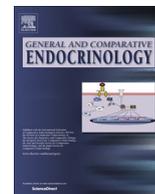




Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Putting the brakes on reproduction: Implications for conservation, global climate change and biomedicine

John C. Wingfield^{a,*}, Nicole Perfito^b, Rebecca Calisi^c, George Bentley^b, T. Ubuka^d, M. Mukai^e, Sara O'Brien^f, K. Tsutsui^d

^a Department of Neurobiology, Physiology and Behavior, University of California, One Shields Avenue, Davis, CA 95616, USA

^b Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

^c Department of Biology, Barnard College, Columbia University, New York, NY 10027, USA

^d Department of Biology, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan

^e Department of Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA

^f Department of Biology, Radford University, Radford, VA 24142, USA

ARTICLE INFO

Article history:

Received 28 May 2015

Accepted 8 October 2015

Available online xxxxx

Keywords:

Reproduction

Birds

Gonadotropins

Gonadotropin-releasing hormone

Gonadotropin-inhibitory hormone

Environmental signals

ABSTRACT

Seasonal breeding is widespread in vertebrates and involves sequential development of the gonads, onset of breeding activities (e.g. cycling in females) and then termination resulting in regression of the reproductive system. Whereas males generally show complete spermatogenesis prior to and after onset of breeding, females of many vertebrate species show only partial ovarian development and may delay onset of cycling (e.g. estrous), yolk deposition or germinal vesicle breakdown until conditions conducive for ovulation and onset of breeding are favorable. Regulation of this “brake” on the onset of breeding remains relatively unknown, but could have profound implications for conservation efforts and for “mismatches” of breeding in relation to global climate change. Using avian models it is proposed that a brain peptide, gonadotropin-inhibitory hormone (GnIH), may be the brake to prevent onset of breeding in females. Evidence to date suggests that although GnIH may be involved in the regulation of gonadal development and regression, it plays more regulatory roles in the process of final ovarian development leading to ovulation, transitions from sexual to parental behavior and suppression of reproductive function by environmental stress. Accumulating experimental evidence strongly suggests that GnIH inhibits actions of gonadotropin-releasing hormones on behavior (central effects), gonadotropin secretion (central and hypophysiotropic effects), and has direct actions in the gonad to inhibit steroidogenesis. Thus, actual onset of breeding activities leading to ovulation may involve environmental cues releasing an inhibition (brake) on the hypothalamo-pituitary-gonad axis.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Understanding the regulation of reproductive function is fundamental not only for biomedicine but also for agriculture, aquaculture, conservation and sustainability of natural bio-resources for harvest. In an age of burgeoning global change, elucidating the mechanisms underlying how organisms respond to environmental cues and regulate reproductive function become more and more important. To do this, it is essential that the reproductive process be analyzed at functional levels so that critical experiments can be designed. In this way we can gain insight into how environmental signals are perceived and then transduced through neural and

neuroendocrine pathways into morphological, physiological and behavioral responses that maximize reproductive success (Visser, 2008; Wingfield, 2008a,b, 2012). Of particular importance is the integration of neuroendocrine and endocrine mechanisms with ecology and evolutionary biology to place neural pathways for environmental signals in context (e.g. MacDougall-Shackleton et al., 2009; Visser et al., 2010). As climate changes and phenology of migrations, breeding seasons, food supply, temperature, precipitation etc. shift, often in different ways, there is an increasing awareness that phenological mismatches are occurring more and frequently. For example, the great tit, *Parus major*, depends upon oak (*Quercus* sp) caterpillars in spring to feed young. The timing of caterpillar emergence in northern Europe is changing and some populations, but not all, of the great tit still breed at the same time resulting in a mismatch of environmental phenology and timing of

* Corresponding author.

E-mail address: jcwingfield@ucdavis.edu (J.C. Wingfield).

breeding (e.g. Visser et al., 2012). Why does this mismatch occur in some populations and not others? The great tits showing a mismatch appear to be unable to respond to changed environmental signals and no longer time egg-laying, and thus the hatching of chicks, to coincide with the most favorable food supply.

Other investigations have shown variation in responsiveness to local predictive cues in terms of gonadal development. Lowland populations of song sparrows, *Melospiza melodia morphna*, are less sensitive to temperature cues affecting photoperiodically-induced gonadal growth than are high altitude populations (Perfito et al., 2005). In other species visual and tactile cues relating to food availability can enhance gonadal development (e.g. Hau et al., 2000; O'Brien and Hau, 2005; Furlonger et al., 2012) indicating that responsiveness, or not, to local environmental cues varies across populations and in some could lead to mismatches of food supply and reproductive function. What components of the “perception–transduction–response” system (Wingfield and Mukai, 2009) fail to adjust to changed phenology?

To answer these important and urgent questions will require careful analysis of phenology and the neural pathways for environmental signals in context (Visser et al., 2010). This review focuses primarily on songbird species, such as the white-crowned sparrow, *Zonotrichia leucophrys*, song sparrow, *Melospiza melodia*, and European starling, *Sturnus vulgaris*. They have been studied in considerable detail both from the perspective of their ecology and behavior under natural conditions and the neuroendocrinology and endocrinology of reproductive function. Such approaches have the potential to allow us to predict which populations will be able to cope with environmental change and which will not. Conservation efforts could thus be focused on the latter.

2. Environmental and endocrine regulation of the reproductive system

At mechanistic levels it is crucial to bear in mind that reproduction involves ontogenetic processes resulting in initial differentiation of the gonads and establishing sex (Crews and Moore, 1986; Wingfield, 2008a,b) followed by development of the reproductive system at puberty, and subsequently in seasonally breeding species, on a yearly basis. The ontogenetic development leading to initial establishment of male or female sex in Aves is not repeatable whereas annual development of the reproductive system in seasonal species is repeatable. This suggests fundamentally different regulatory mechanisms for these different events. However, developing a functional gonad is not the end point of the reproductive process, especially in females of tetrapods. For example, in avian species development of the gonad results in “mature capability” meaning that the individual is then capable of initiating a breeding attempt resulting in ovulation, copulation and in many species, raising of young. Moreover, in species that breed seasonally or periodically, reproductive function is terminated at the end of the breeding season and the gonads show morphological and physiological regression to varying degrees. Reproductive behaviors also frequently decline (Wingfield, 2008a).

A schematic diagram of the organization of the reproductive process in seasonally breeding birds and for many mammals is given in Fig. 1 (see Wingfield, 2006). Note the three phases of reproduction: development, mature capability (actual cycling of breeding attempts) and termination. Each phase may be regulated differently. The mature capability phase is perhaps the most complex because this involves onset of ovarian cycling in females leading to ovulation and mating (Fig. 1). In many avian and mammalian species there follows a period of parental care which can then lead to second breeding attempts, or if the nest and young are lost to predators, a replacement breeding attempt can be initiated

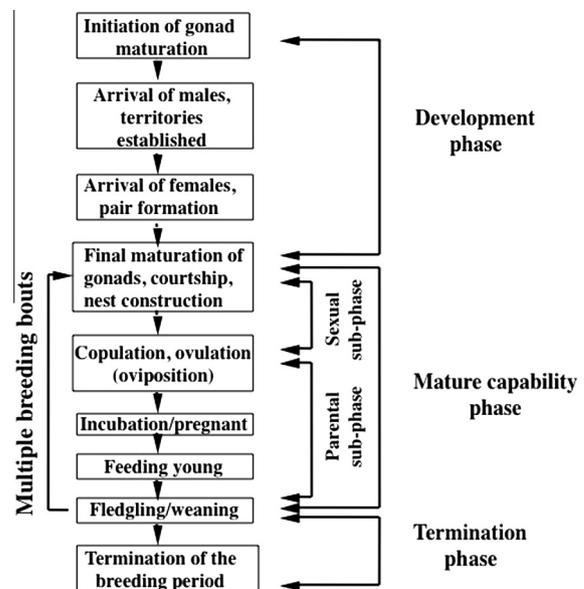


Fig. 1. The reproduction life history stage is made up of three distinct phases. First the development phase in which the gonads reach maturity and the animal is capable of reproduction. Second is the phase when actual onset of reproduction can take place. This may involve onset of estrus in mammals or yolk deposition leading to ovulation in egg-laying vertebrates. Parental care can be included in this phase. The third phase is termination of reproduction when cycling in females ceases and in many seasonally breeding animals the gonads regress. From Wingfield (2006), courtesy of Elsevier.

(Fig. 1). Note that mature capability is entirely dependent upon the reproductive system being in a mature state so these nesting attempts – sexual and parental sub-phases – can be initiated (Fig. 1). There are some exceptions to this rule in mammals and other vertebrates (Crews and Moore, 1986), but this tends to be more uniformly so in birds.

We can now predict that multiple cues from the physical and social environments will regulate complex transitions between sexual and parental sub-phases of mature capability (Fig. 1). When reproductive development is complete and the individual has mature capability, onset of breeding does not necessarily occur immediately. In many species females are able to place a “brake” on final maturation leading to ovulation and subsequently the parental phase, until favorable cues from the physical and social environments release that brake. If those favorable cues do not occur then females can maintain the brake on onset of breeding indefinitely, or until the termination phase of reproductive function sets in. Therefore, it is possible that because of climate change, favorable conditions may not occur in some years, or the individual is no longer matched with those favorable conditions and females may even forego breeding for that year (e.g. Visser et al., 2010, 2012).

What environmental cues are used to regulate, and time, reproductive function? The literature is replete with hundreds, if not thousands, of reports indicating factors from physical and social environments that can influence the reproductive process. Firstly, initial predictive cues such as the seasonal change in day length are very reliable environmental cues that trigger reproductive development, sustain mature capability through the breeding season and then terminate reproductive function usually through gonadal regression (Fig. 2, see Dawson et al., 2001; Bentley et al., 2007; Yoshimura, 2004; Wingfield and Silverin, 2002, 2009). Then there are local predictive cues (supplementary factors) that speed up or slow down effects of initial predictive cues so that mature capability and onset of breeding can be timed accurately. Local

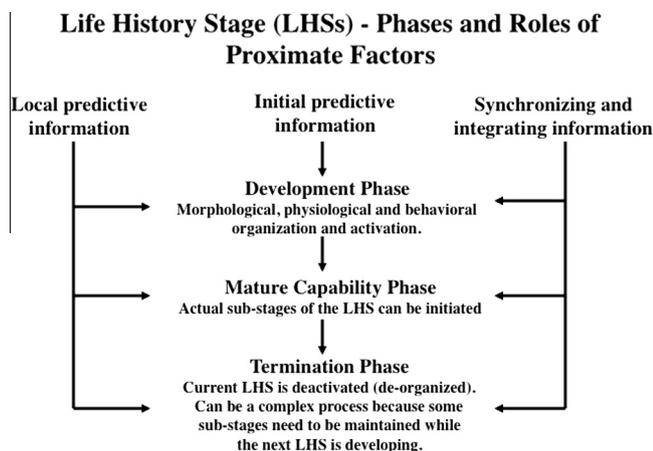


Fig. 2. Different types of environmental signals from the physical environment and from social interactions can influence all three phases of the reproduction life history stage. Initial predictive information initiates gonadal development, maintains reproductive capacity and then terminates the breeding season. Local predictive and synchronizing and integrating information can influence all three phases too. Thus knowing phase of the reproduction life history stage will be an important determinant of how, for example, a given behavioral interactions will affect neuroendocrine and endocrine mechanisms. From Wingfield (2006), courtesy of Elsevier.

predictive cues may also act on the termination phase (Fig. 2, Wingfield, 2006). Social cues serve to synchronize individual reproductive function, such as between mates, and integrate changes in sexual and parental sub-phases such as transition from sexual to parental behavior and the reverse for subsequent breeding attempts (Fig. 2). Note that all of these types of environmental cues may have different mechanisms of action (perception, transduction, response, Wingfield, 2008b, 2012; Wingfield and Mukai, 2009) in each phase of the reproductive life history stage (Fig. 2). It should also be noted that a fourth type of environmental cue, labile perturbation factors also known as modifying factors, include the effects of unpredictable events such as loss of the young or mates to predators and severe weather that disrupts reproductive function (Wingfield et al., 1999; Wingfield and Silverin, 2002, 2009). Individuals responding to perturbation factors interrupt the sexual and parental sub-phases and re-instate the “brake” on breeding until the perturbation passes. If environmental conditions become conducive to onset of breeding once more then the brake is released and a re-nest attempt is initiated (Wingfield and Farner, 1979; Wingfield and Silverin, 2002, 2009).

Overall, field and laboratory investigations suggest that individuals do indeed hold in the mature capability phase of the reproductive system until environmental conditions are favorable for onset of final maturation of the ovary, ovulation and transition to the parental phase, but what is the evidence for a “brake” on onset of breeding?

3. Evidence for a “brake on onset of breeding (reproductive cycling)

Finite-state machine theory points out that all adult life history stages have development, mature capability and termination phases (Jacobs and Wingfield, 2000, 2008a). This indicates that the timing of reproductive development and then onset of actual cycling (breeding) are intimately related but regulated differently. For example, ovarian development in free-living female white-crowned sparrows, *Zonotrichia leucophrys gambelii*, progresses as day length increases in spring (Fig. 3). This reproductive baseline where the ovary reaches mature capability with selected follicles

up to 2 mm in diameter, may be held indefinitely until conditions conducive to breeding then result in final maturation of the ovary (yolking) leading to ovulation and onset of nesting (Figs. 3 and 4). Note that light shading indicates where final maturation of the ovary accompanied by surges of plasma luteinizing hormone (LH) and estradiol levels occurs (Fig. 3). After egg-laying, the ovary and plasma LH and estradiol, return to the breeding baseline (Fig. 3) suggesting that a brake has been reset. Further surges of ovarian final maturation can occur in subsequent broods or after loss of clutch or brood to a perturbation factors (Wingfield and Farner, 1978, 1979, 1993, see Fig. 4). In contrast, testicular development in males progresses to mature capability and remains much more uniform than in females (Fig. 3). In these males the brake is not so much on testicular development but on surges of plasma LH and testosterone that occur in response to sexual receptivity of females and interactions with other males (e.g. Wingfield et al., 1990, 1999).

This brake on final ovarian development can apparently be modified by experience. Female song sparrows, *M. m. melodia*, captured from the wild and then photostimulated to induce ovarian development, do not enter the final maturation of ovarian follicles or ovulate in captivity. The ovary remains in mature capability until they turn photorefractory and the reproductive system involutes. However, if they are taken through a second bout of photostimulation many of these females then ovulate suggesting that there is some form of adjustment to captive conditions allowing release of the brake on onset of breeding in subsequent reproductive cycles (J.C. Wingfield, unpublished). Furthermore, female song sparrows captured as juveniles and raised in captive conditions will also complete final maturation of the ovary when photostimulated (Fig. 5) suggesting that some form of environmental “conditioning” is occurring so that local cues can regulate final onset of breeding. Similar results in white-crowned sparrows show that hand raised females lay eggs in captivity compared with wild caught females (Baptista and Petrinovich, 1986). Males do not show these differences in testicular development with experience of captivity but plasma levels of LH and testosterone may be affected (Wingfield et al., 1990).

It is entirely possible that visual information is an important cue, or set of cues, that females use to regulate final ovarian development, but what is the evidence for a brake? Bilaterally enucleated female white-crowned sparrows show enhanced ovarian development when photostimulated (Yokoyama and Farner, 1976). Indeed many females showed large yolky follicles and plasma levels of LH were elevated over uni-laterally enucleated and intact females (Yokoyama and Farner, 1976, Fig. 6). It was suggested that visual information may impart a tonic inhibition (i.e. a brake) on final maturation of the ovary, and visual cues conducive to onset of nesting then release that inhibition (brake). Disruption of this visual input results in release of the inhibition and a spontaneous increase in gonadotropins and eventually full development of ovarian follicles to near ovulation (Yokoyama and Farner, 1976). These authors were also careful to point out that because the photoreceptors for photoperiodic effects on reproductive state are in the hypothalamus, not the retina, it is possible that exposure of that part of the brain by enucleation may have resulted in hyper-photostimulation and may not be indicative of inhibition by visual information.

To resolve the potential issue of hyper-photostimulation we glued small plastic eye caps to the rim of the eye socket of song and white-crowned sparrows by using Skinbond. First birds were anesthetized with isoflurane and the small feathers of the eye ring removed. Plastic eye caps tailored to fit over the eye ring were then glued in place. In some experiments birds with no caps or with just a rim of plastic were used as controls (i.e. no impairment of vision, $n = 6$). In another experiment we used complete caps with clear

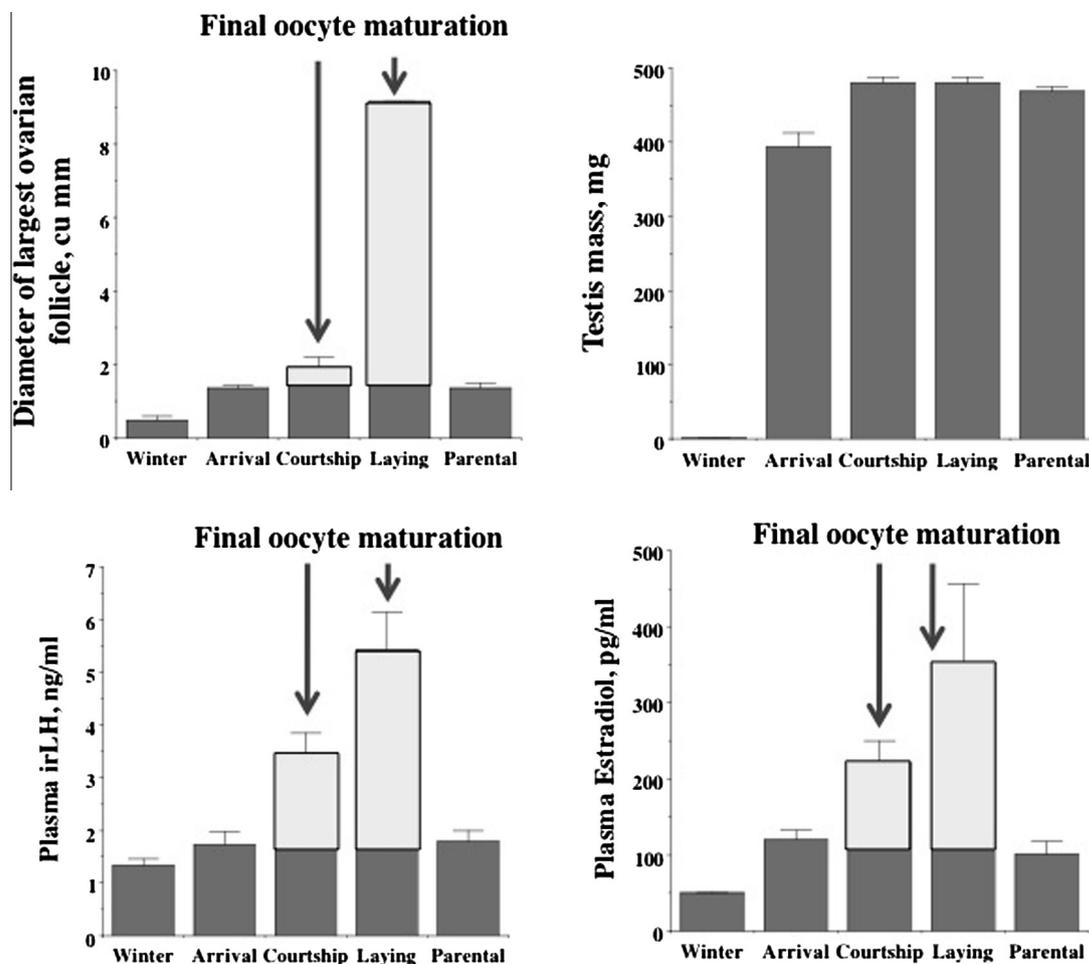


Fig. 3. Ovarian and testicular development, and plasma levels of immunoreactive luteinizing hormone (irLH) and estradiol throughout the year in free-living white-crowned sparrows, *Zonotrichia leucophrys gambelii*. The pale bars represent additional increases in ovarian follicle that occur during onset of nesting. These increases are primarily incorporation of yolk into follicle prior to ovulation. This is the "final oocyte maturation" sub-stage and is also accompanied by further increases in irLH and estradiol. Note that in males, testis size remains relatively stable after arrival and throughout the rest of the breeding season. Redrawn from Wingfield and Farner (1978).

plastic that also allowed full visual input ($n = 6$, Fig. 7). Experimental groups were given plastic eye caps painted black and after gluing to the eye rim were coated with black neoprene glue to reduce scratching and effectively block all light entering the eye ($n = 7$, Fig. 7). Test of their ability to detect shadows revealed that they were effectively blind. Nonetheless, several water bowls and dishes of food were provided so they could easily eat and drink. A third group of birds received opaque plastic eye caps that had been fogged so that light could enter the eye but the bird could not resolve images ($n = 7$, Fig. 7). Again these birds had copious food and water to enable them to eat and drink.

The eye caps were placed after about 20–30 days of photostimulation when ovarian development was nearing mature capability. By the end of the experiment, volumes of the largest developing follicle showed similar ranges from 0.06 to 14.10 cubic mm in both western song sparrows, *M. m. morphna* and white-crowned sparrows, and from 0.06 to 65.40 cubic mm in eastern song sparrows. In both subspecies of song sparrow, follicles tended to be larger in females wearing black caps relative to clear-capped controls (Fig. 8). However, this difference was significant only in western song sparrows, ($H(2) = 6.17$, $P = 0.046$; *M. m. melodia* $H(2) = 4.81$, $P = 0.09$; Fig. 8). Western song sparrow females wearing black caps had significantly larger follicles than birds wearing control caps ($P < 0.05$, Dunn's Method; Fig. 8). Follicle volume in white-crowned sparrows did not differ among treatment groups ($H(2)$

$= 2.99$, $P = 0.22$; Fig. 8). The final maturation of follicles before ovulation includes the deposition of yolk that is visible during laparotomy. None of the females wearing clear caps began to deposit yolk in follicles in any of the three groups, but yolk deposition did begin in females wearing fogged and black caps. Two white-crowned sparrows (one each with fogged and black caps), four eastern song sparrows (two each with fogged and black caps), and four western song sparrows (one fogged and 3 black cap) females had visible yolk in the largest developing follicle (Fisher's exact test, $P = 0.011$, N. Perfito and J.C. Wingfield, unpublished).

At present we cannot explain why Yokoyama and Farner (1976) showed an effect of enucleation on final ovarian maturation in *Z. l. gambelii* whereas our capping experiments on the same subspecies showed only a trend for ovarian follicle development and no effect on plasma LH (Fig. 8). This may be because enucleation does indeed result in hyper-photostimulation, although inhibitory actions of visual information could still be acting. In song sparrows also there were no consistent differences in LH concentration through capping of the eyes (Fig. 8). In *M. m. melodia* there was a trend for higher LH concentration in black-capped birds than in controls, but these differences were not significant (Fig. 8). Since LH acts primarily to induce sex steroid production in the gonad, increased follicular development could occur without changes in LH levels. It is possible that FSH secretion was increased with this manipulation, and is probably a more likely candidate to regulate

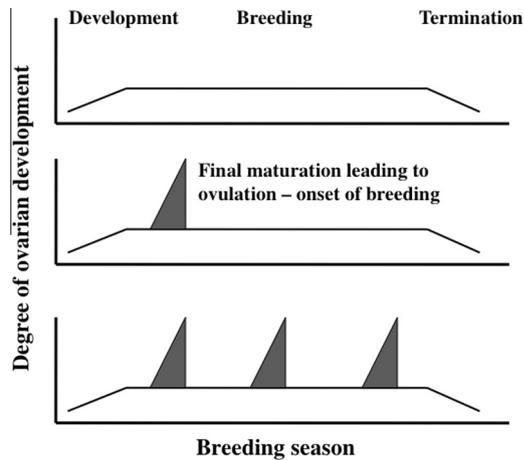


Fig. 4. Schematic diagram showing how development of the ovarian follicles reaches mature capability and then holds for the entire breeding season until termination results in regression of the ovary to a non-breeding state (top panel). The dark triangle indicates when onset of nesting is triggered accompanied by a further increase in ovarian follicle development leading to ovulation (mid panel). Note that after the clutch of eggs is laid, ovarian development returns to that typical of mature capability. The bottom panel shows that this final maturation of ovarian follicles leading to ovulation can occur at any time appropriate during the mature capability phase. This might be production of a second or third clutch after successfully raising young in a previous brood, or could be a re-nesting attempt after an earlier nest was lost to a predator (from Wingfield and Farner (1978, 1979, 1993)).

differences in follicular growth. But the results overall are encouraging, especially in song sparrows, that further exploration of this phenomenon is warranted.

The variation among the responses of species in these capping experiments can be explained in part using the paradigm suggested by Wingfield et al. (1992, 1993). Both sub-species of song sparrow breed at mid-latitudes where spring breeding conditions vary from year to year and onset of laying can vary by weeks or months (Nice, 1937; Wingfield et al., 1992). Under these conditions, individuals should be sensitive to a variety of environmental cues in addition to photoperiod to time breeding. Our data on song

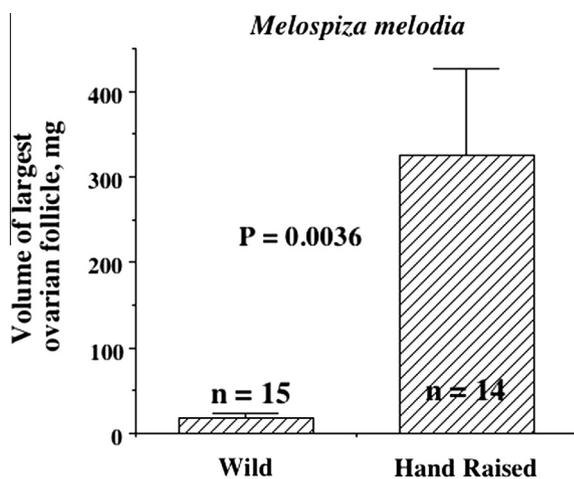


Fig. 5. Volume of the largest ovarian follicle in wild female song sparrows, *Melospiza melodia*, photostimulated in captivity versus females that were hand-raised in captivity and kept over winter in cages prior to photostimulation. Hand raised females showed much larger follicles with yolk suggesting final maturation of follicles had begun. Wild caught females progressed only to the pre-yolk deposition phase of follicular growth suggesting that there is a brake on final maturation. Difference is significant, Mann-Whitney *U* test. J.C. Wingfield, unpublished.

sparrows are consistent with these paradigms, and further suggest that local predictive cues may be perceived in part by the visual system. In contrast, *Z. l. gambelii* breeds at high latitudes in the Arctic where the breeding season is much more rigidly timed. Visual local predictive cues may be less important for final maturation of the ovary and females rely primarily on photoperiodic cues.

Because hyper-photostimulation is not an issue in the eye-capping experiment (eyes are intact) these data are consistent with visual information imparting constant inhibitory signals to the hypothalamo-pituitary-gonad axis (HPG-axis) which can be released by disruption of this system (eye capping) or when the correct visual cues are perceived. Under natural conditions in the field, female songbirds have the ability to interrupt follicular development until favorable breeding conditions arrive and then quickly begin yolk deposition and egg-laying. (King et al., 1966; Kern and King, 1972; Silverin et al., 1989). Individuals that are able to use visual information (e.g. plant flowering, insect abundance, or snow melt) in advance of changes in food intake would perhaps be at an advantage. Hau et al. (2000) have shown that purely visual components of live insect food stimulate singing behavior in male spotted antbirds, *Hylophylax n. naevioides*, before gonadal volume increases. Further, red crossbill, *Loxia curvirostra*, males dramatically increased gonadal volume with perceived increase in food abundance above males continuously fed ad libitum (Hahn, 1995). Similar results were obtained feeding standard pellet diet or supplemented with preferred seeds to American goldfinches, *Spinus tristis*, resulting in greater development of ovarian follicles (Furlonger et al., 2012). These findings indicate that visual input is stimulatory to gonadal development, presumably by removing inhibitory cues present in the laboratory setting. Are there any candidates for neural or neuroendocrine factors that might provide such inhibition?

4. A candidate for the brake: inhibition of onset of breeding

In vertebrates there are several forms of GnRH in the hypothalamus that regulate gonadotropin release from the anterior pituitary (hypophysiotropic effects) or act centrally on reproductive behavior. Two other peptides, kisspeptin and gonadotropin-inhibitory hormone (GnIH), act respectively to increase or decrease efficacy of GnRH (Tsutsui et al., 2000, 2010b; Parhar et al., 2012).

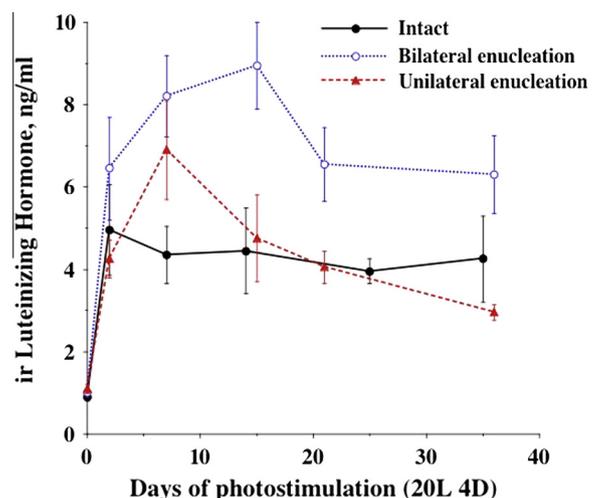


Fig. 6. Visual information may release an inhibition on gonadotropin release in female white-crowned sparrows. Changes in plasma concentrations of LH of unilateral enucleates (red line, *N* = 5), bilateral enucleates (blue line, *N* = 8), and intact females (black line, *N* = 8). Redrawn from Yokoyama and Farner (1976) with permission. Courtesy of Elsevier.

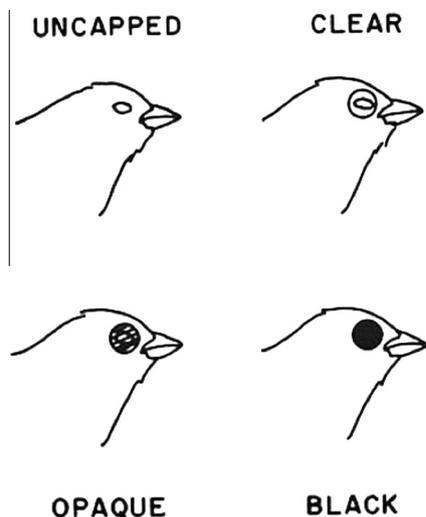


Fig. 7. We manipulated vision by gluing small plastic caps (kindly provided by Prof. Kenneth Able, SUNY, Albany) over both eyes. The feathers were first removed around the eyes and then caps were fitted and secured with Skinbond or Nexaband (veterinary glue (Veterinary Products Laboratories). Neoprene glue (McNett Corp.) applied to the circumference of the caps further fixed the caps in place and prevented birds from loosening caps with their claws. Birds acclimated first to one eye cap, followed the next day to both eyes capped. Caps were checked regularly. The three treatment groups consisted of birds with: (1) black caps that blocked all visual stimuli, (2) fogged caps that allowed light to penetrate, but prevented resolution of images, and (3) clear caps that allowed normal vision (controls). Control caps worn by white-crowned sparrows and western song sparrows were small plastic rings that were the same diameter and glued in the same way as experimental caps, but did not cover the eyes. Eastern song sparrow controls wore caps that were identical to experimental caps, but were clear. Un-manipulated males were kept in individual cages in the same room with female white-crowned sparrows and western song sparrows, but not with eastern song sparrows. N. Perfito and J.C. Wingfield, unpublished.

However, in birds the kisspeptin gene is yet to be identified (Tsutsui et al., 2010b) and here we will focus on GnIH as a primary candidate for the brake on reproductive onset (Tsutsui et al., 2006, 2007, 2012). GnIH and related peptides have now been isolated from the brains of all major vertebrate groups, (Tsutsui, 2009; Tsutsui et al., 2010a,b, 2013) including songbirds (Bentley et al., 2003).

GnIH can inhibit LH release in songbirds both hypophysiotropically and centrally through inhibition of GnRH release (Fig. 9, Osugi et al., 2004; Bentley et al., 2006a,b, 2008; Ubuka et al., 2008b; O'Brien, 2009). It is very clear that GnIH does not affect breeding baseline LH but blocks surges of LH above the breeding baseline possibly associated with GnRH stimulation (Bentley et al., 2003; Osugi et al., 2004), and with onset of breeding in free-living male *Z. l. gambelii* compared with later in the breeding season when LH levels decline to the breeding baseline (O'Brien, 2009). Infusion of GnIH via mini-osmotic pumps results in a sustained decline in plasma levels of LH and increased mRNA for GnIH in the PVN, but had no effects on testis size or spermatogenesis in white-crowned sparrows (O'Brien, 2009).

Although GnIH in the brain is only expressed in neurons in the paraventricular nucleus (Osugi et al., 2004; O'Brien, 2009), its receptors (GnIH-R; Yin et al., 2005) are expressed widely including on GnRH-I and GnRH-II neurons in avian brain (Ubuka et al., 2008a). The GnIH gene and the GnIH-R are also expressed in the ovary and testis suggesting multiple actions on reproductive function at several levels in the HPG axis (Bentley et al., 2008, 2009, 2010; Maddineni et al., 2008). Actions in mammals and other vertebrates are similar (Bentley et al., 2006a,b, 2010; Calisi, 2014) and whereas mechanisms may vary across species evidence is building that GnIH plays a key role in regulation seasonal reproduction and sexual

motivation (O'Brien, 2009; Kriegsfeld et al., 2010, 2015). In birds injection of GnIH into the third ventricle can rapidly decrease female sexual behavior (copulation solicitation displays) stimulated by estradiol implants and GnRH-II (Bentley et al., 2006a). Similar results of GnIH infusion on male sexual behavior have been found in rodents also (Johnson et al., 2007). GnIH may mediate these effects centrally by expressing GnIH-receptors on GnRH-I and GnRH-II neurons through G-protein coupled signaling (Ubuka et al., 2013b). In mice, direct application of mammalian RF-amide related peptide to GnRH cells in cultured brain slices decreases firing rate in a subpopulation of cells, further suggesting a direct action of GnIH on GnRH neurons (Ducret et al., 2009; Wu et al., 2009).

5. Possible role of GnIH in responses to perturbations of the environment

Acute environmental perturbations have long been known to inhibit reproductive function, particularly onset of breeding or even interruption of breeding (e.g. Wingfield et al., 1999). More recently, Deviche et al. (2012) showed that capture and handling stress in the rufous-winged sparrow, *Peucaea carpalis*, resulted in a decline of plasma testosterone in 10–30 min while plasma levels of LH were unaffected. Injection of GnRH increased plasma levels of LH in both stressed and unstressed birds. Similarly ovine LH injections resulted in elevated testosterone regardless of whether they were stressed (Deviche et al., 2010). These authors concluded that the acute stress-induced decline in testosterone is not mediated through reduced sensitivity of the anterior pituitary gland to GnRH or sensitivity of the testis to LH. Circulating levels of corticosterone were elevated during this procedure but it remains unknown whether corticosterone directly inhibited testosterone release at the level of the testis (Deviche et al., 2012). Stress may inhibit reproduction through release of GnIH followed by rapid inhibition of LH release, gonad steroid production and decreased sexual behavior (Calisi et al., 2008; Calisi, 2014). In house sparrows, *Passer domesticus*, acute stress resulted in an increase in hypothalamic cells showing immunoreactive GnIH. Furthermore, this effect was only seen in breeding males and females but not in the non-breeding season suggesting effects of GnIH are restricted to regulation of reproductive function (Calisi et al., 2008). More recent evidence in mammals and birds indicates that glucocorticoid receptors (GRs) are expressed in neurons containing GnIH (Calisi, 2014; Kirby et al., 2009; Son et al., 2014). In rats, adrenalectomy resulted in attenuation of the GnIH response to acute stress (Kirby et al., 2009). Similarly, Geraghty et al. (2015) showed that genetic silencing of RFamide-related peptide 3 (GnIH) reversed chronic stress-induced infertility in female rats. Dexamethasone treatment of immortalized hypothalamic neurons increased GnIH and its receptor expressions through a GR mediated mechanism. Estradiol treatment had no effect suggesting that GnIH function may play an important regulatory role in mediating stress-induced suppression of reproduction (Gojska and Belsham, 2014). Moreover, in Japanese quail, GR mRNA has been localized in GnIH expressing neurons in the PVN (Son et al., 2014). Treatment with exogenous corticosterone increased GnIH expression within the quail diencephalon suggesting that GnIH may mediate some aspects of the inhibitory effects of environmental stress on reproductive function (Son et al., 2014).

In the European starling captive pairs kept in semi-free-ranging conditions in outdoor flight aviaries with nest boxes breed freely compared with pairs held indoor flight cages with a nest box (Dickens and Bentley, 2014). This experimental design is ideal to explore possible mechanisms by which onset of breeding (nest building, egg-laying etc.) may be controlled. Although all males had fully developed testes, regardless of treatment, only females

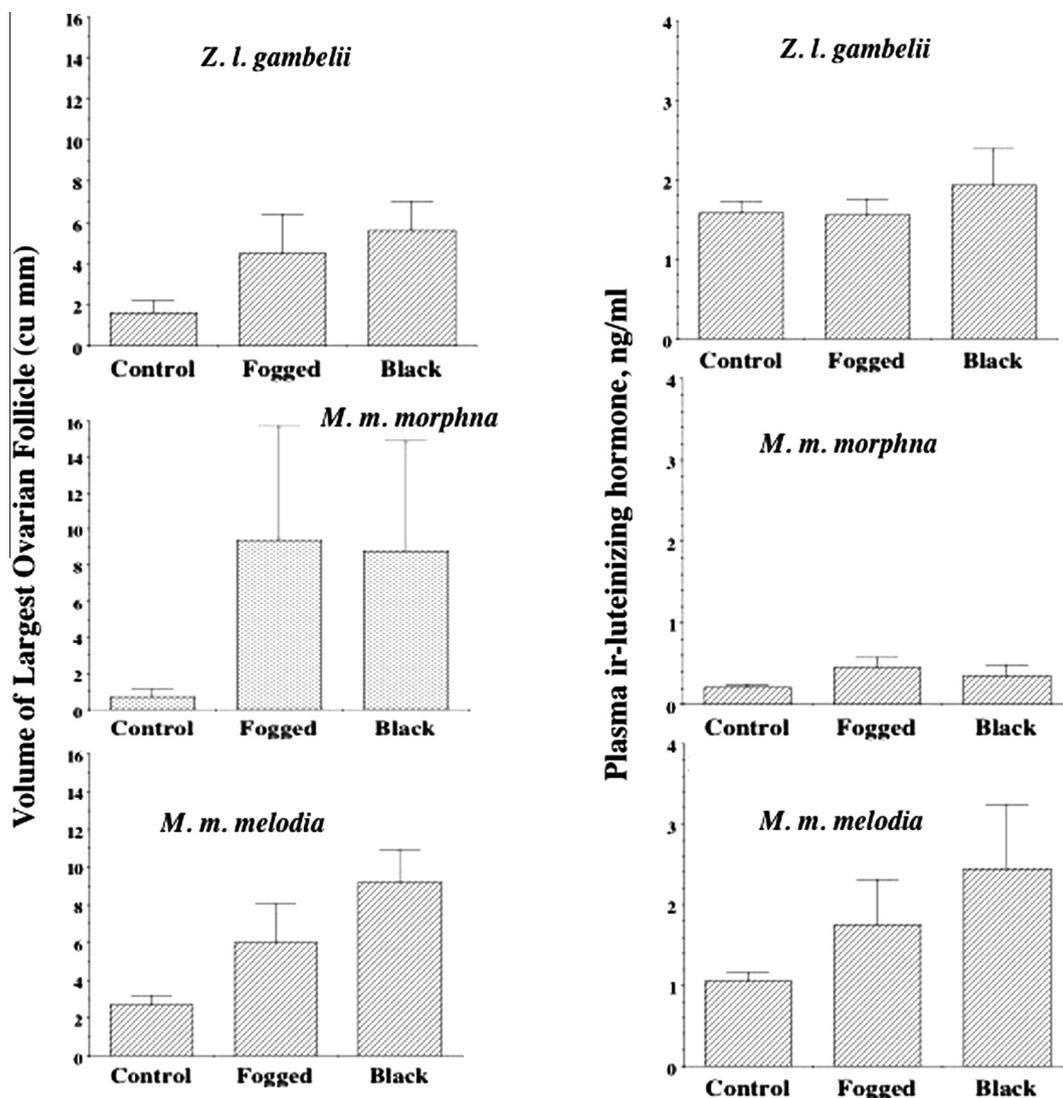


Fig. 8. Differences among groups in the concentration of plasma LH were tested using one-way ANOVA. Differences in follicle volume among treatment groups were tested using with a nonparametric procedure (Kruskal–Wallis ANOVA with Dunn's Method for pairwise comparisons) because variance was unequal among groups, and not normally distributed in some cases. N. Perfito and J.C. Wingfield, unpublished.

in the outdoor aviaries underwent final maturation of ovarian follicles leading to egg laying. Plasma levels of testosterone were generally lower in male starlings housed indoors versus the birds breeding in outdoor aviaries. In both males and females, baseline plasma corticosterone and acute stress-induced levels tended to be lower in the indoor birds compared with those housed outdoors (Dickens and Bentley, 2014). Immunohistochemistry of GnRH cells in the hypothalamus showed an interaction of treatment with higher levels in outdoor males compared to females and indoor males. However, there were no differences in numbers of GnIH positive cells. Furthermore, there were no effects of treatment on GnRH or GnIH gene expression in the hypothalamus. Gene expression of CRH in relation to the stress response and GR as a possible mechanism to inhibit breeding showed no effects in relation to treatment at the hypothalamic level. Moreover, there was an effect of sex on GR, but no effect of treatment was observed (Dickens and Bentley, 2014). In the pituitary gland gene expression for FSH β subunit and GR were the same in outdoor and indoor birds.

Dickens and Bentley (2014) went on to show that there was a significant increase in GnIH gene expression in the ovaries of indoor females but no differences in testes in outdoor and indoor

birds. These authors found no significant differences in gonadotropin receptor expression, aromatase, 17β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 -isomerase (17β -HSD) or GR with treatment in either males or females. This very detailed and extensive study suggests that suppression of onset of breeding in female starlings may be associated with enhanced GnIH actions in the ovary rather than at the central level.

Injection of lipopolysaccharide (LPS) is often used to mimic the effects of a bacterial infection that in turn activates the immune system with downstream effects on the HPA axis. Well known behavioral effects such as reduced appetite and sickness behavior follow and in wild species may be accompanied by an interruption of breeding (Lopes et al., 2012). LPS treatment of male zebra finches, *Taenopygia guttata*, revealed a trend for decreased plasma testosterone levels and elevated corticosterone concentrations but these were not quite significant. LPS injection also decreased hypothalamic GnRH-I gene expression and immunoreactive GnRH but had no effect on GnIH gene expression or immunoreactivity (Lopes et al., 2012).

Intracerebral infusion of GnIH and its related peptides (GnIH-RP-1 and GnIH-RP-2) stimulated an increase in food intake in domestic

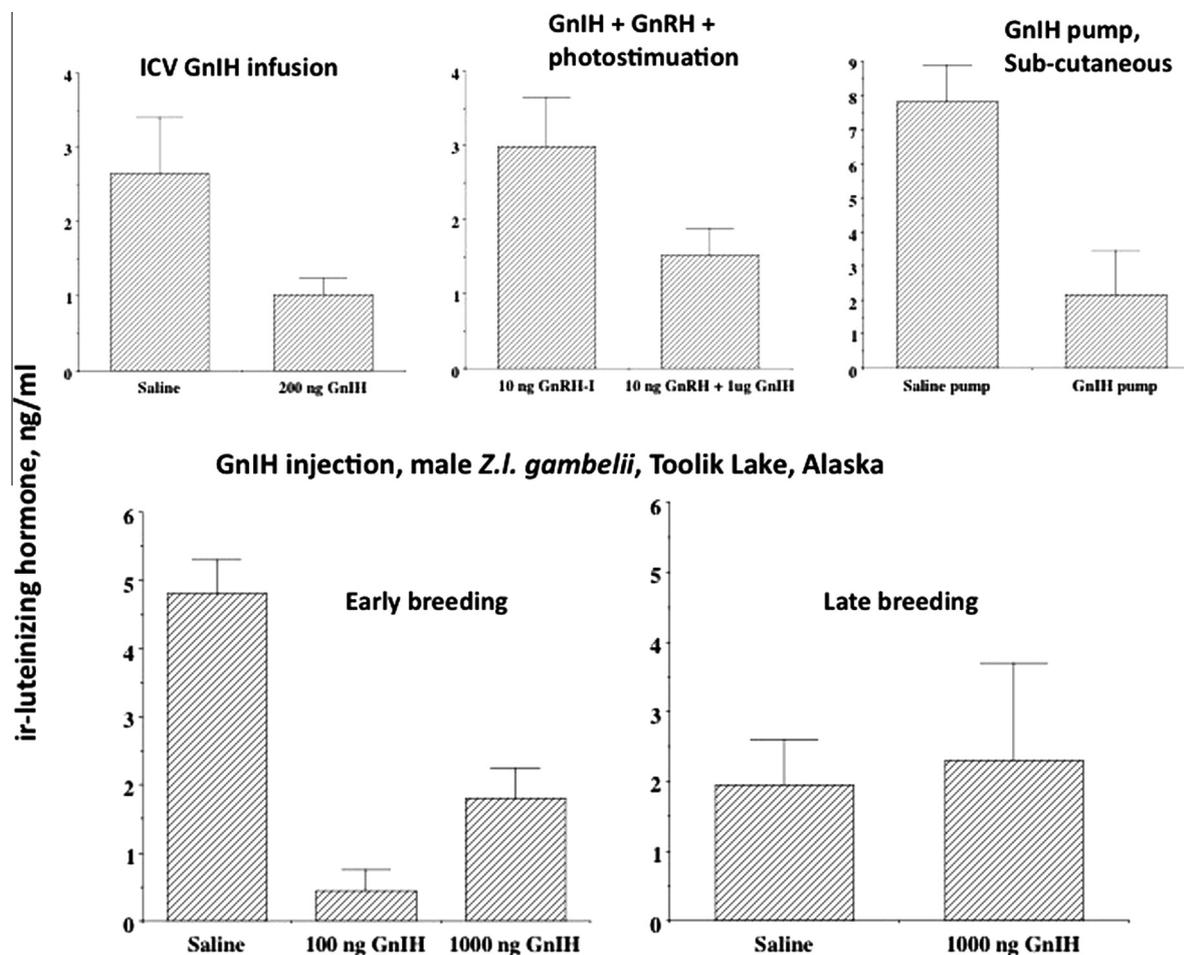


Fig. 9. Effects of various GnIH treatments in plasma levels of immunoreactive luteinizing hormone (irLH) in white-crowned sparrows, *Zonotrichia leucophrys gambelii* (Bentley et al., 2003; Oslugi et al., 2004; O'Brien, 2009). Note that in the center top panel GnIH is most effective in suppressing GnRH-induced surges of irLH. Central injection (top left) or peripheral infusion via a min-osmotic pump (top right) are effective in decreasing breeding baseline plasma irLH. In free-living males breeding in the North Slope of Alaska, highest irLH levels occur early in the breeding season and can be decreased by GnIH injection (both left panel). Breeding baseline of irLH later in the breeding season were not affected by GnIH consistent with GnIH having a "brake" function to inhibit surges of LH associated with onset of nesting. From Bentley et al. (2003) courtesy of the Society for Endocrinology and O'Brien (2009).

fowl chicks, *Gallus gallus*, (Tachibana et al., 2005). Interestingly, these authors showed that infusion of anti-GnIH serum did not suppress feeding when food was provided ad libitum but did inhibit appetite induced by fasting. High ambient temperature can inhibit food intake in domestic fowl, and exposure to high ambient temperature for 24–48 h resulted in elevated expression of GnIH precursor mRNA (Chowdhury et al., 2012). These authors suggest that high temperature-induced reduction in food intake may be opposed by the resulting increase in GnIH expression in the PVN. On the other hand, a 10 h period of fasting in male zebra finches increased circulating levels of corticosterone and decreased plasma testosterone, but hypothalamic gene expression for GnRH-I and GnIH were not affected (Lynn et al., 2015). However, birds with elevated corticosterone showed greater gene expression for GnIH in the testis whereas expression of the StAR and LH receptor (LH-R) genes were decreased indicating reduced sensitivity to circulating LH and lower testosterone synthesis. Clearly fasting effects on reproductive function were mediated through testicular adjustments of GnIH release rather directly through traditional GnRH hypothalamic control. Lynn et al. (2015) suggest that local testicular control of sex steroid release may provide a rapid and flexible response to environmental perturbations such as changes in food supply.

Presence of GR in the gonads suggests direct responsiveness to stress via this pathway thus short circuiting the hypothalamo-

pituitary axis of environmental signal transduction (e.g. McGuire et al., 2013; Lynn et al., 2015). Food cues could also be directly measured through metabolite levels in blood. Using European starling gonads in culture, McGuire et al. (2013) showed that corticosterone and metabolic challenges delivered through 2-deoxy-D-glucose (2DG) and ethyl-2-mercaptoacetate (MA) decreased testosterone and estradiol production even in the presence of LH and FSH. This effect was most pronounced early in the breeding season and may be mediated through up-regulation of GnIH in the gonads. Testes of starlings respond to corticosterone by increasing GnIH expression whereas ovaries respond to metabolic cues by also increasing GnIH expression. GnIH then directly inhibits sex steroid production potentially bypassing regulatory neuroendocrine and endocrine secretions of the HPG axis (McGuire et al., 2013). Furthermore, human gonadotropin-inhibitory hormone ortholog RFamide-related peptide-3 inhibited progesterone production in human granulosa cells incubated with gonadotropin (Oishi et al., 2012). Environmental cues that have direct effects on endocrine tissues rather than be signaled through the brain and hypothalamus may be more frequent than we realize (Wingfield, 2015).

There is growing evidence that glucocorticoid levels during development as well as in response to local environmental conditions, food availability etc. can have profound effects on the timing

of breeding (Schoech et al., 2009). In other words, glucocorticoid secretion may determine current investment of an individual in reproduction (low levels of glucocorticoids) versus self-preservation (higher levels of glucocorticoids, see Angelier et al., 2007; Angelier and Wingfield, 2013).

6. GnIH and transitions between sexual and parental phases of reproduction

European starlings held in semi-natural conditions in a large flight aviary will initiate final maturation of the ovary and lay eggs if pairs have access to nest boxes. At the start of the breeding season, males and females with a nest box had significantly fewer GnIH expressing neurons, smaller GnIH soma sizes and were estimated to be producing less GnIH peptide than birds without a nest box (Amorin and Calisi, 2015; Calisi et al., 2011). Later in the season, at the beginning of the parental phase of nesting the numbers of GnIH expressing neurons were now in greater abundance, GnIH cell soma sizes were larger and the estimated amount of GnIH peptide production had increased in both sexes compared to those without a box, suggesting that GnIH may put a brake on further nesting once there are eggs and young in the nest (Calisi et al., 2011; Amorin and Calisi, 2015). These authors also showed that GnRH content, testosterone and corticosterone were not good indicators of nest box ownership. In European starlings, females housed in semi-natural enclosures isolated from males did not show any final maturation of ovarian follicles. However, females housed with males, or exposed to males for 7 days, showed marked yolk deposition and final ovarian follicle maturation but with no changes in GnIH (Perfito et al., 2014).

The zebra finch is also an excellent model for investigations of environmental control of life cycles because it is able to breed essentially year round – i.e. it is a nomadic and opportunistic breeder. It is thought that in this species there may be tonic expression of GnRH gene with modulation by GnIH expression according to environmental conditions (Perfito et al., 2011). Investigations of wild-caught male zebra finches from southern and central Australia showed that there were no differences in GnRH or GnIH expressions in the hypothalamus with season or age (juveniles). These data suggest that seasonal (periodic) changes in reproductive function, including gonadotropin levels are not mirrored in changes in GnRH and GnIH expressions (Perfito et al., 2011). These results are consistent with the hypothesis that GnRH expression should be tonic in an opportunistically-breeding bird. Perfito et al. (2011) did find that expression of GnRH-II in the mid brain changed with season with breeding males having more and larger GnRH-II expressing neurons. Behavioral effects through GnRH-II may an important additional control point for seasonal/periodic breeding.

Hypothalamic actions of GnIH may be more important for social interactions and gonadal GnIH for breeding and social interactions (through regulation of sex steroids). In Japanese quail, interactions of males with sexually mature females result in a transient decline in LH and testosterone (Cornil et al., 2009). More recently it has been shown that the presence of females increases norepinephrine release in the PVN of males resulting, counter-intuitively, in an elevation of GnIH gene expression and ultimately a decrease in plasma LH (Tobari et al., 2014). In male quail neuroestrogens resulting from direct activation of aromatase in the pre-optic area (POA) appear to inhibit socio-sexual behavior (Ubuka et al., 2014). There is a daily rhythm in expression of sexual behavior in male quail with a peak in the morning hours when neuroestrogen synthesis in the POA is low followed by an increase later in the day and a decline in male sexual behavior. GnIH infusion centrally into male quail in the morning inhibits socio-sexual behavior. Infusion

of estradiol-17 β at this time has a similar effect (Ubuka et al., 2014).

7. Inhibition of GnIH expression

To further investigate the role of GnIH in reproductive function, it is essential to decrease GnIH secretion experimentally. RNA interference experiments in Japanese quail and Gambell's white-crowned sparrows successfully decreased GnIH mRNA in the PVN as well as GnIH peptide (Ubuka et al., 2012, 2013a). GnIH precursor mRNA (GnIH siRNA) infused directly into the third ventricle of white-crowned sparrows resulted in dramatic arousal and expression of complex behavior. This was best summarized as reduced resting time (Ubuka et al., 2012) including spontaneous production of complex vocalizations. Furthermore, a decrease in GnIH expression stimulated brief agonistic vocalizations resembling those of breeding birds, both males and females, during territorial defense and formation of pair bonds. Most striking was a marked increase in copulation solicitations in treated females exposed to playback of recorded male song (Ubuka et al., 2012) consistent with earlier studies showing that GnIH infusion inhibits copulation solicitation in estradiol-treated female sparrows (Bentley et al., 2006a). In Japanese quail, central administration of GnIH siRNA resulted in spontaneous increases in aggressive and sexual behaviors (Ubuka et al., 2013a). Moreover, GnIH administration into GnIH RNAi treated male quail reinstated aggressive and sexual behaviors (Ubuka et al., 2013a).

8. Flexibility and plasticity of control systems: implications for global climate change

In summary, GnIH may have multiple functions as a central nervous system suppressor of social interactions, particularly the control of reproductive behavior and general aggression including, possibly, territorial defense. GnIH may play additional integrating roles in the temporal regulation of reproduction in mammals and birds by environmental factors, physical and social (Parhar et al., 2012; Tsutsui et al., 2013), particularly the onset of actual breeding and transitions between sexual and parental phases of cycling. An additional emerging concept is the action of GnIH expression in the gonads and the presence of GnIH receptors on steroid synthesizing cells that may likely regulate sex steroid production, particularly in females, associated with final ovarian development. This suggests that some responses to environmental cues may bypass the CNS and neural pathways. Taken together, the evidence to date indicates that GnIH acting centrally, hypophysiotropically and at the level of the gonads may be the “brake on onset of cycling” critical for maximizing reproductive success.

This purported role of GnIH has clear implications for biomedicine, such as human fertility, contraception and other reproductive issues. Similarly for reproductive function in agricultural livestock, a role of GnIH will be important. But, there is also a possible important role for GnIH in conservation biology of endangered and threatened vertebrates that fail to reproduce in captive breeding programs, or free-living populations that are declining because of mismatches of environmental phenology and reproductive development. Much more investigation is needed to explore organism-environment interaction and how GnIH is an integrator of physical and social environmental cues that in turn regulate many aspects of the life cycle. For example, GnIH studies may pinpoint populations that are less able to cope with climate change and identify or predict which species conservation efforts should be focused on. For the first time we are able to explore how onset of reproductive cycling is actually triggered in appropriate natural settings.

Acknowledgments

The senior author is grateful for grants RO1 MH65974-01 from the NIH, grants IBN-9905679, IBN-0317141 and IOS-0750540 from the National Science Foundation, and the Endowed Chair in Physiology from the University of California, Davis.

References

- Amorin, Nelson, Calisi, R.M., 2015. Measurements of neuronal soma size and estimated peptide concentration in addition to cell abundance offer a higher resolution of seasonal and reproductive influences of GnRH-I and GnIH in European starlings. *Integr. Comp. Biol.* 55, 1–11.
- Angelier, F., Wingfield, J.C., 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *Gen. Comp. Endocrinol.* 190, 118–128.
- Angelier, F., Weimerskirch, H., Dano, S., Chastel, O., 2007. Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behav. Ecol. Sociobiol.* 61, 611–621.
- Baptista, L.F., Petrinovich, L., 1986. Egg production in hand-raised white-crowned sparrows. *Condor* 88, 379–380.
- Bentley, G.E., Perfito, N., Ukena, K., Tsutsui, K., Wingfield, J.C., 2003. 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.* 15, 794–802.
- Bentley, G.E., Jensen, J.P., Kaur, G.J., Wacker, D.W., Tsutsui, K., Wingfield, J.C., 2006a. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). *Horm. Behav.* 49, 550–555.
- Bentley, G.E., Kriegsfeld, L.J., Osugi, T., Ukena, K., O'Brien, S., Perfito, N., Moore, I.T., Tsutsui, K., Wingfield, J.C., 2006b. Interactions of gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH) in birds and mammals. *J. Exp. Zool.* 305A, 807–814.
- Bentley, G.E., Perfito, N., Ubuka, T., Ukena, K., Osugi, T., O'Brien, S., Tsutsui, K., Wingfield, J.C., 2007. Gonadotropin-inhibitory hormone in seasonally-breeding songbirds: neuroanatomy and functional biology. *J. Ornithol.* 148 (Supplement 2), S521–S526.
- Bentley, G.E., Ubuka, T., McGuire, N.L., Chowdhury, V.S., Morita, Y., Yano, T., Hasunuma, I., Binns, M., Wingfield, J.C., Tsutsui, K., 2008. Gonadotropin-inhibitory hormone and its receptor in the avian reproductive system. *Gen. Comp. Endocrinol.* 156, 34–43.
- Bentley, G.E., Tsutsui, K., Kriegsfeld, L.C., 2010. Recent studies of gonadotropin-inhibitory hormone (GnIH) in the mammalian hypothalamus, pituitary and gonads. *Brain Res.* 1364, 62–67.
- Bentley, G.E., Ubuka, T., McGuire, N.L., Calisi, R., Perfito, N., Kriegsfeld, L.J., Wingfield, J.C., Tsutsui, K., 2009. Gonadotropin-inhibitory hormone: a multifunctional neuropeptide. *J. Neuroendocrinol.* 21, 276–281.
- Calisi, R.M., 2014. An integrative overview of the role of gonadotropin-inhibiting hormone in behavior: applying Tinbergen's four questions. *Gen. Comp. Endocrinol.* <http://dx.doi.org/10.1016/j.ygcen.2014.03.028>.
- Calisi, R.M., Rizzo, N.O., Bentley, G.E., 2008. Seasonal differences in hypothalamic EGR-1 and GnIH expression following capture-handling stress in house sparrows (*Passer domesticus*). *Gen. Comp. Endocrinol.* 157, 283–287.
- Calisi, R.M., Diaz-Muñoz, S.L., Wingfield, J.C., Bentley, G.E., 2011. Social and breeding status are associated with the expression of GnIH. *Genes Brain Behav.* 10, 557–564.
- Chowdhury, V.S., Tomonaga, S., Nishimura, S., Tabata, S., Cockem, J.F., Tsutsui, K., Furuse, M., 2012. Hypothalamic gonadotropin-inhibitory hormone precursor mRNA is increased during depressed food intake in heat-exposed chicks. *Comp. Biochem. Physiol. A* 162, 227–233.
- Cornil, C.A., Stevenson, T.J., Ball, G.F., 2009. Are rapid changes in gonadal testosterone release involved in the fast modulation of brain estrogen effects? *Gen. Comp. Endocrinol.* 163, 298–305.
- Crews, D., Moore, M.C., 1986. Evolution of mechanisms controlling mating behavior. *Science* 231, 121–125.
- Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. *J. Biol. Rhythms* 16, 365–380.
- Deviche, P., Gao, S., Davies, S., Sharp, P.J., Dawson, A., 2012. Rapid stress-induced inhibition of plasma testosterone in free-ranging male rufous-winged sparrows, *Peucaea carpalis*: characterization, time course, and recovery. *Gen. Comp. Endocrinol.* 177, 1–8.
- Deviche, P., Hurley, L.L., Fokoidis, H.B., Lerbour, B., Silverin, B., Silverin, B.J., Sabo, J., Sharp, P.J., 2010. Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: potential site of action and mechanisms. *Gen. Comp. Endocrinol.* 169, 82–90.
- Dickens, M.M.J., Bentley, G.E., 2014. Stress, captivity, and reproduction in a wild bird species. *Horm. Behav.* 66, 685–693.
- Ducret, E., Anderson, G.M., Herbison, A.E., 2009. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. *Endocrinology* 150, 2799–2804.
- Furlonger, A.A., Huyn, L., Luloff, T.W., Schmidt, K.L., MacDougall-Shackleton, S.A., 2012. Effects of supplementation with preferred foods on the reproductive axis of American goldfinches (*Spinus tristis*). *Can. J. Zool.* 90, 203–209.
- Geraghty, A.C., Muroy, S.E., Zhao, S., Bentley, G.E., Kriegsfeld, L.J., Kaufer, D., 2015. Knockdown of hypothalamic RFRP3 prevents chronic stress-induced infertility and embryo resorption. *eLIFE*. <http://dx.doi.org/10.7554/eLIFE.04316>.
- Gojska, N.M., Belsham, D.D., 2014. Glucocorticoid receptor-mediated regulation of *Rfrp* (GnIH) and *Gpr147* (GnIH-R) synthesis in immortalized hypothalamic neurons. *Mol. Cell. Endocrinol.* 384, 23–31.
- Hahn, T.P., 1995. Integration of photoperiodic and food cues to time changes in reproductive physiology by an opportunistic breeder, the red crossbill, *Loxia curvirostra* (Aves: Carduelinae). *J. Exp. Zool.* 272, 213–226.
- Hau, M., Wikelski, M., Wingfield, J.C., 2000. Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird. *J. Exp. Zool.* 286, 494–504.
- Jacobs, J.D., Wingfield, J.C., 2000. Endocrine control of life-cycle stages: a constraint on response to the environment? *Condor* 102, 35–51.
- Johnson, M.A., Tsutsui, K., Fraley, G.S., 2007. 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.* 51, 171–180.
- Kern, M.D., King, J.R., 1972. Testosterone-induced singing in female White-crowned sparrows. *Condor* 74, 204–209.
- King, J.R., Follett, B.K., Farner, D.S., Morton, M.L., 1966. Annual gonadal cycles and pituitary gonadotropins in *Zonotrichia leucophrys gambelii*. *Condor* 68, 476–487.
- Kirby, E.D., Geraghty, A.C., Ubuka, T., Bentley, G.E., Kaufer, D., 2009. Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11324–11329.
- Kriegsfeld, L.J., Gibson, E.M., Williams III, W.P., Mason, A.O., Bentley, G.E., Tsutsui, K., 2010. The roles of RFamide-related peptide-3 in mammalian reproduction and behavior. *J. Neuroendocrinol.* 22, 692–700.
- Kriegsfeld, L.J., Ubuka, T., Bentley, G.E., Tsutsui, K., 2015. Seasonal control of gonadotropin-inhibitory hormone (GnIH) in birds and mammals. *Front. Neuroendocrinol.* <http://dx.doi.org/10.1016/j.yfrne.2014.12.001>, in press.
- Lopes, P.C., Wingfield, J.C., Bentley, G.E., 2012. Lipopolysaccharide injection induces rapid decrease of hypothalamic GnRH mRNA and peptide, but does not affect GnIH in zebra finches. *Horm. Behav.* <http://dx.doi.org/10.1016/j.yhbeh.2012.06.007>.
- Lynn, S.E., Perfito, N., Guardado, D., Bentley, G.E., 2015. Food, stress, and circulating testosterone: cue integration by the testes, not the brain, in male zebra finches (*Taenopygia guttata*). *Gen. Comp. Endocrinol.* 21 (5), 1–9.
- MacDougall-Shackleton, S.A., Stevenson, T.J., Watts, H.E., Pereyra, M.E., Hahn, T.P., 2009. The evolution of photoperiod response systems and seasonal GnRH plasticity in birds. *Integr. Comp. Biol.* <http://dx.doi.org/10.1093/icb/icp048>.
- Maddineni, S.R., Ocón-Grove, O.M., Krzysik-Walker, S.M., Hendricks III, G.L., Ramachandran, R., 2008. Gonadotropin-inhibitory hormone (GnIH) receptor gene is expressed in the chicken ovary: potential role of GnIH in follicular maturation. *Reproduction* 135, 267–274.
- McGuire, N.L., Koh, A., Bentley, G.E., 2013. The direct response of the gonads to cues of stress in a temperate songbird species is season-dependent. *Peer J.* <http://dx.doi.org/10.7717/peerj.139>.
- Nice, M.M., 1937. *Studies in the Life History of the Song Sparrow*. Dover Publications Inc., New York.
- O'Brien, S., 2009. The characterization of gonadotropin-inhibitory hormone (GnIH) and its role in fine-tuning reproduction in the white-crowned sparrow (*Zonotrichia leucophrys*). Ph.D. Thesis, University of Washington.
- O'Brien, S., Hau, M., 2005. Food cues and gonadal development in neotropical spotted antbirds (*Hylophylax naevoides*). *J. Ornithol.* <http://dx.doi.org/10.1007/s10336-005-0013-9>.
- Oishi, H., Klausen, C., Bentley, G.E., Osugi, T., Tsutsui, K., Gilks, C.B., Yano, T., Leung, P. C., 2012. The human gonadotropin-inhibitory hormone ortholog RFamide-related peptide-3 suppresses gonadotropin-induced progesterone production in human granulosa cells. *Endocrinology* 153, 3435–3445.
- Osugi, T., Ukena, K., Bentley, G.F., O'Brien, S., Moore, I.T., Wingfield, J.C., Tsutsui, K., 2004. Gonadotropin-inhibitory hormone in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*): cDNA identification, transcript localization and functional effects in laboratory and field experiments. *J. Endocrinol.* 182, 33–42.
- Parhar, I., Ogawa, S., Kitahashi, T., 2012. RFamide peptides as mediators in environmental control of GnRH neurons. *Prog. Neurobiol.* 98, 176–196.
- Perfito, N., Guardado, D., Williams, T.D., Bentley, G.E., 2014. Social cues regulate switching of hypothalamic Dio2/Dio3 and the transition into final follicle maturation in European starlings (*Sturnus vulgaris*). *Endocrinology*. <http://dx.doi.org/10.1210/en.2014-1450>.
- Perfito, N., Meddle, S.L., Tramontin, A.D., Sharp, P.J., Wingfield, J.C., 2005. Seasonal gonadal recrudescence in song sparrows: response to temperature cues. *Gen. Comp. Endocrinol.* 143, 121–128.
- Perfito, N., Zann, R., Ubuka, T., Bentley, G.E., Hau, M., 2011. Potential roles for GnIH and GnRH-II in reproductive axis regulation of an opportunistically breeding songbird. *Gen. Comp. Endocrinol.* 173, 20–26.
- Schoech, S.J., Rensel, M.A., Bridge, E.S., Boughton, R.K., Wilcoxon, T.E., 2009. Environment, glucocorticoids, and the timing of reproduction. *Gen. Comp. Endocrinol.* 163, 201–207.
- Silverin, B., Viebke, P.A., Westin, J., 1989. An artificial stimulation of the vernal increase in day length and its effects on the reproductive system in three species of tits (*Parus* spp.), and modifying effects of environmental factors – a field experiment. *Condor* 91, 598–608.
- Son, Y.L., Ubuka, T., Narihiro, M., Fukuda, Y., Hasunuma, I., Yamamoto, K., Belsham, D.D., Tsutsui, K., 2014. Molecular basis for the activation of gonadotropin-inhibitory hormone gene transcription by corticosterone. *Endocrinology* 155, 1817–1826.

- Tachibana, T., Sato, M., Takahashi, H., Ukena, K., Tsutsui, K., Furuse, M., 2005. Gonadotropin-inhibiting hormone stimulates feeding behavior in chicks. *Brain Res.* 1050, 94–100.
- Tobari, Y., Son, Y.L., Ubuka, T., Hasegawa, Y., Tsutsui, K., 2014. A new pathway mediating social effects on the endocrine system: female presence acting via norepinephrine release stimulates gonadotropin-inhibitory hormone in the paraventricular nucleus and suppresses luteinizing hormone in quail. *J. Neurosci.* 34, 9803–9811.
- Tsutsui, K., 2009. Review: A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): biosynthesis, mode of action and functional significance. *Prog. Neurobiol.* 88, 76–88.
- Tsutsui, K., Bentley, G.E., Bedecarrats, G.T., Osugi, T., Ubuka, T., Kriegsfeld, L.J., 2010a. Review: Gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. *Front. Neuroendocrinol.* 31, 284–295.
- Tsutsui, K., Bentley, G.E., Kriegsfeld, L.J., Osugi, T., Seong, J.Y., Vaudry, H., 2010b. Review: Discovery and evolutionary history of gonadotropin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. *J. Neuroendocrinol.* 22, 716–727.
- Tsutsui, K., Saigoh, E., Ukena, K., Teranishi, H., Fujisawa, Y., Kikuchi, M., 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. *Biochem. Biophys. Res. Commun.* 275, 661–667.
- Tsutsui, K., Ubuka, T., Bentley, G.E., Kriegsfeld, L.J., 2012. Review: Gonadotropin-inhibitory hormone (GnIH): discovery, progress and prospect. *Gen. Comp. Endocrinol.* 177, 305–314.
- Tsutsui, K., Ubuka, T., Yin, H., Osugi, T., Ukena, K., Bentley, G.E., Ciccone, N., Inoue, K., Chowdhury, V.S., Sharp, P.J., Wingfield, J.C., 2006. Review: Mode of action and functional significance of avian gonadotropin-inhibitory hormone (GnIH). *J. Exp. Zool.* 305A, 801–806.
- Tsutsui, K., Ubuka, T., Yin, H., Osugi, T., Ukena, K., Bentley, G.E., Sharp, P.J., Wingfield, J.C., 2007. Discovery of gonadotropin-inhibitory hormone in a domesticated bird, its mode of action and functional significance. *J. Ornithol. (Supplement 2)*, S515–S520.
- Tsutsui, K., Ubuka, T., Bentley, G.E., Kriegsfeld, L.J., 2013. Review: regulatory mechanisms of gonadotropin-inhibitory hormone (GnIH) synthesis and release in photoperiodic animals. *Front. Neurosci.* 7, 1–11.
- Ubuka, T., Haraguchi, S., Tobari, Y., Narihiro, M., Ishikawa, K., Hayashi, T., Harada, N., Tsutsui, K., 2014. Hypothalamic inhibition of socio-sexual behavior by increasing neuroestrogen synthesis. *Nat. Commun.* <http://dx.doi.org/10.1038/ncomms4061>.
- Ubuka, T., Kim, S., Huang, Y.C., Reid, J., Jiang, J., Osugi, T., Chowdhury, V.S., Tsutsui, K., Bentley, G.E., 2008a. Gonadotropin-inhibitory hormone neurons interact directly with gonadotropin-releasing hormone-I and -II neurons in European starling brain. *Endocrinology* 149, 268–278.
- Ubuka, T., McGuire, N.L., Calisi, R.M., Perfito, N., Bentley, G.E., 2008b. The control of reproductive physiology and behavior by gonadotropin-inhibitory hormone. *Integr. Comp. Biol.* 48, 560–569.
- Ubuka, T., Mizuno, T., Fukuda, Y., Bentley, G.E., Wingfield, J.C., Tsutsui, K., 2013a. RNA interference of gonadotropin-inhibitory hormone gene induces aggressive and sexual behavior in birds. *Gen. Comp. Endocrinol.* 181, 179–186.
- Ubuka, T., Mukai, M., Wolfe, J., Beverly, R., Clegg, S., Wang, A., Hsia, S., Li, M., Krause, J.S., Mizuno, T., Fukuda, Y., Tsutsui, K., Bentley, G.E., Wingfield, J.C., 2012. RNA interference of gonadotropin-inhibitory hormone gene induces arousal in songbirds. *PLoS ONE* 7, e30202.
- Ubuka, T., Son, Y.L., Bentley, G.E., Millar, R.P., Tsutsui, K., 2013b. Gonadotropin-inhibitory hormone (GnIH), GnIH receptor and cell signaling. *Gen. Comp. Endocrinol.* 190, 10–17.
- Visser, M.E., 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proc. R. Soc. B* 275, 649–659.
- Visser, M.E., Caro, S.P., van Oers, K., Schaper, S.V., Helm, B., 2010. Phenology, seasonal timing and circannual rhythms: toward a unified framework. *Philos. Trans. R. Soc. B* 365, 3113–3127.
- Visser, M.E., te Marvelde, L., Lof, M.E., 2012. Adaptive phenological mismatches of birds and their food in a warming world. *J. Ornithol.* 153 (Suppl.), S75–S84.
- Wingfield, J.C., 2006. Communicative behaviors, hormone-behavior interactions, and reproduction in vertebrates. In: Neill, J.D. et al. (Eds.), *Physiology of Reproduction*. Academic Press, New York, pp. 1995–2040.
- Wingfield, J.C., 2008a. Organization of vertebrate annual cycles: implications for control mechanisms. *Philos. Trans. R. Soc. B* 363, 425–441.
- Wingfield, J.C., 2008b. Comparative endocrinology, environment and global change. *Gen. Comp. Endocrinol.* 157, 207–216.
- Wingfield, J.C., 2012. The challenge hypothesis: behavioral ecology to neurogenomics. *J. Ornithol.* <http://dx.doi.org/10.1007/s10336-012-0857-8>.
- Wingfield, J.C., 2015. Coping with change: a framework for environmental signals and how neuroendocrine pathways might respond. *Front. Neuroendocrinol.* <http://dx.doi.org/10.1016/j.fme.2014.11.005>.
- Wingfield, J.C., Doak, D., Hahn, T.P., 1993. Integration of environmental cues regulating transitions of physiological state, morphology and behavior. In: Sharp, P.J. (Ed.), *Avian Endocrinology*. J. Endocrinol. Ltd., Bristol, U.K., pp. 111–122.
- Wingfield, J.C., Farner, D.S., 1978. The endocrinology of a naturally breeding population of the white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol. Zool.* 51, 188–205.
- Wingfield, J.C., Farner, D.S., 1979. Some endocrine correlates of reneating after loss of clutch or brood in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* 38, 322–331.
- Wingfield, J.C., Farner, D.S., 1993. The endocrinology of wild species. In: Farner, D.S., King, J.R., Parkes, K.C. (Eds.), *Avian Biology*, vol. 9. Academic, New York, pp. 163–327.
- Wingfield, J.C., Hahn, T.P., Levin, R., Honey, P., 1992. Environmental predictability and control of gonadal cycles in birds. *J. Exp. Zool.* 261, 214–231.
- Wingfield, J.C., Hegner, R.E., Dufty Jr., A.M., Ball, G.F., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136, 829–846.
- Wingfield, J.C., Jacobs, J.D., Tramontin, A.D., Perfito, N., Meddle, S., Maney, D.L., Soma, K., 1999. Toward and ecological basis of hormone-behavior interactions in reproduction of birds. In: Wallen, K., Schneider, J. (Eds.), *Reproduction in Context*. M.I.T. Press, Cambridge, pp. 85–128.
- Wingfield, J.C., Mukai, M., 2009. Endocrine disruption in the context of life cycles: environmental perturbations, global change and the adrenocortical response to stress. *Gen. Comp. Endocrinol.* 163, 92–96.
- Wingfield, J.C., Silverin, B., 2002. Ecophysiological studies of hormone-behavior relations in birds. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), *Hormones, Brain and Behavior*, vol. 2. Elsevier Science, Amsterdam, pp. 587–647.
- Wingfield, J.C., Silverin, B., 2009. Ecophysiological studies of hormone-behavior relations in birds. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), *Hormones, Brain and Behavior*, 2nd ed., vol. 2. Acad. Press, New York, pp. 587–647.
- Wu, M., Dumalska, I., Morozova, E., van den Pol, A.N., Alreja, M., 2009. Gonadotropin inhibitory hormone inhibits basal forebrain vGluT2-gonadotropin-releasing hormone neurons via a direct postsynaptic mechanism. *J. Physiol.* 587, 1401–1411.
- Yin, H., Ukena, K., Ubuka, T., Tsutsui, K., 2005. A novel G protein-coupled receptor for gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*): identification, expression and binding activity. *J. Endocrinol.* 184, 257–266.
- Yokoyama, K., Farner, D.S., 1976. Photoperiodic responses in bilaterally enucleated female white-crowned sparrows, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* 30, 528–533.
- Yoshimura, T., 2004. Molecular bases for seasonal reproduction in birds. *J. Poultry Sci.* 41, 251–258.