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Neural mechanisms controlling seasonal reproduction: principles derived from the sheep model and its comparison with hamsters

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Abstract

Seasonal reproduction is a common adaptive strategy among mammals that allows for breeding to occur at times of the year when it is most advantageous for the subsequent survival and growth of offspring. A major mechanism responsible for seasonal reproduction is a striking increase in the responsiveness of gonadotropin-releasing hormone (GnRH) neurons to the negative feedback effects of estradiol. The neural and neuroendocrine circuitry responsible for mammalian seasonal reproduction has been primarily studied in three animal models: the sheep, and two species of hamsters. In this review, we first describe the afferent signals, neural circuitry and transmitters/peptides responsible for seasonal reproductive transitions in sheep, and then compare these mechanisms with those derived from studies in hamsters. The results suggest common principles as well as differences in the role of specific brain nuclei and neuropeptides, including that of kisspeptin cells of the hypothalamic arcuate nucleus, in regulating seasonal reproduction among mammals.

Keywords

GnRH; neuroendocrine; seasonality; photoperiod; kisspeptin; dopamine; thyroid hormone

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1. Introduction

Seasonal reproduction has been described as “nature’s contraceptive”, a naturally occurring mechanism to induce a reversible pattern of fertility and infertility in adult mammals (1). The ability of the adult reproductive system to be turned on and off has a distinct selective advantage, by helping to predict a time of year for breeding that ensures young are born when environmental conditions favor the energetic demands of lactation and survival of offspring (2–4). Although annual variations in reproductive function are common among animals living in regions with harsh winter seasons, the fundamental neuroendocrine changes responsible have been studied in only three species of mammals: sheep (*Ovis aries*), Syrian (or Golden) hamsters (*Mesocricetus auratus*), and Siberian (or Djungarian) hamsters (*Phodopus sungorus*). One major advantage of sheep is that hypophysial portal blood samples can be collected from unanesthetized animals so that secretion of GnRH can be directly assessed during different seasons. Moreover because of their large size, sheep have been an ideal model to study changes in episodic LH responsible for seasonal breeding and to map the underlying alterations in the neural circuitry in the hypothalamus controlling GnRH secretion.

Sheep show distinct annual patterns of reproductive activity: in females, these are comprised of periods of ovarian activity and ovulation, termed the breeding season, which alternate with periods of ovarian quiescence and anovulation, referred to as anestrus (2). These seasonal variations in reproductive activity at an ovarian level are the direct result of changes in the brain, and result from two complimentary alterations in the control of hypothalamic function, specifically in the regulation of gonadotropin-releasing hormone (GnRH) secretion. The first is a dramatic increase in the responsiveness to the negative-feedback actions of estradiol (E2) that peaks during anestrus, diminishing as the breeding season begins (6,7). The second is a steroid-independent inhibition of GnRH that is seen in ovariectomized (OVX) animals (3). However, of the two, the increase in E2 negative feedback is accepted as the main mechanism responsible for seasonal reproduction and, thus, has been a key point of interest, leading to development and implementation of the ovine model.

In this review, we will focus primarily on the seasonal regulation of reproduction in sheep, the afferent pathways by which the external environment times seasonal reproductive transitions, and the neural circuitry ultimately responsible for changes in the negative feedback responsiveness in GnRH secretion. In addition, we will compare the basic principles responsible for neuroendocrine control of seasonal reproduction in sheep with recent findings from Syrian and Siberian hamsters. This comparison reveals both similarities and differences in the functional circuitry mediating mammalian seasonal reproduction as well as in the roles of specific neurotransmitters and neuropeptides.

Finally, it should be noted that in addition to sheep and hamsters, studies in birds, such as the Japanese quail, have been extremely valuable in elucidating the mechanisms underlying seasonal reproduction, some of which differ fundamentally from those uncovered in mammals. The present review focuses specifically on mammalian models of seasonal reproduction, while other papers in this Special Issue will cover avian models.

2. Photoperiodic and circannual control of reproduction in sheep

In seasonal mammals, a key environmental signal responsible for the timing of reproductive transitions is daylength or photoperiod (1). In female sheep, the short daylengths of fall and winter are stimulatory to the reproductive neuroendocrine axis leading to ovulatory cycles and fertility; by contrast, the long daylengths of spring and summer, are inhibitory to the reproductive axis in female sheep and produce an anovulatory state (9,10). Photoperiodic information is conveyed to the reproductive neuroendocrine system by way of a neurohormonal pathway that starts with retinal projections to the suprachiasmatic nucleus (SCN), and thence by way of a multi-synaptic circuit that includes neurons in the paraventricular nucleus, intermediolateral spinal cord and superior cervical ganglion, terminating in the innervation of the pineal gland. Seminal studies in sheep and other mammals in the 1980s showed the prominent role of pineal melatonin as an essential signal conveying photoperiodic information to the hypothalamus and rest of the brain (3). Melatonin is normally synthesized and secreted by the pineal gland in a diurnal fashion, with a dramatic increase occurring during the nighttime that returns to near undetectable levels in the morning (3). In sheep, the duration of the nighttime elevation in melatonin serves as an internal code of the external photoperiod, with long duration melatonin coding for short (stimulatory) daylengths, and short duration melatonin coding long (inhibitory) daylengths (4). In species that are “long day” breeders such as hamsters (see Section 4), the roles of these long and short duration melatonin signals are reversed (Fig. 1), with long duration (short day) signals being inhibitory to the HPG axis and short duration (long day) signals being stimulatory (5).

In sheep, melatonin does not directly drive seasonal changes in reproduction but rather appears to synchronize an endogenous circannual rhythm (12–14). This was first demonstrated in long-term studies of pinealectomized female sheep, which show free-running circannual rhythms of reproductive activity and inactivity (6), that can be synchronized by once-yearly, 90-day blocks of melatonin infusions (7). Thus synchronization of the circannual rhythm by melatonin is analogous to the ability of light to entrain circadian rhythms in the SCN and other tissues (8). Moreover, like the entrainment of circadian rhythms, melatonin appears to be most effective during a specific phase of the circannual rhythm: from the summer solstice to the fall equinox (7).

While the physiological actions of melatonin are now well described in sheep, the sites of action of this indolamine remain controversial. The highest concentration of melatonin receptors in sheep, as in other species (9, 10), is in the pars tuberalis (PT) (11), but specific binding sites are also evident in the premammillary region (PMR) of the hypothalamus (11, 12). Early studies using local administration of melatonin via microimplants provided strong evidence that the PMR (12), not the PT (13), was an important site at which melatonin acted to advance the onset of the breeding season. However, the results of more recent studies have pointed to the PT as a critical site of action, at least for the transition to anestrus.

Recent work on the PT grew out of earlier reports demonstrating that thyroid hormones (T3 and T4) are critical for the transition into anestrus – thyroidectomized sheep remain in a persistent breeding season state (14) and T4 replacement is able to restore seasonal

reproductive transitions (15). Interestingly, thyroid hormones are required for the transition from the breeding season to anestrus, but not for the reverse, that is the subsequent transition from the breeding season back to anestrus. These early studies also concluded that circulating T4 is likely a permissive signal since constant low levels are able to reinstate reproductive transitions in sheep (15). However, more recent work has provided strong evidence for the hypothesis that photoperiod-controlled changes in the local conversion of T4 to T3 in the brain are important for the effects of long days (16). Moreover, these changes appear to be due to actions of melatonin in the PT (16, 17), although tests of this hypothesis using melatonin administration have yet to be done in sheep.

According to this hypothesis, which is discussed in detail elsewhere (9, 18), a long-day melatonin pattern increases expression of the transcription factor, eyes absent 3 (Eya3), in the PT (19, 20). Eya3 is a circadian-controlled gene with a peak in expression occurring about 12 hrs after melatonin secretion increases with darkness. The amplitude of this peak increases if melatonin concentrations are low (i.e., as is the case with a long-day melatonin pattern), which stimulates expression of TSH β (19). TSH then diffuses into nearby neural tissue which contains the TSH receptor (16), stimulates the expression of type 2 deiodinase (Dio2) (16), the enzyme that converts T4 to T3, and T3 then acts to induce neural changes that shut down episodic GnRH secretion. Long-day photoperiod has the opposite effect on type 3 deiodinase (Dio3), although this action may not be mediated by TSH (17). Since Dio3 inactivates T4, a decrease in its activity would complement the increase in Dio2. While this is an attractive hypothesis for which there is now significant support, key questions remain, such as whether it applies to the transition to the breeding season and where and how T3 acts to produce its effects.

It is interesting to note that because T3 plays a major role in neuronal plasticity, including effects on neurogenesis (21), one possible mechanism for its action to cause the seasonal increase in responsiveness to E2 negative feedback during anestrus (see below) involves morphological rearrangements in the neural circuitry responsible for conveying this influence onto GnRH neurons. Indeed, seasonal plasticity and synaptic rearrangements have been described at several levels within the neuroendocrine pathways mediating E2 negative feedback in anestrus (22). Examples of this are discussed in more detail in the next section.

3. Neural pathways responsible for seasonal breeding in sheep

There is now a great deal of information on the neural circuitry within the hypothalamus of female sheep responsible for E2-negative feedback during anestrus. As noted in the introduction, seasonal reproductive transitions in the female sheep are a direct result of striking changes in the ability of E2 to inhibit GnRH; specifically, during anestrus, E2 gains the ability to dramatically suppress the frequency of GnRH and LH pulses (2, 28, 29). Because of this increased responsiveness to E2 inhibition, even the low levels of E2 present during anestrus strongly inhibit GnRH activity, preventing the rise in LH necessary for the late follicular-phase peak in E2 that induces the GnRH/LH surge that triggers ovulation (23). Even though ovulation is blocked during anestrus due to increased E2 negative feedback, the surge mechanism is still intact, and exogenous E2 can induce a positive feedback response and produce a GnRH/LH surge (2). Thus E2 negative, but not positive, feedback is under

seasonal control, and an initial approach to uncovering the neuroendocrine changes responsible for seasonal breeding in sheep has focused on understanding how this negative feedback influence is conveyed to GnRH neurons.

Because GnRH neurons lack the alpha isoform of the estrogen receptor (24), the isoform responsible for negative feedback influence of E2 on GnRH secretion (25), much work has focused on identifying the afferent circuitry by which E2 exerts its inhibitory effects on GnRH/LH pulses during anestrus. Early pharmacological studies found that dopamine (DA) D2 receptor antagonists, administered either peripherally or intracerebrally, block the ability of E2 to inhibit GnRH/LH pulses during anestrus in female sheep (26). This implication of the involvement of DA in seasonal breeding, led to the discovery of a key set of dopaminergic neurons within the retrochiasmatic area (RCh), known as the A15 group, that are now believed to play a critical role in seasonal regulation of GnRH secretion (32–37). Several lines of evidence have since supported this hypothesis. First, lesions that include A15 neurons block E2-negative feedback during anestrus in ewes (27); while direct stimulation of A15 neurons inhibits GnRH/LH pulse frequency regardless of season (28). Second, multi-unit activity of the A15 group is decreased prior to GnRH/LH pulse production (28); and increased in the presence of E2 during anestrus (29) when pulses are inhibited. Finally, neural activity studies have shown that E2 increases tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis, (30) and Fos expression (31) in A15 neurons during anestrus but, importantly, not during the breeding season.

However, like GnRH neurons, A15 DA cells do not express ER-alpha; thus, the regulation of A15 DA cells by E2 must be indirect, meaning that estrogen-responsive afferents must be a key part of this circuitry. Studies using local administration of E2 have provided strong evidence identifying two primary areas of estrogen-responsive cells that are activated during estradiol negative feedback, specifically during anestrus: the ventromedial preoptic area (vmPOA) and the retrochiasmatic area (RCh) (31, 39–41). ER-alpha positive cells in both regions provide input to the A15 region (23) and vmPOA and RCh fibers directly contact TH-positive dendrites and somas of the A15 (23). Pharmacological and anatomical studies suggest that this input may be either glutamatergic and/or GABAergic, and E2 stimulates glutamate and inhibits GABA release in the A15, respectively (32). Moreover, there are seasonal changes in the number of glutamatergic, but not GABAergic, input to A15; A15 dopaminergic somas and dendrites receive more glutamatergic inputs during anestrus than the breeding season (32). This difference complements the increased number of total synaptic inputs onto A15 dendrites seen during anestrus (33), as well as an increase in A15 dendritic length and bifurcations (33). Thus, seasonal plasticity in the number of glutamatergic inputs to A15 may account in part for the increased activity of A15 neurons, and the inhibition of GnRH pulse frequency, during anestrus.

The next logical question is what are the projections and pathways by which A15 neurons inhibit GnRH pulse frequency in anestrus? In sheep, a majority of GnRH cell bodies are located in POA, with smaller populations of GnRH neurons being found both in the anterior hypothalamic area (AHA) and the mediobasal hypothalamus (MBH). However, tract-tracing studies have confirmed that the primary projections of A15 neurons are caudally toward the MBH, not rostrally toward the POA or AHA (23). Moreover, dopaminergic terminals have

been shown to be in direct axo-axonic contact with GnRH terminals in the sheep median eminence (ME) during breeding season (34). Likewise, retrograde tracers injected into the ME labeled cells in the anterior hypothalamic area in the region of the A15 (35). These findings suggest that DA released by A15 neurons may directly inhibit GnRH pulses by acting at the level of GnRH axons within the median eminence; however, although D2 dopamine receptors are present in ovine GnRH neurons (36), their trafficking and presence in GnRH axons and terminals within the median eminence is unclear.

Although some GnRH cells may receive direct A15 contacts, recent evidence suggest that A15-mediated regulation of GnRH secretion may occur via an alternative, multi-synaptic pathway with the arcuate nucleus (ARC) at its center. A subpopulation of neurons expressing kisspeptin located in the ARC is an ideal candidate for mediating DA actions on GnRH secretion. Kisspeptin is a key stimulator of GnRH secretion and like A15 cells, has been implicated in seasonal breeding in sheep (37) as well as other species (see below). ARC Kiss cells are unique in that they co-express two other peptides that have also been linked to GnRH regulation, neurokinin B (NKB) and dynorphin (38). The aptly named KNDy cells have become a center of focus for the neuroendocrine regulation of reproduction (39).

Several studies have supported the hypothesis that ARC KNDy neurons are critical to seasonal breeding. The number of kisspeptin peptide (Fig. 2A) and mRNA-containing cells decreases in the ARC during anestrus, largely due to a stronger inhibition by E2 (37). Furthermore there are parallel decreases in the number of kisspeptin-positive inputs to GnRH cells within the MBH in anestrus ewes compared to breeding season (Fig. 2B) (37). This decrease in stimulatory inputs to GnRH neurons may allow for increased inhibition of GnRH pulses by other inputs. In addition, recent studies have found that: i) KNDy cells, using dynorphin as a marker, co-express the D2 dopamine receptor (D2R), the subtype responsible for the seasonal inhibition of GnRH pulses (26) (40); ii) that this co-expression changes with season, with about 80% of KNDy cells colocalizing D2R in anestrus compared to 40% in the breeding season and iii) that intracerebroventricular infusion of a kisspeptin antagonist completely blocks the ability of D2R antagonists to stimulate GnRH pulse frequency during anestrus (36). Thus, it can be inferred that A15 dopamine neurons projecting into the ARC act to inhibit kisspeptin neurons resulting in the inhibition of GnRH secretion during anestrus.

Taken together, we propose the following model (Fig. 3) for the neural circuitry responsible for E2 negative feedback in anestrus ewes. During anestrus, E2 acts upon ER-alpha (ER) containing cells in the vmPOA and RCh. These neurons, in turn, simulate A15 dopamine cells using glutamate as a transmitter. Activated A15 cells project caudally to the mediobasal hypothalamus (MBH), where dopamine is released. Dopamine acts upon GnRH neurons either directly, via axo-axonic contacts in the median eminence, and/or indirectly, via kisspeptin cells in the arcuate nucleus, to inhibit GnRH pulse frequency.

Although there is good evidence that ARC KNDy neurons are a key part of this circuitry in the anestrus ewe, several questions remain. The decrease in kisspeptin within the KNDy population during anestrus may be due to increased DA release from A15 terminals;

however the possibility that this effect may simply be due to decreased expression of D2R, and/or changes in other inputs, has yet to be determined. In our original study, dynorphin was chosen as the KNDy cell marker because, unlike kisspeptin, its expression is believed to remain constant between seasons (36); circumstantial evidence suggests that NKB may also be under seasonal regulation (41) but this needs to be directly tested. Furthermore, despite their expression of D2R, it remains to be demonstrated whether ARC KNDy neurons receive direct contact from A15 neurons, or if there is seasonal plasticity in this portion of the circuitry.

Although the diagram presented in Fig. 3 summarizes the pathways by which E2 ultimately inhibits GnRH and LH pulse frequency during anestrus, it does not address the question of what changes in this pathway might be responsible for the increased responsiveness to E2 seen during anestrus. As noted above, seasonal neuroplasticity has been observed at multiple levels in this circuitry (22). These include increases during anestrus in the total number of synapses (33), as well as specific glutamatergic inputs (32), onto A15 dopamine neurons; increased dendritic length of dopamine A15 neurons (33); increases in the number of kisspeptin inputs onto GnRH neurons in the MBH (37) as well as earlier EM observations of decreased synaptic inputs onto GnRH neurons in the POA (42). Neural plasticity at the level of both ARC and GnRH neurons has long been associated with reproductive function, both development (43) and adulthood (54, 55), consistent with the possibility that synaptic and dendritic plasticity in this circuit may also play an important role in seasonal reproduction.

4. Comparison with neural mechanisms controlling seasonality in hamsters

In addition to sheep, the neuroendocrine mechanisms controlling seasonal breeding have been extensively studied in two other mammals: Syrian (or Golden) and Siberian (or Djungarian) hamsters. It is thus of interest to compare neuroendocrine control of seasonality among these three species. In many respects they are very similar (3, 5, 18): 1) they all use photoperiod as the primary environmental cue to time reproduction, 2) the duration of melatonin secretion from the pineal gland is the key endocrine signal linking external photoperiod to the HPG axis, 3) the neural pathway by which photoperiod controls melatonin secretion appears to be the same, 4) both steroid-dependent and steroid-independent inhibition of gonadotropin secretion can be demonstrated in all three species (3, 44–46), and 5) earlier data pointed to intrahypothalamic sites of action for melatonin in hamsters (47–51), while more recent data supports the hypothesis that melatonin acts to stimulate conversion of T_4 to T_3 in the hypothalamus via TSH production in the PT at the transition from short to long photoperiod (18, 52–54).

Although this broad perspective identified many similarities among these three mammals, a more detailed examination reveals some minor variations. First, steroid-independent inhibition of gonadotropin secretion plays a much more important role in seasonal breeding in hamsters than in ewes (3, 45, 55), a difference that correlates with the much greater suppression of gonadal function by inhibitory photoperiod in hamsters (55). Second, the intrahypothalamic sites of action of melatonin varies among these three; as noted above melatonin appears to act in the PMR in ewes, while sites of melatonin binding (10) and results of studies using local administration of melatonin or lesions indicate that this

indoleamine acts in the dorsomedial hypothalamus (DMH) and SCN in Syrian (47, 48) and Siberian (49–51) hamsters, respectively. Finally, while changes in both Dio2 and Dio3 have been implicated in the response to long days in ewes (16, 17), hamsters appear to be more selective. Thus, long days induced an increase in Dio2 in Syrian hamsters (53, 56, 57), but expression of Dio3 was undetectable in either photoperiod (18). In contrast, changes in Dio3 appear to be important in Siberian hamsters (58, 59), while inconsistent responses were observed in expression of Dio2 (58–60). Readers interested in a more detailed discussion of these topics are referred to other papers on seasonality in hamsters in this Special Issue.

There are also some major differences among these species in seasonal reproductive function. One might expect that these mechanisms would be more closely related in the two species of hamsters than between them and sheep, and this is true for two major aspects of seasonality. First, hamsters are long-day breeders in that they are reproductively active during the long days of spring and summer, while sheep are fertile during the short days of fall and winter (Fig. 1) (5). This difference undoubtedly reflects the duration of pregnancy, which is a few weeks in hamsters and approximately five months in sheep (3). Since seasonal reproductive patterns most likely evolved to ensure young are born in the spring when environmental conditions favor their survival, the differences in durations of pregnancy required the season of fertility, and thus the effects of photoperiod, to differ between hamsters and sheep. The second major difference is that sheep can become photorefractory to both long and short daylength (61, 62), while hamsters can only become photorefractory to short days (5). Thus if male hamsters are maintained on continuous short day length they will first be infertile, but after several months they will undergo testicular recrudescence and then remain reproductively active as long as they are maintained on this “inhibitory” photoperiod (5). In contrast, under similar circumstances, sheep will continue to show fluctuations in reproductive activity of about a year, a phenomenon that reflects the endogenous circannual rhythm in reproduction discussed above (7, 63, 64). It has been suggested that photorefractoriness to short days allows the reproductive axis of male hamsters to begin activity in anticipation of long days so that mature sperm, which take several weeks to produce, are available at the right time of year (5). It is unclear why hamsters have not developed endogenous circannual rhythms similar to those seen in sheep, but one possibility is that this has been precluded by their relatively short life span.

Given these differences between sheep and hamsters, one might expect that the neural mechanisms driving seasonal differences in GnRH secretion would be similar in Syrian and Siberian hamsters, and differ significantly from those in sheep and this is true for the role of A15 DA neurons because there is no evidence that this system plays a role in seasonality in hamsters. However, when one examines the role of kisspeptin, marked differences are evident between Syrian and Siberian hamsters, with the former more closely resembling sheep. Specifically there is strong evidence that changes in kisspeptin are critical for seasonal changes in fertility in male Syrian hamsters (18, 65). Thus, as in the ewe, kisspeptin expression is elevated during stimulatory photoperiod (66, 67) and administration of kisspeptin can restore fertility in hamsters maintained on inhibitory photoperiod (66, 68). In the hamster, as in the sheep, there are two major populations of kisspeptin cells, one in the ARC and the other in the preoptic region (76); in hamsters, the latter population is specifically localized in the anteroventral periventricular nucleus (AVPV). Although

kisspeptin expression increases in both the ARC and AVPV the former is most likely critical for seasonality because it is controlled by melatonin (66, 67) and its expression is not altered by testosterone administration to photosuppressed hamsters (66, 67). In contrast, kisspeptin expression in the AVPV can be induced by testosterone administration to such hamsters and is not affected by pinealectomy (67). Thus the increase in kisspeptin in the AVPV during the breeding season is most likely an effect, rather than a cause, of the reactivation of the hypothalamo-pituitary-testicular axis in Syrian hamsters.

When kisspeptin expression is examined in Siberian hamsters a much different picture emerges: kisspeptin expression in the ARC is inhibited during stimulatory photoperiod, while expression in the AVPV is elevated (69, 70). However, the latter can be explained by the seasonal differences in testosterone because testosterone administration to photosuppressed hamsters increases kisspeptin expression in the AVPV as it does in Syrian hamsters (71). The seasonal changes in the ARC may also reflect changes in endogenous testosterone because this steroid is an inhibitor of kisspeptin expression in the ARC of Siberian hamsters (71), as it is in other species. Finally, exogenous kisspeptin infusion was unable to restore fertility to Siberian hamsters exposed to inhibitory photoperiod (72). This lack of effect might reflect a loss of responsiveness produced by continuous kisspeptin administration as seen in some other species (73), but this seems unlikely because intermittent administration (once/day) was also ineffective in Siberian hamsters (72). It is interesting to note in this regard that constant infusion of kisspeptin was more effective than intermittent (twice/day) injection in inducing ovulation in anestrous ewes (74), and that continuous icv administration of this peptide (6 nmoles/day) to Syrian hamsters exposed to short days stimulated testicular growth (66). On the other hand, a similar dose of kisspeptin (10 nmoles/day) failed to stimulate testicular growth in Syrian hamsters when given sc (68). Taken together, the results from studies in Siberian hamsters support the conclusion that kisspeptin does not play an important role in driving seasonal reproductive function in this species, and instead seasonal changes in kisspeptin expression reflect corresponding changes in circulating testosterone concentrations.

If kisspeptin is not responsible for seasonal changes in reproduction in Siberian hamsters, what system is? Early data raised the possibility that glutamatergic neurons might be involved (75), but more recent work has focused on an interesting alternative: RFamide-related peptide-3 (RFRP-3) (65). RFRP-3, one mammalian analogue of avian gonadotropin-inhibitory hormone, is found primarily in neurons located in the DMH and adjacent structures, and inhibits LH secretion, most likely by suppressing GnRH secretion, under most circumstances (18, 65). Surprisingly given its inhibitory actions, RFRP3 expression (mRNA and protein) is increased by stimulatory photoperiod in both Siberian and Syrian hamsters (76–78). These changes in expression are driven by melatonin (76, 78) and not influenced by castration of reproductively-active hamsters to decrease testosterone or elevating testosterone with exogenous treatment in hamsters whose reproductive activity was inhibited by photoperiod (76, 77). In parallel with this positive correlation with reproductive function, exogenous RFRP-3 stimulates LH secretion in both species of hamsters if administered during inhibitory photoperiod (78, 79); in contrast, during stimulatory photoperiod RFRP-3 inhibits LH secretion in Siberian males (78), but stimulates it in Syrian males (79). These data raise the possibility that long-day photoperiod stimulates

release of RFRP-3 which in turn activates the hypothalamo-pituitary-testicular axis in male hamsters. This hypothesis is supported by the report that prolonged icv administration of RFRP-3 can induce testicular growth in Syrian hamsters (79). Interestingly this treatment also increased the number of kisspeptin-positive cells in the ARC (79) so that RFRP-3 may act via kisspeptin neurons in these hamsters. However, this connection is unlikely to be important in Siberian hamsters because expression of these two peptides can be experimentally dissociated (80). Moreover, as discussed above, kisspeptin probably plays little, or no role, in photoperiod-induced changes in reproduction in these hamsters.

5. Conclusions

In summary, ARC kisspeptin neurons play a key role in the photoperiodic control of GnRH secretion in sheep and Syrian hamsters (Fig. 4), but long days has opposite effects in these two species. Thus in the ewe, long days increase the activity of glutamatergic neurons that innervate A15 DA neurons. DA released from these neurons in turn inhibits the activity of ARC kisspeptin neurons, thus inhibiting GnRH and LH secretion and inducing infertility. In Syrian hamsters, long days stimulate release of RFRP-3 from neurons in the dorsomedial hypothalamus, which increases the activity of ARC kisspeptin neurons (presumably by suppressing an unidentified inhibitory neural system because RFRP-3 acts via an inhibitory G protein). The increase in kisspeptin then stimulates GnRH secretion to cause testicular recrudescence and the resulting increase in testosterone stimulates kisspeptin expression in the AVPV. In contrast, kisspeptin appears to play no role in mediating the effects of photoperiod in male Siberian hamsters. The initial response of this species to long days is similar to that of Syrian hamsters: increased release of RFRP-3. Thereafter the pathway diverges with RFRP-3 suppressing an unknown inhibitory system to stimulate GnRH secretion possibly via glutamatergic neurons. The increase in GnRH ultimately leads to increased testosterone, which, in turn, acts to stimulate kisspeptin expression in the AVPV and inhibit this peptide in the ARC.

If one focuses on the sheep, there are clearly important gaps in our understanding of the mechanisms by which photoperiod controls GnRH secretion. One such gap is the link between melatonin action and changes in the neural circuitry mediating E2 negative feedback. Thus, it is not clear whether the effects of melatonin in the PT can account for both transitions between breeding and anestrus seasons. Early data with melatonin microimplants suggests not (13), but expression of both *Dio2* and *Dio3* changed during prolonged exposure to either long day or short day photoperiod (17), although these alterations did not correlate well with changes in reproductive function. Similarly the sites of thyroid action at the transition to anestrus (81) are not entirely consistent with the current model for induction of type 2 deiodinase, and the specific actions of T3 remain largely unknown. Other important questions relate to the neural circuitry mediating E2 negative feedback in anestrus, including: 1) the identity of the estrogen-responsive and glutamatergic and GABAergic neurons, 2) whether A15 neurons project directly to KNDy neurons, and 3) the specific changes in this circuit that account for transitions between breeding season and anestrus. Although many questions remain, much is now known about the actions of melatonin and the neural circuitry underlying seasonal breeding in the ewe that allows for the design of experimental approaches to address these questions.

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Highlights

- Seasonal reproduction is mediated by gonadotropin-releasing hormone (GnRH) neurons
- Increased responsiveness of GnRH cells to estradiol negative feedback is a key factor
- Arcuate kisspeptin neurons also play an important role in seasonal reproduction
- Similarities and differences exist between sheep and hamsters in central mechanisms

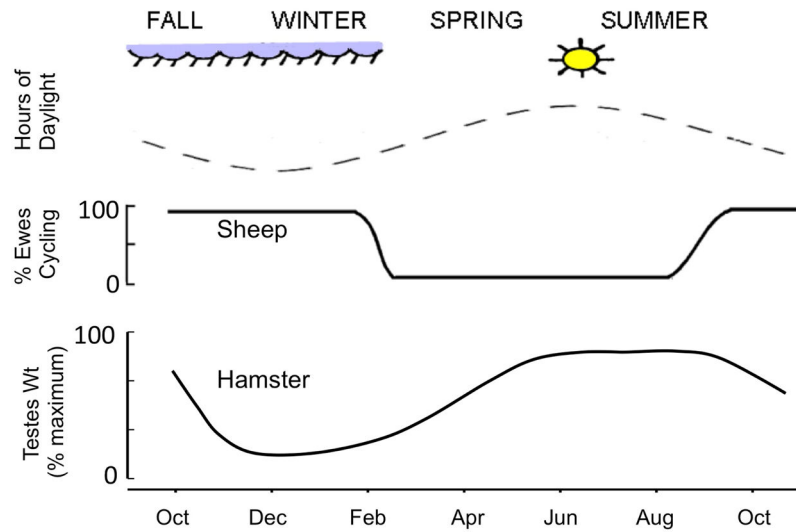


Figure 1.

Comparison of seasonal reproductive patterns in sheep and hamsters. Changes in fertility are controlled by variations in the duration of daylight throughout the year (top panel), but photoperiod has different effects in these species. Short days stimulate reproductive function in sheep, so all ewes have ovulatory cycles during the fall and winter, but become anovulatory in the spring and summer (middle panel). In contrast, reproductive activity in hamsters (illustrated as changes in size of the testes in the bottom panel) is stimulated by long days; thus hamsters are fertile in the spring and summer and reproductive inactive in the fall and winter.

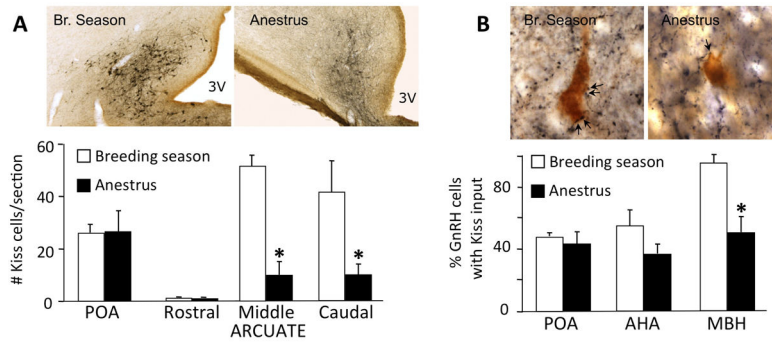


Figure 2.

Seasonal changes in number of ARC kisspeptin cells (A) and their inputs to GnRH neurons (B). A: The number of kisspeptin-immunoreactive (Kiss) neurons in the middle and caudal ARC is decreased during anestrus (* $p < .001$). Images show examples of sections through the caudal ARC immunostained for kisspeptin in ewes perfused during either the breeding season or anestrus. Ewes were ovariectomized and implanted with E2 (OVX+E) to control for seasonal variation in endogenous steroid levels. Note that the number of kisspeptin cells in the POA, another major kisspeptin population (76), does not change seasonally. Bar = 100 μm . B: The percentage of GnRH cells in the MBH that receive one or more kisspeptin (Kiss) close contacts was less in anestrus than in breeding season ewes (* $p < .001$); GnRH cells in the POA or anterior hypothalamic area (AHA) showed no significant seasonal differences. In addition, GnRH cells in the MBH and AHA had significantly fewer Kiss contacts per cell in anestrus than in the breeding season (data not shown). Images show examples of dual immunoperoxidase stained sections in which close contacts (e.g., arrows) are seen between kisspeptin terminals (blue-black) and GnRH somas (brown) in the MBH. Bar = 15 μm . (A and B modified from ref. 48)

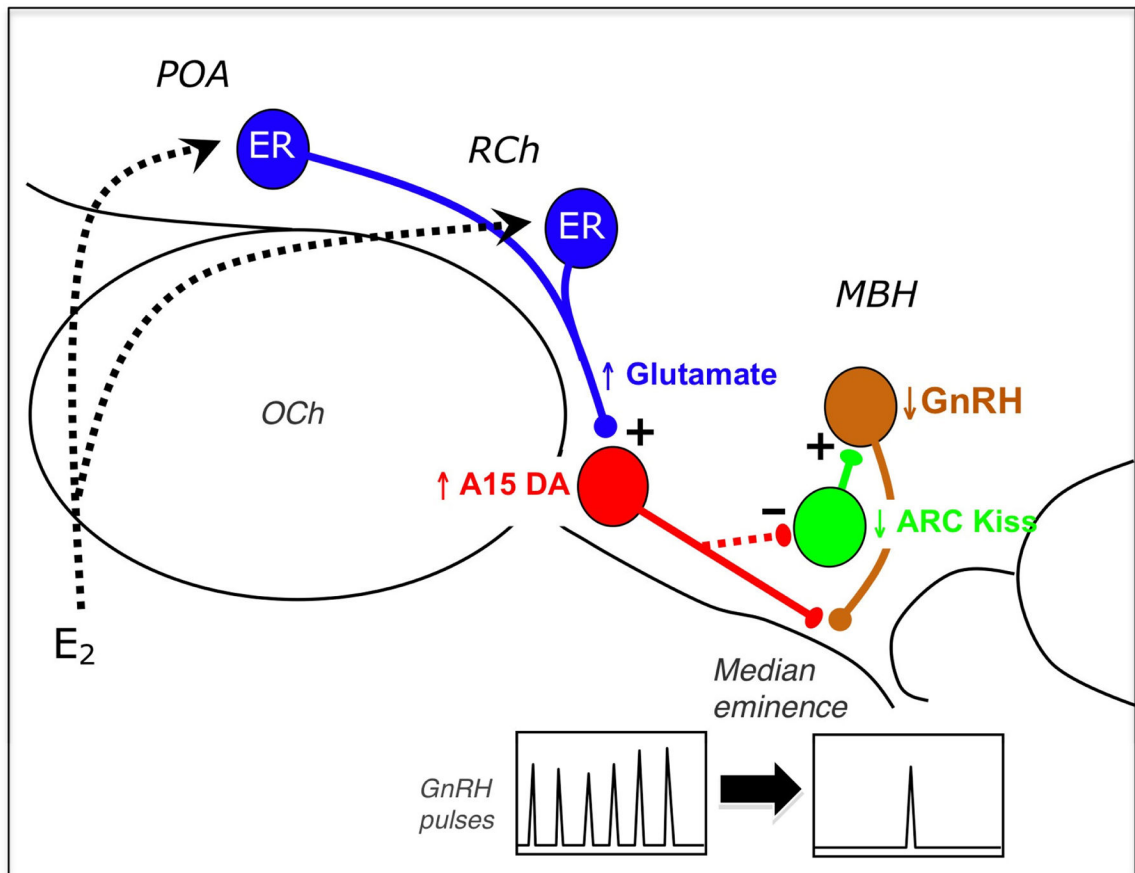


Figure 3.

Schematic depiction of the neural circuitry in the hypothalamus regulating seasonal control of estradiol (E_2) negative feedback. E_2 acts upon ER- α containing cells (blue) in the ventromedial preoptic area (POA) and retrochiasmatic area (RCh); these neurons, in turn, stimulate A15 dopamine cells (red) via glutamatergic inputs. A15 dopamine cells project caudally to the mediobasal hypothalamus (MBH) and inhibit GnRH cells (brown) either directly at the level of their terminals in the median eminence, and/or indirectly via kisspeptin (Kiss) cells (green) in the arcuate nucleus. Seasonal plasticity has been demonstrated at several sites in this circuitry including that of glutamatergic contacts onto A15 neurons (42); the dendritic morphology of dopaminergic A15 neurons (43); and the number of kisspeptin inputs to GnRH neurons in the MBH (48). Such morphological changes may result in a functional “reconnection” of this circuitry during anestrus, and underlie the ability of E_2 to inhibit GnRH pulse frequency at this time of year. OCh = optic chiasm. (Modified from ref. 27).

FEMALE SHEEP:

LD PP → ↑ Glutamate → ↑ A15 DA → ↓ ARC Kiss → ↓ GnRH

MALE SYRIAN HAMSTERS:

LD PP → ↑ DMH RFRP-3 → ↓ ? inhibitory neurons → ↑ ARC Kiss → ↑ GnRH → ↑ Test → ↑ AVPV Kiss

MALE SIBERIAN HAMSTERS:

LD PP → ↑ DMH RFRP-3 → ↓ ? inhibitory neurons → ↑ Glutamate → ↑ GnRH → ↑ Test → $\left\{ \begin{array}{l} \downarrow \text{ARC Kiss} \\ \uparrow \text{AVPV Kiss} \end{array} \right.$

Figure 4.

Comparison of the neural systems mediating the effects of long days on GnRH secretion in sheep, Syrian and Siberian hamsters. Note that long days (LD PP) are inhibitory in sheep, but stimulatory in hamsters. In sheep and Syrian hamsters, changes in ARC kisspeptin play a central role in the photoperiodic control of GnRH release, but different afferent inputs are involved in these two species. In contrast, kisspeptin does not appear to be important for seasonal changes in GnRH secretion in Siberian hamsters; rather the increase in circulating testosterone drives the changes observed in kisspeptin expression. See text for further details.