IEGs) such as *egr-1*, *c-fos*, *jun*, *arc*, and others have been useful tools for identifying activated neurons within reproductive and neuroendocrine circuits (25, 104), but important limitations of this technique are that neuronal activation is not always associated with IEG induction, IEGs are often not expressed in chronically activated neurons, and IEG induction is not typically related to challenge-induced neuropeptide expression (35, 51, 65, 104). Nevertheless, socially relevant reproductive stimuli are known to induce IEG expression within GnRH1 neurons across vertebrates from fishes (11) to mammals (44, 88, 105). In the cichlid fish *A. burtoni* for example, the perception of a social opportunity as a subordinate male switches to a dominant male (i.e., social ascent) is associated with a rapid (20–30 min) induction of the IEG *egr-1* (a transcription factor-encoding gene; also called *zenk*, *zif-268*, *ngfi-a*, *krox-24*, *tis8*) in the preoptic area and in GnRH1 neurons (11), as well as increases in GnRH1 mRNA levels in the brain at 30 min (Maruska et al., unpublished observations; FIGURE 3). This molecular response is likely due to the recognition of a social opportunity because it is not elicited in males who are already dominant. Recent studies in *A. burtoni* also suggest that visual cues alone are not sufficient to fully suppress the reproductive axis of subordinate males and that other senses such as olfaction are likely involved (21, 80). Male mice also show increased GnRH mRNA levels at 90 min after exposure to female mouse bedding (47), and pheromone olfactory cues induce c-fos expression in GnRH neurons of the ewe after 90 min (44). In female rats, steroids (e.g., estrogen and progesterone) can also augment the responsiveness of some GnRH neurons

(as measured by IEG co-expression in GnRH neurons) to sexual (vaginocervical) stimulation (105), suggesting there may be subpopulations of GnRH cells involved in specific social circuitry in some animals. Courtship interactions are also known to rapidly (within 1–2 h) increase GnRH1 mRNA levels and protein synthesis in birds (78), and activate GnRH neurons (as measured by co-localization of IEGs within GnRH cells) in the rodent brain (104, 105, 109, 152). In addition to social signals, there is strong evidence that environmental and seasonal cues such as photoperiod and temperature can also influence IEG expression and mRNA levels within GnRH1 neurons (124, 135), as well as regulate protein levels of GnRH1 (84, 106, 132, 133, 136), which likely functions to coordinate reproduction in seasonally breeding species.

Kisspeptins have recently emerged as critical upstream regulators of GnRH neurons involved in the control of puberty and normal reproductive function across vertebrates (reviewed in Refs. 2, 24, 30, 50, 98, 143). Kisspeptin was shown to activate GnRH neurons via its cognate receptor (*kiss1r*, or *GPR54*) that is expressed in these neurons (reviewed in Refs. 2, 30, 50, 98), rodents with disruption of the *kiss1* or *kiss1r* genes do not undergo puberty and are often infertile (31, 55), and the kisspeptin system is likely important for temporal regulation of the reproductive axis in seasonally breeding animals (114, 129). Despite this recent attention over the last decade, there is still limited information on how kiss1 or its receptor might be influenced by social information. In the cichlid fish *A. burtoni*, dominant males have higher levels of *kiss1r* in whole brain samples compared with subordinate males, but there was no difference in *kiss1r* expression on preoptic area GnRH1 neurons between social states determined via in situ hybridization (49). In a more recent study, however, *kiss1r* mRNA levels in micro-dissected preoptic areas were higher at 30 min after social opportunity compared with both dominant and subordinate males, suggesting that the kisspeptin-signaling system is important during the social transition when suppressed males need to quickly upregulate their reproductive behavior and physiology (Maruska et al., unpublished observations; FIGURE 3). In mammals, studies have also shown that *kiss1* and *kiss1r* mRNA levels can be influenced by stress (61), suckling stimuli during lactation (154), photoperiod, and season (114, 129). For example, exposure to a psychological stressor (restraint) significantly reduced *kiss1* and *kiss1r* mRNA levels in the medial preoptic area of the female rat brain, suggesting that reduced kisspeptin signaling may contribute to the stress-related suppression of LH secretion and reproductive inhibition seen in many animals (61). Furthermore, *kiss1* and *kiss1r* mRNA levels in the arcuate nucleus of the hypothalamus were lower in lactating compared with nonlactating rats, which may contribute to the suppression of LH secretion observed during suckling (154). Future studies are needed to determine the effects of social interactions on the genomic response of the kisspeptin system.

In addition to kisspeptin and GnRH, recent studies have also focused on the role of gonadotropin inhibitory hormone (GnIH) or related RFamide peptides as important "pause buttons" for the HPG axis because they decrease GnRH neuron activity, reduce synthesis and release of LH from the pituitary, reduce testosterone release from the testes, and decrease sexual behaviors (6, 14, 23, 66, 142, 144). Recent studies have also demonstrated that GnIH expression in birds is influenced by social status, breeding condition, and photoperiod (14, 145). For example, in the socially monogamous European starling *Sturnus vulgaris*, birds that out-competed others for nest boxes and therefore had increased breeding opportunities, showed lower numbers of GnIH cells in the brain, suggesting that GnIH serves as a modulator of reproductive function in response to the social environment (14). In seasonally breeding mammals like sheep, terminal projections from GnIH cells to GnRH neurons are increased during the nonbreeding season, suggesting that GnIH might contribute to the translation of environmental signals such as photoperiod into reproductive output on a seasonal basis (130). Since its discovery in the avian brain in 2000, GnIH homologs have been described from fishes to humans (141, 143), and thus this peptide likely plays a conserved role in reproductive modulation across vertebrates, but relatively little is known about how social information might influence the GnIH system. GnIH has not yet been examined in *A. burtoni*, but it is intriguing to hypothesize that it may play an important role in suppressing the HPG axis in subordinate males and that removal of this inhibition on social opportunity allows rapid activation of the reproductive axis. The opposing effects of kisspeptins (stimulatory) and GnIH (inhibitory) on GnRH neuron and HPG axis activity suggests that these two related RFamides are both important components of the neural circuitry involved in integrating social and environmental information with changes in reproductive physiology and behavior.

Social Regulation of Gene Expression in Brain Regions Involved in Reproductive Behavioral Decisions

In comparison to the relatively sparse information on socially induced genomic responses of "direct"

HPG axis players (i.e., kisspeptin, GnRH1, GnIH), there is considerably more information on how reproductively salient social signals elicit genomic responses in brain regions that regulate sexual and other social behaviors. For example, social behaviors in all vertebrates are thought to be controlled by a series of conserved nuclei ("nodes") in the brain (e.g., medial extended amygdala, lateral septum, preoptic area, ventromedial hypothalamus, anterior hypothalamus, periaqueductal gray/tegmentum) that are anatomically interconnected, express sex steroid receptors, and contain neuropeptidergic innervation (46, 95, 96). There is evidence across vertebrate taxa, including social fishes such as *A. burtoni* (11, 34), that detection or perception of social information causes genomic activation (as measured by IEG expression or microarrays) of these and other socially relevant brain regions (3, 52, 64, 102, 104, 117, 120, 123). Since the forebrain regions of this "vertebrate social behavior network" contain many of the neurons directly involved in HPG axis regulation, it is likely that this genomic response is also linked to reproductive activation, but this hypothesis is not typically tested in these studies because the identities of the cells expressing IEG activation are often not known. Thus large-scale technologies such as transcriptomics and epigenomics have allowed simultaneous examination of genomic network changes in response to social information that have advanced our understanding of which brain regions and neural circuits are involved in processing complex social stimuli (29, 67, 93, 112, 113, 118). The challenge from these studies for the future, however, is annotating this differential gene expression and then conducting mechanistic and functional studies to identify the role that each gene, neuron type, or network of genes and neurons plays in the reception of or behavioral response to the social information.

Social Regulation of Gene Expression in the Pituitary: GnRH Receptors and Gonadotropin Hormones

The primary target of the hypothalamic-preoptic area GnRH1 neurons are the gonadotrope-producing cells in the anterior pituitary gland. The released GnRH1 peptide, either delivered directly via neuronal projections in fishes or via the hypothalamic-pituitary portal system in tetrapods, binds to GnRH receptors (members of the large G-protein-coupled receptor superfamily) on these secretory cells to induce synthesis and release of the two gonadotropin hormones, LH and FSH, which then target the gonads (testes or ovaries) to stimulate steroid production and gamete development. Multiple forms of GnRH receptors (i.e., types I, II, III) are found in mammals (89), amphibians (147), and fishes (39, 71, 92, 122), and they often show differential distributions, expression patterns (e.g., across season, reproductive stage, or dominance status), and varying responses to regulation by steroids, GnRH, and monoamines, all of which suggest functional specializations (4, 19, 28, 73, 74). Although there is considerable information on the signal transduction pathways and how different neurohormones and steroids influence gonadotropin synthesis and release (9, 138), little is known about how social information modulates gonadotrope output at the pituitary.

In male *A. burtoni*, pituitary mRNA levels of *GnRH-R1*, but not *GnRH-R2*, are socially regulated such that stable dominant males have higher levels compared with subordinate males, and the increase during the social transition appears to occur more slowly (days) than changes in mRNA levels of other genes that occur within minutes to hours (4, 83) (FIGURES 2 AND 3). However, pituitary mRNA levels of the IEG *egr-1* and of the β-subunits of LH and FSH are increased at just 30 min after social ascent, suggesting that GnRH1 release has quickly activated the pituitary gland (Maruska et al., unpublished observations) (83). In mammals, pulsatile GnRH release and stimulation of GnRH receptors in pituitary gonadotrophs is associated with activation of several IEG transcription factors (*egr-1*, *c-fos*, *c-jun*), which then increase transcription rates of LHβ via mechanisms that involve intracellular signaling pathways and messengers such as PKC, CREB, Elk-1, ERK1/2, MEK1/2, MKP-1, and others (8–10, 87). Furthermore, pulsatile GnRH regulates *egr-1* mRNA levels both in vivo and in cultured anterior pituitary cells (10, 128), and when the *egr-1* gene is inactivated in mice, the animals have small anterior pituitary glands without LHβ expression and are infertile due to defects in hormone regulation (70, 139), demonstrating that *egr-1* is critical for LHβ gene transcription. Microarray and microtranscriptome studies in the LβT2 gonadotrope cell line also showed that multiple different IEGs, late-response genes, and microRNAs were significantly upregulated after GnRH exposure, providing a glimpse into the complex signaling pathways likely involved in gonadotrope production (54, 153, 156). It is also important to note that the majority of these studies mentioned above were performed on cell cultures or in females, and there is increasing evidence for differences in HPG axis function between in vivo and cultured conditions (16) and between genders that requires future study (8, 104). Although there have been many advances in our understanding of the intracellular mechanisms involved in GnRH1 activation of pituitary gonadotrope cells, less is known

about how social signals influence the genomic response at the pituitary level in any taxa.

Sex-changing fishes are also useful models for examining how social information influences genomic changes along the HPG axis because removal of the dominant or terminal phase morph of one sex typically induces the highest ranking individual of the opposite sex to rapidly assume coloration and behaviors typical of that opposite sex, followed by physiological changes to transform the reproductive system from female to male (protogynous) or male to female (protandrous). Although the majority of studies on sex-changing fishes have concentrated on the role of circulating steroids, monoamines, and neuropeptides such as GnRH and arginine vasotocin (AVT) at the protein level (see Ref. 45 for review), several recent studies have begun to investigate the control of sex-change at the genomic level (62, 63). In the sex-changing protogynous grouper fish *Epinephelus merra*, for example, increased pituitary mRNA levels of FSHβ, but not LHβ or the α-gonadotropin subunit, were associated with testis development during the female-to-male transition, suggesting that FSH may trigger the sex change in this species by stimulating both androgen production and spermatogenesis (62). Socially controlled sex change in the wrasse *Pseudolabrus sieboldi* is also associated with distinct gene expression profiles of LH and FSH subunits (α and β) in the pituitary gland, including sex-specific diurnal changes in gonadotropin secretion that may mediate socially induced sexual plasticity in this species (99). Although the use of IEGs has not yet been applied to the study of HPG axis function in sex-changing fishes, this is an area of future direction that should yield important insights into the missing links between detection or perception of social information and genomic response of the reproductive axis.

Social Regulation of Gene Expression in the Testes: Spermatogenesis and Steroid Production

Although many studies have shown how social information including mating opportunities, female presence or attractiveness, and social status can influence testicular function in terms of sperm quality (e.g., velocity, motility, number) from fishes to humans (27, 43, 59, 68, 110), less is known about how social cues induce molecular changes in the testes. In *A. burtoni*, however, perception of social opportunity triggers genomic changes in mRNA levels on both rapid (minutes to hours; FSHR, androgen receptors, corticosteroid receptors) and slower (days; LHR, aromatase, estrogen receptors) time scales (81) (FIGURE 3). During the subordinate-to-dominant male social transition, the morphological and structural changes in testicular cell composition and relative testes size takes several days, whereas many molecular changes in the testes are detected more quickly (81). This rapid genomic response in the most distal component of the HPG axis highlights the sensitivity and plasticity of the entire reproductive system to social information. Furthermore, the quick genomic changes in the testes raise the possibility that there may be additional and parallel signaling pathways that perhaps bypass the traditional linear cascade from brain GnRH1 release to pituitary LH/FSH release to testicular gonadotropin receptor activation scheme. A genomic response to social opportunity was also described in the gonads of the sequential hermaphroditic goby fish *Trimma okinawae*, which can reversibly change sex in both male-to-female and female-to-male directions depending on the composition of the social environment (63, 75, 137). This study demonstrated that perception of the opportunity to change sex is transmitted via unknown channels to the gonads and results in activation of the inactive gonad and inactivation of the active gonad via a mechanism that involves a rapid switching in the mRNA expression levels of the LH and FSH receptors (63). Since testicular function is critical for male reproductive success, future studies should examine how perception of social information is translated into genomic changes in the testes.

Conclusions

Despite the profound influence of social information on the function of the reproductive axis in all vertebrates, less is known about how this social information influences the HPG axis at the molecular level (e.g., changes in gene expression), and there are still many unanswered questions about the links between social behaviors, reproductive axis function, and the genome. For example, what are the neural pathways from reception/perception of an external social cue that lead to changes in gene expression along the HPG axis? What is the function of IEG activation within GnRH1 and other neurons of the conserved social behavior network? How does social information influence the known (e.g., kisspeptin and GnIH neurons) and yet undiscovered upstream afferent inputs to GnRH1 neurons at the molecular level, and are there subpopulations of GnRH1 neurons that serve to specifically process and integrate external social signals that differ from those that might process internal signals like nutrition and hormonal state? Recent advances in large-scale technologies (proteomics, transcriptomics, microtranscriptomics, epigenomics) combined with comparative systems approaches, single-cell analyses, optogenetics,

and transgenic methods should increase our understanding of how the social environment can cause genomic changes that regulate reproduction and fertility. The role of both epigenetic changes and small RNA (e.g., microRNAs) regulation in potentially mediating socially induced changes along the reproductive axis is also an exciting area of future work (53, 108, 119–121) that should provide insights into our understanding of the mechanisms governing social and seasonal reproductive plasticity across taxa. We also propose that the cichlid fish *A. burtoni*, with its complex and experimentally manipulative social system, wealth of background knowledge on the social control of HPG axis function, and the recently available genomic resources should become a valuable vertebrate model system for studying how the social environment influences genomic plasticity and function of the reproductive axis. ■

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