Journal of Neuroendocrinology
From Molecular to Translational Neurobiology

Journal of Neuroendocrinology **21**, 276–281

REVIEW ARTICLE

Gonadotrophin-Inhibitory Hormone: A Multifunctional Neuropeptide

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Journal of Neuroendocrinology

Gonadotrophin-inhibitory hormone (GnIH) was discovered 8 years ago in birds. Its identification raised the possibility that gonadotrophin-releasing hormone (GnRH) is not the sole hypothalamic neuropeptide that directly influences pituitary gonadotrophin release. Initial studies on GnIH focused on the avian anterior pituitary as comprising the only physiological target of GnIH. There are now several lines of evidence indicating that GnIH directly inhibits pituitary gonadotrophin synthesis and release in birds and mammals. Histological studies on projections from hypothalamic GnIH neurones subsequently implied direct actions of GnIH within the brain and in the periphery. In addition to actions on the pars distalis via the median eminence, GnIH axons and terminals are present in multiple brain areas in birds, and the GnIH receptor is expressed on GnRH-I and -II neurones. Furthermore, we have demonstrated the presence of GnIH and its receptor in avian and mammalian gonads. Thus, GnIH can act directly at multiple levels: within the brain, on the pituitary and in the gonads. In sum, our data indicate that GnIH and its related peptides are important modulators of reproductive function at the level of the GnRH neurone, the gonadotroph and the gonads. Here, we provide an overview of the known levels of GnIH action in birds and mammals. In addition, environmental and physiological factors that are involved in GnIH regulation are reviewed.

Key words: GnRH, seasonal breeding, melatonin, HPG axis, RFamide.

doi: 10.1111/j.1365-2826.2009.01851.x

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Gonadotrophin-inhibitory hormone (GnIH) was discovered in birds (1). This neuropeptide was initially found to inhibit the release of pituitary gonadotrophins in quail (*Coturnix japonica*) both *in vitro* and *in vivo* (1, 2). The effect on gonadotrophin release has subsequently been confirmed in rodents and sheep using avian GnIH or homologous mammalian peptides (3–6). Additionally, GnIH inhibits pituitary synthesis of the gonadotrophin common α subunit and the specific β subunits (7). Thus, study of GnIH and its action at the level of the anterior pituitary strongly supports a role for this neuropeptide as a bona fide GnIH. The structure of GnIH, its history and aspects of its functional significance are discussed in detail elsewhere (8), where it is noted that GnIH homologous peptides have been identified in the hypothalamus of a variety of vertebrates, including mammals, reptiles, amphibians and teleosts. These identified GnIH homologous peptides possess a C-terminal LPXRFamide motif (X = L or Q), as does GnIH. Avian GnIH inhibits luteinising hormone (LH) release in hamsters (3), and a mammalian GnIH homologue [RFamide-related peptide (RFRP)-3] inhibits LH release in rats (4, 5) and sheep (6), as well as inhibiting second messenger pathways in mammalian gonadotroph cell lines (Ubuka *et al.*, unpublished data). Thus, we consider avian GnIH and mammalian RFRPs to be mammalian GnIH homologues, both structurally and functionally.

GnIH/RFRP precursor mRNA encodes a polypeptide that is possibly cleaved into three mature peptides in birds and usually two in mammals. The LPXRF (X = L or Q) motif at the C-termini for GnIH/RFRP peptides is followed by glycine as an amidation signal and arginine or lysine as endoproteolytic basic amino acids. Endogenous GnIH/RFRP peptides can be cleaved at the basic amino acids of their N-termini. Three LPXRF-amide (X = L or Q) peptide sequences are encoded in the quail and starling GnIH precursor polypeptide, designated GnIH-related peptide-1 (GnIH-RP-1), GnIH

and GnIH-RP-2 from the N-termini to the C-termini. Of these possible RFRP sequences, quail GnIH (SIKPSAYLPLRF-amide), quail GnIH-RP-2 (SSIQSLLNLPQRF-amide) and starling GnIH (SIKPFANLPLRF-amide) have been identified as mature endogenous peptides by mass spectrometric analysis (1, 9, 10). In the human RFRP precursor polypeptide, two LPXRF-amide (X = L or Q) peptide sequences (human RFRP-1 and RFRP-3) are encoded, and we have identified both as being mature peptides by mass spectrometric analysis (human RFRP-1: MPHSFANLPLRF-amide, human RFRP-3: VPNLPQRF-amide) (11). Interestingly, there is an LPLRS-amide peptide sequence encoded in the GnIH position of the precursor polypeptide, which could be designated as human RFRP-2 (12), although we were unable to isolate this mature peptide in our studies. By contrast, there is no RFRP-2 sequence in the rat RFRP precursor polypeptide, although two LPXRF-amide (X = L or Q) peptide sequences (rat RFRP-1 and RFRP-3) are conserved. Of these two LPXRF-amide (X = L or Q) peptides, only rat RFRP-3 (ANMEAGTMSHFPSLPQRFamide) has been identified as an endogenous mature peptide (13); for alignment of precursor polypeptides in several vertebrate species, see Ubuka *et al.* (14).

Identification and structural analysis of the quail GnIH receptor indicates that it belongs to the G-protein-coupled receptor superfamily (15). Binding analysis reveals that the C-terminal LPXRF-amide (X = L or Q) motif is critical for its binding to the GnIH receptor. The mammalian homologue of the GnIH receptor is GPR147 (OT7T022, NPFF-1) (16). RFRP peptides suppress the production of cAMP in ovarian cells of Chinese hamsters transfected with GPR147, suggesting that the receptor couples to $G_{\alpha i}$. GPR147 mRNA is also expressed in various parts of the brain as well as in the pituitary, suggesting that there are multiple actions within the central nervous system (12).

The present review focuses on the apparent multi-functionality of avian GnIH and its mammalian counterpart, RFRP, its actions at different levels of the hypothalamic-pituitary gonad axis and its modulation by different environmental inputs.

Levels of action of GnIH

Brain

The widespread distribution of GnIH fibres and terminals in the avian brain indicate that, in addition to direct regulation of pituitary hormone release, GnIH might be involved in the direct regulation of neuroendocrine or neurotransmitter systems. In birds, GnIH fibres appear to be in contact with GnRH-I and -II neurones (17) and both populations of GnRH neurones express GnIH receptor mRNA. GnIH infusion to the third ventricle in white-crowned sparrows reduces plasma LH, which is thought to be dependent upon GnRH-I (18). Until now, songbird GnRH had not been cloned, and researchers in this area had been unable to determine whether GnIH administration directly affects synthesis of GnRH or whether GnIH simply inhibits GnRH release. Our recent identification of the songbird GnRH cDNA sequence should allow us to answer this fundamental question (19).

There appear to be conserved properties of GnIH distribution and function in vertebrate brain. GnIH/RFRP makes apparent contact with GnRH neurones not only in birds, but also in hamsters, mice and rats (3, 4), as well as sheep (20). A detailed mapping of GnIH/RFRP in rhesus macaque brain revealed that GnIH/RFRP-immunoreactive (-ir) fibres are in close proximity to GnRH-I, dopamine, β-endorphin and GnRH-II neurones in the preoptic area, intermediate reticular zone, arcuate nucleus of hypothalamus and central gray substance of midbrain, respectively. Thus, GnIH/RFRP neurones in monkeys might regulate several neuropeptide systems and behaviours in addition to influencing pituitary gonadotrophin release (21). The frequency of copulation solicitation display, a GnRH-II-dependent behaviour (22) is also reduced after central GnIH administration, and may occur as a direct result of GnIH binding to its cognate receptor on GnRH-II neurones (10, 17). Central administration of avian GnIH to rodents also reduces plasma LH and the frequency of male sexual behaviours (3, 4). Peripheral administration of the putative ovine homologue of GnIH reduces the amplitude of LH pulses in sheep (6), and reduces LH and follicle-stimulating hormone (FSH) release *in vitro*.

Overall, there are distinct similarities in the distribution of GnIH fibres and their contact with GnRH neurones in all vertebrates studied. There are also similarities in terms of the *in vivo* actions of GnIH and its homologous peptides.

Pituitary

GnIH/RFRP neurones project to the external layer of the median eminence in several bird and mammal species (1, 3, 6, 10, 17). This neuroanatomical distribution is consistent with the first identified action of GnIH (i.e. as a direct inhibitor of pituitary gonadotrophin release) (1). Furthermore, GnIH/RFRP receptor (GnIH-R) is found to be expressed in the pituitary of all avian and mammalian species studied to date: quail (15), chickens (23), European starling (10), rufous-winged sparrow (N. L. McGuire, P. Deviche and G. E. Bentley, unpublished data), Siberian hamster (24), monkey and human (T. Ubuka and G. E. Bentley, unpublished data). GnIH-R is regulated as a function of sexual maturity in chickens; the quantity of GnIH-R mRNA was significantly higher in the pituitaries of sexually immature chickens relative to sexually mature chickens (23). Furthermore, oestradiol or oestradiol plus progesterone treatment caused a significant decrease in the qiantity of pituitary GnIH-R mRNA relative to vehicle controls. At the cellular level, GnIH-R-ir cells were co-localised with LH β subunit mRNA-, and FSH β subunit mRNA-containing cells. In agreement with the GnIH-R expression data, GnIH treatment significantly decreased LH release from anterior pituitary gland slices collected from sexually immature, but not sexually mature, chickens (23). Thus, GnIH-R expression in chickens appears to be down-regulated by circulating oestradiol and progesterone levels because chickens undergo sexual maturation to allow gonadotrophin secretion. GnIH also decreases gonadotrophin α and FSH β subunit expression in the chicken pituitary *in vitro* (25).

Despite the similarities in distribution of GnIH in the median eminence and GnIH-R in the pituitary, there are some anomalies

among the vertebrates. For example, rufous-winged sparrows (*Aimophila carpalis*) do not have detectable immunoreactive GnIH in the median eminence (26) despite expressing GnIH-R mRNA in the pituitary. Nor do peripheral injections of GnIH rapidly inhibit LH secretion in this species (27). This raises the question of the functional significance of GnIH-R that is expressed in the pituitary of this species (N. L. McGuire, Deviche and G. E. Bentley, unpublished data). It also raises the question of whether there is another source of GnIH that can influence pituitary gonadotrophin release? In some avian species, there appear to be GnIH projections to the pars nervosa (G . E. Bentley and J. C. Wingfield, unpublished data); thus, GnIH could potentially be released directly into the peripheral circulation. The gonads are also a source of GnIH synthesis, but it is unclear whether GnIH can pass into the bloodstream from the gonads (see below). A more parsimonious explanation is that there is some form of dynamic regulation of GnIH in the median eminence, and the time-window in which it is transported to the external layer simply has not yet been detected. Further study over a finer temporal scale might help clarify this issue because certainly there is dynamic regulation of GnIH–GnRH contact in the hypothalamus of rufous-winged sparrows, as in mammals (see below).

The presence of GnIH/RFRP terminal boutons in the external layer of the median eminence is somewhat consistent in mammalian species that have been studied, and no dynamic regulation of GnIH in the external median eminence has yet been reported. Thus, rufous-winged sparrows appear to be almost uniquely enigmatic in this regard. Siberian hamsters (24), rhesus macaques (21), sheep (6) and humans (T. Ubuka and G. E. Bentley, unpublished data) all exhibit immunoreactive GnIH in the median eminence. There are reports that there is no detectable GnIH/RFRP in the median eminence of Wistar rats (Rizwan, M.Z., Porteous, R., Herbison, A.E. & Anderson, G.M., unpublished data), although a different antibody was used for this study versus the other mammalian studies mentioned. It is not known whether these rats, similar to rufous-winged sparrows, express GnIH receptor in the pituitary despite a lack of GnIH in the median eminence. Overall, the regulation of pituitary hormone release appears to be well-conserved across vertebrate classes, but there are some interesting anomalies.

Gonads

Many hormones that are classified as neuropeptides are synthesised in vertebrate gonads in addition to the brain. Receptors for these hormones are also expressed in gonadal tissue; thus, there is potential for a highly-localised autocrine or paracrine effect of these hormones on a variety of gonadal functions. We recently provided evidence for the synthesis of GnIH and its receptor in the avian reproductive system, including the gonads and accessory reproductive organs, in studies of two orders of birds: Passeriformes and Galliformes (28). Binding sites for GnIH were initially identified via *in vivo* and *in vitro* receptor fluorography, and were localised in ovarian granulosa cells along with the interstitial layer and seminiferous tubules of the testis. Furthermore, species-specific primers produced clear polymerase chain reaction (PCR) products of GnIH and GnIH receptor GnIH-R in songbird and quail gonadal and other reproductive tissues, such as oviduct, epididymis and vas deferens. Sequencing of the PCR products confirmed their identities. Immunocytochemistry detected GnIH peptide in ovarian thecal and granulosa cells, testicular interstitial cells and germ cells, and pseudostratified columnar epithelial cells in the epididymis. *In situ* hybridisation of GnIH-R mRNA in testes produced a strong reaction product, which was localised to the germ cells and interstitium. In the epididymis, the product was also localised in the pseudostratified columnar epithelial cells. Similar data have been gathered from chickens, and oestradiol and/or progesterone treatment of sexually immature chickens significantly decreased ovarian GnIH-R mRNA abundance (29).

In summary, these results indicate that the avian reproductive system has the capability to synthesise and bind GnIH in several tissues. The distributions of GnIH and its receptor suggest a potential for autocrine/paracrine regulation of gonadal steroid production and germ cell differentiation and maturation.

Mammalian gonads also express GnIH/RFRP. Using semi-quantitative PCR, we found that the GnIH/RFRP precursor is expressed in the testes of Syrian hamsters and other rodent species (30). GnIH/RFRP expression is confined to seminiferous tubules, and approximately one-third of tubules express GnIH in primary and secondary spermatocytes. Quantification of proliferating cell nuclear antigen and GnIH/RFRP expression levels revealed that higher numbers of proliferating germ cells occur in GnIH/RFRP-negative tubules, suggesting an inhibitory effect of this peptide on spermatogenesis. Thus, as in birds, there appears to be an autocrine/paracrine role for GnIH/RFRP in mammalian testis, regulating the proliferation and/or differentiation of gonadal germ cells. We observed a similar distribution of GnIH and GnIH-R in rhesus macaque testis (31). Interestingly, the ovary of a lizard, *Calotes versicolor*, was also reported to express GnIH (32), and the expression of this neuropeptide in the gonads might be a universal property of vertebrates.

Dynamic regulation of the GnIH system

The findings obtained to date from a number of laboratories indicate the dynamic temporal regulation of the GnIH system in all vertebrates under study. Changes in GnIH over time have been studied in isolation, or in relation to changes in its interaction with GnRH.

Development

Ubuka *et al.*, (33) analysed GnIH precursor mRNA and the mature peptide during embryonic and post-hatch ages in quail diencephalon. GnIH precursor mRNA expression occurred on embryonic day 10 (E10) and showed a significant increase at E17, just before hatch. GnIH-like-ir neurones were localised in the PVN on E10, but GnIH-ir fibres did not extend to the median eminence until E17 (just before hatch), when the GnIH-ir neurone number in the PVN was also increased. GnIH content of the diencephalon decreased just after hatch and subsequently increased progressively into adulthood. Thus, it appears that GnIH begins to function around

hatch (33). To our knowledge, no developmental study on mammalian GnIH has yet been performed.

Photoperiod

In a photoperiod manipulation experiment on song sparrows, GnIH-containing neurones were found to be larger in birds at the termination of the breeding season compared to at other times (17). These data are consistent with a role for this neuropeptide in the regulation of seasonal breeding, but it is still unclear what that role is. In quail, GnIH expression and synthesis increase under short days, and appear to be directly regulated by melatonin via the Mel 1c receptor expressed on GnIH neurones (34).

GnIH/RFRP is also regulated by changing photoperiod in seasonally-breeding mammals (again by melatonin). Interestingly, the regulation in mammals appears to be in the opposite direction to that of quail (35). The level of GnIH/RFRP mRNA and the number of GnIH/RFRP-immunoreactive cell bodies were reduced in sexually quiescent Syrian and Siberian hamsters on short days compared to sexually active animals maintained under long-days (LD). No evidence for GnIH/RFRP photoperiodic modulation was seen in Wistar rats. Furthermore, pinealectomy prevented the photoperiodic modulation of GnIH/RFRP in hamsters, and melatonin injections of hamsters during long days reduced GnIH/RFRP expression to short-day levels. Thus, in these hamster species, the GnIH/RFRP neurones appear to be modulated via melatonin signalling, as in quail.

By contrast to quail and hamsters, sheep are short-day breeders. Thus, it might be predicted that the expression of GnIH/RFRP in sheep would decrease under short day lengths. In Blackface sheep, the GnIH/RFRP neurone number decreases by 40% during the short-day breeding season (20), but it is not known whether this regulation is melatonin-dependent, although seasonal breeding in this species is strongly influenced by melatonin. In Soay sheep, another melatonin-responsive seasonally-breeding species, GnIH/RFRP expression is also regulated by photoperiod. GnIH/RFRP expression increased overall with exposure to long days, and increased markedly in the ependymal cells surrounding the base of the third ventricle (36).

In summary, there is consistency in birds of mammals in terms of the regulation of GnIH/RFRP by changing photoperiod. There is also consistency in that this neuropeptide appears to be regulated by melatonin in those birds and mammals that have been studied. The lack of agreement between quail (i.e. the only bird species studied in this regard) and the mammalian species studied is in the direction of regulation of GnIH/RFRP by day length. In quail, melatonin increases GnIH expression. In photoperiodic mammals, melatonin appears to decrease GnIH/RFRP expression. In non-photoperiodic rats, there is no change in expression under different day lengths. Thus, GnIH/RFRP expression appears to be linked to seasonal changes in reproductive activity in vertebrates, but the nature of that link varies according to the breeding strategy of the species under investigation. It is possible that this difference may be related to gonadal development (birds) versus onset of cycling/reproductive activity in an already developed gonad (sheep/other mammals).

Fine-tuning of reproductive cyclicity within the breeding season

The first indication that GnIH neurones make putative contact with the GnRH system came from studies on sparrow species and on quail (17, 37). Double-labelled immunocytochemistry (ICC) with light microscopy and fluorescent ICC with confocal microscopy indicated a high probability of co-localisation of GnIH with GnRH-I and -II neurones and GnRH-I fibres within the avian brain. Subsequently, similar contact between GnIH/RFRP axons and GnRH neurones has been identified in several rodent species (3, 4), European starlings (10), sheep (20), horses (Amstalden, M., Williams, G.L. and Bentley, G.E., unpublished data) and rhesus macaque (21). Thus, GnIH–GnRH contact appears to be a consistent feature of the vertebrate neuroendocrine system and is likely to be an evolutionarily conserved trait.

Regulation of GnIH–GnRH contact as a function of season or breeding condition is also prevalent among those species studied. Rufous-winged sparrows breed during the monsoon season in the Sonoran desert. In this species, there are fewer GnIH-ir fibres in the preoptic area of birds caught during the monsoon season versus birds caught just prior to monsoon. These data suggest that GnIH could inhibit GnRH neuronal activity in the POA prior to the monsoon season (27). Sheep (ovariectomised with oestrogen replacement) also experience changes in GnIH–GnRH contact, with fewer contacts in the breeding season (20). It is likely that this dynamic regulation of GnIH–GnRH contact is prevalent throughout vertebrates, although this remains to be confirmed.

Syrian hamsters exhibit changes in GnIH/RFRP across the oestrous cycle, and these changes are most likely mediated by the suprachiasmatic nucleus (24). During the time of the LH surge, GnIH/RFRP activity is reduced and this reduction of activity is: (i) oestrogen-dependent and (ii) in anti-phase with activity of the GnRH system. Thus, there is circadian control of the GnIH/RFRP system in Syrian hamsters. As GnIH reduces LH in this species, and there are contacts of GnIH axons with GnRH neurones, it is thought that temporal regulation of the GnIH/RFRP system is necessary for removal of inhibitory input to the reproductive system and normal ovulatory function (24).

Stress can have a negative impact upon the reproductive system (38–41). In a capture-handling stress experiment, a significant increase in GnIH-immunoreactive neurones occurred in stressed birds at the onset of the breeding season (42). At the end of the breeding season, there were more GnIH-immunoreactive neurones compared to the spring, and there was no increase in response to stress. These data indicate that: (i) the GnIH system of house sparrows is responsive to stress at the start of the breeding season and (ii) there is seasonal regulation of the GnIH system in this species. The GnIH stress response observed at the beginning of the breeding season may reflect a mechanism by which reproduction is slowed or halted in stressful conditions. The involvement of GnIH would allow for rapid changes in behaviour without the need for long-term inactivation of the reproductive axis (GnRH system), as occurs at the end of the breeding season. There is also some evidence for stress effects upon the GnIH/RFRP system in rats (43), but it

remains to be determined whether this is a universal vertebrate trait.

Summary

In summary, there have been rapid advances in our understanding of GnIH and its mammalian counterpart subsequent to the initial discovery of this novel neuropeptide in 2000 (1). There appears to be general agreement that GnIH acts as a bona fide negative regulator of the reproductive system. The levels of action of GnIH (brain, pituitary, gonads) are generally conserved across the two vertebrate classes that have been studied, and within the mammalian class from rodents to primates. There are some data, however, that do not fit within these generalisations. Those anomalies, along with the data that can be categorised as generalisations, deserve further exploration if we are to understand the origins, functions and regulation of this multi-functional neuropeptide in more detail.

Acknowledgements

Grant support: NSF IOS 0641188 to G.E.B.), Hellmann Family Foundation Fund (to G.E.B.), UC Berkeley COR Junior Faculty Research Grant (to G.E.B.); and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (15207007, 16086206 and 18107002 to K. T.).

Received: 22 July 2008,
revised 29 December 2008,
accepted 29 January 2009

References

1 Tsutsui K, Saigoh E, Ukena K, Teranishi H, Fujisawa Y, Kikuchi M, Ishii S, Sharp PJ. A novel avian hypothalamic peptide inhibiting gonadotropin release. *Biochem Biophys Res Commun* 2000; **275**: 661–667.

2 Osugi T, Ukena K, Bentley GE, O'Brien S, Moore IT, Wingfield JC, Tsutsui K. Gonadotropin-inhibitory hormone in Gambel's white-crowned sparrows: cDNA identification, transcript localization and functional effects in laboratory and field experiments. *J Endocrinol* 2004; **182**: 33–42.

3 Kriegsfeld LJ, Mei DF, Bentley GE, Ubuka T, Mason AA, Inoue K, Ukena K, Tsutsui K, Silver R. Identification and characterization of a gonadotropin-inhibitory system in the brain of mammals. *Proc Natl Acad Sci USA* 2006; **103**: 2410–2415.

4 Johnson MA, Tsutsui K, Fraley GS. Rat RFamide-related peptide-3 stimulates GH secretion, inhibits LH secretion, and has variable effects on sex behavior in the adult male rat. *Horm Behav* 2007; **51**: 171–180.

5 Murakami M, Matsuzaki T, Iwasa T, Yasui T, Irahara M, Osugi T, Tsutsui K. Hypophysiotropic role of RFamide-related peptide-3 in the inhibition of LH secretion in female rats. *J Endocrinol* 2008; **199**: 105–112.

6 Clarke IJ, Sari IP, Qi Y, Smith JT, Parkington HC, Ubuka T, Iqbal J, Li Q, Tilbrook A, Morgan K, Pawson AJ, Tsutsui K, Millar RP, Bentley GE. Potent action of RFRP-3 on pituitary gonadotropes indicative of an hypophysiotropic role in the negative regulation of gonadotropin secretion. *Endocrinology* 2008; **149**: 5811–5821.

7 Ubuka T, Ukena K, Sharp PJ, Bentley GE, Tsutsui K. Gonadotropin-inhibitory hormone inhibits gonadal development and maintenance by decreasing gonadotropin synthesis and release in male quail. *Endocrinology* 2006; **147**: 1187–1194.

8 Tsutsui K, Saigoh E, Yin H, Ubuka T, Chowdhury VS, Osugi T, Ukena K, Sharp PJ, Wingfield JC, Bentley GE. A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH) in birds: discovery, progress and prospect. *J Neuroendocrinol* 2009; **21**: 271–275.

9 Satake H, Hisada M, Kawada T, Minakata H, Ukena K, Tsutsui K. Characterization of a cDNA encoding a novel avian hypothalamic neuropeptide exerting an inhibitory effect on gonadotropin release. *Biochem J* 2001; **354**: 379–385.

10 Ubuka T, Kim S, Huang YC, Reid J, Jiang J, Osugi T, Chowdhury VS, Tsutsui K, Bentley GE. Gonadotropin-inhibitory hormone neurons interact directly with gonadotropin-releasing hormone-I and -II neurons in European starling brain. *Endocrinology* 2008; **149**: 268–278.

11 Ubuka T, Binns M, Cadigan PA, Osugi T, Chowdhury VS, Tsutsui K, Bentley GE. Identification of gonadotropin-inhibitory hormone in human brain. Abstract 658.17 presented at the 36th Annual Meeting for the Society for Neuroscience Atlanta, GA: Society for Neuroscience, Washington, DC, 2006

12 Hinuma S, Shintani Y, Fukusumi S, Iijima N, Matsumoto Y, Hosoya M, Fujii R, Watanabe T, Kikuchi K, Terao Y, Yano T, Yamamoto T, Kawamata Y, Habata Y, Asada M, Kitada C, Kurokawa T, Onda H, Nishimura O, Tanaka M, Ibata Y, Fujino M. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. *Nat Cell Biol* 2000; **2**: 703–708.

13 Ukena K, Iwakoshi E, Minakata H, Tsutsui K. A novel rat hypothalamic RFamide-related peptide identified by immunoaffinity chromatography and mass spectrometry. *FEBS Lett* 2002; **512**: 255–258.

14 Ubuka T, McGuire NL, Calisi RM, Perfito N, Bentley GE. The control of reproductive physiology and behavior by gonadotropin-inhibitory hormone. *Integr Comp Biol* 2008; **48**: 560–569.

15 Yin H, Ukena K, Ubuka T, Tsutsui K. A novel G protein-coupled receptor for gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*): identification, expression and binding activity. *J Endocrinol* 2005; **184**: 257–266.

16 Fukusumi S, Fujii R, Hinuma S. Recent advances in mammalian RFamide peptides: the discovery and functional analyses of PrRP, RFRPs and QRFP. *Peptides* 2006; **27**: 1073–1086.

17 Bentley GE, Perfito N, Ukena K, Tsutsui K, Wingfield JC. Gonadotropin-inhibitory peptide in song sparrows (*Melospiza melodia*) in different reproductive conditions, and in house sparrows (*Passer domesticus*) relative to chicken gonadotropin-releasing hormone. *J Neuroendocrinol* 2003; **15**: 794–802.

18 Bentley GE, Jensen JP, Kaur GJ, Wacker DW, Tsutsui K, Wingfield JC. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). *Horm Behav* 2006; **49**: 550–555.

19 Ubuka T, Bentley GE. *Taeniopygia guttata* gonadotropin-releasing hormone precursor mRNA, complete cds. 2008; Genbank Accession 1145626.

20 Smith JT, Coolen LM, Kriegsfeld LJ, Sari IP, Jaafarzadehshirazi MR, Maltby M, Bateman K, Goodman RL, Tilbrook AJ, Ubuka T, Bentley GE, Clarke IJ, Lehman MN. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. *Endocrinology* 2008; **149**: 5770–5782.

21 Ubuka T, Lai H, Kitani M, Suzuuchi A, Pham V, Cadigan PA, Chowdhury VS, Tsutsui K, Bentley GE. Gonadotropin-inhibitory hormone neurons in rhesus macaque brain. *J Comp Neurol* 2009; unpublished data.

22 Maney DL, Richardson RD, Wingfield JC. Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behavior in a female sparrow. *Horm Behav* 1997; **32**: 11–18.

23 Maddineni S, Ocón-Grove OM, Krzysik-Walker SM, Hendricks GL 3rd, Proudman JA, Ramachandran R. Gonadotrophin-inhibitory hormone receptor expression in the chicken pituitary gland: potential influence of sexual maturation and ovarian steroids. *J Neuroendocrinol* 2008; **20**: 1078–1088.

24 Gibson EM, Humber SA, Jain S, Williams WP 3rd, Zhao S, Bentley GE, Tsutsui K, Kriegsfeld LJ. Alterations in RFamide-related peptide expression are coordinated with the preovulatory luteinizing hormone surge. *Endocrinology* 2008; **149**: 4958–4969.

25 Ciccone NA, Dunn IC, Boswell T, Tsutsui K, Ubuka T, Ukena K, Sharp PJ. Gonadotrophin inhibitory hormone depresses gonadotrophin α and follicle-stimulating hormone β subunit expression in the pituitary of the domestic chicken. *J Neuroendocrinol* 2004; **16**: 999–1006.

26 Small TW, Sharp PJ, Bentley GE, Millar RP, Tsutsui K, Mura E, Deviche P. Photoperiod-independent hypothalamic regulation of luteinizing hormone secretion in a free-living Sonoran desert bird, the Rufous-winged Sparrow (*Aimophila carpalis*). *Brain Behav Evol* 2008; **71**: 127–142.

27 Deviche P, Small T, Sharp P, Tsutsui K. Control of luteinizing hormone and testosterone secretion in a flexibly breeding male passerine, the rufous-winged Sparrow, *Aimophila carpalis*. *Gen Comp Endocrinol* 2006; **149**: 226–235.

28 Bentley GE, Ubuka T, McGuire NL, Chowdhury VS, Morita Y, Yano T, Hasunuma I, Binns M, Wingfield JC, Tsutsui K. Gonadotropin-inhibitory hormone and its receptor in the avian reproductive system. *Gen Comp Endocrinol* 2008; **156**: 34–43.

29 Maddineni SR, Ocón-Grove OM, Krzysik-Walker SM, Hendricks GL 3rd, Ramachandran R. Gonadotropin-inhibitory hormone (GnIH) receptor gene is expressed in the chicken ovary: potential role of GnIH in follicular maturation. *Reproduction* 2008; **135**: 267–274.

30 Zhao S, Bentley GE, Tsutsui K, Kriegsfeld LJ. Local regulation of spermatogenesis by gonadotropin-inhibitory hormone. Abstract 831.9 presented at the 37th Annual Meeting for the Society for Neuroscience, San Diego, CA: Society for Neuroscience, Washington, DC, 2007.

31 McGuire NL, Ubuka T, Kangas K, Bentley GE. A novel neuropeptide system within primate gonads: RFRP and RFRPR in rhesus macaque (*Macaca mulatta*) testis and ovary. 2009; unpublished data. submitted.

32 Singh P, Krishna A, Sridaran R, Tsutsui K. Changes in GnRH I, bradykinin and their receptors and GnIH in the ovary of Calotes versicolor during reproductive cycle *Gen Comp Endocrinol* 2008; **159**: 158–69.

33 Ubuka T, Ueno M, Ukena K, Tsutsui K. Developmental changes in gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*) hypothalamo-hypophysial system. *J Endocrinol* 2003; **178**: 311–318.

34 Ubuka T, Bentley GE, Ukena K, Wingfield JC, Tsutsui K. Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. *Proc Natl Acad Sci USA* 2005; **102**: 3052–3057.

35 Revel FG, Saboureau M, Pévet P, Simonneaux V, Mikkelsen JD. RFamide-related peptide gene is a melatonin-driven photoperiodic gene. *Endocrinology* 2008; **149**: 902–912.

36 Dardente H, Birnie M, Lincoln GA, Hazlerigg DG. RFamide-related peptide and its cognate receptor in the sheep: cDNA cloning, mRNA distribution in the hypothalamus and effect of photoperiod. *J Neuroendocrinol* 2008: **20**: 1252–1259.

37 Ukena K, Ubuka T, Tsutsui K. Distribution of a novel avian gonadotropin-inhibitory hormone in the quail brain. *Cell Tissue Res* 2003; **312**: 73–79.

38 Siegel HS. Physiological stress in birds. *Bioscience* 1980; **30**: 529–534.

39 Moberg GP. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. *J Dairy Sci* 1991; **74**: 304–311.

40 Moore IT, Jessop TS. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm Behav* 2003; **43**: 39–47.

41 Boonstra R. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integr Comp Biol* 2004; **44**: 95–108.

42 Calisi RM, Rizzo NO, Bentley GE. Seasonal differences in hypothalamic EGR-1 and GnIH expression following capture-handling stress in house sparrows (*Passer domesticus*). *Gen Comp Endocrinol* 2008; **157**: 283–287.

43 Kirby ED, Ubuka T, Kaufer D, Bentley GE. Acute immobilization stress increases putative gonadotropin inhibitory hormone expression in the rat hypothalamus. Abstract 831.7 presented at the 37th Annual Meeting for the Society for Neuroscience, San Diego, CA: Society for Neuroscience, Washington, DC, 2007.