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Report

Ancestral TSH Mechanism Signals Summer in a Photoperiodic Mammal

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Summary

In mammals, day-length-sensitive (photoperiodic) seasonal breeding cycles depend on the pineal hormone melatonin, which modulates secretion of reproductive hormones by the anterior pituitary gland [1]. It is thought that melatonin acts in the hypothalamus to control reproduction through the release of neurosecretory signals into the pituitary portal blood supply, where they act on pituitary endocrine cells [2]. Contrastingly, we show here that during the reproductive response of Soay sheep exposed to summer day lengths, the reverse applies: Melatonin acts directly on anterior-pituitary cells, and these then relay the photoperiodic message back into the hypothalamus to control neuroendocrine output. The switch to long days causes melatonin-responsive cells in the pars tuberalis (PT) of the anterior pituitary to increase production of thyrotrophin (TSH). This acts locally on TSH-receptor-expressing cells in the adjacent mediobasal hypothalamus, leading to increased expression of type II thyroid hormone deiodinase (DIO2). DIO2 initiates the summer response by increasing hypothalamic tri-iodothyronine (T3) levels. These data and recent findings in quail [3] indicate that the TSH-expressing cells of the PT play an ancestral role in seasonal reproductive control in vertebrates. In mammals this provides the missing link between the pineal melatonin signal and thyroid-dependent seasonal biology.

Results and Discussion

Seasonal Neuroendocrine Control of the Reproductive and Prolactin Axes in Soay Sheep

The Soay sheep is a primitive breed, showing many characteristics of wild mouflon, and is an excellent example of a photoperiodic mammal [4]. Previously, we have used a "first long day" paradigm in Soay rams to investigate the molecular physiology underlying seasonal regulation of the anterior-pituitary hormone prolactin, which controls lactation and the molting cycle [5]. Here, we extended this approach to consider secretion of the pituitary reproductive hormone follicle-stimulating hormone (FSH), which supports ovarian and testicular function during the winter breeding season [4]. Transfer of Soay rams from short photoperiod (SP, 8 hr of light/day) to long photoperiod (LP, 16 hr of light/day) initiated a decline in FSH secretion within 2 weeks, an effect that is the inverse of that we observed in prolactin regulation. The decline continued for a further 6 weeks, by which time the testes became fully regressed and a reproductively inactive "summer phenotype" was established (Figure 1A).

The secretion of FSH by the pituitary depends on the release of gonadotrophin-releasing hormone (GnRH) by hypothalamic neurosecretory cells into the pituitary portal blood supply (Figure 1B), implying that melatonin-responsive cells in the vicinity of this system relay the reproductive effects of photoperiod [2]. Although experiments conducted in the 1990s employing electrolytic lesions or melatonin microimplants suggest that sites within the mediobasal hypothalamus (MBH) mediate these effects of melatonin [6–9], we were unable to detect expression of type 1 melatonin receptors (MT1) within the MBH of Soay sheep (Figure 1C). Because we have been unable to detect expression of type II melatonin receptors (MT2) in this species, and because the MT2 gene is not functionally expressed in seasonally breeding hamsters [10], this result appears inconsistent with hypothalamic mediation of melatonin actions. Indeed, earlier autoradiographic studies with 2-iodo-melatonin (IMEL) reveal wide species variation in the distribution of hypothalamic melatonin-binding sites [11], and in the ferret, a highly photoperiodic mustelid, no neural IMEL binding has been detected [12]. Contrastingly, in the Soay sheep (Figure 1C) and across all other photoperiodic mammals studied, high levels of MT1 expression are consistently observed in a region of the anterior pituitary surrounding the hypophyseal stalk known as the pars tuberalis (PT) [13].

Previously, the PT has been implicated in the seasonal regulation of prolactin secretion [14]. Because prolactin responses persist in hypothalamopituitary-disconnected sheep and PT cells do not secrete prolactin [15], this role of PT is envisaged to involve a relay function within the pituitary whereby PT cells secrete a paracrine factor that governs the activity of the lactotrophs [13]. The melatonin-receptor-expressing PT cells derive from a thyrotroph lineage, expressing both thyroid-stimulating hormone beta subunit (TSHβ) and the common glycoprotein hormone alpha subunit (αGSU). The biologically active TSH hormone is a heterodimer of these molecules [16].

Unlike classical thyrotrophs in the pars distalis (PD) of the pituitary gland, PT cells lack receptors for both thyrotropin-releasing hormone (TRH) and thyroid hormone (TH) [17], suggesting that PT cells serve a distinctive role. Involvement of PT glycoprotein-hormone production in seasonal photoperiodic responses is suggested by the marked induction of PT αGSU and TSHβ expression in hamsters and Soay sheep

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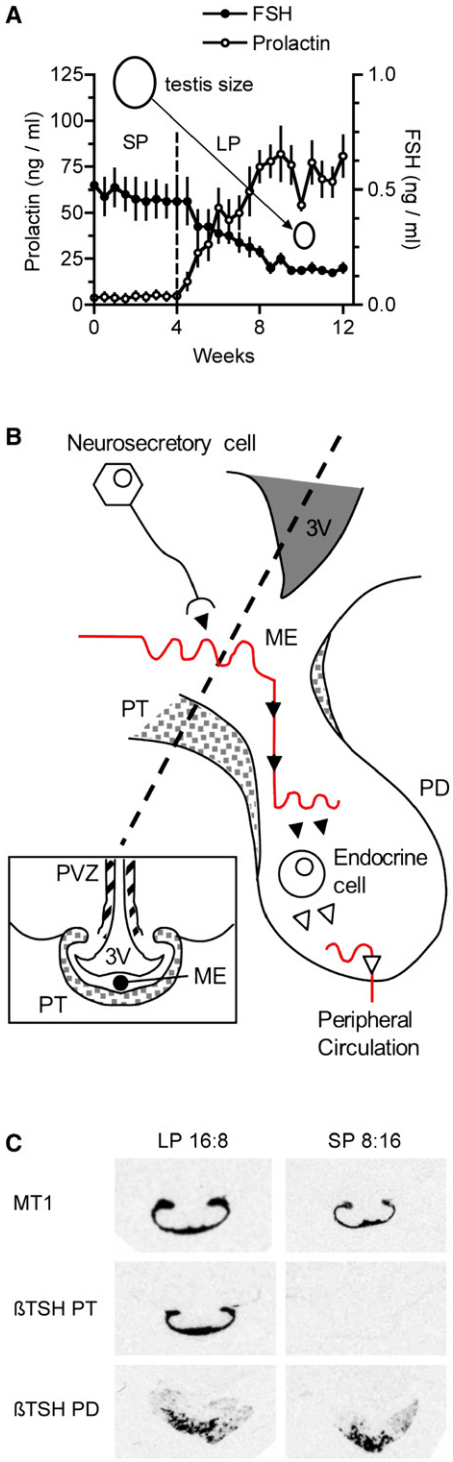

Figure 1. Neuroendocrine Basis to Photoperiodic Responses in the Sheep

(A) Anterior pituitary hormonal profiles from Soay sheep subjected to an abrupt change from short photoperiod (SP, 8 hr light and 16 hr dark per day) to long photoperiod (LP, 16 hr light and 8 hr dark per day); data are mean ± SEM from n = 7 animals/group. Note that within a week, LP begins to activate prolactin and suppress FSH secretion, and testicular regression reaches a maximum at 8–12 weeks under LP (represented schematically).

(B) Schematic drawing of the hypothalamus-anterior-pituitary portal system. Hypothalamic neurosecretory cells release signals into capillaries in the median eminence (ME) that drain into portal vessels (shown in red); further capillary beds in the anterior pituitary allow these signals to reach pituitary endocrine cells, whose hormone secretions reach the peripheral blood circulation. The dashed line indicates the plane of section from which the inset is drawn. This shows a coronal view at the level of the ME, which lies ventral to the third ventricle (3V); cells surrounding the sides of the 3V form a layer known as the paraventricular zone (PVZ), extending down to the ME. The pituitary pars tuberalis (PT) lies adjacent to the ME ventrally.

(C) Representative images of MT1 melatonin receptor and TSHβ gene expression in the PT or PD of sheep acclimated to LP or SP; note MT1 expression on both photoperiods and strong SP suppression of TSHβ expression in the PT, but not in the PD.

exposed to LP [18, 19] (Figure 1C). These effects are not reflected in circulating TSH concentrations [20], probably because of the small size of the PT relative to the PD and the fact that the PD shows no photoperiodic changes in TSHβ expression (Figure 1C). These factors presumably swamp any photoperiodic effect of the PT on systemic TSH titers. We therefore considered local rather than systemic actions of PT-derived TSH a plausible hypothesis for further investigation.

Local Expression of Functional TSH Receptors in the Sheep Neuroendocrine System

We cloned the ovine homolog of the TSH receptor (TSH-R) and performed in situ hybridization in brains from Soay sheep raised on either SP or LP and killed at different times of day. Strikingly, we observed strong expression of TSH-R both within the PT itself and in adjacent cells in the median eminence (ME), extending into the ependymal paraventricular zone (PVZ) surrounding the base of the third ventricle (3V) (Figure 2A). No TSH-R expression was observed in the PD, indicating that PT-derived TSH does not act as the pituitary paracrine signal between melatonin and prolactin secretion. TSH-R expression in PT, but not in the hypothalamus, was subject to a pronounced diurnal variation, depressed in the day (3 hr after lights on) and elevated at night (3 hr after lights off) (Figure 2B). No photoperiodic effects on TSH-R expression were observed.

In the thyroid gland, activation of TSH-Rs stimulates production of the intracellular second messenger cyclic adenosine monophosphate (cAMP) [21], whereas in the PT, activation of melatonin receptors has the opposite effect [22]. We therefore used primary cultures prepared from explants of sheep PT and attached ME to assess the interactive effects of TSH and melatonin on cAMP production (Figure 2C). TSH caused a dose-dependent induction of cAMP within 30 min of application, and this effect was strongly inhibited by cotreatment with melatonin. These data confirm the functionality of the TSH-R RNA expression observed in the region of the PT and ME and suggest that TSH and melatonin interact to control PT function. Because TSHβ is a cAMP-induced gene [23], we speculate that the presence of TSH-R positively coupled to cAMP in PT cells may form the basis for a positive-feedback-loop-based amplification of TSH production with exposure to increasing photoperiods.

Long-Day Induction of Type II Deiodinase in Hypothalamic TSH-R-Expressing Regions

The anatomical distribution of TSH-R expression within the mediobasal hypothalamus of the Soay sheep is strikingly reminiscent of that of type II thyroid hormone deiodinase (DIO2) described in tanycytes of photoperiodic rodents [24–26] and quail [27]. DIO2 is a key enzyme for the control of thyroid-hormone bioactivity that converts thyroxine (T4) into tri-iodothyronine (T3) in various target tissues [28]. In the hypothalamus, DIO2 expression is confined to specialized cells known as

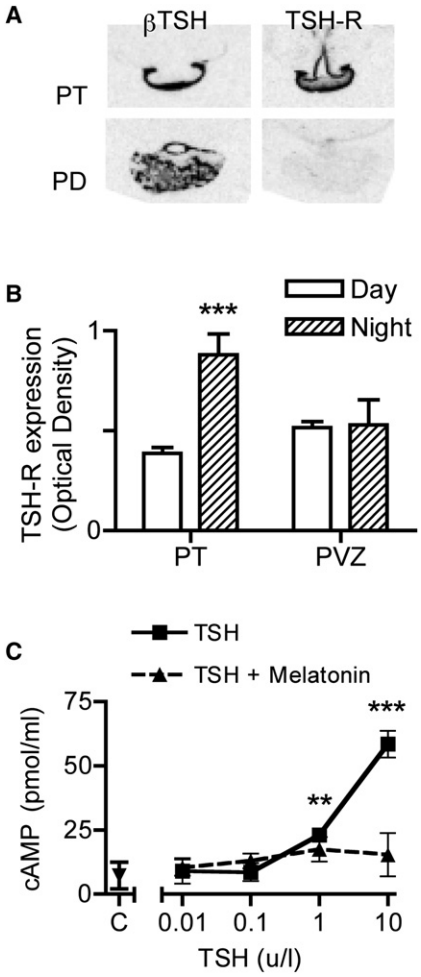

Figure 2. Expression of Functional TSH Receptors in the Sheep Neuroendocrine System

(A) Representative autoradiograhic images taken at two different coronal planes at the level of the PT and PD, showing TSH-R and TSHβ expression patterns observed by in situ hybridization. Note strong TSH-R labeling in the ME and PVZ, and also in the PT itself. Caudally, no TSH-R expression is seen in the PD.

(B) Diurnal regulation of TSH-R expression in the PT, but not in the region of the ME and PVZ; note that expression in the PT is depressed in the light phase. Data are mean ± SEM from n = 8 animals/group.

(C) Interactive effects of TSH and melatonin on cAMP accumulation in primary cultures of sheep PT and attached ME. Cells were stimulated for 20 min in the presence of the indicated concentration of purified ovine TSH with or without melatonin (10 nM). Data are mean ± SEM of triplicate observations in a single experiment that was repeated three times with similar results. **/*** $p < 0.01/0.001$, respectively.

tanycytes that surround the base of the third ventricle [28]. These cells have a characteristic bipolar morphology, with projections to the surface of the 3V and down into the median eminence [29]. In hamsters, SP inhibits hypothalamic expression of DIO2 through a melatonin-dependent pathway [25, 26]. Importantly, the suppressive effects of SP exposure on reproduction and energy metabolism can be overridden by intrahypothalamic T3 microimplants [30], implying that DIO2-mediated effects of melatonin on hypothalamic T3 are crucial for expression of a summer endocrine phenotype. The mechanisms underlying T3-dependent tanycyte actions possibly relate to plasticity in the morphology of their endfeet, which surround and isolate the terminals of the GnRH neurons in the median eminence [31].

We therefore investigated how DIO2 is regulated by photoperiod in Soay sheep and found an unambiguous induction of DIO2 by LP exposure for 6 weeks (Figures 3A and 3B). Furthermore, DIO2 expression was induced within 10 days of transfer to LP, when increased TSHβ expression was first observed (Figures 3C and 3D). This pattern of LP-induced DIO2 expression probably accounts for earlier reports that hypothalamic T4 microimplants are sufficient to override the blocking effects of thyroidectomy on LP-induced gonadotrophin suppression in sheep [32]. Further, the data show that DIO2 induction by LP is a common feature in different photoperiodic mammals, whether they breed under long days as in rodents or short days as in sheep.

Direct Effects of TSH on DIO2 Expression in the Sheep Hypothalamus

This regulation of DIO2, coupled with our studies on TSH-R function in the same local region of the mediobasal hypothalamus, prompted us to consider whether TSH drives photoperiodic changes in DIO2 expression. We stimulated PT/ME cell cultures with purified ovine TSH (0.1 IU/ml) and then assayed cells for DIO2 RNA expression by real-time PCR (Figure 3E). Within 3 hr of stimulation, we observed an acute induction of DIO2 expression, which was also mimicked by forskolin (5 μM), a pharmacological stimulus for cAMP production [33]. These data are consistent with the hypothesis that TSH induces DIO2 RNA expression in the median eminence through a cAMP-dependent pathway.

To investigate whether these in vitro effects reflect in vivo hypothalamic sensitivity to TSH, we performed lateral intracerebroventricular (i.c.v.) cannulations of Soay sheep acclimated to SP with low baseline levels of hypothalamic DIO2 expression (Figure 3F). Animals were then injected twice daily with purified ovine TSH (0.1 IU in 1 ml artificial cerebrospinal fluid) for 5 days (a duration chosen on the basis of the DIO2 response seen in the LP transfer experiment), after which hypothalamic DIO2 expression was assayed by in situ hybridization. Relative to vehicle control injections, TSH infusion caused a clear induction of DIO2 expression, specifically in those areas in which TSH-R expression occurs, producing an expression pattern similar to that seen in LP-acclimated sheep. Blood prolactin concentrations were predictably low in the experimental animals under SP and were unaffected by the i.c.v. infusion of TSH (data not shown).

Conclusions

Collectively, these results support a model for melatonin action in mammals in which the PT acts as a relay to feed photoperiodic information into the hypothalamus, governing local responsiveness to thyroid hormone. This model fills a crucial gap in the understanding of mammalian photoperiodism, providing a link between thyroid signaling in the ependymal PVZ and the major site of melatonin-receptor expression in the pituitary. This pathway shows remarkable similarity to the pathway very recently described in the Japanese quail, the key difference being that in the quail, the photoperiodic reproductive response to LP exposure is more rapid and is believed to depend on an uncharacterized deep-brain photoreceptor [3] rather than melatonin. Although DIO2-dependent changes in hypothalamic T3 levels seem to be crucial for the reproductive response, they are not required for photoperiodic regulation of prolactin secretion in mammals [14]. Hence, we predict that

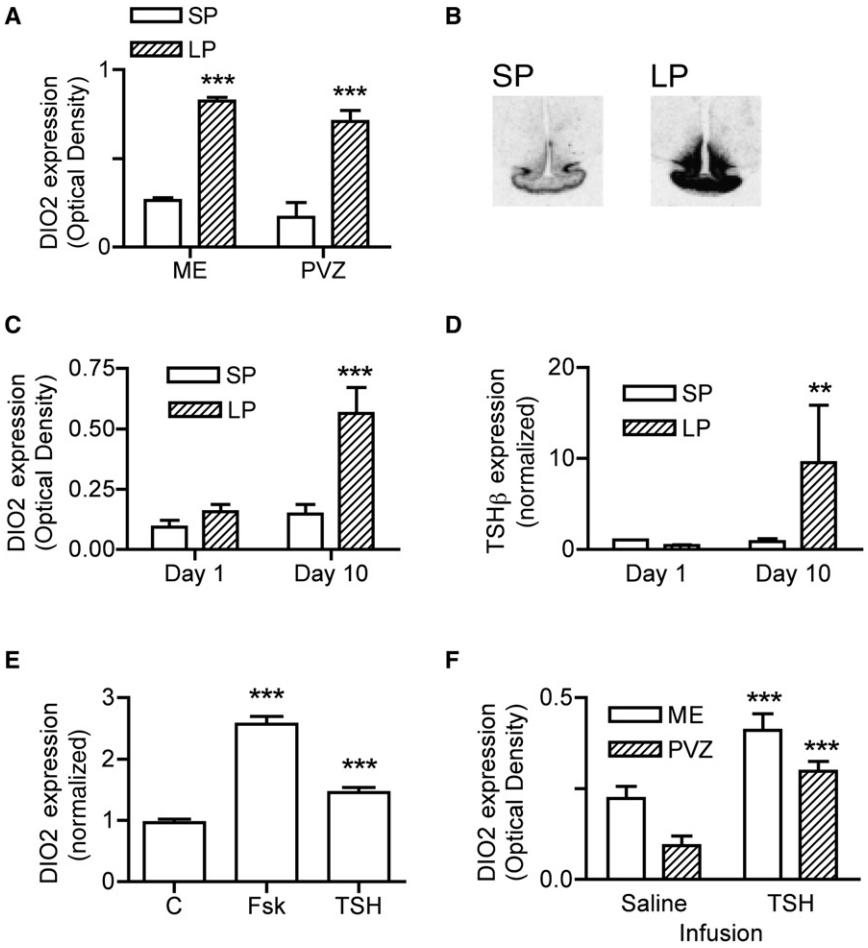

Figure 3. Regulation of Hypothalamic DIO2 Expression by Photoperiod and TSH

(A and B) DIO2 expression in Soay sheep acclimated to SP or LP for 6 weeks. Representative images are shown with densitometric analysis; data are mean ± SEM from n = 8 animals/group.

(C and D) Induction of DIO2 and TSHβ expression by transfer to LP; no expression is seen after 1 day, but both genes are significantly induced by 10 days. Data are mean ± SEM from n = 4 animals/group.

(E) Stimulation of DIO2 expression by TSH (0.1 IU/ml) or forskolin (5 μM) in primary PT/ME cultures. Cells were stimulated for 3 hr prior to assay of expression by real-time PCR. Data are mean ± SEM.

(F) Induction of DIO2 expression in the PVZ and ME by TSH infusion into the lateral ventricles of sheep acclimated to SP. TSH (0.1 IU in 1 ml artificial cerebrospinal fluid) or an equivalent volume of saline was infused twice daily for a period of 5 days prior to sacrifice and assay for DIO2 expression. Data are mean ± SEM for n = 6 animals/group. **/*** $p < 0.01/0.001$, respectively.

the PT produces additional signals, distinct from TSH, through which prolactin secretion is governed.

The data presented here constitute an intriguing reverse of Harris's classic model for hypothalamic control of the anterior pituitary by neurosecretion into the pituitary portal blood supply [34]. Here, a peripheral circulatory signal (melatonin) is decoded in the pituitary to produce photoperiodic control of anterior pituitary hormone (TSH) production; the TSH then acts locally in the adjacent hypothalamus by changing T3 production in the tanycytes, thereby altering the activity of the neurosecretory cells in the hypothalamus that govern seasonal biology.

The unusual direction of information flow described here probably reflects an ancestral mechanism preceding the evolution of a separation between the hypothalamus and pituitary and the development of a local portal blood system linking the tissues. In ancestral vertebrates (Figure 4, left), it is likely that photoreceptor expression in multiple sites in the central nervous

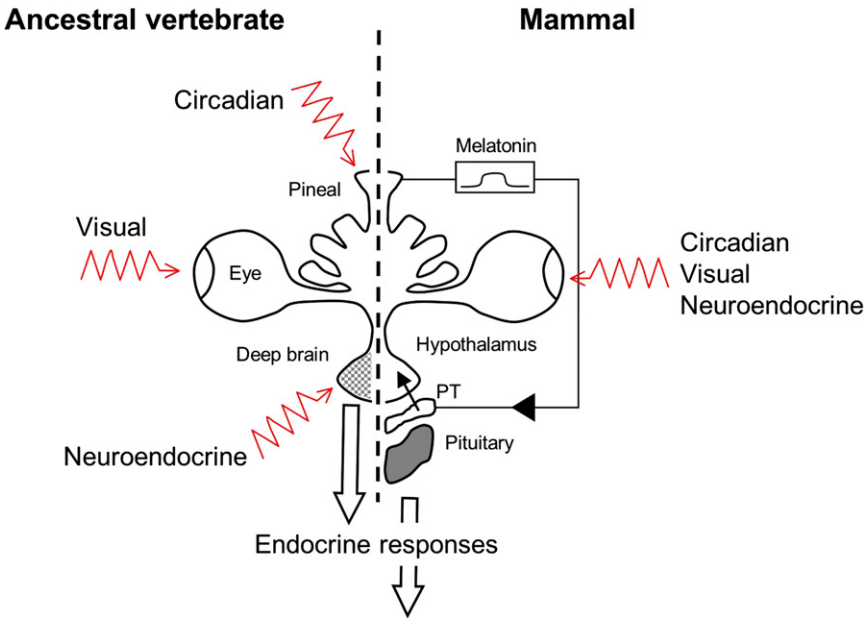

Figure 4. Melatonin Relay Overcomes Evolutionary Loss of Seasonal Photoreceptors

A coronal section through the vertebrate CNS is shown; left of midline shows the situation in an ancestral vertebrate, and right of midline shows the situation in mammals. In ancestral forms, light input (red) to different structures serves different principal functions (overlapping roles are not specifically excluded). The eye deals with vision, and the pineal and deep-brain sites deal with circadian and photoperiodic functions. Within the deep brain of ancestral forms, sites of photoperiodic integration and endocrine secretion (open arrows) overlap (indicated by stippling in the diagram). In mammals, pineal and deep-brain photosensitivity is lost, and the lateral eyes are responsible for all light input. Additionally, the hypothalamus integrates environmental information, and the pituitary gland generates endocrine output. Loss of direct-light sensitivity in these structures leads to the establishment of a photoperiod relay whereby light information perceived by the retina defines the nocturnal pattern of pineal melatonin secretion. This is translated at the level of the PT, which forms an interface between the hypothalamus and pituitary.

system (CNS) served discrete principal functions: control of vision (lateral eyes), circadian rhythms (pineal structures), and photoperiodism (deep brain and pituitary). In mammals (Figure 4, right), photoreceptor loss has led to the lateral eyes' assuming all light-sensing functions, with pineal melatonin secretion becoming a humoral relay for photoperiodic information to pituitary and deep-brain sites. Additionally, distinct regions of the ancestral brain have become specialized for different functions, notably the hypothalamus for integration of environmental cues and the pituitary for hormone production. Our interpretation is that photoperiodic control has been assumed by TSH expression at the PT-brain interface, allowing information encoded in the melatonin signal to reach hypothalamic sites. Birds may be viewed as an intermediate scenario in which compartmentalization of endocrine control into sites of integration (hypothalamus) and output (pituitary) has occurred, but extraretinal photoreceptor sites persist [35]. The highly derived state of the photoperiod-transduction pathway in mammals may well reveal the constraints imposed by their nocturnal ancestry.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/18/15/1147/DC1/.

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