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

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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.^{1–5} 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.⁶ 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.^{3,7} 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.”^{8–11}

2 | PHOTOPERIODIC CONTROL OF MELATONIN

In mammals, melatonin is the endocrine messenger of photoperiod.^{12–15} 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.^{16,17} 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*.^{18–20} 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.^{21–24} 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.¹⁴ Remarkably, the annual change in day length modulates daily electrical activity and clock gene transcription in the SCN,^{25–27} 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.^{14,28} 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.^{12,14,29,30} 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.^{4,31,32}

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.^{33–37} Such findings suggested that seasonal control of GnRH output might be indirect,³⁸ 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.^{15,39,40} In brief, and even though data somehow differed between species and laboratories (mostly for melatonin binding assays), several firm conclusions could be reached.^{41,42}

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.^{43–46} Thus, the highly seasonal Siberian hamster bears a natural knockout of MT2⁴³ 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.⁴⁷ 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.⁴⁸ Finally, recent evidence from transgenic mouse models confirms that the MT2 is not expressed in the PT.⁴⁷ 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.^{41,42,49,50} 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 bloodstream.

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.^{50–55} 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,^{55–58} 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.⁵⁴ 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.^{59–62} 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.^{61,63}

Seasonal control of *Tshb* expression and TSH production in the PT was first demonstrated in the Siberian hamster in the late 1980s.⁶⁴ 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.^{65,66} 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)⁶⁷ and are therefore mostly sensitive to melatonin because they express MT1 receptor.^{68,69} 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.^{7,66,70–72} 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.^{7,73–76} 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.^{15,77} In particular, it was found that T3 plays a permissive role⁷⁸ through a central mechanism located in the MBH. In parallel, great advances in our understanding of TH action have been made.^{79–82} 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.⁸³ 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.^{84–88}

Using quail as a model, Yoshimura et al.⁸⁹ 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.”¹⁵ In a further twist, work in quail,⁹⁰ sheep⁹¹ and hamsters⁹² 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*⁹³ and *in vitro*⁹⁴ 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.^{95–101} 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.⁵ 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,⁶⁷ murine PT-TSH displays a glycosylation pattern distinct from PD-TSH, which would account for its specific action on TSHR in tanycytes.¹⁰² 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.^{90–94,103,104} 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 α tanycytes lining the walls of the third ventricle.^{86,88} 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.^{88,105}

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.^{2,15,42,106} 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.¹⁰⁷ Therefore, because seasonal timing is based upon accurate daily timing, orchestrated by a melatonin-dependent circadian clock,^{108,109} 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.^{15,42}

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 U⁹³). 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.^{66,110–115} 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.¹¹⁵ 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.^{114–119} Furthermore, recent RNA-sequencing analyses in sheep have identified hundreds of genes that display strong photoperiodic and circannual control of expression.^{62,66,70,72,120} These inner switches in expression seem to be accounted for by epigenetic changes at the gene promoter level, as shown for *Tshb* and *Eya3*.⁶² Furthermore, the PT-expressed histone methyltransferases *Ezh2* and *Suv39H2* display a robust increase in expression under long days compared to short days.⁷⁰ 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.^{70,121} Preliminary data in voles and sheep show a moderate impact of food-restriction on *Tshb* and *Eya3* expression within the PT¹²²; 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,^{123,124} 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^{5,50,112,115,125,126} (Figure 1). Second, the TSH-driven DIO2/T3 signal also regulates seasonal changes in body weight and torpor.^{92,127,128} 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,⁸⁶ and, on the other side, the TSH-driven T3 signal may control specific metabolic targets, including somatostatin and pro-opiomelanocortin neurons.^{7,92,127} Therefore tanycytes appear as an important integration site of multiple cues (photoperiod, food,

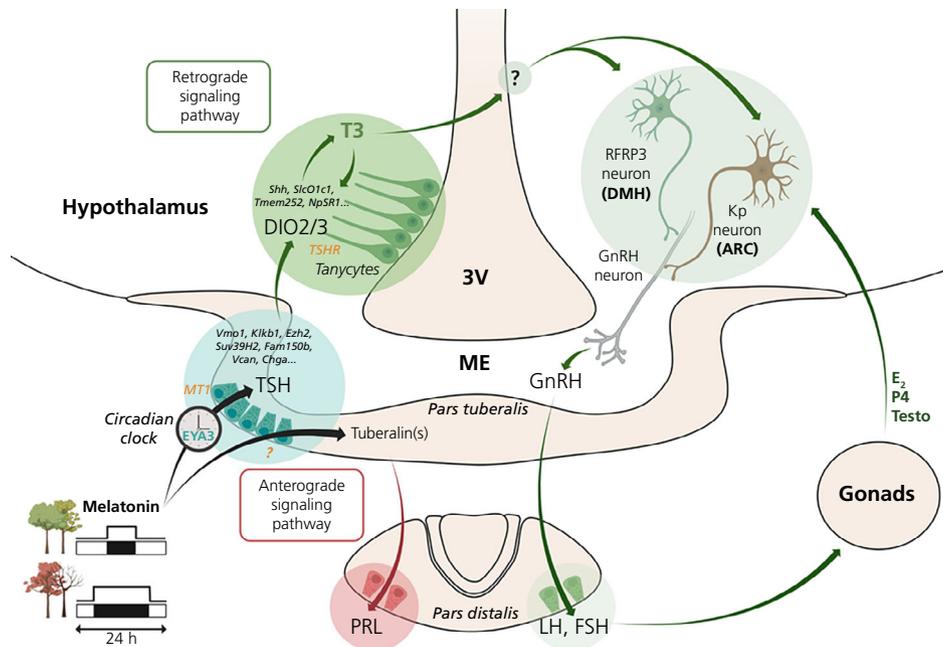


FIGURE 1 A simplified framework for the photoperiodic control of seasonal breeding. Melatonin acts at the level of the *pars tuberalis* (through the MT1 melatonin receptor) to control both anterograde and retrograde signaling pathways. The anterograde pathway involves *pars tuberalis*-derived tuberalin(s) 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.^{84–88}

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,⁷⁷ more recent experiments have demonstrated that chronic intracerebral administration of T3 or TSH in short-day adapted sexually inactive quails⁸⁹ or hamsters^{92,127} 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.¹²⁹ 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.^{5,130}

Therefore, the identification of cellular and molecular targets of T3 is a priority for understanding seasonal breeding. T3 receptors, TR α and, to a lesser extent, TR β are ubiquitously distributed in the central nervous system, making it difficult to identify these targets. Notably, a recent work identified TR α expression in mouse tanycytes, which could participate in transmitting the seasonal signal towards the hypothalamus.¹³¹ Another recent work in ewes has identified TMEM252, a gene specifically expressed by β 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.⁷⁰ Therefore, *Tmem252* and a few other genes also expressed by tanycytes, such as *Shh* and *Dct*,^{70,71} 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.^{86,132} 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,¹³³ and T3 implantation reverses the short-day phenotype by reducing encasement of GnRH nerve terminals.¹³³ Siberian hamster¹³⁴ and sheep⁶⁶ 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.¹³⁵⁻¹³⁸ Therefore, former studies have attempted to unravel the key upstream regulator(s) of GnRH neurons responsible for the seasonal release of GnRH.^{2,139} A possible upstream regulator of GnRH neurons could be the dopaminergic A15 nucleus.¹³⁹ 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.^{139,140} 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.¹⁴¹ Likewise, the A15 nucleus is not involved in breeding seasonality in hamsters.¹³⁹ 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^{3,142,143} (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,¹⁴⁴ were associated with idiopathic hypogonadotropic hypogonadism in humans¹⁴⁵ and a lack of puberty in mice.¹⁴⁶ 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.¹⁴⁷

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.¹⁴⁸⁻¹⁵⁴ 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.^{155,156} *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.¹⁵⁷⁻¹⁶¹ In rodents, studies indicate that ARC Kp fibers contact GnRH nerve endings and AVPV Kp fibers contact GnRH cell bodies,^{162,163} whereas, in sheep and primates, Kp fibers mostly contact GnRH cell bodies.¹⁶⁴ Almost all GnRH neurons express KISS1R and are activated by a low dose (< 1 nmol, administered in the intracerebroventricular space) of Kp.¹⁶⁵⁻¹⁶⁸ 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,^{150,167-172} including man and woman.^{173,174} Furthermore, a specific deletion of KISS1R in GnRH neurons impairs mouse fertility, which is restored when the receptor is selectively reexpressed in GnRH neurons.^{175,176} One striking feature of Kp neurons is that, in contrast to GnRH neurons, they express a high density of $ER\alpha$.^{149,152,153,157,177} 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.^{147,152,153,157,158,178} 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.¹⁷⁹ 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,¹⁸⁰⁻¹⁸² 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.^{183,184} 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.^{172,185–187} The AVPV/POA Kp neurons are involved in the preovulatory GnRH/LH surge in females and are up-regulated by the sex steroid feedback.^{169,188–191}

The similarity of the gonadal phenotype between KISS1R KO mice¹⁴⁶ 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 hamster¹⁹² and ewe^{154,193} 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,^{194,195} European hamster,¹¹⁴ Libyan jird,¹⁹⁶ jerboa,¹⁹⁷ red deer,¹⁹⁸ elephant-shrew,¹⁹⁹ Brandt's voles,²⁰⁰ dromedary camel,²⁰¹ wild mouse²⁰² and water vole.²⁰³ Interestingly, neurokinin B and dynorphin co-expressed in ARC Kp neurons also exhibit seasonal variation in Syrian hamster²⁰⁴ and sheep.²⁰⁵ 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.^{15,192} A similar inhibitory effect of melatonin on ARC Kp was also reported in the Turkish²⁰⁶ and striped²⁰⁷ hamsters and in the rat.²⁰⁸ In sheep, long days inhibit ARC *Kiss1* expression through an E₂-dependent mechanism.¹²¹ It is likely that melatonin is not acting directly on Kp neurons because ARC Kp neurons do not express MT1, at least in sheep.²⁰⁹ 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,⁹² and exogenous T3 administration also restored the long-day phenotype of hypothalamic Kp and gonadal activity to photo-inhibited Siberian hamsters.²¹⁰ In the same line, thyroidectomy in sheep severely dampens the seasonal variation in *Kiss1* expression.⁷⁰ 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 α (at least in sheep²¹¹), 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.²¹¹ Circulating sex steroids are also part of the seasonal synchronization of breeding activity.¹³ Thus, a seasonal change in the sex steroid feedback may regulate Kp neurons, which express a high

density of ER α . Actually, gonadectomy with/without testosterone or E₂ 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.^{114,121,154,192,195,212,213} 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.^{114,195} 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.²¹⁴ 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 hamsters¹⁹² or male and female Siberian hamsters^{195,215} 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.²¹⁶ 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.²¹⁷ In the mare, chronic Kp injection also triggered a modest increase in gonadotropin secretion, but failed to induce ovulation.²¹⁸ 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-448²¹⁹; C6,²²⁰ Bz-Arg-Trp-NH₂.²²¹ Among those, C6 delivered *i.m.* triggered fertile ovulations in anoestrus ewes and goats.^{220,222} 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 eCG²²³).

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

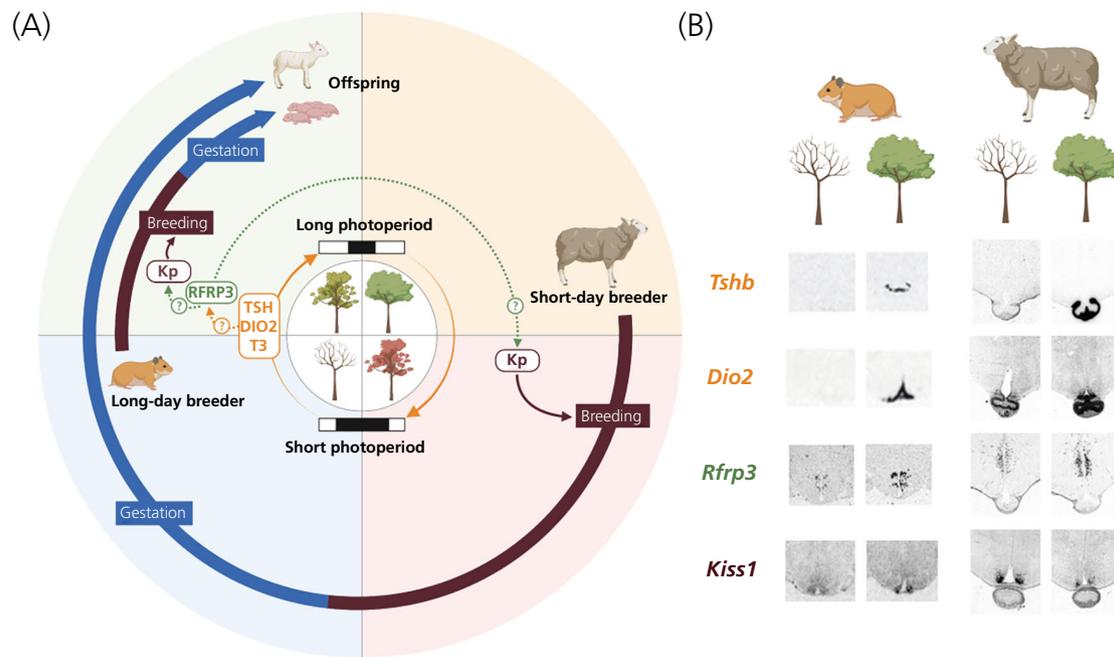


FIGURE 2 Neurons expressing kisspeptin (Kp) and RFRP3 as penultimate neuroendocrine controllers of the retrograde signaling pathway in seasonal breeders. A, A model to account for opposite breeding phenologies in long-day and short-day breeders – Longer days of spring and summer trigger induction of 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.²²⁴ 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.²²⁵ 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.^{226–228}

In mammals, *Npvf*-expressing neurons are mainly located in the dorsomedial and ventromedial nuclei of the hypothalamus (DMH/VMH) in multiple species, including rodents,^{225,228–230} sheep,^{231,232} humans,²³³ non-human primates,^{181,234} cattle,²³⁵ horses²³⁶ and camels.²⁰¹ 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.^{228,237,238} 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.^{228,232,237–240} 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.^{237,240} 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.^{241,242} Using a GnRH neuronal cell line, RFRP-3 application was found to reduce GnRH mRNA expression by approximately 60%.²⁴³ Early in vivo experiments reported that intracerebral injection of RFRP-3 inhibits LH secretion in female Syrian hamsters,²²⁸ as well as male and female rats,^{244–246} and that systemic injection of RFRP-3 reduces LH levels in

ewes,²⁴⁷ 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.²⁴⁸ 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.²³⁹ Third, multiple protocols of either central or i.v. administration of RFRP-3 failed to modulate LH secretion in ewes.^{249,250} 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^{245,248,251,252} and humans.²⁵³ Of note, several studies indicated that RFRP-3 could act on the HPG axis via a direct role on pituitary gonadotrophic cells.¹⁴² 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,²²⁸ sheep,²⁵⁴ cattle,²⁵⁵ rats²⁴⁶ and pigs.²⁵⁶ However, further studies in rodents, ewes, pigs and horses did not yield any support for a hypophysiotropic effect of RFRP-3.^{218,236,245,248,257} Furthermore, although RFRP-3 is detected in the ewe portal blood, its circulating levels show no correlation with LH levels in the peripheral blood.²⁵⁸ Importantly, ablation of the *Npffr2* gene does not compromise mice fertility, although several minor to moderate effects were reported.²⁵⁹ 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^{245,251–253}; for a review, see also Angelopoulou et al.¹⁴²

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.²³⁰ 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,^{195,230,239,251,260} female Turkish hamsters,²⁰⁶ male European hamsters,¹¹⁴ male and female Jerboas,¹⁹⁷ female Brandt's voles,²⁰⁰ male water voles²⁶¹ and male wild-derived mice,²⁰² but also for short-day breeders such as ewes,^{231,232} female brushtail possums,²⁶² female goats,²⁶³ and male and female dromedary camels.²⁰¹ Additionally, the number of RFRP-3 fibers contacting GnRH neurons in the Siberian hamster and ewe^{232,239} and *Npffr2* expression in the Syrian hamster²³⁸ 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.²⁶⁴ In Syrian,²³⁰ Siberian²³⁹ and Turkish²⁰⁶ 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,²⁶⁵ and albeit a 24-h melatonin incubation slightly increases *Npvf* expression in an hypothalamic cell line,²⁶⁶ a direct impact of melatonin on RFRP-3 neurons appears very unlikely.¹⁰⁷ 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.⁹² 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 days²¹⁰ and thyroidec-tomy blunts the seasonal change in *Npvf* expression in ewes.⁷⁰ Finally, in TR α null mice, melatonin no longer inhibits RFRP-3 expression.¹³¹ Altogether, these data suggest that the melatonin-driven seasonal change in hypothalamic T3 regulates RFRP-3 neurons via TR α . However, it is doubtful that T3 directly acts on RFRP-3 neurons because only a low number of RFRP-3 neurons expresses TR α , and melatonin still inhibits RFRP-3 expression in mice bearing a neuron-specific mutation of TR α .¹³¹ Yet, the possibility that T3 regulates RFRP-3 neurons through TR β 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 α , respectively.^{228,267,268} E₂ treatment after ovariectomy has been shown to either increase c-FOS in RFRP-3 neurons in Syrian hamsters,²²⁸ to decrease *Npvf* mRNA in mice²⁶⁷ or to have no effect on *Npvf* expression in rat.²⁶⁹ Finally, manipulating testosterone or E₂ 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,^{195,230,251} suggesting a sex steroid independent seasonal regulation of RFRP-3 synthesis in rodents. By contrast, there is evidence that E₂ is required for the seasonal regulation of *Npvf* expression in the ewe.¹²¹

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.^{248,251} 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.²¹⁵

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 GJ14,²⁷⁰ RF313²⁷¹ or RF3286,²⁷² 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.

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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.

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DATA AVAILABILITY STATEMENT

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

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