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Kisspeptins and the neuroendocrine control of reproduction: Recent progress and new frontiers in kisspeptin research

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ABSTRACT

In late 2003, a major breakthrough in our understanding of the mechanisms that govern reproduction occurred with the identification of the reproductive roles of kisspeptins, encoded by the *Kiss1* gene, and their receptor, Gpr54 (aka, Kiss1R). The discovery of this unsuspected reproductive facet attracted an extraordinary interest and boosted an intense research activity, in human and model species, that, in a relatively short period, established a series of basic concepts on the physiological roles of kisspeptins. Such fundamental knowledge, gathered in these early years of kisspeptin research, set the scene for the more recent in-depth dissection of the intimacies of the neuronal networks involving Kiss1 neurons, their precise mechanisms of regulation and the molecular underpinnings of the function of kisspeptins as pivotal regulators of all key aspects of reproductive function, from puberty onset to pulsatile gonadotropin secretion and the metabolic control of fertility. While no clear temporal boundaries between these two periods can be defined, in this review we will summarize the most prominent advances in kisspeptin research occurred in the last ten years, as a means to provide an up-dated view of the state of the art and potential paths of future progress in this dynamic, and ever growing domain of Neuroendocrinology.

1. Introduction: Key concepts in the early years of the kisspeptin era (2003–2012)

As essential for perpetuation of the species, reproductive function has been the subject of active research, with seminal developments occurring in the last decades of the 20th century. These are exemplified by the identification of the hypothalamic driver of the reproductive axis, the gonadotropin releasing hormone (GnRH), and neurons producing it, in 1970's (Knobil, 1992; Conn and Crowley, 1994), as well as the initial characterization, mainly during 1980's and 90's, of a complex network of central transmitters and peripheral hormones, ranging from glutamate and GABA to nitric oxide and leptin, as major modulators of puberty and fertility (Grumbach, 2002; Dhandapani and Brann, 2000; Terasawa and Fernandez, 2001). In fact, by the turn of the Millennium, there was the perception that the fundamentals of the neuroendocrine systems governing the reproductive axis had been already exposed, thus leaving little room for major conceptual developments in this apparently exhausted field of contemporary Endocrinology. Reality, however, proved to be much more exiting, and the last twenty years have witnessed ground-breaking findings in this area that have changed our understanding of the mechanisms of reproductive control, and boosted an extraordinary interest in reproductive research, both basic and translational.

Undoubtedly, one of the major recent breakthroughs in reproductive neuroendocrinology was the identification of kisspeptins, as major gatekeepers of virtually all aspects of reproductive maturation and function. Kisspeptins, initially catalogued as metastasis-suppressing factors (Ohtaki et al., 2001; Kotani et al., 2001), are encoded by the

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Kiss1 gene and operate via the G-protein coupled receptor, Gpr54 (aka Kiss1R) (Kotani et al., 2001). Disclosure of their reproductive dimension came in late 2003, when two seminal publications reported in a lapse of few weeks that inactivating mutations in humans and rodents affecting Gpr54 caused hypogonadism of central origin (de Roux et al., 2003; Seminara et al., 2003). The fact that this genetic form of hypogonadotropic hypogonadism (HH) differed from other developmental types of HH, such as Kallmann syndrome, in which early maturation and migration of GnRH neurons is perturbed (Boehm et al., 2015), pointed out that kisspeptin signaling is rather involved in fundamental aspects of the regulation of GnRH neurosecretion, a totally unsuspected facet of kisspeptin biology that immediately drew much attention and research efforts.

Indeed, the years following the disclosure of the reproductive dimension of kisspeptins were of intense research activity, aiming not only to uncover the biological effects of kisspeptins on the reproductive axis in different species, including humans, but also to disclose their major physiological roles and mechanisms of action on key aspects of reproduction, from brain sex differentiation and puberty to the regulation of gonadotropin secretion and the hormonal control of ovulation. In fact, in the period of (grossly) ten years between 2003 and 2012, the major principles of the reproductive roles of kisspeptins were set, in one of the most fertile decades of reproductive research ever. Extensive recapitulation of such features exceeds the focus of this review, and can be found in authoritative reviews published during these years (Popa et al., 2008; Oakley et al., 2009; d'Anglemont de Tassigny and Colledge, 2010; Hameed et al., 2011; Colledge, 2009; Roa et al., 2008; Navarro and Tena-Sempere, 2011; Pinilla et al., 2012). Anyhow, a bulleted summary of some salient findings of these early years are provided below, as a means to set the basis for the contents of later sections of this review. For specific references, see the review articles quoted above.

- Albeit rare, inactivating mutations of the genes encoding kisspeptins (*KISS1*) and their receptor (*GPR54*) cause HH in humans; mice with genetic inactivation of *Kiss1* or *Gpr54* are a phenocopy of affected patients.
- Neurons expressing *Kiss1* are found in the hypothalamus, with two prominent populations: one in the arcuate nucleus (ARC) and another in the preoptic/rostral hypothalamic area, mainly the anteroventral periventricular area (AVPV) in rodents, the latter being much larger in females.
- ARC and AVPV Kiss1 neuronal populations are oppositely regulated by sex steroids, which repress *Kiss1* expression in the ARC but stimulate it in the AVPV, as basis for their negative and positive feedback regulation of gonadotropins, respectively.
- During puberty, Kiss1 neurons undergo a complex maturational program, with increased number of appositions and excitatory actions onto GnRH neurons; blockade of kisspeptin signaling during the pubertal transition prevents normal pubertal timing.
- Kisspeptins potently activate GnRH neurons and elicit robust gonadotropin responses in different species, including humans. A large subset of ARC Kiss1 neurons, named KNDy neurons, are shown to co-express neurokinin B (NKB) and dynorphin, as reciprocal stimulatory and inhibitory signals, respectively, for the fine control of kisspeptin output to GnRH neurons.
- In rodents, AVPV Kiss1 neurons play a key role in the generation of the pre-ovulatory surge of gonadotropins, as major hormonal driver of ovulation; blockade of kisspeptin actions prevents this surge.
- Kiss1 neurons are modulated by nutritional and metabolic cues and are proposed to participate in the metabolic control of puberty and reproduction
- While initial reports documented a direct role of key metabolic signals, as leptin, in the control of Kiss1 neurons, additional evidence was presented supporting an indirect mode of action of leptin on Kiss1 neurons.

Collectively, these seminal findings set the scene for more in-depth analyses, covering different aspects of kisspeptin physiology, which were implemented in the following years. This review aims to provide a summary of the most crucial developments in this area during the last decade (2012–2021), which for sake of simplicity will be referred to as "recent progress" in the following sections, as a means to offer an updated guide to navigate the ever growing field of kisspeptin research.

2. Recent progress on the roles of kisspeptins -and their partners- in the control of puberty

The paramount importance of puberty within the cascade of events leading to the attainment of reproductive capacity drew immediate attention to the putative pubertal roles of kisspeptins (Avendano et al., 2017; Vazquez et al., 20192019), once the reproductive phenotype of patients with alterations of kisspeptin signaling was disclosed. In fact, these patients (and their mouse counterparts) suffered impuberism, supporting a role for kisspeptins in pubertal maturation. Indeed, active research between 2004 and 2011 set the contention that, even if developmental compensation may permit pubertal progression in extreme conditions of congenital ablation of Kiss1 neurons (Mayer and Boehm, 2011), kisspeptin signaling in the hypothalamus plays an indispensable role in the physiological timing of puberty (Pinilla et al., 2012). Notwithstanding, while initial studies suggested that kisspeptins might be the long-sought trigger of puberty, later analyses suggested that kisspeptins act in concert with other central transmitters and rather operate as amplifier of the cascade of events leading to full activation of GnRH neurosecretion at puberty (Clarkson et al., 2009). This section summarizes major findings in the last decade regarding the pubertal roles of kisspeptins, their partners and mode of action.

2.1. Kisspeptin partners and the control of puberty: Roles of tachykinins

Some of the major developments in the last ten years in this area are related with the characterization of the pubertal roles of different co-transmitters and neuropeptide partners of kisspeptins, which cooperate in the fine tuning of pubertal timing. On this point, however, it is important to stress that, as is also the case for kisspeptins, most of these regulatory partners are not specific of puberty, but rather participate both in the activation of the reproductive axis at puberty and its modulation in adulthood. This is clearly the case of the member of the tachykinin family, NKB, and the endogenous opioid, Dyn, expressed also in ARC KNDy neurons, which have been actively studied not only in the context of puberty, but also in terms of control of adult GnRH/gonadotropin secretion, as described in detail in Section 3. Regarding puberty, in 2012, two independent studies, in rats and mice, respectively, documented a putative role of NKB signaling in the control of female puberty (Navarro et al., 2012; Gill et al., 2012). Interestingly, elevation of hypothalamic expression of *Tac2*, encoding NKB in rodents, preceded the pubertal rise of *Kiss1* expression (Navarro et al., 2012; Gill et al., 2012), whereas chronic administration of a NKB receptor (NK3R) antagonist moderately delayed puberty onset in female rats (Navarro et al., 2012). These findings were later confirmed by independent studies using pharmacological tools (i.e., NK3R antagonist in rats) (Li et al., 2014) or functional genomics (i.e., Tac2 KO mice) (True et al., 2015). On the latter, while male Tac2 KO mice failed to show any sign of altered puberty, null female mice displayed clear phenotypic signs of delayed puberty, suggesting sex differences on the pubertal roles of NKB signaling (True et al., 2015). In the same vein, our pharmacological studies demonstrated a salient sex dimorphism in the responses to NKB stimulation, with male rats becoming unresponsive to the NKB agonist, senktide, from puberty onwards, while females displayed persistent LH responses to senktide all though postnatal maturation until adulthood (Ruiz-Pino et al., 2012). Overall, given the stimulatory role proposed for NKB on kisspeptin neurosecretion (Navarro et al., 2009), and the fact that the gonadotropin-releasing effects of NKB require preserved

kisspeptin signaling (Garcia-Galiano et al., 2012), these observations are compatible with a role of NKB as activator of kisspeptin secretion to stimulate puberty onset. Conversely, and in keeping with the proposed inhibitory role of Dyn on kisspeptin output by KNDy neurons, chronic blockade of the canonical Dyn receptor, kappa-opioid receptor (KOR), has been reported to cause an advancement of puberty onset, together with enhancement of pulsatile LH secretion, in female rats (Nakahara et al., 2013). Therefore, the reciprocal interplay between NKB (stimulator) and Dyn (inhibitor) on kisspeptin output by KNDy cells, and thereby, GnRH secretion seems to be operative also during the pubertal transition. Further details on the function of KNDy neurons as master regulators of GnRH can be found in Section 3.

More recently, the putative pubertal roles of other members of the tachykinin (TAC) family, and their interplay with kisspeptin, have been actively explored using pharmacological probes and genetic models. Again, this function is not specific of puberty and encompasses also the modulation of adult reproductive axis. Initial studies by the Navarro's lab demonstrated a role of Substance P (SP), encoded by *Tac1*, which operates via the receptor, NK1R, in the control of puberty. Hypothalamic *Tac1* expression peaked before puberty and central injection of a NK1R agonist stimulated gonadotropin secretion and advanced puberty onset in immature female mice (Simavli et al., 2015), whereas *Tac1* KO mice of both sexes displayed delayed puberty (Simavli et al., 2015; Maguire et al., 2017). The interplay of SP with kisspeptin signaling is likely to occur at two sites: (i) half of Kiss1 neurons show electrophysiological responses to SP, suggesting that SP can enhance kisspeptin output to GnRH neurons, and (ii) NK1R and Gpr54 can heterodimerize and, actually, *Tac1* KO mice show decreased responses to kisspeptin, therefore suggesting an additional layer of TAC/kisspeptin interaction, at the level of GnRH neurons (Maguire et al., 2017).

In the same vein, another member of the TAC family, neurokinin A (NKA), has been suggested to play a role in the modulation of puberty, since repeated activation of its putative receptor, NK2R, in pre-pubertal female mice accelerated the onset of puberty, with a stimulatory effect on LH release that was dependent on preserved kisspeptin signaling in the presence of sex steroids (Leon et al., 2019). Since both SP and NKA are encoded by *Tac1*, it is tenable that at least part of the pubertal phenotype of *Tac1* null mice is due to the lack of NKA actions. Interestingly, despite pharmacological evidence for a pubertal role of NKA, congenital ablation of its receptor, NK2R, failed to totally suppress LH responses and to alter pubertal timing in mice (Torres et al., 2021), suggesting some degree of physiological redundancy across TAC receptors, that has been also suggested for SP and NKB. Anyhow, the above experimental evidence collectively supports a role of different tachykinins in the modulation of puberty, via interplay with kisspeptin signaling.

2.2. Other kisspeptin partners and the control of puberty

Other central transmitters, besides kisspeptins and tachykinins, do play a role in pubertal control, as illustrated by multiple studies, produced before and after kisspeptin discovery. In recent years, evidence of the potential interplay between kisspeptins and some of these transmitters in the precise control of puberty has been presented, even though these are not restricted to the pubertal transition and are operative also in adulthood. As illustrative example, the pubertal roles of RFRP-3, the mammalian orthologue of the gonadotropin-inhibiting hormone (GnIH) (Ubuka et al., 2016), as putative inhibitory counterpart of kisspeptins, have been explored. Of note, RFRP-3 has been suggested to negatively modulate a subset of Kiss1 neurons (Poling et al., 2013), supporting a putative interaction between these two systems in the control of puberty. However, while RFRP-3 expression and neuronal activation is suppressed in the dorsomedial hypothalamus of female mice during the period preceding puberty (Semaan and Kauffman, 2015; Xiang et al., 2015), mice lacking the RFRP-3 receptor, NPFF1R, did not show altered pubertal timing (Leon et al., 2014), suggesting a dispensable role in the modulation of puberty. On the other hand, congenital ablation of NPFF1R failed to reverse the hypogonadal and impubertal state of *Gpr54* KO mice (Leon et al., 2014). Yet, prepubertal NPFF1R KO mice displayed elevated LH levels, whereas ARC *Kiss1* expression was increased in adult NPFF1R null mice, pointing out a repressive action of RFRP-3 on kisspeptin, with a potential role in the control of the gonadotropic axis also at puberty. As another example, neurons expressing proopiomelanocortin (POMC), producing α-MSH (for melanocyte-stimulating hormone), have been suggested to stimulate puberty onset and gonadotropin secretion via modulation of ARC Kiss1 neurons (Manfredi-Lozano et al., 2016). The physiological relevance of α-MSH signaling, via melanocortin receptor 3 (MC3R), in the control of puberty has been further documented by human and rodent studies (Lam et al., 2021), for further details see Section 5.1. Likewise, a recent study has demonstrated that neurons in the ventral premammillary nucleus (PMV) expressing PACAP (for pituitary adenylate cyclase activating polypeptide) project to, and activate a subset of ARC and AVPV Kiss1 neurons, whereas targeted deletion of this PMV neuronal population delayed puberty (Ross et al., 2018), suggesting a novel PACAP/ kisspeptin pathway for the modulation of puberty.

2.3. Novel genetic determinants of puberty and interaction with Kiss1

Other key genetic determinants of puberty have been disclosed in recent years, which may operate, at least partially, via modulation of Kiss1 neurons. This is the paradigmatic case of the makorin ring-finger protein 3, which is encoded by maternally-imprinted gene, MKRN3. In 2013, four different heterozygous mutations of MKRN3 were reported to cause precocious puberty in humans (Abreu et al., 2013); a finding that has been confirmed and expanded by numerous genetic studies in recent years (Maione et al., 2020; Seraphim et al., 2021), therefore suggesting a physiological role of MKRN3 as repressor of puberty. In line with this contention, hypothalamic expression of *Mkrn3* has been shown to decline during postnatal maturation in mice and rats (Abreu et al., 2013; Heras et al., 2019). However; the intimate mechanisms whereby this factor suppresses puberty have remain unfolded until recently, when *Mkrn3* was found to be expressed in Kiss1 neurons (Heras et al., 2019; Abreu et al., 2020); and MKRN3 was proven to have repressor activity at the human *KISS1* promoter (Abreu et al., 2020). Interestingly; the developmental decline of *Mkrn3* expression in the hypothalamus, as permissive for puberty to proceed, is driven, at least partially, by enhanced expression of the microRNA, miR-30b, which is also expressed in Kiss1 neurons (Heras et al., 2019). Thus, the miR-30/Mkrn3 tandem appears as novel regulatory element of *Kiss1*, and thereby of the tempo of puberty. Admittedly, however, MKRN3 is endowed also with ubiqutinase activity, which may contribute , in a kisspeptin-dependent or -independent manner, to the modulation of puberty (Abreu et al., 2020; Liu et al., 2017). Whether MKRN3 contributes to the control of reproductive function beyond puberty remains unexplored.

2.4. Kiss1 neuronal populations and impact of endocrine disruptors: Implications for puberty

Additional progress has taken place recently in the characterization of the molecular phenotype and specific contribution of the various Kiss1 neuronal populations in the control of puberty. While initial evidence documented a complex developmental program of ARC and AVPV Kiss1 neurons during the pubertal transition, the relative contribution each population in pubertal control began to be disclosed more recently, when interference studies showed that 37% knockdown of kisspeptin expression in the AVPV resulted in delayed puberty onset in female rats, whereas a 32% suppression in the ARC failed to alter pubertal timing (Hu et al., 2015). In addition, the pubertal roles of a third population of Kiss1 neurons, located in the amygdala (Pineda et al., 2017), has been explored recently. Thus, blockade of kisspeptin signaling in the posterodorsal medial amygdala (MePD), by chronic infusion of a specific

antagonist during the juvenile period, delayed puberty onset in female rats (Adekunbi et al., 2017), while chemo-genetic activation of this MePD population of Kiss1 neurons has been reported to elicit LH secretion in mice (Fergani et al., 2018). Collectively, these data suggest that kisspeptin signaling at or originating from the amygdala might contribute to the modulation of puberty, although its physiological relevance is yet to be fully disclosed. On the other hand, an intriguing pubertal shift has been recently shown in the molecular phenotype of a subset of ARC Kiss1 neurons, which changes from negligible co-expression with the gene encoding the growth hormone-releasing hormone (GHRH) in prepubertal female mice to >45% co-expression in adult females. This novel GHRH/Kiss1 dual-phenotype neurons might contribute to the interplay between the growth and gonadotropic axes during puberty, and its modulation by sex steroids (Garcia-Galiano et al., 2020).

As final note to this section, recent studies have documented that Kiss1 neurons are targets of the actions of endocrine disrupting chemicals (EDC) and likely contribute to mediate at least part of their impact on puberty onset. This is the case of bisphenol A (BPA), for which gestational exposures to low, environmentally relevant doses cause a divergent impact on the postnatal development of ARC vs. AVPV Kiss1 neurons in mice, with lower *Kiss1* expression in the ARC, but enhanced numbers of kisspeptin cells in the AVPV, which were associated to earlier puberty onset (Ruiz-Pino et al., 2019). Likewise, developmental exposure to a mixture of EDC has been shown to transgenerationally delay puberty in female rats and to disturb the hypothalamic expression of key genes for pubertal control, including *Kiss1* (Lopez-Rodriguez et al., 2021). For further details on some of the most salient developments occurred in the last decade in our knowledge about the roles of kisspeptins, their partners and regulatory mechanisms in the context of the control of puberty, see Fig. 1.

3. Recent progress on KNDy neurons and their role in the control of pulsatile GnRH secretion

As mentioned in a previous section, by 2012, the basis of the so-called KNDy hypothesis had been set, based mostly on expression and pharmacological studies in rodent and ovine species (Navarro et al., 2009; Lehman et al., 2010). These studies documented not only the co-expression of kisspeptin, NKB and Dyn in a majority of ARC Kiss1 neurons, but also the predominant stimulatory and inhibitory effects of NKB and Dyn, respectively, on gonadotropin secretion, as proxy marker of their actions on kisspeptin and, thereby, GnRH neurosecretion. Even if some sex- and species-differences were noted, e.g., the proportion of KNDy neurons is greater in rodents than in humans (for further details of KNDy neuronal network in humans, see Section 3.2), and possibly larger in females than in males (Hrabovszky et al., 2012), the KNDy model was enormously appealing and attracted considerable interest, as it provided

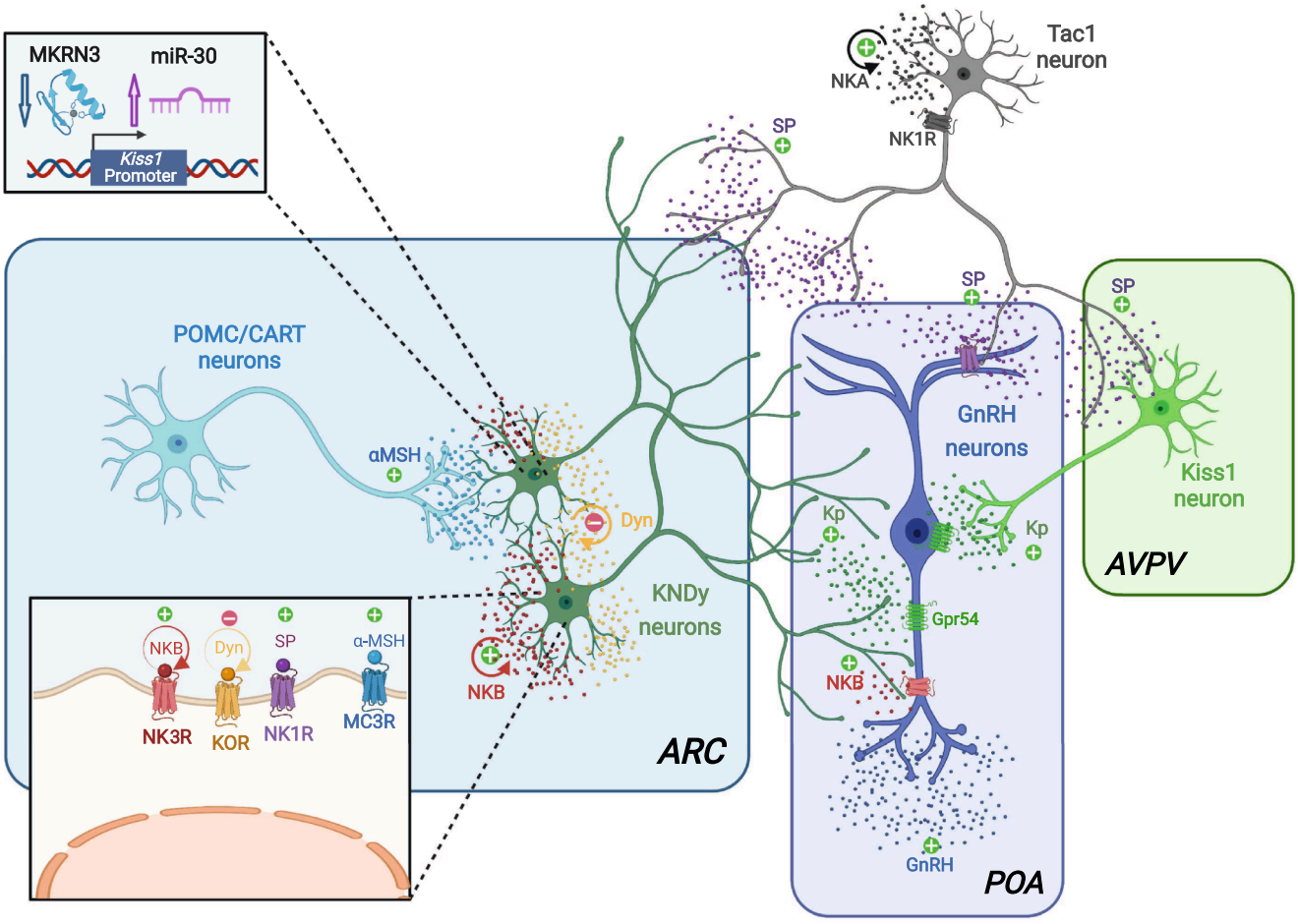

Fig. 1. *Neuroendocrine pathways controlling Kiss1 neurons and puberty*. A schematic is presented depicting some of the neuronal networks and molecular mechanisms, identified in recent years, involved in the control of pubertal activation of Kiss1 neuronal populations. A major focus is placed in Kiss1 neurons of the ARC, co-expressing NKB and Dynorphin. The roles of other tachykinins, such as Substance P (SP), and the product of POMC neurons, α-MSH, acting via its receptor, MC3R, are indicated. Likewise, the putative role of Mkrn3 and its regulator, miR-30b, in the control of Kiss1 and puberty is also depicted. For sake of clarity, other neuroendocrine and molecular mechanisms, as well as other Kiss1 neuronal populations (e.g., amygdala) are not depicted, but are described in detail in Section 2. Figure created with BioRender.

the conceptual basis for better understanding of the neuro-endocrine mechanisms that dictate the pulsatile release of GnRH. However, these early studies did not provide a complete functional interrogation of the organization and functionality of the KNDy circuits, and the co-transmitters involved, aspects that have been further exposed by more recent research, which is summarized in this section.

3.1. Assessing the KNDy hypothesis: Pharmacological studies

Pharmacological studies, conducted in the last decade in wild-type and genetically modified murine models, have reinforced the contention that NKB operates mainly via stimulation of kisspeptin output to GnRH neurons (Leon and Navarro, 2019), analyses that allowed also to ponder the relative contribution of NKB (co-expressed in Kiss1 neurons) and other tachykinins, such as SP and NKA, in the control of GnRH and gonadotropin secretion. These studies have been partially reviewed in Section 2.1, as they pertain also to pubertal control, and collectively point out some degree of redundancy and overlapping across TAC signaling, that ensures an appropriate regulation of KNDy neurons in different conditions (Leon and Navarro, 2019; Moore et al., 2018). It must be noted, though, that the degree of redundancy in TAC signaling may vary across species, it being higher in rodents than in the sheep and primates (Moore et al., 2018). Pharmacological work in rodents has also provided evidence for a differential effect of NKB, and other tachykinins, depending on the gonadal status, the developmental stage, the sex and even the gonadotropin (LH vs. FSH) considered (Ruiz-Pino et al., 2012; Ruiz-Pino et al., 2015; Navarro et al., 2015), therefore surfacing the polyhedral nature of NKB actions in the control of kisspeptin neurosecretion, which goes beyond a mere and uniform stimulation across the lifespan and functional states of the reproductive axis. The physiological meaning of such dynamic and even opposite regulation is yet to be fully exposed.

3.2. Assessing the KNDy hypothesis: Neuroanatomical studies

The advent of more incisive methods has also allowed progress in the characterization of the neuro-anatomical and molecular properties of the population of KNDy neurons in different species, and their connections. These have included the use of three-dimensional imaging using optical brain tissue clearing followed by multiple-label immunocytochemistry, which has permitted comparative analyses of KNDy populations between the rat and sheep brain (Moore et al., 2018), with better characterization of the anatomical distribution of KNDy cells within the rat hypothalamus, which was more abundant in the caudal vs. rostral ARC, and the identification of novel populations of KNDy neurons (e.g., in the lateral area of the mediobasal hypothalamus) in the sheep (Moore et al., 2018). Likewise, the recent use of viral-based monosynaptic tract tracing in mice has allowed the disclosure of the proximal connectome of KNDy cells, with >90% mono-synaptic inputs coming from the hypothalamus, of which a great majority originates from non-KNDy ARC neurons, as those expressing POMC (Moore et al., 2019). This technique has permitted also to identify sex differences, with a higher number of connections in the female mouse coming from estrogen-responsive neurons in the peri-ventricular nucleus and medial preoptic area (Moore et al., 2019). Finally, in a very recent report, Moore and co-workers have applied multi-fluorescent in situ hybridization, using the RNAscope technology, to simultaneously detect up to 12 different transcripts in KNDy cells, an approach that has permitted identification of divergent changes in gene expression of the androgen and progesterone receptor, as well as dynorphin, in KNDy neurons in a mouse model of polycystic ovary syndrome (Moore et al., 2021). The combined use of the above techniques in other physiological (e.g., puberty, senescence) and pathological (e.g., obesity, stress) conditions will be instrumental to further disclose the features of KNDy neurons.

In the same context, advances have taken place in the characterization of the human counterpart of KNDy neurons, which, despite operational limitations, is endowed with substantial translational interest. Departing from the pioneering work of his group in 2010 (Hrabovszky et al., 2010); Hrabovszky and colleagues have further disclosed the features of this neuronal population in the human hypothalamus, by assessing both sexes and different ages (Hrabovszky et al., 2019; Borsay et al., 2014). These analyses have not only documented that the degree of co-expression of Dyn in human ARC Kiss1/NKB neurons is much lower than in rodents (Hrabovszky et al., 2012), but have also substantiated interesting species differences in the set of co-transmitters of Kiss1 neurons, so that while in contrast to mice, human Kiss1 neurons do not co-express galanin, they express SP, proenkephalin-derived opioids and CART (for cocaine- and amphetamine-regulated transcript), which are not found in the ARC Kiss1 neurons of rodents (Skrapits et al., 2015). These features suggest potential functional differences across species, e.g., in the signals for modulation of ARC Kiss1 neurons and GnRH pulse termination (see below), which warrant further investigation.

3.3. Assessing the KNDy hypothesis: Molecular studies

Regarding the characterization of their whole set of co-transmitters, KNDy neurons have been recently shown to be glutamatergic also, at least in mice (Nestor et al., 2016). Thus, single-cell qPCR studies revealed that up to 88% of ARC Kiss1 neurons express the gene encoding the vesicular glutamate transporter 2 (VGLUT-2), and low-frequency optogenetic stimulation of ARC Kiss1 neurons resulted in fast glutamatergic inward current in neighboring POMC and AgRP neurons (Nestor et al., 2016). This transmission, however, does not likely participate in the control of GnRH neurosecretion but rather is relevant for conveying estrogenic modulation to these neuronal populations, with key roles in metabolic homeostasis, via KNDy projections, in a kisspeptin-independent manner. In fact, KNDy cells express high levels of estrogen receptor alpha (ERα) and enhanced Kiss1 neuron excitability and glutamate transmission, driven by estrogen, appears to play a relevant role in feeding control in mice (Qiu et al., 2018). Interestingly, KNDy neurons have been shown also to receive glutamatergic inputs in rodents and sheep, which are more abundant during the LH surge in the ewe and are modulated by estrogens (Porter et al., 2021).

In addition, several studies have been produced in recent years applying transcriptomics and single-cell RNA sequencing for the molecular characterization of hypothalamic neuron populations in mice, either in the whole hypothalamus (Romanov et al., 2017), or in specific hypothalamic areas, such as the lateral hypothalamus (Mickelsen et al., 2019), the preoptic area (POA) (Moffitt et al., 2018), or the ARC (Campbell et al., 2017). While these studies were not focused on Kiss1 or KNDy neurons, in as much they targeted sensitive hypothalamic areas, they have helped to disclose some molecular features of ARC Kiss1 neurons. It must be noted, though, that since in these unsupervised analyses the cell types are defined on the basis of their expression patterns, and considering that Kiss1 neurons are relatively scarce and co-express other transmitters, they are classified either as Kiss1/Tac2 neurons, in studies targeting the ARC (Campbell et al., 2017), or as glutamatergic cells in single-cell RNA sequencing studies in the whole mouse hypothalamus (Chen et al., 2017). In any event, these analyses have surfaced interesting differences in the molecular phenotype of ARC vs. AVPV Kiss1 neurons, as the latter were classified as dopaminergic cells (Moffitt et al., 2018). Very recently, the whole set of actively-translated mRNAs of Kiss1 neurons in the AVPV region of female mice has been reported, allowing identification of factors co-expressed in this neuronal population (Stephens and Kauffman, 2021). Future single-cell RNA sequencing analyses in isolated populations of Kiss1 neurons will permit disclosure of the molecular phenotype and heterogeneity of KNDy neurons.

3.4. Assessing the KNDy hypothesis: Functional studies

The advent of modern techniques for functional cellular

manipulation in vivo, coupled with progress in the mapping of the physical interplay between ARC Kiss1 fibers and GnRH neurons, have allowed also to provide direct evidence for the mode of action and physiological relevance of KNDy neurons in the control of pulsatile GnRH secretion. The pioneering work of the Herbison's lab documented the peculiar morphology of GnRH neurons in mice, endowed with bipolar dendron structures, as they integrate both axonal (transmission of action potentials) and dendritic (synaptic contacts) features (Herbison, 2018). These dendrons project down to the median eminence, where they were reported to receive apparent synaptic contacts from fibers of ARC Kiss1 neurons, at the edge of the median eminence (Herbison, 2018; Moore et al., 2018). This dense innervation at the distal dendron appears crucial for the pulsatile control of GnRH neurosecretion at the median eminence, and thus for the pulsatile control of gonadotropin secretion. Of note, however, a very recent report has documented that while KNDy neurons make multiple appositions with GnRH dendrons in mice, these are non-synaptic in nature, therefore suggesting that kisspeptins signal preferentially via volume transmission, rather than classical synapses, to activate terminal GnRH fibers (Liu et al., 2021).

In this scenario, functional interrogation of the role of ARC KNDy neurons in the generation of GnRH pulses has advanced substantially in recent years, by combination of optogenetic techniques, genetic mouse models and the development of super-sensitive analytical tools for measurement of LH levels in minute blood volumes (Steyn et al., 2013), allowing repeated sampling of conscious animals in vivo, and accurate detection of LH pulses. Using these approaches, Clarkson and co-workers provided conclusive evidence in mice that a subset of ARC Kiss1 neurons, tenably KNDy cells, are the core of the so-called GnRH pulse generator, as major extrinsic driver of GnRH pulses (Clarkson et al., 2017). These studies not only documented a perfect synchronization between optogenetic activation or inactivation of ARC Kiss1 neurons and the induction or suppression of LH pulses, respectively, but also that spontaneous LH peaks are correlated with synchronized episodes of calcium activity in the mouse ARC Kiss1 neuronal population (Clarkson et al., 2017). As a whole, these studies not only supported the contention that ARC Kiss1 neurons are the GnRH pulse generator, but disclosed also a non-linear relationship between pulse generator frequency and LH frequency, with inhibition of pulsatile LH secretion after ultra-high frequency stimulation (Han et al., 2020).

The use of additional genetic mouse models has recently provided further details about the role of KNDy neurons in GnRH pulse generation and synchronization. Thus, rescue experiments in female *Kiss1* KO rats, in which *Kiss1* expression was reinstated in NKB-expressing cells in the ARC, have very recently suggested that retaining >20% of KNDy neurons is sufficient to maintain LH pulsatility, whereas ablation of >90% *Kiss1* expression in the ARC totally suppressed LH pulses (Nagae et al., 2021). Interestingly, a very recent study in mice has documented that, while synchronization of KNDy neurons in the ARC occurs in a kisspeptin-independent manner, and is possibly driven by the auto-synaptic feedback of NKB and Dyn, full LH pulses evoked by KNDy

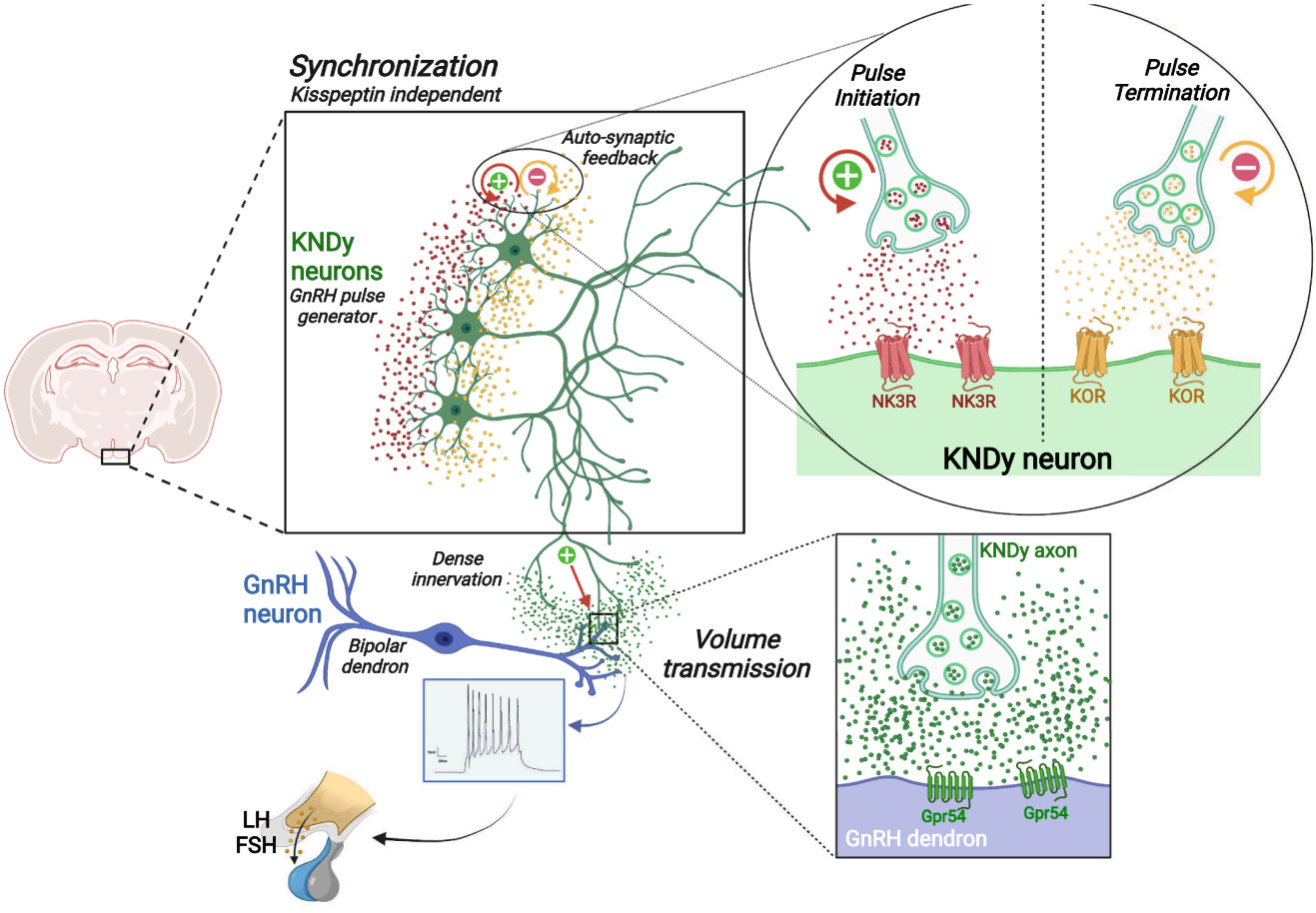

Fig. 2. *KNDy neurons and GnRH pulse generation.* A schematic is shown on the basic mechanisms for KNDy-mediated GnRH pulse synchronization, generation and termination. Due to large number of collaterals, KNDy neurons form a dense network in which communication across this cell population allows synchronization of kisspeptin discharges on GnRH neurons, which drive GnRH pulse generation. Regarding pulse generation, NKB plays a major role in initiation of kisspeptin pulses, while Dyn conducts an opposite effect, being responsible for pulse termination. Of note, while KNDy neuronal synchronization occurs in a kisspeptin-independent manner, generation of full GnRH pulses absolutely requires kisspeptin input to GnRH cells. Recent evidence suggests that contacts between KNDy neurons and GnRH dendrons at the boundary of median eminence are not classical synapses, and hence transmitter communication is likely to occur as volume transmission. For further details, see text of Section 3. Figure created with BioRender.

activation fully depend on appropriate kisspeptin output onto GnRH dendrons (Liu et al., 2021), therefore confirming the validity of the KNDy hypothesis. For further details, see Fig. 2.

As final note to this section, functional manipulation of ARC KNDy neurons have been recently used also to document functions of this neuronal population, other than GnRH pulse generation. Chemo- (DREADDs) and optogenetic activation of ARC Kiss1 neurons in mice has recently confirmed that KNDy neurons are a key component for the generation of hot flushes, acting in an NKB-dependent manner (Padilla et al., 2018). Likewise, as mentioned earlier, optogenetic tools disclosed that, via glutamatergic inputs to POMC and AGRP neurons, KNDy neurons participate in mediating the effects of estrogen on feeding behavior (Qiu et al., 2018).

4. Recent progress on the regulatory mechanisms of kisspeptin expression and actions

Shortly after the disclosure of the reproductive dimension of kisspeptins in late 2003, the patterns of hypothalamic expression of *Kiss1* (and to a lesser extent Gpr54) began to be explored. These initial analyses led to the recognition of a major role of sex steroids as key regulators of *Kiss1* transcription (Garcia-Galiano et al., 2012), and drew much attention to elucidation of the mechanisms for the transcriptional regulation of *Kiss1* expression. Indeed, efforts in these early years were devoted to unveil the mechanisms for the differential transcriptional control of *Kiss1* by estrogens in the ARC (i.e., repression of *Kiss1* via non-classical ERα pathways) vs. the AVPV (i.e., induction of *Kiss1* via classical ERα pathways) using appropriate mouse models (Gottsch et al., 2009). In parallel, emphasis was made on the characterization of the *Kiss1* promoter, and different activators (e.g., TTF1) and repressors (e.g., EAP1) of the human *KISS1* gene were reported already in 2011 (Mueller et al., 2011). More recently, other transcriptional factors controlling *Kiss1* have been identified, including Runx3, which is expressed in the hypothalamus and whose congenital ablation results in decreased *Kiss1* expression in mice (Ojima et al., 2016), VAX1, which represses *Kiss1* expression in the ARC of male mice (Lavalle et al., 2021), and Nhlh2, which has binding sites in the *Kiss1* promoter, enhances human *KISS1* promoter activity, and whose ablation causes a male-specific suppression of ARC *Kiss1* expression in mice in vivo (Leon et al., 2021).

4.1. Regulatory mechanisms of Kiss1: Emerging roles of epigenetics

Despite these and other advances on the characterization of the mechanisms for the transcriptional control of *Kiss1*, possibly the most relevant conceptual progress in our recent understanding of the mechanisms controlling *Kiss1* expression came with the recognition of the roles of different epigenetic mechanisms in this phenomenon. Admittedly, although a substantial fraction of these mechanisms have been described in the context of puberty, these are summarized in this section in order to provide a global view of the epigenetic pathways controlling *Kiss1*, assuming that some of these pubertal mechanisms are likely to operate also at later functional stages of the reproductive axis.

Experimental data gathered in the last two decades have solidly documented that a wide range of reproductive phenomena are modulated by epigenetic mechanisms (Piferrer, 2013; Lomniczi et al., 2015), which provide an additional layer of sophistication to *classical* transcriptional regulatory events. In this context, a number of studies produced in the last ten years have shown not only that changes in DNA methylation, histone modifications and/or miRNA regulatory pathways are putatively involved in the regulation of key aspects of reproductive maturation and function, but also that part of this epigenetic regulation occurs at the level of Kiss1 neurons. Admittedly, however, epigenetic regulation of reproduction goes beyond kisspeptins, and influences not only other neuronal populations (e.g., GnRH neurons) (Kurian et al., 2016), but also other reproductive tissues, such as the pituitary and the gonads (Yosefzon et al., 2017; Eguizabal et al., 2016).

4.2. Epigenetic control of Kiss1: Roles in early maturational events

While most epigenetic studies in this area have focused on puberty, the putative involvement of epigenetic regulatory mechanisms in the control of earlier events, such as Kiss1 neuronal maturation that takes places well before puberty, has been also analyzed, with particular attention to the generation of sex differences in the AVPV population of Kiss1 neurons. Thus, it has been reported that neonatal inhibition of histone deacetylase (HDAC), a major epigenetic modulator of chromatin, enhanced the number of AVPV Kiss1 neurons in mice, but failed to eliminate sex differences in the magnitude of this neuronal population, which remained larger in females (Semaan et al., 2012). This suggests that histone deacetylation may modulate the development of the population of Kiss1 neurons in the rostral hypothalamus, but is not a major driver for its sex differences. In addition, the methylation status of the *Kiss1* promoter in the AVPV of male and female mice was markedly different, with an overall trend for higher methylation levels in females (Semaan et al., 2012). Admittedly, however, whether such changes actually contribute to the emergence of sex differences in the population of Kiss1 neurons in the AVPV is yet to be clarified.

4.3. Epigenetic control of Kiss1: Roles in pubertal regulation

The epigenetic control of *Kiss1* has been mostly analyzed in the context of puberty, specially by the work of Ojeda, Lomniczi and colleagues, who disclosed a dynamic interplay between repressive and activator epigenetic marks that operate mainly in the ARC population of Kiss1 neurons to finely control *Kiss1* expression during the pubertal transition. The first (repressive) member of this system that was disclosed was the Polycomb Group (PcG) of gene silencers (Grossniklaus and Paro, 2014). In 2013, two members of the PcG, EED and CBX7, were found to silence *Kiss1* expression during the early juvenile period, by inducing a repressive histone configuration linked to increased Histone 3 (H3) methylated levels (H3k27me3). In turn, at the time of puberty, methylation of *Eed* and *Cbx7* promoters augments in female rats, causing a decrease in their expression. This leads to a switch in chromatin configuration, from repressive to permissive, due to the histone modifications, H3K9/14ac and H3K4me3, that ultimately elevates *Kiss1* expression (Lomniczi et al., 2013).

The repressive actions of PcG are counterbalanced by the effects of members of the Trithorax group (TrxG) of epigenetic regulators, which globally operate as activators of gene expression. Two members of the TrxG, mixed-lineage leukemia 1 (MLL1) and MLL3, have been found to interact with the *Kiss1* promoter at puberty, helping to cause a change in chromatin configuration, from repressive to active, which permits the increase of *Kiss1* expression at puberty (Toro et al., 2018). Conversely, suppression of MLL1 in the ARC caused a reduction of *Kiss1* expression, and delayed puberty in female rats (Toro et al., 2018). Of note, MML1 seems to operate also as activator of *Tac2* (encoding NKB), a phenomenon that may contribute to its effects on pubertal control. Other elements putatively involved in this reciprocal interaction between repressive and activator epigenetic marks are KDM6B, a histone demethylating enzyme that removes methylation of H3 at k27, which is a repressive mark, and CHD7, another member of the TrxG. KDM6B is repressed by the PcG member, EED, during the infantile period in female rats, a phenomenon that contributes to keep high H3 methylation, which in turn maintains a repressive configuration on various genes, including *Kiss1* (Wright et al., 2021). Regarding CHD7, genetic inactivation in humans has been shown to cause central hypogonadism (Kim et al., 2008). Yet, its direct role in the control of *Kiss1* is yet to be defined. Finally, GATAD1, which belongs to the ZNF family of transcriptional repressors, has been shown to represses human *KISS1* transcription by recruitment of a histone demethylase (KDM1a), which, in turn, reduces the activating histone mark (H3K4me2) at the promoter level (Lomniczi et al., 2015).

4.4. Epigenetic control of Kiss1: Roles in the regulation of adult reproductive axis

Albeit less well studied, epigenetic regulatory mechanisms seem to operate also in Kiss1 neurons in adulthood for the regulation of key features of reproductive function. As illustrative examples, it has been reported that the rise of estradiol levels that drives the pre-ovulatory surge of gonadotropins, responsible for ovulation, evokes opposite changes in the levels of histone acetylation at the *Kiss1* promoter of AVPV vs. ARC Kiss1 neurons. In detail, estradiol increased acetyl-H3 levels in the AVPV, which appeared to be determinant for the rise of *Kiss1* expression in the rostral hypothalamus at the pre-ovulatory period (Tomikawa et al., 2012). Conversely, estrogen caused H3 deacetylation in the ARC, which was associated to decreased *Kiss1* expression. Rather than stable, these epigenetic changes occur in a dynamic manner in these two hypothalamic regions to precisely control *Kiss1* expression in conditions of positive and negative feedback. Other putative regulator of the epigenetic machinery in Kiss1 neurons is *Rbbp7*, encoding the retinoblastoma binding protein 7 (RBBP7), which is a member of histone modification and chromatin remodeling complexes that is abundantly expressed in ARC and AVPV Kiss1 neurons. Recent studies have documented that Rbbp7 may contribute to enhance *Kiss1* expression in rodents, in an estrogen-independent manner (Horihata et al., 2020). In addition, changes in DNA methylation have been also linked to the regulation of *Kiss1* expression in adulthood. As example, the level of methylation of *Kiss1* promoter in the preoptic hypothalamic region, where AVPV Kiss1 neurons are located, is up-regulated in a sex-steroid dependent manner in female vs. male mice. However, since AVPV expression of *Kiss1* in higher in females, this phenomenon would be opposite to the expected repressive role of hyper-methylation on gene transcription, and suggest the participation of complex regulatory mechanisms, e.g., involving inhibition of transcriptional repressors of Kiss1 (Semaan et al., 2012).

As final note to this section, other epigenetic mechanisms, involving non-coding RNAs, have been shown also to participate in the regulation of various aspects of reproductive function. This is particularly the case of microRNAs (miRNAs), which are reported to operate at different levels to modulate various facets of reproductive function. However, despite the recognition of miRNA-based mechanisms for the control of puberty, and their regulatory role in the activation program of GnRH neurons during prepubertal stages (Messina et al., 2016), the putative function of miRNA-regulatory mechanisms in Kiss1 neurons remains largely unfolded. However, fragmentary evidence suggests that specific miRNAs may operate in the control of *Kiss1* expression in other tissue or cellular contexts. For instance, our recent data suggest a repressive functional interaction between the 3′UTR region of human *KISS1* and miR-324-3p, which might be relevant for the observed suppression of kisspeptin expression in ectopic pregnancy (Romero-Ruiz et al., 2019). On the other hand, in the context of cancer, miR-345 has been shown to down-regulate *KISS1* expression in human-derived, brain metastatic subclones of breast cancer cell lines (Ulasov et al., 2020). However, the putative functional role of miR-324 or miR-345 in the control of hypothalamic *Kiss1* expression has not been explored. Anyhow, our data (see Section 2.3) suggest a role of miR-30b as repressor of Mkrn3, potentially in Kiss1 neurons in female rats, and we have evidence for a late-onset, profound hypogonadal state caused by congenital ablation of Dicer, the enzyme responsible for generation of mature miRNAs, selectively in Kiss1 neurons in mice (Roa & Tena-Sempere, in preparation). Altogether, this fragmentary evidence supports a putative role of miRNA-regulatory mechanisms in the control of hypothalamic *Kiss1* expression and Kiss1 neurons, whose physiological relevance is yet to be disclosed.

5. Recent progress on the roles of kisspeptins in the metabolic control of reproduction

Disclosure of the reproductive dimension of kisspeptins drew immediate attention to their putative function in the modulation of the reproductive axis by metabolic cues, which profoundly influence puberty and fertility (Manfredi-Lozano et al., 2018). Thus, from 2006 to 2011, a number of studies documented that conditions of metabolic stress, such as subnutrition, suppress hypothalamic *Kiss1* mRNA levels and that key metabolic hormones, such as leptin, modulate the hypothalamic expression of *Kiss1* (Navarro and Tena-Sempere, 2011). This evidence, together with data from expression and functional studies in various models (e.g., *ob/ob* mouse) and species (rats, mice, guinea pig, sheep) led to the initial hypothesis that leptin directly regulate Kiss1 neurons to influence GnRH secretion. However, contemporary studies provided also evidence that functional leptin receptors are low or null in Kiss1 neurons, and dispensable for mediating leptin actions on puberty and fertility (at least in congenital models), therefore suggesting indirect or independent actions of leptin, findings that rose considerable debate on the actual mode of action of metabolic hormones on Kiss1 neurons (Navarro and Tena-Sempere, 2011; Pinilla et al., 2012).

On the above basis, in the last decade, considerable research efforts have been devoted to unveil the network of central transmitters that may connect metabolic and nutritional status with Kiss1 neurons, aiming to elucidate at least part of the molecular mediators and energy sensors that are likely to contribute to the metabolic control of these neurons (Navarro, 2020). Much progress in this domain has taken place in the context of puberty, as key maturational period of the reproductive axis that is highly sensitive to metabolic cues; yet, these pubertal regulatory mechanisms might operate also in adulthood. Accordingly, advancements in this area, covering different age-periods, are integrally reviewed in this section, as a mean to provide a comprehensive view of our current, as yet incomplete knowledge on the signals and mechanisms for the metabolic control of Kiss1 neurons.

5.1. Central neuropeptides/transmitters and the metabolic control of Kiss1 neurons

In line with an indirect mode of action of key hormonal signals, such as leptin, in the modulation of Kiss1 neurons, a number of studies produced over the last ten years have documented the potential interactions between Kiss1 neurons and other neuronal populations with key roles in energy and metabolic homeostasis, including prominently POMC/CART and neuropeptide Y (NPY)/Agouti related peptide (AGRP) neurons. Both neuronal populations are located in the ARC, are sensitive to leptin and other hormonal signals, and play a reciprocal role in the control of energy balance: POMC/CART neurons are anorexigenic and activated in conditions of energy excess, while NPY/AgRP neurons are orexigenic and activated in conditions of energy deficit (Navarro, 2020). Of note, there is not only evidence for physical contacts between these neuronal populations and Kiss1 neurons, but also that at least some subsets of Kiss1, POMC and AgRP neurons may share cellular lineage, since POMC-expressing progenitors in the ARC were found to differentiate not only into POMC and AgRP neurons, but also into Kiss1 cells in mice (Sanz et al., 2015). To what extent this phenomenon may link early nutritional conditions and the maturation of metabolic- and reproductive-controlling pathways in the hypothalamus is yet to be defined.

POMC neurons in the ARC are a central element for metabolic homeostasis. In addition, this neuronal population has been shown to modulate reproductive function, and its major product, α-MSH, acting via melanocortin receptors 3 (MC3R) and MC4R, can directly modulate GnRH neurosecretory activity (Manfredi-Lozano et al., 2018). By a combination of expression and functional analyses in rats and mice, we have provided evidence for a putative melanocortin/kisspeptin cross-talk, which contributes to mediate the permissive effects of leptin on puberty onset (Manfredi-Lozano et al., 2016). Thus, blockade of MC3/

4R signaling not only delayed puberty and prevented the permissive effects of leptin on pubertal timing, but suppressed also ARC *Kiss1* expression in pubertal female rats (Manfredi-Lozano et al., 2016). Moreover, congenital elimination of Gpr54 or DREADD-induced suppression of ARC Kiss1 neurons blunted gonadotropic responses to α-MSH agonist in mice (Manfredi-Lozano et al., 2016). These observations, together with the demonstration of close contacts between POMC fibers and Kiss1 neurons in the ARC (Manfredi-Lozano et al., 2016), strongly suggest that POMC neurons transmit at least part of the modulatory effects of leptin on Kiss1 neurons. This contention has been very recently confirmed by a series of studies documenting that melanocortin signaling, particularly via MC3R, plays a key role in the control of puberty and the modulation of reproductive function by nutritional cues, as denoted by delayed pubertal timing and lack of impact of nutritional deprivation on reproductive cycle length in *Mcr3* null mice (Lam et al., 2021). The fact that *Mcr3* expression is enriched in ARC KNDy neurons in mice further supports a putative melanocortin-kisspeptin pathway for the nutritional control of the reproductive axis. This pathway seem to operate also in humans, since patients with loss-of-function mutations of *MC3R* suffer later pubertal onset (Lam et al., 2021). Interestingly, the other main product of POMC neurons, CART, has been proposed to modulate also Kiss1 neurons. CART-positive fibers are found in close contact with ARC and AVPV Kiss1 neurons, as well as GnRH neurons, and CART has been shown to excite Kiss1 and GnRH neurons in mice (True et al., 2013). Notably, caloric restriction can suppress CART-immunoreactivity in the ARC, and CART mRNA levels in the AVPV, suggesting that defective CART signaling may contribute to suppressed *Kiss1* expression and reproductive function in conditions of energy deficit (True et al., 2013).

NPY/AgRP neurons not only antagonize the function of POMC neurons in terms of food intake and metabolic control, but likely conduct also opposite effects in terms of control of reproductive function. Padilla et al. recently demonstrated a direct connection between AgRP/NPY and Kiss1 cells, and showed that stimulation of AgRP fibers causes a direct inhibition of Kiss1 neurons in the ARC and AVPV in mice (Padilla et al., 2017). In addition, indirect evidence for an AgRP pathway controlling ARC Kiss1 neurons has been very recently presented in the sheep (Merkley et al., 2021). In the same vein, NPY has been recently shown to conduct direct inhibitory effects on ARC Kiss1 neurons in male and female mice, acting via NPY 1 receptors (Hessler et al., 2020). Since NPY/AgRP neurons seem to drive a net inhibitory signal on Kiss1 neurons and are potently suppressed by leptin, it is tenable to hypothesize that inhibition of this neuronal population may contribute to mediate at least part of the positive effects of leptin on Kiss1 neurons. For further details, see Fig. 3.

GABA has been proposed as another putative mediator of leptin actions on Kiss1, based on studies in a mouse model of congenital ablation of leptin receptors in GABAergic neurons, which displayed delayed or absent puberty onset and anovulatory hypogonadism (Martin et al., 2014). In this model, *Kiss1* expression in the ARC was reduced, but gonadotropin responses to kisspeptin were preserved, suggesting a primary defect at the level of Kiss1 neurons due to blunted leptin signaling

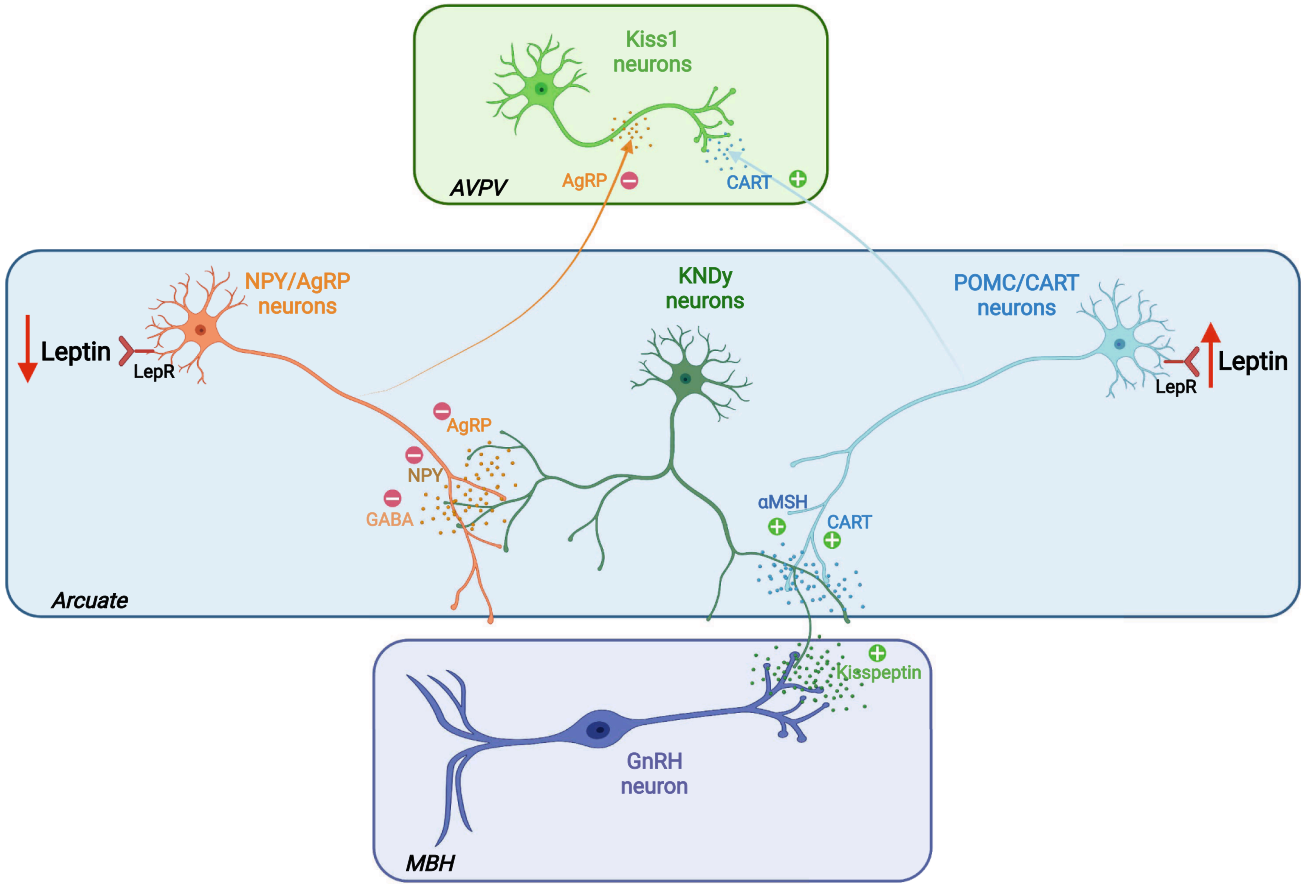

Fig. 3. *Control of Kiss1 neurons via metabolically-relevant transmitters*. A schematic is presented of the hypothalamic circuits putatively involved in transmitting metabolic information to Kiss1 neurons. Both NPY/AgRP and POMC/CART neurons, located in the ARC, have been reported to project to and modulate Kiss1 neurons, in an opposite manner, congruent with their major roles as metabolic regulators. NPY/AgRP neurons are activated in conditions of energy deficit and low leptin levels; AgRP and NPY have been shown to inhibit Kiss1 neurons. In contrast, POMC neurons are activated in conditions of excess of energy stores and high leptin levels; α-MSH and CART have been reported to stimulate Kiss1 neurons. Both populations may contribute to transmit the effects of leptin to KNDy neurons, and thereby, modulate GnRH neurons. For further details, see text of Section 5.1. Figure created with BioRender.

in GABAergic cells. It must be noted, though, that NPY/AgRP neurons produce also GABA. Thus, it is tenable that part of reproductive phenotype of this mouse line derives from elimination of the suppressive effect of leptin on ARC NPY/AgRP neurons. In addition, PACAP neurons from the PMV may also contribute to convey leptin effects onto Kiss1 neurons, as deletion of leptin receptors in PACAP cells caused infertility in mice (Ross et al., 2018).

5.2. Metabolic hormones other than leptin and the control of Kiss1 neurons

In addition to leptin, studies conducted over the last decade have addressed the potential regulatory actions of other key metabolic hormones in the control of Kiss1 neurons. This is the case of insulin and ghrelin. Despite initial evidence supporting a lack of effect of insulin on hypothalamic expression of *Kiss1* mRNA in rats (Castellano et al., 2006), and the fact that congenital ablation of insulin receptors from Kiss1 neurons has virtually null impact on the reproductive axis in mice (Evans et al., 2014), insulin receptor expression has been detected in mouse Kiss1 neurons (Campbell et al., 2017), and insulin has been shown to activate TRPC5 channels in Kiss1, as well as POMC, neurons in guinea pigs, to excite these cell types (Qiu et al., 2014). On the other hand, initial studies evidenced that the orexigenic hormone, ghrelin, inhibits hypothalamic *Kiss1* expression, as putative mechanism for ghrelin-induced suppression of LH pulsatility in rats (Forbes et al., 2009). More recent studies in mice have demonstrated co-expression of the ghrelin receptor, as well as ERα, in a subset of Kiss1 neurons, and documented that Kiss1 neuronal responsiveness to ghrelin is modulated by estradiol (Frazao et al., 2014).

5.3. Central energy sensors and the metabolic control of Kiss1 neurons

In the last decade, progress has also taken place regarding characterization of the role of cell energy sensors in the metabolic control of the Kiss1 system. Initial work of our group, published back in 2009, documented that the serine, threonine kinase, mammalian target of rapamycin (mTOR), is a modulator of *Kiss1* expression, and contributes to transmit the permissive effects of leptin on puberty onset in female rats (Roa et al., 2009). Whether mTOR operates within Kiss1 neurons and/or their afferents for this function has not been fully clarified. In any event, genetic models have been used recently to explore the roles of putative up-stream factors in the mTOR pathway in the control of Kiss1 neurons. Thus, genetic inactivation in Kiss1 neurons of the catalytic subunit of PI3K, a common node for transmission of the metabolic effects of key hormones, such as leptin, has been reported to reduce kisspeptin immunoreactivity in the ARC and to suppress markers of reproductive function in mice, preferentially in females (Beymer et al., 2014). Very recently, using a similar genetic approach, the role of phosphatase and tensin homolog (PTEN) in Kiss1 neurons has been explored. PTEN is known to block PI3K. Congenital ablation of PTEN in Kiss1 cells in mice resulted in Kiss1 neuronal hypertrophy and increased kisspeptin fiber density, together with hyper-activation of mTOR, preferentially in females (Negron et al., 2020). This resulted in a state of resistance to the inhibitory impact of fasting on the gonadotropic axis, suggesting that PTEN can restrain the PI3K/mTOR pathway in Kiss1 neurons to suppress the gonadotropic axis in conditions of energy deficit.

The putative role of AMPK in the metabolic control of the reproductive axis, and particularly of Kiss1 neurons, has been also explored in recent years. Of note, AMPK is regulated and operates in an opposite manner to mTOR, hence, AMPK becomes activated in conditions of energy deficit and is inhibited by leptin (Vazquez et al., 20192019). Initial fragmentary evidence documented that activation of central AMPK resulted not only in increased food intake, but also in perturbed ovarian cyclicity in rats (Coyral-Castel et al., 2008), while adiponectin was reported to inhibit *Kiss1* expression in the GnRH cell line, GT1-7, via activation of AMPK (Wen et al., 2012); yet, the relevance of the latter was shadowed by the fact that GnRH neurons do not normally express Kiss1. Anyhow, evidence for a physiological role of AMPK in the metabolic control of Kiss1 neurons has been provided recently. Thus, Torsoni and colleges showed that congenital elimination of the AMPKα2 subunit from Kiss1 cells partially impaired the capacity of adult female mice to cope with the metabolic stress associated to fasting, so that the alteration in estrous cyclicity caused by food deprivation was not detected in these conditional KO mice (Torsoni et al., 2016). More recently, our group has documented a role of AMPK signaling in Kiss1 neurons in the metabolic control of puberty, since not only central activation of AMPK resulted in reduced *Kiss1* expression in the ARC and delayed puberty, but also conditional ablation of AMPKα1 subunit in Kiss1 neurons protected immature female mice from the delay in puberty onset caused by subnutrition (Roa et al., 2018). Overall, these data point out that conditions of energy insufficiency can inhibit the reproductive axis, at least partially, by activating AMPK signaling in Kiss1 neurons. Notwithstanding, recent evidence from our group has documented that AMPK drives also an inhibitory signal at the level of GnRH neurons in conditions of subnutrition, as supported by findings in a mouse model with conditional ablation AMPKα1 subunit in GnRH cells (Franssen et al., 2021). This is in line with previous data suggesting that AMPK participates in conveying the inhibitory actions of low glucose in GnRH neurons (Roland and Moenter, 2011).

SIRT1 is another metabolic sensor that has been recently pointed out as putative modulator of Kiss1 neurons. This is a member of the Sirtuin family that operate as NAD^+-dependent class III deacetylase, acting on a wide range of targets, such as histones and p53, to modulate numerous biological processes (Nogueiras et al., 2012; Giblin et al., 2014). As is the case for AMPK, SIRT1 can function as a cell energy sensor, that is activated by conditions of energy restriction, signaled in this case by an increased NAD+/NADH ratio (Nogueiras et al., 2012). SIRT1 expression is found in ARC and fasting causes a moderate increase of its levels (Ramadori et al., 2008), whereas over-expression of SIRT1 in the brain prolongs lifespan in mice (Satoh et al., 2013). In collaboration with the group of Ojeda and Lomniczi, we have provided evidence for a major repressive role of SIRT1 in ARC Kiss1 neurons as transducer of the influence of body weight and nutritional status on puberty onset. Thus, not only were hypothalamic SIRT1 protein levels changed in an opposite manner vs. *Kiss1* expression during the infantile-to-pubertal transition in female rats, but also hypothalamic SIRT1 levels were reduced in models of advanced puberty caused by early-onset obesity (Vazquez et al., 2018). In contrast, prepubertal subnutrition evoked an increase of SIRT1 content (Vazquez et al., 2018). Furthermore, SIRT1 levels specifically in Kiss1 neurons displayed similar changes in conditions of obesity (decrease) or undernutrition (increase), while SIRT1 activation by pharmacological or genetic means decreased in *Kiss1* expression and delayed puberty in female rats, suggesting that SIRT1 is a repressor of *Kiss1*. Chromatin-immunoprecipitation (ChIP) assays confirmed this possibility, providing a link between nutritional-driven variations in SIRT1 activity and changes in the epigenetic machinery controlling the *Kiss1* promoter (Vazquez et al., 2018), which moves from a repressive to a permissive configuration during the pubertal progression. Subnutrition protracts this SIRT1-dependent repressive configuration, whereas early-onset obesity accelerates the eviction of SIRT1 from the *Kiss1* promoter, thereby favoring advancement of puberty. Interestingly, a recent study has reported that over-expression of a deacetylase-deficient SIRT1 mutant in astrocytes resulted in reduced *Kiss1* expression and markers of impaired reproductive function in mice, suggesting that SIRT1 activity in glial cells may also indirectly modulate Kiss1 expression (Choi et al., 2019). For a synoptic view of the roles and mechanisms of action of different cell energy sensors in the control of Kiss1 neurons, see Fig. 4.

As final note to this section, we have very recently provided evidence for a novel pathway that putatively contributes to mediate the effects of obesity on puberty onset, involving *de novo* ceramide synthesis in the paraventricular nucleus (PVN) and the modulation of the sympathetic

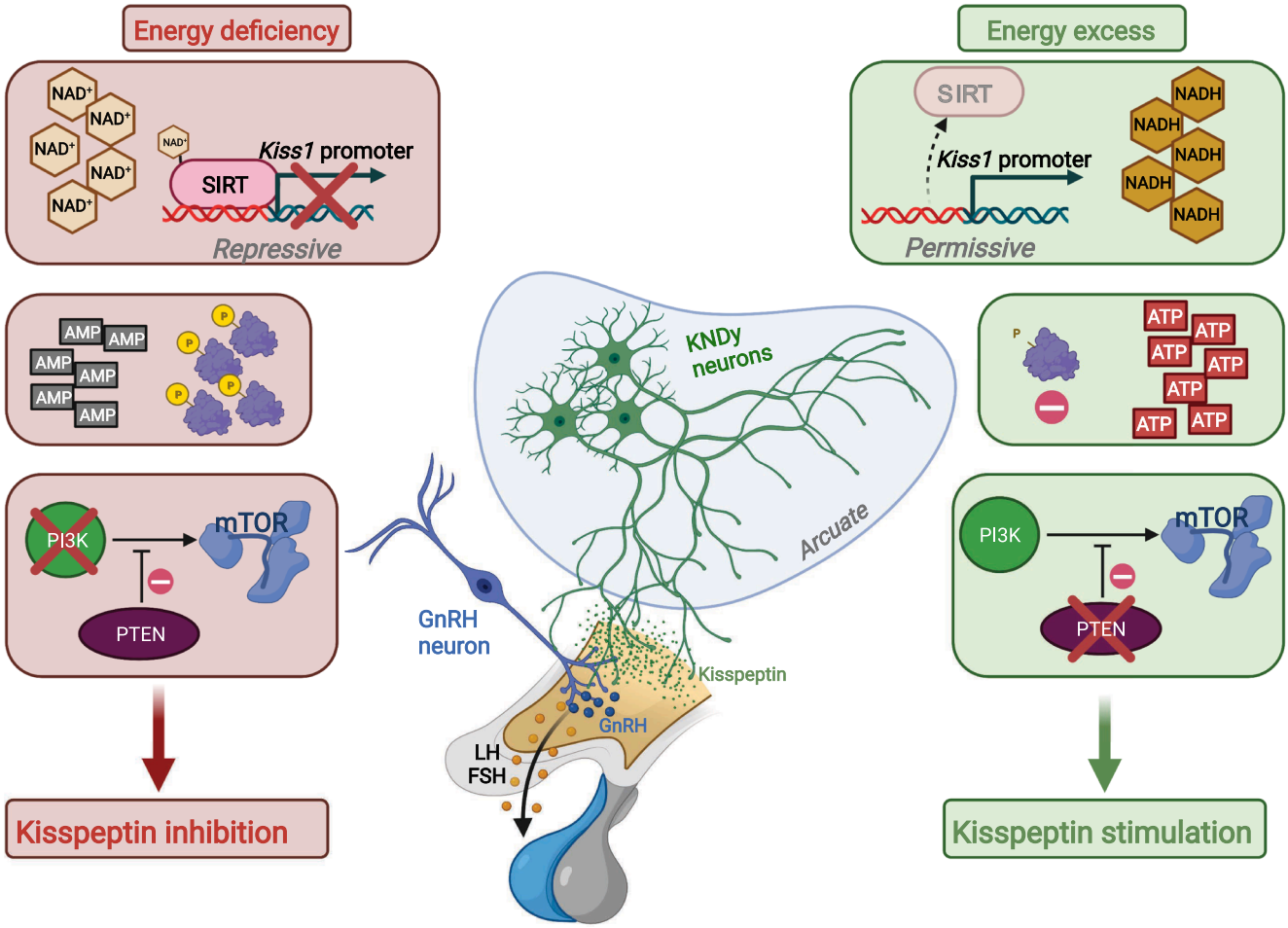

Fig. 4. *Control of Kiss1 neurons by relevant cell energy sensors*. A schematic is presented of the potential contribution of various cell energy sensors, including SIRT1, AMPK and mTOR, and related up-stream elements (PI3K, PTEN), to directly or indirectly modulate *Kiss1* expression in conditions of energy deficiency or energy excess. For further details, see text of Section 5.2. Figure created with BioRender.

output to the ovary in rats. While this pathway does not seem to directly modulate Kiss1 neurons, the promoting effects of kisspeptin on puberty onset do require preserved central ceramide synthesis to occur, and kisspeptin fibers project to the PVN, where ceramide-producing neurons are abundant, with increased expression of the ceramide-synthesizing enzyme, serine palmitoyl transferase, in conditions of obesity (Heras et al., 2020). These data suggest that Kiss1 neurons, possibly from the ARC, are upstream afferents and putative modulators of this novel ceramide pathway, mediating at least part of the effects of obesity on female pubertal timing.

6. Recent progress on the roles of peripheral kisspeptins

As reviewed in previous sections, there is total consensus that the primary site of expression and actions of kisspeptins for the control of the reproductive axis is the hypothalamus. However, there is ample evidence for the expression of *Kiss1*, kisspeptins and/or Gpr54 in multiple peripheral, reproductive and non-reproductive tissues, where kisspeptins were claimed to participate in a wide array of biological functions (Bhattacharya and Babwah, 2015). These included not only a putative role as metastasis suppressors in different cancers, but also local modulation of pituitary and gonadal function, regulation of human placenta, and the control of pancreatic insulin secretion, vascular tone and kidney development, just to mention some examples. While most of this evidence was accumulated more than ten years ago and was extensively reviewed elsewhere (Pinilla et al., 2012), in this section, we will briefly summarize more recent progress in our understanding on the physiological roles of kisspeptin actions in peripheral tissues, with major focus on reproductive actions.

6.1. Gonadal expression and actions of kisspeptins

While direct pituitary actions of kisspeptins, including direct control of gonadotrope cells, were documented in a variety of species already more than a decade ago (Gahete et al., 2016), moderate progress has taken place in recent years in our understanding on the physiological relevance of such pituitary effects, which are still under considerable debate. In contrast, even if initial data had already demonstrated the expression of the elements of the Kiss1 system in the gonads (Pinilla et al., 2012), a number of studies have been produced over the last decade addressing the roles of local kisspeptins in the modulation of gonadal function. This is clearly the case of the ovary. While before 2010, it was already set that *Kiss1*, kisspeptin and Gpr54 are expressed in the mammalian ovary, including the rat, monkey and human, evidence for a potential role of such local kisspeptin system has been presented only in the last ten years (Ruohonen et al., 2020). Collectively, these studies, conducted in rodents and humans, have suggested a variety of functions for ovarian kisspeptins, ranging from the control of puberty in rats (Ricu et al., 2012), to the modulation of human granulosa lutein cells (Owens et al., 2018) and rat ovarian ageing (Hu et al., 2017; Fernandois et al., 2016). In this context, pharmacological studies, using kisspeptin antagonists, have demonstrated that blockade of kisspeptin actions locally in the rat ovary can not only perturb ovarian maturation at puberty (Ricu et al., 2012), but also reduce the number of corpora

lutea, as marker of ovulation, while direct ovarian injection of kisspeptin evoked opposite effects (Fernandois et al., 2016). In the same vein, using various genetic mouse models, we presented indirect evidence for a role of kisspeptin signaling in the oocyte, as modulator of ovulation and determinant of proper ovarian ageing (Dorfman et al., 2014; Gaytan et al., 2014). We have recently refined these observations, showing that selective ablation of Gpr54 from oocytes causes an state of premature ovulatory failure in mice (Ruohonen et al., 2022), therefore suggesting a major physiological role of oocyte kisspeptin signaling in the modulation of ovulatory and follicular dynamics. It must be stressed, though, that such local actions of kisspeptins are subordinated to their central gonadotropin functions, and that the ovulatory failure caused by global absence of kisspeptin signaling can be rescued, at least qualitatively, by proper gonadotropin priming in humans and rodents.

Regarding testicular expression and actions, evidence has been presented for the presence of kisspeptin in Leydig cells, i.e., the androgen producing cells of the testis, but not in Sertoli cells in mice (Salehi et al., 2015). In keeping with the fundamental role of LH in stimulating Leydig function and hormone secretion, Leydig kisspeptin levels are modulated by LH (Salehi et al., 2015). In turn, kisspeptin has been shown to augment the magnitude of testosterone responses to the agonist of LH, human choriogonadotropin (hCG) in monkeys in vivo (Irfan et al., 2014), while kisspeptin antagonism significantly attenuated basal and hCG-stimulated testosterone secretion by purified Leydig cells from goats in vitro (Samir et al., 2018). Moreover, kisspeptin signaling have been recently shown to modulate gonadotropin responsiveness and steroidogenic function in the murine Leydig tumor cell line, MA-10 (Hsu et al., 2020). Yet, lack of effects of kisspeptin on basal testosterone secretion has been also reported in monkeys (Tariq and Shabab, 2017). In addition, fragmentary evidence has suggested the expression of Gpr54 in mouse seminiferous tubules, possibly in spermatids (Chiang et al., 2020), as well as in primate Sertoli cells (Irfan et al., 2016), and a putative role of kisspeptins in modulating spermatogenesis has been proposed, albeit data are not conclusive. In any event, the physiological role, if any, of local kisspeptins in the control of testicular function remains controversial and yet to be fully defined (Sharma et al., 2020).

6.2. Placental and endometrial expression of kisspeptins

While evidence gathered before 2010 had solidly documented the expression of kisspeptins in the human placenta and the dramatic elevation of circulating kisspeptin levels during pregnancy (Horikoshi et al., 2003), different studies conducted over the last ten years have addressed the putative function of kisspeptin signaling in the control of uterine physiology and key gestational phenomena, such as decidualization and embryo implantation. While extensive recapitulation of this facet of kisspeptin biology goes beyond the scope of this review, it is worth to stress that functional studies, using genetic mouse models, have documented a specific role of kisspeptin signaling in embryo implantation (Calder et al., 2014), likely via induction of uterine LIF (leukemia inhibitory factor) expression and endometrial adenogenesis (Leon et al., 2016); endometrial glandular development being a pre-requisite for reproductive competence. Very recent data using a mouse model of conditional ablation of Gpr54 in the uterus have further supported a functional role of uterine kisspeptin signaling, causing down-regulation of ERα-mediated transcriptional activity at the pre-implantation window, which seems to be crucial for acquisition of endometrial receptivity (Schaefer et al., 2021). Despite this evidence, *Kiss1* and *Gpr54* KO mice can become pregnant if properly primed to rescue ovulation, and the ultra-structural features of such mutant placentas, deficient in either *Kiss1* or *Gpr54*, appear to be preserved (Herreboudt et al., 2015). This suggest that, even if conducting discernible roles in uterine function and implantation, kisspeptin signaling might be partially dispensable (or compensable) in terms of placental development and function.

6.3. Other functions of peripheral kisspeptins: Putative roles in metabolism

As final note to this section, it is worth mentioning that considerable attention has been drawn recently by the possibility that kisspeptin signaling in peripheral tissues may participate in the control of different aspects of metabolism, from body weight and glucose homeostasis to brown adipose tissue thermogenesis (Hudson and Kauffman, 2021; Velasco et al., 2019). Indeed, cumulative evidence supports putative roles of kisspeptins or kisspeptin signaling in key metabolic tissues, such as the liver, pancreas and adipose tissue. Admittedly, the actual physiological (and eventual pathophysiological) relevance of such kisspeptin signaling in these metabolic tissues is yet to be fully defined. On the other hand, part of the metabolic effects of kisspeptins are likely to stem from their actions upon central pathways, which, for instance, are known to contribute to mediate at least part of the effects of estrogen on body weight (Mittelman-Smith et al., 2012). Anyhow, given that this review is mainly focused on reproductive aspects of kisspeptins, we will not extend on this interesting facet of kisspeptin physiology, and refer the reader to the excellent review article by Hudson and Kauffman, for an updated summary of the actions of kisspeptins in the control of metabolism (Hudson and Kauffman, 2021).

7. Conclusions: New frontiers in kisspeptin research

Discovery of the reproductive dimension of kisspeptins, and the collective research efforts devoted thereafter to the characterization of their physiological functions and mechanisms of action in the control of the reproductive axis, can be considered as one of the most relevant breakthroughs in contemporary Neuroendocrinology. Thus, kisspeptin research has attracted great attention, as reflected by the ever growing number of articles produced on this topic. As a consequence, in a relatively short period of time (since late 2003), there has been an astonishing progress of our knowledge of key facets of kisspeptin physiology. This review article aimed to provide a synoptic view of some of the most salient developments in this area occurred during the last ten years. In doing so, we do not only intend to offer a succinct summary of current knowledge, but also help to set the basis for identification of future paths of development of this field, some of which are forecasted below, according to the educated predictions of the authors.

While the anatomical distribution and some functional features of the main populations of Kiss1 neurons have been well set in the last two decades, key aspects regarding not only the precise lineage of the different neuronal populations (ARC, AVPV, amygdala, eventually others), but also their detailed molecular phenotype, are yet to be fully disclosed. Admittedly, initial efforts in this front have materialized into the characterization of the origin of some Kiss1 populations (Sanz et al., 2015), and the transcriptomic signatures of Kiss1 neurons have begun to be disclosed in the context of larger studies for molecular mapping of all hypothalamic cell types (see Section 3.3). Yet, the molecular heterogeneity of ARC and AVPV Kiss1 neurons, their differential origin, and their dynamic transcriptional patterns, depending on sex, developmental state and functional status of the reproductive axis, remain largely unknown. Similarly, defining the correlates between the transcriptomic landscape, and the profiles of miRNAs and other epigenomic marks, in Kiss1 neurons would provide a very valuable tool for better understanding (and interrogating) the actual roles of kisspeptins in the central control of the reproductive axis, and related functions, along the lifespan.

In a related context, there has been an extraordinary progress in the characterization of the functional roles of KNDy neurons in the synchronization and generation of GnRH pulses, as cornerstone for reproductive function. These studies have benefited from the combined use of genetic models and contemporary techniques of neuronal monitoring and functional manipulation, including fiber photometry, optogenetics and pharmacogenetics. Additional efforts are anticipated in this front,

which may involve the use of more sophisticated genetic models, based on either classical gene recombination approaches or more recent tools for gene editing in vivo, for individual dissection of the triad of KNDy peptides in the modulation of GnRH secretion and reproductive maturation and function. A very recent example is the generation of a novel mouse line with conditional ablation of *Kiss1* in dynorphin-expressing cells, which due to massive elimination of *Kiss1* from the ARC, recapitulated the hypogonadal phenotype of global *Kiss1* KO, albeit with a later onset (Nandankar et al., 2021). In the same vein, we have produced recently the first Tac2-specific *Kiss1* KO mouse line, in which kisspeptin output is selectively ablated from NKB-expressing cells (Velasco, Franssen & Tena-Sempere, in preparation). Phenotypic and hormonal characterization of this line, which is to be completed very soon, is allowing us to disclose important differences in KNDy-born kisspeptins between sexes, and their roles not only in terms of control of gonadotropin secretion and fertility, but also in terms of metabolic modulation.

In the same vein, better characterization of the contribution of kisspeptins to the neuroendocrine mechanisms for GnRH pulse generation will permit to further disclose their differential roles in the control of LH vs. FSH secretion, which may be relevant for physiological or pathological conditions in which the secretion of the two gonadotropins dissociates. Admittedly, FSH secretion has been found to be more constitutive than LH, and hence, LH has been widely used as surrogate marker for the effects of kisspeptins on GnRH neurosecretion. It must be noted, though, that preferential LH vs. FSH secretion is dictated, to a large extent, by the pattern of pulsatile GnRH release: high frequency favors LH secretion while FSH secretion is mainly driven by low-frequency GnRH pulses (Stamatiades and Kaiser, 2018). Thus, in-depth analyses of changes in FSH secretory profiles in response to functional manipulation of KNDy neurons may allow better definition of the mechanisms whereby kisspeptin signaling contributes to adapt gonadotropin secretion to different normal or pathological conditions. Of note, precise monitoring of dynamic changes in FSH secretory profiles in laboratory rodents had been hampered by the lack of sensitive-enough assays for accurate determination of FSH concentrations in minute blood aliquots, as those required for repeated sampling. The recent development of a super-sensitive immunometric assay for FSH is likely to circumvent this major limitation in the near future (Ongaro et al., 2021).

In recent years, the epigenetic mechanisms responsible for the control of *Kiss1* expression, and thereby key phenomena, such as puberty, have been actively investigated, thus providing a complement to previous knowledge on the transcriptional control of *Kiss1*. While much progress has taken place in this domain, further developments are expected, e.g., regarding the characterization of the whole set of epigenetic regulatory mechanisms operating in Kiss1 neurons involved in the control of adult reproductive function (as most advancements have occurred in the context of puberty), and how these may transmit the influence of multiple modulators onto brain reproductive centers. Incorporation of novel tools from single-cell epigenomic analyses may prove very helpful in this context, especially if applied to the study of the epigenetic landscape of Kiss1 neurons in different developmental periods and functional states of the reproductive axis. On the latter, evaluation of the epigenetic mechanisms acting on Kiss1 neurons after different forms of nutritional challenge, from subnutrition to obesity (Vazquez et al., 2018), and exposures to endocrine disrupting compounds (Lopez-Rodriguez et al., 2021), are expected to attract interest in the near future. Similarly, epigenetic mechanisms other than chromatin modifications or changes in DNA methylation are likely to operate also in Kiss1 neurons, e.g., involving miRNAs, but these remain largely unfolded and are predicted to be the subject of active investigation in the coming years.

In a related front, although our knowledge on the molecular mechanisms for the control of Kiss1 neurons by metabolic and nutritional cues has substantially enlarged in recent years, additional progress is expected in this area ,e.g., on the characterization of the precise interplay between various metabolic sensors for the regulation of Kiss1 neurons at puberty and adulthood, and the definition of the whole set of up-stream modulators and co-transmitters of kisspeptins that cooperate in the metabolic control of the reproductive axis. This is illustrated by recent developments in the field, as the identification of new afferent pathways transmitting metabolic cues to Kiss1 neurons, e.g., PACAP neurons in the PMV (Ross et al., 2018). In this context, it will be relevant not only to identify individual players, but also to define the hierarchy of these metabolic regulatory pathways, as a means to define which signals are necessary, sufficient or eventually redundant for the metabolic gating of puberty and fertility. Likewise, further efforts are needed to characterize novel down-stream targets of kisspeptins that may contribute to alterations in pubertal timing in conditions of metabolic distress, such as obesity (Heras et al., 2020). Similarly, the putative contribution of deregulated *Kiss1* expression in conditions linked to perturbed gonadal and metabolic function, as obesity-induced hypogonadism or polycystic ovary syndrome (PCOS), has been fragmentarily studied also, but it is likely to be the focus of additional research in the coming years.

Studies on preclinical models and clinical samples may also help to define the potential use of kisspeptins either as biomarkers of disease or novel targets of pharmacological intervention. On the former, much attention has concentrated on the eventual value of changes in circulating kisspeptins as markers of gestational pathologies, antenatal complications, risk of miscarriage and ectopic pregnancy; as very recent examples, see (Romero-Ruiz et al., 2019a; Abbara et al., 2022; Abbara et al., 2021), and additional studies in this broad area are expected. On the other, the neuroendocrine properties of kisspeptins make them tenable candidates for pharmacological intervention, as illustrated by studies in a number of conditions, ranging from oocyte stimulation in *in vitro* fertilization to gonadotropin stimulation and ovulatory induction in PCOS or hypothalamic amenorrhea (Romero-Ruiz et al., 2019b; Abbara et al., 2020). While recent progress in this area has focused more on co-transmitters of kisspeptins, such as NKB analogs, the progressive recognition of the whole array of biological functions of kisspeptins, which now include not only the control of various aspects of metabolism, but also the central modulation of emotional and sex behavioral responses (Comninos et al., 2017; Comninos et al., 2018), as well as estrogen-dependent modulation of bone formation (Herber et al., 2019), just to mention some prominent examples, certainly expands the therapeutic options of kisspeptins, whose medical application, though, requires further experimental and clinical testing.

In summary, departing from consensus knowledge set during the first ten years of active investigation following the disclosure of the reproductive dimension of kisspeptins, we have provided here an update on the major progress occurred in the last ten years in various domains of kisspeptin research. In addition, we have included some educated predictions for the expected developments of this active field of Neuroendocrinology. Based on the experience of the last two decades, and the interest and activities taking place currently in this area, the future promises to bring even more exciting and groundbreaking findings, that may help to reshape our current understanding of the neurohormonal mechanisms for the control of reproduction and related functions.

Data availability statement

This is a narrative review based on published data. Accordingly, specific data sharing is not applicable to this article as no new datasets were generated or analyzed during the current study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors have nothing to disclose in relation to the contents of this work.

References

Abbara, A., Eng, P.C., Phylactou, M., Clarke, S.A., Richardson, R., Sykes, C.M., Phumsatitpong, C., Mills, E., Modi, M., Izzi-Engbeaya, C., Papadopoulou, D., Purugganan, K., Jayasena, C.N., Webber, L., Salim, R., Owen, B., Bech, P., Comninos, A.N., McArdle, C.A., Voliotis, M., Tsaneva-Atanasova, K., Moenter, S., Hanyaloglu, A., Dhillo, W.S., 2020. Kisspeptin receptor agonist has therapeutic potential for female reproductive disorders. J. Clin. Invest. 130, 6739–6753.

Abbara, A., Al-Memar, M., Phylactou, M., Kyriacou, C., Eng, P.C., Nadir, R., Izzi-Engbeaya, C., Clarke, S.A., Mills, E.G., Daniels, E., Huo, L., Pacuszka, E., Yang, L., Patel, B., Tan, T., Bech, P., Comninos, A.N., Fourie, H., Kelsey, T.W., Bourne, T., Dhillo, W.S., 2021. Performance of plasma kisspeptin as a biomarker for miscarriage improves with gestational age during the first trimester. Fertil. Steril. 116, 809–819.

Abbara, A., Al-Memar, M., Phylactou, M., Daniels, E., Patel, B., Eng, P.C., Nadir, R., Izzi-Engbeaya, C., Clarke, S.A., Mills, E.G., Hunjan, T., Pacuszka, E., Yang, L., Bech, P., Tan, T., Comninos, A.N., Kelsey, T.W., Kyriacou, C., Fourie, H., Bourne, T., Dhillo, W.S., 2022. Changes in circulating kisspeptin levels during each trimester in women with antenatal complications. J. Clin. Endocrinol. Metab. 107, e71–e83.

Abreu, A.P., Dauber, A., Macedo, D.B., Noel, S.D., Brito, V.N., Gill, J.C., Cukier, P., Thompson, I.R., Navarro, V.M., Gagliardi, P.C., Rodrigues, T., Kochi, C., Longui, C. A., Beckers, D., de Zegher, F., Montenegro, L.R., Mendonca, B.B., Carroll, R.S., Hirschhorn, J.N., Latronico, A.C., Kaiser, U.B., 2013. Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl. J. Med. 368, 2467–2475.

Abreu, A.P., Toro, C.A., Song, Y.B., Navarro, V.M., Bosch, M.A., Eren, A., Liang, J.N., Carroll, R.S., Latronico, A.C., Ronnekleiv, O.K., Aylwin, C.F., Lomniczi, A., Ojeda, S., Kaiser, U.B., 2020. MKRN3 inhibits the reproductive axis through actions in kisspeptin-expressing neurons. J. Clin. Invest. 130, 4486–4500.

Adekunbi, D.A., Li, X.F., Li, S., Adegoke, O.A., Iranloye, B.O., Morakinyo, A.O., Lightman, S.L., Taylor, P.D., Poston, L., O'Byrne, K.T., Vaudry, H., 2017. Role of amygdala kisspeptin in pubertal timing in female rats. PLoS One 12 (8), e0183596.

Avendano, M.S., Vazquez, M.J., Tena-Sempere, M., 2017. Disentangling puberty: novel neuroendocrine pathways and mechanisms for the control of mammalian puberty. Hum. Reprod. Update 23, 737–763.

Beymer, M., Negrón, A.L., Yu, G., Wu, S., Mayer, C., Lin, R.Z., Boehm, U., Acosta-Martínez, M., 2014. Kisspeptin cell-specific PI3K signaling regulates hypothalamic kisspeptin expression and participates in the regulation of female fertility. Am. J. Physiol. Endocrinol. Metab. 307 (11), E969–E982.

Bhattacharya, M., Babwah, A.V., 2015. Kisspeptin: beyond the brain. Endocrinology 156 (4), 1218–1227.

Boehm, U., Bouloux, P.M., Dattani, M.T., de Roux, N., Dode, C., Dunkel, L., Dwyer, A.A., Giacobini, P., Hardelin, J.P., Juul, A., Maghnie, M., Pitteloud, N., Prevot, V., Raivio, T., Tena-Sempere, M., Quinton, R., Young, J., 2015. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. Nat. Rev. Endocrinol. 11, 547–564.

Borsay, B.A., Skrapits, K., Herczeg, L., Ciofi, P., Bloom, S.R., Ghatei, M.A., Dhillo, W.S., Liposits, Z., Hrabovszky, E., 2014. Hypophysiotropic gonadotropin-releasing hormone projections are exposed to dense plexuses of kisspeptin, neurokinin B and substance p immunoreactive fibers in the human: a study on tissues from postmenopausal women. Neuroendocrinology 100, 141–152.

Calder, M., Chan, Y.M., Raj, R., Pampillo, M., Elbert, A., Noonan, M., Gillio-Meina, C., Caligioni, C., Berube, N.G., Bhattacharya, M., Watson, A.J., Seminara, S.B., Babwah, A.V., 2014. Implantation failure in female Kiss1-/- mice is independent of their hypogonadic state and can be partially rescued by leukemia inhibitory factor. Endocrinology 155, 3065–3078.

Campbell, J.N., Macosko, E.Z., Fenselau, H., Pers, T.H., Lyubetskaya, A., Tenen, D., Goldman, M., Verstegen, A.M., Resch, J.M., McCarroll, S.A., Rosen, E.D., Lowell, B. B., Tsai, L.T., 2017. A molecular census of arcuate hypothalamus and median eminence cell types. Nat. Neurosci. 20, 484–496.

Castellano, J.M., Navarro, V.M., Fernandez-Fernandez, R., Roa, J., Vigo, E., Pineda, R., Dieguez, C., Aguilar, E., Pinilla, L., Tena-Sempere, M., 2006. Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes 55, 2602–2610.

Chen, R., Wu, X., Jiang, L., Zhang, Y., 2017. Single-cell RNA-Seq reveals hypothalamic cell diversity. Cell Rep. 18, 3227–3241.

C.M. Chiang, H.Y. Chiu, D.S. Jong, L.S. Wu, Y.J. Lee, and C.H. Chiu, Role of the Kisspeptin/KISS1 Receptor System in the Testicular Development of Mice. J Chin Med Assoc (2020) doi: 10.1097.

Choi, I., Rickert, E., Fernandez, M., Webster, N.J.G., 2019. SIRT1 in astrocytes regulates glucose metabolism and reproductive function. Endocrinology 160, 1547–1560.

Clarkson, J., Boon, W.C., Simpson, E.R., Herbison, A.E., 2009. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. Endocrinology 150, 3214–3220.

Clarkson, J., Han, S.Y., Piet, R., McLennan, T., Kane, G.M., Ng, J., Porteous, R.W., Kim, J. S., Colledge, W.H., Iremonger, K.J., Herbison, A.E., 2017. Definition of the hypothalamic GnRH pulse generator in mice. Proc. Natl. Acad. Sci. USA 114 (47), E10216–E10223.

Colledge, W.H., 2009. Kisspeptins and GnRH neuronal signalling. Trends Endocrinol Metab 20 (3), 115–121.

Comninos, A.N., Wall, M.B., Demetriou, L., Shah, A.J., Clarke, S.A., Narayanaswamy, S., Nesbitt, A., Izzi-Engbeaya, C., Prague, J.K., Abbara, A., Ratnasabapathy, R., Salem, V., Nijher, G.M., Jayasena, C.N., Tanner, M., Bassett, P., Mehta, A., Rabiner, E.A., Honigsperger, C., Silva, M.R., Brandtzaeg, O.K., Lundanes, E., Wilson, S.R., Brown, R.C., Thomas, S.A., Bloom, S.R., Dhillo, W.S., 2017. Kisspeptin modulates sexual and emotional brain processing in humans. J. Clin. Invest. 127, 709–719.

Comninos, A.N., Demetriou, L., Wall, M.B., Shah, A.J., Clarke, S.A., Narayanaswamy, S., Nesbitt, A., Izzi-Engbeaya, C., Prague, J.K., Abbara, A., Ratnasabapathy, R., Yang, L., Salem, V., Nijher, G.M., Jayasena, C.N., Tanner, M., Bassett, P., Mehta, A., McGonigle, J., Rabiner, E.A., Bloom, S.R., Dhillo, W.S., 2018. Modulations of human resting brain connectivity by kisspeptin enhance sexual and emotional functions. JCI Insight 3 (20), e121958.

Conn, P.M., Crowley Jr., W.F., 1994. Gonadotropin-releasing hormone and its analogs. Annu. Rev. Med. 45, 391–405.

Coyral-Castel, S., Tosca, L., Ferreira, G., Jeanpierre, E., Rame, C., Lomet, D., Caraty, A., Monget, P., Chabrolle, C., Dupont, J., 2008. The effect of AMP-activated kinase activation on gonadotrophin-releasing hormone secretion in GT1-7 cells and its potential role in hypothalamic regulation of the oestrous cyclicity in rats. J. Neuroendocrinol. 20, 335–346.

d'Anglemont de Tassigny, X., Colledge, W.H., 2010. The role of kisspeptin signaling in reproduction. Physiology (Bethesda) 25, 207–217.

de Roux, N., Genin, E., Carel, J.C., Matsuda, F., Chaussain, J.L., Milgrom, E., 2003. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc. Natl. Acad. Sci. USA 100, 10972–10976.

Dhandapani, K.M., Brann, D.W., 2000. The role of glutamate and nitric oxide in the reproductive neuroendocrine system. Biochem. Cell Biol. 78, 165–179.

Dorfman, M.D., Garcia-Rudaz, C., Alderman, Z., Kerr, B., Lomniczi, A., Dissen, G.A., Castellano, J.M., Garcia-Galiano, D., Gaytan, F., Xu, B., Tena-Sempere, M., Ojeda, S. R., 2014. Loss of Ntrk2/Kiss1r signaling in oocytes causes premature ovarian failure. Endocrinology 155, 3098–3111.

Eguizabal, C., Herrera, L., De Onate, L., Montserrat, N., Hajkova, P., Izpisua Belmonte, J. C., 2016. Characterization of the epigenetic changes during human gonadal primordial germ cells reprogramming. Stem Cells 34, 2418–2428.

Evans, M.C., Rizwan, M., Mayer, C., Boehm, U., Anderson, G.M., 2014. Evidence that insulin signalling in gonadotrophin-releasing hormone and kisspeptin neurones does not play an essential role in metabolic regulation of fertility in mice. J. Neuroendocrinol. 26, 468–479.

Fergani, C., Leon, S., Padilla, S.L., Verstegen, A.MJ., Palmiter, R.D., Navarro, V.M., 2018. NKB signaling in the posterodorsal medial amygdala stimulates gonadotropin release in a kisspeptin-independent manner in female mice. Elife 7, e40476.

Fernandois, D., Na, E., Cuevas, F., Cruz, G., Lara, H.E., Paredes, A.H., 2016. Kisspeptin is involved in ovarian follicular development during aging in rats. J. Endocrinol. 228, 161–170.

Forbes, S., Li, X.F., Kinsey-Jones, J., O'Byrne, K., 2009. Effects of ghrelin on Kisspeptin mRNA expression in the hypothalamic medial preoptic area and pulsatile luteinising hormone secretion in the female rat. Neurosci. Lett. 460, 143–147.

Franssen, D., Barroso, A., Ruiz-Pino, F., Vazquez, M.J., Garcia-Galiano, D., Castellano, J. M., Onieva, R., Ruiz-Cruz, M., Poutanen, M., Gaytan, F., Dieguez, C., Pinilla, L., Lopez, M., Roa, J., Tena-Sempere, M., 2021. AMP-activated protein kinase (AMPK) signaling in GnRH neurons links energy status and reproduction. Metabolism 115, 154460.

Frazao, R., Lemko, H.M.D., da Silva, R.P., Ratra, D.V., Lee, C.E., Williams, K.W., Zigman, J.M., Elias, C.F., 2014. Estradiol modulates Kiss1 neuronal response to ghrelin. Am. J. Physiol. Endocrinol. Metab. 306 (6), E606–E614.

Gahete, M.D., Vazquez-Borrego, M.C., Martinez-Fuentes, A.J., Tena-Sempere, M., Castano, J.P., Luque, R.M., 2016. Role of the Kiss1/Kiss1r system in the regulation of pituitary cell function. Mol. Cell Endocrinol. 438, 100–106.

Garcia-Galiano, D., Pinilla, L., Tena-Sempere, M., 2012. Sex steroids and the control of the Kiss1 system: developmental roles and major regulatory actions. J. Neuroendocrinol. 24, 22–33.

Garcia-Galiano, D., van Ingen Schenau, D., Leon, S., Krajnc-Franken, M.A., Manfredi-Lozano, M., Romero-Ruiz, A., Navarro, V.M., Gaytan, F., van Noort, P.I., Pinilla, L., Blomenrohr, M., Tena-Sempere, M., 2012. Kisspeptin signaling is indispensable for neurokinin B, but not glutamate, stimulation of gonadotropin secretion in mice. Endocrinology 153, 316–328.

Garcia-Galiano, D., Cara, A.L., Tata, Z., Allen, S.J., Myers Jr., M.G., Schipani, E., Elias, C. F., 2020. ERalpha signaling in GHRH/Kiss1 dual-phenotype neurons plays sex-specific roles in growth and puberty. J. Neurosci. 40, 9455–9466.
Gaytan, F., Garcia-Galiano, D., Dorfman, M.D., Manfredi-Lozano, M., Castellano, J.M., Dissen, G.A., Ojeda, S.R., Tena-Sempere, M., 2014. Kisspeptin receptor haplo-insufficiency causes premature ovarian failure despite preserved gonadotropin secretion. Endocrinology 155, 3088–3097.
Giblin, W., Skinner, M.E., Lombard, D.B., 2014. Sirtuins: guardians of mammalian healthspan. Trends Genet 30 (7), 271–286.
Gill, J.C., Navarro, V.M., Kwong, C., Noel, S.D., Martin, C., Xu, S., Clifton, D.K., Carroll, R.S., Steiner, R.A., Kaiser, U.B., 2012. Increased neurokinin B (Tac2) expression in the mouse arcuate nucleus is an early marker of pubertal onset with differential sensitivity to sex steroid-negative feedback than Kiss1. Endocrinology 153, 4883–4893.
Gottsch, M.L., Navarro, V.M., Zhao, Z., Glidewell-Kenney, C., Weiss, J., Jameson, J.L., Clifton, D.K., Levine, J.E., Steiner, R.A., 2009. Regulation of Kiss1 and dynorphin gene expression in the murine brain by classical and nonclassical estrogen receptor pathways. J. Neurosci. 29, 9390–9395.
Grossniklaus, U., Paro, R., 2014. Transcriptional silencing by polycomb-group proteins. Cold Spring Harb Perspect Biol 6 (2014) a019331.
Grumbach, M.M., 2002. The neuroendocrinology of human puberty revisited. Horm. Res. 57 (Suppl 2), 2–14.
Hameed, S., Jayasena, C.N., Dhillo, W.S., 2011. Kisspeptin and fertility. J Endocrinol 208, 97–105.
Han, S.Y., Cheong, I., McLennan, T., Herbison, A.E., 2020. Neural determinants of pulsatile luteinizing hormone secretion in male mice. Endocrinology 161, bqz045.
Heras, V., Sangiao-Alvarellos, S., Manfredi-Lozano, M., Sanchez-Tapia, M.J., Ruiz-Pino, F., Roa, J., Lara-Chica, M., Morrugares-Carmona, R., Jouy, N., Abreu, A.P., Prevot, V., Belsham, D., Vazquez, M.J., Calzado, M.A., Pinilla, L., Gaytan, F., Latronico, A.C., Kaiser, U.B., Castellano, J.M., Tena-Sempere, M., Ong, K.K., 2019. Hypothalamic miR-30 regulates puberty onset via repression of the puberty-suppressing factor, Mkrn3. PLoS Biol 17 (11), e3000532.
Heras, V., Castellano, J.M., Fernandois, D., Velasco, I., Rodriguez-Vazquez, E., Roa, J., Vazquez, M.J., Ruiz-Pino, F., Rubio, M., Pineda, R., Torres, E., Avendano, M.S., Paredes, A., Pinilla, L., Belsham, D., Dieguez, C., Gaytan, F., Casals, N., Lopez, M., Tena-Sempere, M., 2020. Central ceramide signaling mediates obesity-induced precocious puberty. Cell. Metab. 32, 951–966.
Herber, C.B., Krause, W.C., Wang, L., Bayrer, J.R., Li, A., Schmitz, M., Fields, A., Ford, B., Zhang, Z., Reid, M.S., Nomura, D.K., Nissenson, R.A., Correa, S.M., Ingraham, H.A., 2019. Estrogen signaling in arcuate Kiss1 neurons suppresses a sex-dependent female circuit promoting dense strong bones. Nat. Commun. 10, 163.
Herbison, A.E., 2018. The gonadotropin-releasing hormone pulse generator. Endocrinology 159, 3723–3736.
Herreboudt, A.M., Kyle, V.R., Lawrence, J., Doran, J., Colledge, W.H., 2015. Kiss1 mutant placentas show normal structure and function in the mouse. Placenta 36, 52–58.
Hessler, S., Liu, X., Herbison, A.E., 2020. Direct inhibition of arcuate kisspeptin neurones by neuropeptide Y in the male and female mouse. J. Neuroendocrinol. 32 (5) https://doi.org/10.1111/jne.v32.510.1111/jne.12849.
Horihata, K., Inoue, N., Uenoyama, Y., Maeda, K.I., Tsukamura, H., 2020. Retinoblastoma binding protein 7 is involved in Kiss1 mRNA upregulation in rodents. J. Reprod. Dev. 66, 125–133.
Horikoshi, Y., Matsumoto, H., Takatsu, Y., Ohtaki, T., Kitada, C., Usuki, S., Fujino, M., 2003. Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. J. Clin. Endocrinol. Metab. 88, 914–919.
Hrabovszky, E., Ciofi, P., Vida, B., Horvath, M.C., Keller, E., Caraty, A., Bloom, S.R., Ghatei, M.A., Dhillo, W.S., Liposits, Z., Kallo, I., 2010. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur. J. Neurosci. 31, 1984–1998.
Hrabovszky, E., Sipos, M.T., Molnar, C.S., Ciofi, P., Borsay, B.A., Gergely, P., Herczeg, L., Bloom, S.R., Ghatei, M.A., Dhillo, W.S., Liposits, Z., 2012. Low degree of overlap between kisspeptin, neurokinin B, and dynorphin immunoreactivities in the infundibular nucleus of young male human subjects challenges the KNDy neuron concept. Endocrinology 153, 4978–4989.
Hrabovszky, E., Takacs, S., Gocz, B., Skrapits, K., 2019. New perspectives for anatomical and molecular studies of kisspeptin neurons in the aging human brain. Neuroendocrinology 109, 230–241.
Hsu, M.C., Wu, L.S., Jong, D.S., Chiu, C.H., 2020. KISS1R signaling modulates gonadotropin sensitivity in mouse Leydig cell. Reproduction 160, 843–852.
Hu, M.H., Li, X.F., McCausland, B., Li, S.Y., Gresham, R., Kinsey-Jones, J.S., Gardiner, J. V., Sam, A.H., Bloom, S.R., Poston, L., Lightman, S.L., Murphy, K.G., O'Byrne, K.T., 2015. Relative importance of the arcuate and anteroventral periventricular kisspeptin neurons in control of puberty and reproductive function in female rats. Endocrinology 156, 2619–2631.
Hu, K.L., Zhao, H., Chang, H.M., Yu, Y., Qiao, J., 2017. Kisspeptin/Kisspeptin Receptor System in the Ovary. Front. Endocrinol. (Lausanne) 8, 365.
Hudson, A.D., Kauffman, A.S., 2021. Metabolic actions of kisspeptin signaling: effects on body weight, energy expenditure, and feeding. Pharmacol. Ther. 107974. https://doi.org/10.1016/j.pharmthera.2021.107974.
Irfan, S., Ehmcke, J., Wahab, F., Shahab, M., Schlatt, S., 2014. Intratesticular action of kisspeptin in rhesus monkey (Macaca mulatta). Andrologia 46, 610–617.
Irfan, S., Ehmcke, J., Shahab, M., Wistuba, J., Schlatt, S., 2016. Immunocytochemical localization of kisspeptin and kisspeptin receptor in the primate testis. J. Med. Primatol. 45, 105–111.
Kim, H.G., Kurth, I., Lan, F., Meliciani, I., Wenzel, W., Eom, S.H., Kang, G.B., Rosenberger, G., Tekin, M., Ozata, M., Bick, D.P., Sherins, R.J., Walker, S.L., Shi, Y., Gusella, J.F., Layman, L.C., 2008. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am. J. Hum Genet. 83, 511–519.
Knobil, E., 1992. Remembrance: the discovery of the hypothalamic gonadotropin-releasing hormone pulse generator and of its physiological significance. Endocrinology 131, 1005–1006.
Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J.M., Le Poul, E., Brezillon, S., Tyldesley, R., Suarez-Huerta, N., Vandeput, F., Blanpain, C., Schiffmann, S.N., Vassart, G., Parmentier, M., 2001. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J. Biol. Chem. 276, 34631–34636.
Kurian, J.R., Louis, S., Keen, K.L., Wolfe, A., Terasawa, E., Levine, J.E., 2016. The methylcytosine dioxygenase ten-eleven translocase-2 (tet2) enables elevated gnrh gene expression and maintenance of male reproductive function. Endocrinology 157, 3588–3603.
Lam, B.Y.H., Williamson, A., Finer, S., Day, F.R., Tadross, J.A., Goncalves Soares, A., Wade, K., Sweeney, P., Bedenbaugh, M.N., Porter, D.T., Melvin, A., Ellacott, K.L.J., Lippert, R.N., Buller, S., Rosmaninho-Salgado, J., Dowsett, G.K.C., Ridley, K.E., Xu, Z., Cimino, I., Rimmington, D., Rainbow, K., Duckett, K., Holmqvist, S., Khan, A., Dai, X., Bochukova, E.G., Genes, Health Research, T., Trembath, R.C., Martin, H.C., Coll, A.P., Rowitch, D.H., Wareham, N.J., van Heel, D.A., Timpson, N., Simerly, R.B., Ong, K.K., Cone, R.D., Langenberg, C., Perry, J.R.B., Yeo, G.S., O'Rahilly, S., 2021. MC3R links nutritional state to childhood growth and the timing of puberty. Nature 599, 436-441.
Lavalle, S.N., Chou, T., Hernandez, J., Naing, N.C.P., Tonsfeldt, K.J., Hoffmann, H.M., Mellon, P.L., 2021. Kiss1 is differentially regulated in male and female mice by the homeodomain transcription factor VAX1. Mol. Cell Endocrinol. 534, 111358.
Lehman, M.N., Coolen, L.M., Goodman, R.L., 2010. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 151, 3479–3489.
Leon, S., Garcia-Galiano, D., Ruiz-Pino, F., Barroso, A., Manfredi-Lozano, M., Romero-Ruiz, A., Roa, J., Vazquez, M.J., Gaytan, F., Blomenrohr, M., van Duin, M., Pinilla, L., Tena-Sempere, M., 2014. Physiological roles of gonadotropin-inhibitory hormone signaling in the control of mammalian reproductive axis: studies in the NPFF1 receptor null mouse. Endocrinology 155, 2953–2965.
Leon, S., Fernandois, D., Sull, A., Sull, J., Calder, M., Hayashi, K., Bhattacharya, M., Power, S., Vilos, G.A., Vilos, A.G., Tena-Sempere, M., Babwah, A.V., 2016. Beyond the brain-Peripheral kisspeptin signaling is essential for promoting endometrial gland development and function. Sci. Rep. 6, 29073.
Leon, S., Fergani, C., Talbi, R., Simavli, S., Maguire, C.A., Gerutshang, A., Navarro, V.M., 2019. Characterization of the role of NKA in the control of puberty onset and gonadotropin release in the female mouse. Endocrinology 160, 2453–2463.
Leon, S., Navarro, V.M., 2019. Novel biology of tachykinins in gonadotropin-releasing hormone secretion. Semin. Reprod. Med. 37, 109–118.
Leon, S., Talbi, R., McCarthy, E.A., Ferrari, K., Fergani, C., Naule, L., Choi, J.H., Carroll, R.S., Kaiser, U.B., Aylwin, C.F., Lomniczi, A., Navarro, V.M., 2021. Sex-specific pubertal and metabolic regulation of Kiss1 neurons via Nhlh2. Elife 10, e69765.
Li, S.Y., Li, X.F., Hu, M.H., Shao, B., Poston, L., Lightman, S.L., O'Byrne, K.T., 2014. Neurokinin B receptor antagonism decreases luteinising hormone pulse frequency and amplitude and delays puberty onset in the female rat. J. Neuroendocrinol. 26, 521–527.
Liu, H., Kong, X., Chen, F., 2017. Mkrn3 functions as a novel ubiquitin E3 ligase to inhibit Nptx1 during puberty initiation. Oncotarget 8, 85102–85109.
Liu, X., Yeo, S.H., McQuillan, H.J., Herde, M.K., Hessler, S., Cheong, I., Porteous, R., Herbison, A.E., 2021. Highly redundant neuropeptide volume co-transmission underlying episodic activation of the GnRH neuron dendron. Elife 10, e62455.
Lomniczi, A., Loche, A., Castellano, J.M., Ronnekleiv, O.K., Bosch, M., Kaidar, G., Knoll, J.G., Wright, H., Pfeifer, G.P., Ojeda, S.R., 2013. Epigenetic control of female puberty. Nat. Neurosci. 16, 281–289.
Lomniczi, A., Wright, H., Castellano, J.M., Matagne, V., Toro, C.A., Ramaswamy, S., Plant, T.M., Ojeda, S.R., 2015. Epigenetic regulation of puberty via Zinc finger protein-mediated transcriptional repression. Nat. Commun. 6, 10195.
Lomniczi, A., Wright, H., Ojeda, S.R., 2015. Epigenetic regulation of female puberty. Front. Neuroendocrinol. 36, 90–107.
Lopez-Rodriguez, D., Aylwin, C.F., Delli, V., Sevrin, E., Campanile, M., Martin, M., Franssen, D., Gerard, A., Blacher, S., Tirelli, E., Noel, A., Lomniczi, A., Parent, A.S., 2021. Multi- and transgenerational outcomes of an exposure to a mixture of endocrine-disrupting chemicals (EDCs) on puberty and maternal behavior in the female rat. Environ. Health Perspect. 129, 87003.
Maguire, C.A., Song, Y.B., Wu, M., Leon, S., Carroll, R.S., Alreja, M., Kaiser, U.B., Navarro, V.M., 2017. Tac1 signaling is required for sexual maturation and responsiveness of GnRH neurons to kisspeptin in the male mouse. Endocrinology 158, 2319–2329.
Maione, L., Naule, L., Kaiser, U.B., 2020. Makorin RING finger protein 3 and central precocious puberty. Curr. Opin. Endocr. Metab. Res. 14, 152–159.
Manfredi-Lozano, M., Roa, J., Ruiz-Pino, F., Piet, R., Garcia-Galiano, D., Pineda, R., Zamora, A., Leon, S., Sanchez-Garrido, M.A., Romero-Ruiz, A., Dieguez, C., Vazquez, M.J., Herbison, A.E., Pinilla, L., Tena-Sempere, M., 2016. Defining a novel leptin-melanocortin-kisspeptin pathway involved in the metabolic control of puberty. Mol. Metab. 5, 844–857.
Manfredi-Lozano, M., Roa, J., Tena-Sempere, M., 2018. Connecting metabolism and gonadal function: Novel central neuropeptide pathways involved in the metabolic control of puberty and fertility. Front. Neuroendocrinol. 48, 37–49.

Martin, C., Navarro, V.M., Simavli, S., Vong, L., Carroll, R.S., Lowell, B.B., Kaiser, U.B., 2014. Leptin-responsive GABAergic neurons regulate fertility through pathways that result in reduced kisspeptinergic tone. J. Neurosci. 34, 6047–6056.
Mayer, C., Boehm, U., 2011. Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat. Neurosci 14, 704–710.
Merkley, C.M., Shuping, S.L., Sommer, J.R., Nestor, C.C., 2021. Evidence that agouti-related peptide may directly regulate kisspeptin neurons in male sheep. Metabolites 11, 138.
Messina, A., Langlet, F., Chachlaki, K., Roa, J., Rasika, S., Jouy, N., Gallet, S., Gaytan, F., Parkash, J., Tena-Sempere, M., Giacobini, P., Prevot, V., 2016. A microRNA switch regulates the rise in hypothalamic GnRH production before puberty. Nat. Neurosci. 19, 835–844.
Mickelsen, L.E., Bolisetty, M., Chimileski, B.R., Fujita, A., Beltrami, E.J., Costanzo, J.T., Naparstek, J.R., Robson, P., Jackson, A.C., 2019. Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. Nat. Neurosci. 22, 642–656.
Mittelman-Smith, M.A., Williams, H., Krajewski-Hall, S.J., Lai, J., Ciofi, P., McMullen, N. T., Rance, N.E., 2012. Arcuate kisspeptin/neurokinin B/dynorphin (KNDy) neurons mediate the estrogen suppression of gonadotropin secretion and body weight. Endocrinology 153, 2800–2812.
Moffitt, J.R., Bambah-Mukku, D., Eichhorn, S.W., Vaughn, E., Shekhar, K., Perez, J.D., Rubinstein, N.D., Hao, J., Regev, A., Dulac, C., Zhuang, X., 2018. Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science 362 (6416). https://doi.org/10.1126/science:aau5324.
Moore, A.M., Lucas, K.A., Goodman, R.L., Coolen, L.M., Lehman, M.N., 2018. Three-dimensional imaging of KNDy neurons in the mammalian brain using optical tissue clearing and multiple-label immunocytochemistry. Sci. Rep. 8, 2242.
Moore, A.M., Coolen, L.M., Porter, D.T., Goodman, R.L., Lehman, M.N., 2018. KNDy cells revisited. Endocrinology 159, 3219–3234.
Moore, A.M., Prescott, M., Czieselsky, K., Desroziers, E., Yip, S.H., Campbell, R.E., Herbison, A.E., 2018. Synaptic innervation of the GnRH neuron distal dendron in female mice. Endocrinology 159, 3200–3208.
Moore, A.M., Coolen, L.M., Lehman, M.N., 2019. Kisspeptin/Neurokinin B/Dynorphin (KNDy) cells as integrators of diverse internal and external cues: evidence from viral-based monosynaptic tract-tracing in mice. Sci. Rep. 9, 14768.
Moore, A.M., Lohr, D.B., Coolen, L.M., Lehman, M.N., 2021. Prenatal androgen exposure alters KNDy neurons and their afferent network in a model of polycystic ovarian syndrome. Endocrinology 162, bqab158.
Mueller, J.K., Dietzel, A., Lomniczi, A., Loche, A., Tefs, K., Kiess, W., Danne, T., Ojeda, S. R., Heger, S., 2011. Transcriptional regulation of the human KiSS1 gene. Mol. Cell Endocrinol. 342 (1-2), 8–19.
Nagae, M., Uenoyama, Y., Okamoto, S., Tsuchida, H., Ikegami, K., Goto, T., Majarune, S., Nakamura, S., Sanbo, M., Hirabayashi, M., Kobayashi, K., Inoue, N., Tsukamura, H., 2021. Direct evidence that KNDy neurons maintain gonadotropin pulses and folliculogenesis as the GnRH pulse generator. Proc Natl Acad Sci USA 118 (2021) e2009156118.
Nakahara, T., Uenoyama, Y., Iwase, A., Oishi, S., Nakamura, S., Minabe, S., Watanabe, Y., Deura, C., Noguchi, T., Fujii, N., Kikkawa, F., Maeda, K., Tsukamura, H., 2013. Chronic peripheral administration of kappa-opioid receptor antagonist advances puberty onset associated with acceleration of pulsatile luteinizing hormone secretion in female rats. J. Reprod. Dev. 59, 479–484.
Nandankar, N., Negrón, A.L., Wolfe, A., Levine, J.E., Radovick, S., 2021. Deficiency of arcuate nucleus kisspeptin results in postpubertal central hypogonadism. Am. J. Physiol. Endocrinol. Metab. 321 (2), E264–E280.
Navarro, V.M., 2020. Metabolic regulation of kisspeptin - the link between energy balance and reproduction. Nat. Rev. Endocrinol. 16, 407–420.
Navarro, V.M., Gottsch, M.L., Chavkin, C., Okamura, H., Clifton, D.K., Steiner, R.A., 2009. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J. Neurosci. 29, 11859–11866.
Navarro, V.M., Ruiz-Pino, F., Sanchez-Garrido, M.A., Garcia-Galiano, D., Hobbs, S.J., Manfredi-Lozano, M., Leon, S., Sangiao-Alvarellos, S., Castellano, J.M., Clifton, D.K., Pinilla, L., Steiner, R.A., Tena-Sempere, M., 2012. Role of neurokinin B in the control of female puberty and its modulation by metabolic status. J. Neurosci. 32, 2388–2397.
Navarro, V.M., Tena-Sempere, M., 2011. Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. Nat. Rev. Endocrinol. 8, 40–53.
Navarro, V.M., Bosch, M.A., Leon, S., Simavli, S., True, C., Pinilla, L., Carroll, R.S., Seminara, S.B., Tena-Sempere, M., Ronnekleiv, O.K., Kaiser, U.B., 2015. The integrated hypothalamic tachykinin-kisspeptin system as a central coordinator for reproduction. Endocrinology 156, 627–637.
Negron, A.L., Yu, G., Boehm, U., Acosta-Martinez, M., 2020. Targeted deletion of PTEN in kisspeptin cells results in brain region- and sex-specific effects on kisspeptin expression and gonadotropin release. Int. J. Mol. Sci. 21, 2107.
Nestor, C.C., Qiu, J., Padilla, S.L., Zhang, C., Bosch, M.A., Fan, W., Aicher, S.A., Palmiter, R.D., Ronnekleiv, O.K., Kelly, M.J., 2016. Optogenetic stimulation of arcuate nucleus Kiss1 neurons reveals a steroid-dependent glutamatergic input to POMC and AgRP neurons in male mice. Mol. Endocrinol. 30, 630–644.
Nogueiras, R., Habegger, K.M., Chaudhary, N., Finan, B., Banks, A.S., Dietrich, M.O., Horvath, T.L., Sinclair, D.A., Pfluger, P.T., Tschop, M.H., 2012. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. Physiol. Rev. 92, 1479–1514.
Oakley, A.E., Clifton, D.K., Steiner, R.A., 2009. Kisspeptin signaling in the brain. Endocr. Rev. 30, 713–743.
Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., Terao, Y., Kumano, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Onda, H., Nishimura, O., Fujino, M., 2001. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 411, 613–617.
Ojima, F., Saito, Y., Tsuchiya, Y., Kayo, D., Taniuchi, S., Ogoshi, M., Fukamachi, H., Takeuchi, S., Takahashi, S., 2016. Runx3 transcription factor regulates ovarian functions and ovulation in female mice. J. Reprod. Dev. 62, 479–486.
Ongaro, L., Alonso, C.A.I., Zhou, X., Brule, E., Li, Y., Schang, G., Parlow, A.F., Steyn, F., Bernard, D.J., 2021. Development of a highly sensitive ELISA for measurement of FSH in serum, plasma, and whole blood in mice. Endocrinology 162 (4), bqab014.
Owens, L.A., Abbara, A., Lerner, A., O'Floinn, S., Christopoulos, G., Khanjani, S., Islam, R., Hardy, K., Hanyaloglu, A.C., Lavery, S.A., Dhillo, W.S., Franks, S., 2018. The direct and indirect effects of kisspeptin-54 on granulosa lutein cell function. Hum. Reprod. 33, 292–302.
Padilla, S.L., Qiu, J., Nestor, C.C., Zhang, C., Smith, A.W., Whiddon, B.B., Ronnekleiv, O. K., Kelly, M.J., Palmiter, R.D., 2017. AgRP to Kiss1 neuron signaling links nutritional state and fertility. Proc. Natl. Acad. Sci. USA 114, 2413–2418.
Padilla, S.L., Johnson, C.W., Barker, F.D., Patterson, M.A., Palmiter, R.D., 2018. A neural circuit underlying the generation of hot flushes. Cell Rep 24, 271–277.
Piferrer, F., 2013. Epigenetics of sex determination and gonadogenesis. Dev. Dyn. 242, 360–370.
Pineda, R., Plaisier, F., Millar, R.P., Ludwig, M., 2017. Amygdala kisspeptin neurons: putative mediators of olfactory control of the gonadotropic axis. Neuroendocrinology 104, 223–238.
Pinilla, L., Aguilar, E., Dieguez, C., Millar, R.P., Tena-Sempere, M., 2012. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol. Rev. 92, 1235–1316.
Poling, M.C., Quennell, J.H., Anderson, G.M., Kauffman, A.S., 2013. Kisspeptin neurones do not directly signal to RFRP-3 neurones but RFRP-3 may directly modulate a subset of hypothalamic kisspeptin cells in mice. J. Neuroendocrinol. 25, 876–886.
Popa, S.M., Clifton, D.K., Steiner, R.A., 2008. The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. Annu. Rev. Physiol. 70, 213–238.
Porter, D.T., Goodman, R.L., Hileman, S.M., Lehman, M.N., 2021. Evidence that synaptic plasticity of glutamatergic inputs onto KNDy neurones during the ovine follicular phase is dependent on increasing levels of oestradiol. J. Neuroendocrinol. 33 (3), e12945.
Qiu, J., Zhang, C., Borgquist, A., Nestor, C.C., Smith, A.W., Bosch, M.A., Ku, S., Wagner, E.J., Ronnekleiv, O.K., Kelly, M.J., 2014. Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels. Cell Metab. 19, 682–693.
Qiu, J., Rivera, H.M., Bosch, M.A., Padilla, S.L., Stincic, T.L., Palmiter, R.D., Kelly, M.J., Rønnekleiv, O.K., 2018. Estrogenic-dependent glutamatergic neurotransmission from kisspeptin neurons governs feeding circuits in females. Elife 7, e35656.
Ramadori, G., Lee, C.E., Bookout, A.L., Lee, S., Williams, K.W., Anderson, J., Elmquist, J. K., Coppari, R., 2008. Brain SIRT1: anatomical distribution and regulation by energy availability. J. Neurosci. 28, 9989–9996.
Ricu, M.A., Ramirez, V.D., Paredes, A.H., Lara, H.E., 2012. Evidence for a celiac ganglion-ovarian kisspeptin neural network in the rat: intraovarian anti-kisspeptin delays vaginal opening and alters estrous cyclicity. Endocrinology 153, 4966–4977.
Roa, J., Aguilar, E., Dieguez, C., Pinilla, L., Tena-Sempere, M., 2008. New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. Front. Neuroendocrinol. 29, 48–69.
Roa, J., Garcia-Galiano, D., Varela, L., Sanchez-Garrido, M.A., Pineda, R., Castellano, J. M., Ruiz-Pino, F., Romero, M., Aguilar, E., Lopez, M., Gaytan, F., Dieguez, C., Pinilla, L., Tena-Sempere, M., 2009. The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology 150, 5016–5026.
Roa, J., Barroso, A., Ruiz-Pino, F., Vázquez, M.J., Seoane-Collazo, P., Martínez-Sanchez, N., García-Galiano, D., Ilhan, T., Pineda, R., León, S., Manfredi-Lozano, M., Heras, V., Poutanen, M., Castellano, J.M., Gaytan, F., Diéguez, C., Pinilla, L., López, M., Tena-Sempere, M., 2018. Metabolic regulation of female puberty via hypothalamic AMPK-kisspeptin signaling. Proc. Natl. Acad. Sci. USA 115 (45), E10758–E10767.
Roland, A.V., Moenter, S.M., 2011. Glucosensing by GnRH neurons: inhibition by androgens and involvement of AMP-activated protein kinase. Mol. Endocrinol. 25, 847–858.
Romanov, R.A., Zeisel, A., Bakker, J., Girach, F., Hellysaz, A., Tomer, R., Alpar, A., Mulder, J., Clotman, F., Keimpema, E., Hsueh, B., Crow, A.K., Martens, H., Schwindling, C., Calvigioni, D., Bains, J.S., Mate, Z., Szabo, G., Yanagawa, Y., Zhang, M.D., Rendeiro, A., Farlik, M., Uhlen, M., Wulff, P., Bock, C., Broberger, C., Deisseroth, K., Hokfelt, T., Linnarsson, S., Horvath, T.L., Harkany, T., 2017. Molecular interrogation of hypothalamic organization reveals distinct dopamine neuronal subtypes. Nat. Neurosci. 20, 176–188.
Romero-Ruiz, A., Avendano, M.S., Dominguez, F., Lozoya, T., Molina-Abril, H., Sangiao-Alvarellos, S., Gurrea, M., Lara-Chica, M., Fernandez-Sanchez, M., Torres-Jimenez, E., Perdices-Lopez, C., Abbara, A., Steffani, L., Calzado, M.A., Dhillo, W.S., Pellicer, A., Tena-Sempere, M., 2019a. Deregulation of miR-324/KISS1/kisspeptin in early ectopic pregnancy: mechanistic findings with clinical and diagnostic implications. Am. J. Obstet. Gynecol. (e1–480), e17
Romero-Ruiz, A., Skorupskaite, K., Gaytan, F., Torres, E., Perdices-Lopez, C., Mannaerts, B.M., Qi, S., Leon, S., Manfredi-Lozano, M., Lopez-Rodriguez, C., Avendano, M.S., Sanchez-Garrido, M.A., Vazquez, M.J., Pinilla, L., van Duin, M., Kohout, T.A., Anderson, R.A., Tena-Sempere, M., 2019b. Kisspeptin treatment induces gonadotropic responses and rescues ovulation in a subset of preclinical models and women with polycystic ovary syndrome. Hum. Reprod. 34, 2495–2512.
Ross, R.A., Leon, S., Madara, J.C., Schafer, D., Fergani, C., Maguire, C.A., Verstegen, A. M.J., Brengle, E., Kong, D., Herbison, A.E., Kaiser, U.B., Lowell, B.B., Navarro, V.M.,

2018. PACAP neurons in the ventral premammillary nucleus regulate reproductive function in the female mouse. Elife 7, e35960.
Ruiz-Pino, F., Navarro, V.M., Bentsen, A.H., Garcia-Galiano, D., Sanchez-Garrido, M.A., Ciofi, P., Steiner, R.A., Mikkelsen, J.D., Pinilla, L., Tena-Sempere, M., 2012. Neurokinin B and the control of the gonadotropic axis in the rat: developmental changes, sexual dimorphism, and regulation by gonadal steroids. Endocrinology 153, 4818–4829.
Ruiz-Pino, F., Garcia-Galiano, D., Manfredi-Lozano, M., Leon, S., Sanchez-Garrido, M.A., Roa, J., Pinilla, L., Navarro, V.M., Tena-Sempere, M., 2015. Effects and interactions of tachykinins and dynorphin on FSH and LH secretion in developing and adult rats. Endocrinology 156, 576–588.
Ruiz-Pino, F., Miceli, D., Franssen, D., Vazquez, M.J., Farinetti, A., Castellano, J.M., Panzica, G., Tena-Sempere, M., 2019. Environmentally relevant perinatal exposures to bisphenol A disrupt postnatal Kiss1/NKB neuronal maturation and puberty onset in female mice. Environ. Health Perspect 127, 107011.
Ruohonen, S.T., Gaytan, F., Gaudi, A.U., Velasco, I., Kukoricza, K., Perdices-Lopez, C., Franssen, D., Guler, I., Mehmood, A., Elo, L.L., Ohlsson, C., Poutanen, M., Tena-Sempere, M., 2022. Selective loss of kisspeptin signaling in oocytes causes progressive premature ovulatory failure. Hum Reprod (*Accepted; In press*).
Ruohonen, S.T., Poutanen, M., Tena-Sempere, M., 2020. Role of kisspeptins in the control of the hypothalamic-pituitary-ovarian axis: old dogmas and new challenges. Fertil Steril 114 (3), 465–474.
Salehi, S., Adeshina, I., Chen, H., Zirkin, B.R., Hussain, M.A., Wondisford, F., Wolfe, A., Radovick, S., 2015. Developmental and endocrine regulation of kisspeptin expression in mouse Leydig cells. Endocrinology 156, 1514–1522.
Samir, H., Nagaoka, K., Watanabe, G., 2018. Effect of kisspeptin antagonist on goat in vitro Leydig cell steroidogenesis. Theriogenology 121, 134–140.
Sanz, E., Quintana, A., Deem, J.D., Steiner, R.A., Palmiter, R.D., McKnight, G.S., 2015. Fertility-regulating Kiss1 neurons arise from hypothalamic POMC-expressing progenitors. J. Neurosci. 35, 5549–5556.
Satoh, A., Brace, C.S., Rensing, N., Cliften, P., Wozniak, D.F., Herzog, E.D., Yamada, K.A., Imai, S., 2013. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 18, 416–430.
Schaefer, J., Vilos, A.G., Vilos, G.A., Bhattacharya, M., Babwah, A.V., 2021. Uterine kisspeptin receptor critically regulates epithelial estrogen receptor alpha transcriptional activity at the time of embryo implantation in a mouse model. Mol. Hum Reprod. 27, gaab060.
Semaan, S.J., Kauffman, A.S., 2015. Daily successive changes in reproductive gene expression and neuronal activation in the brains of pubertal female mice. Mol. Cell Endocrinol. 401, 84–97.
Semaan, S.J., Dhamija, S., Kim, J., Ku, E.C., Kauffman, A.S., 2012. Assessment of epigenetic contributions to sexually-dimorphic Kiss1 expression in the anteroventral periventricular nucleus of mice. Endocrinology 153, 1875–1886.
Seminara, S.B., Messager, S., Chatzidaki, E.E., Thresher, R.R., Acierno Jr., J.S., Shagoury, J.K., Bo-Abbas, Y., Kuohung, W., Schwinof, K.M., Hendrick, A.G., Zahn, D., Dixon, J., Kaiser, U.B., Slaugenhaupt, S.A., Gusella, J.F., O'Rahilly, S., Carlton, M.B., Crowley Jr., W.F., Aparicio, S.A., Colledge, W.H., 2003. The GPR54 gene as a regulator of puberty. N Engl. J. Med. 349, 1614–1627.
Seraphim, C.E., Canton, A.P.M., Montenegro, L., Piovesan, M.R., Macedo, D.B., Cunha, M., Guimaraes, A., Ramos, C.O., Benedetti, A.F.F., de Castro Leal, A., Gagliardi, P.C., Antonini, S.R., Gryngarten, M., Arcari, A.J., Abreu, A.P., Kaiser, U.B., Soriano-Guillen, L., Escribano-Munoz, A., Corripio, R., Labarta, J.I., Travieso-Suarez, L., Ortiz-Cabrera, N.V., Argente, J., Mendonca, B.B., Brito, V.N., Latronico, A.C., 2021. Genotype-phenotype correlations in central precocious puberty caused by MKRN3 mutations. J. Clin. Endocrinol. Metab. 106, 1041–1050.
Sharma, A., Thaventhiran, T., Minhas, S., Dhillo, W.S., Jayasena, C.N., 2020. Kisspeptin and Testicular function-is it necessary? Int. J. Mol. Sci. 21, 2958.
Simavli, S., Thompson, I.R., Maguire, C.A., Gill, J.C., Carroll, R.S., Wolfe, A., Kaiser, U.B., Navarro, V.M., 2015. Substance p regulates puberty onset and fertility in the female mouse. Endocrinology 156, 2313–2322.
Skrapits, K., Borsay, B.A., Herczeg, L., Ciofi, P., Liposits, Z., Hrabovszky, E., 2015. Neuropeptide co-expression in hypothalamic kisspeptin neurons of laboratory animals and the human. Front. Neurosci. 9, 29.
Stamatiades, G.A., Kaiser, U.B., 2018. Gonadotropin regulation by pulsatile GnRH: Signaling and gene expression. Mol. Cell. Endocrinol. 463, 131–141.
Stephens, S.B.Z., Kauffman, A.S., 2021. estrogen regulation of the molecular phenotype and active translatome of AVPV kisspeptin neurons. Endocrinology 162, bqab080.
Steyn, F.J., Wan, Y., Clarkson, J., Veldhuis, J.D., Herbison, A.E., Chen, C., 2013. Development of a methodology for and assessment of pulsatile luteinizing hormone secretion in juvenile and adult male mice. Endocrinology 154, 4939–4945.
Tariq, A.R., Shabab, M., 2017. Effect of kisspeptin challenge on testosterone and inhibin secretion from in vitro testicular tissue of adult male rhesus monkey (Macaca mulatta). Andrologia 49 (1). https://doi.org/10.1111/and.12590.
Terasawa, E., Fernandez, D.L., 2001. Neurobiological mechanisms of the onset of puberty in primates. Endocr. Rev. 22, 111–151.
Tomikawa, J., Uenoyama, Y., Ozawa, M., Fukanuma, T., Takase, K., Goto, T., Abe, H., Ieda, N., Minabe, S., Deura, C., Inoue, N., Sanbo, M., Tomita, K., Hirabayashi, M., Tanaka, S., Imamura, T., Okamura, H., Maeda, K.-i., Tsukamura, H., 2012. Epigenetic regulation of Kiss1 gene expression mediating estrogen-positive feedback action in the mouse brain. Proc. Natl. Acad. Sci. USA 109 (20), E1294–E1301.
Toro, C.A., Wright, H., Aylwin, C.F., Ojeda, S.R., Lomniczi, A., 2018. Trithorax dependent changes in chromatin landscape at enhancer and promoter regions drive female puberty. Nat. Commun. 9, 57.
Torres, E., Velasco, I., Franssen, D., Heras, V., Gaytan, F., Leon, S., Navarro, V.M., Pineda, R., Candenas, M.L., Romero-Ruiz, A., Tena-Sempere, M., 2021. Congenital ablation of Tacr2 reveals overlapping and redundant roles of NK2R signaling in the control of reproductive axis. Am. J. Physiol. Endocrinol. Metab 320 (3), E496–E511.
Torsoni, M.A., Borges, B.C., Cote, J.L., Allen, S.J., Mahany, E., Garcia-Galiano, D., Elias, C.F., 2016. AMPKalpha2 in Kiss1 neurons is required for reproductive adaptations to acute metabolic challenges in adult female mice. Endocrinology 157, 4803–4816.
True, C., Verma, S., Grove, K.L., Smith, M.S., 2013. Cocaine- and amphetamine-regulated transcript is a potent stimulator of GnRH and kisspeptin cells and may contribute to negative energy balance-induced reproductive inhibition in females. Endocrinology 154, 2821–2832.
True, C., Nasrin Alam, S., Cox, K., Chan, Y.M., Seminara, S.B., 2015. Neurokinin B is critical for normal timing of sexual maturation but dispensable for adult reproductive function in female mice. Endocrinology 156, 1386–1397.
Ubuka, T., Son, Y.L., Tsutsui, K., 2016. Molecular, cellular, morphological, physiological and behavioral aspects of gonadotropin-inhibitory hormone. Gen. Comp. Endocrinol. 227, 27–50.
Ulasov, I., Borovjagin, A., Fares, J., Yakushov, S., Malin, D., Timashev, P., Lesniak, M.S., 2020. MicroRNA 345 (miR345) regulates KISS1-E-cadherin functional interaction in breast cancer brain metastases. Can. Lett. 481, 24–31.
Vazquez, M.J., Velasco, I., Tena-Sempere, M., 2019. Novel mechanisms for the metabolic control of puberty: implications for pubertal alterations in early-onset obesity and malnutrition. J. Endocrinol. 242 (2019) R51-R65.
Vazquez, M.J., Toro, C.A., Castellano, J.M., Ruiz-Pino, F., Roa, J., Beiroa, D., Heras, V., Velasco, I., Dieguez, C., Pinilla, L., Gaytan, F., Nogueiras, R., Bosch, M.A., Ronnekleiv, O.K., Lomniczi, A., Ojeda, S.R., Tena-Sempere, M., 2018. SIRT1 mediates obesity- and nutrient-dependent perturbation of pubertal timing by epigenetically controlling Kiss1 expression. Nat. Commun. 9, 4194.
Velasco, I., Leon, S., Barroso, A., Ruiz-Pino, F., Heras, V., Torres, E., Leon, M., Ruohonen, S.T., Garcia-Galiano, D., Romero-Ruiz, A., Sanchez-Garrido, M.A., Olhsson, C., Castellano, J.M., Roa, J., Poutanen, M., Pinilla, L., Vazquez, M.J., Tena-Sempere, M., 2019. Gonadal hormone-dependent vs. -independent effects of kisspeptin signaling in the control of body weight and metabolic homeostasis. Metabolism 98, 84–94.
Wen, J.P., Liu, C., Bi, W.K., Hu, Y.T., Chen, Q., Huang, H., Liang, J.X., Li, L.T., Lin, L.X., Chen, G., 2012. Adiponectin inhibits KISS1 gene transcription through AMPK and specificity protein-1 in the hypothalamic GT1-7 neurons. J. Endocrinol. 214, 177–189.
Wright, H., Aylwin, C.F., Toro, C.A., Ojeda, S.R., Lomniczi, A., 2021. Polycomb represses a gene network controlling puberty via modulation of histone demethylase Kdm6b expression. Sci. Rep. 11, 1996.
Xiang, W., Zhang, B., Lv, F., Ma, Y., Chen, H., Chen, L., Yang, F., Wang, P., Chu, M., 2015. The inhibitory effects of rfamide-related peptide 3 on luteinizing hormone release involves an estradiol-dependent manner in prepubertal but not in adult female mice. Biol. Reprod. 93, 30.
Yosefzon, Y., David, C., Tsukerman, A., Pnueli, L., Qiao, S., Boehm, U., Melamed, P., 2017. An epigenetic switch repressing Tet1 in gonadotropes activates the reproductive axis. Proc. Natl. Acad. Sci. USA 114, 10131–10136.