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Emerging ideas about kisspeptin–GPR54 signaling in the neuroendocrine regulation of reproduction

Alexander S. Kauffman[1], Donald K Clifton[2] and Robert A. Steiner[1,2]

[1] Departments of Physiology and Biophysics, University of Washington, Seattle, WA 98195, USA
[2] Departments of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, USA

Neurons that produce gonadotropin-releasing hormone (GnRH) drive the reproductive axis, but the molecular and cellular mechanisms by which hormonal and environmental signals regulate GnRH secretion remain poorly understood. Kisspeptins are products of the *Kiss1* gene, and the interaction of kisspeptin and its receptor GPR54 plays a crucial role in governing the onset of puberty and adult reproductive function. This review discusses the latest ideas about kisspeptin–GPR54 signaling in the neuroendocrine regulation of reproduction, with special emphasis on the role of *Kiss1* and kisspeptin in the negative and positive feedback control of gonadotropin secretion by sex steroids, timing of puberty onset, sexual differentiation of the brain and photoperiodic regulation of seasonal reproduction.

Discovery, structure, and localization of GPR54 and kisspeptins

In 1999, the gene for an orphan G-protein-coupled membrane receptor, termed GPR54, was discovered in the rat and subsequently identified in the human genome [1–3]. The *GPR54* gene is expressed in several peripheral tissues (placenta, pancreas, kidney, testis and pituitary) and the brain, most notably the hypothalamus, preoptic area (POA), midbrain, hippocampus, amygdala and medulla [1–3]. Less commonly known as AXOR12 or hOT7T175, the GPR54 protein is about 40% homologous to the galanin family of receptors but does not bind either galanin or galanin-like peptide (GALP) [2].

In 2001, it was discovered that kisspeptins, encoded by the *Kiss1* gene, represent natural high-affinity ligands for GPR54 [1,3,4]. *Kiss1* encodes a 145 amino acid protein, which is proteolytically processed to produce a 54 amino acid peptide called kisspeptin-54 [5]. Kisspeptin-54 has also been termed 'metastin', based on early work identifying *Kiss1* as a cancer metastasis suppressor gene [6,7]. In addition to kisspeptin-54, several other smaller peptide fragments derived from the precursor protein were identified (kisspeptin-14, -13, -10), which all share a distinct structural RF-amide motif (Arg-Phe-NH_2) in their C-terminal region. Although each of these kisspeptin peptides can bind and activate GPR54 with similar efficacy [1,3,4], their relative importance *in vivo* remains to be determined. In rodents, sheep and primates, *Kiss1* mRNA has been detected by either *in situ* hybridization or RT–PCR in discrete regions of the forebrain, including the arcuate nucleus (ARC), the anteroventral periventricular nucleus (AVPV) and the anterodorsal preoptic nucleus (APN), as well as in the bed nucleus of the stria terminalis and amygdala [3,8–10]. In a similar way to *GPR54*, *Kiss1* is also expressed in several peripheral tissues, most notably, the placenta, ovary, testis, pancreas and liver [1–4].

The relationship between the kisspeptin–GPR54 system and reproduction

In 2003, several groups reported that humans and mice with either spontaneous or genetically targeted mutations in the *GPR54* gene display striking impairments in reproductive function, including a failure of pubertal development, low levels of sex steroids, impaired gametogenesis and a lack of estrous or menstrual cyclicity [11–13]. Mutations and deletions of *GPR54* are also associated with a severe deficiency in gonadotropin (LH and FSH) secretion, which was ultimately traced to diminished secretion of gonadotropin-releasing hormone (GnRH) [13–15]; similar impairments have recently been reported for *Kiss1* knockout (KO) mice [16,17]. GnRH neurons are the final common pathway through which the brain regulates the secretion of pituitary gonadotropins, and hence, all of reproduction. Emerging evidence supports the idea that kisspeptin–GPR54 signaling directly regulates GnRH secretion. In rodents, sheep and primates (including humans), exogenous kisspeptin treatment elicits rapid increases in plasma levels of LH and FSH [8,9,15,18–22]. Although *GPR54* is expressed both in the pituitary and GnRH neurons [9,15], evidence suggests that the stimulation of gonadotropin secretion by kisspeptin reflects direct activation of GnRH neurons and not pituitary gonadotropes. First, in rodents and monkeys, the kisspeptin-induced increase in gonadotropin secretion can be blocked with GnRH antagonists [8,9,20,22]. Second, in rodents, kisspeptin induces Fos expression and prolonged firing of action potentials in GnRH neurons [9,23,24]. Third, in sheep, kisspeptin infusions increase the concentration of GnRH in the hypothalamo–pituitary portal circulation [15]. Finally, in all species examined so far, GnRH neurons express *GPR54* [9,15,24,25]. It is also noteworthy that kisspeptin administration cannot induce

Corresponding author: Steiner, R.A. (steiner@u.washington.edu).
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LH secretion or nuclear Fos production in GnRH neurons in *GPR54* KO mice [15,23], suggesting that the effects of kisspeptin on the GnRH axis are mediated specifically by GPR54 (and not another receptor).

Kisspeptin–GPR54 signaling is clearly essential for maintaining GnRH secretory activity, but whether gonadotropes are also targets for kisspeptin action remains unresolved. *GPR54* is expressed in the human pituitary [1,3], and kisspeptin stimulates gonadotropin release *in vitro* from cultured rat, ovine and bovine primary pituitary cells [21,26,27]. Notwithstanding, other studies have shown no apparent effect of kisspeptins on *in vitro* LH or FSH secretion in cultured primary rat pituitary cells or anterior pituitary fragments [20,28]. The explanation for these conflicting findings is unclear and might reflect differences in experimental design; however, recent studies in sheep indicate that GnRH is required for the stimulatory effect of kisspeptin on gonadotropin secretion *in vivo*, bolstering the argument that GnRH neurons are the primary targets for the action of kisspeptin in the neuroendocrine reproductive axis.

Steroidal regulation of the *Kiss1* system: implications for positive and negative feedback

The secretion of GnRH is regulated by sex steroids (i.e. positive and negative feedback), but the cellular and molecular mechanism subserving this regulation remains unclear. GnRH neurons do not express the receptors thought to mediate steroidal feedback effects [neither estrogen receptor α (ERα) nor the androgen receptor (AR)] [29], suggesting that other steroid-sensitive neurons 'upstream' of GnRH neurons receive and transmit sex steroid signals to the reproductive axis. Given the potent stimulatory effects of kisspeptin on GnRH secretion, *Kiss1* neurons are plausible candidates to be these 'upstream' steroid-sensitive neurons. Indeed, *Kiss1* neurons in the forebrain are direct targets of sex steroids: in rodents, almost all hypothalamic *Kiss1* neurons express ERα and AR, and *Kiss1* mRNA is strongly regulated by both estradiol and testosterone [30,31]. Similarly, in sheep, most hypothalamic *Kiss1* cells express ERα, as well as the progesterone receptor [32]. The co-expression of *Kiss1* and the progesterone receptor has not been addressed in rodents, whereas the expression of AR in *Kiss1* neurons has not been studied in any species except the mouse [31]. Of note, ERβ is also expressed in about 30% of hypothalamic *Kiss1* neurons; however, ERßKO mice show no discernable impairments in GnRH/LH secretion and display appropriate regulation of *Kiss1* expression in response to estrogen [30]. Thus, the functional significance of ERβ in *Kiss1* neurons is unclear, although it is possible that other genes coexpressed in some *Kiss1* neurons (e.g. genes encoding dynorphin and neurokinin B) might be regulated through ERβ.

The effects of sex steroids on *Kiss1* gene expression in the brain are region-specific, at least in rodents. In the ARC, estrogen and testosterone *inhibit* the expression of *Kiss1*, whereas in the AVPV, these same steroids *stimulate* *Kiss1* expression (Figure 1) [30,31,33,34]. Similarly, in gonadectomized rodents (i.e. having very low sex steroids), levels of *Kiss1* mRNA are increased in the ARC and decreased in the AVPV [30,31,33,34]. The differential

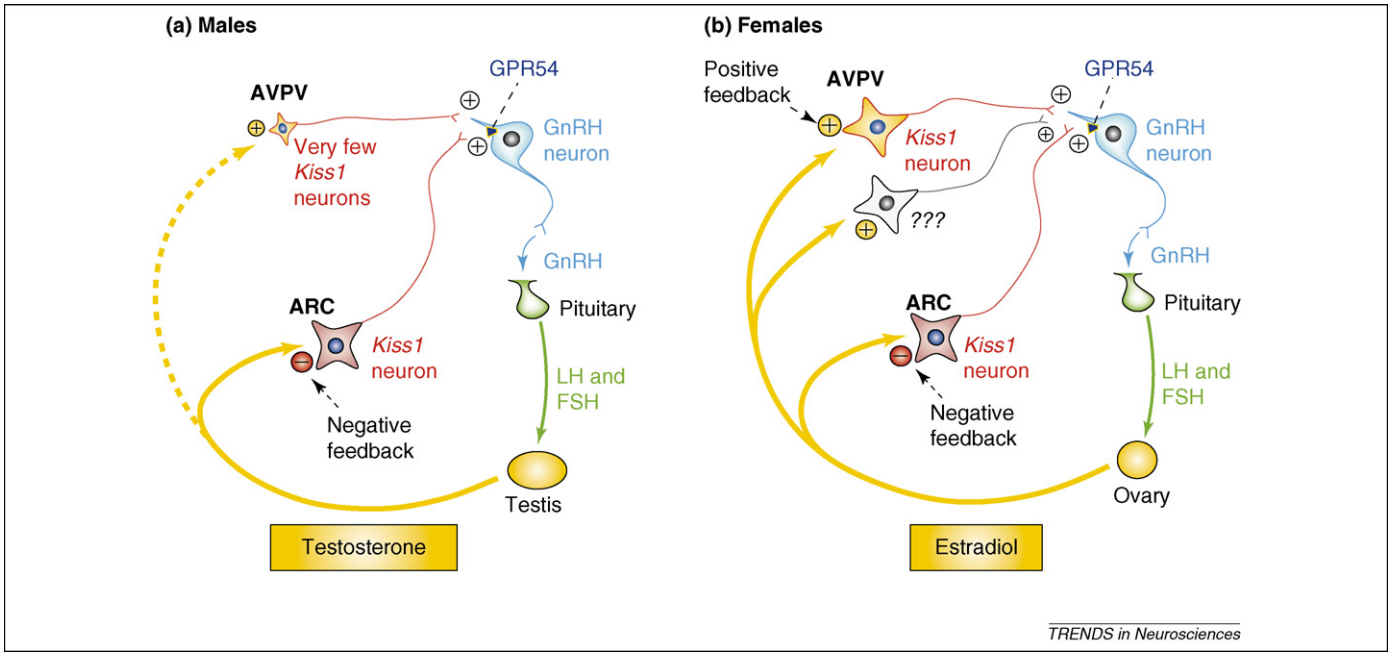

Figure 1. Model of kisspeptin–GPR54 signaling in the brains of male and female rodents. In both **(a)** males and **(b)** females, *Kiss1* neurons in the arcuate (ARC) probably provide tonic stimulatory input to GnRH neurons and are negatively regulated by gonadal sex steroids, thereby providing the cellular basis for the negative feedback effects of testosterone and estrogen on GnRH secretion (and hence, LH and FSH secretion from the pituitary). In the anteroventral periventricular nucleus (AVPV), males possess very few *Kiss1* neurons, even when levels of sex steroids are high, whereas females possess numerous *Kiss1* cells in this region; this sex difference in *Kiss1* expression in the AVPV is sexually differentiated by sex steroids early in perinatal life. In contrast to the ARC, *Kiss1* expression in the AVPV is stimulated by the presence of sex steroids, providing the cellular basis for the positive feedback induction by estrogen of the preovulatory GnRH surge in females. In addition, an unidentified population of neurons, possibly in the AVPV, can mediate an estrogen-induced GnRH/LH surge in females in the absence of functional kisspeptin–GPR54 signaling (as determined in estrogen-treated *GPR54* KO female mice). It should be noted that this rodent model of kisspeptin–GPR54 signaling might not apply to sheep and primates in which positive feedback signaling is probably derived from kisspeptin neurons in the medial basal hypothalamus (a region including the ARC) rather than the more anterior AVPV region.

effects of estrogen and testosterone on *Kiss1* gene expression in the ARC and AVPV suggest that *Kiss1* neurons might be involved in the steroid-mediated positive and negative feedback control of GnRH neurons (Figure 1); however, the molecular mechanism for this differential regulation of *Kiss1* by sex steroids is unknown.

In rodents, sheep and primates, the ARC comprises the neural elements that mediate the negative feedback regulation of reproduction [35–37], and *Kiss1* neurons in this region might provide the cellular mechanism orchestrating this phenomenon. Similar to findings in castrated rodents, gonadectomized sheep and monkeys (and post-menopausal women having low plasma levels of estrogen) also show elevated *Kiss1* expression in the ARC (or its homologue in the primate, the infundibular nucleus) [32,38,39], correlating with increased LH secretion. Notably, this 'disinhibition' or release from sex-steroid-dependent negative feedback control of gonadotropin secretion in gonadectomized animals is not observed in gonadectomized *GPR54* KO mice. Although *Kiss1* gene expression in the ARC of *GPR54* KO mice increases after gonadectomy, as in wild-type mice, *GPR54* KO mice show no post-castration rise in LH. Collectively, these findings support the contention that steroid-mediated inhibition of kisspeptin–GPR54 signaling arising from ARC *Kiss1* neurons mediates the negative feedback effects of sex steroids on GnRH secretion (Figure 1). However, proof of the validity of this model awaits further studies establishing direct connections between ARC *Kiss1* neurons and GnRH neurons and showing that targeted deletions of *Kiss1* in ARC cells disrupt negative feedback.

In contrast to the classical negative feedback effects of sex steroids on GnRH/LH secretion that occur in both sexes, estrogen (above a certain plasma threshold) can also act in females to exert a so-called 'positive feedback' effect, which stimulates GnRH/LH secretion and triggers ovulation. In rodents, the AVPV is the anatomical nodal point for generating the preovulatory GnRH/LH surge [40–42]. Lesions of the AVPV block the spontaneous and steroid-induced surge [43–46], and placement of estrogen into the AVPV (but not other areas) elicits an LH surge [47]. A compelling line of evidence suggests that *Kiss1* neurons in the AVPV drive the sexually differentiated estrogen-induced GnRH/LH surge in rodents. First, kisspeptin is a potent secretagogue for GnRH, and GnRH neurons express *GPR54* [8,9,20,24,48]. Second, *Kiss1* expression in the AVPV increases at the time of the LH surge, coincident with increased coexpression of the transcription factor Fos in *Kiss1* neurons [34]. Third, central infusion of kisspeptin antiserum blocks the spontaneous and estrogen-induced LH surges in female rats [49]. Fourth, ERα is thought to mediate the stimulatory effects of estrogen on the surge mechanism [50], and almost all *Kiss1* neurons in the AVPV express ERα [30,31]. Finally, the GnRH/LH surge is sexually differentiated, and only females are capable of displaying a surge in response to estrogen [51–55]. Similarly, only females possess significant numbers of *Kiss1* cells in the AVPV [33]; even if adult males are treated with high levels of estrogen (or testosterone), they display few *Kiss1* neurons in this region. Thus, *Kiss1* neurons in the AVPV of female rodents probably serve as the cellular conduit for integrating and relaying estrogen signals to GnRH neurons to generate the sexually differentiated preovulatory LH surge (Figure 1). However, the interaction between *Kiss1* neurons in the AVPV and other factors that regulate the GnRH/LH surge (e.g. circadian signals and progesterone) remains unexplored. Moreover, although it has become clear that kisspeptin–GPR54 signaling provides an important element of the neural surge-generating mechanism in rodents, it is not the entire story. Recent data indicate that female *GPR54* KO mice retain the ability to produce a GnRH/LH surge in response to estrogen, suggesting possible redundancy or developmental compensation in the circuitry that generates the positive feedback event in the murine brain. This possibility for redundancy of GnRH regulation is echoed in the finding that *GPR54* KO mice have plasma gonadotropin levels that, although exceedingly low, are not completely absent (although these gonadotropin concentrations are often at or near the low level of detection of the radioimmunoassay, precluding a definitive interpretation of their functionality).

In sheep, the population of *Kiss1* neurons in the AVPV is relatively small (containing only a few *Kiss1* cells relative to the ARC) and is not regulated by sex steroids [32]. Furthermore, in ewes, the mediobasal hypothalamus (containing the ventromedial nucleus [VMN] and ARC), not the POA, is believed to contain the circuitry necessary for mediating the positive feedback effects of estrogen on GnRH neurons [56–59]. It is perhaps not surprising then that the expression of *Kiss1* in the ovine ARC is regulated by sex steroids and markedly increased before and during the preovulatory LH surge [32,60]. Thus, in the ewe, both positive and negative feedback effects of sex steroids might be mediated by *Kiss1* neurons in the medial basal hypothalamus (i.e. the ARC). Whether these positive and negative feedback effects are mediated by the *same* ARC *Kiss1* neurons or by *separate* subpopulations of *Kiss1* cells within the ARC warrants additional study. Similar to sheep, in primates (including humans), the distribution of *Kiss1* neurons is concentrated in the medial basal hypothalamus and infundibular nucleus (the ARC homologue) [22,38]. However, the role, if any, of the infundibular *Kiss1* system in generating the preovulatory surge in primates is unexplored.

Sexual differentiation and the *Kiss1* system

In mammals, the brain is anatomically and physiologically differentiated between the sexes [55,61,62]. This phenomenon develops during the perinatal critical period [55] and reflects the organizational effects of perinatal sex steroids on the developing brain. In rodents and sheep, one important sexually differentiated trait is the ability of adult females, but not adult males, to display an estrogen-induced, circadian-dependent GnRH/LH surge (i.e. positive feedback) [55,63,64]. In male rodents, exposure to testosterone or its estrogenic metabolites during perinatal life (or during prenatal life in the sheep) permanently alters the circuitry in the developing forebrain [62], averting its ability as an adult to generate a GnRH/LH surge in response to estrogen [62]. Because the brain of the normal female is not exposed to testosterone during the perinatal

period, it retains (or develops) the circuitry necessary to generate a GnRH/LH surge in adulthood [42,55]. Male rodents that are castrated during the critical period can produce a GnRH/LH surge as adults, just like normal females [55,63]; similarly, females that are exposed to testosterone during the perinatal period lose their ability to generate GnRH/LH surges as adults [55,63]. However, the population of forebrain neurons that drives the GnRH/LH surge, which develops in females and regresses in males, has not been fully elucidated.

Estrogen-sensitive cells in the AVPV are thought to play a key role in generating the GnRH/LH surge. Neuronal populations in the AVPV are sexually differentiated, and females possess more neurons overall than do males, as well as greater numbers of tyrosine hydroxylase (TH-)containing (dopaminergic) cells and GABA/glutamate cells [42,65,66]. *Kiss1* neurons in the AVPV are also sexually differentiated, and adult females possess more *Kiss1* cells than do males (Figure 1) [33,67]. In rats, the number of *Kiss1* neurons in the AVPV of adult females is as much as 25 times greater than in males [33], and similar sex differences in kisspeptin protein levels in the AVPV [as determined by immunocytochemistry (ICC)] have also been reported in the mouse [67]. Although sex steroids in adulthood induce *Kiss1* gene expression in the AVPV [34,68], the gender difference in AVPV *Kiss1* neurons is not attributable to sex differences in circulating levels of testosterone or estrogen in adulthood. Gonadectomized male and female rats receiving identical sex steroid treatments as adults still display sex differences in *Kiss1* expression in the AVPV [33]. By contrast, the perinatal hormonal milieu affects the sex difference in *Kiss1* neurons; female rats treated perinatally with androgen do not exhibit a GnRH/LH surge and possess very few *Kiss1* neurons in the AVPV as adults, similar to adult males [33]. These observations indicate that the *Kiss1* system in the AVPV is sexually differentiated early in development under the influence of sex steroids, thereby producing robust gender differences in *Kiss1* expression in the AVPV in adulthood (and probably accounting for the gender-specific ability of female rodents to produce an LH surge). It remains to be determined whether these developmental effects of sex steroids on *Kiss1* neurons in the AVPV are mediated by either AR- or ER-dependent pathways and whether the sexually dimorphic *Kiss1* population in the AVPV is the same set of cells as other sexually dimorphic neurons in the region, such as GABA/glutamate [65] or TH [42,55]. (For example, in the rat, the sexually dimorphic *Kiss1* and *TH* populations in the AVPV appear to represent two separate sexually differentiated populations [33].)

In contrast to the AVPV, the ARC displays no gender-based differences in either the number of *Kiss1* neurons or the content of *Kiss1* mRNA per cell. Adult male and female rats display similar levels of enhanced *Kiss1* expression in the ARC after gonadectomy and similarly reduced *Kiss1* expression after sex hormone replacement (testosterone or estrogen) [33]. Likewise, in mice, kisspeptin immunoreactivity in the ARC is similar between gonad-intact adult males and females [67]. We have suggested that *Kiss1* cells in the ARC provide tonic stimulatory input to GnRH neurons and relay the negative feedback effects of sex steroids to GnRH secretion, in both sexes [69]. So, it is not surprising that, in rodents, there is no sexual differentiation of *Kiss1* neurons in the ARC. Sex differences in *Kiss1* expression in the ARC of other species have yet to be examined.

Recent findings indicate that the process of sexual differentiation is itself dependent upon functional kisspeptin–GPR54 signaling. Specifically, sexual differentiation towards the male phenotype is impaired in the genotypic male *GPR54* KO mouse [23]: despite testosterone replacement in adulthood, male *GPR54* KO mice display female-like characteristics in several sexually dimorphic traits, including olfactory partner preference, the number of TH- and *Kiss1*-expressing neurons in the AVPV, and the number of motoneurons in the spinal cord [23]. These findings indicate that kisspeptin–GPR54 signaling during the perinatal period is essential for proper sexual differentiation in normal males, probably through direct stimulation of the GnRH–LH–testosterone axis during the developmental 'critical period'. Although a role of peripheral kisspeptin–GPR54 signaling within the perinatal testes is also conceivable, the foregoing observations suggest that central kisspeptin–GPR54 signaling probably regulates GnRH secretion during early postnatal life, just as it does in adulthood.

Kisspeptin signaling and puberty

In mammals, activation of GnRH neurons is the key event gating the onset of puberty; however, the mechanisms that trigger GnRH secretion at puberty remain one of the enigmas of modern science [70]. Intriguingly, sexual maturation is impaired in humans and mice with targeted deletions or spontaneous mutations in the *GPR54* gene [11–13], suggesting that kisspeptin–GPR54 signaling is essential for pubertal maturation. Moreover, exogenous kisspeptin administered to prepubertal rodents and monkeys initiates various aspects of precocious puberty (such as LH secretion or vaginal opening) [20,22,24,71]. Similarly, neural *Kiss1* gene expression increases in both male and female rats, mice and monkeys from pre- to post-puberty [10,22,24]; however, the specific *Kiss1* population(s) (ARC versus AVPV) that changes with puberty is equivocal and might reflect species-specific mechanisms that differ between primates and rodents [72,73]. Regardless, changes in the activity of *Kiss1* neurons appear to represent a seminal and perhaps common event in the timing of puberty in many species.

Whether changes in the expression/activity of GPR54 are also involved in pubertal maturation is less clear: *GPR54* expression increases from pre- to post-puberty in rats of both sexes and in female monkeys [10,22], but not in male mice and monkeys [22,24]. In mice, the number of kisspeptin-containing fibers that appose GnRH neurons increases at puberty [67], suggesting that pubertal maturation might also include the completion of developmental circuitry coupling *Kiss1* and GnRH neurons. Collectively, these findings indicate that hypothalamic-derived kisspeptin–GPR54–GnRH signaling is intimately involved in the mechanism(s) that initiate puberty, but precisely how this signaling is triggered remains a mystery.

The role of *Kiss1* neurons in seasonal breeding: effects of photoperiod and melatonin

Many mammals living in non-equatorial zones have mechanisms that synchronize reproduction with the external environment, thereby optimizing reproductive success. Most seasonal breeders modulate reproductive activity by responding to photoperiodic cues (i.e. day length) [74,75]. In mammals, photoperiodic information is transmitted from the retina to the circadian oscillator in the suprachiasmatic nucleus (SCN), which in turn regulates the secretion of melatonin from the pineal gland via a multisynaptic pathway (Figure 2) [74]. Melatonin is secreted exclusively at night and in direct proportion to the dark portion of the light–dark cycle. It is the duration of daily melatonin secretion, rather than its amplitude, that encodes day length information. In hamsters, a long duration melatonin signal (about 10 h per day, indicative of a long night and short day) induces a short-day (SD) 'winter' phenotype (i.e. inhibited reproductive axis), whereas a short duration melatonin signal (5–6 h per day) induces a long-day (LD) 'summer' phenotype (i.e. activated reproduction) (Figure 2) [75–77].

The neural mechanisms by which melatonin signals are decoded and transmitted to the reproductive axis are unknown, but melatonin does not appear to have direct actions on GnRH neurons [78–81]. Little or no melatonin binding is observed in forebrain regions that contain GnRH neurons [82], suggesting that the regulatory actions of melatonin are upstream of GnRH neurons. Autoradiographic studies have identified several brain areas of hamsters (Syrian and Siberian) that bind melatonin, most notably, the SCN, pars tuberalis, paraventricular nucleus of the thalamus, nucleus reuniens, and the medial-basal hypothalamus (includes the ARC and dorsomedial regions) [82]; however, it remains ambiguous where and how melatonin signals are decoded and transmitted to GnRH neurons.

Because kisspeptins are integral to the regulation of GnRH activity, it seems plausible that the photoperiodic control of reproduction involves direct or indirect modulation of the *Kiss1* system. Recently, *Kiss1* mRNA and kisspeptin protein levels were reported to be decreased in the ARC of SD Syrian hamsters, correlating with decreased reproductive activity (Figure 2) [83]. In Syrian hamsters, melatonin receptors are located in the ARC and dorsal–medial hypothalamus, suggesting that the actions of melatonin on *Kiss1* neurons in the ARC could be direct. However, it is also conceivable that ARC *Kiss1* neurons are indirectly regulated by melatonin via projections from other melatonin-responsive sites. In contrast to the findings in Syrian hamsters, kisspeptin immunoreactivity in Siberian hamsters appears to be upregulated in the ARC of SD animals [19]. Considering differences in the neural sites of action of melatonin between Syrian and Siberian

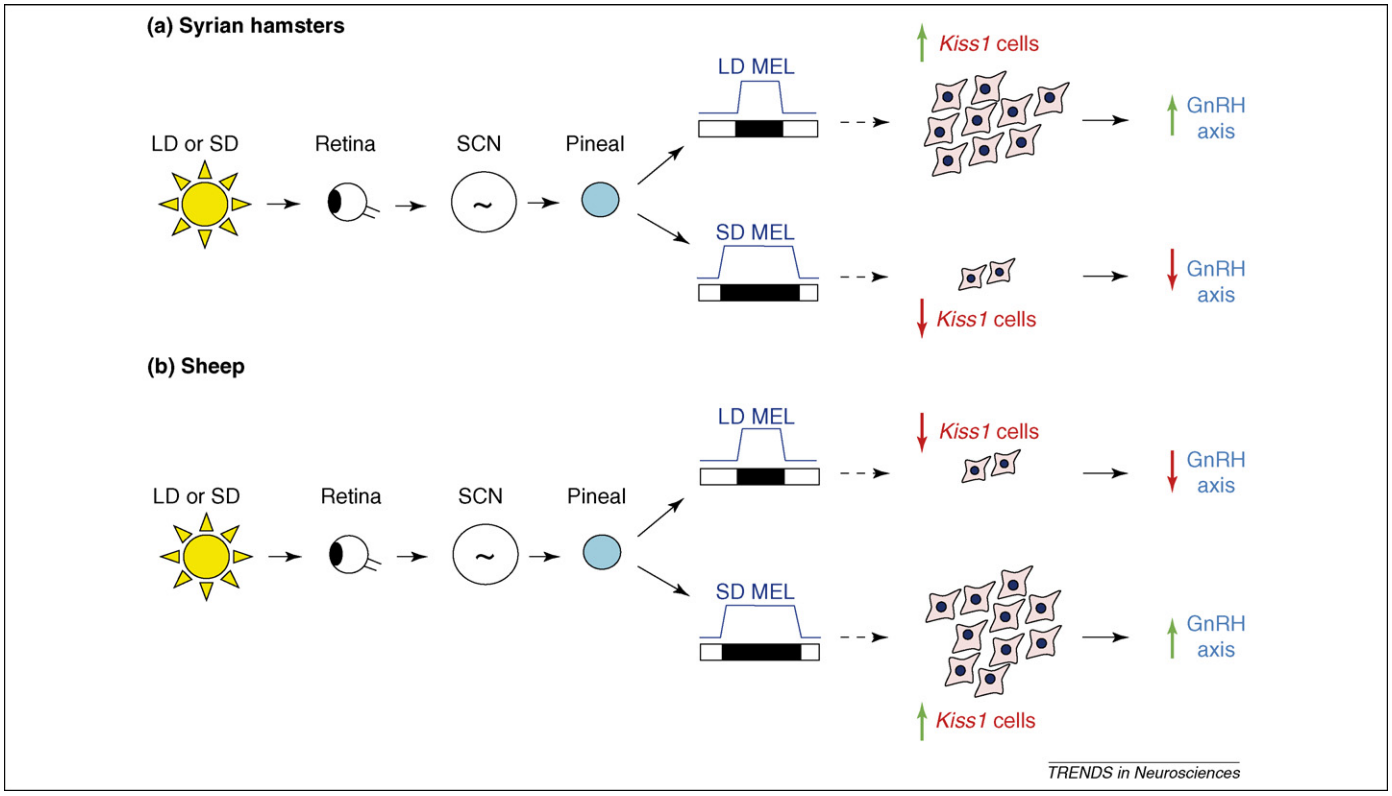

Figure 2. Species-specific effects of photoperiod and melatonin (MEL) on *Kiss1* neurons in the arcuate nucleus. Photoperiod information is received by the retina and transmitted to the suprachiasmatic nucleus (SCN; i.e. the circadian pacemaker), which then directs the secretion of pineal MEL by way of an indirect, multisynaptic pathway. Long duration MEL signals are indicative of short days (SD; winter) whereas short duration MEL signals are indicative of long days (LD; summer). **(a)** Syrian hamsters are LD breeders, with reproduction activated by short-duration MEL signals. **(b)** By contrast, sheep are SD breeders, with reproduction activated by long duration MEL signals. In both species, *Kiss1* gene expression in the ARC is substantially inhibited in the non-breeding season (SD in Syrian hamsters, LD in sheep). An inhibition of the ARC *Kiss1* system presumably results in decreased tonic stimulation of GnRH neurons, thereby inhibiting the reproductive axis. It remains to be determined whether the effects of MEL on *Kiss1* neurons are direct or indirect, as well as how the same MEL signal (SD MEL) stimulates *Kiss1* expression in the ARC of one species (sheep) but inhibits it in the other (Syrian hamster).

hamsters [74], species differences in the organization or regulation of the *Kiss1* system might account for the contradictory results. However, many published accounts of kisspeptin protein levels (as reflected by ICC) have involved different kisspeptin antisera, which in many cases lack specificity for kisspeptin and might therefore confound interpretation of the results. It is also conceivable that elevated kisspeptin immunoreactivity in the ARC of SD Siberian hamsters reflects increased storage (decreased release) of kisspeptin protein. This explanation would reconcile the apparently incongruous finding of increased levels of kisspeptin (which is stimulatory to the reproductive axis) during SDs (when reproduction is inhibited).

In contrast to hamsters, which breed in LDs, sheep are SD seasonal breeders. Thus, SDs stimulate the ovine GnRH axis whereas LDs inhibit it (Figure 2). Interestingly, *Kiss1* expression in the ARC of ovariectomized ewes increases during the transition between the anestrous, non-breeding season and the breeding season [32], reminiscent of findings in Syrian hamsters [83]. Autoradiographic studies have revealed that, in sheep, melatonin binds in many brain areas, including the pars tuberalis, POA, and the ventromedial and dorsomedial nuclei [84,85]; however, little melatonin binding is observed in the ovine ARC, suggesting an indirect effect of melatonin on ARC *Kiss1* neurons. Notwithstanding, a kisspeptin-mediated mechanism for regulating seasonal reproduction might exist in all photoperiodic mammals (Figure 2), although the manner in which the same photoperiodic signal (long melatonin duration) produces the opposite effect on *Kiss1* expression in SD and LD breeders warrants further investigation.

Conclusions and future perspectives

Recent investigations in many species have yielded a wealth of information detailing the role of kisspeptin–GPR54 signaling in the regulation of reproduction, including its involvement in sexual differentiation of the brain, onset of puberty, regulation of estrous cyclicity and the LH surge, and seasonal reproduction. Despite these advances, many challenges remain. Among these are: (i) mapping the afferent and efferent connections of the different populations of *Kiss1* neurons, in the mouse, rat, sheep and primate; (ii) dissecting the molecular mechanisms that explain how estradiol can induce the expression of *Kiss1* in the AVPV and inhibit its expression in the ARC; (iii) learning how sex steroids influence the development of *Kiss1* neurons in the forebrain; (iv) understanding the role of kisspeptin–GPR54 signaling in timing the onset of puberty; and (v) revealing the functional significance of co-transmitters in discrete populations of *Kiss1* neurons (e.g. dynorphin in the ARC).

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