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An integrative view of mammalian seasonal neuroendocrinology

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Abstract

Seasonal neuroendocrine cycles that govern annual changes in reproductive activity, energy metabolism and hair growth are almost ubiquitous in mammals that have evolved at temperate and polar latitudes. Changes in nocturnal melatonin secretion regulating gene expression in the *pars tuberalis* (PT) of the pituitary stalk are a critical common feature in seasonal mammals. The PT sends signal(s) to the *pars distalis* of the pituitary to regulate prolactin secretion and thus the annual moult cycle. The PT also signals in a retrograde manner via thyrotropin stimulating hormone (TSH) to tanycytes, which line the ventral wall of the third ventricle in the hypothalamus. Tanycytes show seasonal plasticity in gene expression and play a pivotal role in regulating local thyroid hormone (TH) availability. Within the medio-basal hypothalamus, the cellular and molecular targets of TH remain elusive. However, two populations of hypothalamic neurons, which produce the RF-amide neuropeptides Kisspeptin and RFRP3, are plausible relays between TH and the GnRH-pituitary-gonadal axis. In contrast, the ways through which TH also impinges on hypothalamic systems regulating energy intake and expenditure remains unknown. Here, we review the neuroendocrine underpinnings of seasonality and identify several areas which warrant further research.

Introduction

Daily and seasonal cycles have shaped the evolution of life on Earth. Migration, hibernation, aestivation, diapause, pelage moult, reproductive status and changing ingestive behaviour are all examples of key adaptive strategies, which have been implemented in a species-specific manner. These strategies ensure an optimal temporal use of a diversity of environmental niches. The underlying processes, which include extensive morphological, physiological and behavioural changes, typically take weeks to months to complete. Therefore, the ability to keep track of the time of year to anticipate upcoming changes is crucial. The annual change in day length (photoperiod) is the most predictive signal (noise-free) for these seasonal changes, so has been selected as the main driver of seasonal programs in most species living at temperate and polar latitudes. Animals have evolved to use changes in photoperiod in concert with endogenous long-term timers, known as circannual clocks, to synchronize seasonal functions.

The underlying central cellular and molecular mechanisms governing seasonality and circannual timing are still poorly understood. However, recent advances have highlighted a conserved neuroendocrine pathway across vertebrates. This pathway, and its molecular components, are involved in photoperiod measurement and might also be an integral part of the elusive circannual clock. The aim of this review is to summarize our current understanding of the mechanisms which underlie mammalian seasonality, providing a unique integrative view of research in multiple mammalian models to unravel commonalities and highlight open questions. We will mostly focus on breeding and metabolic aspects of seasonal programs since these have received particular attention. The current model [Figure 1] emphasizes the role of TSH produced by the *pars tuberalis* (PT) of the pituitary in the seasonal control of thyroid hormone (TH) deiodinases (*Dio2-Dio3*) expressed in tanycytes, and in turn TH levels within the neighbouring medio-basal hypothalamus (MBH) [Figure 1].

We wish to emphasize that this molecular pathway seems conserved in a wide array of species, whether they are usually categorized as short-day breeders (exemplified by sheep) or long-day breeders (exemplified by hamsters and quail). Therefore, species-specific divergence downstream of this common pathway is anticipated, as pointed recently by Helfer *et al*[1]. Indeed, our understanding of downstream pathways – from triiodothyronine (T3) production to physiological seasonal outputs – remains limited. This undoubtedly constitutes the major unanswered question in the field, which should drive future research. Here, we further discuss the potential role of newly described "seasonal genes" that are expressed by the *pars tuberalis* (PT) and tanycytes and consider how dynamic cellular and tissue-specific seasonal remodelling in the hypothalamus and pituitary might be implicated in seasonal timing. We also revisit the concept that control of LH/FSH and prolactin (PRL) are likely governed in a coordinated manner by the PT, but with distinct pathways/messengers (retrograde vs anterograde, [Figure 1]). Finally, we discuss recent findings on the roles of neuropeptides involved in seasonal metabolism and breeding. We focus on two neuropeptidergic systems involved in seasonal breeding: the family of Kisspeptins (KP; encoded by the gene *Kiss1*), which are produced from proteolytic cleavage of a common precursor and only differ on the length of their N-Term, and the RFRP3 neuropeptide (RF-amide Related Peptide 3, encoded by the *Npvf* gene) [Figure 1].

Photoperiodism and circannual rhythmicity

In mammals, duration of the nightly melatonin production by the pineal gland transduces the photoperiodic information to the body[2–6]. Pineal gland removal (i.e. pinealectomy, PX) blocks both reproductive and metabolic responses to photoperiod in multiple species, including sheep and hamsters[7–9], while timed melatonin infusions in PX animals are sufficient to mimic photoperiodic responses[10–12].

Endogenous long-term timers are coupled to photoperiod sensing, but there are marked differences in the nature and persistence of the endogenous rhythm, which led to the discrete categorization of species as being either photoperiodic or circannual. Circannual species are defined by the persistence of full annual cycles of physiology in constant conditions. In contrast, photoperiodic species do exhibit endogenous rhythms, which represent only half an annual cycle. Small short-lived seasonal species such as Syrian and Siberian hamsters exemplify photoperiodic species: the activation of reproduction in spring takes place even though animals are maintained on a fixed short photoperiod (SP); it is independent from increasing daylength, even though premature exposure to long photoperiod (LP) triggers reproductive recrudescence. Therefore, initiation of the spring reproductive phenotype reflects refractoriness to the prevailing SP rather than LP activation, which is a hallmark of an endogenous timing device. However, reproductively active hamsters do not spontaneously revert to the reproductively inactive phenotype. This switch in physiology requires exposure to photoperiods with a duration shorter than the critical photoperiod (~12.5h; see[13]). Refractory mechanisms are common to virtually all seasonally breeding mammals which are sensitive to photoperiodic change, including marsupial lineages[14].

In contrast, longer-lived species may display circannual cycles when maintained on a fixed photoperiod. In this case, animals display recurrent spontaneous switches to the opposite physiological status over time. These switches usually occur at rather stable time intervals even though the amplitude of the cycles dampens with time, depending on the species and the photoperiodic condition under which animals are maintained [Figure 2]. Therefore, refractoriness occurs in both photoperiodic and circannual species, which suggest mechanistic similarities as detailed before[3,6,15–17]. The molecular and cellular substrates of this divergence – the ability to show refractoriness only once or repeatedly over time – are

unknown but we speculate they reflect varying degrees of "plasticity" in the neuroendocrine circuits downstream of photoperiod decoding, which in turn allow for differences in life history.

Circannual rhythms, an ancestral trait expressed in a large range of organisms[18], can persist for many cycles in constant conditions, even in the absence of a pineal gland[19,20], but these rhythms are no longer entrained to the solar year and depend on prior photoperiodic history. The importance of melatonin in endogenous rhythms has been questioned as the refractory state and/or circannual cycles occur without changes in the melatonin signal[21,22]. However, in these cases it is clear that the photoperiodic history of the animal has an effect. For example, in sheep and golden-mantled ground squirrels, a rhythmic melatonin signal is required for the generation of circannual rhythms[9,23], though this signal can be given for only 90 days (and in a summer-like melatonin profile) and still entrain the whole circannual cycle. In PX European hamsters, circannual rhythms persist under constant photoperiods[24] and some PX animals can also entrain to a 6-month accelerated natural photoperiod cycle[25], arguing for independence of the circannual rhythm from melatonin. However, there is a clear season-dependent impact of PX, which suggests that photoperiodic history impacts the trajectory of the rhythms. Furthermore, the emergence of circannual rhythms appear to require prior exposure to LP and persistence of these rhythms is much more obvious when animals are housed under constant LP[26–29]. Overall, exposure to LP seems to be both necessary and sufficient to prime then drive circannual cycles.

In a natural setting, the endogenous seasonal program is also manifested during the polar night and day and in response to equinoctial daylengths, which do not provide information regarding the direction of change. Here too, prior photoperiodic experience determines the appropriate biological response at each time of the year[30,31]. In arctic species, rhythmic melatonin secretion is halted during long periods around the summer and winter solstices. In spite of this, the seasonal rhythms of these species remain synchronized to the sidereal year[32–35]. These findings suggest that only part of the yearly photoperiodic information is meaningful to synchronise circannual rhythms, which is congruent with earlier observations in sheep[9]. The impact of photoperiodic history on physiology has also been evidenced in a developmental paradigm mimicking equinoctial responses in offspring[36–40]. The trajectories of both reproductive and metabolic development drastically diverge according to the season of birth in order to ensure proper alignment of physiology with environmental constraints and opportunities. This phenotypic flexibility is set during gestation by maternal melatonin, which crosses the placental barrier to provide photoperiodic information to the foetuses. Importantly, this early photoperiodic history affects juvenile offspring's own photoperiodic interpretation demonstrating the 'programming' effect of maternal melatonin[36–40].

Seasonality in the *pars tuberalis* (PT)

The PT and the hypothalamic tanycytes (specialised ependymal cells) are critical sites for integration of photoperiodic information and history and their transmission to neuroendocrine pathways controlling physiology[5,17,20,26,41,42]. In the search for neuroendocrine sites controlling seasonality, attention initially focused on the PT as it is the only consistent site of melatonin binding across a wide range of seasonally breeding mammalian species[43]. Here, melatonin receptors are expressed in PT-specific thyrotrophs[44–46]. In addition, the positioning of the PT, between the hypothalamus and the pituitary, in direct contact with the median

eminence (ME), is ideal for coordinating both anterograde (towards the *pars distalis* of the pituitary, PD) and retrograde (back to the hypothalamus) pathways governing seasonal physiology[27]. Similarly, endogenous circannual rhythms in PT-pituitary and PT-hypothalamic pathways keep on ticking in the absence of changing photoperiodic and melatonin conditions in seasonal mammals, leading to the proposal that the PT is pivotal to the generation of circannual rhythms[17,20,26].

Anterograde seasonal regulation: from the PT to the anterior pituitary

The first clear demonstration of an anterograde pathway from the PT to the PD came from studies of the effects of surgical disconnection of the pituitary from the hypothalamus (hypothalamo-pituitary disconnection; HPD) in sheep. This surgery damages the ME and arcuate nucleus, effectively removing the hypothalamic drive from GnRH neurons to gonadotrophs, which leads to a hypogonadal state[47]. However, seasonal rhythms in PRL secretion that control seasonal changes in pelage in birds and mammals[48] remain photoperiodic in HPD rams[49]. Moreover, HPD rams keep on exhibiting circannual rhythmicity in PRL secretion[26]. Co-culture of ovine PT and PD cells revealed that PT cells stimulates PRL production by lactotrophs, suggesting that PT cells produce an unknown PRL releasing factor, which was then dubbed "tuberalin"[50]. Similar findings were reported in Syrian hamsters[51]. A hypothetical model was proposed for tuberalin regulation of PRL production via melatonin[52], based on the observed inhibitory effects of melatonin on cAMP production in pituitary cell cultures initially stimulated by forskolin[53]. This model requires an unknown endogenous stimulator of cAMP within the PT, which the authors termed "Stim X"[52]. The crux of the model is the balance between "Stim X" activation and melatonin-mediated inhibition of cAMP production, which would direct seasonal expression of tuberalin – predicted to be a CRE-dependent gene – and in turn PRL secretion.

The cell signalling mechanisms used to interpret the seasonal melatonin signal remain unclear. Indeed, melatonin onset not only acts as an inhibitor but also stimulates the expression of a range of genes in the PT, which further complicates the model[54–58]. In the PD, dopamine acting through D2 receptors on lactotrophs inhibits cAMP and PRL[59–61]. The D1 receptor on the other hand stimulates cAMP production via activation of adenylate cyclase in neurons (reviewed in[62]). In the ovine PT, only the D1 receptor is expressed[41,63]. Furthermore in an acute melatonin infusion paradigm, D1 receptor is one of the most highly differentially expressed genes in the PT[58]. This suggests that D1 receptors in the PT and dopamine signalling via these receptors could increase cAMP and fulfil the predicted role of Stim X.

The contribution of dopamine to seasonal PRL secretion has been dismissed in a study focusing on the D2 receptor (and therefore PD lactotrophs)[61]. One study showed that D1 receptor analogues stimulated PRL secretion in sheep, however the site of action was not defined[64]. Evidence for an action of D1 receptor signalling in the PT comes from studies on *Npas4*, a gene that is acutely responsive to melatonin[58] and de-repressed in response to D1 receptor signaling[65]. NPAS4 is also known for its roles in regulating cellular plasticity[65]. If dopamine signalling via D1 receptor and downstream cAMP signalling are important for seasonal PRL regulation, then searching for differentially expressed genes in these pathways might constitute a first step towards elucidation of the mechanisms used to interpret the seasonal message carried by melatonin.

More than 30 different factors are known to trigger PRL secretion[66]. In this context, identification of a PT-specific factor (i.e. tuberalin) is even more challenging. Over the years, several candidates have been put forth, such as tachykinin 1 (TAC1) and neurokinin A (NKA) peptides in sheep[67] or endocannabinoids in hamsters[68,69]. Specifically, the

endocannabinoid 2-arachidonoylglycerol (2-AG) produced by the PT increases PRL release in the presence of adenosine or forskolin in Syrian hamsters[69,70]. Strikingly, receptors for both NKA and 2-AG are not expressed by lactotrophs but by folliculostellate (FS) cells of the pituitary gland[67–69]. Therefore, folliculostellate cells might be an important relay for transducing seasonal information towards lactotrophs[17]. However, as it stands, it is plausible that the identity of the "true tuberalin(s)" remain(s) to be disclosed. In this context, it is noteworthy that RNA-seq in sheep identified multiple PT-secreted factors of yet-to-be-determined functions[41,63] (see below).

Seasonal pituitary remodelling, differentiation and histogenesis

A current model for long-term internal timekeeping mechanisms proposes individual cell binary switching in the PT, leading to a progressive tissue level response and subsequent physiology cycles[71]. This model is based on a recent study in sheep showing that individual PT thyrotrophs exist either in a winter or a summer state [Figure 2], defined by the expression of chromogranin A (CHGA) or TSH, respectively[41]. Whether this model is present in other circannual species is unknown, but it illustrates that mechanisms pertinent to cell and tissue plasticity might be involved in timekeeping devices. Indeed, a large number of genes involved in cellular plasticity and differentiation are differentially expressed according to the season in PT and MBH[41,63]. It has been proposed that seasonal timing relies on histogenic processes[72]. However, plasticity at the level of the PT, rather than histogenesis, may be key[71]. While these are not mutually exclusive explanations[71], as histogenesis appears to be a strong seasonal feature of the MBH, the evidence for histogenesis in the PT is inconsistent[41,73,74] (see below).

Cellular differentiation and development are regulated by epigenetic processes. Interestingly, a number of enzymes involved in chromatin remodelling are expressed in the ovine PT, where their expression is increased under LP[41,63]. The histone methyltransferase EZH2, a member of the PRC2 complex that lays down the repressive H3K27me3 mark, is one of these. EZH2 is required for proper differentiation of lung secretory cells during development[75] and promotes neuronal differentiation in adults[76], which make EZH2 an attractive candidate for the regulation of seasonal cycles of differentiation. SUV39H2, another PT-expressed histone methyltransferase, also displays a large increase in expression under LP as compared to SP[41,63]. Overall, at least 20 different chromatin and histone modifiers show differential seasonal expression in the ovine PT[41]. Seasonal changes in expression of a reduced number of chromatin modifiers have also been observed in the hypothalamus (see below). While a role for seasonal differentiation cycles and epigenetics are distinct possibilities[71], defining the seasonal chromatin landscape of the PT will be required before a functional role for epigenetic processes in seasonal timing can be assumed.

The gross anatomy of the sheep PT shows seasonal changes at the cellular level, including junctional contacts between FS cells and PT-specific thyrotrophs[41] [Figure 2]. PT thyrotrophs increase in size, increase rough endoplasmic reticulum, gain a secretory phenotype and reorganise into networks on LP, presumably to coordinate TSH secretion[41]. Clearly, the PT region undergoes seasonal remodelling, but the distinction between morphological remodelling as a consequence of the new physiological function to be fulfilled, or remodelling that drives a timer process, remains to be determined.

Conserved seasonal retrograde pathways: from the PT to the hypothalamus

The current model of photoperiodic entrainment focuses on a conserved retrograde pathway involving secretion of TSH from the PT which, acting on the deiodinase-expressing tanycytes cell layer surrounding the ventral third ventricle (3V) of the hypothalamus, governs local TH metabolism [Figure 1 & Figure 3]. Seasonal regulation of TH is crucial for the expression of seasonal rhythms in multiple vertebrate species (reviewed in:[3–5,17]). Most of our current understanding on the PT-hypothalamus retrograde pathway revolves around the observation that the changing nocturnal duration of melatonin governs local expression of *Tshß* from the PT[77]. The current model of photoperiodic entrainment emphasizes that changes in melatonin signal are transduced by a circadian based "coincidence timer" in the PT[77,78] (reviewed in:[3,17]). This timer uses the duration of the melatonin signal to dictate the amplitude of expression of the transcriptional co-activator EYA3 that impinges on *Tshß* expression[77]. In LP, increased expression of *Eya3* leads to the upregulation of *Tshß*, while this system is tuned down in SP[77,78] [Figure 3A].

Although this model is based on melatonin-induced changes in *Eya3* expression driving the changes in *Tshß* expression, endogenous switches in the expression of these genes have also been observed in the PT of sheep maintained in a constant photoperiodic environment, with an unchanging melatonin pattern[41,79]. Photoperiodic synchronization of *Tshß* expression can also occur in the absence of melatonin, as recently observed in PX European hamsters[80]. A recent study in reindeer showed that exposure to constant light or constant darkness do not prevent seasonal life history to proceed as anticipated[81], which suggests that circadian rhythmicity may not be a prerequisite for seasonal rhythmicity in this species. The need for circadian clock(s) to drive seasonal rhythms has long been established[12,82,83], but current data favour a dual model in which the "generation of long-term cycles depends on the interaction

between a circadian-based, melatonin-dependent timer that drives the initial photoperiodic response and a non-circadian-based timer that drives circannual rhythmicity in long-lived species"[22,84]. Current data suggest that the EYA3/TSH/DIO "seasonal backbone" is a crucial component of both the melatonin-dependent photoperiodic input pathway and the melatonin-independent circannual timer[20,41,79]. Therefore, we anticipate that insights into the regulation of these genes, and how they link photoperiod decoding to circannual timing, will shed light on the nature and organization of seasonal timers.

Seasonality in tanycytes

Seminal work in quail[85,86] and sheep[87] demonstrated a key role for tanycytes in the control of seasonal breeding. Through their expression of TSH receptor (TSHR), tanycytes sense PT-specific TSH[88], which translates into an opposite seasonal regulation of *Dio2* and *Dio3*. DIO2 converts circulating thyroxine (T4) into the more biologically active T3 while DIO3 degrades T4 and T3 to inactive reverse T3[89]. Importantly, in all seasonal species studied, LP has the same effect towards an increase in the *Dio2/Dio3* ratio, which translates into an increased local T3 production under LP in quail[85] and Syrian hamster[90], but not in F344 rats[91]. Furthermore, we acknowledge that direct evidence for an LP-induced increase in T3 levels in the MBH of short-day breeders such as sheep or goats is still missing. Bearing these caveats in mind, we assume that local T3 levels are increased under LP whatever the species' seasonal physiology and reproductive season. This assumption implies that mechanisms downstream of LP-induced T3 production likely diverge in order to produce the full repertoire of reproductive outputs: from inhibition in sheep, to activation in hamsters, and no overt effect in most strains of mice and rats.

Square wave changes in photoperiod and melatonin are sufficient to regulate *Dio2* and *Dio3* expression in tanycytes[79,87,92–94]. TSH directly up-regulates the expression of *Dio2*[87,92], and while there is evidence for *Dio3* down-regulation as well, the underlying mechanism remains unknown[92,95]. Strong evidence in sheep and hamsters show that rapid activation/deactivation of this axis is sufficient to prime long-term seasonal changes in physiology[27,96,97]. However, the long-term dynamics of these responses differ between species. Taking this into consideration, levels of expression along the TSH/DIO/T3 axis (especially at the level of *Dio2*/*Dio3* expression in tanycytes) might not necessarily be congruent with the physiological output. This observation implies that "unexpected" level of expression of any of these markers is not sufficient to dismiss or undermine the role of this axis. However, the differing temporal relationship between *Dio2* and *Dio3* in Siberian hamsters under square wave or natural photoperiods may indicate that additional PT-derived signals regulate *Dio3* expression, and perhaps also modify *Dio2* expression[96].

Another PT-derived candidate is neuromedin U (NMU), which is governed by photoperiod in juvenile Fischer 344 rats[95] [Figure 3B]. The NMU-R2 receptor is highly expressed in the ependymal cell layer containing tanycytes in rodents[98], and intracerebroventricular infusion of NMU in F344 rats upregulates *Dio2* but does not affect *Dio3*[95]. Whilst further studies on PT-tanycyte signalling are needed, it is established that changes in tanycyte gene expression ensure a local hypothalamic metabolism of TH –disconnected from the traditional hypothalamo-pituitary thyroid axis– and bring together the long-recognized roles of melatonin and TH in seasonal breeding[3,63,88,94,99].

Tanycytes: different subtypes and different roles?

Tanycytes are a specialized type of ependymal cells, which line the walls of the 3V and send long processes toward hypothalamic nuclei and the median eminence (ME)/PT region [Figure 3]. The strategic location of tanycytes at the interface between the cerebrospinal fluid and the pituitary blood flow at the ME suggests key functions in the blood-brain barrier and in the selective transport of molecules between compartments (see[100,101], and in nutrient sensing[102,103]). Even though these cells were described over a century ago, and their morphology has been extensively studied, comparatively little is known regarding their functions[101]. Below we briefly address recent findings which shed new light on the role of tanycytes in the control of seasonal functions.

Tanycytes are usually classified according to their location along the dorso-ventral axis of the 3V: α1 and α2 tanycytes occupy the most dorsal positions, while β1 and β2 tanycytes line the infra-lateral and basal parts of the 3V[100,101,104,105]. The α tanycytes send their processes towards the dorsomedial/ventromedial nuclei of the hypothalamus, β1 towards the ventro-medial/Arcuate nuclei of the hypothalamus and β2 tanycytes towards the ME/PT region. While this classification has been useful, recent data show that it largely undermines the diversity of tanycytes. Single cell RNAseq and hierarchical clustering applied to the MBH reveals many more molecular phenotypes, both for neurons and glial cells, than usually recognized[106–108]. This has been perfectly summarized by Chen *et al*[106] who analysed tanycytes in some detail: "Notably, although specific marker genes (or combinations of marker genes) can be used to roughly separate tanycyte subtypes, many genes exhibited a gradient, rather than a clear-cut distribution across tanycyte subpopulations consistent with the notion that tanycytes may be composed of continuous cell trajectory with transition zones between different subtypes." Although all three single-cell RNAseq studies were performed

in the mouse, there is no *a priori* reason to believe such complexity would not apply to other species. It is worth keeping in mind the wide variety of tanycytes and their current simplified classification to interpret future studies, especially when using classical approaches (ie. qRT-PCR or ISH). For further discussion on this topic the reader is referred to the review by Prévot *et al*[101].

Novel seasonal markers for tanycytes in sheep: a role for autocrine/paracrine thyroid hormone feed-back?

Amongst the strongest seasonal markers identified by our recent RNAseq analysis in sheep, many were found to be expressed exclusively in the PT, but a few were also found to be expressed specifically in tanycytes as revealed by *in situ* hybridization[63,109] [Figure 3]. Apart from *Dio2* and the TH transporters MCT8 (*Slc16a2)* and Oatp1c1 (*SlcO1c1*), we further identified *Shh*, *Tmem252, NpSR1* and *Dct* as novel tanycyte-specific markers regulated by photoperiod and TH, as suggested by the outcome of experiments in which chronic lack of TH (5-6 months) was achieved through surgical thyroidectomy (THX). These 4 genes appear to be exclusively expressed by tanycytes located in the infra-lateral walls and bottom of the 3V, which suggests they are ß tanycytes. These genes show specific response to photoperiod and TH: *Shh*, *Dct* and *Tmem 252* show higher expression under LP, while *NpSR1* is a SP marker. Interestingly, expression of *Shh* and *Dct* – but also of *Dio2* and *SlcO1c1* – is induced by acute exposure to LP and is increased by THX, irrespective of photoperiod. In contrast, *Tmem252* is also induced by acute exposure to LP but this induction is severely blunted in THX animals[63,109], which suggests *Tmem252* plays a specific role as relay of the LP message carried by TH. Finally, expression of *NpSR1* is not induced by acute exposure to LP and THX leads to constant intermediate levels. We also note that the impact of TH on expression of some of these genes might reflect longer-term effects since it is not seen in animals studied

one month after THX[63]. A strategy of TH replacement, perhaps through the use of hypothalamic implants, in THX animals should be used to clarify the role of TH.

Most importantly, there is strong evidence that SHH, DCT and NPSR1 are involved in processes linked to plasticity and cell proliferation[110–114]. A potential role for TMEM252 remains to be investigated as there is virtually no data in the literature for this gene. This seems to put the emphasis back (again) on the potential role of cell proliferation and histogenesis in long-term timing programs[72].

Seasonal structural remodelling in tanycytes

Tanycytes show a remarkable seasonal remodelling of their cytoplasmic processes and cytoskeletal composition. Studies using Japanese quail – long-day breeders – revealed seasonal remodelling of tanycyte endfeet at the level of the PT[115], such that GnRH terminal fields specifically contact the pericapillary space only during the breeding season (LP). This remodelling is also observed in SP-kept sheep – short-day breeders – or sheep endogenously reactivating their reproductive axis in a constant photoperiod[41] [Figure 2]. Tanycyte end-feet retraction is also associated to an altered sex steroid milieu during the transition to estrous in rats[116], situating this phenomenon as part of the reproductive output and not of the photoperiodic response itself.

In tanycytes, the cytoskeletal proteins vimentin and neural cell adhesion molecule (NCAM) show reduced expression in SP as compared to LP in hamsters[117,118], associated with changes in melatonin but not sex steroids. Morphological studies in sheep have demonstrated increased expression of these structural markers during the winter season instead, associated to an increase in the thickness of the tanycytic nuclear layer, junctions between cells and

tanycytes protrusions into the 3V at the arcuate nucleus level (ß1 tanycytes)[119], reinforcing the view that such changes occur as a consequence of the seasonal response associated to the season of breeding (i.e. sex steroid dependent process).

Akin to what has been observed for the PT (see above), several genes related to the modification of chromatin structure (e.g. DNA methyltransferases and histone deacetylases) undergo seasonal and photoperiodic variation in tanycytes of Siberian hamster and F344 rats, which suggests that epigenetic changes occur in a coordinated manner in PT and tanycytes[120–122].

Tanycytes as stem cells – does hypothalamic cell proliferation play a role in circannual rhythms?

Since the initial demonstration in mice and rats that tanycytes comprise a population of stem cells that can be induced to proliferate (as assessed by BrdU incorporation) by growth factors such as bFGF and CNTF[123,124], the stem cell niche of the MBH has been described in other mammals, including sheep and humans[73,74,119,125] [Figure 3B]. This topic has been extensively reviewed recently[101,105]. Here we briefly consider the potential relevance of local cell proliferation to circannual rhythmicity and photoperiodic responses. Fate-mapping studies in mouse, aimed at identifying which population of tanycytes truly are stem-cells, have pointed either to α tanycytes[126] or ß tanycytes[127]. Potential stem cells have also been identified within the hypothalamic parenchyma, rather than among tanycytes[128]. At least in sheep, BrdU-labelled cells are also found within the PT/ME[73,74], but a high proportion of these might be microglia[74]. Therefore, it seems safe to conclude that the location of stem-cells within the MBH is still a matter of debate and that species-specificity in proliferation processes is plausible.

Unsurprisingly, most of the studies investigating hypothalamic cell proliferation have been performed in laboratory strains of mice and rats, which are not overtly photoperiodic. However, there is good evidence that hypothalamic cell proliferation is increased under SP in sheep[73,74,129], and there is limited evidence for a heightened number of dividing cells under SP than LP in the golden hamster[130] and the F344 rats[131]. What might trigger seasonal cell proliferation? As mentioned above, cell proliferation can be prompted by a variety of growth factors including bFGF, CNTF or IGF1[123,124,132]. We note that expression of *Areg*, a ligand of the EGFR, was transiently up-regulated in the MBH of ewes sampled in August[63]. Several members of the IGF1 signalling pathway also appeared to be regulated by season[63]. Whether EGFR or IGF1 signalling also play roles in seasonal timing and cell proliferation remains unknown. Placing these considerations in the perspective of seasonal timing, one may envision a model in which various growth factors sequentially activate (or repress) proliferation of different subsets of stem cells, at different location in the ventricular walls or in the median eminence or within the hypothalamic parenchyma. To the best of our knowledge the potential direct role of other secreted factors such as TSH or NMU[95,133] on cell proliferation in the MBH has not been investigated [Figure 3B].

Recent evidence obtained in sheep, using infusion of the anti-mitotic compound Ara-C at the bottom of the 3V, hints at a functional role for tanycytic cell proliferation in the timing of the breeding season[129]. How cell proliferation may impact seasonal timing remains unknown[72]. Do newborn cells integrate specific circuits? Alternatively, since tanycytes play an important barrier role, is it possible that proliferation leads to transient disorganization/reorganization of the barrier properties? In other words, is proliferation *per se* the important factor?

These questions arise from the limited number of newly generated cells observed under SP and the differences in seasonal programs between species. As mentioned above, cell proliferation is increased under SP in both sheep and golden hamster, thus appears to be a conserved mechanism, as is the photoperiodic regulation of the TSH-DIO axis[3,86,134]. Although photoperiodic regulation of cell proliferation has not been evaluated in non-photoperiodic rodents, we may anticipate an increase under SP if proliferation is coupled to the TSH-DIO axis. While we agree that species-specific differences in seasonal programs likely arise downstream of this axis, it is not immediately obvious that cell proliferation (alone) could account for the wide spectrum of seasonal phenotypes. In other words, these new cells would govern (i) short-day breeding without notable changes in body weight for sheep, (ii) long-day breeding with a fast 30% body weight loss during autumn and preparation for winter torpor cycles in the Siberian hamster, and (iii) long-day breeding with weight gain and preparation for hibernation in the golden hamster. To summarize, a functional role for cell proliferation/histogenesis in seasonal timing seems plausible, but current data are not sufficient to draw firm conclusions regarding mechanisms.

Sensitization of TSHR signalling pathway in tanycytes

Seasonal changes in tanycyte sensitivity to TSH stimulation may be integral to internal timing mechanisms. While there is evidence that the endogenous downregulation of the TSH-DIO axis may be central to the transition from summer to winter physiology in sheep[41,63,79], data are inconsistent with a converse increase in *Tshß* expression during the intrinsic transition to summer physiology observed in different species[20,42,79,97,135]. This does not necessarily mean that this switch is TSH-independent. Instead, our recent work in hamsters and sheep reveals changes in sensitivity to TSH in response to prior photoperiod and thus exposure to *Tshß* history [Figure 4].

When Siberian hamsters with either LP- (ie. high TSH) or SP- (ie. low TSH) history are raised in intermediate photoperiod (14L:10D), they show similar intermediate levels of PT-*Tshß* expression, as assessed by *in situ* hybridization. In contrast, expression of *Dio2* is highly increased only in those animals with a SP-history (ie. low TSH-history) as compared to animals with a LP history (ie. high TSH-history)[40] [Figure 4A]. Furthermore, when juveniles with different photoperiodic history received small intracerebroventricular doses of TSH, juveniles with a SP-history had higher *Dio2* expression as compared to those with a LP-history, demonstrating a difference in TSH signalling sensitivity dependent on the animals' previous photoperiodic history[40,42].

Similarly, using step-wise increases in photoperiod after exposure to SP in sheep, we recently showed that a small increase in photoperiod, thus a small increase in PT-*Tshß* expression, leads to sub-maximal *Dio2* expression (i.e. identical to LP expression) [Figure 4B]. We believe this reveals sensitization of the TSHR signalling pathway after prolonged deprivation of TSH during winter months. Moreover, this shows that *Tshß* expression can be increased by photoperiods considered as short (~11h;[136]; Dardente and Lomet, unpublished). Collectively, this indicates that sensitization/desensitization of signalling pathways in tanycytes (and PT perhaps) plays a significant role in seasonal cycles. We propose that sensitization of the TSH signalling pathway –at the TSHR or downstream– might be a key component of the photoperiodic history in mammals [Figure 4C].

Integration of seasonality into metabolic physiology

Perhaps, the greatest remaining challenge for the field is to establish how changes in tanycyte-directed plasticity and signalling in the MBH ultimately impact on the known neuroendocrine pathways that underpin fertility and energy balance [Figure 3B]. The

experimental observations that direct placement of TH-releasing microimplants in the MBH can induce reproductive and metabolic physiology mimicking the LP state in hamsters[94,137] and sheep[138] is consistent with the studies reviewed above indicating enhanced *Dio2* expression and thus local TH availability in LP. However, this same signal elicits activation of the GnRH secretory system in hamsters but inhibition in sheep. Moreover, in the melatonin-producing strain of CBA/N mice, changes in photoperiod elicited TSH-dependent regulation of *Dio2* in tanycytes, but this did not translate to any effect on the reproductive axis, at least within the short time frame of the study[92]. One potential explanation for these paradoxes is that we do not yet know the direct targets of TH (also see above). There are likely to be multiple targets: a study in a hypothyroid rat model identified >100 genes that were up- or down-regulated in the hypothalamus following TH replacement[139]. Perhaps differences in these targets between species may explain the evolution of different seasonal timing.

A second explanation is that although under experimental conditions central manipulation of TH is sufficient to modify seasonal cycles, it seems likely that multiple tanycyte-derived signals change seasonally, that then also modify neuroendocrine responses. For example, studies in both Siberian hamsters and F344 rats have identified upregulation of genes encoding retinoic acid transporters, binding proteins and receptors in tanycytes under LP[140,141] [Figure 3B]. Given that both TH and retinoic acid signalling act in concert to regulate initial brain development, it seems likely that this is also the case for directing seasonal plasticity and change in function in the adult brain[131]. In these species, expression of several elements of the Wnt signalling pathway in the MBH are upregulated under LP, but also by leptin and NMU administration, suggesting that this developmental pathway might also be involved in seasonal body weight regulation[95,142].

An initial expectation that followed the identification of the PT as central to photoperiodic signalling in mammals was that the downstream effects of seasonal changes in appetite and energy expenditure would be the well-researched peptidergic pathways (NPY, AgRP, POMC/αMSH, CART) identified in the MBH that are critical in short-term homeostatic control[143]. Some studies in jerboas[144,145] or in sheep support this conjecture, for example increased expression of the "orexigenic gene" *Npy* has been found in rams and ewes in the non-breeding season when appetite increases[146,147], but recent studies in red deer present a much more complex picture with opposite seasonal regulation of NPY in male and female animals[148]. Moreover, extensive studies in Siberian hamsters from three different research groups found a consistent decrease in POMC gene expression despite showing a consistent weight loss in SP, but failed to find photoperiodic changes in these peptidergic systems that correlate with altered appetite[149–151]. While POMC appears to be involved in the long-term timing of energy balance, we clearly need to look beyond these peptidergic systems to understand long-term rheostatic control of appetite.

One particularly interesting candidate is the VGF system, which is not only one of the most widely and highly expressed genes in the hypothalamus[152], but also shows clear seasonal regulation in the arcuate nucleus of Siberian hamsters[153]. Importantly, it is a TH-regulated gene so a potential direct target of altered tanycyte signaling[154], and upregulation of gene expression in the hamster hypothalamus (using a recombinant adeno-associated viral vector) increased energy expenditure and reduced body weight gain[155]. Unfortunately, processing of the proVGF precursor is complex and tissue-specific, comparable to the biology of POMC processing, thus overexpression of *Vgf* resulted in increased hypothalamic content of a variety of VGF-derived peptides[155], some with orexigenic activity (eg NERP2), and others with anorectic and catabolic actions (e.g. TLQP21,[156]). Clearly, more sophisticated

experimental tools will be necessary to understand better the seasonal function of this peptidergic system. Another peptidergic system worthy of further study is somatostatin, as hypothalamic expression of this gene decreases markedly in LP in Siberian hamsters, then increases in SP[96], and expression is downregulated by intracerebroventricular infusion of TSH in hamsters – suggesting again that it is a target of tanycyte-produced TH[90]. Somatostatin is a key inhibitor of pituitary growth hormone so likely contributes to seasonal growth cycles via this route. However, given that treatment of hamsters with the somatostatin agonist pasireotide can promote a wide range of SP responses in addition to growth/metabolic adaptations, such as gonadal involution[157] and enhanced frequency of torpor bouts[158], it seems likely that somatostatin has additional central mechanisms of action.

Connecting tanycytes and GnRH: the neuropeptides Kisspeptin and RFRP3

The conserved TSH-dependent retrograde pathway discussed above is primarily involved in the regulation of seasonal breeding. However, neurons producing GnRH are located within the hypothalamic preoptic area, rostrally to the MBH. The question arises as to how T3 produced within the MBH impacts GnRH secretion, hence LH/FSH production by the PD. It is now obvious that the KP family of neuropeptides, encoded by the *Kiss1* gene, expressed in the arcuate nucleus of the hypothalamus, play a central role in the seasonal control of breeding, being strongly modulated by melatonin[159], and also by sex steroids[160,161]. The neuropeptide RFRP3 (RF-amide Related Peptide 3), encoded by the *Npvf* gene, which is expressed in the dorsomedial/ventromedial nuclei of the hypothalamus, is strongly downregulated by melatonin[162,163] and may also play a role [Figure 1 & Figure 3A]. *Kiss1* and *Npvf* display large opposite seasonal variation in expression, modulated in a sex and species-specific manner: while *Kiss1* expression is generally – but not always[20,164] – higher in the breeding season, there is a conserved downregulation of *Npvf* expression in short days, in

all long- and short-day breeders studied[165]. The role of these neuropeptides in seasonal breeding has been extensively reviewed over the last years[165–168].

KP has emerged as the most potent GnRH secretagogue, and its role in the central control of all aspects of breeding, from puberty onset to regular oestrus cycles through to seasonal breeding, is unequivocal[169]. In contrast, a role for RFRP3 in the control of breeding is still controversial. It was initially proposed that KP and RFPR3 play opposite roles towards the gonadal axis (the "yin/yang model"[170,171]). However, recent findings are inconsistent with such a simple scenario: studies in hamsters disclose a stimulatory role for RFRP3 in SP-kept male hamsters, but an inhibitory role in LP[163,172,173]; mice KO for the Npffr1 receptor (RFRP3 receptor;[174]) had no overall fertility deficits. In sheep, RFRP3 has been reported to have no effect[175] or to inhibit gonadotropin secretion[176]; and in horse, RFRP3 has no effect upon GnRH-mediated LH release (Thorson et al 2014). *Npvf* expression might be regulated by metabolic cues[177] and temperature[178], somehow obscuring the impact of photoperiod. Central TSH infusion[90] or TH implants[179] in long-day breeders kept under SP, consistently impacted the expression of *Kiss1* and *Npvf*, which then reverted to LP-like profiles. No data are available for any short-day breeder.

Overall, current data place these two cell populations in a local hypothalamic circuit downstream of T3 production by tanycytes. While divergence downstream of T3 is anticipated (see[1], and above), we deem it likely that differential control of these two cell populations – by mechanisms which remain to be characterized – might explain the wide array of reproductive seasonal outputs; i.e. the neuropeptides KP and RFRP3 might constitute the common conduit towards GnRH control (at least in mammals since birds lack a *Kiss1* gene). Further studies will be required to clarify (i) whether *Kiss1*- and *Npvf*-expressing cells

establish (reciprocal) synaptic communication, (ii) the impact of sex steroids, photoperiod, temperature and metabolic status upon the expression of both genes and (iii) the anticipated role of KP at the level of the GnRH neuron endfeet in the ME. To be meaningful, these goals will have to be met in multiple species, since seasonal timing of breeding is in essence a comparative question.

Challenges and insights

Our current knowledge of the central mechanisms underlying seasonality highlights a conserved neuroendocrine pathway involving PT TSH-mediated regulation of tanycyte DIO2/DIO3 balance, which in turn drives seasonal switches of T3 availability in the MBH. As mentioned before, whether the seasonal changes in deiodinase expression actually lead to corresponding modulation of T3 levels across species is contentious, especially because data are not available for short-day breeders. If we assume that the LP-triggered increase of T3 levels in the MBH is a conserved feature – i.e. present in both long-day and short-day breeders – it follows that this pathway alone cannot explain the divergence in seasonal breeding and metabolic strategies[1]. At this stage, there is no simple explanation to this, but we might emphasize several plausible scenarios, which are not mutually exclusive.

First, it is very likely that several species-specific paracrine/autocrine circuits operate in parallel. For instance, TSH and NMU, WNT or retinoic acid might provide complementary signalling, which lead to long-day activation of the HPG axis in hamsters. Notably, there is no conspicuous seasonal changes in expression of members of NMU, WNT or retinoic acid signalling pathways in sheep[63], as already pointed by others[1]. As mentioned earlier, species-specific combinations of specific growth factors (or others), acting at the level of tanycytes or elsewhere, might also be involved. In addition, different responsiveness to these signals

might be driven by species-specific gene regulatory elements. Second, one might consider a simpler explanation, which involves hypothalamic populations expressing *Kiss1* and *Npvf*. In mouse, ~90% of neurons expressing *Kiss1* in the arcuate nucleus are glutamatergic[106,180,181], even though a substantial fraction may also use GABA[180]. In sheep, *Kiss1*-expressing neurons are also mostly glutamatergic[182]. In mouse, there is good evidence that *Npvf* neurons are glutamatergic too[108], and that distinct subpopulations of *Npvf* neurons may exist[183]. No data are available in sheep or hamsters regarding the neurochemical identity of *Npvf*-expressing neurons or the existence of neuronal subpopulations. Overall, we know very little regarding neurotransmitter content and fine organization of neurons producing KP and RFRP3 in seasonal species. Could these neuronal (sub)populations use different neurotransmitters in different species? What about potential neuronal connections between these two neuronal populations? An effort will have to be made to provide answers to these questions in the different photoperiodic models. Third, there is strong evidence that the seasonal circuit controlling seasonal breeding in sheep involves the dopaminergic A15 nucleus[184,185], which does not exist in hamsters. Therefore, species-specific circuitry downstream of T3 might also explain the plasticity in timing of seasonal breeding.

A fourth point concerns the impact of sex steroids upon the seasonal cycle of LH/FSH and the expression of *Kiss1* and *Npvf*. In ewes, it is obvious that E2 is required for the seasonal switches in LH/FSH[186,187]; it might indeed be permissive to the impact of T3 (see above and[27]). In contrast, castration in mares[188], female quail[189] or snowshoe hares of both sexes[190] does not blunt seasonal fluctuations in LH/FSH. Therefore, the role played by sex steroids in the seasonal organization is species-specific. Interestingly, sex steroids dampen *Kiss1* expression in neurons of the arcuate nucleus in virtually all mammals studied and this is recognized as a key feature for the control of seasonal breeding (reviewed in[166,168,191]). The

sex steroid sensitivity of *Npvf*-expressing neurons has comparatively received little attention and available data are discordant[165]. However, gonadectomy does not appear to affect *Npvf* expression in Syrian, Siberian or European hamsters[20,162,164,173], while it affects the expression of *Kiss1*. This suggests that *Npvf*-expressing neurons are not *bona fide* targets of sex steroids and also weakens the hypothesis that the two subpopulations are synaptically connected, at least in hamsters. In contrast, our unpublished data in ewes comparing intact, OVX (ovariectomized) and OVX+E2 implanted animals in May and November (seasons of anestrus and breeding, respectively) reveals a profound and almost opposite impact of sex steroids on the expression of *Kiss1* and *Npvf* (Dardente and Lomet, unpublished). This illustrates a species-specific response of *Kiss1* and *Npvf* to sex steroids and suggests an anatomical connection (direct or indirect) between these neuronal populations in sheep. In conclusion, we surmise that species-specific temporal organization beyond the TSH/DIO/T3 axis may be due to the use of multiple signals, a differential use of neurotransmitters, a distinctive neuroanatomical organization in circuits involving neurons produce KP and RFRP3, and/or a varying degree of sex steroid responsiveness of these populations or other neuronal or glial populations involved in the pathway (e.g. tanycytes).

How phylogenetically conserved is the TSH/DIO/T3 axis? Thus far, compelling evidence has been gathered in multiple species of birds and mammals. There are no data about the conservation of this pathway in reptiles and amphibians, but these vertebrates have a distinct PT and show a roughly similar organization of the MBH region[192], which provides neuroanatomical ground for conservation. The fish pituitary instead does not appear to include a PT-like region[192]. There is some evidence for the existence of a specific TSH/DIO/T3 axis in fish, but with substantial differences from the mammalian models. In salmon, the *Tshß*/*Dio2* response to LP is conserved, but this occurs in another directly

photoreceptive structure called the *saccus vasculosus*[4,193]. In addition, genome duplication in fish may have allowed for some level of plasticity through specialization of paralogues along the putative TSH/DIO/T3 axis. Fleming *et al* reported expression of two distinct *Tshß* subunits in the salmon pituitary, one of which (*Tshßb)* exhibits a marked induction as daylength increases from late winter onwards and a specific pattern of expression in the dorsal region near the pituitary stalk, a location comparable to the PT in mammals[194]. Differential tissue expression and response to photoperiod have also been reported for *Dio2* paralogs in salmon[195]. In stickleback, *Tshß* expression in the pituitary is acutely, but very transiently, induced by LP exposure[196]. The transient nature of the response may explain the lack of difference in *Tshß* expression observed by others in sticklebacks adapted to SP or LP[197] From a general standpoint this finding calls for a cautious (re)interpretation of prior data, which examined and compared this axis in animals maintained under LP or SP for various durations. These gaps in our knowledge on the phylogenetic conservation of the TSH/DIO/T3 axis have to be filled to enlighten the evolution of photoperiodic read-out mechanisms.

We thus believe that comparative physiology is key to further our understanding of seasonal time-keeping mechanisms. The ever-increasing availability of sequenced and annotated genomes in vertebrates along with the development and relative affordability of large-scale approaches in transcriptomics (RNAseq/single-cell RNAseq/ChIP-seq, etc) and proteomics now makes it possible to address questions at the genome-wide level in non-model species. Such approaches should be applied to the MBH of multiple species under a range of photoperiodic manipulations to gain insights into the level of conservation of the TSH/DIO/T3 axis and other pathways. One might predict a low level of conservation, limited to a few key components, as demonstrated for circadian clocks (and clock genes) across

species and tissues (e.g.[198]). Pharmacological approaches should also be developed to investigate the seasonal change of tanycyte sensitivity to TSH signalling (and other newly identified diffusible factors, see below) since this might be central to the organization of circannual timing.

The role of alternative signalling pathways in hamsters (e.g. NMU, WNT or RA) and recently identified secreted factors in sheep (e.g. Vmo1, Fam150b, Areg, Shh; see ref[63]) in seasonal physiology might be explored by long-term intracerebroventricular infusions or hypothalamic implants, as previously done for other peptides[90,137,159,172,173] or the use of recombinant viral vectors, which are effective in Siberian hamsters[155]. CRISPR/Cas9 technology (e.g. in hamsters[199]) instead, would be beneficial to explore the requirement of any of these genes for the seasonal response. For instance, deleting *Dio3* would allow a direct test of the hypothesis that a "hypothyroid MBH" state is required for the transition to winter physiology. However, the use of CRISPR/Cas9 in hamsters and sheep is arguably limited due to technical challenges, time (especially true for long-lived species), financial issues and, crucially, the fact that such an approach produces systemic mutations, which complicates data interpretation. Clearly, commercially available strains of hamsters and sheep to perform intersectional genetics, akin to the CRE-LoxP system in mouse, is way beside the point. However, the use of genetically modified mouse models could be occasionally beneficial for interrogating signalling pathways to complement studies in seasonal species (e.g.[57,92,200]). Even though our understanding of the cellular and molecular underpinnings of seasonality and circannual clocks improved significantly over the last decade, there are great challenges and many more surprises ahead of us.

Data Availability Statement

Data sharing is not applicable as no new data were created or analysed in this article.

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Figure legends

Figure 1: Neuroendocrine pathways of seasonality

A. In mammals, the photic input pathway from the retina to the suprachiasmatic nuclei (SCN) drives rhythmic melatonin production from the pineal gland. This melatonin signal provides an internal endocrine representation for external photoperiod. Short (winter) photoperiods are represented by increased duration of melatonin and long (summer) photoperiods by short duration of melatonin.

B. Retrograde action of TSH on ependymal cells in the hypothalamus (blue box): The prime site of melatonin action is the pituitary *pars tuberalis*. PT-derived TSH is translocated back to the hypothalamus where it binds to TSH receptors (TSHR) expressed in tanycytes lining the third ventricle. This regulates the expression of deiodinases (Dio2 and Dio3), which in turn control the local metabolism of thyroid hormone (T4 to T3 conversion). Changes in T3 availability modulate energy metabolism and reproductive circuits. RF-amide peptides (i.e. Kisspeptin and RFRP3) likely serve as neuroendocrine intermediates in the regulation of reproduction. Anterograde action (red box) is believed to control seasonal prolactin (PRL) secretion from lactotrophic cells in the *pars distalis*, which drives the pelage/moult cycle. The pathway is stimulated through secretion of low molecular weight molecules (collectively termed “tuberalins”) produced in the PT and transported to the PD through the portal blood system. To date, several tuberalin candidates have been proposed including Tachykinins (TAC1) and endocannabinoids (2-AG).

Figure 2: The binary switch model for PT cells

A. The binary switch model proposes that an endogenous timer switches TSHß/EYA3 expression in the PT thyrotroph cells, driving TSH and hypothalamic TH metabolism

independently of photoperiod. Individual PT thyrotroph cells are either in a long (TSH/EYA3+) or short (CHGA+) photoperiod state, and the relative proportion of these binary-state cells determines the phase of the circannual cycle. Also shown are the cellular remodelling that occurs with season, thyrotrophs get bigger in summer and reorganise to increase junctional contacts. In winter, folliculostellate cells form a network with increased junctional contacts and thyrotrophs are isolated from each other. After data from[41].

B. Vimentin immunostaining for tanycytes (brown) of coronal section of the sheep mediobasal hypothalamus (upper panels). Scale bar = 100µm & 20µm respectively. PT - pars tuberalis, Me - median eminence, 3V - third ventricle, HYP – hypothalamus. 3D render series of IHC images showing GnRH (red), vimentin (green) and DAPI (blue) in SP and LP. Scale bar = 50µm. After from[41].

Figure 3: Key roles for PT and tanycytes in the seasonal control of breeding and food intake

A. Model for the seasonal control of the gonadal axis by the PT-DIO axis in sheep. Under LP, low melatonin action in the PT translates into up-regulation of the *Eya3*/*Tshß*/*Dio2* axis. We recently identified novel PT-expressed genes which display photoperiodic variations. Their respective roles and their potential control by EYA3 are unknown. Within tanycytes, TSH triggers *Dio2* expression and T3 production. We identified several novel genes which display large photoperiodic variation and regulation by autocrine T3 feed-back. These genes might govern seasonal GnRH output, perhaps by acting at the level of the median eminence. Finally, the expression of both *Kiss1* and *Rfrp*, modulators of GnRH, are also subject to photoperiodic control; whether this depends upon input from tanycytes or factors coming from the PT remains unknown (question marks). The circannual clock might be located in the PT; it might also comprise tanycytes. After data from[63,109].

B. Tanycytes are a hub for a host of environmental signals towards the regulation of food intake and metabolism. Not only photoperiod, but also nutritional status and various endocrine and paracrine signals impinge on tanycytes. These signals interact to regulate the DIO2/DIO3 balance, hence T3 signaling within the hypothalamus. At least in hamsters, retinoic acid (RA) signaling might modulate T3 signaling. This complex network finely tunes various aspects of metabolism. Lower panels: tanycytes comprise a population of stem cells and directly sense nutrients.

Figure 4: Sensitization of TSHR signalling in tanycytes is affected by photoperiodic history A. Siberian hamsters with LP (16 h light/day) or SP (8 h light per day) history show a similar level of PT *TSHß* expression when raised in intermediate photoperiod (14 h light/day). However, *dio2* gene expression and in turn testis size are highly increased (red arrowheads) in animals with short photoperiodic history (8→14) as compared to animals with long photoperiodic history (16→14). After data from[40].

B. Sheep with a history of SP (8h light/day) exposed to step-wise increases in photoperiod show increases in *dio2* gene expression with minimal or no change in *TSHß* expression. This change is reflected in testosterone levels that switch over photoperiods in the range from 11.75 to 12.5 h (red arrowheads). After data from[136].

C. Photoperiodic-history affects tanycyte sensitivity to TSH signalling at a level that remains to be determined (question mark), leading to differential *dio2* gene expression in response to a given *pars tuberalis* TSH signal.