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Melatonin and Seasonal Synchrony in Mammals

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ABSTRACT

In mammals, seasonal opportunities and challenges are anticipated through programmed changes in physiology and behavior. Appropriate anticipatory timing depends on synchronization to the external solar year, achieved through the use of day length (photoperiod) as a synchronizing signal. In mammals, nocturnal production of melatonin by the pineal gland is the key hormonal mediator of photoperiodic change, exerting its effects via the hypothalamopituitary axis. In this review/perspective, we consider the key developments during the history of research into the seasonal synchronizer effect of melatonin, highlighting the role that the *pars tuberalis*–tanycyte module plays in this process. We go on to consider downstream pathways, which include discrete hypothalamic neuronal populations. Neurons that express the neuropeptides kisspeptin and (Arg)(Phe)–related peptide-3 (RFRP-3) govern seasonal reproductive function while neurons that express somatostatin may be involved in seasonal metabolic adaptations. Finally, we identify several outstanding questions, which need to be addressed to provide a much thorough understanding of the deep impact of melatonin upon seasonal synchronization.

1 | Introduction

Throughout his 50-year research career, Paul Pévet's abiding interest was to unravel the secret ("percer le secret") (Figure 1) of mammalian pineal physiology. From his first paper published in 1974 on the cytology of pinealocytes in the European mole (*Talpa europeana*) [1], Paul has published numerous papers describing the structure, ultrastructure, and biochemistry of the pineal gland in various vertebrate species. He also explored how various inputs, such as serotonin, gonadal hormones, and several peptides, regulate the structure and biochemical pathways leading to the rhythmic synthesis of the methylated pineal products, among which is melatonin. The physiological role of the seasonal rhythm in melatonin was another of his passionate scientific interests.

In the closing address of a recent symposium celebrating his research life (https://www.neurex.org/events/archives/item/594-from-the-pineal-to-biological-rhythms-through-melatonin), Paul highlighted the mixed reception of one of his lesser-known works as a source of frustration. The paper in question, lead-authored by Bruno Pitrosky [2], is not among the most prominent papers of a long and illustrious career. So, why did Paul return to this work in summing up more than five decades and 500 publications' worth of research on pineal biology?

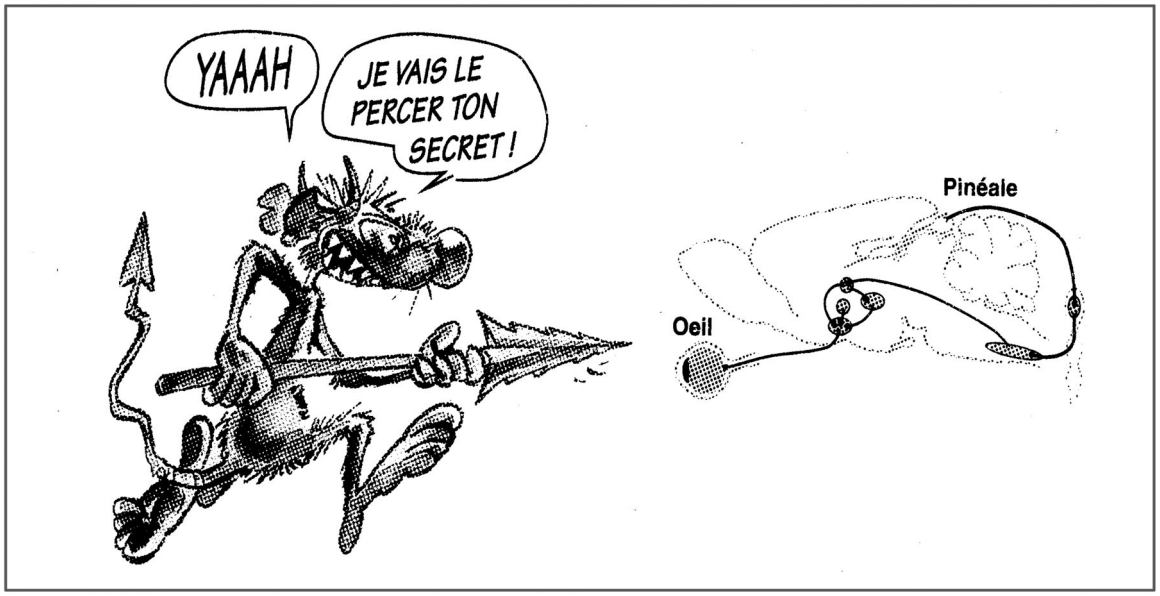

FIGURE 1 | Cartoon illustrates Paul Pévet's research quest over his 50-year scientific career—understanding how melatonin secretion by the pineal is controlled and how it governs seasonal output in physiology. Some secrets have been revealed, but large parts of the mystery remain! *Source:* Artwork by Paul Pevet/Anne Duittoz, inspired by Ptiluc´s´ Rat´s´ series.

2 | Melatonin Signal Duration and Photoperiodic Time Measurement

To answer this, we must look at this paper and where it sits in the development of our understanding of the role of melatonin in seasonal synchrony (i.e., in determining when in the year seasonal life-history transitions such as initiation and termination of breeding, molting, and hibernation occur). This framing of melatonin action sees seasonal life-history transitions as the outputs of internal annual timekeeping processes. Dependent on species, this may amount to self-sustained (*circannual*) rhythms, as seen in ground squirrels, European hamsters (*Cricetus cricetus*), and sheep or may require exposure to photoperiodic change for completion of the internal annual cycle, as seen in Syrian and Siberian hamsters (*Mesocricetus auratus* and *Phodopus sungorus*, respectively) [3]. Whether "truly circannual" and "photoperiodic" rhythms emerge from qualitatively different underlying mechanisms is highly doubtful, and they more likely represent "variations on a common theme", as initially proposed by Follett and Nicholls [4–6]. Across this continuum, it appears that melatonin is the key chronobiotic mediator of synchronization to photoperiod. This gives rise to the question of how, mechanistically, melatonin exerts its annual entrainment effects.

The Pitrosky et al. paper deals with the attributes of the pineal melatonin signal through which it serves an annual synchronization function [2]. So far as mechanism goes at a tissue or cellular level, readout is considered to take place in a black box, into which the melatonin signal is fed to drive a shift in circannual phase. This framing of the problem treats the melatonin secretory profile as analogous to the light–dark cycle in the external environment: dusk as akin to the circadian upswing in pineal melatonin secretion; dawn as akin to the circadian downswing. Accordingly, it appeared logical to ask whether a circadian-based readout system was responsible for interpretation of melatonin as a seasonal synchronizer, analogous to the circadian-based readout of photoperiod as formulated in the external coincidence timer model originally proposed by Erwin Bünning [7].

The key tenet of coincidence timer models is that it is the circadian phase during which light or a chronobiotic is present that determines the response rather than continuous duration of presence per se. This leads to the prediction that short durations of exposure to light (or melatonin) delivered at the appropriate phase will elicit a seasonal photoperiodic response. At the time of the Pitrosky study, several prior experiments using melatonin injections or subcutaneous infusions appeared inconsistent with a Bünning-analogous coincidence timer model for the seasonal synchronization function of melatonin. First, while it appeared that the time of day at which single melatonin injections were given in pineal-intact hamsters determined whether they entered reproductive arrest (i.e., an autumn response to declining photoperiod) [8], this phase dependence disappeared in pinealectomized hamsters, to be replaced by a requirement for three successive injections separated by 3 h, with no specific phase requirement relative to the daily light–dark cycle [9]. This stimulated the emergence of the concept that it was the continuous duration of the melatonin signal that determined whether the black box readout system interpreted it as a long or short photoperiod signal. This concept was further supported by experiments using timed subcutaneous infusions of melatonin in pinealectomized hamsters and in sheep, which appeared to show that continuous infusion duration was the key variable and that discontinuous melatonin infusion profiles, loosely analogous to skeleton photoperiods in light experiments, were ineffective as short day signals [10–13].

Against this consensus, Pitrosky et al. [2] reported that an infusion pattern comprising two 2.5-h melatonin infusions separated by an interval of 3 h was able to induce gonadal

regression in a similar fashion to a single 10-h continuous melatonin infusion. Since their measures of plasma melatonin demonstrated a clear return of circulating melatonin to daytime baseline levels, the only interpretation open was that signal continuity was not a requirement, and the authors proposed that the seasonal synchronization function of melatonin depended on a readout mechanism involving a "rhythm of sensitivity to melatonin, driven by melatonin itself." While some contemporary authors were skeptical of this interpretation [14, 15], subsequent analyses addressing the readout problem at the cellular and molecular level are broadly consistent with this hypothesis.

3 | Melatonin Receptors

Understanding of the chronobiotic actions of melatonin at the cellular level took off with the development of a high specific activity radioanalogue of melatonin (2-^{125}I-melatonin, IMEL) with pharmacological properties similar to those of melatonin itself [16, 17]. This allowed screening of candidate tissues for melatonin binding sites, the establishment of in vitro models for probing the cellular actions of melatonin, and in vivo studies designed to understand the role of specific tissues in mediating circadian or circannual effects of melatonin.

The *pars tuberalis* (PT) of the anterior pituitary quickly emerged as one of several candidate sites for the seasonal chronobiotic actions of melatonin. This was based both on its anatomical location at the interface between the mediobasal hypothalamus (MBH) and the endocrine cells of the *pars distalis* and on the high concentration of IMEL binding sites consistently found in this tissue in mammals [18, 19]. Within the PT, IMEL binding was shown to be GTP-dependent and pertussis toxin-sensitive, and cell culture–based assays showed that melatonin acted as an inhibitor of adenylate cyclase–mediated cAMP production [20]. This led to the hypothesis that melatonin acts via inhibitory G-protein coupled receptors (GPCRs), which was confirmed by cloning a family of vertebrate melatonin GPCRs as well as the orphan GPCR, GPR50 [21–23]. Originally known as the "melatonin-related receptor," GPR50 shares an approximately 50% amino acid identity/similarity to type 1 or type 2 melatonin receptors (MT1 and MT2, respectively) but is not directly sensitive to melatonin [22, 24]. For a comprehensive, up-to-date account of the history of melatonin receptor cloning, readers are directed to the review by Gautier et al. [25].

As well as the PT, mappings of IMEL binding in the brain of several seasonal mammals also revealed binding sites within the hypothalamus [26], and this stimulated lesioning and local exogenous melatonin treatment experiments designed to resolve the relative contributions of these different sites to the annual zeitgeber actions of melatonin [27–29]. Collectively, these led to a generic "two-site" model in which seasonal reproductive modulation was seen as due to actions of melatonin on cells within the basal hypothalamus [30], while, based on data from hypothalamopituitary disconnected rams, seasonal control of prolactin was seen as mediated by the PT [31]. While the close proximity of basal hypothalamic sites to the PT inevitably left room for doubt due to the possibility of off-target effects [32], the two-site model persisted until about 2003, after which new developments firmly placed the PT in a lynchpin position, mediating annual synchronization effects of melatonin not only on prolactin secretion but also on reproduction, energy metabolism, and hibernation.

4 | PT–Tanycyte Module

This model revision stemmed first from the demonstration that changes in thyroid hormone deiodinase enzyme activity and hence triiodothyronine (T3) concentration within the MBH were the key driver of changes in seasonal reproductive and metabolic function in birds and mammals [33–35] and secondarily that these changes stemmed from photoperiod-dependent effects on TSH secretion by PT cells coupled to TSH sensitivity in tanycytes lining the wall of the basal third ventricle [36–39]. Hence, while the textbook thyrotrophic function of TSH is endocrine and mediated by TSHR in thyroid follicles, this novel photoperiodic role of TSH depended on a TSHR–mediated paracrine action between two tissues in direct contact with each other. The mammalian conservation of this central TSHR–mediated effect has been shown by central infusion experiments in sheep and hamsters and by analysis of the photoperiodic response in *Tshr*–knockout mice [37–39]. The TSH-TSHR-deiodinase pathway is photoperiod sensitive in Siberian hamsters [40–42], despite this species being a natural knockout for the MT2 melatonin receptor [43]. Contrastingly, transgenic knockout of MT1 in mice abolishes melatonin sensitivity of the TSH-TSHR-deiodinase pathway [44]. Hence, available evidence all points to MT1 being necessary and sufficient for the photosensitivity of the PT–tanycyte module.

The characterization of an intercellular communication pathway linking melatonin to seasonal changes in hypothalamic function opened the previously impenetrable black box of melatonin signal processing—essentially to explore the extent to which the dynamics of the melatonin response at the level of the PT could account for seasonal changes in production of TSH. Based on a range of light and melatonin manipulation experiments in sheep and in rodents, the current working model sees production of TSH by PT cells as being controlled via transcriptional changes at the promoter region of the TSH beta subunit gene (*Tshb*), dependent on interactions between positively and negatively acting transcriptional regulators under the control of the circadian machinery within PT cells. This machinery shows a high degree of plasticity in response to melatonin input [45-47]. Indeed, in the sheep, exogenous melatonin injection induces expression of the clock gene *Cry1* independent of the phase at which melatonin is applied [48]. Hence, *Cry1* expression is a marker of night/melatonin onset. At the same time, whenever melatonin is present, other positive and negative elements of the PT circadian machinery show suppressed expression [48]. Functionally, this property means that transcriptional actions of the circadian machinery in the PT are highly dependent on the waveform of the melatonin signal produced by the pineal gland. Whether the PT of seasonal species such as sheep and hamsters harbors a classical self-sustained circadian clock (running without the requirement for daily melatonin resetting through *Cry1* induction) is still

contentious and might remain so in years to come. Indeed, the lack of transgenic models (e.g., circadian clock gene reporter genes) in these non-model species hampers our ability to address this question directly.

How is coupling of melatonin to the circadian transcriptional machinery in the PT envisaged to control transcription of *Tshb*? In 2010, independent studies in sheep and mice led to the discovery that the transcriptional coactivator EYA3 plays a pivotal role in this [49, 50]. This finding has led to a working model for the photoperiodic control of *Tshb* expression in PT cells, summarized briefly here: melatonin sets the phase of the PT clock through *Cry1* induction [46–53], which in turn dictates the phase of expression of clock-controlled genes. *Eya3* is one of those genes, as its expression is primarily controlled by the CLOCK/BMAL1 heterodimer through several conserved E-Box motifs in its promoter region [49]. Recent data confirm this and further show that other clock components, namely BMAL2 and DEC1, also intervene in the transcriptional control of *Eya3* [54]. This control by the circadian clock leads to a sharp peak of *Eya3* expression that invariably occurs ~12 h after *Cry1* induction (Figure 2). Similar to several clock components (see above), the expression of *Eya3* is blunted by melatonin. This leads to a dual control of *Eya3* transcription by the circadian clock and melatonin: under long days (> 12 h), hence a short melatonin signal, the circadian clock drives peak expression of *Eya3*, which is not blunted by melatonin; in contrast, under short days (< 12 h), the peak of *Eya3* occurs at a time when melatonin is present, and its amplitude—hence the amount of EYA3 protein—is drastically reduced.

How does EYA3 impinge upon *Tshb* transcription in PT cells? It appears that another clock component of the PAR-bZIP family, known as TEF (thyrotroph embryonic factor) promotes *Tshb* expression through direct binding at a D-Box motif located near the transcription start site [49]. As defined by in vitro assays, this transcriptional activation is robust; yet, it can be significantly increased through the recruitment of two transcriptional coactivators, SIX1 and EYA3, which—on their own—do not have any transcriptional impact upon *Tshb*. This molecular switch, driven by increased *Eya3* expression under long days, leads to heightened expression of *Tshb* and promotes the spring/summer phenotype. It is noteworthy that definitive functional evidence for the implication of EYA3 in the seasonal

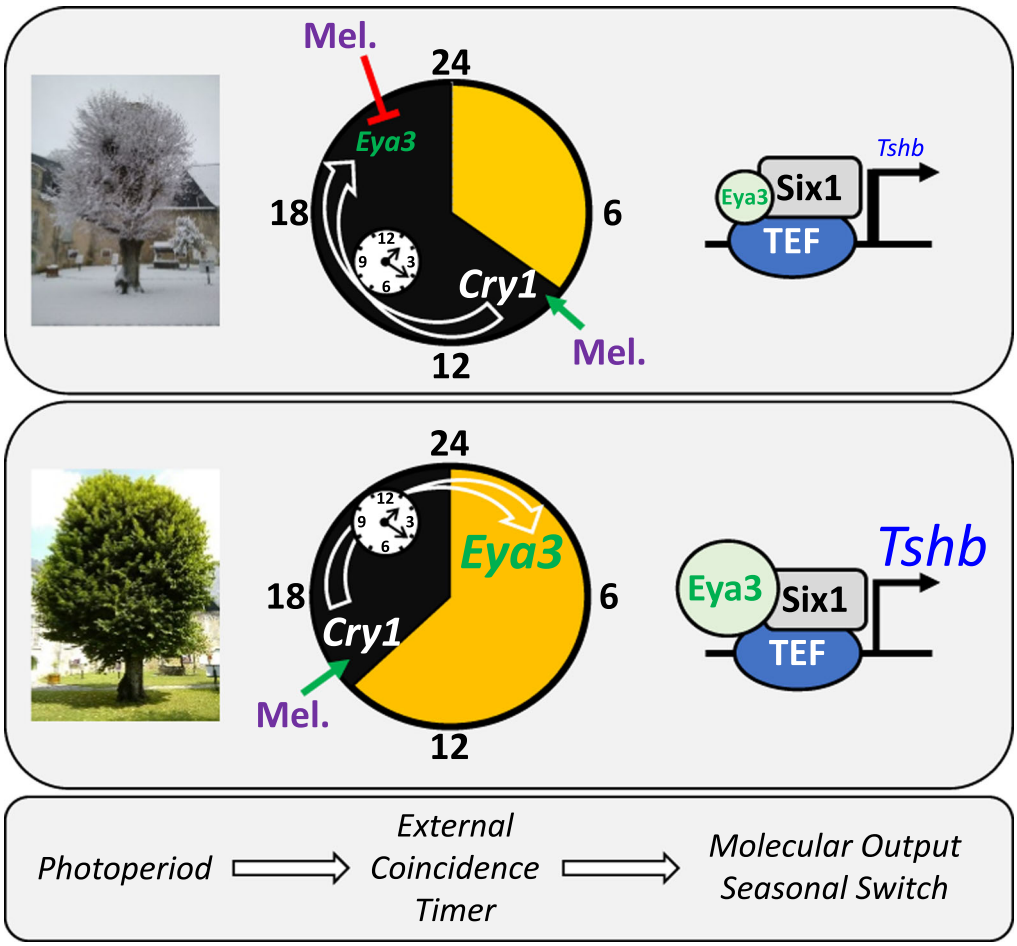

FIGURE 2 | Current (simplified) model for the photoperiodic, melatonin-dependent, circadian clock-based, external coincidence timer that drives seasonal switches in reproduction. During short days (upper panel; here illustrated by SP 8:16) melatonin blunts peak expression of the clock-controlled gene *Eya3*, which leads to reduced EYA3 production. As EYA3 forms a heterotrimeric complex with SIX1 and the DNA–bound TEF at the *Tshb* promoter, this yields low transcriptional activity (right part of the panel) and imposes the "winter phenotype." As daylength increases (middle panel; here illustrated by LP 16:8), phase-locked *Eya3* transcription gets unimpeded by melatonin, yielding a much larger peak, hence enhanced *Tshb* transcription (see text for further details).

response in mammals is still missing, as PT–specific deletion of *Eya3* in sheep, hamsters, or any other seasonal mammal is beyond our reach. However, we note that EYA has recently been implicated in the photoperiodic response in *Drosophila melanogaster* [55, 56], which suggests conservation in some molecular underpinnings of photoperiodism throughout the animal kingdom.

So, does this transcriptional regulation–based model for melatonin action amount to direct evidence for a melatonin-driven rhythm of sensitivity to melatonin, as envisaged by Pitrosky and Pévet some 30 years ago? What is clear is that the model sees the timing of termination of the melatonin signal relative to its onset during the preceding evening as the key determinant of the photoperiodic readout. Since these processes seem to involve molecular players that are controlled by circadian oscillators in other contexts, it is plausible to suggest that *continuous* melatonin presence until its withdrawal at dawn is not essential for the model: circadian molecular oscillators proceed through a sequence of states *independently* of outside input. Nevertheless, definitive evidence that this is indeed the case for the molecular events described by Dardente, Masumoto, Wood, and colleagues [49, 50, 54] is still lacking and would require in vivo designs in which the effects of discontinuous melatonin signals on PT gene expression rhythms could be assessed (see Section 7).

Besides seasonal variations in *Tshb* expression in the PT [57, 58] and photoperiodic regulation of deiodinases in tanycytes [59], described over 15 years ago, recent transcriptomics data (RNAseq) have shed light on the extent of control exerted by photoperiod and melatonin upon the PT–tanycyte module. And this control is extensive indeed. Studies in sheep have revealed that 8%–10% of the transcriptome in the MBH—including PT and tanycytes—is impacted by melatonin and thyroid hormone [54, 60–63]. Most genes, if not all, that are differentially expressed according to photoperiod are markers of either PT cells or tanycytes [64, 65]. Current data point to a high degree of plasticity in the local circuitry, from both the epigenetics/transcriptional and the cellular/extracellular matrix perspectives [54, 60, 61, 66]. While this somehow leads back to the proposal that cyclical histogenesis is the basis for seasonal/circannual timing [67], cellular proliferation at the level of the PT and/or tanycytes appears rather modest [68–70]. All in all, the function of many seasonally expressed genes within the PT–tanycyte module remains unknown, and the degree to which cellular and molecular plasticity, and/or cell proliferation, contribute to the overall seasonal phenotype remains unknown. The use of single nuclei RNAseq in these species should help us decipher how photoperiod impacts transcription within the multiple cell types that comprise this region.

5 | Downstream of the PT–Tanycyte Module

While the consequences of TSH–mediated seasonal photoperiodic changes in tanycyte function are not fully understood, recent insights into the role of tanycytes in reproductive and metabolic regulation in nonseasonal animal models (i.e., mice) [71–73] give considerable grounds for optimism. The essential function of tanycytes seems to be as an interface between feedback signals reaching the CNS and hypothalamic neuronal networks controlling reproduction and energy homeostasis. This casting applies both to the modulation of thyroid hormone bioavailability through deiodinase expression and to the plethora of effects reported for tanycytes in non-seasonal animals, including the dynamic of the preovulatory LH surge in female rodents [74] and sensing of nutrient metabolites [75] and peripheral hormones [76]. A recent laser capture microdissection/RNAseq experiment in Siberian hamsters revealed marked photoperiod-dependent changes in the density of sensory cilia in the tanyctic region [66], suggesting that modulation of tanycytic relay of feedback signals to neighboring neuronal circuits may be the general mechanism whereby tanycytes serve a seasonal "gatekeeper" function [34]. This gatekeeper function is presumed to modulate hypothalamic interneuronal circuits that govern reproduction and energy homeostasis, and efforts to characterize these have focussed attention on three neuropeptidergic signals in particular: (Arg)(Phe)–related peptide-3 (RFRP-3, encoded by the *Npvf* gene), kisspeptins (encoded by the *Kiss1* gene), and somatostatin (encoded by the *SST* gene) [65].

RFRP-3 neurons, located in the ventro/dorsomedial hypothalamus, are of particular interest because *Npvf* shows a consistent pattern of reduced expression on declining photoperiod in all seasonal species investigated [77–82]. This regulation probably depends on PT–mediated changes in hypothalamic thyroid hormone metabolism, as pinealectomy or TSH central infusion in Syrian and Siberian hamsters prevent the short-day–induced decrease in RFRP-3 [39, 77, 83] and because the melatonin-induced inhibition of RFRP-3 is abolished in mice devoid of functional T3 receptors [84]. The notion of a direct link between RFRP-3 and hypothalamic T3 levels is further supported by the relative insensitivity of *Npvf* expression to sex steroid feedback [77, 78, 85].

The role of RFRP-3 in seasonal function remains unclear, however, and is probably highly species and sex-dependent. The initial observation that this peptide inhibits LH secretion in quail [86], giving rise to its description as gonadotropin-inhibitory hormone (GnIH), is not corroborated by studies in seasonal mammals. For instance, central administration of RFRP-3 restores reproductive activation in Syrian hamsters exposed to short photoperiod [87, 88], and acute central injection of RFRP-3 increases LH secretion in Siberian hamsters exposed to short days [83], even though chronic central administration of RFRP-3 fails to rescue gonadal activity in this species [89]. Similarly, various protocols of RFRP-3 administration were unable to alter seasonal reproduction in sheep [90], suggesting that the observed changes in RFRP-3 expression may be involved in other aspects of seasonal physiology. Indeed in rats [91, 92] and in a variety of seasonal species (sheep [93], female jerboas [94], male [but not female] Siberian hamsters [89, 95]), RFRP-3 administration increases food intake and body weight. The complexity of the emerging picture probably reflects species- and sex-dependent differences in RFRP-3 network organization.

Arcuate kisspeptin neurons, which co-express neurokinin B and dynorphin (known as KNDy neurons), constitute the "GnRH pulse generator," which governs reproductive activation [96].

In all seasonal mammals investigated, kisspeptin expression shows seasonal variation in expression [78, 80, 81, 85, 97–102]. In photo-inhibited Syrian and Siberian hamsters [85, 89, 97] or female sheep [103], central infusion of kisspeptin rescues reproductive activity, pointing to a critical role of kisspeptin in the seasonal regulation of reproduction.

In Syrian and Siberian hamsters, TSH increases and melatonin inhibits kisspeptin expression [39, 85, 97]. It is worth noting that kisspeptin neuronal activity may be directly modulated by RFRP-3 since in the mouse and the rhesus monkey arcuate kisspeptin neurons express RFRP-3 receptors and receive RFRP-3 neuronal projections [81, 104, 105]. Furthermore, kisspeptin expression is increased in the RFRP-3–induced reactivation of gonadal activity in short-day adapted Syrian hamsters [87, 88]. Additionally, circulating sex steroids in photo-activated hamsters [80, 85, 97] and sheep [78, 99] inhibit arcuate kisspeptin expression, indicating that expression levels reflect the combined influences of downstream gonadal feedback as well as direct PT–dependent seasonal circuit modulation.

Somatostatin, known for its inhibition of growth hormone secretion [106], is implicated in seasonal body weight regulation. Somatostatin exhibits an increased expression in the posterior arcuate nucleus of Siberian hamsters adapted to short days [39, 89, 107], and this is driven by the melatonin-controlled T3 signal [39]. In long day-adapted male Siberian hamsters, subcutaneous infusion of the somatostatin agonist, pasireotide, decreases body weight and circulating IGF-1 and prevents body weight gain in animals switched from short to long days [108].

The mechanisms discussed above provide a working model for the hypothalamic network involved in the melatonin-driven regulation of reproduction (Figure 3). Current hypotheses point to RFRP-3 neurons as being the primary hypothalamic integrator of photoperiod, independently of species and sex. In a few species, there are anatomical and pharmacological evidence that this photoperiodic RFRP-3 signal is forwarded to kisspeptin neurons, which are also modulated by the negative sex steroid feedback and ultimately govern GnRH release, hence seasonal reproductive activity. In species showing seasonal changes in body weight, only a few metabolic peptides are reported to exhibit photoperiodic changes [109, 110], of which somatostatin is regulated by the T3 signal and drives the body weight loss in short days. Proving the validity of such a model will require the adaptation of new genetic tools to unconventional seasonal species, notably by applying Crispr-Cas9 technology to knockout putative candidate genes.

6 | Circannual Properties

The mechanisms discussed above provide a working model for decoding of photoperiodic information from pineal melatonin secretion but leave open the nature of the core circannual timer upon which this signal exerts its annual chronobiotic effects. Three basic formulations can be considered: (1) the circannual timer resides within the PT itself (“tissue autonomous timer hypothesis”) and (2) circannual time emerges from an interaction between the PT and the neighboring tanycytes (“PT–tanycyte circannual timer module hypothesis”) or (3) circannual rhythms are a multi-tissue emergent property (“system

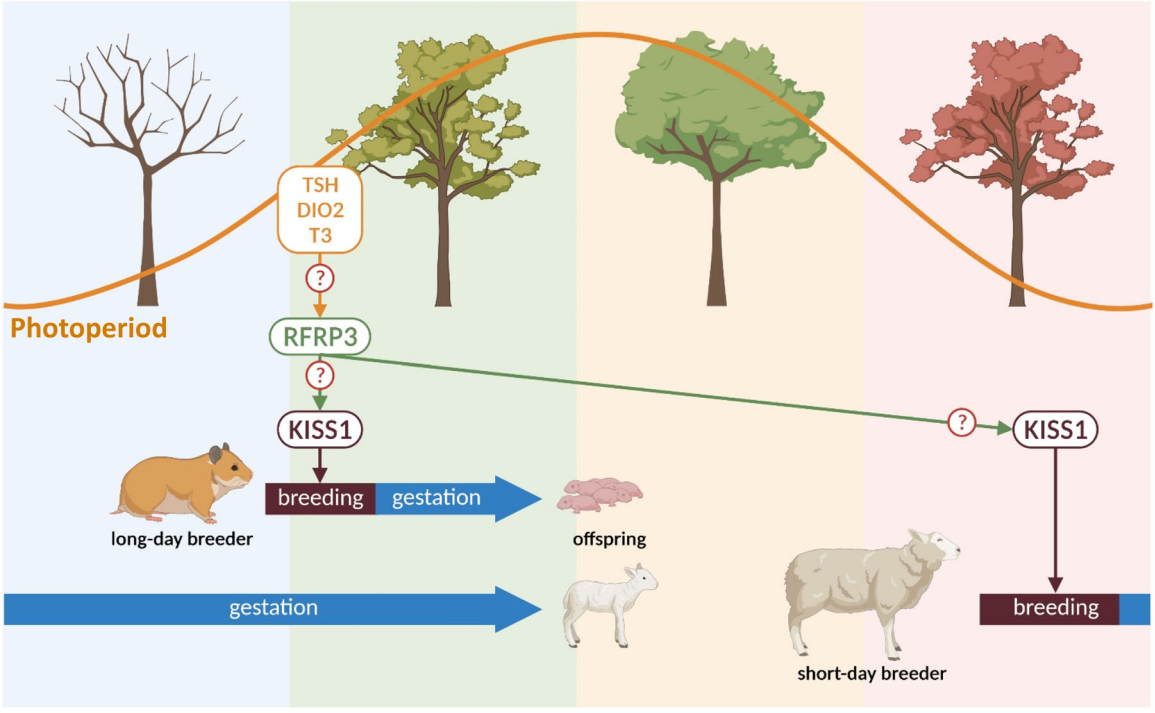

FIGURE 3 | Schematics illustrating our current understanding of the neuroendocrine control of breeding in a long-day (Syrian hamster) and a short-day breeder (sheep). In both species, long days trigger an increase of TSH production (see Figure 2) by the PT, leading to increased *Dio2* expression and local T3 production within the MBH. This is responsible for increased RFRP-3 production, through unknown mechanisms, which in turn would govern opposite seasonal phasing of kisspeptin production (here again, unknown mechanisms). This allows for delivery of the offspring at the end of winter/beginning of spring, when environmental conditions are the most conducive to survival and growth of both parents and offspring.

property hypothesis"). At the time of writing, there is no definitive evidence favoring one of these formulations, although evidence from hypothalamopituitary-disconnected rams [111] and from castrated, steroid-implanted sheep [112, 113] indicates that intact endocrine feedback is probably not required for rhythm generation, which makes the system property hypothesis less plausible.

Paul Pévet's contribution to circannual timekeeping, along with his wife, Mireille, derives from their maintenance of a breeding colony of European hamsters (*Cricetus cricetus*) at their Strasbourg laboratory [114], which provided both a valuable research resource and, through the release of captively bred animals, a means to support dwindling wild populations in eastern France [115].

European hamsters held under constant photoperiod show free-running rhythms of gonadal activation, body weight changes, and hibernation physiology similar to those seen in many species, and the Pévet lab has used this model to explore several aspects of circannual timekeeping. These include the limits of entrainment of the innate circannual rhythm to different photoperiod cycles [116], the importance of melatonin as a mediator of circannual entrainment [117], and the relationship between gene expression changes within the photoneuroendocrine system and physiological circannual phase [80]. This last aspect includes the demonstration that during melatonin-independent circannual cycles, expression of *Tshb* in the PT and *Dio2* in the hypothalamic tanycytic region both align with circannual phase: high expression in the "subjective" summer phase and reduced expression in the "subjective" winter phase. This finding echoes results from studies in sheep [118] and Siberian hamsters [66], which collectively suggest that the PT and the tanycytes may form a regulatory module through which endocrine control of long-term changes in physiology including circannual status is exerted. Whether one or other of these two tissues is more important for circannual rhythm generation and the extent to which they act cell or tissue autonomously remains unclear. Indeed, there is much more work to be done on this front before firm conclusions can be drawn, and basic questions about the fundamental mechanisms leading to cycles with circannual time constants remain unanswered [119].

7 | Conclusions and Future Perspectives

It has been a long journey from identification of melatonin as a key pineal product to recognition that it was crucial for photoperiod decoding. A few more decades were necessary to achieve cloning of melatonin receptors, localize their expression within the brain, and decipher how melatonin duration (rather than amplitude or phase), which drives the photoperiodic response. Then, identification of the PT as the main melatonin target in the control of the seasonal response and of the retrograde TSH/DIO2/T3 pathway have been milestones. Finally, evidence linking this pathway to the control of kisspeptin and RFRP-3, hence breeding, has accumulated at a fast pace, yielding the current model for the photoperiodic control of seasonal functions. Yet, the journey is not over. There are still many outstanding questions to be answered. What is the signal transduction pathway leading to *Cry1* induction by melatonin? We already know that melatonin induction depends upon EGR1–like factors [51] and NPAS4 [53], rather than CLOCK/BMAL1 [120]; yet, we have no clue regarding how long it takes after melatonin withdrawal to reinstate sensitivity of *Cry1* induction to melatonin, hence resetting of the putative PT circadian clock. This is no trivial issue as it might explain earlier conflicting findings using pinealectomized hamsters and timed infusion of melatonin, as illustrated by the Pitrosky et al. study [2]. Are PT–thyrotrophs the circannual timers or does a PT–tanycyte module comprise this timing system? What is the exact nature of the still elusive circannual clock? How does it connect to the circadian clock? What is the role of cellular plasticity and cell proliferation in circannual timing? What do all the seasonally secreted factors identified in the PT actually do? Could one of these be the "still-to-be-defined" tuberalin that is thought to drive prolactin seasonal output? How is it that the same long-day–induced TSH/DIO2/T3 axis drives opposite breeding phenologies in seasonal sheep and hamsters? There are also interesting comparative issues remaining. How has evolution led to the dichotomy between melatonin-dependent seasonal synchrony in mammals and deep-brain photoreceptor-dependent synchronization in birds? [121] Is the evolutionary loss of GPR50 sensitivity to melatonin seen in eutherian and metatherian mammals but not in monotremes [122], indicative of a fundamental shift in melatonin function at the base of the mammalian lineage, and does the highly prolactin-centric mode of melatonin action seen in marsupials [123, 124] somehow relate to this? Answering these questions will require the development of effective genetic toolboxes in relevant seasonal species.

Finally, assessing the impact of global warming on seasonal species, which comprise the majority of living organisms on Earth, will not be achieved with the use of non-photoperiodic mice. A paradigm shift, with the recognition that seasonal biology is the most pervasive feature of virtually every living organism, is now required to address these unanswered issues.

Acknowledgments

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H.D.: This review is dedicated to Paul and Mireille. As a PhD student, I spent 4 years (1999–2003) in Paul's lab, "Neurobiologie des Rythmes," in Strasbourg, under the supervision of Mireille Masson-Pévet. I remember bits of my first encounter—a formal interview—with Mireille and then briefly with Paul who forcefully conveyed his main message (mantra, so to speak): physiology, pineal, and photoperiodism.

It seems fair to say that Paul has never been fond of genetics and molecular biology. How could these compare to physiology? No way. Yet, I was allowed to perform some basic "molecular biology" experiments; as long as these were heavily seasoned with melatonin, of course. This freedom to develop my own ideas has been most precious to my PhD training. In this regard, I wish to thank Mireille who helped me so much with scientific reasoning and writing, matters that take ages and ultimate patience to teach properly, which she did—and that was hard work I'm afraid. Special thanks are also due for her attentive

ear throughout my PhD. This made a huge difference. All these are ingredients for a very sweet PhD training, but Paul's lab had definitely more than this on offer: an unparalleled friendly, if not familial, atmosphere. This feeling was perhaps reinforced by the unusual localization of the lab in a late 19th century German-built massive cobblestone, high ceilinged, with wooden floors, marble pillars, and never-ending corridors. Quite a classy research environment—much closer to a large home than a basic lab.

Paul did not only focus on running the lab, but he has always been eager to serve the community at large. He was instrumental in the creation of a strong PhD associative network, which is still running strong some 20 years later. He created the Neurex network, which generates fantastic opportunities for PhD students to go abroad and organize varied scientific events directed toward the public. Paul also gave conferences to the lay audience, trying to bring down multiple outrageous and silly beliefs surrounding the "melatonin madness" of the 1990s by showing the real data, putting back science on the central stage (already quite a feat in the 1990s and probably a lost cause nowadays). Paul has been ahead of his time on all these fronts, realizing that scientific communication was much more than an asset: a necessity to keep things running by attracting attention from those who make the decisions and provide financial support.

Sheer enthusiasm and pure positive energy, this is what defines Paul best, to me. And, of course, his unique ability to spread this energy; a human battery of sorts. Not plain dedication to his work, but real passion for his favorite hobby—scientific research. For someone as pessimistic and easily discouraged as myself, Paul will always be a wonder.

V.S.: It is no understatement to say that my career has been strongly influenced by my encounter with Paul Pévet during the course of my PhD. Finishing a PhD on osmoregulation in diadromous fishes, I had planned to continue with a postdoctoral fellowship in Germany to study ionic exchanges in the digestive system under physiological and pathological conditions, a project that did little to motivate me as it took me further and further away from my initial training in marine biology. It was during this period of doubt that stimulating discussions with a very persuasive Paul Pévet led me to consider redirecting my research to his favorite molecule, melatonin. This option was made all the more attractive by Paul's optimism, who foresaw a simple and effective course: do a first-year postdoc in his team to develop a pineal gland perifusion system to analyze the regulation of melatonin secretion; do a second postdoc abroad to acquire knowledge and expertise in pharmacology; apply to a CNRS position to join his laboratory. Although, with hindsight, I realize that Paul must have had a super-power of persuasion to make me naively adhere to this plan for my future research years, in reality this is what actually happened, as I was recruited to CNRS 4 years after my PhD.

Although Paul was instrumental in reorienting my research, from the moment I was recruited, I enjoyed a great deal of freedom to conduct my own research, even if this was strongly influenced by the frequent discussions around the "sacred" coffee break where researchers, technicians, and students got together to debate about research but also political and societal issues. Paul, with Mireille, had indeed succeeded in creating a stimulating laboratory ecosystem open to many foreign researchers and students, where ideas flowed and were discussed with passion. They created a working atmosphere that was both intense and caring. I learned a lot from both Paul and Mireille about positive management and the ability to catalyze emerging ideas. Paul's energetic and passionate contribution to local and international research policies also paved my way for involvement in the management of research and education.

I would like to end with a personal note from a time when women were struggling to find an equal place with men in the workplace. Paul, even though he could sometimes make comments that we might call "typically masculine," was equally supportive of his female and male colleagues; moreover, he made sure that periods of maternity leave did not negatively affect our careers.

D.G.H.: Although I had first observed Paul and Mireille as a very green PhD student attending the 1990 Guildford European Pineal Study Group meeting organized by Jo Arendt, I first really got to know them during a sabbatical stay in Strasbourg in 2003. For one reason and another, this was "a strange time in my life," and the stay in Strasbourg served as a most enjoyable and eye-opening retreat. There was much that was unusual about the Pévet lab: its imposing but dated setting, its first-generation "chronobiotron"—to the untrained eye; a garden blockhouse shaded by trees and invaded by cockroaches; its array of filter coffee machines about which the entire lab would orbit. But it worked. To echo what has already been said, Paul and Mireille had established a lively, internationally welcoming research culture and fostered an environment of enthusiasm and scientific debate. An excellent template for an academic research group and for a life scientific. I am grateful for my time there.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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