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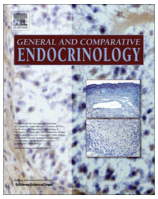

Review

Photoperiod-dependent regulation of gonadotropin-releasing hormone 1 messenger ribonucleic acid levels in the songbird brain

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ABSTRACT

Annual changes in day length induce marked changes in reproductive function in temperate zone vertebrates. In many avian species, in contrast to other seasonally breeding animals, plasticity in hypothalamic gonadotropin-releasing hormone – 1 (GnRH1) expression rather than (or in addition to) release governs changes in pituitary–gonadal activity. Investigations of the cellular and molecular mechanisms that govern GnRH1 plasticity were previously hindered by a collective inability of scientists in the field to characterize the *gnrh1* cDNA in songbirds. We finally overcame this roadblock after data from the zebra finch (*Taeniopygia guttata*) genome project enabled us to rapidly clone the *gnrh1* cDNA from hypothalamic RNA of zebra finches and European starlings (*Sturnus vulgaris*). Here, we review the original data that identified GnRH1 protein plasticity in the songbird brain and discuss earlier failed attempts to clone *gnrh1* in these animals. Then, we present recent efforts, including our own, that successfully characterized *gnrh1* in zebra finch and starling, and demonstrated dynamic regulation of *gnrh1* mRNA expression, particularly in sub-populations of preoptic area neurons, in the latter. Overall, this paper highlights GnRH1 plasticity in the avian brain, and weaves into the narrative the previously untold story of the challenges to sequencing *gnrh1* in songbirds.

1. Historical perspective

The annual change in day length, commonly referred to as photoperiod, is a key environmental signal driving seasonal breeding in many temperate (Baker, 1938; Cockrem, 1993; Dawson, 2001; Hahn and MacDougall-Shackleton, 2008; MacDougall-Shackleton et al., 2009; Moore, 1983; Rowan, 1926; Wingfield and Farner, 1980; Wingfield and Farner, 1993) and some tropical birds (Hau, 2001). Traditionally, the primary measure used to determine the stage of the annual breeding cycle was gonadal volume (Wingfield et al., 1992). In the 1970s and the 1980s, the development of radioimmunoassays for gonadotropin and sex steroid hormones in songbirds (Passeriformes) provided a method to obtain a read-out as to how the brain changed seasonally in its regulation of seasonal cycles in gonadal volume and reproductive function (Follett et al., 1972; Wingfield and Farner, 1975). Studies employing hormone measurement in combination with more traditional analyses of gonadal volume led to a clearer understanding of the way in which variation in photoperiod regulates the timing of reproduction in songbirds species such as the European starling and white-crowned sparrow (Dawson, 2005; Nicholls et al., 1988). During the 1980s, work in the starling illustrated that the neuropeptide gonadotropin-releasing hormone (GnRH1) exhibited marked changes in hypothalamic content across different photoinduced reproductive states (Dawson et al., 1985). This finding provided evidence that a discrete hypothalamic neuropeptide played a significant role for the seasonal regulation of reproductive physiology.

In this review, we will discuss the relationship between photoperiodism and GnRH1 plasticity in songbirds. Then we will review some of the prior attempts by multiple laboratories to determine the genetic sequence for *gnrh1* in songbirds. Lastly, we will present recent work that successfully identified the sequence for *gnrh1* and establish the photoperiodic regulation of *gnrh1* mRNA expression in starlings.

2. Photoperiodic time measurement in songbirds: a focus on absolute photorefractoriness

Birds have evolved a range of photoperiodic responses (reviewed in MacDougall-Shackleton et al. (2009)). In seasonally breeding songbirds that inhabit the temperate zone, the annual

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change in day length provides a predictive cue for the appropriate timing of reproduction when environmental conditions are optimal (Dawson, 2001; Stevenson and Ball, 2011). Seasonally breeding birds exhibit dramatic involution (regression) in their reproductive systems and successful reproduction requires that they initiate gonadal growth (recrudescence) well before the appropriate conditions have occurred (Dawson et al., 2001). Birds that exhibit this photoperiodic response progress through three distinct reproductive states (Fig. 1A). The vernal increase in day length stimulates gonadal recrudescence, increased gonadotropin and testosterone concentrations as well as an increase in the rate of reproductive behaviors. Birds that are in this state are referred to as *photostimulated* and are in breeding condition. Although long day lengths stimulate gonadal growth, prolonged exposure paradoxically lead to gonadal involution and ultimately renders the birds in a non-breeding state through a process referred to as *photorefractoriness*. That is, the long days that initially stimulated reproductive physiology eventually become inhibitory to hypothalamic–pituitary–gonadal (HPG) activity. Birds in this state are reproductively non-responsive to long day lengths (including constant light) as well as to other supplementary environmental cues that can stimulate gonadal growth at other times of the year (Dawson et al., 2001; Dawson and Sharp, 2007; Nicholls et al., 1988). Two photorefractory responses have been described in bird: absolute and relative photorefractoriness. In the interest of brevity, this review only discusses birds that exhibit absolute photorefractoriness. There are recent views that include a description of relative photorefractoriness and GnRH1 plasticity (see Hahn and MacDougall-Shackleton, 2008; MacDougall-Shackleton et al., 2009). Two criteria that have been applied to examine absolute photorefractoriness include: (1) gonadal involution after prolonged exposure to previously stimulatory long day lengths, and (2) the maintenance of gonadal regression in the presence of an even longer photoperiod (Hahn and MacDougall-Shackleton, 2008; Nicholls et al., 1988). In the laboratory, when birds are transferred from a short day (e.g., 9L:15D) to long day (e.g., 16L:8D), gonadal volumes will increase within 3 weeks and after approximately 6–7 weeks, there is an onset of the photorefractory state and gonadal involution ensues (Fig. 1B). In order to restore physiological sensitivity (i.e., responsiveness) to long day lengths, birds must first experience short days similar to those that occur naturally during the late fall and early winter. Thus, short day lengths lead to the re-sensitization of the neuroendocrine axis; birds in this condition are termed *photosensitive* or pre-breeding.

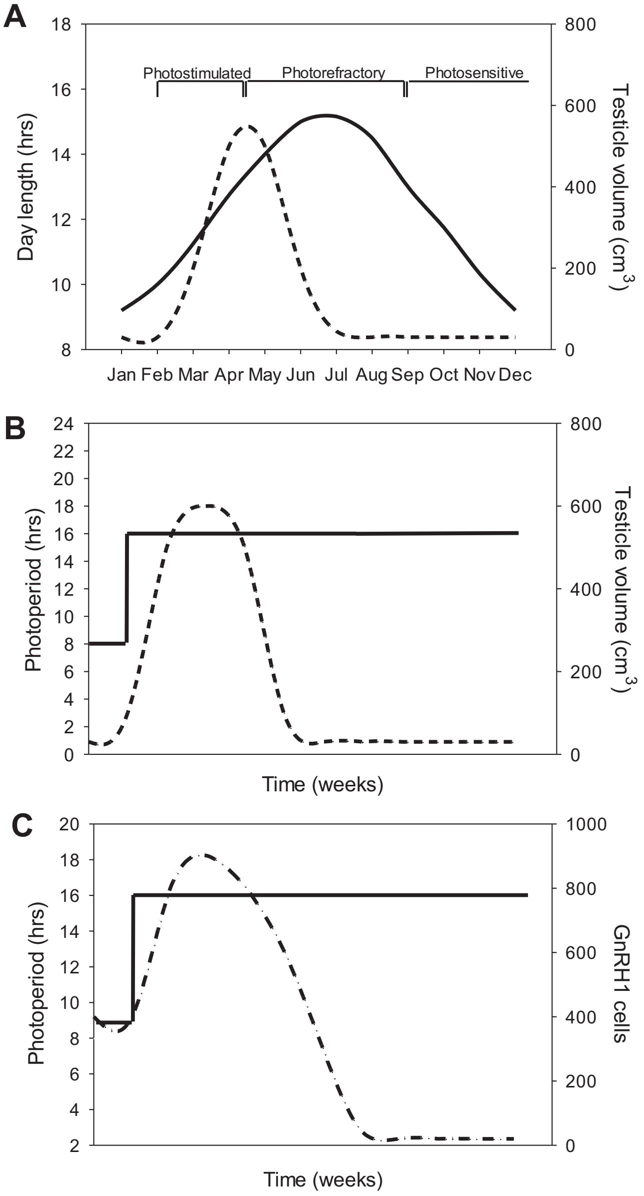

Fig. 1. Photoperiodic regulation of reproduction. (A) The vernal increase in day length stimulates gonadal development. In many avian species, the onset of photorefractoriness is associated with gonadal involution, even in the presence of stimulatory days. The late fall and early winter short days are required for the termination of a photorefractory state and the development of a photosensitive state. (B) In laboratory settings, long days will stimulate gonadal recrudescence in photosensitive birds. Continued exposure to long days results in photorefractoriness and gonadal involution. (C) Photosensitive birds have moderate levels of GnRH1 expression that are further increased after stimulation. The onset of photorefractoriness is associated with a decrease in GnRH1 expression. The lack of parallel patterns between gonadal state (B) and GnRH1 content (C) is due to the dual role of the photoperiod: regulating GnRH1 synthesis and release. Data was adapted from Dawson et al. (2001).

3. The avian photoperiodic response is governed by the brain

Systematic experiments were performed to test whether photorefractoriness was explained by a decrease in the ability of gonadotropins to increase gametogenesis and/or steroidogenesis or via a change in sensitivity of the pituitary to hypothalamic stimulation (reviewed in Nicholls et al. (1988)). The conclusion derived from these studies is that the gonads and the pituitary in photorefractory birds remain responsive to exogenous treatment with, respectively, gonadotropins and gonadotropin-releasing hormone (GnRH1) so that it was assumed that the site of photorefractoriness was in the hypothalamic-preoptic region at the site of the GnRH1 system or of the upstream neural systems that regulate it (Nicholls et al., 1988). Studies illustrating that castrated birds responded to photoperiod in a manner identical to intact birds further supported the notion that changes in the regulation of the neuroendocrine axis at the level of the brain were critical for the control of the photoperiodic response and turned the focus away from gonadal or pituitary mechanisms (Ball, 1993; Nicholls et al., 1988). Indeed, the annual change in pituitary luteinizing hormone (LH) concentrations emerged as the surrogate marker of hypothalamic (GnRH1) output (Ball, 1993). Unfortunately, follicle-stimulating hormone (FSH) remains difficult to reliably measure due to technical problems but it is unlikely that changes in pituitary sensitivity to factors regulating FSH synthesis (e.g., GnRH1, activins, or inhibins) are a major factor driving photoperiodic responses (Nicholls et al., 1988).

More recent studies do indicate that it is an oversimplification to merely state that the pituitary and gonadal is responsive