



**HAL**  
open science

# GnRH and the photoperiodic control of seasonal reproduction: Delegating the task to kisspeptin and RFRP-3

Hugues Dardente, Valérie Simonneaux

► **To cite this version:**

Hugues Dardente, Valérie Simonneaux. GnRH and the photoperiodic control of seasonal reproduction: Delegating the task to kisspeptin and RFRP-3. *Journal of Neuroendocrinology*, 2022, 34 (5), pp.e13124. 10.1111/jne.13124 . hal-04279910

**HAL Id: hal-04279910**

**<https://hal.science/hal-04279910>**

Submitted on 13 Nov 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# GnRH and the photoperiodic control of seasonal reproduction: Delegating the task to kisspeptin and RFRP-3

Hugues Dardente<sup>1</sup> | Valérie Simonneaux<sup>2</sup> 

<sup>1</sup>CNRS, IFCE, INRAE, Université de Tours, PRC, Nouzilly, France

<sup>2</sup>Institute for Cellular and Integrative Neuroscience, University of Strasbourg, Strasbourg, France

## Correspondence

Hugues Dardente, CNRS, IFCE, INRAE, Université de Tours, PRC, F-37380, Nouzilly, France.

Email: hugues.dardente@inrae.fr

Valérie Simonneaux, Institute for Cellular and Integrative Neuroscience, University of Strasbourg, F-67000 Strasbourg, France. Email: simonneaux@inci-cnrs.unistra.fr

## Funding information

ANR, Grant/Award Numbers: GMO-Phen ANR-20-CE14-0031-01, Repramide ANR-13-BSV1-001

## Abstract

Synchronization of mammalian breeding activity to the annual change of photoperiod and environmental conditions is of the utmost importance for individual survival and species perpetuation. Subsequent to the early 1960s, when the central role of melatonin in this adaptive process was demonstrated, our comprehension of the mechanisms through which light regulates gonadal activity has increased considerably. The current model for the photoperiodic neuroendocrine system points to pivotal roles for the melatonin-sensitive pars tuberalis (PT) and its seasonally-regulated production of thyroid-stimulating hormone (TSH), as well as for TSH-sensitive hypothalamic tanycytes, radial glia-like cells located in the basal part of the third ventricle. Tanycytes respond to TSH through increased expression of thyroid hormone (TH) deiodinase 2 (*Dio2*), which leads to heightened production of intrahypothalamic triiodothyronine (T3) during longer days of spring and summer. There is strong evidence that this local, long-day driven, increase in T3 links melatonin input at the PT to gonadotropin-releasing hormone (GnRH) output, to align breeding with the seasons. The mechanism(s) through which T3 impinges upon GnRH remain(s) unclear. However, two distinct neuronal populations of the medio-basal hypothalamus, which express the (Arg)(Phe)-amide peptides kisspeptin and RFamide-related peptide-3, appear to be well-positioned to relay this seasonal T3 message towards GnRH neurons. Here, we summarize our current understanding of the cellular, molecular and neuroendocrine players, which keep track of photoperiod and ultimately govern GnRH output and seasonal breeding.

## KEYWORDS

medio-basal hypothalamus, melatonin, pars tuberalis, pineal gland, tanycyte

## 1 | INTRODUCTION

The axial approximately 23.5° tilt of the rotating Earth along with its yearly revolution around the Sun generate immutable annual changes in solar radiation. These recurrent fluctuations in photoperiod, rainfall, temperature and subsequent overall food availability are more marked as one moves away from the equator towards either pole. Nevertheless, plants and animals managed to colonize almost every biotope, even the most challenging. Biological seasonal rhythms are a critical

feature of most organisms inhabiting the Earth. Hibernation/torpor, migration, body weight fluctuations and switches in reproductive states are iconic examples of seasonal functions, which bear high adaptive value because they all support survival of the individual and perpetuation of the species.<sup>1–5</sup> These rhythms should not be considered independently because they are all part of a rather strict species-specific temporal program. For example, to survive the harsh winter season, the Siberian hamster (*Phodopus sungorus*) reduces its energy demand by inhibiting reproductive activity, reducing body weight,

changing pelage and displaying daily torpor, which are all reversed in late winter to breed and give birth to the offspring during spring. Then, in autumn, the animal undergoes a drastic inhibition of reproduction and loses weight, before entering into torpor again.<sup>6</sup> This adaptive process requires animals to keep track of solar time, which, through resetting of endogenous clocks, will help anticipating upcoming changes and adjusting biological functions to seasons.

At temperate latitudes, photoperiod is the main cue driving seasonality in physiological functions. Other cues may further modulate the impact of photoperiod upon seasonal functions.<sup>3,7</sup> This is particularly well documented for seasonal breeding, which can be modulated by food availability and quality, temperature, social interactions and various stressful conditions. For example, positive or negative metabolic challenges usually translate into a slightly increased or decreased duration of the breeding season, respectively, whereas interaction with a sexually active ram/buck may allow punctual out-of-season ovulation and breeding in ewes/goats, a feature known as the “male effect.”<sup>8–11</sup>

## 2 | PHOTOPERIODIC CONTROL OF MELATONIN

In mammals, melatonin is the endocrine messenger of photoperiod.<sup>12–15</sup> Even though melatonin can be produced by other tissues, the pineal gland is solely responsible for the nightly increase in melatonin plasma levels. The pineal gland integrates the photoperiodic message through the so-called retino-hypothalamic tract. This tract consists of intrinsically-photosensitive retinal ganglion cells (ipRGCs), expressing the photopigment melanopsin, which project to the hypothalamic suprachiasmatic nuclei (SCN), where the main biological clock is located.<sup>16,17</sup> The ipRGCs projections release pituitary adenylate-cyclase-activating polypeptide and glutamate to the retino-recipient, ventro-lateral region of the SCN, which in turn modulates the circadian molecular clockwork through a cAMP/P-CREB mediated transcriptional activation of the clock genes *Per1/Per2*.<sup>18–20</sup> This mechanism is responsible for the photic resetting of the SCN master clock, which then synchronizes a myriad of secondary central and peripheral circadian clocks through neural and/or humoral pathways.<sup>21–24</sup> The SCN connects to the pineal gland through a multi-synaptic neural pathway, leading to the nocturnal activation of melatonin synthesis and its immediate release.<sup>14</sup> Remarkably, the annual change in day length modulates daily electrical activity and clock gene transcription in the SCN,<sup>25–27</sup> so that, in turn, the SCN-driven melatonin secretion exhibits changes in the duration of its nocturnal production, which carry time-of-year information to melatonin-responsive target tissues. The duration of the nocturnal melatonin peak (and neither its amplitude, nor its phase) is the critical feature that drives seasonal breeding.<sup>14,28</sup> In mammals, lack of melatonin, following either pinealectomy or disruption of the SCN-to-pineal pathway, renders the animals blind to photoperiod and the reproductive output no longer shows synchronized alternations of activity and quiescence.<sup>12,14,29,30</sup> Of special note, birds (and most likely other non mammalian vertebrates) appear to use pineal-independent mechanisms to drive seasonal rhythms, notably through photoreceptive cells located in the hypothalamus itself.<sup>4,31,32</sup>

Because gonadotropin-releasing hormone (GnRH) neurons, which are scattered throughout a rostro-caudal continuum extending from the preoptic area (POA) to the mediobasal hypothalamus (MBH), constitute the pinnacle of the hypothalamic-pituitary gonadal (HPG) axis, the simplest scenario for the neuroendocrine control of seasonal breeding would have been one in which GnRH neurons are directly responsive to melatonin. However, and akin to that found later for sex steroids, early findings showed that neither the number of detectable GnRH neurons, nor *GnRH* gene transcription are drastically affected by photoperiod, even though the data are arguably at variance.<sup>33–37</sup> Such findings suggested that seasonal control of GnRH output might be indirect,<sup>38</sup> and achieved through a controlled release of the decapeptide at nerve terminals of the median eminence, rather than through transcriptional control of the *GnRH* gene at hypothalamic cell bodies.

## 3 | TARGET TISSUES OF MELATONIN

Studies using radioactive iodo-melatonin as a highly specific ligand shed some light on the possible central target tissues for the hormone. These early studies were shortly followed by cloning of two high-affinity G-protein coupled receptors for melatonin, now known as MT1 and MT2, which allowed in situ detection of MT1/MT2 transcripts.<sup>15,39,40</sup> In brief, and even though data somehow differed between species and laboratories (mostly for melatonin binding assays), several firm conclusions could be reached.<sup>41,42</sup>

First, it is indeed the MT1, and not the MT2, that is involved in the seasonal control of breeding, and most likely other seasonal functions as well.<sup>43–46</sup> Thus, the highly seasonal Siberian hamster bears a natural knockout of MT2<sup>43</sup> and, in most species, MT2 is expressed to much lower levels and by fewer brain nuclei and structures compared to MT1; however, see also Klosien et al.<sup>47</sup> Second, across all vertebrate species, be they seasonal or not, only a single structure displayed consistent melatonin binding and MT1 expression: the pars tuberalis (PT), an endocrine tissue which constitutes the most rostral part of the adenohypophysis. Furthermore, melatonin binding in the brain of the ferret, a highly seasonal species, is exclusively present in the PT.<sup>48</sup> Finally, recent evidence from transgenic mouse models confirms that the MT2 is not expressed in the PT.<sup>47</sup> These findings indicate that the PT might be the hotspot for the neuroendocrine control of seasonality through the MT1 melatonin receptor.

## 4 | THE PT IS THE MELATONIN TARGET TISSUE DRIVING SEASONALITY

Subsequent to the discovery that MT1 melatonin receptors are highly expressed in the PT, evidence has accumulated to convincingly demonstrate that this tissue is the master-controller of seasonal breeding in mammals.

The MT1 is heavily expressed in the PT and findings from the early 1990s, often obtained using ovine primary cell cultures, showed that

melatonin exerts an acute time-dependent inhibition upon cAMP signaling.<sup>41,42,49,50</sup> These findings led to an initial model for photoperiodic time-measurement (PTM) in which melatonin keeps cAMP-dependent pathways silent during the night but allows a strong rebound in activation in the morning as melatonin gets cleared from the bloodflow.

The PT rhythmically expresses multiple clock genes, with consistent phase relationships between their peaks of expression, thereby substantiating the notion that the PT harbors a functional circadian clock.<sup>50–55</sup> Importantly, the amplitude of the morning peak of *Per1* mRNA displays a marked decrease in short days compared to long days. Furthermore, it was found that the expression of *Cry1*, a key transcriptional repressor of the CLOCK/BMAL1 heterodimer, is directly induced by melatonin in the PT,<sup>55–58</sup> leading to a second refined model for PTM in the (ovine) PT, based upon the time interval between the *Per1* peak, phase-locked to dawn, and the *Cry1* peak, phase-locked to dusk.<sup>54</sup> The current, third PTM model, posits that melatonin resetting of the PT circadian clock (through *Cry1* induction) also phase-locks peak expression of a clock-controlled gene, *Eya3*, which occurs approximately 12 h after *Cry1*. Because *Eya3* expression is also blunted by melatonin, this leads to a classic “external coincidence model” in which *Eya3* phase is dictated by the clock while its amplitude depends on the presence (short days, hence long nights) or absence (long days, hence short nights) of melatonin.<sup>59–62</sup> This melatonin-driven seasonal switch in the pattern of *Eya3* expression is crucial because the EYA3 protein acts as co-activator of two other transcription factors, SIX1 and TEF, which together control transcription of the *Tshb* gene, and hence seasonal thyroid-stimulating hormone (TSH) output from the PT.<sup>61,63</sup>

Seasonal control of *Tshb* expression and TSH production in the PT was first demonstrated in the Siberian hamster in the late 1980s.<sup>64</sup> PT thyrotropes are anatomically and functionally different from the thyrotropes of the pars distalis (PD). PT thyrotropes are round-shaped cells that display profound seasonal changes in their ultra-structure and organization, as assessed by electron microscopy.<sup>65,66</sup> By contrast to PD thyrotropes, which are under the control of the thyroid axis, PT thyrotropes lack receptors for thyrotropin-releasing hormone and triiodothyronine (T3)<sup>67</sup> and are therefore mostly sensitive to melatonin because they express MT1 receptor.<sup>68,69</sup> Recent transcriptomics studies in sheep have demonstrated that, beyond clock genes, *Tshb* and *Eya3*, there are indeed several hundreds of PT-expressed genes that display large seasonal switches in expression level.<sup>7,66,70–72</sup> To conclude, there is overwhelming evidence that the PT is sensitive to the photoperiodic melatonin signal, which impacts its structure, function, molecular signature and endocrine output.

## 5 | LINKING PT-TSH SEASONAL OUTPUT TO GNRH CONTROL: AN UNEXPECTED ROLE FOR LOCAL THYROID HORMONE METABOLISM AND SIGNALING

Back in the 1930s, it was found that thyroid hormones (TH) may be pivotal for seasonal functions because thyroidectomy impacts seasonal

breeding in birds.<sup>7,73–76</sup> More precisely, it was found that lack of TH impairs photorefractoriness, a process by which an animal no longer responds to the prevailing photoperiod so that its physiological status spontaneously reverts to the opposite reproductive phenotype.

This impact of thyroidectomy upon photorefractoriness suggests that T3 (i.e., the active form of TH compared to thyroxine [T4]) is involved in the control of seasonal breeding. Evidence for this has accumulated in the 1980s and 1990s using sheep as a model.<sup>15,77</sup> In particular, it was found that T3 plays a permissive role<sup>78</sup> through a central mechanism located in the MBH. In parallel, great advances in our understanding of TH action have been made.<sup>79–82</sup> First, and in contrast to prior belief, T4/T3 do not passively cross membranes but need dedicated transmembrane carriers, which implies that TH do not passively diffuse into the brain. Second, TH metabolism might be achieved locally to finely impact cellular events during development and in adulthood.<sup>83</sup> Within the brain, this local regulation is performed by enzymes known as TH deiodinases (DIO). DIO2 converts T4 into active T3, whereas DIO3 converts T4 into rT3 and T3 into T2. Therefore, the DIO2/DIO3 balance governs local TH signaling pathways. Interestingly, although *Dio2* and *Dio3* are expressed in only few brain regions, both are enriched in tanycytes, radial glia-like cells that line the walls of the third ventricle with endfeet processes found close to the median eminence and in contact with neurons of the arcuate nucleus. Furthermore, specific TH transporters such as MCT8 (Slc16a2) and OATP1C1 (SlcO1c1) are also expressed in tanycytes. Tanycytes are therefore endowed with the ability to regulate TH signaling at the brain-periphery interface, which might be key to the regulation of a wide array of functions, from reproduction to inflammation and metabolism.<sup>84–88</sup>

Using quail as a model, Yoshimura et al.<sup>89</sup> demonstrated that *Dio2* and *Dio3* display opposite photoperiodic regulation in tanycytes, with high *Dio2* in long days and high *Dio3* in short days. This photoperiodic balance leads to a local increase in T3 production under long days, consistent with the role of T3 as a “mediator of long days.”<sup>15</sup> In a further twist, work in quail,<sup>90</sup> sheep<sup>91</sup> and hamsters<sup>92</sup> demonstrated that the long-day increase in *Dio2* expression results from PT-TSH activation of its cognate receptor, TSHR, highly expressed by tanycytes, via a Gs-induced increase in cAMP levels. Similarly, the long-day decrease in *Dio3* appears driven by TSH because both *in vivo*<sup>93</sup> and *in vitro*<sup>94</sup> studies in rats have shown that TSH inhibits tanycytic *Dio3* expression, although the cellular mechanism involved is still unknown. The photoperiodic changes in TSH and DIO2/DIO3 have subsequently been described in almost every bird and mammal species examined to date.<sup>95–101</sup> Even though there are species difference in the level of *Dio2* and *Dio3* expression, the TSH-induced DIO2/DIO3 switch always results in increased T3 in long days. Whether this axis is also present in other vertebrates, especially in fish, remains contentious.<sup>5</sup> Because TSH produced by the PD of the pituitary is also present in the blood flow, one may wonder how it is prevented from acting upon tanycytes, which would seriously interfere with photoperiod decoding. Although *Tshb* mRNA is identical between the PT and PD,<sup>67</sup> murine PT-TSH displays a glycosylation pattern distinct from PD-TSH, which would account for its specific action on TSHR in tanycytes.<sup>102</sup> However, TSHR in the tanycytes of multiple species is readily activated by TSH from ovine and bovine

pituitary (i.e. mostly PD-TSH) extracts that are unlikely to contain significant amounts of PT-TSH.<sup>90–94,103,104</sup> An unresolved question is how PT-TSH reaches TSHR in tanycytes. One plausible explanation is that it is retrogradely taken up by the endfeet of  $\beta 2$  tanycytes (at the bottom of the third ventricle) then released into the cerebrospinal fluid, where it eventually acts on  $\beta 1$  and  $\alpha$  tanycytes lining the walls of the third ventricle.<sup>86,88</sup> However, assaying TSH in cerebrospinal fluid samples collected at the bottom of the third ventricle of ewes throughout the year failed to reveal the presence of TSH (H. Dardente & D. Lomet, unpublished data). Another possibility is that TSH acts upon tanycytic processes and endfeet, through the specific ME-MBH microcirculation.<sup>88,105</sup>

## 6 | CONCLUSIONS: THE PT AS A SEASONAL HUB

Early studies in the 1990s, proposed that distinct hypothalamic nuclei, including the dorso-median hypothalamus (DMH) and the area of the premammillary hypothalamus, are pivotal for the control of seasonal breeding because these nuclei exhibit binding sites for iodo-melatonin and studies using lesional approaches and chronic melatonin implants reported significant effects on seasonal breeding.<sup>2,15,42,106</sup> However, definitive evidence for a functional role of melatonin for seasonal breeding in these areas has not been obtained. First, considering the MBH as a whole, expression of clock genes is mostly restricted to the PT because the expression elsewhere is at best very faint and never rhythmic, at least in sheep. Second, apart from the PT, the hypothalamic nuclei of the MBH exhibit no (i.e., undetectable) mRNA encoding MT1 and these are likely “soaked” in high melatonin levels throughout the day.<sup>107</sup> Therefore, because seasonal timing is based upon accurate daily timing, orchestrated by a melatonin-dependent circadian clock,<sup>108,109</sup> it is very unlikely that these nuclei could discriminate day from night and, consequently, long days from short days. Furthermore, a number of serious shortcomings associated with the use of melatonin binding assays, melatonin implants and electrolytic lesions, have been exposed and explained previously.<sup>15,42</sup>

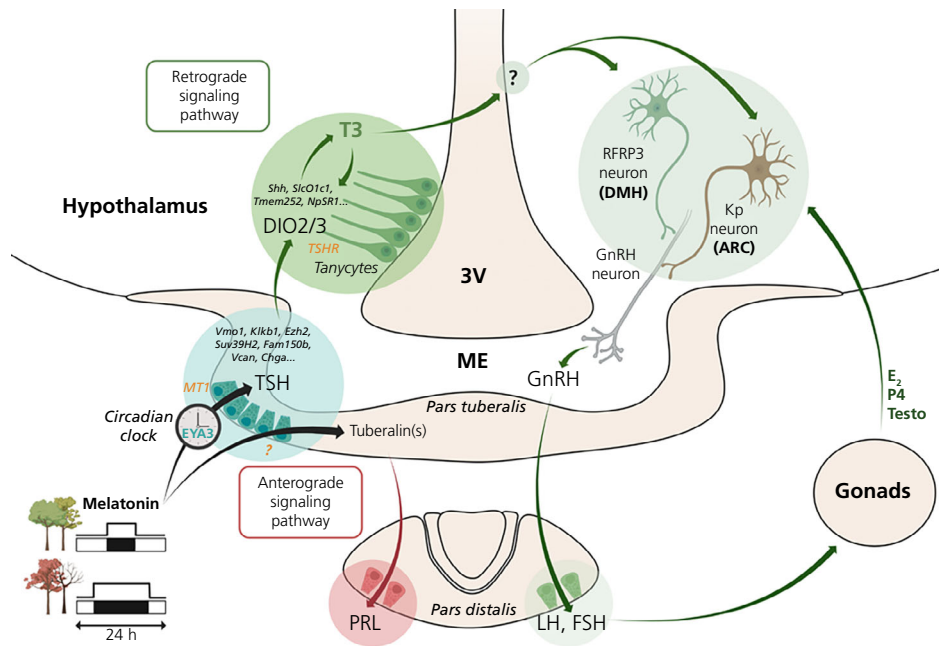
Currently, there is a general consensus that the PT possesses all the cardinal features of a tissue involved in the seasonal control of breeding. It connects melatonin-dependent photoperiodic input to the T3-dependent GnRH output through seasonal regulation of TSH, and perhaps other secreted factors (such as neuromedin U<sup>93</sup>). This mechanism includes a major intermediary role for tanycytes in translating the TSH input into a long-day T3 signal. Therefore, in contrast to expectations, the photoperiodic control of the gonadotropic axis does not rely on an anterograde signaling pathway because it implicates the intervention of hypothalamic tanycytes, and most likely populations of hypothalamic neurons (see below). Therefore, this axis is often referred to as “retrograde signaling”, going from the pituitary back to the hypothalamus (Figure 1).

Although it is indisputable that the PT seasonal activity is driven by melatonin, there is also evidence that the PT sustains endogenous long-term cycles with a period close to 10 months, which may in turn

drive circannual rhythms of physiology.<sup>66,110–115</sup> Circannual rhythms have been described in some, but not all, seasonal species, because they likely are more important for species exhibiting hibernation/torpor or migration. Indeed, in species unable to detect upcoming environmental changes (e.g., living in burrows), this mechanism is responsible for continuation of seasonal phenotypes, such as breeding reactivation or exit of hibernation.<sup>115</sup> There is now strong evidence that the PT hosts an endogenous circannual timer. Thus, in animals kept under a constant photoperiod for several weeks, the expression of some PT markers exhibits spontaneous induction or repression, in a pattern congruent with what would occur if photoperiod had been changed. The PT displays circannual fluctuations in *Tshb* and *Eya3* expression in sheep and hamsters, which are accompanied by changes in *Dio2/Dio3* expression and concomitant modifications of both reproductive and metabolic phenotypes.<sup>114–119</sup> Furthermore, recent RNA-sequencing analyses in sheep have identified hundreds of genes that display strong photoperiodic and circannual control of expression.<sup>62,66,70,72,120</sup> These inner switches in expression seem to be accounted for by epigenetic changes at the gene promoter level, as shown for *Tshb* and *Eya3*.<sup>62</sup> Furthermore, the PT-expressed histone methyltransferases *Ezh2* and *Suv39H2* display a robust increase in expression under long days compared to short days.<sup>70</sup> Therefore, evidence for circannual timing in the PT can be tracked down to the epigenetic level.

Given the prevailing role of the PT in the synchronization of seasonal reproduction, one might wonder whether additional signals, other than the photoperiodic melatonin, impact its activity. RNA-sequencing has led to the identification of dozens of reliable molecular markers, which can be used to probe this hypothesis. Our current findings show that T3 and estradiol ( $E_2$ ) do not have any noticeable impact upon the expression of PT markers of photoperiod.<sup>70,121</sup> Preliminary data in voles and sheep show a moderate impact of food-restriction on *Tshb* and *Eya3* expression within the PT<sup>122</sup>; H. Dardente & D. Lomet, unpublished data). Finally, a melatonin-independent effect of photoperiod on PT-TSH and downstream seasonal phenotypes was recently reported in the European hamster,<sup>123,124</sup> but the mechanisms involved remains to be elucidated.

Aside from the control of the HPG axis, the implication of the PT in the control of other seasonal functions, is slowly emerging. First, the PT is involved in anterograde signaling for the seasonal control of prolactin secretion through molecule(s) known as “tuberalin(s)”, as reviewed elsewhere<sup>5,50,112,115,125,126</sup> (Figure 1). Second, the TSH-driven DIO2/T3 signal also regulates seasonal changes in body weight and torpor.<sup>92,127,128</sup> These findings are in line with the emerging pivotal role played by tanycytes in conveying peripheral metabolic cues to the hypothalamic centers that regulate food intake and energy status. On the one side, the peripheral metabolic cues showing seasonal variations, such as leptin, may use the tanycyte conduit to regulate seasonal metabolism,<sup>86</sup> and, on the other side, the TSH-driven T3 signal may control specific metabolic targets, including somatostatin and pro-opiomelanocortin neurons.<sup>7,92,127</sup> Therefore tanycytes appear as an important integration site of multiple cues (photoperiod, food,



**FIGURE 1** A simplified framework for the photoperiodic control of seasonal breeding. Melatonin acts at the level of the *pars tuberalis* (through the MT1 melatonin receptor) to control both anterograde and retrograde signaling pathways. The anterograde pathway involves *pars tuberalis*-derived tuberalin(s) whose identity remain(s) unknown. These tuberalins are released at the ME and trigger the seasonal production of prolactin (PRL) by the *pars distalis*. The retrograde pathway requires seasonal transcription of *Tshb*, which is governed through a CRY1/EYA3-dependent timing mechanism. TSH then acts (through TSHR) on tanycytes to govern a DIO2/DIO3 switch, which directs long-day production of T3 in the hypothalamic parenchyma. The downstream mechanisms of action of T3 are likely to comprise (most likely indirectly) the RFRP3 neurons in the dorsomedial hypothalamus (DMH) and kisspeptin (Kp) neurons in the arcuate nucleus (ARC), which in turn control GnRH output at the median eminence (ME), hence LH/FSH production by the *pars distalis*. In a short autocrine feedback loop T3 impacts transcription of select genes in tanycytes. Neither T3 nor E2 impact seasonal transcription in the *pars tuberalis* (not shown here). Hundreds of genes display seasonal/circannual expression in the *pars tuberalis* and tanycytes, the identity of some of these is provided in italics

inflammation), which occupy a strategic location in contact with the PT/median eminence and key hypothalamic nuclei.<sup>84–88</sup>

## 7 | FROM THE TSH/T3 SIGNAL TO THE SEASONAL RELEASE OF GNRH AND BREEDING ACTIVITY: STILL A LONG ROAD

In addition to the previous thyroidectomy experiments indicating a role of circulating TH in ovine seasonal breeding,<sup>77</sup> more recent experiments have demonstrated that chronic intracerebral administration of T3 or TSH in short-day adapted sexually inactive quails<sup>89</sup> or hamsters<sup>92,127</sup> restores their long day sexually active phenotype. Therefore, the current PT-TSH/tanycyte-DIOs/hypothalamus-T3 model, as presented above, appears both necessary and sufficient to dictate the seasonal control of GnRH release. However, there are at least two major caveats to such an “Occam's razor model.” First, considering the seasonal control of GnRH release as being either “on or off” is a crude over-simplification. To be efficient, GnRH must be released in a pulsatile manner and photoperiod must control both frequency and amplitude of GnRH/luteinising hormone (LH) pulses.<sup>129</sup> The second caveat concerns the timing of breeding. Small vertebrates, with short gestation/incubation duration, such as hamsters and quails,

usually breed during spring/summer, whereas larger vertebrates, with long gestation duration, such as sheep, goats and camels, breed during summer/autumn. And yet all these species rely on a similar TSH/DIO2/T3 axis, which is invariably activated by long days. Therefore, to explain the opposite breeding phenology one has to invoke a mechanism located downstream of T3.<sup>5,130</sup>

Therefore, the identification of cellular and molecular targets of T3 is a priority for understanding seasonal breeding. T3 receptors, TR $\alpha$  and, to a lesser extent, TR $\beta$  are ubiquitously distributed in the central nervous system, making it difficult to identify these targets. Notably, a recent work identified TR $\alpha$  expression in mouse tanycytes, which could participate in transmitting the seasonal signal towards the hypothalamus.<sup>131</sup> Another recent work in ewes has identified TMEM252, a gene specifically expressed by  $\beta$ 2 tanycytes, as a genuine T3 target because the approximately 10-fold increase in *Tmem252* expression in long days compared to short days is fully suppressed by thyroidectomy.<sup>70</sup> Therefore, *Tmem252* and a few other genes also expressed by tanycytes, such as *Shh* and *Dct*,<sup>70,71</sup> are potential T3 targets mediating the impact of long days towards GnRH neurons. Therefore, it is interesting that tanycytes, which translate long days into a local T3 output, are themselves responsive to T3, thus defining a peculiar autocrine feedback loop that deserves further investigation. At this point, it is still unclear how seasonal changes in hypothalamic



T3 synchronize breeding activity, although various studies indicate that T3 acts at the morphological and/or neuronal levels to control GnRH release.

Looking carefully at local morphology, the PT/median eminence/tanycyte complex appears to be ideally placed to play a key role in the regulation of the release of GnRH and other secretagogues. The PT extends along the ventral aspect of the median eminence, surrounds the pituitary stalk in its most caudal part, and is in contact with nerve endings of the median eminence and capillaries of the pituitary portal system. Furthermore, tanycyte endfeet and GnRH nerve terminals are closely intermingled and show an  $E_2$ -dependent remodeling, which is involved in shaping the GnRH surge during the estrous cycle.<sup>86,132</sup> In quail, GnRH nerve terminals are in close proximity to the basal lamina under long day conditions, whereas they are encased by tanycyte endfeet under short-day conditions,<sup>133</sup> and T3 implantation reverses the short-day phenotype by reducing encasement of GnRH nerve terminals.<sup>133</sup> Siberian hamster<sup>134</sup> and sheep<sup>66</sup> tanycytes also exhibit a marked photoperiodic remodeling. Thus, there is a clear photoperiodic remodeling of the tanycyte endfeet/GnRH nerve endings interaction at the median eminence, supporting the hypothesis that T3 participates to the photoperiodic GnRH secretion via neuro-glial plasticity. However, whether this mechanism is key for the seasonal GnRH release is yet to be demonstrated. Indeed, although Siberian hamsters and sheep are opposite seasonal breeders, both show a higher density in tanycyte vimentin under short days.

As mentioned earlier, GnRH neurons do not appear to be directly responsive to the seasonal change in sex steroid feedback. Indeed, GnRH neurons do not express the key estrogen receptor  $ER\alpha$  and only express low levels of  $ER\beta$  which, when selectively removed from GnRH neurons, do not alter electrophysiological properties and fail to result in any gross reproductive dysfunction.<sup>135-138</sup> Therefore, former studies have attempted to unravel the key upstream regulator(s) of GnRH neurons responsible for the seasonal release of GnRH.<sup>2,139</sup> A possible upstream regulator of GnRH neurons could be the dopaminergic A15 nucleus.<sup>139</sup> However, and akin to GnRH neurons, these neurons do not express  $ER\alpha$ , and would receive information on the  $E_2$  state through afferents arising from the POA and/or retrochiasmatic area.<sup>139,140</sup> In turn, DA neurons of the A15 would impact the functioning of *Kiss1* expressing neurons (see below) to modulate GnRH release. Although *Kiss1* neurons appear responsive to DA, evidence for a direct implication of the A15 is still scarce.<sup>141</sup> Likewise, the A15 nucleus is not involved in breeding seasonality in hamsters.<sup>139</sup> Finally, considering that *Kiss1* neurons are themselves  $E_2$ -responsive, the rationale for an additional upstream  $E_2$ -dependent regulator is unclear (see below). The mystery surrounding the identity of the  $E_2$ -responsive neuronal population(s) driving GnRH release was solved in the early 2000s, with the identification of two hypothalamic neuropeptides belonging to the family of (Arg)(Phe)-amide peptides, kisspeptin (Kp) and RFamide-related peptide (RFRP)-3. Soon enough, both appeared as promising candidates to fill the role of the missing link(s) between T3 and GnRH output, hence seasonal breeding<sup>3,142,143</sup> (Figure 1).

## 8 | EVIDENCE FOR A ROLE OF KISSPEPTIN NEURONS IN SEASONAL BREEDING

In 2003, mutations of the orphan receptor GPR54 (now known as KISS1R), to which Kp is a natural ligand,<sup>144</sup> were associated with idiopathic hypogonadotropic hypogonadism in humans<sup>145</sup> and a lack of puberty in mice.<sup>146</sup> Subsequent to the discovery of the critical role played by Kp/KISS1R signaling in reproductive function, numerous studies have reported that *Kiss1*, the gene encoding Kp, is expressed by hypothalamic neurons acting upstream of GnRH neurons, and is required for sexual differentiation and maturation, as well as for normal adult reproductive activity in many mammalian species, including humans.<sup>147</sup>

Kp neurons are mainly found in the arcuate nucleus (ARC) and more rostrally either in the anteroventral periventricular area around the third ventricle (AVPV) in rodents or the POA in non-rodent mammals.<sup>148-154</sup> In the ARC, Kp neurons also express neurokininB and dynorphin (and are thereafter dubbed KNDy neurons), which act as activatory and inhibitory inputs, respectively, onto Kp neurons.<sup>155,156</sup> *Kiss1* expression in the AVPV/POA is sexually dimorphic, with higher levels in females than in males, whereas, in the ARC, sexual dimorphism appears to depend on the species, being observed in sheep but not in rodents.<sup>157-161</sup> In rodents, studies indicate that ARC Kp fibers contact GnRH nerve endings and AVPV Kp fibers contact GnRH cell bodies,<sup>162,163</sup> whereas, in sheep and primates, Kp fibers mostly contact GnRH cell bodies.<sup>164</sup> Almost all GnRH neurons express KISS1R and are activated by a low dose ( $< 1$  nmol, administered in the intracerebroventricular space) of Kp.<sup>165-168</sup> Consequently, in vivo exogenous administration of Kp or optogenetic activation of Kp neurons induces GnRH release, LH and follicle-stimulating hormone (FSH) secretion, and sex steroid production in all mammalian species investigated so far,<sup>150,167-172</sup> including man and woman.<sup>173,174</sup> Furthermore, a specific deletion of KISS1R in GnRH neurons impairs mouse fertility, which is restored when the receptor is selectively reexpressed in GnRH neurons.<sup>175,176</sup> One striking feature of Kp neurons is that, in contrast to GnRH neurons, they express a high density of  $ER\alpha$ .<sup>149,152,153,157,177</sup> Remarkably, the  $E_2$  feedback depends on Kp neuron localization because  $E_2$  stimulates *Kiss1* expression in the AVPV/POA but inhibits *Kiss1* expression in the ARC.<sup>147,152,153,157,158,178</sup> It appears that distinct molecular and epigenetic control of *Kiss1* expression by  $E_2$  within these two nuclei are responsible for this striking opposite response.<sup>179</sup> To add further complexity, in non-rodent species, particularly sheep and primates, there is evidence that  $E_2$  can indeed stimulate a subset of caudal ARC Kp neurons during the preovulatory period,<sup>180-182</sup> which suggests the existence of two subpopulations of ARC Kp neurons. Notably, mice lacking  $ER\alpha$  in Kp neurons display an advanced puberty and irregular estrous cycles, highlighting the role of Kp neurons in conveying the  $E_2$  feedback towards the GnRH neurons.<sup>183,184</sup> Altogether, it is now recognized that reproductive activity in mammals requires Kp action at the KISS1R on GnRH neurons, with different functions for the two Kp neuronal populations. The ARC KNDy neurons form the GnRH pulse generator in males and females and are down-regulated by the sex

steroid feedback.<sup>172,185–187</sup> The AVPV/POA Kp neurons are involved in the preovulatory GnRH/LH surge in females and are up-regulated by the sex steroid feedback.<sup>169,188–191</sup>

The similarity of the gonadal phenotype between KISS1R KO mice<sup>146</sup> and photo-inhibited seasonal rodents, prompted studies to test the hypothesis that Kp neurons link the photoperiodic melatonin signal to seasonal breeding. Indeed, early studies reported that male Syrian hamster<sup>192</sup> and ewe<sup>154,193</sup> exhibit a clear photoperiodic variation in ARC *Kiss1* expression, with higher values being associated with the breeding season; notably, under long days for the Syrian hamster and under short days for the ewe. Subsequently, photoperiodic/seasonal variations in Kp expression in the ARC and AVPV/POA have been reported for many more species, including Djungarian hamster,<sup>194,195</sup> European hamster,<sup>114</sup> Libyan jird,<sup>196</sup> jerboa,<sup>197</sup> red deer,<sup>198</sup> elephant-shrew,<sup>199</sup> Brandt's voles,<sup>200</sup> dromedary camel,<sup>201</sup> wild mouse<sup>202</sup> and water vole.<sup>203</sup> Interestingly, neurokinin B and dynorphin co-expressed in ARC Kp neurons also exhibit seasonal variation in Syrian hamster<sup>204</sup> and sheep.<sup>205</sup> Intriguingly, although seasonal variation of ARC Kp is conserved among seasonal species, a high Kp level is not always associated with the breeding season as exemplified in Siberian and European hamsters, which both exhibit higher ARC Kp in photo-inhibited conditions.

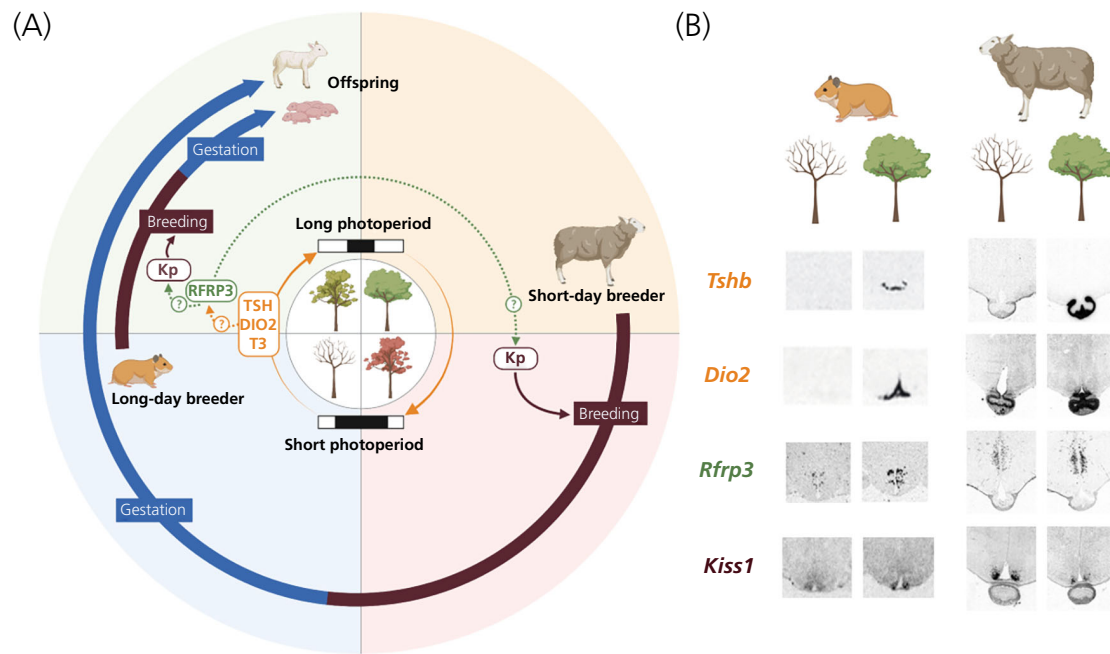
To test the suspected role of the seasonal change in circulating melatonin on hypothalamic *Kiss1* expression, early experiments performed pinealectomy under short-day conditions or melatonin administration under long-day conditions. In male and female Syrian hamsters, the short-day melatonin signal inhibited *Kiss1* expression in the ARC but not in the AVPV.<sup>15,192</sup> A similar inhibitory effect of melatonin on ARC Kp was also reported in the Turkish<sup>206</sup> and striped<sup>207</sup> hamsters and in the rat.<sup>208</sup> In sheep, long days inhibit ARC *Kiss1* expression through an E<sub>2</sub>-dependent mechanism.<sup>121</sup> It is likely that melatonin is not acting directly on Kp neurons because ARC Kp neurons do not express MT1, at least in sheep.<sup>209</sup> Because melatonin acts at the PT to synchronize seasonal breeding via local TSH/T3 signaling, it was predicted that TSH/T3 modifications would impact upon ARC Kp. Actually, a chronic central infusion of TSH in short day-adapted sexually inactive Syrian and Siberian hamsters rescued a long-day phenotype of ARC *Kiss1* expression and testicular activity,<sup>92</sup> and exogenous T3 administration also restored the long-day phenotype of hypothalamic Kp and gonadal activity to photo-inhibited Siberian hamsters.<sup>210</sup> In the same line, thyroidectomy in sheep severely dampens the seasonal variation in *Kiss1* expression.<sup>70</sup> At this point, the mechanisms through which hypothalamic T3 acts on ARC Kp neurons are a matter of debate. Although Kp neurons, similar to most other hypothalamic neurons, express the T3 receptor TR $\alpha$  (at least in sheep<sup>211</sup>), studies favor an indirect impact of T3 on *Kiss1* expression because a similarly high T3 signal (in long summer days) induces an opposite effect on ARC *Kiss1* expression in short-day (sheep) and long-day (Syrian) breeders. Furthermore, a recent *in vitro* study failed to demonstrate an effect of T3 on *Kiss1* expression in hypothalamic explants.<sup>211</sup> Circulating sex steroids are also part of the seasonal synchronization of breeding activity.<sup>13</sup> Thus, a seasonal change in the sex steroid feedback may regulate Kp neurons, which express a high

density of ER $\alpha$ . Actually, gonadectomy with/without testosterone or E<sub>2</sub> replacement in males and females of seasonal species demonstrated that sex steroids inhibit *Kiss1* expression in the ARC but increases *Kiss1* expression in the AVPV/POA.<sup>114,121,154,192,195,212,213</sup> Therefore, ARC Kp neurons are regulated by a strong dual mechanism, which comprises a melatonin-driven T3 seasonal signal on one side and sex steroids on the other side. From current experiments, it appears that melatonin-driven T3 induces an opposite effect between long-day and short-day breeders, whereas the sex steroid feedback is always inhibitory, although with varying potency among species. Overall, this would explain why variation in ARC *Kiss1* expression is not always phased with the seasonal change in breeding activity (Figure 2). Thus, in Siberian and European hamsters, it is likely that ARC *Kiss1* expression is inhibited by the strong inhibitory feedback of sex steroids once individuals are adapted to the photo-stimulatory long day.<sup>114,195</sup> By contrast, seasonal variation in AVPV/POA Kp neurons appears to be solely driven by the positive sex steroid feedback. Additional hormonal signals exhibiting seasonal changes, notably leptin or insulin, also regulate ARC Kp neurons.<sup>214</sup> However, whether these metabolic hormones regulate ARC *Kiss1* expression in a seasonal context has not yet been studied. This issue would deserve further investigation as there is a tight coordination between energy states and reproductive functions, notably in species exposed to seasonal changes in food availability.

The relevance of seasonal variation in hypothalamic *Kiss1* expression was investigated through chronic Kp administration in photo-inhibited male and female animals. Thus, chronic (4–5 weeks) *i.c.v.* infusion of a low Kp dose in short-day adapted sexually inactive male Syrian hamsters<sup>192</sup> or male and female Siberian hamsters<sup>195,215</sup> fully restored reproductive activity, with gonadal mass and circulating sex steroid levels being similar to those of the long-day adapted sexually active hamsters. Interestingly, repeated *i.p.* injections also reactivated rodent reproduction, which indicates that Kp could act at GnRH nerve terminals to regulate seasonal breeding.<sup>216</sup> In the ewe as well, a 48-h long intravenous infusion of a low dose of Kp in anestrus ewes produced surge levels of LH and ovulation.<sup>217</sup> In the mare, chronic Kp injection also triggered a modest increase in gonadotropin secretion, but failed to induce ovulation.<sup>218</sup> The converging data reporting that exogenous Kp can reverse seasonal inhibition of reproduction has led to recent research on the identification of Kp analogs that could be used for the control of seasonal breeding in farms. In recent years, several KISS1R agonists with a longer half-life than the natural Kp10 peptide have been designed: TAK-448<sup>219</sup>; C6,<sup>220</sup> Bz-Arg-Trp-NH<sub>2</sub>.<sup>221</sup> Among those, C6 delivered *i.m.* triggered fertile ovulations in anoestrus ewes and goats.<sup>220,222</sup> Therefore, C6, and possibly other Kp analogs may be used as a more ethical alternatives to the use of pregnant mare serum gonadotropin (PMSG, also known as eCG<sup>223</sup>).

In conclusion, all seasonal species investigated so far exhibit seasonal variation in hypothalamic *Kiss1* expression. In ARC Kp neurons, this variation is mainly driven by both the melatonin-driven T3 signal and negative sex steroid feedback, whereas, in Kp neurons of the AVPV/POA, it mostly depends on positive sex steroid feedback. In





**FIGURE 2** Neurons expressing kisspeptin (Kp) and RFRP3 as penultimate neuroendocrine controllers of the retrograde signaling pathway in seasonal breeders. **A**, A model to account for opposite breeding phenologies in long-day and short-day breeders – Longer days of spring and summer trigger induction of the TSH/DIO2 pathway leading to enhanced T3 levels in the MBH. RFRP3 expression is also increased in long days, which suggests it is driven by T3 – the mechanism remains unknown. In contrast, the level of Kp expression is typically tied to the reproductive status of the seasonal species considered, which suggests a switch between RFRP3 and Kp neurons. Here again, the mechanism is unknown. Both long-day and short-day breeders (e.g. Syrian hamster and sheep) give birth in spring, when environmental conditions are most favourable to the survival of the mother and the offspring. **B**, Examples of autoradiograms depicting seasonal variation of expression for *Tshb*, *Dio2*, *Rfrp3* and *Kiss1* in the Syrian hamster (left) and sheep (right). Note that *Tshb*, *Dio2* and *Rfrp3* display increased expression under long days in both animal models (however, see text for further information regarding the sex steroid status) while *Kiss1* expression is higher under long days in hamsters but under short days in the ewe (here again, see text for a more weighted view)

photo-inhibited breeders, exogenous application of Kp reinstates reproductive activity, thus strengthening the physiological relevance of Kp in the regulation of seasonal breeding. Yet, the final demonstration that seasonal changes in hypothalamic Kp are necessary and sufficient for regulating seasonal breeding will probably require the use of other tools (specific antagonists, directed viral neuronal inhibition, clustered regularly interspaced palindromic repeat [CRISPR]/CRISPR-associated protein 9 [Cas9] knockout) to inhibit the Kp/KISS1R system in seasonal species not traditionally used for transgenic studies.

## 9 | A DISPUTED ROLE OF RFRP-3 IN THE SEASONAL REGULATION OF BREEDING ACTIVITY

In 2000, a novel hypothalamic peptide inhibiting gonadotropin secretion was discovered in quail.<sup>224</sup> This peptide, belonging to the family of (Arg)(Phe)-amide peptides, was named gonadotropin-inhibitory hormone (GnIH). Around the same time, cDNAs that encode novel RFamide peptides similar to GnIH have been detected in mammalian brains.<sup>225</sup> The existence of an orthologous *Npvf* gene, encoding two active peptides called RFamide-related peptides RFRP-1 and RFRP-3, was thereafter reported in various mammalian species.<sup>226–228</sup>

In mammals, *Npvf*-expressing neurons are mainly located in the dorsomedial and ventromedial nuclei of the hypothalamus (DMH/VMH) in multiple species, including rodents,<sup>225,228–230</sup> sheep,<sup>231,232</sup> humans,<sup>233</sup> non-human primates,<sup>181,234</sup> cattle,<sup>235</sup> horses<sup>236</sup> and camels.<sup>201</sup> RFRP-3 fibers project to various intra- and extra-hypothalamic areas where the specific RFRP-3 receptor GPR147 (also known as NPFFR2) is also found, providing divergent neural pathways by which RFRP-3 might influence various biological functions.<sup>228,237,238</sup> Notably, RFRP-3 fibers project to the POA and form close contact with 20%–40% GnRH neurons, and approximately one-third of GnRH neurons expresses NPFFR2.<sup>228,232,237–240</sup> Additionally, RFRP-3 neuron projections form close contact with Kp neurons, with approximately 10% (AVPV) to 25% (ARC) of Kp neurons expressing NPFFR2 in mice.<sup>237,240</sup> Thus, RFRP-3 may, directly or indirectly, influence GnRH neuronal activity.

Electrophysiological studies show that RFRP-3 application inhibits neuronal activity in a majority of GnRH neurons (40%–70%), but also increases it in approximately 12% of GnRH neurons.<sup>241,242</sup> Using a GnRH neuronal cell line, RFRP-3 application was found to reduce GnRH mRNA expression by approximately 60%.<sup>243</sup> Early in vivo experiments reported that intracerebral injection of RFRP-3 inhibits LH secretion in female Syrian hamsters,<sup>228</sup> as well as male and female rats,<sup>244–246</sup> and that systemic injection of RFRP-3 reduces LH levels in

ewes,<sup>247</sup> in line with a gonadotropin inhibitory action of RFRP-3. However, further studies made it obvious that the effect of RFRP-3 on gonadotropin secretion is more complex than this. First, a study in male Syrian hamsters showed that central injection of RFRP-3 activates GnRH neurons and increases LH, FSH and testosterone production.<sup>248</sup> Second, in the male Siberian hamster, central RFRP-3 injection stimulates LH in short day-adapted animals, but inhibits it in long day-adapted animals.<sup>239</sup> Third, multiple protocols of either central or i.v. administration of RFRP-3 failed to modulate LH secretion in ewes.<sup>249,250</sup> Fourth, in some species, RFRP-3 appears to show a clear sex difference in its impact, being inhibitory in female but stimulatory, or without any effect, in male rodents<sup>245,248,251,252</sup> and humans.<sup>253</sup> Of note, several studies indicated that RFRP-3 could act on the HPG axis via a direct role on pituitary gonadotrophic cells.<sup>142</sup> RFRP-3 fibers are found in the outer layer of the median eminence, although with various density according to the species and study. RFRP-3 application on GnRH-stimulated cultured pituitary cells was reported to inhibit gonadotropin secretion in Syrian hamsters,<sup>228</sup> sheep,<sup>254</sup> cattle,<sup>255</sup> rats<sup>246</sup> and pigs.<sup>256</sup> However, further studies in rodents, ewes, pigs and horses did not yield any support for a hypophysiotropic effect of RFRP-3.<sup>218,236,245,248,257</sup> Furthermore, although RFRP-3 is detected in the ewe portal blood, its circulating levels show no correlation with LH levels in the peripheral blood.<sup>258</sup> Importantly, ablation of the *Npffr2* gene does not compromise mice fertility, although several minor to moderate effects were reported.<sup>259</sup> Altogether, a number of studies have reported significant, albeit often moderate, effects of RFRP-3 on the HPG axis, with differences according to species, sex and environmental conditions. Yet, most studies are consistent to indicate that in female mammals, RFRP-3 inhibit elevated LH secretion, notably at the time of the GnRH/LH surge<sup>245,251–253</sup>; for a review, see also Angelopoulou et al.<sup>142</sup>

In 2008, the use of an anti-Kp antibody revealed an unexpectedly marked photoperiodic variation in immunoreactivity in cells located in the DMH of male Syrian hamsters, with enhanced labeling under long days compared to short days.<sup>230</sup> Further analyses demonstrated that this labeling did not correspond to Kp cells (i.e. cross-reactivity) but to cells expressing another RF-amide peptide, which turned out to be RFRP-3. Thereafter, a number of studies reported a very similar photoperiodic pattern of RFRP-3 mRNA/protein expression in many seasonal mammals. This is true not only for long-day breeders such as male and female Syrian and Siberian hamsters,<sup>195,230,239,251,260</sup> female Turkish hamsters,<sup>206</sup> male European hamsters,<sup>114</sup> male and female Jerboas,<sup>197</sup> female Brandt's voles,<sup>200</sup> male water voles<sup>261</sup> and male wild-derived mice,<sup>202</sup> but also for short-day breeders such as ewes,<sup>231,232</sup> female brushtail possums,<sup>262</sup> female goats,<sup>263</sup> and male and female dromedary camels.<sup>201</sup> Additionally, the number of RFRP-3 fibers contacting GnRH neurons in the Siberian hamster and ewe<sup>232,239</sup> and *Npffr2* expression in the Syrian hamster<sup>238</sup> are lower in short- compared to long-day conditions. Altogether, these neuroanatomical data demonstrate that the high expression under long days and the short day/winter decrease in the RFRP-3/NPFFR2 system is the same among seasonal mammals, which points to conserved mechanisms for the seasonal regulation of *Npvf* expression, regardless of whether the animals are male or female, or long or short day breeders (Figure 2).

In the quail, which, in contrast to mammals, shows higher GnIH expression under short-day conditions, melatonin acts directly on GnIH neurons via the Mel1C receptor to induce GnIH expression.<sup>264</sup> In Syrian,<sup>230</sup> Siberian<sup>239</sup> and Turkish<sup>206</sup> hamsters, the number of RFRP-3 expressing neurons is increased by pinealectomy and decreased by melatonin injections. Therefore, *Npvf* expression and RFRP-3 production are tightly regulated by melatonin. Although melatonin binding sites were previously found in the DMH of Syrian hamsters,<sup>265</sup> and albeit a 24-h melatonin incubation slightly increases *Npvf* expression in an hypothalamic cell line,<sup>266</sup> a direct impact of melatonin on RFRP-3 neurons appears very unlikely.<sup>107</sup> Therefore, further experiments aimed to test the hypothesis that melatonin uses the PT-TSH/hypothalamus-T3 pathway to regulate RFRP-3 expression. Indeed, a chronic central infusion of TSH in short day-adapted Syrian and Siberian hamsters brings the number of RFRP-3 expressing neurons to a level comparable to that observed in long day-adapted animals, and this effect comes along with restoration of gonadal activity.<sup>92</sup> In the same line, T3 injection results in a long day-like restoration of the number of RFRP-3 neurons, as well as testis size, in Syrian hamster exposed to short days<sup>210</sup> and thyroidectomy blunts the seasonal change in *Npvf* expression in ewes.<sup>70</sup> Finally, in TR $\alpha$  null mice, melatonin no longer inhibits RFRP-3 expression.<sup>131</sup> Altogether, these data suggest that the melatonin-driven seasonal change in hypothalamic T3 regulates RFRP-3 neurons via TR $\alpha$ . However, it is doubtful that T3 directly acts on RFRP-3 neurons because only a low number of RFRP-3 neurons expresses TR $\alpha$ , and melatonin still inhibits RFRP-3 expression in mice bearing a neuron-specific mutation of TR $\alpha$ .<sup>131</sup> Yet, the possibility that T3 regulates RFRP-3 neurons through TR $\beta$  cannot be excluded. By contrast to Kp neurons for which sex steroid feedback is well documented, the issue of gonadal hormone regulation of *Npvf* expression, notably in the seasonal context, is controversial. In female mouse and Syrian hamster, 20%–40% of RFRP-3 neurons express ER $\alpha$ , respectively.<sup>228,267,268</sup> E<sub>2</sub> treatment after ovariectomy has been shown to either increase c-FOS in RFRP-3 neurons in Syrian hamsters,<sup>228</sup> to decrease *Npvf* mRNA in mice<sup>267</sup> or to have no effect on *Npvf* expression in rat.<sup>269</sup> Finally, manipulating testosterone or E<sub>2</sub> levels by gonadectomy with or without sex steroid replacement in the male and female Syrian and Siberian hamsters had no significant effect on the photoperiodic regulation of *Npvf* expression,<sup>195,230,251</sup> suggesting a sex steroid independent seasonal regulation of RFRP-3 synthesis in rodents. By contrast, there is evidence that E<sub>2</sub> is required for the seasonal regulation of *Npvf* expression in the ewe.<sup>121</sup>

The large and conserved melatonin-driven variation in *Npvf* expression raised the issue of the role of RFRP-3 in seasonal breeding. In both male and female short-day adapted Syrian hamsters, chronic central administration of RFRP-3 restored gonadal activity, as well as ARC *Kiss1* expression, to levels observed in long day-adapted animals.<sup>248,251</sup> These results suggest that the seasonal change in hypothalamic RFRP-3 drives seasonal reproduction via an up-regulation of ARC Kp. However, RFRP-3 failed to rescue reproductive activity of short-day adapted male and female Siberian hamsters.<sup>215</sup>

In conclusion, a long-day increase in hypothalamic *Npvf* expression and RFRP-3 synthesis is a hallmark of seasonality, irrespective of

species, sex and breeding strategy (Figure 2). This might be triggered by T3, although the mechanisms through which T3 regulates RFRP-3 neurons remain unclear, albeit these are most likely indirect. Overall, there is strong evidence that RFRP-3 modulates the HPG axis activity but the effects vary significantly across species, sex and environmental conditions. The reasons for this remain unknown, and it can not be excluded that hypothalamic RFRP-3 regulates other seasonal functions. Therefore, studies in other seasonal species, the use of more selective NPFFR2 ligands, such as GJ14,<sup>270</sup> RF313<sup>271</sup> or RF3286,<sup>272</sup> and the development of novel genetically modified seasonal models are required to disclose the extent of the complex physiological role(s) played by RFRP-3 in the control of seasonal breeding.

## 10 | CONCLUSIONS

When it comes to seasonal breeding, it is obvious that GnRH neurons do not get first hand information. Instead, their seasonal activity relies on a complex neuroendocrine pathway, which involves many participants. Seasonal change in photoperiod is conveyed by melatonin to the PT of the pituitary, which translates it into increased TSH output under long days. In turn, TSH triggers activation of DIO2 and inhibition of DIO3 in tanycytes in order to increase local intrahypothalamic T3 levels. There is no doubt that such local control of T3 is key to the organisation of seasonal breeding, but downstream cellular and molecular targets of T3 remain unclear (Figure 1). However, in terms of neuronal populations, there are two very strong contenders. On the one hand are *Kiss1*-expressing neurons of the ARC. *Kiss1* expression is governed by photoperiod and further submitted to negative sex steroid feedback, with higher *Kiss1* levels observed during the breeding season in most species (Figure 2). *Kp* is unquestionably the major GnRH secretagogue in all mammalian species, whatever the breeding phenology. This makes it unlikely that T3 directly impinges onto *Kp* neurons and instead suggests the existence of one or several relays. On the other hand are RFRP3-producing neurons of the DMH/VMH. They display increased *Npvf* expression upon long days, whatever the breeding phenology of the species (Figure 2), indicating that they are targets of T3 albeit, here again, direct control seems unlikely. It is tempting to speculate that the RFRP-3 neuronal population is the hypothalamic site for the integration of photoperiod, independently of the breeding phenology, and that in turn these neurons regulate *Kp* synthesis and release with an opposite effect in long and short day breeders. However, the actual effect of the RFRP-3 on *Kp*, GnRH and gonadotropin release remains obscure. Therefore, further work should focus on unveiling the missing link between T3 and RFRP3 neurons, defining the neurochemical identity of RFRP3 neurons in long-day and short-day breeders and investigating a potential cross-talk between *Kp* and RFRP-3 neurons.

## ACKNOWLEDGMENTS

Part of the work presented in this review was supported by the French National Research Agency (ANR, Repramide ANR-13-BSV1-001;GMO-Phen ANR-20-CE14-0031-01). Authors are very grateful

to Marie-Az lie Moralia and Marine Simonneaux for their help in designing the figures.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Val rie Simonneaux:** Conceptualization; project administration; writing – original draft. **Hugues Dardente:** Conceptualization; writing – original draft.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jne.13124>.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review because no new data were created or analyzed.

## ORCID

Val rie Simonneaux  <https://orcid.org/0000-0002-6004-7850>

## REFERENCES

- Lincoln GA, Short RV. Seasonal breeding: nature's contraceptive. *Recent Prog Horm Res*. 1980;36:1-52.
- Malpoux B. Chapter 41: seasonal regulation of reproduction in mammals. In: Neill JD, ed. *Knobill & Neill's Physiology of Reproduction*. 3rd ed. Elsevier; 2006:2231-2281.
- Hazlerigg DG, Simonneaux V. Seasonal regulation of reproduction in mammals. In: Plant TM, Zeleznic AJ, eds. *Knobill and Neill's Physiology of Reproduction*. Vol 2. 4th ed; 2015:1575-1604.
- Follett BK. "seasonal changes in the neuroendocrine system": some reflections. *Front Neuroendocrinol*. 2015;37:3-12.
- Dardente H, Wood S, Ebling F, Saenz de Miera C. An integrative view of mammalian seasonal neuroendocrinology. *J Neuroendocrinol*. 2019;31:e12729.
- Scherbarth F, Steinlechner S. Endocrine mechanisms of seasonal adaptation in small mammals: from early results to present understanding. *J Comp Physiol B*. 2010;7:935-952.
- Dardente H, Migaud M. Chapter 4: thyroid hormone and hypothalamic stem cells in seasonal functions. *Vitamins & Hormones*. Vol 116. Elsevier Inc; 2021:91-131.
- Martin GB, Rodger J, Blache D. Nutritional and environmental effects on reproduction in small ruminants. *Reprod Fertil Dev*. 2004; 4:491-501.
- Salazar-Ortiz J, Camous S, Briant C, Lardic L, Chesneau D, Guillaume D. Effects of nutritional cues on the duration of the winter anovulatory phase and on associated hormone levels in adult female welsh pony horses (*Equus caballus*). *Reprod Biol Endocrinol*. 2011;9:130.
- Menassol JB, Collet A, Chesneau D, Malpoux B, Scaramuzzi RJ. The interaction between photoperiod and nutrition and its effects on seasonal rhythms of reproduction in the ewe. *Biol Reprod*. 2012; 2:52.
- Delgadoillo JA, Hernandez H, Abecia JA, Keller M, Chemineau P. Is it time to reconsider the relative weight of sociosexual relationships compared with photoperiod in the control of reproduction of small ruminant females? *Domest Anim Endocrinol*. 2020;73: 106468.

12. Reiter RJ. Evidence for refractoriness of the pituitary-gonadal axis to the pineal gland in golden hamsters and its possible implications in annual reproductive rhythms. *Anat Rec.* 1972;3:365-371.
13. Karsch FJ, Bittman EL, Foster DL, Goodman RL, Legan SJ, Robinson JE. Neuroendocrine basis of seasonal reproduction. *Recent Prog Horm Res.* 1984;40:185-232.
14. Simonneaux V, Ribelayga C. Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol Rev.* 2003;2:325-395.
15. Dardente H. Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. *J Neuroendocrinol.* 2012;2:249-266.
16. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science.* 2002;295:1065-1070.
17. Lucas RJ. Mammalian inner retinal photoreception. *Curr Biol.* 2013;3:R125-R133.
18. Hannibal J, Fahrenkrug J. Neuronal input pathways to the brain's biological clock and their functional significance. *Adv Anat Embryol Cell Biol.* 2006;182:1-71.
19. Dardente H, Cermakian N. Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiol Int.* 2007;2:195-213.
20. Moore RY. Chapter 1: the Suprachiasmatic nucleus and the circadian timing system. *Progress in Molecular Biology and Translational Science.* Vol 119. Elsevier; 2013:1-28.
21. Buijs RM, Kalsbeek A. Hypothalamic integration of central and peripheral clocks. *Nat Rev Neurosci.* 2001;7:521-526.
22. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature.* 2002;6901:935-941.
23. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu Rev Physiol.* 2010;72:517-549.
24. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet.* 2017;3:164-179.
25. Tournier BB, Menet JS, Dardente H, et al. Photoperiod differentially regulates clock genes' expression in the suprachiasmatic nucleus of Syrian hamster. *Neuroscience.* 2003;118:317-322.
26. Coomans CP, Ramkisoensing A, Meijer JH. The suprachiasmatic nuclei as a seasonal clock. *Front Neuroendocrinol.* 2015;37:29-42.
27. Dardente H, Wyse CA, Lincoln GA, Wagner GC, Hazlerigg DG. Effects of photoperiod extension on clock gene and Neuropeptide RNA expression in the SCN of the Soay sheep. *PLoS One.* 2016;7:e0159201.
28. Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res.* 1993;4:161-190.
29. Bittman EL, Dempsey RJ, Karsch FJ. Pineal melatonin secretion drives the reproductive response to daylength in the ewe. *Endocrinology.* 1983;6:2276-2283.
30. Lincoln GA, Libre EA, Merriam GR. Long-term reproductive cycles in rams after pinealectomy or superior cervical ganglionectomy. *J Reprod Fertil.* 1989;2:687-704.
31. Garcia-Fernandez JM, Cernuda-Cernuda R, Davies WI, et al. The hypothalamic photoreceptors regulating seasonal reproduction in birds: a prime role for VA opsin. *Front Neuroendocrinol.* 2015;37:13-28.
32. Shinomiya A, Shimmura T, Nishiwaki-Ohkawa T, Yoshimura T. Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. *Front Endocrinol.* 2014;5:12.
33. Urbanski HF, Doan A, Pierce M. Immunocytochemical investigation of luteinizing hormone-releasing hormone neurons in Syrian hamsters maintained under long or short days. *Biol Reprod.* 1991;4:687-692.
34. Webster JR, Moenter SM, Barrell GK, Lehman MN, Karsch FJ. Role of the thyroid gland in seasonal reproduction. III. Thyroidectomy blocks seasonal suppression of gonadotropin-releasing hormone secretion in sheep. *Endocrinology.* 1991;3:1635-1643.
35. Ronchi E, Krey LC, Pfaff DW. Steady state analysis of hypothalamic GnRH mRNA levels in male Syrian hamsters: influences of photoperiod and androgen. *Neuroendocrinology.* 1992;2:146-155.
36. Kawamoto K, Tanaka S, Kawano M, Hayashi T, Tsuchiya K. Effects of photoperiod and ambient temperature on the gonadotropin-releasing hormone neuronal system in the gray hamster, *Tscherskia triton*. *Neuroendocrinology.* 2000;5:284-292.
37. Porkka-Heiskanen T, Khoshaba N, Scarbrough K, et al. Rapid photoperiod-induced increase in detectable GnRH mRNA-containing cells in Siberian hamster. *Am J Physiol.* 1997;6:R2032-R2039.
38. Malpoux B, Thiery JC, Chemineau P. Melatonin and the seasonal control of reproduction. *Reprod Nutr Dev.* 1999;3:355-366.
39. Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron.* 1994;5:1177-1185.
40. Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol Rev.* 2010;3:343-380.
41. Morgan PJ, Barrett P, Howell HE, Helliwell R. Melatonin receptors: localization, molecular pharmacology and physiological significance. *Neurochem Int.* 1994;2:101-146.
42. Morgan PJ, Hazlerigg DG. Photoperiodic signalling through the melatonin receptor turns full circle. *J Neuroendocrinol.* 2008;6:820-826.
43. Weaver DR, Liu C, Reppert SM. Nature's knockout: the Mel1b receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. *Mol Endocrinol.* 1996;11:1478-1487.
44. Liu C, Weaver DR, Jin X, et al. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron.* 1997;1:91-102.
45. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Melatonin transmits photoperiodic signals through the MT1 melatonin receptor. *J Neurosci.* 2009;9:2885-2889.
46. Prendergast BJ. MT1 melatonin receptors mediate somatic, behavioral, and reproductive neuroendocrine responses to photoperiod and melatonin in Siberian hamsters (*Phodopus sungorus*). *Endocrinology.* 2010;2:714-721.
47. Klosien P, Lapmanee S, Schuster C, et al. MT1 and MT2 melatonin receptors are expressed in nonoverlapping neuronal populations. *J Pineal Res.* 2019;1:e12575.
48. Weaver DR, Reppert SM. Melatonin receptors are present in the ferret pars tuberalis and pars distalis, but not in brain. *Endocrinology.* 1990;5:2607-2609.
49. Hazlerigg DG, Gonzalez-Brito A, Lawson W, Hastings MH, Morgan PJ. Prolonged exposure to melatonin leads to time-dependent sensitization of adenylate cyclase and down-regulates melatonin receptors in pars tuberalis cells from ovine pituitary. *Endocrinology.* 1993;1:285-292.
50. Dardente H. Does a melatonin-dependent circadian oscillator in the pars tuberalis drive prolactin seasonal rhythmicity? *J Neuroendocrinol.* 2007;8:657-666.
51. Sun ZS, Albrecht U, Zhuchenko O, Bailey J, Eichele G, Lee CC. RIGUI, a putative mammalian ortholog of the drosophila period gene. *Cell.* 1997;6:1003-1011.
52. Messenger S, Ross AW, Barrett P, Morgan PJ. Decoding photoperiodic time through Per1 and ICER gene amplitude. *Proc Natl Acad Sci USA.* 1999;17:9938-9943.



53. Messenger S, Garabette ML, Hastings MH, Hazlerigg DG. Tissue-specific abolition of Per1 expression in the pars tuberalis by pineal-ectomy in the Syrian hamster. *Neuroreport*. 2001;3:579-582.
54. Lincoln G, Messenger S, Andersson H, Hazlerigg D. Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. *Proc Natl Acad Sci USA*. 2002;21:13890-13895.
55. Dardente H, Menet JS, Poirel VJ, et al. Melatonin induces Cry1 expression in the pars tuberalis of the rat. *Brain Res Mol Brain Res*. 2003;2:101-106.
56. Hazlerigg DG, Andersson H, Johnston JD, Lincoln G. Molecular characterization of the long-day response in the Soay sheep, a seasonal mammal. *Curr Biol*. 2004;4:334-339.
57. Johnston JD, Tournier BB, Andersson H, Masson-Pevet M, Lincoln GA, Hazlerigg DG. Multiple effects of melatonin on rhythmic clock gene expression in the mammalian pars tuberalis. *Endocrinology*. 2006;2:959-965.
58. Dupre SM, Burt DW, Talbot R, et al. Identification of melatonin-regulated genes in the ovine pituitary pars tuberalis, a target site for seasonal hormone control. *Endocrinology*. 2008;149:5527-5539.
59. Dupre SM, Miedzinska K, Duval CV, et al. Identification of Eya3 and TAC1 as long-day signals in the sheep pituitary. *Curr Biol*. 2010;9:829-835.
60. Dardente H, Wyse CA, Birnie MJ, et al. A molecular switch for photoperiod responsiveness in mammals. *Curr Biol*. 2010;24:2193-2198.
61. Dardente H, Hazlerigg DG, Ebling FJ. Thyroid hormone and seasonal rhythmicity. *Front Endocrinol (Lausanne)*. 2014;5:19.
62. Wood SH, Hindle MM, Mizoro Y, et al. Circadian clock mechanism driving mammalian photoperiodism. *Nat Commun*. 2020;11:4291.
63. Masumoto KH, Ukai-Tadenuma M, Kasukawa T, et al. Acute induction of Eya3 by late-night light stimulation triggers TSHbeta expression in Photoperiodism. *Curr Biol*. 2010;20:2199-2206.
64. Wittkowski W, Bergmann M, Hoffmann K, Pera F. Photoperiod-dependent changes in TSH-like immunoreactivity of cells in the hypophysial pars tuberalis of the Djungarian hamster, *Phodopus sungorus*. *Cell Tissue Res*. 1988;1:183-187.
65. Wittkowski W, Hewing M, Hoffmann K, Bergmann M, Fechner J. Influence of photoperiod on the ultrastructure of the hypophysial pars tuberalis of the Djungarian hamster, *Phodopus sungorus*. *Cell Tissue Res*. 1984;1:213-216.
66. Wood SH, Christian HC, Miedzinska K, et al. Binary switching of calendar cells in the pituitary defines the phase of the circannual cycle in mammals. *Curr Biol*. 2015;20:2651-2662.
67. Bockmann J, Bockers TM, Winter C, et al. Thyrotropin expression in hypophyseal pars tuberalis-specific cells is 3,5,3'-triiodothyronine, thyrotropin-releasing hormone, and pit-1 independent. *Endocrinology*. 1997;138:1019-1028.
68. Klosen P, Bienvenu C, Demarteau O, et al. The mt1 melatonin receptor and RORbeta receptor are co-localized in specific TSH-immunoreactive cells in the pars tuberalis of the rat pituitary. *J Histochem Cytochem*. 2002;12:1647-1657.
69. Dardente H, Klosen P, Pevet P, Masson-Pevet M. MT1 melatonin receptor mRNA expressing cells in the pars tuberalis of the European hamster: effect of photoperiod. *J Neuroendocrinol*. 2003;8:778-786.
70. Lomet D, Cognie J, Chesneau D, Dubois E, Hazlerigg D, Dardente H. The impact of thyroid hormone in seasonal breeding has a restricted transcriptional signature. *Cell Mol Life Sci*. 2018;5:905-919.
71. Dardente H, Lomet D. Photoperiod and thyroid hormone regulate expression of l-dopachrome tautomerase (Dct), a melanocyte stem-cell marker, in tanycytes of the ovine hypothalamus. *J Neuroendocrinol*. 2018;9:e12640.
72. Xia Q, Chu M, He X, et al. Identification of photoperiod-induced lncRNAs and mRNAs in pituitary pars Tuberalis of sheep. *Front Vet Sci*. 2021;3:644474.
73. Nicholls TJ, Follett BK, Goldsmith AR, Pearson H. Possible homologies between photorefractoriness in sheep and birds: the effect of thyroidectomy on the length of the ewe's breeding season. *Reprod Nutr Dev*. 1988;2B:375-385.
74. Sharp PJ. Photoperiodic regulation of seasonal breeding in birds. *Ann N Y Acad Sci*. 2005;1040:189-199.
75. Yoshimura T. Thyroid hormone and seasonal regulation of reproduction. *Front Neuroendocrinol*. 2013;3:157-166.
76. Dawson A. Annual gonadal cycles in birds: modeling the effects of photoperiod on seasonal changes in GnRH-1 secretion. *Front Neuroendocrinol*. 2015;37:52-64.
77. Karsch FJ, Dahl GE, Hachigian TM, Thrun LA. Involvement of thyroid hormones in seasonal reproduction. *J Reprod Fertil Suppl*. 1995;49:409-422.
78. Dahl GE, Evans NP, Thrun LA, Karsch FJ. Thyroxine is permissive to seasonal transitions in reproductive neuroendocrine activity in the ewe. *Biol Reprod*. 1995;3:690-696.
79. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev*. 2002;1:38-89.
80. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol*. 2011;1:1-14.
81. Muller R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev*. 2014;2:355-382.
82. Bernal J, Guadano-Ferraz A, Morte B. Thyroid hormone transporters--functions and clinical implications. *Nat Rev Endocrinol*. 2015;7:406-417.
83. Lopez M, Alvarez CV, Nogueiras R, Dieguez C. Energy balance regulation by thyroid hormones at central level. *Trends Mol Med*. 2013;19:418-427.
84. Bolborea M, Dale N. Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. *Trends Neurosci*. 2013;2:91-100.
85. Lewis JE, Ebling FJ. Tanycytes as regulators of seasonal cycles in neuroendocrine function. *Front Neurosci*. 2017;8:79.
86. Prevot V, Dehouck B, Sharif A, Ciofi P, Giacobini P, Clasadonte J. The versatile Tanycyte: a hypothalamic integrator of reproduction and energy metabolism. *Endocr Rev*. 2018;3:333-368.
87. Rodriguez E, Guerra M, Peruzzo B, Blazquez JL. Tanycytes: a rich morphological history to underpin future molecular and physiological investigations. *J Neuroendocrinol*. 2019;3:e12690.
88. Prevot V, Nogueiras R, Schwaninger M. Tanycytes in the infundibular nucleus and median eminence and their role in the blood-brain barrier. *Handb Clin Neurol*. 2021;180:253-273.
89. Yoshimura T, Yasuo S, Watanabe M, et al. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. *Nature*. 2003;6963:178-181.
90. Nakao N, Ono H, Yamamura T, et al. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature*. 2008;7185:317-322.
91. Hanon EA, Lincoln GA, Fustin JM, et al. Ancestral TSH mechanism signals summer in a photoperiodic mammal. *Curr Biol*. 2008;15:1147-1152.
92. Klosen P, Sebert ME, Rasri K, Laran-Chich MP, Simonneaux V. TSH restores a summer phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. *FASEB J*. 2013;7:2677-2686.
93. Helfer G, Ross AW, Morgan PJ. Neuromedin U partly mimics thyroid-stimulating hormone and triggers Wnt/beta-catenin signaling in the photoperiodic response of F344 rats. *J Neuroendocrinol*. 2013;12:1264-1272.
94. Stoney PN, Helfer G, Rodrigues D, Morgan PJ, McCaffery P. Thyroid hormone activation of retinoic acid synthesis in hypothalamic tanycytes. *Glia*. 2016;64:425-439.
95. Ono H, Hoshino Y, Yasuo S, et al. Involvement of thyrotropin in photoperiodic signal transduction in mice. *Proc Natl Acad Sci USA*. 2008;47:18238-18242.



96. Yasuo S, Yoshimura T, Ebihara S, Korf HW. Photoperiodic control of TSH-beta expression in the mammalian pars Tuberalis has different impacts on the induction and suppression of the Hypothalamo-Hypophysial gonadal Axis. *J Neuroendocrinol.* 2010;1:43-50.
97. Hanon EA, Routledge K, Dardente H, Masson-Pevet M, Morgan PJ, Hazlerigg DG. Effect of photoperiod on the thyroid-stimulating hormone neuroendocrine system in the European hamster (*Cricetus cricetus*). *J Neuroendocrinol.* 2010;1:51-55.
98. Ross AW, Helfer G, Russell L, Darras VM, Morgan PJ. Thyroid hormone signalling genes are regulated by photoperiod in the hypothalamus of F344 rats. *PLoS One.* 2011;6:e21351.
99. Krol E, Douglas A, Dardente H, et al. Strong pituitary and hypothalamic responses to photoperiod but not to 6-methoxy-2-benzoxazolinone in female common voles (*Microtus arvalis*). *Gen Comp Endocrinol.* 2012;2:289-295.
100. Dunn IC, Wilson PW, Shi Y, Burt DW, Loudon ASI, Sharp PJ. Diurnal and photoperiodic changes in thyrotrophin-stimulating hormone beta expression and associated regulation of deiodinase enzymes (DIO2, DIO3) in the female juvenile chicken hypothalamus. *J Neuroendocrinol.* 2017;29:e12554.
101. Appenroth D, Melum VJ, West AC, Dardente H, Hazlerigg DG, Wagner GC. Photoperiodic induction without light-mediated circadian entrainment in a high Arctic resident bird. *J Exp Biol.* 2020;223:jeb.220699.
102. Ikegami K, Liao XH, Hoshino Y, et al. Tissue-specific posttranslational modification allows functional targeting of thyrotropin. *Cell Rep.* 2014;3:801-810.
103. Unfried C, Ansari N, Yasuo S, Korf HW, von Gall C. Impact of melatonin and molecular clockwork components on the expression of thyrotropin beta-chain (Tshb) and the Tsh receptor in the mouse pars tuberalis. *Endocrinology.* 2009;10:4653-4662.
104. Bolborea M, Helfer G, Ebling FJ, Barrett P. Dual signal transduction pathways activated by TSH receptors in rat primary tanycyte cultures. *J Mol Endocrinol.* 2015;3:241-250.
105. Ciofi P, Garret M, Lapirot O, et al. Brain-endocrine interactions: a microvascular route in the mediobasal hypothalamus. *Endocrinology.* 2009;12:5509-5519.
106. Malpoux B, Migaud M, Tricoire H, Chemineau P. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *J Biol Rhythms.* 2001;4:336-347.
107. Legros C, Chesneau D, Boutin JA, Barc C, Malpoux B. Melatonin from cerebrospinal fluid but not from blood reaches sheep cerebral tissues under physiological conditions. *J Neuroendocrinol.* 2014;3:151-163.
108. Pittendrigh CS. Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. *Proc Natl Acad Sci USA.* 1972;9:2734-2737.
109. Appenroth D, Wagner GC, Hazlerigg DG, West AC. Evidence for circadian-based photoperiodic timekeeping in Svalbard ptarmigan, the northernmost resident bird. *Curr Biol.* 2021;12:2720-2727.
110. Lincoln GA, Clarke IJ. Photoperiodically-induced cycles in the secretion of prolactin in hypothalamo-pituitary disconnected rams: evidence for translation of the melatonin signal in the pituitary gland. *J Neuroendocrinol.* 1994;3:251-260.
111. Morgan PJ. The pars tuberalis: the missing link in the photoperiodic regulation of prolactin secretion? *J Neuroendocrinol.* 2000;4:287-295.
112. Lincoln GA, Andersson H, Hazlerigg D. Clock genes and the long-term regulation of prolactin secretion: evidence for a photoperiod/circannual timer in the pars tuberalis. *J Neuroendocrinol.* 2003;4:390-397.
113. Lincoln GA, Clarke IJ, Hut RA, Hazlerigg DG. Characterizing a mammalian circannual pacemaker. *Science.* 2006;5807:1941-1944.
114. Saenz de Miera C, Monecke S, Bartzen-Sprauer J, et al. A circannual clock drives expression of genes central for seasonal reproduction. *Curr Biol.* 2014;13:1500-1506.
115. Lincoln G. A brief history of circannual time. *J Neuroendocrinol.* 2019;3:e12694.
116. Watanabe T, Yamamura T, Watanabe M, et al. Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. *Am J Physiol Regul Integr Comp Physiol.* 2007;1:R568-R572.
117. Herwig A, de Vries EM, Bolborea M, et al. Hypothalamic ventricular ependymal thyroid hormone deiodinases are an important element of circannual timing in the Siberian hamster (*Phodopus sungorus*). *PLoS One.* 2013;4:e62003.
118. Saenz de Miera C, Hanon EA, Dardente H, et al. Circannual variation in thyroid hormone deiodinases in a short-day breeder. *J Neuroendocrinol.* 2013;4:412-421.
119. Milesi S, Simonneaux V, Klosen P. Downregulation of deiodinase 3 is the earliest event in photoperiodic and photorefractory activation of the gonadotropic axis in seasonal hamsters. *Sci Rep.* 2017;7:1-10.
120. Dardente H. Circannual biology: the double life of the seasonal Thyrotroph. *Curr Biol.* 2015;20:R988-R991.
121. Lomet D, Druart X, Hazlerigg D, Beltramo M, Dardente H. Circuit-level analysis identifies target genes of sex steroids in ewe seasonal breeding. *Mol Cell Endocrinol.* 2020;512:110825.
122. van Rosmalen L, Hut RA. Food and temperature change photoperiodic responses in two vole species: different roles for hypothalamic genes. *J Exp Biol.* 2021;224:jeb.243030.
123. Monecke S, Sage-Ciocca D, Wollnik F, Pevet P. Photoperiod can entrain circannual rhythms in pinealectomized European hamsters. *J Biol Rhythms.* 2013;4:278-290.
124. Saenz de Miera C, Sage-Ciocca D, Simonneaux V, Pevet P, Monecke S. Melatonin-independent photoperiodic entrainment of the circannual TSH rhythm in the pars Tuberalis of the European hamster. *J Biol Rhythms.* 2018;33:302-317.
125. Wood S, Loudon A. Clocks for all seasons: unwinding the roles and mechanisms of circadian and interval timers in the hypothalamus and pituitary. *J Endocrinol.* 2014;2:R39-R59.
126. Ebling FJP, Fletcher J, Hazlerigg DG, Loudon ASI. Gerald Lincoln: a man for all seasons. *J Neuroendocrinol.* 2021;5:e12968.
127. Barrett P, Ebling FJ, Schuhler S, et al. Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology.* 2007;8:3608-3617.
128. Murphy M, Jethwa PH, Warner A, et al. Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinology.* 2012;1:101-112.
129. Barrell GK, Moenter SM, Caraty A, Karsch FJ. Seasonal changes of gonadotropin-releasing hormone secretion in the ewe. *Biol Reprod.* 1992;6:1130-1135.
130. Helfer G, Barrett P, Morgan PJ. A unifying hypothesis for control of body weight and reproduction in seasonally breeding mammals. *J Neuroendocrinol.* 2019;3:e12680.
131. Quignon C, Beymer M, Gauthier K, Gauer F, Simonneaux V. Thyroid hormone receptors are required for the melatonin-dependent control of Rfrp gene expression in mice. *FASEB J.* 2020;9:12072-12082.
132. Prevot V, Bellefontaine N, Baroncini M, et al. Gonadotrophin-releasing hormone nerve terminals, tanycytes and neurohaemal junction remodelling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. *J Neuroendocrinol.* 2010;7:639-649.
133. Yamamura T, Hirunagi K, Ebihara S, Yoshimura T. Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail. *Endocrinology.* 2004;9:4264-4267.
134. Bolborea M, Laran-Chich MP, Rasri K, et al. Melatonin controls photoperiodic changes in tanycyte vimentin and neural cell adhesion molecule expression in the Djungarian hamster (*Phodopus sungorus*). *Endocrinology.* 2011;10:3871-3883.

135. Hrabovszky E, Shughrue PJ, Merchenthaler I, et al. Detection of estrogen receptor-beta messenger ribonucleic acid and 125I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain. *Endocrinology*. 2000;9:3506-3509.
136. Wintermantel TM, Campbell RE, Porteous R, et al. Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. *Neuron*. 2006;2:271-280.
137. Kenealy BP, Keen KL, Terasawa E. Rapid action of estradiol in primate GnRH neurons: the role of estrogen receptor alpha and estrogen receptor beta. *Steroids*. 2011;9:861-866.
138. Cheong RY, Porteous R, Chambon P, Abraham I, Herbison AE. Effects of neuron-specific estrogen receptor (ER) alpha and ERbeta deletion on the acute estrogen negative feedback mechanism in adult female mice. *Endocrinology*. 2014;155:1418-1427.
139. Weems PW, Goodman RL, Lehman MN. Neural mechanisms controlling seasonal reproduction: principles derived from the sheep model and its comparison with hamsters. *Front Neuroendocrinol*. 2015;37:43-51.
140. Nestor CC, Bedenbaugh MN, Hileman SM, Coolen LM, Lehman MN, Goodman RL. Regulation of GnRH pulsatility in ewes. *Reproduction*. 2018;3:R83-R99.
141. Goodman RL, Maltby MJ, Millar RP, et al. Evidence that dopamine acts via kisspeptin to hold GnRH pulse frequency in check in anestrus ewes. *Endocrinology*. 2012;12:5918-5927.
142. Angelopoulou E, Quignon C, Kriegsfeld LJ, Simonneaux V. Functional implications of RFRP-3 in the central control of daily and seasonal rhythms in reproduction. *Front Endocrinol*. 2019;10:183.
143. Simonneaux V. A Kiss to drive rhythms in reproduction. *Eur J Neurosci*. 2020;1:509-530.
144. Kotani M, Dethoux M, Vandenbogaerde A, et al. The metastasis suppressor gene KISS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*. 2001;37:34631-34636.
145. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KISS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA*. 2003;19:10972-10976.
146. Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med*. 2003;17:1614-1627.
147. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol Rev*. 2012;3:1235-1316.
148. Cravo RM, Margatho LO, Osborne-Lawrence S, et al. Characterization of Kiss1 neurons using transgenic mouse models. *Neuroscience*. 2011;173:37-56.
149. Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y, Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. *Neurosci Lett*. 2006;3:225-230.
150. Gottsch ML, Cunningham MJ, Smith JT, et al. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*. 2004;9:4073-4077.
151. Hrabovszky E, Ciofi P, Vida B, et al. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. *Eur J Neurosci*. 2010;11:1984-1998.
152. Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*. 2005;9:3686-3692.
153. Smith JT, Dungan HM, Stoll EA, et al. Differential regulation of KISS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology*. 2005;7:2976-2984.
154. Smith JT, Clay CM, Caraty A, Clarke IJ. KISS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. *Endocrinology*. 2007;3:1150-1157.
155. Goodman RL, Lehman MN, Smith JT, et al. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin a and neurokinin B. *Endocrinology*. 2007;12:5752-5760.
156. Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology*. 2010;8:3479-3489.
157. Adachi S, Yamada S, Takatsu Y, et al. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. *J Reprod Dev*. 2007;2:367-378.
158. Ansel L, Bolborea M, Bentsen AH, Klosen P, Mikkelsen JD, Simonneaux V. Differential regulation of kiss1 expression by melatonin and gonadal hormones in male and female Syrian hamsters. *J Biol Rhythms*. 2010;2:81-91.
159. Cheng G, Coolen LM, Padmanabhan V, Goodman RL, Lehman MN. The kisspeptin/neurokinin B/dynorphin (KNDy) cell population of the arcuate nucleus: sex differences and effects of prenatal testosterone in sheep. *Endocrinology*. 2010;1:301-311.
160. Clarkson J, Herbison AE. Postnatal development of kisspeptin neurons in mouse hypothalamus: sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology*. 2006;12:5817-5825.
161. Kauffman AS, Gottsch ML, Roa J, et al. Sexual differentiation of Kiss1 gene expression in the brain of the rat. *Endocrinology*. 2007;4:1774-1783.
162. Yeo SH, Herbison AE. Projections of arcuate nucleus and rostral periventricular kisspeptin neurons in the adult female mouse brain. *Endocrinology*. 2011;6:2387-2399.
163. Yip SH, Boehm U, Herbison AE, Campbell RE. Conditional viral tract tracing delineates the projections of the distinct Kisspeptin neuron populations to gonadotropin-releasing hormone (GnRH) neurons in the mouse. *Endocrinology*. 2015;7:2582-2594.
164. Goodman RL, Lehman MN. Kisspeptin neurons from mice to men: similarities and differences. *Endocrinology*. 2012;11:5105-5118.
165. Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci*. 2005;49:11349-11356.
166. Herbison AE, de Tassigny X, Doran J, Colledge WH. Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology*. 2010;1:312-321.
167. Irwig MS, Fraley GS, Smith JT, et al. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KISS-1 mRNA in the male rat. *Neuroendocrinology*. 2004;4:264-272.
168. Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci USA*. 2005;5:1761-1766.
169. Kinoshita M, Tsukamura H, Adachi S, et al. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. *Endocrinology*. 2005;10:4431-4436.
170. Navarro VM, Castellano JM, Fernandez-Fernandez R, et al. Developmental and hormonally regulated messenger ribonucleic acid expression of KISS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KISS-1 peptide. *Endocrinology*. 2004;10:4565-4574.
171. Plant TM, Ramaswamy S, Dipietro MJ. Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges. *Endocrinology*. 2006;2:1007-1013.
172. Han SY, McLennan T, Czielesky K, Herbison AE. Selective optogenetic activation of arcuate kisspeptin neurons generates pulsatile luteinizing hormone secretion. *Proc Natl Acad Sci USA*. 2015;42:13109-13114.

173. Dhillon WS, Chaudhri OB, Patterson M, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab.* 2005;12:6609-6615.
174. Dhillon WS, Chaudhri OB, Thompson EL, et al. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *J Clin Endocrinol Metab.* 2007;10:3958-3966.
175. Kirilov M, Clarkson J, Liu X, et al. Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. *Nat Commun.* 2013;4:2492.
176. Leon S, Barroso A, Vazquez MJ, et al. Direct actions of Kisspeptins on GnRH neurons permit attainment of fertility but are insufficient to fully preserve gonadotropin Axis activity. *Sci Rep.* 2016;6:19206.
177. Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH, Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. *J Neurosci.* 2008;35:8691-8697.
178. Gottsch ML, Navarro VM, Zhao Z, et al. Regulation of Kiss1 and dynorphin gene expression in the murine brain by classical and non-classical estrogen receptor pathways. *J Neurosci.* 2009;29:9390-9395.
179. Uenoyama Y, Inoue N, Nakamura S, Tsukamura H. Kisspeptin neurons and estrogen-estrogen receptor alpha signaling: unraveling the mystery of steroid feedback system regulating mammalian reproduction. *Int J Mol Sci.* 2021;17:9229. doi:10.3390/ijms22179229
180. Estrada KM, Clay CM, Pompolo S, Smith JT, Clarke IJ. Elevated KISS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/luteinizing hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. *J Neuroendocrinol.* 2006;10:806-809.
181. Smith JT, Shahab M, Pereira A, Pau KY, Clarke IJ. Hypothalamic expression of KISS1 and gonadotropin inhibitory hormone genes during the menstrual cycle of a non-human primate. *Biol Reprod.* 2010;4:568-577.
182. Merkley CM, Porter KL, Coolen LM, et al. KNDy (kisspeptin/neurokinin B/dynorphin) neurons are activated during both pulsatile and surge secretion of LH in the ewe. *Endocrinology.* 2012;11:5406-5414.
183. Greenwald-Yarnell ML, Marsh C, Allison MB, et al. ERalpha in Tac2 neurons regulates puberty onset in female mice. *Endocrinology.* 2016;157:1555-1565.
184. Mayer C, Acosta-Martinez M, Dubois SL, et al. Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. *Proc Natl Acad Sci USA.* 2010;52:22693-22698.
185. Choe HK, Kim HD, Park SH, et al. Synchronous activation of gonadotropin-releasing hormone gene transcription and secretion by pulsatile kisspeptin stimulation. *Proc Natl Acad Sci USA.* 2013;14:5677-5682.
186. Clarkson J, Han SY, Piet R, et al. Definition of the hypothalamic GnRH pulse generator in mice. *Proc Natl Acad Sci USA.* 2017;47:E10216-E10223.
187. Kanasaki H, Tselmeg M, Oride A, Sukhbaatar U, Hara T, Kyo S. Pulsatile kisspeptin effectively stimulates gonadotropin-releasing hormone (GnRH)-producing neurons. *Gynecol Endocrinol.* 2017;9:721-727.
188. Caraty A, Franceschini I, Hoffman GE. Kisspeptin and the preovulatory gonadotrophin-releasing hormone/luteinizing hormone surge in the ewe: basic aspects and potential applications in the control of ovulation. *J Neuroendocrinol.* 2010;7:710-715.
189. Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. *J Neurosci.* 2006;25:6687-6694.
190. Watanabe Y, Uenoyama Y, Suzuki J, et al. Oestrogen-induced activation of preoptic kisspeptin neurones may be involved in the luteinizing hormone surge in male and female Japanese monkeys. *J Neuroendocrinol.* 2014;12:909-917.
191. Beltramo M, Dardente H, Cayla X, Caraty A. Cellular mechanisms and integrative timing of neuroendocrine control of GnRH secretion by kisspeptin. *Mol Cell Endocrinol.* 2014;1:387-399.
192. Revel FG, Saboureaux M, Masson-Pevet M, Pevet P, Mikkelsen JD, Simonneaux V. Kisspeptin mediates the photoperiodic control of reproduction in hamsters. *Curr Biol.* 2006;17:1730-1735.
193. Wagner GC, Johnston JD, Clarke IJ, Lincoln GA, Hazlerigg DG. Redefining the limits of day length responsiveness in a seasonal mammal. *Endocrinology.* 2008;1:32-39.
194. Greives TJ, Mason AO, Scotti MA, et al. Environmental control of kisspeptin: implications for seasonal reproduction. *Endocrinology.* 2007;3:1158-1166.
195. Rasri-Klosen K, Simonneaux V, Klosen P. Differential response patterns of kisspeptin and RFamide-related peptide to photoperiod and sex steroid feedback in the Djungarian hamster (*Phodopus sungorus*). *J Neuroendocrinol.* 2017;9:e12529.
196. Boufermes R, Richard N, Le Moguen K, Amirat Z, Khammar F, Kottler ML. Seasonal expression of KISS-1 and the pituitary gonadotropins LHbeta and FSHbeta in adult male Libyan jird (*Meriones libycus*). *Anim Reprod Sci.* 2014;1-2:56-63.
197. Talbi R, Klosen P, Laran-Chich MP, El Ouezzi S, Simonneaux V. Coordinated seasonal regulation of metabolic and reproductive hypothalamic peptides in the desert jerboa. *J Comp Neurol.* 2016;18:3717-3728.
198. Barrell GK, Ridgway MJ, Wellby M, Pereira A, Henry BA, Clarke IJ. Expression of regulatory neuropeptides in the hypothalamus of red deer (*Cervus elaphus*) reveals anomalous relationships in the seasonal control of appetite and reproduction. *Gen Comp Endocrinol.* 2016;229:1-7.
199. Medger K, Bennett NC, Chimimba CT, Oosthuizen MK, Mikkelsen JD, Coen CW. Analysis of gonadotrophin-releasing hormone-1 and kisspeptin neuronal systems in the non-photoregulated seasonally breeding eastern rock elephant-shrew (*Elephantulus myurus*). *J Comp Neurol.* 2018;15:2388-2405.
200. Wang D, Li N, Tian L, et al. Dynamic expressions of hypothalamic genes regulate seasonal breeding in a natural rodent population. *Mol Ecol.* 2019;15:3508-3522.
201. Ainani H, El Bousmaki N, Poirer VJ, et al. The dromedary camel displays annual variation in hypothalamic kisspeptin and Arg-Phe-amide-related peptide-3 according to sex, season, and breeding activity. *J Comp Neurol.* 2020;1:32-47.
202. Saenz de Miera C, Beymer M, et al. Photoperiodic regulation in a wild-derived mouse strain. *J Exp Biol.* 2020;223:jeb217687.
203. Poissenot K, Moussu C, Chesneau D, et al. Field study reveals morphological and neuroendocrine correlates of seasonal breeding in female water voles, *Arvicola terrestris*. *Gen Comp Endocrinol.* 2021;311:113853.
204. Bartzten-Sprauer J, Klosen P, Ciofi P, Mikkelsen JD, Simonneaux V. Photoperiodic co-regulation of kisspeptin, neurokinin B and dynorphin in the hypothalamus of a seasonal rodent. *J Neuroendocrinol.* 2014;8:510-520.
205. Weems P, Smith J, Clarke IJ, Coolen LM, Goodman RL, Lehman MN. Effects of season and estradiol on KNDy neuron peptides, Colocalization with D2 dopamine receptors, and dopaminergic inputs in the ewe. *Endocrinology.* 2017;4:831-841.
206. Piekarski DJ, Jarjisian SG, Perez L, et al. Effects of Pinealectomy and Short day lengths on reproduction and neuronal RFRP-3, Kisspeptin, and GnRH in female Turkish hamsters. *J Biol Rhythms.* 2014;3:181-191.
207. Li SN, Xue HL, Zhang Q, et al. Photoperiod regulates the differential expression of KISS-1 and GPR54 in various tissues and sexes of striped hamster. *Genet Mol Res.* 2015;4:13894-13905.
208. Oliveira AC, Andreotti S, Sertie RAL, et al. Combined treatment with melatonin and insulin improves glycemic control, white adipose

- tissue metabolism and reproductive axis of diabetic male rats. *Life Sci.* 2018;199:158-166.
209. Li Q, Rao A, Pereira A, Clarke IJ, Smith JT. Kisspeptin cells in the ovine arcuate nucleus express prolactin receptor but not melatonin receptor. *J Neuroendocrinol.* 2011;10:871-882.
  210. Henson JR, Carter SN, Freeman DA. Exogenous T(3) elicits long day-like alterations in testis size and the RFamides Kisspeptin and gonadotropin-inhibitory hormone in short-day Siberian hamsters. *J Biol Rhythms.* 2013;3:193-200.
  211. Dufourny L, Gennetay D, Martinet S, Lomet D, Caraty A. The content of thyroid hormone receptor alpha in ewe Kisspeptin Neurons is not season-dependent. *J Neuroendocrinol.* 2016;2:12344.
  212. Greives TJ, Humber SA, Goldstein AN, Scotti MA, Demas GE, Kriegsfeld LJ. Photoperiod and testosterone interact to drive seasonal changes in kisspeptin expression in Siberian hamsters (*Phodopus sungorus*). *J Neuroendocrinol.* 2008;12:1339-1347.
  213. Greives TJ, Kriegsfeld LJ, Demas GE. Exogenous kisspeptin does not alter photoperiod-induced gonadal regression in Siberian hamsters (*Phodopus sungorus*). *Gen Comp Endocrinol.* 2008;3:552-558.
  214. Ronnekleiv OK, Qiu J, Kelly MJ. Arcuate Kisspeptin neurons coordinate reproductive activities with metabolism. *Semin Reprod Med.* 2019;3:131-140.
  215. Cazarez-Marquez F, Milesi S, Laran-Chich MP, Klosen P, Kalsbeek A, Simonneaux V. Kisspeptin and RFRP3 modulate body mass in *Phodopus sungorus* via two different neuroendocrine pathways. *J Neuroendocrinol.* 2019;4:e12710.
  216. Ansel L, Bentsen AH, Ancel C, et al. Peripheral kisspeptin reverses short photoperiod-induced gonadal regression in Syrian hamsters by promoting GNRH release. *Reproduction.* 2011;3:417-425.
  217. Caraty A, Smith JT, Lomet D, et al. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. *Endocrinology.* 2007;11:5258-5267.
  218. Decourt C, Caraty A, Briant C, et al. Acute injection and chronic perfusion of kisspeptin elicit gonadotropins release but fail to trigger ovulation in the mare. *Biol Reprod.* 2014;90:36.
  219. Yoshida N, Nishizawa N, Matsui H, et al. Development and validation of sensitive sandwich ELISAs for two investigational non-peptide metastin receptor agonists, TAK-448 and TAK-683. *J Pharm Biomed Anal.* 2012;70:369-377.
  220. Decourt C, Robert V, Anger K, et al. A synthetic kisspeptin analog that triggers ovulation and advances puberty. *Sci Rep.* 2016;6:26908.
  221. Doebelin C, Bertin I, Schneider S, et al. Development of Dipeptidic hGPR54 agonists. *ChemMedChem.* 2016;19:2147-2154.
  222. Decourt C, Robert V, Lomet D, et al. The kisspeptin analog C6 is a possible alternative to PMSG (pregnant mare serum gonadotropin) for triggering synchronized and fertile ovulations in the alpine goat. *PLoS One.* 2019;3:e0214424.
  223. Beltramo M, Robert V, Decourt C. The kisspeptin system in domestic animals: what we know and what we still need to understand of its role in reproduction. *Domest Anim Endocrinol.* 2020;73:106466.
  224. Tsutsui K, Saigoh E, Ukena K, et al. A novel avian hypothalamic peptide inhibiting gonadotropin release. *Biochem Biophys Res Commun.* 2000;2:661-667.
  225. Hinuma S, Shintani Y, Fukusumi S, et al. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. *Nat Cell Biol.* 2000;10:703-708.
  226. Fukusumi S, Habata Y, Yoshida H, et al. Characteristics and distribution of endogenous RFamide-related peptide-1. *Biochim Biophys Acta.* 2001;3:221-232.
  227. Ukena K, Iwakoshi E, Minakata H, Tsutsui K. A novel rat hypothalamic RFamide-related peptide identified by immunoaffinity chromatography and mass spectrometry. *FEBS Lett.* 2002;1-3:255-258.
  228. Kriegsfeld LJ, Mei DF, Bentley GE, et al. Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proc Natl Acad Sci USA.* 2006;7:2410-2415.
  229. Ukena K, Tsutsui K. Distribution of novel RFamide-related peptide-like immunoreactivity in the mouse central nervous system. *Neurosci Lett.* 2001;3:153-156.
  230. Revel FG, Saboureau M, Pevet P, Simonneaux V, Mikkelsen JD. RFamide-related peptide gene is a melatonin-driven photoperiodic gene. *Endocrinology.* 2008;3:902-912.
  231. Dardente H, Birnie M, Lincoln GA, Hazlerigg DG. RFamide-related peptide and its cognate receptor in the sheep: cDNA cloning, mRNA distribution in the hypothalamus and the effect of photoperiod. *J Neuroendocrinol.* 2008;11:1252-1259.
  232. Smith JT, Coolen LM, Kriegsfeld LJ, et al. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. *Endocrinology.* 2008;11:5770-5782.
  233. Ubuka T, Morgan K, Pawson AJ, et al. Identification of human GnIH homologs, RFRP-1 and RFRP-3, and the cognate receptor, GPR147 in the human hypothalamic pituitary axis. *PLoS One.* 2009;12:e8400.
  234. Ubuka T, Lai H, Kitani M, et al. Gonadotropin-inhibitory hormone identification, cDNA cloning, and distribution in rhesus macaque brain. *J Comp Neurol.* 2009;6:841-855.
  235. Tanco VM, Whitlock BK, Jones MA, Wilborn RR, Brandebourg TD, Foradori CD. Distribution and regulation of gonadotropin-releasing hormone, kisspeptin, RF-amide related peptide-3, and dynorphin in the bovine hypothalamus. *PeerJ.* 2016;4:e1833.
  236. Thorson JF, Prezotto LD, Cardoso RC, et al. Hypothalamic distribution, adenohypophyseal receptor expression, and ligand functionality of RFamide-related peptide 3 in the mare during the breeding and nonbreeding seasons. *Biol Reprod.* 2014;2:28.
  237. Rizwan MZ, Poling MC, Corr M, et al. RFamide-related peptide-3 receptor gene expression in GnRH and kisspeptin neurons and GnRH-dependent mechanism of action. *Endocrinology.* 2012;8:3770-3779.
  238. Henningsen JB, Poirel VJ, Mikkelsen JD, Tsutsui K, Simonneaux V, Gauer F. Sex differences in the photoperiodic regulation of RFamide related peptide (RFRP) and its receptor GPR147 in the Syrian hamster. *J Comp Neurol.* 2016;9:1825-1838.
  239. Ubuka T, Inoue K, Fukuda Y, et al. Identification, expression, and physiological functions of Siberian hamster gonadotropin-inhibitory hormone. *Endocrinology.* 2012;1:373-385.
  240. Poling MC, Quennell JH, Anderson GM, Kauffman AS. Kisspeptin neurons do not directly signal to RFRP-3 neurones but RFRP-3 may directly modulate a subset of hypothalamic kisspeptin cells in mice. *J Neuroendocrinol.* 2013;10:876-886.
  241. Ducret E, Anderson GM, Herbison AE. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. *Endocrinology.* 2009;6:2799-2804.
  242. Wu M, Dumalska I, Morozova E, van den Pol AN, Alreja M. Gonadotropin inhibitory hormone inhibits basal forebrain vGluT2-gonadotropin-releasing hormone neurons via a direct postsynaptic mechanism. *J Physiol.* 2009;7:1401-1411.
  243. Gojska NM, Friedman Z, Belsham DD. Direct regulation of gonadotropin-releasing hormone (GnRH) transcription by RFamide-related peptide-3 and kisspeptin in a novel GnRH-secreting cell line, mHypoA-GnRH/GFP. *J Neuroendocrinol.* 2014;12:888-897.
  244. Johnson MA, Tsutsui K, Fraley GS. Rat RFamide-related peptide-3 stimulates GH secretion, inhibits LH secretion, and has variable effects on sex behavior in the adult male rat. *Horm Behav.* 2007;1:171-180.
  245. Anderson GM, Relf HL, Rizwan MZ, Evans JJ. Central and peripheral effects of RFamide-related peptide-3 on luteinizing hormone and prolactin secretion in rats. *Endocrinology.* 2009;4:1834-1840.
  246. Pineda R, Garcia-Galiano D, Sanchez-Garrido MA, et al. Characterization of the inhibitory roles of RFRP3, the mammalian ortholog of



- GnIH, in the control of gonadotropin secretion in the rat: in vivo and in vitro studies. *Am J Physiol Endocrinol Metab.* 2010;1:E39-E46.
247. Clarke IJ, Sari IP, Qi Y, et al. Potent action of RFamide-related peptide-3 on pituitary gonadotropes indicative of a hypophysiotropic role in the negative regulation of gonadotropin secretion. *Endocrinology.* 2008;11:5811-5821.
248. Ancel C, Bentsen AH, Sebert ME, Tena-Sempere M, Mikkelsen JD, Simonneaux V. Stimulatory effect of RFRP-3 on the gonadotrophic axis in the male Syrian hamster: the exception proves the rule. *Endocrinology.* 2012;3:1352-1363.
249. Caraty A, Blomenrohr M, Vogel GM, Lomet D, Briant C, Beltramo M. RF9 powerfully stimulates gonadotrophin secretion in the ewe: evidence for a seasonal threshold of sensitivity. *J Neuroendocrinol.* 2012;5:725-736.
250. Decourt C, Anger K, Robert V, et al. No evidence that RFamide-related peptide 3 directly modulates LH secretion in the ewe. *Endocrinology.* 2016;4:1566-1575.
251. Henningsen JB, Ancel C, Mikkelsen JD, Gauer F, Simonneaux V. Roles of RFRP-3 in the daily and seasonal regulation of reproductive activity in female Syrian hamsters. *Endocrinology.* 2017;3:652-663.
252. Ancel C, Inglis MA, Anderson GM. Central RFRP-3 stimulates LH secretion in male mice and has cycle stage-dependent inhibitory effects in females. *Endocrinology.* 2017;9:2873-2883.
253. George JT, Hendrikse M, Veldhuis JD, Clarke IJ, Anderson RA, Millar RP. Effect of gonadotropin-inhibitory hormone on luteinizing hormone secretion in humans. *Clin Endocrinol (Oxf).* 2017;5:731-738.
254. Sari IP, Rao A, Smith JT, Tilbrook AJ, Clarke IJ. Effect of RF-amide-related peptide-3 on luteinizing hormone and follicle-stimulating hormone synthesis and secretion in ovine pituitary gonadotropes. *Endocrinology.* 2009;12:5549-5556.
255. Kadokawa H, Shibata M, Tanaka Y, et al. Bovine C-terminal octapeptide of RFamide-related peptide-3 suppresses luteinizing hormone (LH) secretion from the pituitary as well as pulsatile LH secretion in bovines. *Domest Anim Endocrinol.* 2009;4:219-224.
256. Zmijewska A, Czelejewska W, Dziekonski M, Gajewska A, Franczak A, Okrasa S. Effect of kisspeptin and RFamide-related peptide-3 on the synthesis and secretion of LH by pituitary cells of pigs during the estrous cycle. *Anim Reprod Sci.* 2020;214:106275.
257. Thorson JF, Desaulniers AT, Lee C, White BR, Ford JJ, Lents CA. The role of RFamide-related peptide 3 (RFRP3) in regulation of the neuroendocrine reproductive and growth axes of the boar. *Anim Reprod Sci.* 2015;159:60-65.
258. Smith JT, Young IR, Veldhuis JD, Clarke IJ. Gonadotropin-inhibitory hormone (GnIH) secretion into the ovine hypophyseal portal system. *Endocrinology.* 2012;7:3368-3375.
259. Leon S, Garcia-Galiano D, Ruiz-Pino F, et al. Physiological roles of gonadotropin-inhibitory hormone signaling in the control of mammalian reproductive axis: studies in the NPFF1 receptor null mouse. *Endocrinology.* 2014;155:2953-2965.
260. Cazarez-Marquez F, Laran-Chich MP, Klosen P, Kalsbeek A, Simonneaux V. RFRP3 increases food intake in a sex-dependent manner in the seasonal hamster *Phodopus sungorus*. *J Neuroendocrinol.* 2020;5:e12845.
261. Poissenot K, Chorfa A, Moussu C, et al. Photoperiod is involved in the regulation of seasonal breeding in male water voles (*Arvicola terrestris*). *J Exp Biol.* 2021;224:jeb242792.
262. Harbid AA, McLeod BJ, Caraty A, Anderson GM. Seasonal changes in RFamide-related peptide-3 neurons in the hypothalamus of a seasonally breeding marsupial species, the brushtail possum (*Trichosurus vulpecula*). *J Comp Neurol.* 2013;13:3030-3041.
263. Jafarzadeh Shirazi MR, Zamiri MJ, Salehi MS, et al. Differential expression of RFamide-related peptide, a mammalian gonadotrophin-inhibitory hormone orthologue, and kisspeptin in the hypothalamus of Abadeh ecotype does during breeding and anoestrous seasons. *J Neuroendocrinol.* 2014;3:186-194.
264. Ubuka T, Bentley GE, Ukena K, Wingfield JC, Tsutsui K. Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. *Proc Natl Acad Sci USA.* 2005;8:3052-3057.
265. Maywood ES, Bittman EL, Hastings MH. Lesions of the melatonin- and androgen-responsive tissue of the dorsomedial nucleus of the hypothalamus block the gonadal response of male Syrian hamsters to programmed infusions of melatonin. *Biol Reprod.* 1996;2:470-477.
266. Gingerich S, Wang X, Lee PK, et al. The generation of an array of clonal, immortalized cell models from the rat hypothalamus: analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. *Neuroscience.* 2009;4:1134-1140.
267. Molnar CS, Kallo I, Liposits Z, Hrabovszky E. Estradiol down-regulates RF-amide-related peptide (RFRP) expression in the mouse hypothalamus. *Endocrinology.* 2011;152:1684-1690.
268. Poling MC, Kim J, Dhamija S, Kauffman AS. Development, sex steroid regulation, and phenotypic characterization of RFamide-related peptide (Rfrp) gene expression and RFamide receptors in the mouse hypothalamus. *Endocrinology.* 2012;4:1827-1840.
269. Quennell JH, Rizwan MZ, Relf HL, Anderson GM. Developmental and steroidogenic effects on the gene expression of RFamide related peptides and their receptor in the rat brain and pituitary gland. *J Neuroendocrinol.* 2010;4:309-316.
270. Kim JS, Brownjohn PW, Dyer BS, et al. Anxiogenic and stressor effects of the hypothalamic neuropeptide RFRP-3 are overcome by the NPFFR antagonist GJ14. *Endocrinology.* 2015;11:4152-4162.
271. Elhabazi K, Humbert JP, Bertin I, et al. RF313, an orally bioavailable neuropeptide FF receptor antagonist, opposes effects of RF-amide-related peptide-3 and opioid-induced hyperalgesia in rodents. *Neuropharmacology.* 2017;118:188-198.
272. Quillet R, Schneider S, Utard V, et al. Identification of an N-acylated-(D)Arg-Leu-NH<sub>2</sub> dipeptide as a highly selective neuropeptide FF1 receptor antagonist that potently prevents opioid-induced hyperalgesia. *J Med Chem.* 2021;11:7555-7564.

**How to cite this article:** Dardente H, Simonneaux V. GnRH and the photoperiodic control of seasonal reproduction: Delegating the task to kisspeptin and RFRP-3. *J Neuroendocrinol.* 2022;e13124. doi:10.1111/jne.13124