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Tachykinin signaling in the control of puberty onset.

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Abstract

The tachykinin family of peptides has emerged as a critical component of the central control of the reproductive axis. Mounting evidence suggests that neurokinin B (NKB) plays an essential role in sexual maturation and fertility by directly stimulating the release of kisspeptin, with the contribution of additional tachykinins (neurokinin A [NKA] and substance P [SP]) in the fine tuning of the activity of Kiss1 neurons. The expression of tachykinins increases in the hypothalamus before puberty and, therefore, they are considered as initiators of pubertal development by stimulating the awakening of Kiss1 neurons. This is supported by studies showing delayed or absent puberty onset in humans and mice devoid of tachykinin signaling, and the advancement of puberty onset in rodents subjected to chronic activation of tachykinin receptors. This review compiles the current knowledge on the role of tachykinins in the control of puberty onset.

Keywords

tachykinins; neurokinin; kisspeptin; puberty

1. What are tachykinins?

Tachykinins are a family of peptides that includes substance P (SP), neurokinin A (NKA), and neurokinin B (NKB)¹. While neurokinins are mostly restricted to the central nervous system, SP is also present in a number of peripheral tissues¹. These tachykinins bind preferentially to NK1R, NK2R and NK3R, respectively, although a large degree of cross-activation has been documented for all three ligand-receptor tachykinin systems^{1,2}. A number of neuropathophysiological processes have been related to the malfunction of tachykinin systems (mostly SP and NKB) in anxiety, learning, fear conditioning or cognitive behavior¹. However, a less characterized role of tachykinins pertained to their action within

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the hypothalamic-pituitary-gonadal (HPG) axis. A seminal publication in 2009 reporting that the loss of function of the genes encoding for NKB or NK3R in humans (*TAC3/TACR3*) led to hypogonadotropic hypogonadism, with a severe impairment in these patients to achieve puberty onset and fertility³, rapidly placed tachykinins in the spotlight for reproductive biologists.

Over the last decade, the characterization of the reproductive role of tachykinins has grown exponentially^{4,5}. Because of the human studies cited above, the NKB/NK3R system has received most of the attention in this area. This interest was further increased by the observation that NKB and its receptor, NK3R, co-localize in the majority of Kiss1 neurons of the arcuate nucleus of most studied mammalian species, including sheep, mice, primates and humans^{6–9}. The importance of this finding lies in the critical role of Kiss1 neurons for reproductive function.

Kiss1 neurons are located mostly in two distinct hypothalamic areas, the arcuate nucleus (ARH) (infundibular nucleus in humans) and the anteroventral periventricular area (AVPV/PeN)-almost exclusive to the female brain¹⁰. Studies from our lab and others have documented that while the co-expression of NKB and its receptor in Kiss1^{ARH} neurons is >90%, NKB is absent in Kiss1^{AVPV/PeN} neurons, with only a fourth of this population expressing NK3R^{8,11}. This distribution is indicative of the role of tachykinins in reproduction. Kiss1^{ARH} are considered the master regulatory component of the HPG axis⁴, particularly in the mediation of the negative feedback of sex steroids and the generation of the tonic (pulsatile) release of gonadotropin-releasing hormone (GnRH)⁴ from GnRH neurons. Regarding SP and NKA, because they are encoded by the same gene (*Tac1*), both peptides are cleaved from a precursor peptide in the same neurons, which are different from any of the Kiss1 neuron populations^{4,11}. Rather, *Tac1* neurons are sparse within the ARH but abundant in the adjacent ventral hypothalamic area (VMH)¹¹. While evidence that the VMH is the source of the SP and NKA that control GnRH release is currently missing, given the proximity to the ARH and their regulation by sex steroids in a similar manner to Kiss1^{ARH} neurons¹¹, i.e. inhibited, it is likely that the reproductive role of *Tac1* is played by, at least, VMH neurons. Importantly, ~50% of Kiss1^{ARH} neurons and ~25% of Kiss1^{AVPV/PeN} neurons express NK1R, while none of them express NKA's receptor (NK2R)¹¹, suggesting a) that SP likely plays a direct role on Kiss1 neurons, and b) the existence of yet unknown intermediate neurons in the action of NKA in reproduction. An important factor in the overall reproductive role of tachykinins is the limited expression of their receptors in GnRH neurons. Only a small fraction of these neurons express NK1R and NK3R¹¹ which, together with the absence of any effect on gonadotropin release in the absence of kisspeptin signaling^{11–13}, strongly supports a role for all tachykinins on, or above, Kiss1^{ARH} neurons.

In this context, these three tachykinins have been reported to have an overall stimulatory action on LH release in several species^{9,14–21}. However, the gonadotropic stimulatory action of NKA and NKB (but not SP) is dependent on the existence of circulating sex steroids, which turns inhibitory in their absence, e.g. after gonadectomy, when the central components of the HPG axis are hyper-stimulated by the absence of the inhibitory action of sex steroids^{5,11,21,22}. This phenomenon appears to be mediated by the increase in the inhibitory tone of dynorphin A^{5,21,23}. NKB appears to play the most fundamental role of all

tachykinins by serving as the “accelerator” of the GnRH pulse generator within Kiss1^{ARH} neurons in a process that is dependent on the level of sex steroids. NKB acts aut synaptically on Kiss1^{ARH} neurons to induce the release of a kisspeptin pulse. Every pulse is then ceased by the inhibitory action of dynorphin A on the same neurons. A number of chemo and opto-genetic studies have confirmed this role of Kiss1^{ARH} neurons as the GnRH pulse generator^{24–26}, which is critical for the attainment and maintenance of reproductive function, since it is highly dependent on the pattern of pulsatile GnRH release.

Given this significant regulatory action of tachykinins on gonadotropin release and the fact that humans and mouse models with inactivating mutations in one of these genes have delayed or absent puberty onset^{3,27–29}, there is currently a general consensus that tachykinin action is necessary for the proper maturation of the reproductive axis (i.e. puberty onset), however, the underlying mechanisms and overall contribution of tachykinins to this developmental process remain largely unknown.

2. What drives puberty onset?

Unraveling the molecular and physiological mechanisms that trigger puberty onset has been a major goal for neurobiologists and pediatricians for the last decades. Important advances have been made since the identification of the “reawakening” of GnRH pulsatility during the late juvenile period as the main event to kickstart sexual maturation³⁰. Since then, an extensive number of publications have characterized an ever-growing number of excitatory signals that get activated, and inhibitory signals that reduce their expression and tone on the HPG axis, either right before puberty onset, or during earlier developmental periods through the induction of epigenetic modifications that affect pubertal progression³¹.

As mentioned earlier, GnRH neurons are devoid of most of the necessary regulatory elements and receptors to accurately determine the time of puberty onset³⁰. Therefore, the existing consensus is that GnRH neurons are surrogated to the action of Kiss1 neurons during this process. So, *what determines the timing of the activation of Kiss1 neurons?* It is worth mentioning that while Kiss1 neurons are necessary for puberty onset, there is no clear evidence as to which population (ARH vs AVPV/PeN) plays a more determinant role in this process and even reports of puberty onset in the absence of Kiss1 neurons have been described³². In this context, it is important to highlight the fact that the population of Kiss1 neurons is highly redundant and as few as 5% of these neurons are sufficient to drive sexual maturation³³, which further emphasizes the need for a complete characterization of the role of each Kiss1 neuron population in this process. In rodents, within the whole hypothalamus, *Kiss1* expression increases as the timing of puberty onset approaches³⁴, however, more precise studies investigating the ARH vs the AVPV/PeN have demonstrated that *Kiss1* expression in the ARH does not vary significantly across puberty³⁵, while it increases in the preoptic area³⁶. Yet, this increase in *Kiss1* expression in the preoptic area may be a secondary effect of the rise in circulating sex steroids. In any case, it is possible that the increase in the kisspeptidergic tone on GnRH neurons happens only at the level of kisspeptin release without significant changes in the expression of the gene in the ARH. This hypothesis remains to be investigated.

3. What is the role of tachykinins in puberty onset?

Despite the lack of discernable changes in *Kiss1* expression in the ARH across puberty, that of *Tac1* and *Tac2* (encoding NKB in rodents) in the ARH have been documented to increase^{28,35}, leading to the hypothesis that tachykinins impose the initial activation of Kiss1 neurons to ramp up the pulsatile release of kisspeptin (and therefore GnRH and LH). This role would explain why NKB signaling deficient patients fail to undergo proper pubertal transition^{3,27}. However, unlike in the absence of kisspeptin signaling, where the hypogonadism prevents any possibility of reproduction in humans and mice^{37–39}, the reproductive phenotype of NKB signaling deficiency in these species is milder^{29,40}. First, a number of the human patients described above experience sporadic activation of the HPG axis that, in some cases, may even allow successful pregnancies⁴¹. Second, *Tac2* knockout (KO) and *Tacr3*KO mice display delayed puberty onset but overall preserved fertility^{29,40}. Together, these studies suggest that NKB signaling may be critical for the acute timing of puberty onset but, in its absence, compensatory mechanisms take place to overcome its deficit. However, there is evidence that specific NK3R antagonists inhibit the tonic release of LH release in male and female monkeys as well as in normal men and pre- and post-menopausal women^{42–46}, indicating that in these species compensation to the acute blockade of NKB signaling does not take place. While species differences between the primate and rodent models cannot be excluded, these data suggest that compensation for the absence of NKB signaling in primates only occurs developmentally after congenital deficiency.

In fact, we have observed that humans and mice with NKB deficiency retain the ability to release LH pulses^{47,48}, suggesting that Kiss1 neurons are still able to produce kisspeptin pulses in the face of NKB deficiency. Interestingly, *Tac2*KO mice present a higher frequency and amplitude of pulses in their gonad intact state and lower frequency and amplitude after gonadectomy (*unpublished data*). This reversed regulation of the LH pulse frequency (i.e. faster frequency in intact NKB deficient individuals) is similarly observed in *TAC3* mutant patients^{41,47}. Therefore, in situations of low sex steroid milieu, such as prepubertally or after gonadectomy, and absent NKB signaling, the GnRH pulse generator manifests a deceleration that leads to delayed puberty onset and delayed compensatory rise of LH after gonadectomy, at least in the mouse^{21,29,48}.

A 2019 optogenetic study in mice further validated the pivotal role of NKB and Dyn in the initiation and termination, respectively, of each kisspeptin/GnRH/LH pulse²⁶. This study demonstrated that NKB is sufficient to induce a kisspeptin pulse in the presence of sufficient basal activity of the neuron. However, it also presented the possibility that additional factors, from outside of Kiss1^{ARH} neurons, could modify this basal activity and, therefore, modulate the response of the neuron to NKB. Among these factors, we can include SP and NKA.

Because SP and NKA can stimulate gonadotropin release through the action on NK1R and NK2R, respectively, and through the documented cross-reactivity of the ligand-receptor systems (e.g. SP can also bind NK3R)², a plausible hypothesis is that NKB deficient patients and mice attained reproductive capabilities via the compensatory action of the remaining tachykinin systems. Interestingly, through the generation of double *Tac1/Tac2*KO mice, our

lab recently documented that under the complete absence of tachykinin signaling, the profile of LH release closely resembles that of *Tac2KO* mice, i.e. the presence of rudimentary LH pulses^{47,48}. Nonetheless, these pulses are sufficient to induce (delayed) puberty onset in males (preputial separation) and females (vaginal opening)^{29,48}. These makers suggest the existence of rising sex steroid levels, typical of pubertal progression. However, females failed to exhibit first estrus, which indicates a central deficit in pubertal transition⁴⁸. Altogether, these data suggest that without tachykinin signaling, compensatory mechanisms develop to allow a basal level of activity in Kiss1 neurons that leads to sufficient GnRH pulses to activate the HPG axis, albeit with a delay in timing compared to controls. Moreover, tachykinins may also play a role in the induction of ovulation and likely menarche in humans, which may be compensated between the different tachykinin systems, and therefore only exposed after the complete removal of all tachykinins⁴⁸.

4. Summary and conclusions

Overall, and despite initial expectations of the pivotal role of NKB (and by extension the other tachykinins) as a fundamental piece of the GnRH pulse generator, recent evidence from humans and rodent genetic models points to a less fundamental role of tachykinins than what surmises from the initial publications. While it is true that the reproductive impairments in human patients bearing NKB signaling deficiency are significantly more severe than in the equivalent mouse models, reversal of this phenotype is relatively frequent^{41,47}, and mice largely preserve fertility in the absence of NKB signaling^{29,40}. On one hand, this suggests that either compensatory mechanisms develop (which are independent of tachykinins, at least for the tonic release of GnRH) or that Kiss1 neurons are equipped with an intrinsic pulsatile mechanism that allows for a basal tone of GnRH release, sufficient to eventually induce gonadal activity. This latter contention is supported by the fact that Kiss1 neurons present a pattern of activity that is consistent with a pacemaker type of neuron⁴⁹. On the other hand, this ability of the central elements of the HPG axis to overcome the absence of a potent factor in the fine tuning of the activity of the Kiss1 and, by extension, GnRH neurons, speaks to the evolutionary forces that have led to the existence of a number of failsafe mechanisms to ensure the successful attainment of reproduction, crucial for the perpetuation of the species. Moreover, this “wobble room” in the action (and activation) of the different tachykinin systems between them and the participation of unknown factors that maintain the activity of Kiss1 neurons in their absence, opens the door to the development of novel approaches to treat reproductive impairments of central origin, offering new strategies to eventually modify the output of kisspeptin and GnRH release.

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REFERENCES

1. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. *Physiol Rev.* 2014;94(1):265–301. [PubMed: 24382888]

2. Parnet P, Mitsuhashi M, Turck CW, Kerdelhue B, Payan DG. Tachykinin receptor cross-talk. Immunological cross-reactivity between the external domains of the substance K and substance P receptors. *Brain Behav Immun*. 1991;5(1):73–83. [PubMed: 1712653]
3. Topaloglu AK, Reimann F, Guclu M, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*. 2009;41(3):354–358. [PubMed: 19079066]
4. Fergani C, Navarro VM. Expanding the Role of Tachykinins in the Neuroendocrine Control of Reproduction. *Reproduction*. 2016;153(1):R1–R14. [PubMed: 27754872]
5. Leon S, Navarro VM. Novel Biology of Tachykinins in Gonadotropin-Releasing Hormone Secretion. *Semin Reprod Med*. 2019;37(3):109–118. [PubMed: 31869838]
6. Goodman RL, Lehman MN, Smith JT, et al. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology*. 2007;148(12):5752–5760. [PubMed: 17823266]
7. Hrabovszky E, Ciofi P, Vida B, et al. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. *Eur J Neurosci*. 2010;31(11):1984–1998. [PubMed: 20529119]
8. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci*. 2009;29(38):11859–11866. [PubMed: 19776272]
9. Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM. Neurokinin B stimulates GnRH release in the male monkey (*Macaca mulatta*) and is colocalized with kisspeptin in the arcuate nucleus. *Endocrinology*. 2010;151(9):4494–4503. [PubMed: 20573725]
10. Gottsch ML, Cunningham MJ, Smith JT, et al. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*. 2004;145(9):4073–4077. [PubMed: 15217982]
11. Navarro VM, Bosch MA, Leon S, et al. The integrated hypothalamic tachykinin-kisspeptin system as a central coordinator for reproduction. *Endocrinology*. 2015;156(2):627–637. [PubMed: 25422875]
12. Garcia-Galiano D, van Ingen Schenau D, Leon S, et al. Kisspeptin signaling is indispensable for neurokinin B, but not glutamate, stimulation of gonadotropin secretion in mice. *Endocrinology*. 2012;153(1):316–328. [PubMed: 22067321]
13. Grachev P, Li XF, Lin YS, et al. GPR54-dependent stimulation of luteinizing hormone secretion by neurokinin B in prepubertal rats. *PLoS One*. 2012;7(9):e44344. [PubMed: 23028524]
14. Billings HJ, Connors JM, Altman SN, et al. Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. *Endocrinology*. 2010;151(8):3836–3846. [PubMed: 20519368]
15. de Croft S, Boehm U, Herbison AE. Neurokinin B activates arcuate kisspeptin neurons through multiple tachykinin receptors in the male mouse. *Endocrinology*. 2013;154(8):2750–2760. [PubMed: 23744641]
16. Endo N, Tanaka T. Effects of senktide, a neurokinin 3 receptor agonist, on luteinizing hormone secretion and follicular development in anestrous Shiba goats: a pilot study. *BMC Res Notes*. 2014;7:773. [PubMed: 25362998]
17. Garcia JP, Guerriero KA, Keen KL, Kenealy BP, Seminara SB, Terasawa E. Kisspeptin and Neurokinin B Signaling Network Underlies the Pubertal Increase in GnRH Release in Female Rhesus Monkeys. *Endocrinology*. 2017;158(10):3269–3280. [PubMed: 28977601]
18. Goodman RL, Coolen LM, Lehman MN. A role for neurokinin B in pulsatile GnRH secretion in the ewe. *Neuroendocrinology*. 2014;99(1):18–32. [PubMed: 24008670]
19. Jayasena CN, Comninou AN, De Silva A, et al. Effects of neurokinin B administration on reproductive hormone secretion in healthy men and women. *J Clin Endocrinol Metab*. 2014;99(1):E19–27. [PubMed: 24170109]
20. Nestor CC, Briscoe AM, Davis SM, Valent M, Goodman RL, Hileman SM. Evidence of a role for kisspeptin and neurokinin B in puberty of female sheep. *Endocrinology*. 2012;153(6):2756–2765. [PubMed: 22434087]

21. Leon S, Fergani C, Talbi R, et al. Characterization of the Role of NKA in the Control of Puberty Onset and Gonadotropin Release in the Female Mouse. *Endocrinology*. 2019;160(10):2453–2463. [PubMed: 31504389]
22. Fergani C, Leon S, Padilla SL, Verstegen AM, Palmiter RD, Navarro VM. NKB signaling in the posterodorsal medial amygdala stimulates gonadotropin release in a kisspeptin-independent manner in female mice. *Elife*. 2018;7.
23. Grachev P, Li XF, Kinsey-Jones JS, et al. Suppression of the GnRH pulse generator by neurokinin B involves a kappa-opioid receptor-dependent mechanism. *Endocrinology*. 2012;153(10):4894–4904. [PubMed: 22903614]
24. Clarkson J, Han SY, Piet R, et al. Definition of the hypothalamic GnRH pulse generator in mice. *Proc Natl Acad Sci U S A*. 2017;114(47):E10216–E10223. [PubMed: 29109258]
25. Han SY, McLennan T, Czielesky K, Herbison AE. Selective optogenetic activation of arcuate kisspeptin neurons generates pulsatile luteinizing hormone secretion. *Proc Natl Acad Sci U S A*. 2015;112(42):13109–13114. [PubMed: 26443858]
26. Voliotis M, Li XF, De Burgh R, et al. The Origin of GnRH Pulse Generation: An Integrative Mathematical-Experimental Approach. *J Neurosci*. 2019;39(49):9738–9747. [PubMed: 31645462]
27. Young J, Bouligand J, Francou B, et al. TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. *J Clin Endocrinol Metab*. 2010;95(5):2287–2295. [PubMed: 20194706]
28. Simavli S, Thompson IR, Maguire CA, et al. Substance p regulates puberty onset and fertility in the female mouse. *Endocrinology*. 2015;156(6):2313–2322. [PubMed: 25856429]
29. True C, Nasrin Alam S, Cox K, Chan YM, Seminara S. Neurokinin B is critical for normal timing of sexual maturation but dispensable for adult reproductive function in female mice. *Endocrinology*. 2015;en20141862.
30. Herbison AE, Porteous R, Pape JR, Mora JM, Hurst PR. Gonadotropin-releasing hormone neuron requirements for puberty, ovulation, and fertility. *Endocrinology*. 2008;149(2):597–604. [PubMed: 18006629]
31. Ojeda SR, Lomniczi A, Sandau U, Matagne V. New concepts on the control of the onset of puberty. *Endocr Dev*. 2010;17:44–51. [PubMed: 19955755]
32. Mayer C, Boehm U. Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. *Nat Neurosci*. 2011;14(6):704–710. [PubMed: 21516099]
33. Popa SM, Moriyama RM, Caligioni CS, et al. Redundancy in Kiss1 expression safeguards reproduction in the mouse. *Endocrinology*. 2013;154(8):2784–2794. [PubMed: 23736293]
34. Navarro VM, Castellano JM, Fernandez-Fernandez R, et al. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. *Endocrinology*. 2004;145(10):4565–4574. [PubMed: 15242985]
35. Gill JC, Navarro VM, Kwong C, et al. 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*. 2012;153(10):4883–4893. [PubMed: 22893725]
36. Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. *Endocrinology*. 2009;150(7):3214–3220. [PubMed: 19299459]
37. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A*. 2003;100(19):10972–10976. [PubMed: 12944565]
38. Funes S, Hedrick JA, Vassileva G, et al. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochem Biophys Res Commun*. 2003;312(4):1357–1363. [PubMed: 14652023]
39. Seminara SB, Messenger S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med*. 2003;349(17):1614–1627. [PubMed: 14573733]
40. Yang JJ, Caligioni CS, Chan YM, Seminara SB. Uncovering novel reproductive defects in neurokinin B receptor null mice: closing the gap between mice and men. *Endocrinology*. 2012;153(3):1498–1508. [PubMed: 22253416]

41. Gianetti E, Tusset C, Noel SD, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab.* 2010;95(6):2857–2867. [PubMed: 20332248]
42. Fraser GL, Hoveyda HR, Clarke IJ, et al. The NK3 Receptor Antagonist ESN364 Interrupts Pulsatile LH Secretion and Moderates Levels of Ovarian Hormones Throughout the Menstrual Cycle. *Endocrinology.* 2015;156(11):4214–4225. [PubMed: 26305889]
43. Skorupskaite K, George JT, Veldhuis JD, Anderson RA. Neurokinin B Regulates Gonadotropin Secretion, Ovarian Follicle Growth, and the Timing of Ovulation in Healthy Women. *J Clin Endocrinol Metab.* 2018;103(1):95–104. [PubMed: 29040622]
44. Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Neurokinin 3 receptor antagonism decreases gonadotropin and testosterone secretion in healthy men. *Clin Endocrinol (Oxf).* 2017;87(6):748–756. [PubMed: 28802064]
45. Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Neurokinin 3 Receptor Antagonism Reveals Roles for Neurokinin B in the Regulation of Gonadotropin Secretion and Hot Flashes in Postmenopausal Women. *Neuroendocrinology.* 2018;106(2):148–157. [PubMed: 28380486]
46. Fraser GL, Ramael S, Hoveyda HR, Gheyle L, Combalbert J. The NK3 Receptor Antagonist ESN364 Suppresses Sex Hormones in Men and Women. *J Clin Endocrinol Metab.* 2016;101(2):417–426. [PubMed: 26653113]
47. Lippincott MF, Leon S, Chan YM, et al. Hypothalamic Reproductive Endocrine Pulse Generator Activity Independent of Neurokinin B and Dynorphin Signaling. *J Clin Endocrinol Metab.* 2019.
48. Leon S, Fergani C, Talbi R, et al. Tachykinin signaling is required for the induction of the preovulatory LH surge and normal LH pulses. *Neuroendocrinology.* 2020.
49. Gottsch ML, Popa SM, Lawhorn JK, et al. Molecular properties of Kiss1 neurons in the arcuate nucleus of the mouse. *Endocrinology.* 2011;152(11):4298–4309. [PubMed: 21933870]

ANNOTATED BIBLIOGRAPHY

1. Clarkson J, Han SY, Piet R, McLennan T, Kane GM, Ng J, Porteous RW, Kim JS, Colledge WH, Iremonger KJ, Herbison AE. Definition of the hypothalamic GnRH pulse generator in mice. *Proc Natl Acad Sci U S A.* 2017;114(47):E10216–E10223. [PubMed: 29109258] This study is fundamental to understand the role of Kiss1 neurons as the GnRH pulse generator. Through a series of elegantly designed optogenetic studies in genetic mouse models, Clarkson J and colleagues demonstrate that changes in the activity of arcuate Kiss1 neurons closely correlate with changes in the pulsatile release of GnRH and LH.
2. Garcia JP, Guerriero KA, Keen KL, Kenealy BP, Seminara SB, Terasawa E. Kisspeptin and Neurokinin B Signaling Network Underlies the Pubertal Increase in GnRH Release in Female Rhesus Monkeys. *Endocrinology.* 2017;158(10):3269–3280. [PubMed: 28977601] This study clearly demonstrates that the tachykinin systems undergo developmental regulation in monkeys. Prepubertally, NKB and kisspeptin act independently to stimulate GnRH release. However, during puberty, NKB and kisspeptin form an interdependent system that requires the signaling of both systems in order to elicit proper GnRH release.
3. Fergani C, Leon S, Padilla SL, Verstegen AM, Palmiter RD, Navarro VM. NKB signaling in the posterodorsal medial amygdala stimulates gonadotropin release in a kisspeptin-independent manner in female mice. *Elife.* 2018;7. In this manuscript, the existence of a kisspeptin independent action of NKB to elicit gonadotropin release is demonstrated in a genetic mouse model. This action is dependent on the circulating level of sex steroids in females and takes place through the activation of NKB receptors in the medial amygdala. These findings may be critical to understand the mechanisms of tachykinin action and may offer the platform to overcome reproductive disorders with a central origin.
4. Leon S, Fergani C, Talbi R, Simavli S, Maguire CA, Gerutshang A, Navarro VM. Characterization of the Role of NKA in the Control of Puberty Onset and Gonadotropin Release in the Female Mouse. *Endocrinology.* 2019;160(10):2453–2463. [PubMed: 31504389] This study characterizes the role of NKA in the control of gonadotropin release, showing the similarities and differences with NKB and SP and demonstrating the ability of NKA to activate the HPG axis.

5. Lippincott MF, Leon S, Chan YM, Fergani C, Talbi R, Farooqi IS, Jones CM, Arlt W, Stewart SE, Cole TR, Terasawa E, Hall JE, Shaw ND, Navarro VM, Seminara SB. Hypothalamic Reproductive Endocrine Pulse Generator Activity Independent of Neurokinin B and Dynorphin Signaling. *J Clin Endocrinol Metab.* 2019. In this manuscript, the major finding of the existence of LH pulses in the absence of NKB and dynorphin signaling is presented in humans and rodent models. This study presents the challenging notion that kisspeptin and GnRH pulses may happen without the need of NKB and dynorphin activity, albeit at a lower (basal) level.