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COMMENTARY

Revisiting the specific and potentially independent role of the gonad in hormone regulation and reproductive behavior

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ABSTRACT

Gonadal sex steroid hormones are well-studied modulators of reproductive physiology and behavior. Recent behavioral endocrinology research has focused on how the brain dynamically responds to – and may even produce – sex steroids, but the gonadal tissues that primarily release these hormones receive much less attention as a potential mediator of behavioral variation. This Commentary revisits mechanisms by which the reproductive hypothalamic–pituitary–gonadal (HPG) axis can be modulated specifically at the gonadal level. These mechanisms include those that may allow the gonad to be regulated independently of the HPG axis, such as receptors for non-HPG hormones, neural inputs and local production of conventional 'neuropeptides'. Here, I highlight studies that examine variation in these gonadal mechanisms in diverse taxa, with an emphasis on recent transcriptomic work. I then outline how future work can establish functional roles of gonadal mechanisms in reproductive behavior and evaluate gonad responsiveness to environmental cues. When integrated with neural mechanisms, further investigation of gonadal hormone regulation can yield new insight into the control and evolution of steroid-mediated traits, including behavior.

KEY WORDS: Gene expression, Ovaries, Reproduction, Sex steroids, Steroidogenesis, Testes

Introduction

Gonadal sex steroid hormones regulate fertility, physiology and behavior to coordinate reproductive state in adult vertebrates. These hormones, such as testosterone, 17β-estradiol and progesterone, promote gamete production and/or maturation in the testes and ovaries (McKenna, 2015). To maximize the success of those gametes, sex steroids potentiate changes in reproductive behavior through actions on target tissues ranging from the musculature, adipose tissue and immune system to the brain (reviewed in Graham and Clarke, 1997; Hammes and Levin, 2019; Fuxjager et al., 2023). Given their pleiotropic effects (see Glossary) and their ability to respond dynamically to reproductive contexts and cues (Gleason et al., 2009; Goymann et al., 2019; Wingfield et al., 1990), sex steroids are often considered to be phenotypic integrators (see Glossary; Lipshutz et al., 2019; Martin et al., 2011). Thus, understanding the physiological basis of variation in sex steroid-mediated traits, such as reproductive behaviors, has been a central question in behavioral and evolutionary endocrinology (Rosvall, 2022; Zera et al., 2007).

The expression of sex steroid-mediated traits depends on both sex steroid hormone production – coordinated by the hypothalamic–pituitary–gonadal (HPG) axis – and target tissue responsiveness. Although the adrenal gland can also release sex steroids into the circulation (Kater et al., 2022), the gonads are the primary source of most plasma sex steroids in breeding adults. Gonadal steroid release depends on: (1) cascading endocrine signals from the hypothalamus and pituitary, (2) the availability of receptors for each of those signals across each level of the HPG axis and (3) steroidogenic enzyme activity in the gonads (De Kretser, 2018; Kanda, 2019). In the circulation, the bioavailability of sex steroids can be affected by plasma steroid hormone-binding globulins (see Glossary; Hammond, 1990; Hammond, 2011) and clearance enzymes (Auchus, 2015). Then, at the target tissue, variation in steroid hormone receptors and their intracellular cofactors (see Glossary) can affect the degree of response (McKenna, 2015; Schuppe and Fuxjager, 2019). Further, these mechanisms do not act in a vacuum; other hormonal and neural systems can affect sex steroid release and response (e.g. Viau, 2002).

All of these mechanisms can be targets of selection on steroid-mediated traits (Fuxjager et al., 2018). Thus, whether sex steroid hormone production and target tissue sensitivity, if considered as nodes in a hormone regulatory network (Lipshutz et al., 2019), are regulated or evolve in a concerted manner (i.e. phenotypic integration), or whether specific tissues may diverge in regulation from overall HPG axis activity at certain stages or over evolutionary time (i.e. phenotypic independence) (Ketterson et al., 2009; see also Schuppe and Fuxjager, 2019; Hau, 2007; McGlothlin and Ketterson, 2008) has been a central question in evolutionary and behavioral endocrinology.

Most recent work on the mechanisms of signal production and target tissue sensitivity, especially in a behavioral context, has focused on the brain. This focus is logical; the brain transduces internal and external cues into behavioral outputs (Adkins-Regan, 2008; Levine, 2000). The brain thus integrates environmental stimuli into overall HPG axis activation through release of neuropeptides (see Glossary) such as gonadotropin releasing hormone (GnRH) or gonadotropin inhibitory hormone (GnIH) (Tsutsui et al., 2015). As a target tissue, the brain can respond independently of systemic circulating steroid concentrations through various mechanisms. Such mechanisms include differences in hormone receptor density (Alward et al., 2020; Denney et al., 2024; Feng and Bass, 2017; Freiler et al., 2024; Shaw and Kennedy, 2002), intracellular receptor cofactors (Schuppe and Fuxjager, 2019) and even extra-gonadal steroidogenic enzymes in the brain (Jalabert et al., 2021; London et al., 2009; Schlinger and Remage-Healey, 2012), which may allow animals to produce steroid-mediated behaviors, such as aggression, beyond periods of HPG activation (Soma et al., 2003, 2008). These neural mechanisms may underlie divergence in reproductive traits both within individuals and across species.

Importantly, peripheral tissue signaling can vary independently of the greater endocrine axis to regulate reproductive behavior. Indeed, sex steroid receptors vary across reproductive contexts and

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Glossary

Alternative reproductive tactic
Discrete and consistent variation in reproductive behaviors and morphology within one sex of a single species (Schradin, 2019).

Autocrine
A form of hormone action whereby hormones secreted by a cell have an action on the receptors of the same cell.

Capture–restraint methods
Established methods for inducing acute stress responses, including elevation of glucocorticoid hormones, which involve catching, handling and immobilizing animals for a defined period of time.

Cofactors
Proteins that either activate or repress the ability of intracellular steroid hormone receptors to initiate gene transcription.

Neuropeptides
Proteins typically released by neurons outside the synapse that can have prolonged modulatory actions on target cells/neurons.

Paracrine
A form of hormone action whereby hormones secreted by one cell have local actions on neighboring cells in the same gland or tissue.

Peptidergic nerves
Nerves that secrete neuropeptides, such as vasoactive intestinal peptide or neuropeptide Y, onto the tissues they innervate.

Phenotypic integrator
Physiological mechanisms that link and coordinate multiple phenotypic traits by activating specific gene modules in response to environmental stimuli (Martin et al., 2011).

Pleiotropic effects
In the context of endocrinology, when one hormonal signal can affect multiple phenotypes.

Sex-dynamic species
Species where primary gonad type (i.e. testes or ovaries) and associated morphology and behavior can change in response to the social environment during the adult lifetime (Smiley et al., 2024).

Steroid hormone-binding globulins
Extracellular proteins with specific affinity for steroids that aid in transport through plasma to target tissues and can affect the availability of steroids to bind their hormone receptors.

species in tissues ranging from vocal organs to pectoral muscles (e.g. Anderson et al., 2021; Barske et al., 2019; Fuxjager et al., 2022; Schuppe and Fuxjager, 2019; Schuppe et al., 2017; Veney and Wade, 2004). Further, steroidogenesis in peripheral glands can be targeted directly by natural selection on behaviors, as illustrated by the presence of steroidogenic enzymes in muscle (Schuppe et al., 2022) and the recent discovery of novel steroidogenic cell types in the adrenal gland (Niepoth et al., 2024).

Despite growing attention on peripheral tissues and their role in steroid-mediated traits, only a minority of these studies specifically investigated the gonad. Hormonal regulation in the gonad merits direct study for several important reasons. First, the gonad represents the nexus of the HPG endocrine axis and fertility (through gamete production and maturation). In this role, the gonad then must integrate information about local reproductive state with signals received from upstream in the endocrine axis, thus coordinating reproductive phenotypes through sex steroids in a 'bottom-up' manner. Second, because of this role, the gonad must be able to respond to other systemic cues that may be relevant to reproduction. Third, the gonad is not only a site of hormone production but also a target tissue of both HPG and other endocrine axes. Given all this, the gonad is likely to be able to respond dynamically to changes in the social and physiological environment. The dynamic nature of the gonad is obvious given changes in gonad size in seasonal breeders or sex-dynamic species (see Glossary), and recent transcriptomic studies suggest that even more subtle metrics, such as the number of gonadal genes differentially expressed, can vary in response to environmental manipulations (Austin et al., 2021; Bentz et al., 2021, 2022; Calisi et al., 2018; Lipshutz et al., 2022). Although gonadal steroidogenesis is the main output of the HPG axis, equating the gonad with circulating sex steroids misses the opportunity to evaluate whether and how hormone production may be modulated and responsive within the gonad itself.

In this Commentary, I hope to provide researchers interested in the mechanisms of reproductive behavior with a list of mechanisms of gonadal hormone regulation that may be environmentally responsive and underlie reproductive steroidogenic acute regulatory traits. I first review gonad-specific mechanisms within the classic HPG framework, including variation in steroidogenic enzymes and sensitivity to hypothalamic and pituitary signals. Then, I discuss potentially HPG-independent mechanisms, such as sensitivity to non-HPG hormones, direct innervation and local production of conventional neuropeptides. Throughout, I highlight insights from recent transcriptomic studies in diverse taxa that reveal novel and dynamic gene expression in the gonads. Lastly, I discuss how future studies can establish the functional role of these mechanisms and link them to sex steroid concentrations and, ultimately, reproductive behavior. Overall, my goal is to provide researchers already studying brains and behavior in various systems with a starting place for hypotheses centered on the gonads.

Gonad-specific mechanisms within the HPG framework

Gonadal steroids are the ultimate output of HPG axis activation. Nonetheless, variation in mechanisms specific to the gonad – namely, (1) steroidogenic enzyme expression and/or activity, and (2) gonadal sensitivity to upstream hypothalamic and pituitary signals – presents routes by which HPG activity can be regulated directly at the gonadal level (Fig. 1; left), as discussed below.

Steroidogenic machinery

Within the gonad, steroid production and release depends on the levels and activity of multiple steroidogenic enzymes, as well as transporters that shuttle cholesterol to these enzymes in the inner mitochondrial membrane (steroidogenic acute regulatory protein, StAR; Fig. 2). Further, the intracellular location of enzymes, the presence of cofactors (such as nicotine adenine dinucleotide) and post-translational modifications can also affect enzyme activity (reviewed in Bremer and Miller, 2014).

Enzyme and transporter expression along the steroidogenic pathway may indicate the gonads' steroidogenic 'priorities'. For example, increased expression of proteins responsible for rate-limiting steps (such as StAR and CYP11A1) may indicate increases in overall steroidogenesis. Multiple species with alternative reproductive tactics (see Glossary) exhibit this pattern, where males that show steroid-mediated breeding coloration, hold territories and court females – when compared with sneaker males that mimic females – tend to have both higher levels of circulating androgens (Knapp, 2003) and higher *StAR* gene expression (Kvarnemo et al., 2023; Todd et al., 2018; but see Loveland et al., 2021). Similarly, subordinate male African cichlids (*Astatotilapia burtoni*) increase androgen production and testicular *StAR* mRNA when the opportunity to ascend a dominance hierarchy arises (Huffman et al., 2012).

Alternatively, differences in specific enzymes along the pathway may represent a shift in specific steroid profiles or ratios. For example, increased *HSD11B* expression could suggest higher production of potent androgens such as 11-ketotestosterone, as

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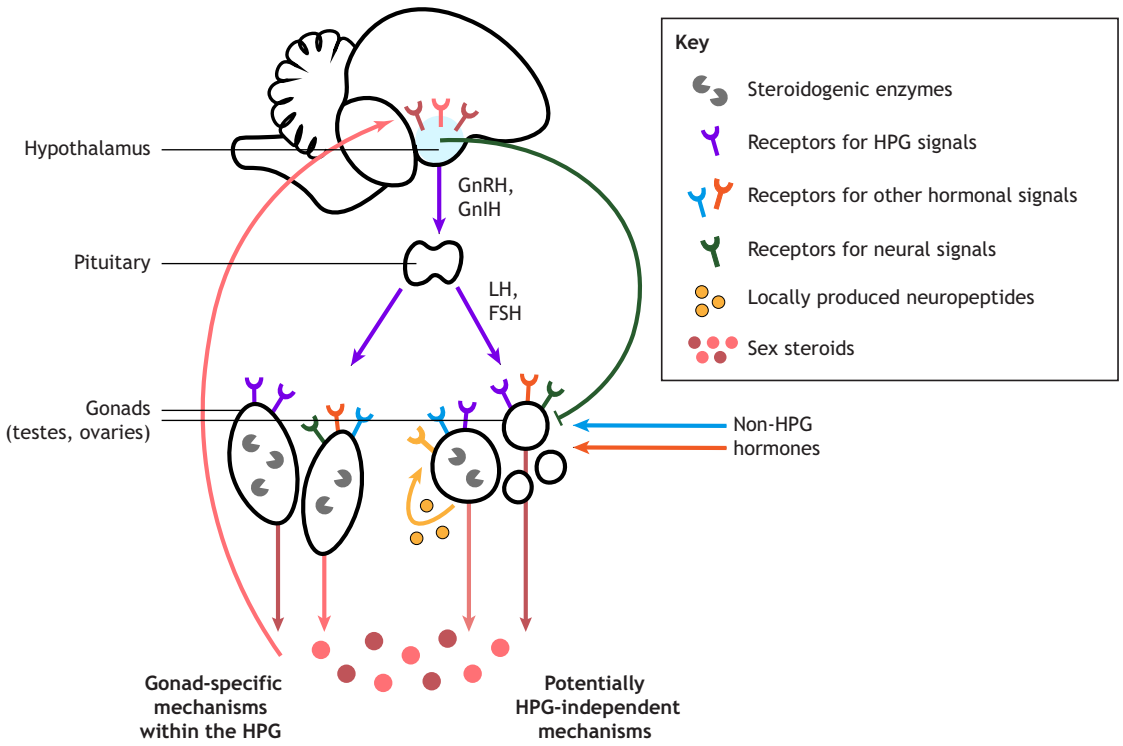

Fig. 1. A conceptual diagram of a generic hypothalamic–pituitary–gonadal (HPG) axis highlighting gonad-specific mechanisms of hormone regulation. Within the classic HPG framework (left), sex steroid release by the gonads (red circles/arrows) can be regulated within the gonad through steroidogenic enzymes (gray symbols) and HPG hormone receptor expression (purple Y shapes). Other mechanisms, which may give the gonad some independence from top-down HPG axis control (right), include other hormone receptors (orange and blue Y shapes), receptors for neural signals from direct innervation (green Y shapes) and local production of neuropeptides that may act in a paracrine or autocrine manner (yellow circles). Circulating sex steroids released by the gonads can then act on steroid receptors in the brain (red Y shapes) to negatively feed back on the HPG axis and affect behavior.

seen in territorial midshipman fish (*Porichthys notatus*) compared with sneaker males (Arterbery et al., 2010). Increased CYP19A1 (aromatase) could indicate increases in conversion of androgens to estrogens. Such shifts from androgen-biased to estrogen-biased steroid profiles occur in some sex-dynamic teleost fish species (Gemmell et al., 2019; Todd et al., 2016). Indeed, as Red Sea clownfish (*Amphiprion bicinctus*) transition from male to female, *CYP19A1* gene expression increases and *HSD17B* expression decreases (Casas et al., 2016). Bluehead wrasses (*Thalassoma bifasciatum*), which transition from female to male when social opportunity arises, similarly show decreased *CYP19A1* gene expression as ovaries transform into testes (Todd et al., 2019). Of course, these interpretations assume that the levels of gene transcripts correlate strongly with functional protein levels, which is often, but not always, the case (Koussounadis et al., 2015; Liu et al., 2016). Other techniques, such as assays of enzymatic conversion of precursors from tissues *in vitro* (e.g. Pradhan et al., 2014), could directly test whether enzymatic conversion rates differ across reproductive stages and contexts.

Sensitivity to gonadotropins

The gonads may also vary in their sensitivity to upstream HPG signals such as gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). If steroid concentrations vary across reproductive contexts, variation in LH receptor expression could modulate the amount of steroids produced, as LH receptor activation in gonadal endocrine cells stimulates StAR, cholesterol intake and enzyme expression (Eacker et al., 2008; Eckstrum and Raetzman, 2018). FSH receptors can also activate steroidogenesis – especially estrogen synthesis via CYP19A1 in females – across various taxa (Eckstrum and Raetzman, 2018; Johnson, 2015; Kumar et al., 2011; Trudeau, 2022). Expression of these two receptors has been shown to vary in response to environmental or physiological contexts, and is also associated with changes in steroidogenesis and/or gonadal size. For example, testicular LH and FSH receptor gene expression increases as African cichlids ascend a dominance hierarchy (Maruska and Fernald, 2011), potentially underlying the higher levels of circulating androgens observed in dominant males compared with subordinates (Parikh et al., 2006). Hormonal manipulations may also affect gonadal gonadotropin receptors; for example, exogenous prolactin increases gonadal LH and FSH receptor expression in male, but not female, rock doves (*Columba livia*), which may explain larger testes mass in prolactin-treated males (Farrar et al., 2022b). Together, variation in these two receptors could render the gonads more or less sensitive to pituitary control, potentially modulating steroidogenic responses to upstream HPG signals.

Evidence for HPG modulation at the gonadal level: key insights from songbirds

Through variation in the two mechanisms discussed above – variation in steroidogenic enzymes and gonadotropin receptor expression – the gonad represents a specific node through which HPG axis activity can vary both within individuals during reproduction and across reproductive phenotypes. Evidence for such gonad-specific modulation of sex steroid concentrations comes from key studies in songbirds. Through elegant experiments, Rosvall, Ketterson, Kimmitt and colleagues (see below) have linked

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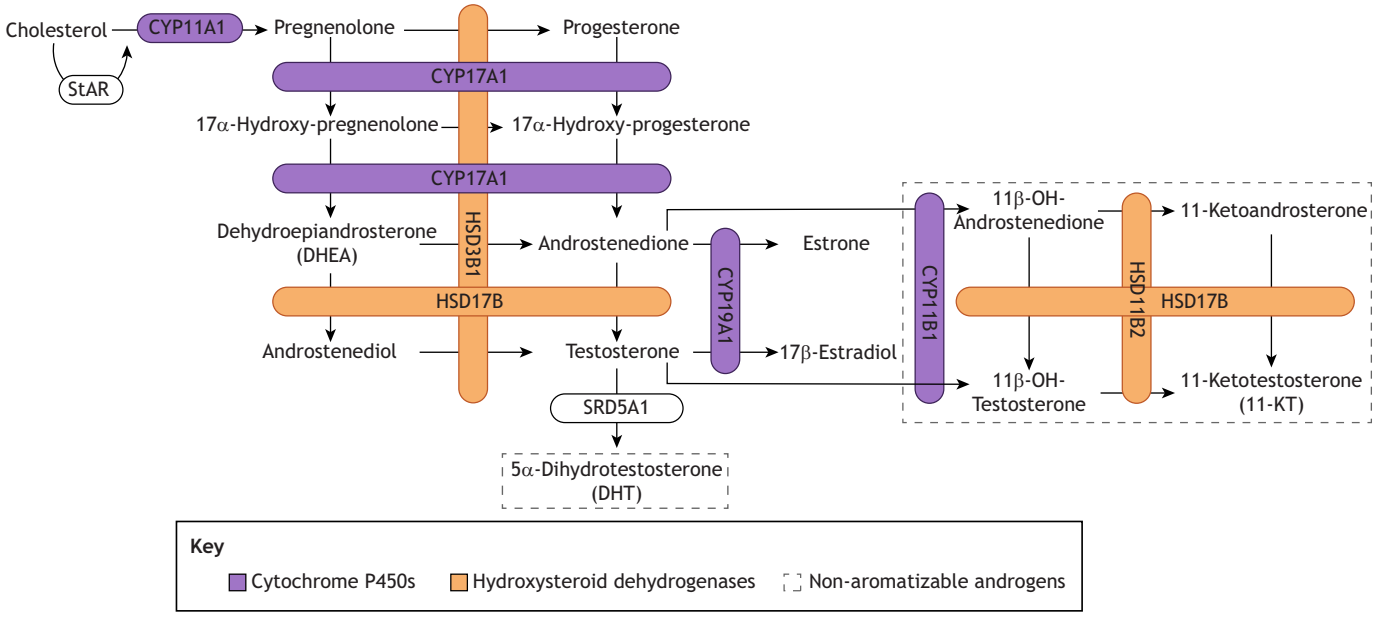

Fig. 2. Overview of the main enzymes and regulatory proteins involved in gonadal steroidogenesis. During steroidogenesis, cholesterol is shuttled by steroidogenic acute regulatory (StAR) protein (Clark and Stocco, 1996) from the outer mitochondrial membrane to the inner mitochondrial membrane, where it is converted to pregnenolone by cholesterol side-chain cleavage enzyme (CYP11A1). Pregnenolone can be converted to active sex steroids by enzymes from the cytochrome P450 (purple) and hydroxysteroid dehydrogenase (orange) families. Non-aromatizable androgens are highlighted with dashed gray boxes. Progesterone, 17β-estradiol, testosterone and the more-potent androgen dihydrotestosterone (DHT) are the primary sex steroids in most breeding mammals, birds, reptiles and amphibians (Deviche et al., 2011; Kumar et al., 2011; Zirkin et al., 2011). In many teleost fish, the primary androgens are the non-aromatizable 11-oxygenated testosterones, especially 11-ketotestosterone (Borg, 1994; Knapp and Carlisle, 2011; Tenugu et al., 2021), although fish can also produce testosterone and non-aromatizable DHT (Martyniuk et al., 2013). Note that these non-aromatizable androgens have also been detected in humans (Imamichi et al., 2016). CYP11A1, cholesterol side-chain cleavage enzyme; CYP11B1, 11-beta-hydroxylase; CYP17A1, steroid 17-alpha-hydroxylase or 17,20-lyase; CYP19A1, aromatase; HSD11B2, 11-beta-hydroxysteroid dehydrogenase 2; HSD17B, 17-beta-hydroxysteroid dehydrogenases; HSD3B1, 3-beta-hydroxysteroid dehydrogenase 1; SRD5A1, steroid 5-alpha reductase; StAR: steroidogenic acute regulator protein.

differences in circulating sex steroids to differences in gonadal gene expression across breeding stages in female tree swallows (*Tachycineta bicolor*) and across populations of dark-eyed juncos (*Junco hyemalis*) that have diverged in reproductive behaviors and/or timing. These studies highlight how direct attention to the gonad can reveal novel mechanisms underlying divergence in HPG activity.

In both species, researchers first evaluated differences in circulating sex steroids (specifically, testosterone) across breeding stages and between divergent populations. In tree swallows, females compete for nest sites and establish territories early in the breeding season, leading to higher baseline testosterone associated with this period of high aggression compared with that of females incubating eggs later in the breeding season (George and Rosvall, 2018). In dark-eyed juncos, subspecies from North Dakota and Virginia that have diverged in androgen-mediated traits, such as male feather ornamentation and aggression (Nolan et al., 2020), surprisingly do not differ significantly in baseline testosterone concentrations (Bergeon Burns et al., 2014; Rosvall et al., 2016). Similarly, female junco subspecies that differ in reproductive timing also do not differ in baseline testosterone (Kimmitt et al., 2020).

However, baseline testosterone concentrations can be modulated at multiple levels of the HPG axis. Thus, to specifically evaluate whether peripheral mechanisms underlie differences in testosterone profiles and their relationship to behavior, researchers used GnRH challenges. By stimulating birds with exogenous GnRH and measuring corresponding changes in plasma testosterone, GnRH challenges allow evaluation of the responsiveness and hormone-production capacity of the pituitary and, ultimately, the gonad (e.g. Jawor et al., 2006, 2007). As well as having higher baseline testosterone concentrations, female tree swallows establishing territories also have significantly higher GnRH-induced changes in testosterone than incubating females (George and Rosvall, 2018). In juncos, more-aggressive North Dakota males release testosterone more rapidly in response to GnRH and sustain elevated testosterone for longer compared with the Virginia subspecies (Rosvall et al., 2016). Female juncos that show earlier onset of breeding also produce higher testosterone in response to GnRH than later-breeding subspecies (Kimmitt, 2020; Kimmitt et al., 2020). The stronger responses to GnRH in these systems suggest that changes in androgen profiles, and potentially behavior, are related to changes in steroidogenic capacity and/or responsiveness to upstream signals at the gonadal level.

In both systems, gonad-specific mechanisms vary across breeding stages and populations, potentially underlying variation in HPG activity and responsiveness. For example, ovarian *CYP19A1* gene expression is significantly higher, and *StAR* and *HSD3B* trend towards higher expression in tree swallows during territory establishment compared with during incubation (Bentz et al., 2019). In dark-eyed juncos, testicular gene expression of LH receptor (*LHR*) (Bergeon Burns et al., 2014), *CYP11A1* (a rate-limiting step of steroidogenesis) and *CYP17* enzymes (Rosvall et al., 2016) is higher in the aggressive North Dakota birds than in the less-aggressive population. Similar trends are observed in females, where earlier-breeding birds have higher *LHR* and *CYP19A1* expression compared with later breeders (Kimmitt et al., 2019). These patterns of gonadal gene expression correspond with, and potentially underlie, variation in HPG responsiveness in these systems.

Taken together, these elegant studies underscore how gonad-specific mechanisms may modulate HPG axis outputs dynamically during reproduction and/or be targeted by selection. Divergence at the gonadal level could produce both sex- and population-specific differences in HPG activity, lending support to the hypothesis of phenotypic independence (Ketterson et al., 2009). Although these studies tested hypotheses squarely within the HPG framework, the gonad receives inputs from other endocrine axes and nerves, as well as autocrine and paracrine signals (see Glossary). In the following section, I further detail mechanisms that may modulate gonadal function independently of HPG axis signals.

Potentially 'HPG-independent' mechanisms in the gonad

Beyond the conventional HPG framework, gonadal sex steroids may be affected by (1) other, non-HPG hormones, (2) neural signals, including those potentially from direct innervation, and (3) local production of conventional neuropeptides (Fig. 1; right). These mechanisms are not intended to be an exhaustive list but instead illustrative examples of factors that may directly modulate gonadal function.

As gonadal steroids are the ultimate output of HPG axis activation, any mechanism that may affect gonadal steroidogenesis likely cannot be considered truly independent from the HPG framework. Instead, in a narrow view of independence, these mechanisms may allow the gonad to integrate information beyond that conveyed by hypothalamic GnRH, GnIH and pituitary gonadotropins. Such mechanisms may allow the gonad to transduce cues from physiological systems into changes in HPG axis activity (i.e. sex steroid release). In this section, I consider the evidence for potentially HPG-independent mechanisms of gonadal hormonal regulation in diverse taxa.

Sensitivity to non-HPG hormones

Beyond gonadotropins, other non-HPG hormones may also affect gonadal function. In fact, the gonad is a target tissue of other endocrine axes. For example, prolactin, glucocorticoids and nonapeptides (oxytocin, vasopressin and homologs) have been implicated in reproductive transitions (Champagne and Curley, 2012; Dulac et al., 2014; Lynn, 2016; Young and Insel, 2002), and receptors for these hormones have been detected in the gonads of many species (e.g. prolactin: Freeman et al., 2000; Farrar et al., 2022a; glucocorticoids: Lattin and Romero, 2013; Tetsuka et al., 1999; nonapeptides: Saller et al., 2010; Thackare et al., 2006). The presence of receptors for these hormones in the gonads suggests that they potentially have direct effects on gonadal function.

Studies of hormone receptor expression suggest that gonadal sensitivity to non-HPG hormones can vary during reproduction. For example, gonadal prolactin receptor gene expression decreases as rock doves begin incubation and when birds are exposed to chicks mid-incubation (Farrar et al., 2022a). These transitions are not accompanied by changes in plasma prolactin (Farrar et al., 2022a), suggesting the gonad may be modulating its response to circulating prolactin (although this remains untested).

To mediate effects of stress or metabolic changes on the HPG axis, glucocorticoid hormones can also affect the gonad. Across seasons, neither gonadal glucocorticoid receptor (GR) nor mineralocorticoid receptor (MR) binding differs in male house sparrows (*Passer domesticus*; Lattin and Romero, 2013), despite the role of glucocorticoids in mediating metabolic demand during breeding (Patterson et al., 2011; Wingfield and Sapolsky, 2003). However, acute stress induced via capture–restraint methods (see Glossary) leads to upregulation of testicular GR gene expression in big brown bats (*Eptesicus fuscus*; Alonge et al., 2023). These results, and others, suggest the gonad can respond rapidly to stressors (Alonge et al., 2023; Hu et al., 2008). Beyond receptors, the gonads can also modulate glucocorticoid responses by inactivating glucocorticoids through HSD11B enzymes (Whirledge and Cidlowski, 2010). Such mechanisms may underlie the rapid, stress-induced changes in circulating sex steroids observed in various taxa (Moore et al., 1991; Sapolsky, 1982; Shors et al., 1999; Van Hout et al., 2010).

Thus, gonadal sensitivity to the hormonal milieu may vary with reproductive context and sex, and may be able to change rapidly in response to the environment. However, how changes in sensitivity to non-HPG hormones may affect steroidogenesis requires further investigation.

Sensitivity to direct innervation and neural signals

In addition to sensitivity to circulating hormones, the gonads can also vary in sensitivity to neural signals. Although hypothalamic and pituitary endocrine signals are the primary regulators of gonadal steroidogenesis, studies in rats suggest that direct hypothalamic–testicular innervation can stimulate steroidogenesis separately from HPG signals (Gerendai et al., 2000; Lee et al., 2002). Additionally, the gonads also can be innervated – though sparsely, compared with other tissues (Gerendai et al., 2000) – by both branches of the autonomic nervous system and other peptidergic nerves (see Glossary; reviewed in Gerendai et al., 2005; Felix et al., 2023). Such neural inputs may present routes by which gonad function can be regulated independently of the HPG axis. Variation in expression of receptors for the relevant neuropeptides across reproductive states or phenotypes could imply differences in how the gonad integrates direct neural signals versus input from conventional HPG endocrine routes. Although reviewing the potential effects of each of these neuropeptides is beyond the scope of this paper, researchers are encouraged to consider receptors for these neuropeptides as potential candidates underlying variation in gonadal function and steroidogenesis. Receptors for neuropeptides known to innervate mammalian gonads, such as vasoactive intestinal peptide (Alm et al., 1980; Bruno et al., 2011; Zhu et al., 1995), have been detected in recent transcriptomic studies (Table S1) (Austin et al., 2021; Bens et al., 2018; Bentz et al., 2022; Boonanuntanasarn et al., 2020; Calisi et al., 2018; Lipshutz et al., 2022; Liu et al., 2015; Liu et al., 2018; MacManes et al., 2017; Tang et al., 2021).

Local production of conventional neuropeptides

Beyond being sensitive to neural signals, the gonad may also locally produce conventional neuropeptides. Across multiple species, gonadal expression has been detected for many peptides thought to originate in the brain (reviewed in McGuire, 2010). Peptides expressed in the gonad include conventional HPG axis neurohormones such as GnRH (Choi et al., 1994; Desaulniers et al., 2017) and GnIH (Bentley et al., 2008, 2017), oxytocin (Stadler et al., 2020) and pituitary peptide hormones such as prolactin (Marano and Ben-Jonathan, 2014). Transcriptomic studies across taxa from the last decade have further uncovered gonadal gene expression of these neuropeptides and others, such as corticotropin releasing hormone, growth hormone releasing hormone or galanin (Table S1).

Although the functionality of these transcripts remains undetermined, locally produced neuropeptides may regulate gonadal steroidogenesis. For instance, GnIH (or RFRP3 in mammals) can reduce circulating testosterone (McGuire and Bentley, 2010; Squicciarini et al., 2018) and potentially increase

progesterone production (Wilsterman et al., 2019) in the gonads of birds and mammals *in vitro*. The expression of receptors for these neuropeptides in the gonads (e.g. Desaulniers et al., 2017; Ubuka et al., 2013) (Table S1) when levels of circulating releasing hormones should be too low to be biologically active in the periphery (Smith et al., 2012) further suggests potential local functions.

Additionally, gonadal neuropeptides may mediate direct gonadal responses to environmental cues or physiological states. *In vivo* gonadal GnIH mRNA increases significantly in response to acute stressors such as classic capture–restraint methods (Alonge et al., 2023; Ernst et al., 2016) and food restriction (Lynn et al., 2015), chronic stress exposure (transition to captivity; Dickens and Bentley, 2014) and changing photoperiod (McGuire et al., 2011). These effects on gonadal GnIH expression may be partially mediated by a direct gonadal response to circulating hormones or metabolic cues, as *in vitro* studies suggest (McGuire et al., 2011, 2013). Beyond GnIH, other peptides conventionally produced in the brain or pituitary can be differentially expressed in the gonads in response to environmental input (Table S1). For example, gonadal prolactin mRNA significantly increases when parental rock doves are exposed to chicks during mid-incubation (Farrar et al., 2022a), suggesting gonadal responsiveness to offspring cues.

Overall, local gonadal neuropeptide production provides another example of what Schmidt et al. (2008) refer to as the 'Balkanization' of the endocrine system, where target tissues can locally synthesize hormones and thus gain some independence from the greater endocrine axis. This idea was first proposed for neurosteroids, with a focus on their role in the shifting regulation of aggression between breeding and non-breeding contexts (Schmidt et al., 2008). Like neural steroidogenesis, gonadal neuropeptide expression highlights how tissues can shift from reliance on systemic HPG signals (in the case of the gonads, circulating gonadotropins, which indirectly reflect cue integration by neural GnRH and GnIH) to independent regulation through local signal production and paracrine/autocrine responses. Schmidt et al. (2008) also suggest that tissue reliance on systemic versus locally produced signals may be context dependent. To date, our understanding of gonadal neuropeptide expression has largely been focused on stress responses (Bentley et al., 2017); future research should examine how these peptides respond to other reproductive cues and contexts. The potential for the gonad to directly respond to and integrate cues through neuropeptide production is exciting and calls for further functional tests of the relationship between gonadal neuropeptides and steroidogenesis and fertility.

Integrating gonad-specific mechanisms with hormones and behavior: avenues for future research

Overall, the mechanisms highlighted in this paper represent routes by which HPG axis activity and sex steroid release may be modulated specifically at the gonadal level, including potentially independently of hypothalamic and pituitary signals. Although the studies reviewed here provide preliminary evidence that these genes in the gonad can dynamically respond to hormonal and environmental cues, or are targets of selection on reproductive phenotypes, more research is needed to functionally link gonad-specific mechanisms with sex steroids and, ultimately, sex steroid-mediated traits such as behavior.

An important next step in understanding the function of these gonadal mechanisms is to integrate them with measures of sex steroid concentrations, to establish that they do modulate HPG axis outputs. Many of the studies reviewed here characterize gonadal gene expression, but do not link this expression to sex steroid concentrations in the same animal. Researchers could, for example, correlate gonadal steroid-converting enzyme expression with the levels of precursor and steroid hormone measured using liquid chromatography–tandem mass spectrometry (e.g. Jalabert et al., 2021) as a proxy for enzyme activity. This approach would provide a first correlational view of whether these mechanisms do relate to steroidogenesis. Correlations with sex steroid-mediated traits, such as behavioral phenotypes or fertility, could then be made. Ultimately, though, a causal relationship between gonadal mechanisms and sex steroids *in vivo* will need to be established.

In addition, the dynamic and responsive nature of mechanisms of gonadal hormone regulation can be tested using physiological, environmental and social manipulations. Such manipulations highlighted here include responses to GnRH challenges, exogenous prolactin, glucocorticoids, opportunities for social ascent, competition for nest sites and exposure to stressors. However, many other contexts remain untested; for example, after gaining reproductive experience, loss of a partner or response to infection. Examining gonadal responses to diverse manipulations would provide insight into the lability of gonad-specific mechanisms and their potential to be environmentally and socially responsive.

Importantly, the mechanisms of gonadal hormone regulation presented here do not have mutually exclusive actions, nor do they represent an exhaustive list of routes by which gonadal steroidogenesis may be modulated. Both HPG-specific and potentially HPG-independent regulatory mechanisms undoubtedly interact to modulate gonadal responses. For example, steroidogenic enzyme expression could be altered by gonadotropins or other hormones acting through their receptors. Similarly, paracrine or autocrine actions of gonadal neuropeptides may affect the expression of hormone receptors or steroidogenic machinery. Ideally, researchers should test hypotheses about multiple mechanisms, including those not reviewed here, such as the role of gonadally produced sex hormone-binding globulins (that can affect steroid release and bioavailability; Bobe et al., 2008; Hammond, 2011), gonadal immune factors (Bhushan et al., 2020) and others (Meccariello et al., 2014). Unbiased approaches, such as transcriptomics, can unveil interactions between multiple *a priori*-defined candidates as well as other understudied or unexpected mechanisms.

Indeed, transcriptomic profiles of the gonads have provided key insights into gonad-specific hormone regulatory mechanisms reviewed here, and emerging technologies can further refine our understanding. Gene expression data from bulk gonad homogenates, although insightful, are limited; we cannot determine whether gene expression is specific to endocrine versus gametogenic cells (or other cell types). Incorporating spatial context using histology, such as immunohistochemistry or *in situ* hybridization, can address these issues, but only for a few target molecules. By contrast, single-cell transcriptomic approaches can yield cell-type resolution (Suzuki, 2023). This approach not only makes it possible to specifically focus on endocrine cells across reproductive states (Ben Yaakov et al., 2023; Wu et al., 2023) but also allows comparisons of hormone sensitivity or neuropeptide expression across cell types. When combined with spatially resolved transcriptomics (Marx, 2021; Zhang et al., 2023), these approaches can specify where differential gene expression occurs within the heterogeneous gonad. Single-cell and spatial transcriptomic approaches are beginning to be used to understand the neural basis of behavior (Johnson et al., 2023). Their application in peripheral tissues (as in Niepoth et al., 2024) will provide a high-resolution view into hormonal regulation at the cellular level.

However, functional manipulations of specific genes or proteins are necessary to understand how gonadal mechanisms may affect reproduction and, potentially, behavior. *In vitro* studies have established a causal relationship between neuropeptides, for instance, and gonadal steroidogenic activity (e.g. McGuire et al., 2011; Wilsterman et al., 2019). However, tissues do not operate in a vacuum: *in vivo* manipulations are critical to understanding how gonad-specific molecules/mechanisms could potentially affect more complex traits such as behavior. Gene-editing technologies, such as CRISPR-Cas9, hold promise for functionally manipulating candidate genes in non-model organisms (Alward and Juntti, 2023; Alward et al., 2020). Genetically modified lines, however, tend to over- or under-express genes systemically, so isolating the tissue-specific effects of gene manipulations can be difficult. Gonad-specific gene manipulation could be achieved by direct surgical delivery of RNA interference or viral transgenic vectors to the gonad in reproductive adults. Similar techniques have been used to manipulate neural gene expression (Ubuka et al., 2012) or to access oocytes for CRISPR transgenesis in non-model organisms (Rasys et al., 2019). When applied specifically to the gonad, such methods could establish causality between gonadal gene expression, hormone production and, ultimately, behavior.

Conclusions

Through the mechanisms presented here, the gonad emerges as a dynamic, and potentially independent, player in endocrine regulation. Thus, the gonad can be considered not only the main effector of the HPG axis but also a key node through which environmental and physiological stimuli are integrated into hormonal outputs. Although emerging technologies hold promise for functional tests, foundational work characterizing variation in these gonadal mechanisms (and others) across contexts is still needed. I encourage researchers to consider investigating the gonad in parallel with the brain and circulating hormones, especially in response to social, hormonal and environmental manipulations or in a comparative context. Such integrative analyses will yield unprecedented insights into the peripheral regulation of behavior.

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Competing interests

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