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SYMPOSIUM

Neural Versus Gonadal GnIH: Are they Independent Systems? A Mini-Review

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Synopsis Based on research in protochordates and basal vertebrates, we know that communication across the first endocrine axes likely relied on diffusion. Because diffusion is relatively slow, rapid responses to some cues, including stress-related cues, may have required further local control of axis outputs (e.g., steroid hormone production by the gonads). Despite the evolution of much more efficient circulatory systems and complex nervous systems in vertebrates, production of many "neuro"transmitters has been identified outside of the hypothalamus across the vertebrate phylogeny and these neurotransmitters are known to locally regulate endocrine function. Our understanding of tissue-specific neuropeptide expression and their role coordinating physiological/behavioral responses of the whole organism remains limited, in part, due to nomenclature and historic dogma that ignores local regulation of axis output. Here, we review regulation of gonadotropin-inhibitory hormone (GnIH) across the reproductive axis in birds and mammals to bring further attention to context-dependent disparities and similarities in neuropeptide production by the brain and gonads. We find that GnIH responsiveness to cues of stress appears conserved across species, but that the response of specific tissues and the direction of GnIH regulation varies. The implications of differential regulation across tissues remain unclear in most studies, but further work that manipulates and contrasts function in different tissues has the potential to inform us about both organism-specific function and endocrine axis evolution.

Evolution of endocrine axes

Classic endocrine axes appear to have become established early during the evolution of vertebrates. For example, the most basal vertebrates, *Agnatha* (jawless fishes such as hagfish and lamprey species that have existed for 500 MY), possess a functional and differentiated adenohypophysis and neurohypophysis. The lamprey adenohypophysis tends to be much better differentiated than in hagfishes; it has distinct pars intermedia in close contact with the pars nervosa. The pars distalis is separated into two regions, based on histology. In contrast, the neurohypophysis of lampreys is not as well developed as in hagfish. It has a thin anterior section that could be viewed as simply the floor of the diencephalon. Its posterior part is thickened and is innervated by neurons whose cell bodies are in the preoptic area (POA) in the brain. Thus, it is similar to a neurohypophysis (also known as pars nervosa or posterior pituitary) in other vertebrates. In both lampreys and hagfishes, a true median eminence is strictly absent. In other words, for neuropeptides to influence hormone

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release from the adenohypophysis in *Agnatha*, they must travel from the brain via diffusion rather than being transported via a pituitary portal system (Fig. 1B)

Further back in vertebrate evolutionary history, cephalochordates such as *Amphioxus* spp. (protochordates) possess what has been called a morphological-equivalent of the vertebrate hypothalamo-hypophysial system. Amphioxus does not possess a median eminence, a hypothalamo-hypophysial portal system or a pituitary gland (adenohypohysis or neurohypophysis) in the modern sense. However, Aubrey Gorbman (Gorbman et al. 1999), one of the founders of the field of Comparative Endocrinology, demonstrated that in *Amphioxus* spp. there is what he called a morphologically equivalent neuro-epithelial complex that resembles the hypothalamo-hypophysial system of vertebrates. A ventral lobe of the brain, the infundibulum, that extends downward along the right side of the notochord, and ends near the dorsal surface of a Rathke's pouch-like structure known as Hatschek's pit (Gorbman et al. 1999). Hatschek's pit is immunoreactive for vertebrate LH-like gonadotropin. Gorbman concluded that the infundibulum–Hatschek's pit system of amphioxus may be involved in regulating the seasonal reproductive cycle, a function shared by the hypothalamo-hypophysial neurosecretory system of modern day vertebrates (Fig. 1).

As a result of Gorbman's observations, one can envisage a rudimentary hypothalamo-pituitary-gonad (HPG) axis. The infundibulum of the brain releases a substance to Hatschek's pit, causing release of a gonadotropin to the body cavity. Diffusion would then carry the gonadotropin to bind to receptors on the gonads. One can also envisage that because the gonads of *Amphioxus* are not innervated by the brain or by a vascular system, they might be directly responsive to other cues that provide information about metabolic status or environmental conditions. Similarly, cues of stress (nutritional/metabolic status, glucocorticoids, etc.) can influence gonadal activity directly in birds without input from the brain (McGuire and Bentley 2010a, 2010b; McGuire et al. 2011, 2013); Gorbman's observations imply that this phenomenon may well have arisen very early on during vertebrate evolution.

Neuropeptides in the gonads—limitations of nomenclature and dogma

Study of Amphioxus and *Agnatha* can help us understand the origins of endocrine axes. It can also help us understand why, despite exquisite control via the brain, the gonads can be directly regulated by environmental cues independently of the brain, such as cues of day length and stress (McGuire et al. 2011, 2013). One aspect of the physiology of vertebrate gonads that is harder to understand is how and why they evolved to synthesize neuropeptides. Further, why was this feature retained once evolution of the HPG axis allowed for precise control of gonad function by the brain—in other words, what relevant function do these local neuropeptides play?

One issue that we need to be aware of is that the way we think about hormone action and interaction can be limited by their nomenclature and the way we teach or learn about hormone axes. For example, prolactin is named for its lactation-inducing effects in mammals, yet it has a diverse array of functions (400+) across vertebrate classes. Another example is the prolactin hypothalamic secretagogue, which has the incongruous name vasoactive intestinal polypeptide. It was so named because of where in the mammalian body it was first discovered and the first function ascribed to it. Now consider the neuropeptide gonadotropin-releasing hormone (GnRH): we all learn (and teach) that it is a neuropeptide produced by the hypothalamus and that it causes the release of gonadotropins. These are both facts, yet when considered in isolation they give rise to the dogma that GnRH is *only* produced in the brain and it *only* is involved in the release of gonadotropins. However, it is quite possible that GnRH initially evolved to influence muscle contraction (particularly the heart as is seen in octopus—(Iwakoshi-Ukena et al. 2004) and eventually was co-opted for the regulation of reproduction. Furthermore, GnRH and its receptor are found in a variety of gonadal and non-gonadal tissues in vertebrates. For example, in the heart in fish and humans (Habibi and Pati 1993; Kakar and Jennes 1995; White and Fernald 1998), in the liver, skeletal muscle, kidney, placenta, and pituitary in humans (Kakar and Jennes 1995), and in the bladder (Bahk et al. 2008). GnRH may also act on/in the skin and adrenal gland (Berger et al. 1993; Xing et al. 2009).

Thus, it is quite possible that early during the evolution of vertebrates, our "pet" neuropeptides were simply hormones produced by a multitude of tissues and likely had a variety of effects, acting via different second messenger systems according to tissue type. When considered in this broader context, it is quite easy to see how and why the gonads are able to synthesize neuropeptides independently of the brain, and that these gonadal neuropeptides may well have functions that differ from those in the brain. This mini-review examines the production

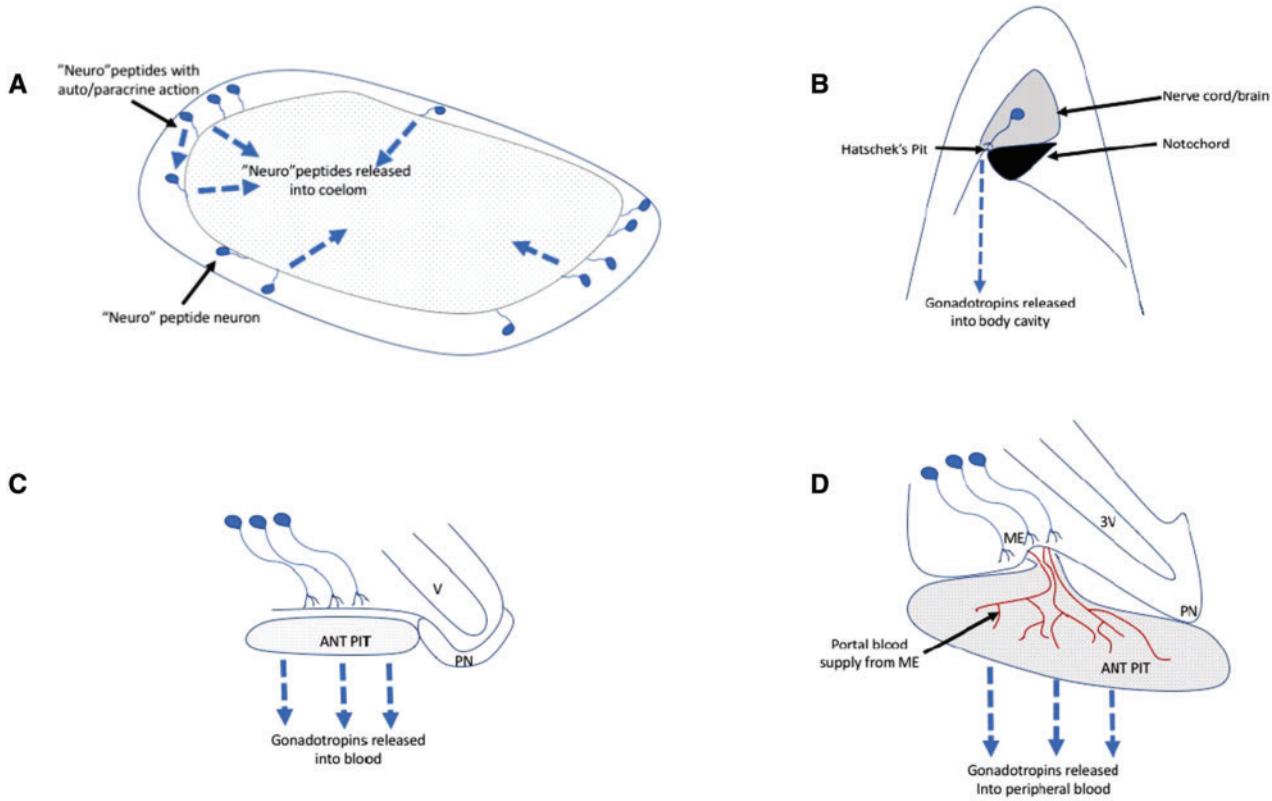

Fig. 1 Diagrammatic representation of putative steps in the evolution of the hypothalamo-pituitary-gonad axis. (**A**) Primitive metazoan with cells producing hormones that we now refer to as neuropeptides. These hormones are produced from a variety of tissues and diffuse throughout the coelom, having autocrine and paracrine actions. (B) Section through the head of *Amphioxus* sp. indicating part of the brain descending to Hatschek's pit, where neuropeptides are released, diffuse across connective tissue and cause gonadotropins to be released into the body cavity. (**C**) Section through agnathan (lamprey) pituitary, showing a well-developed anterior pituitary (ANT PIT) and pars nervosa (PN), but no vascularized median eminence. Neurons release neuropeptides which diffuse across connective tissue to the anterior pituitary, causing pituitary gonadotropin release. V = ventricle. (**D**) Section through avian pituitary, showing well-developed pituitary and hypothalamo-hypophysial portal system, which transports neuropeptides from the median eminence (ME) to the anterior pituitary. 3V = third ventricle.

and function of one neuropeptide, gonadotropin-inhibitory hormone (GnIH), in the brain and gonads in the context of various environmental stimuli. We suggest that GnIH provides an excellent case study for identifying the challenges for and approaches needed to advance our understanding of "neuro"peptide function and importance outside of the hypothalamus.

GnIH as a candidate for modulation of stress-induced reproductive dysfunction

Soon after the initial discovery of GnIH (Tsutsui et al. 2000), GnIH neurons were shown to project to GnRH neurons in birds (Bentley et al. 2003). This neuroanatomical interaction has since been demonstrated in all vertebrates studied, including fish, frogs, hamsters, mice, rats, sheep, monkeys, naked mole-rats, and humans (Kriegsfeld et al. 2006; Smith et al. 2008; Ubuka et al. 2009a, 2009b; Pinelli et al. 2015; Choi et al. 2017; Peragine et al. 2017). GnRH neurons also express GnIH receptor (GnIH-R) (Ubuka et al. 2008, 2012). Thus, GnIH has the potential to influence HPG activity via action on GnRH neurons. Along these lines, there is some evidence that hypothalamic GnIH can act to inhibit firing of GnRH neurons (Ducret et al. 2009) and GnIH can reduce GnRH transcription *in vitro* (Gojska et al. 2014).

GnIH neurons express glucocorticoid receptor (GR) (Kirby et al. 2009; Gojska and Belsham 2014), and elevated glucocorticoids (CORT) cause an increase in GnIH expression *in vivo* and *in vitro* (Kirby et al. 2009; Gojska and Belsham 2014; Son et al. 2014). GnIH neurons project to GnRH neurons (which themselves express GnIH-R), then the neuroanatomical infrastructure is in place for stress-induced changes in circulating CORT concentrations to impact the HPG axis via action on GnIH neurons. In a similar vein, treatment of neonatal

mice with the synthetic glucocorticoid dexamethasone (DEX) delayed pubertal onset and was associated with increased GnIH cell number and GnIH-R expression in the hypothalamus (Soga et al. 2012). Thus, the GnIH system is activated in response to stress or cues of stress and results in decreased HPG activity.

Even though there have been conflicting reports for some species as to whether GnIH is hypophysiotropic in addition to acting on GnRH neurons directly (Murakami et al. 2008; Rizwan et al. 2009), GnIH can inhibit gonadotropin synthesis and release from the adenohypophysis in several species and LH release in humans (Tsutsui et al. 2000; Ciccone et al. 2004; Clarke et al. 2008; Kadokawa et al. 2009; Sari et al. 2009; George et al. 2017). However, this effect is not universal and GnIH might actually increase gonadotropin release under certain circumstances (Henningsen et al. 2017). In general, stress has the potential to act on pituitary gonadotropin synthesis and release via action of glucocorticoids on GnIH neurons (increasing GnIH release to the anterior pituitary).

Finally, GnIH can also be found in the gonads. The first demonstration of GnIH and its receptor in the gonads was in birds (Bentley et al. 2008). As is the case for the GnIH–GnRH interaction in the brain, the gonadal GnIH system exists in all vertebrates studied so far, including primates (McGuire and Bentley 2010b; Zhao et al. 2010). The gonads also tend to express GR (McGuire et al. 2013) and thus they have the potential to respond directly to changes in circulating glucocorticoids independently from the brain.

Differential responses of neural versus gonadal GnIH in different contexts—a classic trade-off?

In the following sections, we will highlight a few studies in which neural and gonadal GnIH do not respond to a stimulus in a consistent manner. In a way, this differential response of gonadal and neural GnIH can be viewed as a trade-off (Stearns 1989). It is quite possible that increased action of GnIH in the brain might be differentially costly in terms of energetics from that of increased action of GnIH in the gonads. The adaptive value or cost to the individual of altering GnIH in the gonads and/or brain may also depend on the context in which the change occurs (i.e., depending on the particular species, its sex, the particular stimulus, the life-history stage or substage), the organism involved might benefit more from changing GnIH action in the brain versus the gonad. More concretely, we can ask whether a transient (rather than sustained) cessation of reproductive behavior in response to a stressful stimulus is more adaptive earlier in the breeding season, because then the animal may resume reproduction in a relatively short space of time. Perhaps at a different stage of reproduction, when a seasonal breeder has already invested a great deal in that year's reproductive effort, an alteration in behavior might be more beneficial than an inhibition of the HPG axis. In the same vein, differential investment in reproduction by the different sexes might cause a different response of gonadal vs. neural GnIH. Life history traits, such as the breeding strategy of a species (opportunistic versus strictly seasonal) might also influence such differences. Thus, as with all trade-offs, the ecological and physiological context of changes in neural or gonadal GnIH are likely to be critical determinants of how an organism responds to a particular physiological or ecological environment at any one time.

Acute stress in opportunistically-breeding birds

Restraint

In a study on zebra finches, a species which breeds opportunistically and independently of photoperiod (Perfito et al. 2008, 2011; Perfito and Bentley 2009), 60 min of restraint stress significantly increased plasma corticosterone in males and females (Ernst et al. 2016). There were also significantly fewer GnIH immunoreactive (−ir) cell bodies in the stressed birds compared to controls. Although GnIH-ir cell number did not differ between the sexes in either treatment or control animals, there was a significant decrease in *FSH-beta* expression in pituitary glands of stressed females. No difference in GnRH expression was observed, suggesting GnIH did not affect synthesis of GnRH. Thus, the reduction in observed GnIH immunoreactivity in females is consistent with increased GnIH release to the hypothalamo-hypophysial portal system, which could then directly inhibit pituitary gonadotropin synthesis.

In the gonads of zebra finches that experienced restraint stress, there was a sex difference in GnIH responsiveness (Ernst et al. 2016). In males, stress increased testicular GnIH expression but in females there was no significant change in ovarian GnIH. This result could indicate a true sex difference in responsiveness to restraint stress, where male hypothalamic and gonadal GnIH respond to the stressor but females only respond with a change in hypothalamic

GnIH. Alternatively, the lack of effect on ovarian GnIH could simply be a sampling issue. Data from ovaries were pooled in this study and given that GnIH has been implicated in follicular development (Maddineni et al. 2008), it is quite possible that differential expression of GnIH occurs in follicles across different developmental stages—which is not detectable when ovaries are analyzed as a whole. Either way, the hypothalamic GnIH system of both sexes responds to acute restraint stress.

Fasting

Fasting or food stress in zebra finches induces elevated corticosterone (Lynn et al. 2010, 2015). In an experiment where male birds were fasted for 10 h (an ecologically-relevant food stress (Lynn et al. 2015), there was no difference in hypothalamic GnIH expression or immunoreactivity between fasted and control birds. However, elevated corticosterone was positively correlated with increased GnIH mRNA expression in the testes. In addition, circulating testosterone was negatively correlated with testicular GnIH mRNA expression. Overall, birds with higher corticosterone had higher testicular GnIH expression and lower testosterone. Consistent with the idea that GnIH might impact steroidogenesis in the gonads, expression of mRNA for steroidogenic acute regulatory protein and LH receptor (StAR and LHR) was lower in the testes of fasted birds (Lynn et al. 2015). Thus, the decrease in circulating testosterone was likely mediated by direct actions of fasting and/or corticosterone on the testes to upregulate GnIH, indicating that the testes can integrate and respond to cues of stress directly—as has been shown in European starlings (McGuire et al. 2013). Such local inhibition of testosterone synthesis may allow for rapid and reversible changes in physiology and behavior when conditions are inappropriate for breeding.

Immune challenge

Immune challenge is also a stressor that activates the hypothalamo-pituitary-adrenal (HPA) axis. Injection of lipopolysaccharide to induce sickness behavior in zebra finches increased circulating corticosterone (CORT) concentration and decreased hypothalamic GnRH-I mRNA expression and immunoreactivity, but did not change GnIH expression or immunoreactivity (Lopes et al. 2012). Gonadal GnIH was not investigated in this study, but in another study involving LPS injection (Lopes et al. 2013), there was no effect on testicular GnIH. In this latter study, LPS did not induce statistically significant changes in hypothalamic GnIH immunoreactivity, but the data indicate that use of a larger sample size would likely have demonstrated such an effect (Lopes et al. 2013). It remains to be seen whether induction of sickness behavior in this way is related to changes in ovarian GnIH.

How the neural and gonadal GnIH systems of this opportunistically-breeding species responds to other stressors in their natural environment remains to be seen. Zebra finches maintain reproductive flexibility year-round (Lynn et al. 2010; Perfito et al. 2006, 2011) despite unpredictable availability of resources. A direct role for GnIH in regulating timing of reproduction in this species in the wild has yet to be identified.

Stress in seasonally-breeding birds

Acute restraint stress

In male and female house sparrows (*Passer domesticus*) at the start and end of the breeding season (spring and fall), acute restraint stress for 60 min caused an increase in hypothalamic GnIH immunoreactivity only in the spring (Calisi et al. 2008). In the fall, baseline (control) GnIH immunoreactivity was as high as stress-induced immunoreactivity in the spring, thus it is quite possible that seasonal regulation of the hypothalamic GnIH system either masked any effects of stress on GnIH in the fall, or precluded any further increase in GnIH immunoreactivity at this time of year. Another possible explanation for the lack of change in GnIH in the fall is that animals have an attenuated stress response at the end of the breeding season (Breuner and Orchinik 2001; Romero 2002), and the GnIH stress response in the hypothalamus may also follow this pattern.

The GnIH stress response seen at the beginning of the breeding season may reflect a mechanism by which reproduction is temporarily halted in stressful conditions. The involvement of GnIH would allow for rapid changes in behavior without the need for long-term inactivation of the reproductive axis, as occurs at the end of the breeding season. Unfortunately, gonadal GnIH was not studied in this experiment (the study was performed at a time when the gonadal GnIH system had only recently been discovered (Bentley et al. 2008) and there was no knowledge of its response to stress). Nevertheless, the seasonal differences in hypothalamic GnIH immunoreactivity and its response to stress highlight the importance of considering the ecological, temporal and physiological contexts of a stimulus.

Housing stress

In a study on male and female European starlings (*Sturnus vulgaris*), birds were divided into two mixed-sex groups and housed in an outdoor, semi-natural environment (Dickens and Bentley 2014). A short time prior to when birds were predicted (based on historical data) to begin laying eggs one group was transferred into an indoor flight aviary and the other remained in the outdoor aviary. After ten days, the birds showed remarkable differences in breeding behavior and HPA activity. Outdoor birds exhibited increases in baseline and stress-induced CORT and progressed into active breeding (pairing, nest building, egg laying, etc.). In contrast, indoor birds displayed no change in baseline or stress-induced CORT and few signs of active breeding. As soon as the outdoor birds had begun to lay eggs, tissues were collected for analysis. None of the indoor birds laid eggs, nor did they have any yolking follicles, whereas outdoor birds either laid eggs or had developing follicles. Testosterone was lower in the indoor males, and oviduct size (a proxy for circulating estradiol concentrations) tended to be smaller in indoor females. Outdoor males had elevated hypothalamic GnRH immunoreactivity, but overall there were no differences in hypothalamic *GnRH* or *GnIH* expression between housing conditions. There was, however, elevated hypothalamic *GnIH* expression in females in both conditions relative to their male counterparts, suggesting that at this time of year, hypothalamic GnIH is differentially expressed in male and female starlings.

Perhaps the most critical finding of this study was that the ovaries of indoor females, which exhibited little to no follicular development, had significantly higher gonadal *GnIH* expression relative to outdoor females (which were apparently exhibiting normal ovarian development for that time of year). Thus, it is possible that reproductive suppression in indoor females was mediated by increased ovarian GnIH production. Given that GnIH can mediate follicular development in chickens (Maddineni et al. 2008), this is a plausible scenario, but direct manipulation of ovarian GnIH will be needed to determine a causal link. There was no difference in testicular *GnIH* expression between indoor versus outdoor males.

The findings on CORT from this study are intriguing. Moving birds to an indoor, unnatural housing environment suppressed seasonal elevation in HPA axis activity in European starling. Similar effects of captivity on seasonal CORT profiles have been shown in Gambel's white-crowned sparrows (*Z. l. gambelii)* (Romero and Wingfield 1999). Thus, HPA dysfunction, rather than acute activation as is seen following capture-handling stress is associated with HPG dysfunction, including elevation of ovarian GnIH. Overall, these findings indicate again that ecological and physiological context are important considerations when designing experiments and interpreting the resulting data.

Housing can also impact hypothalamic GnIH content at different stages of reproduction in seasonally-breeding European starlings. Calisi et al. (2011) experimentally restricted nesting opportunities for pairs of birds. Birds which outcompeted others for nest boxes ("winners") had significantly fewer numbers of GnIH peptide-producing cells prior to egg-laying and incubation than birds unable to secure nest boxes ("losers"). This relationship reversed as birds with nests had laid eggs such that "winners" exhibited an increase in GnIH immunoreactivity to greater amounts than early-season "losers", whereas "losers" had no change in GnIH relative to the early season. No gonadal GnIH data were collected in this experiment, but clearly, ecological and physiological context are important even within the breeding season for the regulation of hypothalamic GnIH in this species.

Cues of stress on gonads from seasonal breeders *in vitro*

Gonads from European starlings respond directly to fluctuations in corticosterone and metabolic fuels by decreasing sex steroid secretion (McGuire et al. 2013). This study indicates that the gonads of this species can respond directly to fluctuations in corticosterone and metabolic fuels by modulating sex steroid secretion. Physiologically-relevant concentrations of corticosterone and metabolic stress (via use of the glucose utilization inhibitor 2-deoxy-D-glucose and the fatty acid oxidation inhibitor ethyl 2-mercaptoacetate (2DG/MA)) decreased testosterone and estradiol secretion from testes and ovaries, even when stimulated with luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Further, this study demonstrated a seasonal response to treatment. Prior to the breeding season, there was a significant decrease in gonadal steroid release from testes and ovaries in response to corticosterone and 2DG/MA. Within the breeding season there was a clear sex difference in response: the testes did not respond to these cues of stress, while the ovaries responded only to corticosterone.

The seasonal difference in response may in part result from the response of the gonads to melatonin, and the interaction of this hormone with gonadal GnIH production. Gonads from birds collected

earlier in the breeding season (during shorter days) had experienced a longer melatonin signal. As the gonadal GnIH system is responsive to melatonin (McGuire et al. 2011), gonads collected during shorter days (earlier in the breeding season) might be primed by melatonin to have a responsive GnIH system which could in turn impact gonadal steroid release. Thus, even though the gonads can directly respond to fluctuations in corticosterone and metabolic fuels during a time of critical importance to the onset of breeding, the response appears to be highly temporally dynamic.

Stress in mammals

Restraint stress

In adult male Sprague–Dawley rats (Kirby et al. 2009), both acute and chronic immobilization stress led to an up-regulation of RF-amide-related peptide (RFRP, mammalian ortholog of avian GnIH) expression and immunoreactivity in the dorsomedial hypothalamus (DMH). After acute stress (3 h), there was a 1.4-fold increase in the number of hypothalamic RFRP immunoreactive cells. This effect had dissipated after 24 h. Chronic stress (3 h per day for 14 days) induced a 1.8-fold increase in hypothalamic RFRP mRNA, a 1.9-fold increase in the number of hypothalamic RFRP immunoreactive cells, and a reduction in circulating LH. Importantly, adrenalectomy blocked the stress-induced increase in RFRP expression, further supporting the hypothesis that adrenal glucocorticoids stimulate RFRP expression in the hypothalamus. Despite a clear impact on HPG function at the level of the hypothalamus and pituitary, there was no effect of these stressors on testicular expression of RFRP or its receptor. It is possible that the reproductive physiology and strategy of male rats (continuous breeder) requires a reduced sensitivity of the gonads to stressors, or that the testicular RFRP system performs a function different from the testicular GnIH system in birds.

Chronic restraint stress (3 h/day for 18 days) applied to female Sprague–Dawley rats caused elevated hypothalamic RFRP expression, even after cessation of stress (after 4 days of no stress exposure) (Geraghty et al. 2015). Even though stress did not affect estrous cyclicity, expression of RFRP and its receptor in the hypothalamus were significantly elevated at all estrus cycle stages—even 4 days after the stressor was terminated. This chronic stress exposure negatively impacted reproductive success, with fewer successful copulation events, fewer pregnancies in those that successfully mated, and increased embryo resorption in stressed animals. Genetic silencing of RFRP in the hypothalamus during the application of stress using an inducible-targeted shRNA completely alleviated this stress-induced reproductive dysfunction, resulting in mating and pregnancy success rates indistinguishable from non-stress controls. Thus, as for male rats, it appears that the effects of stress on HPG function are probably not mediated via RFRP action in the ovaries or peripheral reproductive structures. Rather, in this continuously breeding mammal, stress/RFRP-induced reproductive dysfunction may well be mediated entirely by the brain. Whether this lack of effect of stress on the gonadal GnIH/RFRP system applies to other mammals remains to be seen, and raises the question of the function of gonadal GnIH/RFRP system in mammals (but see section on reproductive aging below).

Reproductive aging and gonadal GnIH/RFRP

Although not a stressor *per* se, aging has a significant impact on reproductive function. In a recent study, a role for RFRP in reproductive senescence was investigated in female Long-Evans rats (Geraghty et al. 2016). Rats of different ages were used: regularly cycling young (3 months old), regularly cycling middle aged (8 months old), irregularly cycling (10 months old), and acyclic (12 months old). Females exhibited a transient increase in hypothalamic RFRP mRNA expression in middle age prior to changes in estrous cycle length. This transient increase in RFRP was followed by decreases in hypothalamic kisspeptin (KiSS1) and gonadotropin-releasing hormone (GnRH) mRNA expression, and an increase in expression of RFRP and its receptor in the ovaries. Thus, it is possible that reproductive senescence in female rats is initiated by alterations in a network of regulatory neuropeptides upstream of the GnRH system, including RFRP. Further, a precise role for the ovarian RFRP system in reproductive senescence is unclear from this study, but the correlative data indicate that this gonadal neuropeptide is regulated as a function of reproductive dysfunction as rat's age.

Conclusions

The evolution of the endocrine system has resulted in the classic top-down axes that are regulated by neuropeptides. In addition, peripheral structures such as the gonads have their own compartmentalized autocrine/paracrine endocrine physiology that is regulated by identical "neuropeptides" that are synthesized in the peripheral structures themselves. In terms of modulation of GnIH by stressors, it is clear that the brain and the gonads do not always respond

in concert. Whether the brain or the gonad up- or down-regulates GnIH synthesis and how its reproductive physiology responds depends on the stressor, the sex/species of the animal, reproductive status (not simply breeding/non-breeding, but also substages within), photoperiod, nutritional status, age, and the ecological context within which an animal experiences a stressor. An open question is why should the brain's GnIH system respond to a stressful stimulus when the gonads do not, and vice-versa? Why are there sex differences in terms of whether the brain or gonads respond to an identical stimulus? It is apparent that we do not fully understand the role of gonadal GnIH, nor its regulation. Gonadal neuropeptides are often ignored in studies on reproduction, but, as with the relatively recent call for study of both sexes (Beery and Zucker 2011), perhaps there will be more studies that include the gonadal GnIH system in addition to that of the brain. Such studies may potentially provide an understanding of their importance in different ecological and physiological contexts, but also may provide further information regarding the evolutionary history of these complex endocrine axes.

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