At the Cutting Edge

Neuro
-endocrinology

Neuroendocrinology 2015;102:200–215
DOI: 10.1159/000431021

Received: December 15, 2014
Accepted after revision: April 29, 2015
Published online: May 7, 2015

Shaping the Reproductive System: Role of Semaphorins in Gonadotropin-Releasing Hormone Development and Function

Paolo Giacobini

Inserm, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Jean-Pierre Aubert Research Centre, U1172, School of Medicine, University of Lille, and Institut de Médecine Prédictive et de Recherche Thérapeutique, IFR114, Lille, France

Key Words
Gonadotropin-releasing hormone · Reproduction · Neuronal plasticity · Cell migration · Development

Abstract
The semaphorin proteins, which contribute to the morphogenesis and homeostasis of a wide range of systems, are among the best-studied families of guidance cues. Much recent research has focused on the role of semaphorins in the development and adult activity of hormone systems and, reciprocally, how circulating reproductive hormones regulate their expression and function. Specifically, several reports have focused on the molecular mechanisms underlying the effects of semaphorins on the migration, survival and structural and functional plasticity of neurons that secrete gonadotropin-releasing hormone (GnRH), essential for the acquisition and maintenance of reproductive competence in mammals. Alterations in the development of this neuroendocrine system lead to anomalous or absent GnRH secretion, resulting in heterogeneous reproductive disorders such as congenital hypogonadotropic hypogonadism (CHH) or other conditions characterized by infertility or subfertility. This review summarizes current knowledge of the role of semaphorins and their receptors on the development, differentiation and plasticity of the GnRH system. In addition, the involvement of genetic deficits in semaphorin signaling in some forms of CHH in humans is discussed.

Introduction

The normal development of the central nervous system depends on the accurate migration of neurons from their site of production to their final location and their appropriate integration into functional networks. Among the numerous classes of proteins that guide this neuronal migration, one of the largest, that of the semaphorins, is phylogenetically conserved across species from nematodes and insects to vertebrates, including humans. Despite their initial identification in the nervous system, the semaphorins and their receptors, the plexins and neuropilins, are involved in a wide variety of developmental and pathological processes, including the development of the cardiovascular system, the immune response and tumor progression [reviewed in 1–9]. Among their various functions, semaphorins and their receptors play a key role in the central neuroendocrine regulation of reproduction by controlling the establishment of the neural circuitry

KARGER 125

0028–3835/15/1023–0200$39.50/0

E-Mail karger@karger.com
www.karger.com/nen

Paolo Giacobini
Inserm Unit 1172, Bâtiment Biserte
Place de Verdun, FR–59045 Lille Cedex (France)
E-Mail paolo.giacobini@inserm.fr

responsible for the secretion of gonadotropin-releasing hormone (GnRH), a decapeptide that acts as the 'master molecule' controlling fertility.

GnRH-secreting neurons in vertebrates originate outside the brain, in the nasal placode [10], during embryonic life, and migrate into the brain along the olfactory/vomeronasal and terminal nerves to their principal target region, the preoptic area of the hypothalamus [11, 12] (fig. 1a). These neurons are then integrated into the network of neurons and glia responsible for the timely secretion of GnRH into the pituitary portal circulation, which carries the neurohormone into the anterior pituitary, where it stimulates the release of gonadotropins from specialized cells, the gonadotropes. The gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone in turn act on peripheral reproductive organs to regulate the onset of puberty, gametogenesis and estrous cycling [13]. The abnormal development or function of this hypothalamic-pituitary-gonadal axis leads to GnRH deficiency in humans, i.e. congenital hypogonadotropic hypogonadism (CHH), a condition characterized by incomplete or absent puberty and infertility [14]. Understanding the mechanisms regulating the correct development and functioning of the GnRH neural network is thus key to understanding the pathogenesis of human reproductive disorders and devising appropriate therapeutic strategies.

In this review, I will provide an overview of current knowledge regarding the involvement of semaphorins and their receptors in the establishment of the rodent and human GnRH system, and specifically the motility and survival of these neurons as well as the periodic growth and retraction of their axons, necessary for the coordinated release of GnRH into pituitary portal blood vessels during appropriate phases of the estrous cycle. In addition, I will present evidence for the regulation of semaphorin expression in the hypothalamus by reproductive hormones, and the significance of these findings to our understanding of the functional plasticity of the GnRH system and the pathophysiology of reproductive disorders.

Semaphorin Expression and Role in the Development of the Olfactory/GnRH Systems

The GnRH neuronal migratory process is one of the best-characterized examples of axonophilic migration in the forebrain [15]. GnRH neurons complete their differentiation within the olfactory/vomeronasal placode during early embryonic stages and migrate along the nasal septum and the cribriform plate, and proceed into the forebrain along the vomeronasal nerves (VNNs)/terminal nerves (TNs) [11, 12, 16] (fig. 1a). From there, they send projections to the median eminence (ME), where they secrete their neurohormone into the pituitary portal circulation for the activation of pituitary gonadotropes.

The list of potential signaling molecules responsible for the correct migratory process and targeting of GnRH neurons to the final hypothalamic target areas has lengthened during the last decade [10, 17, 18]. However, even the large number of molecules identified so far likely underestimates the complexity of the potential interactions involved. Indeed, GnRH neurons spatially and temporally travel across areas (e.g. the nasal region, nasal-forebrain junction and forebrain; fig. 1a), each containing a variety of guidance molecules and factors. In addition, many molecules defy anatomical boundaries by functioning in multiple areas and may induce different responses depending on the receptor complexes expressed by GnRH neurons as a function of time (embryonic stage) and space (anatomical localization).

The development of the olfactory/vomeronasal system and of the GnRH system are intimately intertwined, and several semaphorins are expressed in the developing olfactory/vomeronasal system and along the GnRH migratory route during embryonic life (fig. 1b) [19–29]. Indeed, it is well established that the guidance provided by the olfactory/vomeronasal axonal pathway is an important prerequisite for the establishment of an adult pattern of GnRH neuron distribution [10]. However, little is known as to what controls the complex spatiotemporal events involved in coordinating these diverse signals produced by olfactory/vomeronasal axons and the response of GnRH neurons to them, or even how the expression of semaphorin receptors is regulated in these neurons, although recent studies have begun to elucidate some of the complex molecular mechanisms involved (fig. 1c).

Class 3 Semaphorins

The well-characterized class 3 secreted semaphorins act as chemorepellents for specific yet partially overlapping populations of developing neurons. These semaphorins bind to neuropilins (Nrp), which act as ligand-binding semaphorin co-receptors, and signal through another class of receptors, the plexins. In addition, the semaphorin receptor complex includes other modulatory elements, resulting in the potential for unique and context-specific signaling properties despite the overlapping expression of several related molecules [5]. Four class 3 semaphorins, Sema3A, Sema3B, Sema3C and Sema3F, are expressed in and around the developing olfactory/vomeronasal system

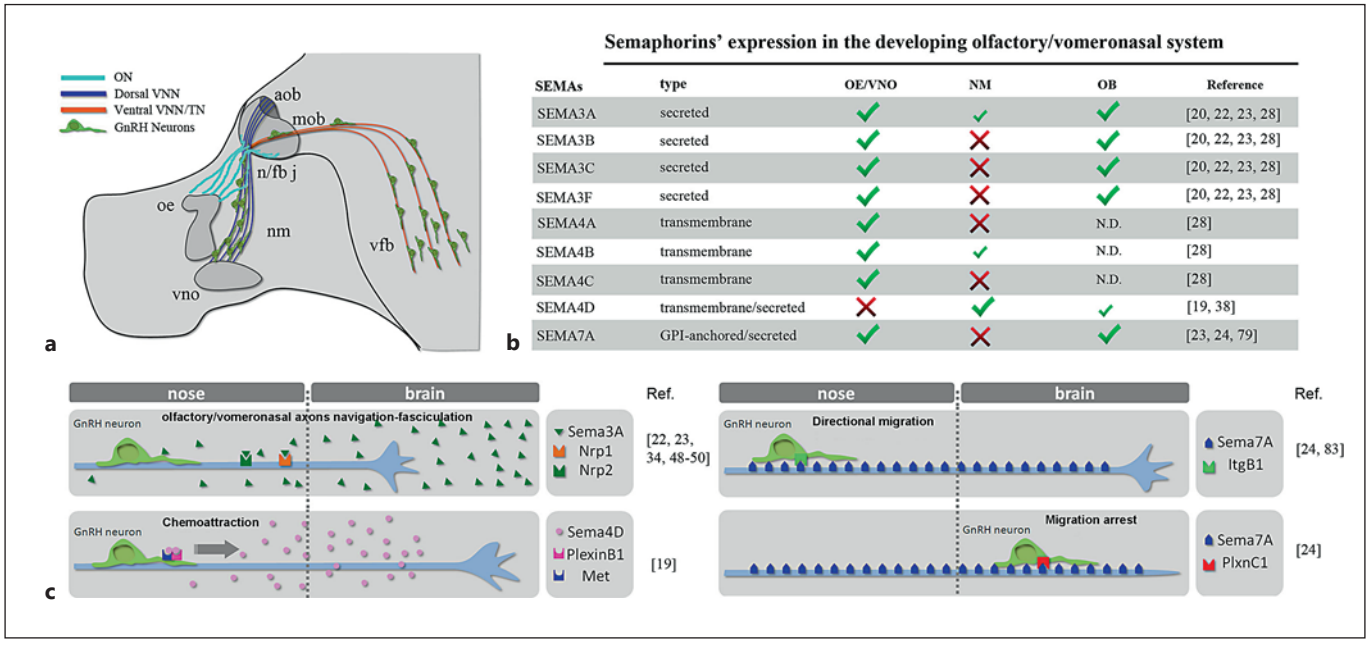


Fig. 1. The migratory route of GnRH neurons and expression/role of semaphorins. **a** Schematic representation of the head of a mouse embryo at E14.5, depicting the scaffold formed by the olfactory nerve (ON) and VNN/TN, along which GnRH cells migrate from the nose to the ventral forebrain. oe = Olfactory epithelium; vno = vomeronasal organ; nm = nasal mesenchyme; n/fb j = nasal/forebrain junction; aob = accessory olfactory bulb; mob = main olfactory bulb; vfb = ventral forebrain. **b** Different semaphorins expressed in the developing nasal region. **c** Mechanisms of action of the semaphorins indicated on GnRH neuron motility and/or navigation along olfactory/vomeronasal nerves. Adapted from Messina and Giacobini [145] with permission.

[20, 22, 23, 28] as well as in the target regions of the olfactory and vomeronasal nerves, namely the main and accessory olfactory bulb (fig. 1b). The neuropilins and plexins are concomitantly expressed in the olfactory system: Nrp1 and 2, the specific co-receptors of the class 3 semaphorins, are expressed by sensory neurons in the main and accessory olfactory epithelia (OE) of rodents and zebrafish [20–22, 30], while PlexinA1 is robustly expressed in the vomeronasal organ (VNO) and the VNNs [31]. It has become clear that repulsive guidance mechanisms play an essential role in axonal pathfinding and target recognition, and several studies have implicated the class 3 semaphorins in the guidance and fasciculation of olfactory and vomeronasal neurons [22, 23, 28, 32, 33]. These semaphorins, which are secreted, can thus act by local diffusion, steering growing axons out of regions in which they are released and thereby channeling them to the correct target areas. Interestingly, in the absence of signaling of class 3 semaphorins through their receptors, the structure and function of the GnRH system are altered. For instance, mice knocked out for Nrp2 *(Nrp2*$^{-/-}$*)*, the receptor for secreted Sema3F [34], display an abnormal accumulation of GnRH neurons in the nasal compartment, potentially due to the defasciculation of olfactory/vomeronasal axons [21, 34] and the resulting failure of the neurons to migrate to their forebrain destinations (fig. 1c). Consistent with this deficit of GnRH neurons at their final location, these *Nrp2*$^{-/-}$ mice are typically infertile [21, 35].

Sema3A is a secretory protein with repulsive effects on primary olfactory axons expressing the co-receptor Nrp1 [23, 33, 36]. It is strongly expressed in the developing OE and vomeronasal epithelium, in the olfactory bulb and, to a lesser extent, in the nasal mesenchyme (fig. 1b). Interestingly, in this region, Sema3A is also expressed by olfactory ensheathing cells (OECs), which enwrap and guide olfactory nerves toward the olfactory bulbs [23, 37]. During embryonic development, GnRH neurons travel together with other neuronal cells apposed to growing fibers and OECs both in vivo and in vitro, forming the so-called migratory mass that emerges from the presumptive VNO [38–40].

Recently, two groups have shown that OECs are neural crest derivatives [40–42], challenging the dogma that the olfactory system is composed of only placodal derivatives and offering new insights into human reproductive pathologies such as Kallmann's syndrome (KS), an inherit-

ed developmental disease that often includes multiple neural crest defects. Today, it is well established that some forms of KS involve the failure of olfactory/TN fibers to establish proper contact with the forebrain. In this context, it is clear that the neural crest-derived OECs could be important players in both normal and abnormal olfactory development and GnRH neuronal migration. As such, the role of these cells in the etiology of the defects observed in KS needs to be further investigated. Notably, several guidance molecules known to be crucial for controlling GnRH neuronal migration, such as NELF/Jacob, SDF-1α, Sema3A and Sema4D [23, 43–45], are expressed by OECs [38, 46, 47] and this list is likely to lengthen in the coming years.

Sema3A is also expressed by migratory GnRH neurons in rodents, while the olfactory/vomeronasal axons along which they migrate into the brain express Nrp1 [48, 49], as does the caudal branch of the VNN/TN [50]. We have recently not only confirmed these findings in the E14.5 mouse embryos, but extended them to humans, with the observation of similar immunofluorescence patterns in the brain of a 9-week-old human fetus [50] (fig. 2a). Nevertheless, much still needs to be elucidated regarding the role of semaphorins in the navigation of GnRH neurons and of the VNNs during embryonic life.

We and others have recently shown, using in vitro experiments and mouse genetics, that Sema3A signals through both Nrp1 and Nrp2 to control the development of the GnRH system [48–50]. The lack of either of these receptors or Sema3A leads to a fetal KS-like phenotype in mice [51], where GnRH neurons and vomeronasal axons fail to enter the brain but accumulate at the dorsal surface of the cribriform plate [48–50]. The aberrant projection of the VNNs in the absence of Sema3A signaling leads to considerable abnormal cell migration in these mutants [50] (fig. 2b, c). In addition, in mice lacking a functional semaphorin binding domain in Nrp1 (*Nrp1*$^{sema/sema}$ mice; fig. 2d–f), the labeling of axons with DiI at E14.5 reveals the abnormal projections of the VNN/TN in the ventral forebrain. The normal distribution of GnRH neurons between the nose and brain and their adult numbers in conditional mutant mice lacking Nrp1 only in GnRH neurons (*GnRH::cre;Nrp1*$^{loxP/loxP}$ mice) [50] further confirms that the defective migration of GnRH neurons in these embryos is due to the abnormal routing of VNN/TN into the ventral forebrain, as remarked above, and is not a cell-autonomous trait (fig. 2d, f). Moreover, in *Nrp1*$^{sema/sema}$ newborn mice, many axons of olfactory receptor neurons also remain stuck at the dorsal aspect of the cribriform plate and do not project into the olfactory bulb glomeruli (fig. 2f, g), a characteristic that resembles the hallmark olfactory defects of KS.

Neuropilins, in addition to their role in semaphorin signaling, also act as receptors for vascular endothelial growth factor (VEGF) [52–54], a molecule that plays a key role in vascular development and angiogenesis under both physiological and pathological conditions [55–58]. It has been shown that Sema3A-mediated axon guidance cooperates with the alternative Nrp1 ligand VEGF164, which ensures that migrating GnRH neurons reach the brain by mediating neuronal survival [49]. However, while it was previously assumed that (1) given the lack of an intracellular catalytic domain, Nrp1 used KDR as a co-receptor for the transduction of VEGF-mediated signals [59], and (2) the neuronal survival-promoting effects of VEGF were thus mediated by KDR [60–62], these assumptions have been overturned by a study by Cariboni et al. [49] demonstrating that this survival signaling relies on neuronal and not endothelial Nrp1 expression and occurs independently of KDR, the main VEGF receptor in blood vessels. Instead, VEGF164 signaling in migrating GnRH neurons and its promotion of their survival occur via the co-activation of ERK and AKT signaling pathways through Nrp1.

Class 4 Semaphorins

The role of the transmembrane semaphorins Sema4A, Sema4B and Sema4C in the development of the olfactory system is unclear at present, even though these molecules are highly expressed in the main OE toward the end of embryonic development in rodents (E16–E19) [28]. Given their temporal and spatial distribution, it has been hypothesized that they might regulate the timing of olfactory axon entry into the olfactory bulb and/or the formation of synapses between olfactory receptor neurons and mitral cells [28]. This is probably true for Sema4C, which is known to bind to proteins involved in neurite outgrowth and synapse formation [63, 64].

Sema4D exists in two forms, a membrane-bound form and a soluble active form that is 'shed' into the extracellular space by the proteolytic cleavage of the membrane-bound form [65, 66]. Among its many known roles, it acts as a signal triggering axonal growth cone collapse [67] and induces the chemotaxis of epithelial and endothelial cells. In addition, through the coupling of its receptor PlexinB1 with Met tyrosine kinase, the receptor for hepatocyte growth factor (HGF), it can also function as a pro-angiogenic factor [68–70]. Sema4D is present in the nasal mesenchyme, although its expression is higher at the nasal/forebrain junction (fig. 1b), while its receptor PlexinB1 is highly expressed not only in the developing nasal

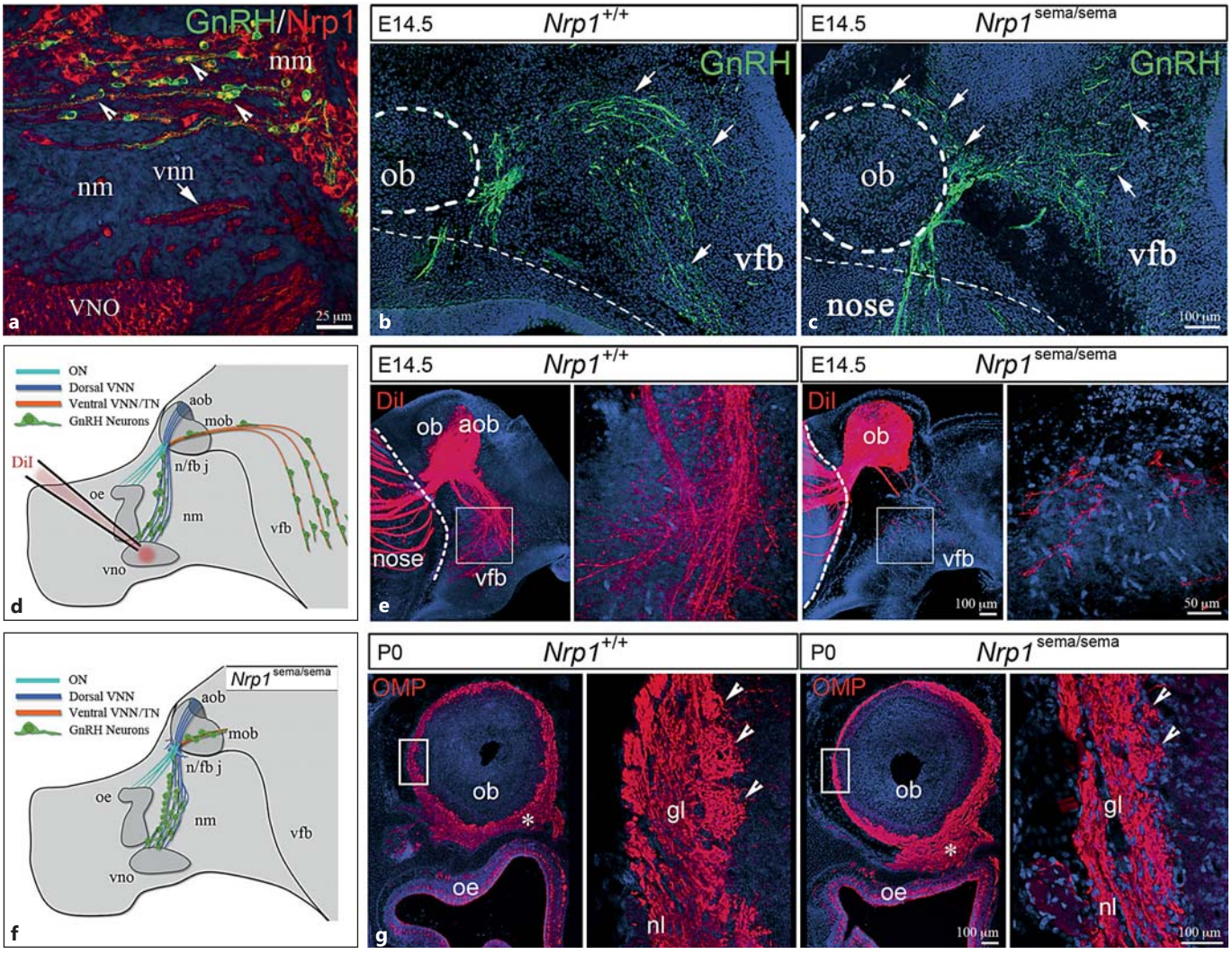


Fig. 2. Involvement of Nrp1 in GnRH neuron migration. **a** Sagittal section of the frontonasal region in a 9-week-old human fetus. In this region, GnRH cells (green) migrate in close contact with Nrp1-immunoreactive axons (red). Nrp1 immunoreactivity is also detectable in the VNO, along the vomeronasal nerve (vnn). Moreover, migrating GnRH cells (arrowheads) as well as other cellular elements belonging to the migratory mass (mm) are also Nrp1-immunoreactive. **b**, **c** Defects in GnRH cell migration in *Nrp1*$^{sema/sema}$ mutant mice at E14.5. **d** A crystal of the DiI lipophilic fluorescent dye was placed in the VNO lumen to anterogradely label vomeronasal axons. The VNN extends across the medial aspect of the olfactory bulb and projects both dorsally, to the accessory olfactory bulb, and caudally, to the ventral forebrain (vfb). **e** Sagittal sections of the rostral and ventral forebrain regions (left panels), and detail of the caudal branch of the VNN (right panels) in *Nrp1*$^{+/+}$ and *Nrp1*$^{sema/sema}$ E14.5 mouse embryos, 3 weeks later DiI injection. In mutant mice, fibers in the caudal branch are scarce compared to those in wild-type mice. **f** Schematic representation of the head of an *Nrp1*$^{sema/sema}$ mouse embryo at E14.5, summarizing the alterations in the olfactory nerve (ON)/VNN scaffold along which GnRH cells migrate from the nose to the ventral forebrain. **g** Coronal sections of the olfactory epithelium (oe) and olfactory bulb (ob; left panels), and detail of the olfactory bulb showing the olfactory nerve layer (nl) and glomerular layer (gl; right panels) in newborn (P0) *Nrp1*$^{+/+}$ and *Nrp1*$^{sema/sema}$ mice. Axons of olfactory receptor neurons are labeled (red) using an antibody directed against the olfactory marker protein (OMP). In the *Nrp1*$^{sema/sema}$ mouse, the immunolabeling is both more extensive beneath the ventromedial aspect of the olfactory bulb (asterisks) and markedly reduced in the glomerular layer (arrowheads) compared to wild-type mice. For other abbreviations, see the legend to figure 1. Adapted from Hanchate et al. [50] with permission.

placode but also by olfactory axons and GnRH cells during embryonic life [19] (fig. 1c). In addition, it has recently been shown that besides the nasal mesenchyme, the OECs represent a major source of Sema4D production at these anatomical locations [38].

Sema4D has been proposed to be involved in the guidance of GnRH neurons from the olfactory placode toward the forebrain through its binding to PlexinB1 [19]. An analysis of PlexinB1-deficient mice has revealed altered migration of GnRH neurons, although no abnormalities were found in the development or organization of olfactory axons [19], which suggests that the migratory defect might be cell-autonomous rather than dependent on alterations of the olfactory axonal pathway. Interestingly, it has been shown that reproduction is also impaired in Sema4D-knockout mice as a consequence of the significant decrease in hypothalamic GnRH cell population and/or reduced ovarian follicle maturation observed in these mutants [71]. Finally, in vitro functional experiments show that Sema4D promotes the directional migration of immortalized GnRH cells by coupling PlexinB1 with the activation of Met tyrosine kinase, the receptor for HGF (fig. 1c) [19], which has been previously shown to play an important role in ensuring correct GnRH neuronal migration [72]. Notably, the expression pattern of HGF in the nasal region of mouse embryos parallels that of Sema4D [72] and, indeed, an additive effect of HGF and Sema4D on Met activation and cell motility has been demonstrated [19]. These results suggest that in vivo, HGF and Sema4D might act in a combinatorial manner to allow the spatial fine-tuning of GnRH migration.

Semaphorin 7A

Semaphorin 7A (Sema7A) is the only glycophosphatidylinositol-linked member of the semaphorin family [reviewed in 73]. The pleiotropic nature of semaphorins is particularly evident for Sema7A, whose roles in immune function [74] and cancer biology [75–77] have been extensively studied. In addition, a few reports have addressed its role in neuronal development [78–82]. A study performed in our laboratory has revealed a role for Sema7A and its two receptors, PlexinC1 and β_1-integrin, in the regulation of GnRH cell motility [24]. Sema7A binds to PlexinC1 to decrease integrin-mediated cell attachment and spreading [76], and its interaction with β_1-integrin induces integrin clustering and the activation of MAPK pathways [80]. We have also shown that Sema7A is highly expressed in the nasal pit, where GnRH neurons begin their migration into the brain, and along the olfactory/vomeronasal scaffold during embryonic development in mice [24]. Moreover, the expression pattern of the two Sema7A receptors in GnRH neurons appears to be spatiotemporally regulated: at early stages, migrating GnRH neurons only express β_1-integrin, whereas they begin to express PlexinC1 during subsequent developmental stages and in anatomical areas where these cells stop migrating [24].

Semaphorin signaling is multifaceted, with subsets of these ligands (e.g. Sema4D, Sema6D and Sema7A) eliciting such diverse effects as integrin activation/cell-substrate adhesion, axon outgrowth and cell chemotaxis under distinct conditions [2]. While the molecular mechanisms underlying these mutually antagonistic activities have not yet been fully elucidated, they appear to be mediated by distinct signaling pathways that differ depending on the cell type targeted and the composition of their receptor complexes. For example, Sema7A increases directional migration in immortalized GnRH cells through a β_1-integrin-dependent pathway by stimulating the rapid phosphorylation of FAK and ERK1/2 (fig. 1c). In contrast, the overexpression of PlexinC1 in GnRH neurons stops their migration [24] (fig. 1c). Moreover, in vitro also, primary GnRH neurons differentially express β_1-integrin and PlexinC1 as a function of migratory stage, with PlexinC1 being upregulated in postmigratory neurons [24]. This switch may be essential for the proper guidance of migrating neurons into the hypothalamus. It is unknown how PlexinC1 expression is induced in migratory GnRH neurons. One possibility is that molecular cues presented by intermediate targets such as the cribriform plate regulate this switch in receptor expression.

The relevance of Sema7A signaling in the correct development of the GnRH system has been confirmed by in vivo studies showing that both the loss of *Sema7A* expression and the conditional inactivation of β_1-integrin in GnRH neurons impact the development of this system, resulting in the significant reduction of the GnRH neuronal population in the brain of adult mice, as well as reduced gonadal size and altered fertility [24, 83].

Semaphorin Mutations in Human Hypogonadotropic Hypogonadism

As mentioned earlier, the abnormal development or function of the hypothalamic-pituitary-gonadal axis leads to hypogonadotropic hypogonadism in humans, characterized by the absence of GnRH secretion and subfertility or infertility. Several disorders affecting this axis are inheritable or congenital. Congenital GnRH deficiency, i.e. CHH, is characterized by absent or incomplete sexual mat-

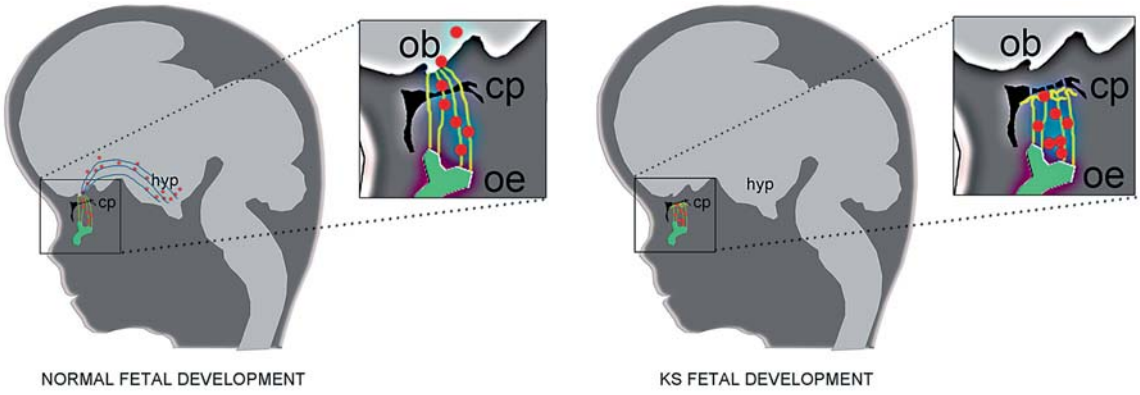
uration and low circulating levels of gonadotropins and sex steroid hormones; however, the structure (in imaging studies) and function of the pituitary remain normal [84, 85]. While in some forms of CHH, the sense of smell remains unaffected (normosmic hypogonadotropic hypogonadism), patients with KS also display anosmia/hyposmia. Both types of hypogonadism are marked by anomalies in the embryonic development of the GnRH system, which shares a common ontogenic history with the olfactory system (fig. 3a). In this respect, the use of animal models has been tremendously helpful in showing that semaphorin signaling is crucial for the migration, survival and maturation of GnRH neurons, and that several genes of the semaphorin family may be mutated in individuals affected by different forms of reproductive insufficiency.

Mutations affecting several disease-causing genes have been shown to be associated with the onset of CHH or KS [86], and these include Anosmin-1 (or *KAL1*) [87, 88], *FGFR1* [89], *FGF8* [90], *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, *FLRT3* [91], *GNRH1* [92, 93]/*GNRHR* [84, 94], *KISS1* [95]/*KISS1R* [96, 97], *TAC3/TACR3* [98], *NELF* [99], *PROK2*, *PROKR2* [100, 101], *CHD7* [102], *HS6ST1* [103], *WDR11* [104], *FEZF1* [105], *SOX10* [106], *SEMA3A* [50, 107, 108] and *SEMA7A* [108].

However, these mutations account for only 30–40% of CHH/KS patients [109]. Efforts are therefore ongoing to identify other genes that could contribute to this disorder, in particular by undertaking the study of genetically modified mice that reproduce the human KS phenotype. For instance, as mentioned above, $Nrp1^{sema/sema}$ mutant mice, which lack the semaphorin binding domain in Nrp1, possess a KS-like phenotype. Concordantly, inadequate Sema3A signaling appears to contribute to human KS [50], with 8 different mutations in the *SEMA3A* gene being identified in 24 of the 386 KS patients studied (approx. 6%). Interestingly, these mutations were consistently observed in the heterozygous state, and 5 patients carried additional heterozygous mutations in other identified KS-related genes: *PROKR2*, *PROK2*, *KAL1* and *FGFR1* (fig. 3b). Three missense changes in *SEMA3A* have also been identified recently in 3 probands with KS belonging to a Finnish cohort [108], of which 2 were identical to mutations previously reported by us [50] (fig. 3b). In the same study, these authors also reported two rare heterozygous variants of the *SEMA7A* gene in 1 CHH patient with a previously identified *KISS1R* non-sense variant and 1 KS patient carrying a mutation in *KAL1* [108].

Young et al. [107] have reported a large heterozygous deletion of 213 kb encompassing 11 of the 17 exons in *SEMA3A* in 2 siblings and their clinically affected father (fig. 3b). These authors have proposed that the heterozygous *SEMA3A* deletion might be sufficient to cause KS since no additional mutations were detected and the deletion co-segregated within the family with an apparent autosomal dominant transmission of the KS phenotype [107]. On the other hand, in our previous study, we concluded that monoallelic mutations in *SEMA3A* were not sufficient to cause the disease phenotype based on the fact that all the missense variants detected were previously reported in the EVS database and that some patients also carried mutations in other known KS genes [50]. These findings indicate that *SEMA3A* might be a novel contributory gene in KS, and further substantiate the oligogenic pattern of inheritance in this developmental disorder [110, 111]. This hypothesis has been further confirmed in a recent study in which nonsynonymous *SEMA3A* variations have also been identified in CHARGE patients [112]. CHARGE syndrome, thought to be caused by mutations in chromodomain helicase DNA binding protein-7 *(CHD7)*, includes eye coloboma, heart malformations, atresia of the choanae, retardation of growth/development, genital anomalies and ear abnormalities [113]. However, CHARGE patients may present with anosmia and/or hypogonadism, features that overlap with CHH and KS. Similarly, some CHH/KS patients also display certain CHARGE features. It has therefore been hypothesized that KS represents a milder allelic variation of CHARGE syndrome, a hypothesis supported by the identification of heterozygous *CHD7* mutations in CHH/KS individuals [102, 113]. However, as with genes identified in CHH/KS, in 5–10% of typical CHARGE patients, no *CHD7* mutation has been detected [114]. Recently, Schulz et al. [112] have reported nonsynonymous *SEMA3A* variations in 3 out of 45 *CHD7*-negative CHARGE patients (fig. 3b), and have suggested that *CHD7* mutations alone are not sufficient to produce the CHARGE phenotype. Instead, they propose an important modifier role for *SEMA3A* in the pathogenesis of this multiple malformative syndrome. Indeed, in the same work these authors have also undertaken a genome-wide microarray expression analysis of wild-type and Chd7-deficient ($Chd7^{Whi/+}$ and $Chd7^{Whi/Whi}$) mouse embryos at day 9.5, a time point important for neural crest cell migration, and have identified 98 differentially expressed genes between wild-type and $Chd7^{Whi/Whi}$ embryos. Many of the misregulated genes are involved in neural crest cell migration, guidance and ectoderm/neural crest cell interactions, including genes such as *Sema3A*, *Sema3C*, *Sema3D* and the Ephrins [112].

Finally, a mutation in another class 3 semaphorin, *SEMA3E*, has also been reported in an individual with



a NORMAL FETAL DEVELOPMENT KS FETAL DEVELOPMENT

	Gene	Nucleotide change (c)/ Deletion (del)/ Translocation (t)	Amino Acid change	Reproductive phenotype	Nonreproductive phenotype	Additional genes mutated in the patient	Reference
	SEMA3A	c.197C>T	p.R66W	KS			[50]
	SEMA3A	c.458A>G	p.N153S	KS	Cleft lip and dental agenesis	*FGFR1*	[50, 108]
	SEMA3A	c.1198A>G	p.I400V	KS		*PROKR2*	[50]
	SEMA3A	c.1303G>A	p.V435I	KS		*PROKR2 PROK2 FGFR1*	[50, 108]
	SEMA3A	c.del1613_1626	p.D538fsX31	KS		*Not detected*	[50]
	SEMA3A	c.2062A>G	p.T688A	KS		*KAL1*	[50]
	SEMA3A	c.2189G>A	p.R730Q	KS		*Not detected*	[50]
	SEMA3A	c.2198G>A	p.R733H	KS		*Not detected*	[50]
	SEMA3A	213 kb at 7q21.11 (Del. Exons 6-17)		KS		*Not detected*	[107]
	SEMA3A	c.1253A>G	p.N418S	KS		*FGFR1*	[108]
	SEMA3A	c.196C>T	p.R66W	CHARGE		*Not detected*	[112]
	SEMA3A	c.2002A>G	p.I668V	CHARGE		*Not detected*	[112]
	SEMA3A	c.2062A>G	p.T688A	CHARGE		*Not detected*	[112]
	SEMA3E	t(2;7)(p14;q21.11)		CHARGE		*Not detected*	[115]
	SEMA3E	c.2108C>T	p.S703L	CHARGE		*Not detected*	[115]
b	*SEMA7A*	c.1421G>A	p.R474Q	KS		*KAL1*	[108]

Fig. 3. Semaphorin mutations. **a** Distribution of GnRH-1-immunoreactive cells (red dots) in the frontonasal region and forebrain of normal human fetuses and in KS fetuses. Curved lines indicate the path of the olfactory (yellow lines) and vomeronasal/terminal (blue lines [pers. obs.]) nerve fibers. In control fetuses, , GnRH-1-expressing cells are distributed all along the migratory route from the frontonasal regions to the presumptive hypothalamus. In KS fetuses, GnRH-1 cells accumulate along the discontinued path of olfactory and terminal nerve fibers that do not make contact with the forebrain. Few or no neuroendocrine cells reach the preoptic/hypothalamic region. **b** Mutations detected in semaphorin genes in humans affected by KS and CHARGE syndrome. Mutations in *SEMA3A*, *SEMA3E* and *SEMA7A* have been reported in KS and CHARGE patients.

CHARGE syndrome [115], further strengthening the relevance of semaphorin signaling in both neural crest cell and axon guidance.

Semaphorins and Neuroglial Plasticity in the Adult Hypothalamic Median Eminence

Over the past two decades, it has become clear that GnRH terminals of the ME undergo dynamic transformations as a function of gonadectomy [116] as well as of fluctuating physiological conditions that influence the distance between GnRH terminals and the basal lamina [117].

Remarkably, both GnRH neurons and the multiple neuronal networks involved in the control of GnRH secretion are subject to direct modulation by peripheral gonadal steroids [118–121]. During the ovarian cycle, under conditions of low gonadotropin output, GnRH-secreting axon terminals are distant from the pericapillary space of the ME, thus impairing the access of the neurohormone to the pituitary portal circulation, but they undergo extensive axonal growth toward the vascular wall at the onset of the preovulatory surge, when massive GnRH release has to occur to trigger ovulation [122].

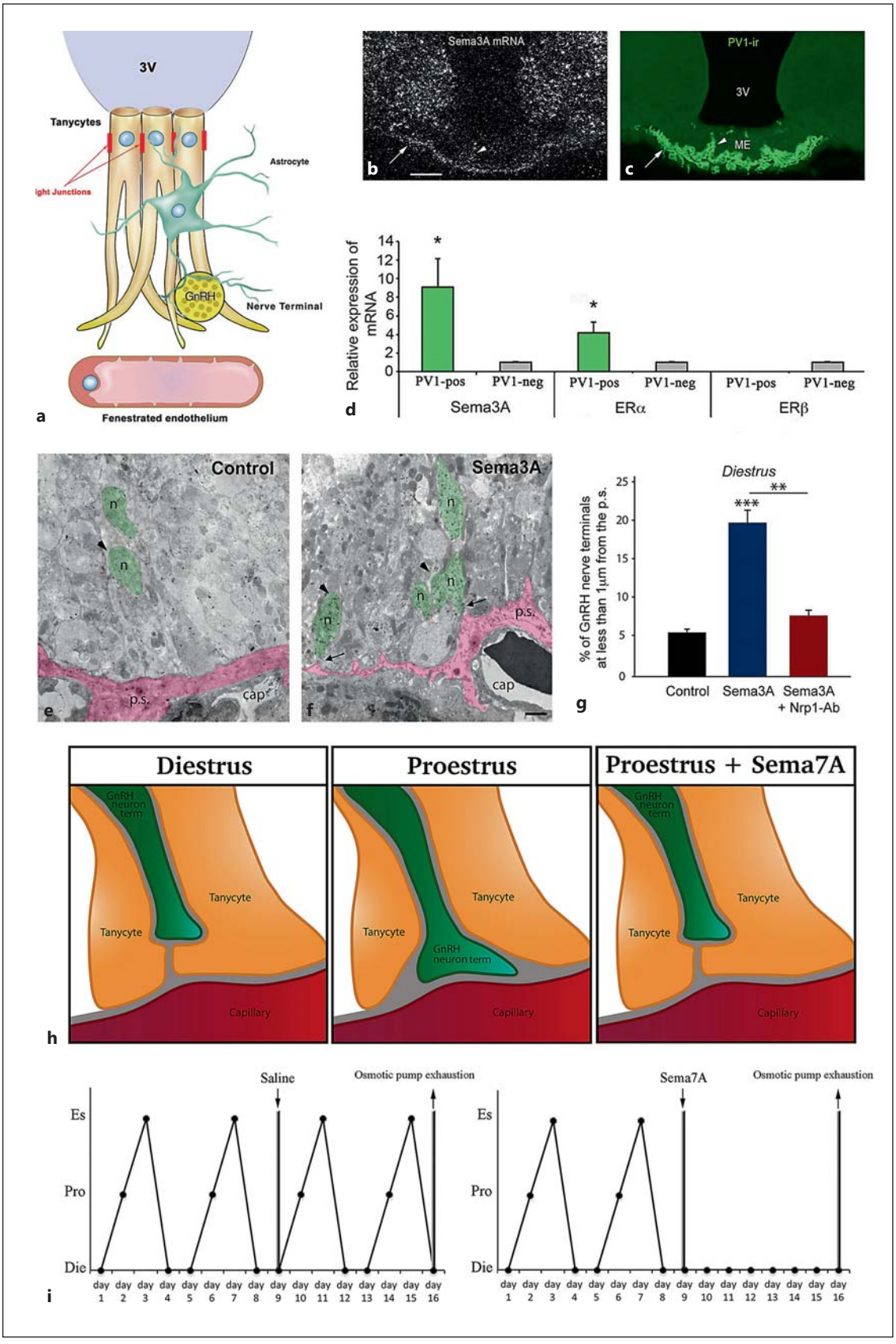
There is now a growing body of evidence indicating that cell-cell interactions involving nonneuronal cells such as vascular endothelial cells, astrocytes and specialized ependymoglial cells named tanycytes, which ensheathe the terminals of GnRH neurons, might be of critical importance in the regulation of GnRH secretion [123–126] (fig. 4a). Very recently, we have started to shed light on the molecular mechanisms responsible for this neuroglial plasticity and for the progression of the estrous cycle in rodents.

In the brain, endothelial cells are positioned to sense peripheral inputs and ideally suited to convey signals that could influence neuronal structure and synaptic plasticity. During development, blood vessels and axons employ similar mechanisms and follow common guidance cues for growth and navigation [127, 128]. Moreover, blood vessels aid axonal trajectories to reach the appropriate destinations [129]. In the developing embryo, endothelial cells release chemotropic signals such as Sema3A [130, 131] that regulate neuronal migration and axon guidance.

However, whether endothelial cells in the adult brain retained the ability to secrete molecules that influence

Fig. 4. a Schematic representation of the cell types (tanycytes, astrocytes and endothelial cells) and neuronal elements (neuroendocrine terminals) that reside within the ME of the hypothalamus. Reprinted with permission from Prevot et al. [122]. **b**, **c** Representative dark-field photomicrographs of a coronal section of an adult female rat ME showing *Sema3A* mRNA localized using a radioactive probe (bright dots indicating silver grains, top panel). Note the presence of *Sema3A* mRNA in the capillary zone of the ME (white arrow) and in intrainfundibular capillary loops (arrowhead) containing PV1-immunoreactive fenestrated endothelial cells (right panel, green immunofluorescence), and its relative paucity in the parenchyma. *Sema3A* mRNA expression is also seen in various nuclei of the mediobasal hypothalamus that lie adjacent to the ME but do not contain PV1-immunoreactive blood vessels. 3V = Third ventricle. Scale bar = 100 μm. **d** Isolation of PV1-positive cell (PV1-pos) by FACS (schematic diagram and dot plot, top) and real-time PCR analysis of PV1, Sema3A, estrogen receptor alpha (ERα) and ERβ transcripts. **e–g** Sema3A-Nrp1 signaling promotes GnRH axonal growth in the ME of the adult female rodent brain. **e**, **f** Representative electron micrographs of GnRH-immunoreactive axon terminals (green) from diestrous female rat hypothalamic explants containing the ME, incubated for 30 min in the presence (**f**) or absence (**e**) of Sema3A. **e** Under basal unstimulated conditions, GnRH nerve endings (n, arrowhead, green) are distant from the pericapillary space (p.s., pink). **f** Sema3A treatment causes GnRH axon terminals to advance towards the pericapillary space (p.s., pink), from which they remain separated by only a few nanometers (arrows). Cap = Pituitary portal blood capillaries. Scale bar = 1 μm. **g** Quantitative analysis of the percentage of GnRH nerve terminals located <1 μm from the pericapillary space in the external zone of the ME in explants from diestrous (left panel) and proestrous (right panel) rats treated with Sema3A, a Nrp1-neutralizing antibody (Nrp1-Ab) and in controls. Illustrations in **b–g** were adapted with permission from Giacobini et al. [132]. **h** Schematic highlighting morphological changes in GnRH terminals and tanycytic end feet during the different phases of the ovulatory cycle. In diestrus, under conditions of low gonadotropin output, GnRH-secreting axon terminals (green) are distant from the pericapillary space and tanycytes (yellow) enwrap GnRH nerve endings, thus impairing access of the neurohormone to the pituitary portal circulation. During proestrus, GnRH nerve endings sprout toward the basal lamina delineating the pericapillary space, with which they eventually make direct contact, while tanycytes retract. Sema7A treatment of rat female ME explants at proestrus induces morphological changes that mimic the diestrus state. **i** Sema7A infusion in the ME in vivo impairs adult reproductive function in rats. Sema7A was infused (0.2 μg/μl, 0.5 μl/h for 7 days) by stereotaxic implantation of a 28-gauge infusion cannula connected to a subcutaneously implanted mini-osmotic pump in the ME of cycling female rats. Representative estrous cycle profiles showing the disruption of estrous cyclicity by the infusion of Sema7A but not of PBS into the ME. Infusion was started on day 9 (downward arrow) and ended 7 days later (upward arrow), when pump contents were exhausted. Die = Diestrus; Pro = proestrus; Es = estrus. Adapted with permission from Parkash et al. [139].

(For figure see next page.)



4

neuronal function was still unknown until recently. A study from our group has shown that in the adult rodent brain, vascular endothelial cells of the ME express and release Sema3A (fig. 4b–d) and that the amount released is regulated by the ovulatory cycle [132]. In particular, this study highlights a new mechanism through which the fenestrated endothelial cells of the ME release the 65-kDa isoform of Sema3A (p65-Sema3A) with precise timing during the ovarian cycle, being maximal during proestrus under the influence of circulating estradiol (E_2), and that Nrp1 is expressed in GnRH axons. Ultrastructural experiments performed in this study have revealed that Sema3A-Nrp1 signaling is required for the extension of GnRH axon terminals toward the vascular plexus on the day of the preovulatory surge (fig. 4e–g). The molecular pathways that underlie this Sema3A-Nrp1-mediated activity are unknown, although they appear to be intrinsic to GnRH neurons since Sema3A promotes GnRH neurite outgrowth both in tissue explants and in isolated cell cultures [132]. In addition, the conditional deletion of Nrp1 in GnRH neurons counteracts Sema3A-induced axonal sprouting, while the localized intracerebral infusion of Nrp1-neutralizing antibodies in vivo disrupts the ovarian cycle, likely by perturbing the pulsatile, coordinated delivery of GnRH into the hypothalamo-hypophyseal portal system. Because ovarian cycle-regulated GnRH axonal elongation in the adult brain is likely to depend on the coordinated actions of many extracellular factors, endothelial p65-Sema3A may work in concert with other secreted molecules including nitric oxide, TGF-β_1 and BDNF, which are particularly enriched in the capillary zone of the ME [133–135] and may influence axonal plasticity by modulating neuronal expression of or responsiveness to semaphorins [136–138].

These results suggest a model in which vascular endothelial cells are dynamic signaling components that relay peripheral information to the brain to control key physiological functions, including the survival of the species. Moreover, they raise the intriguing possibility that vascular semaphorins may play important and unexpected roles in the adult neural plasticity underlying several other key physiological processes such as learning, the stress response and the control of energy homeostasis.

In addition to endothelial cells, ultrastructural studies by our group have revealed that under conditions of low gonadotropin output, such as in diestrus, tanycytic processes ensheathe GnRH nerve terminals in the external layer of the ME and prevent them from directly contacting the perivascular space [117] (fig. 4h). However, the molecular cues responsible for these dynamic morphological changes have not been elucidated so far. We have recently demonstrated a novel mechanism for this plasticity in the ME of adult female rodents, where tanycytes express Sema7A and this expression varies as a function of the hormonal state of the animal during the estrous cycle, being maximal at the onset of the diestrous phase [139]. We hypothesize that Sema7A released by hypothalamic tanycytes cyclically induces GnRH neurons to retract their terminals from the pericapillary space through PlexinC1 signaling and concomitantly promotes tanycytic end feet expansion via β_1-integrin activation, making the pericapillary space inaccessible to GnRH nerve terminals (fig. 4h, 5). This mechanism regulates neuropeptide release at key stages of the ovarian cycle, such as at diestrus, when GnRH secretion into the portal circulation is low. Indeed, when Sema7A is infused into the ME of female rats via a cannula connected to a subcutaneously implanted osmotic minipump for 7 days, there is a disruption of regular estrous cyclicity (fig. 4i).

In the same study, we have also shown that on the 1st day of diestrus, when progesterone secretion reaches peak values [140, 141] while estrogen levels are low [140, 141], progesterone stimulates Sema7A expression and secretion in tanycytes. It is tempting to speculate that in mammalian species in which progesterone has been shown to terminate the GnRH/LH surge [142, 143] it may arrest GnRH release by promoting the Sema7A-mediated engulfment of GnRH nerve terminals by tanycytic end feet.

This study also highlights how tanycytes are remodeled in response to Sema7A-β_1-integrin signaling and further substantiates the idea that the signaling pathways and effects of individual guidance molecules vary as a function of cellular context. Interestingly, β_1-integrin deletion in adult tanycytes leads to an alteration of the estrous cycle with a predominance of estrous stages with elevated circulating levels of LH [139].

Altogether, these studies shed light on the molecular mechanisms responsible for the progression of the estrous cycle in rodents and suggest that this phenomenon relies, at least in part, on the antagonistic effects of two ME semaphorins whose expression is periodically influenced by circulating sex hormones (fig. 5). Alterations in the mechanisms responsible for this estrous cycle-mediated plasticity of tanycytes could thus underlie some forms of hypothalamic infertility, independently of changes occurring during the developmental period. This hypothesis is supported by recent findings that mutations in the *SEMA7A* gene can be found in CHH patients [108] and that gonadal steroids promote structural changes in the hypothalamus of young women during the menstrual

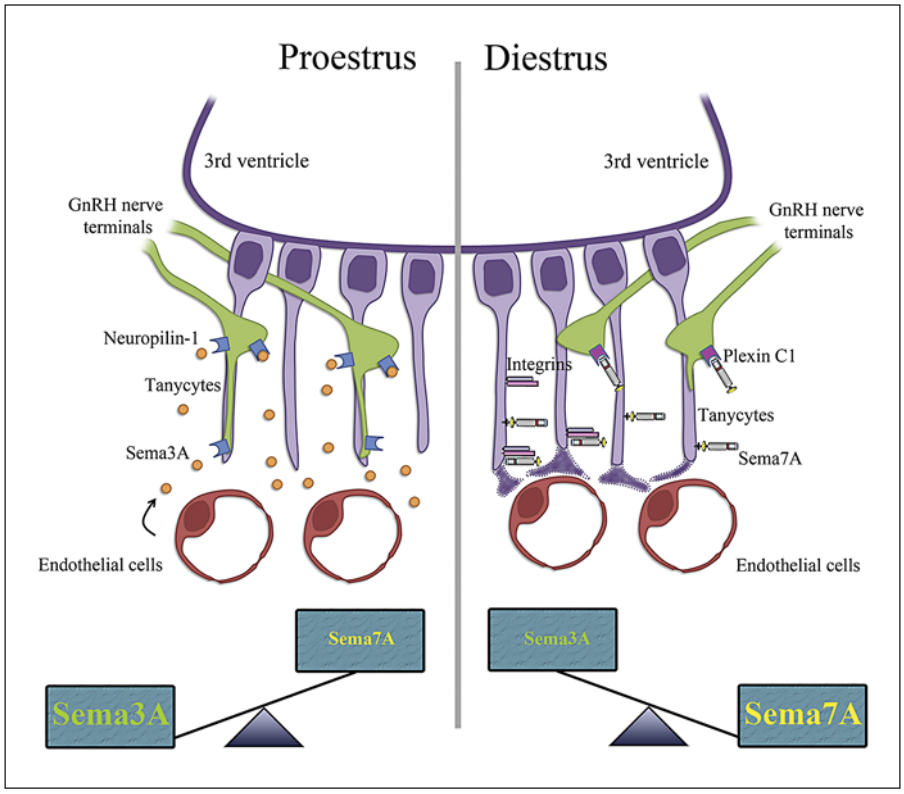


Fig. 5. Schematic representation summarizing the expression levels and actions of Sema3A and Sema7A on the morphological plasticity of GnRH neurons and tanycytes during proestrus and diestrus in female rodents. During proestrus, high levels of circulating estradiol increase the expression and secretion of Sema3A by fenestrated endothelial cells of the ME. Sema3A then binds to its cognate receptor, Nrp1, which is expressed by GnRH axon terminals, and induces the extension of GnRH nerve endings towards the vascular plexus to facilitate GnRH release into the portal blood and thus modulate the amplitude of the preovulatory LH surge [132]. During diestrus, when progesterone secretion reaches peak values, while estrogen levels are low, progesterone stimulates Sema7A expression in tanycytes. Through a bifunctional mechanism of action, Sema7A induces, through β_1-integrin activation, the expansion of tanycytic end feet, which ensheathe GnRH nerve terminals, and via PlexinC1, the retraction of GnRH nerve endings, thus preventing the free diffusion of the neurohormone into the pericapillary space [139].

cycle [144]. Identifying and characterizing such changes could thus be of use for the development of new therapeutic strategies for human disorders involving the central loss of reproductive competence and, conversely, to design novel contraceptive methods.

Conclusion

In addition to their effects on cellular morphology in a wide variety of systems, the semaphorins and their receptors play a pivotal role in the structural and functional development of the nervous system. In this review, we have focused on the intricate involvement of this large and diverse family of guidance cues on the development and operation of the neuroendocrine system underlying fertility. These activities underlie a complex developmental process, from the migration of neurons that control fertility from the nose to their final destination in the brain, to the wiring of the neuroendocrine network controlling neurohormone release. The recent finding that several patients with infertility linked to a developmental failure of the GnRH axis harbor mutations in semaphorin genes illustrates the importance of these semaphorins in the establishment of reproductive competence. Furthermore, semaphorin expression persists in adulthood, and the experimental evidence that these signals regulate the neuroglial plasticity responsible for timely and adequate GnRH release indicates that they also serve to maintain homeostatic set points that enable the survival of individuals and species. The identification of semaphorins and their receptors as modulators of both the development and adult functional plasticity of this neuroendocrine system provides new avenues for research not only from a fundamental mechanistic point of view but also from the point of view of human therapeutics.

Acknowledgements

This work was supported by the Agence Nationale de la Recherche, ANR, France (grant No.: ANR-2010-JCJC-1404-01 and ANR-2014-CE12 RoSes and GnRH), the Institut National de la Santé et de la Recherche Médicale, Inserm, France (grant No.: U1172), and the University of Lille 2, Lille, France (grant: Appel à Projets du Conseil Scientifique de l'Université Lille 2).

I am grateful to Dr. Andrea Messina and Mr. Samuel Malone (Inserm, Jean-Pierre Aubert Research Center, Unit 1172, Lab: Development and Plasticity of the Neuroendocrine Brain) for their graphical assistance and Dr. S. Rasika for the editing of the manuscript.

References

1 Kruger RP, Aurandt J, Guan KL: Semaphorins command cells to move. Nat Rev Mol Cell Biol 2005;6:789–800.
2 Casazza A, Fazzari P, Tamagnone L: Semaphorin signals in cell adhesion and cell migration: functional role and molecular mechanisms. Adv Exp Med Biol 2007;600:90–108.
3 Tran TS, Kolodkin AL, Bharadwaj R: Semaphorin regulation of cellular morphology. Annu Rev Cell Dev Biol 2007;23:263–292.
4 Perala N, Sariola H, Immonen T: More than nervous: the emerging roles of plexins. Differentiation 2012;83:77–91.
5 Pasterkamp RJ: Getting neural circuits into shape with semaphorins. Nat Rev Neurosci 2012;13:605–618.
6 Neufeld G, Kessler O: The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nat Rev Cancer 2008;8:632–645.
7 Zhou Y, Gunput RA, Pasterkamp RJ: Semaphorin signaling: progress made and promises ahead. Trends Biochem Sci 2008;33:161–170.
8 Capparuccia L, Tamagnone L: Semaphorin signaling in cancer cells and in cells of the tumor microenvironment – two sides of a coin. J Cell Sci 2009;122:1723–1736.
9 Ch'ng ES, Kumanogoh A: Roles of Sema4D and Plexin-B1 in tumor progression. Mol Cancer 2010;9:251.
10 Wray S: From nose to brain: development of gonadotrophin-releasing hormone-1 neurones. J Neuroendocrinol 2010;22:743–753.
11 Schwanzel-Fukuda M, Pfaff DW: Origin of luteinizing hormone-releasing hormone neurons. Nature 1989;338:161–164.
12 Wray S, Grant P, Gainer H: Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci USA 1989;86:8132–8136.
13 Ojeda SR, Skinner MK: Physiology of the gonadotropin-releasing hormone neuronal network; in Knobil E, Neill JD (eds): Physiology of Reproduction, ed 3. New York, Elsevier, 2006, pp 2061–2126.
14 Gonzalez-Martinez D, Hu Y, Bouloux PM: Ontogeny of GnRH and olfactory neuronal systems in man: novel insights from the investigation of inherited forms of Kallmann's syndrome. Front Neuroendocrinol 2004;25:108–130.
15 Wray S: Development of gonadotropin-releasing hormone-1 neurons. Front Neuroendocrinol 2002;23:292–316.
16 Yoshida K, Tobet SA, Crandall JE, Jimenez TP, Schwarting GA: The migration of luteinizing hormone-releasing hormone neurons in the developing rat is associated with a transient, caudal projection of the vomeronasal nerve. J Neurosci 1995;15:7769–7777.
17 Wierman ME, Kiseljak-Vassiliades K, Tobet S: Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. Front Neuroendocrinol 2011;32:43–52.
18 Schwarting GA, Wierman ME, Tobet SA: Gonadotropin-releasing hormone neuronal migration. Semin Reprod Med 2007;25:305–312.
19 Giacobini P, Messina A, Morello F, Ferraris N, Corso S, Penachioni J, Giordano S, Tamagnone L, Fasolo A: Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex. J Cell Biol 2008;183:555–566.
20 Giger RJ, Wolfer DP, De Wit GM, Verhaagen J: Anatomy of rat semaphorin III/collapsin-1 mRNA expression and relationship to developing nerve tracts during neuroembryogenesis. J Comp Neurol 1996;375:378–392.
21 Giger RJ, Cloutier JF, Sahay A, Prinjha RK, Levengood DV, Moore SE, Pickering S, Simmons D, Rastan S, Walsh FS, Kolodkin AL, Ginty DD, Geppert M: Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron 2000;25:29–41.
22 Cloutier JF, Giger RJ, Koentges G, Dulac C, Kolodkin AL, Ginty DD: Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. Neuron 2002;33:877–892.
23 Schwarting GA, Kostek C, Ahmad N, Dibble C, Pays L, Puschel AW: Semaphorin 3A is required for guidance of olfactory axons in mice. J Neurosci 2000;20:7691–7697.
24 Messina A, Ferraris N, Wray S, Cagnoni G, Donohue DE, Casoni F, Kramer PR, Derijck AA, Adolfs Y, Fasolo A, Pasterkamp RJ, Giacobini P: Dysregulation of Semaphorin7A/β1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility. Hum Mol Genet 2011;20:4759–4774.
25 Pasterkamp RJ, Kolk SM, Hellemons AJ, Kolodkin AL: Expression patterns of semaphorin7A and plexinC1 during rat neural development suggest roles in axon guidance and neuronal migration. BMC Dev Biol 2007;7:98.
26 de Castro F, Hu L, Drabkin H, Sotelo C, Chedotal A: Chemoattraction and chemorepulsion of olfactory bulb axons by different secreted semaphorins. J Neurosci 1999;19:4428–4436.
27 Ebert AM, Lamont RE, Childs SJ, McFarlane S: Neuronal expression of class 6 semaphorins in zebrafish. Gene Expr Patterns 2012;12:117–122.
28 Williams-Hogarth LC, Puche AC, Torrey C, Cai X, Song I, Kolodkin AL, Shipley MT, Ronnett GV: Expression of semaphorins in developing and regenerating olfactory epithelium. J Comp Neurol 2000;423:565–578.
29 Wu H, Fan J, Zhu L, Liu S, Wu Y, Zhao T, Wu Y, Ding X, Fan W, Fan M: Sema4C expression in neural stem/progenitor cells and in adult neurogenesis induced by cerebral ischemia. J Mol Neurosci 2009;39:27–39.
30 Yu HH, Houart C, Moens CB: Cloning and embryonic expression of zebrafish neuropilin genes. Gene Expr Patterns 2004;4:371–378.
31 Murakami Y, Suto F, Shimizu M, Shinoda T, Kameyama T, Fujisawa H: Differential expression of plexin-A subfamily members in the mouse nervous system. Dev Dyn 2001;220:246–258.
32 Kobayashi H, Koppel AM, Luo Y, Raper JA: A role for collapsin-1 in olfactory and cranial sensory axon guidance. J Neurosci 1997;17:8339–8352.
33 Pasterkamp RJ, De Winter F, Holtmaat AJ, Verhaagen J: Evidence for a role of the chemorepellent semaphorin III and its receptor neuropilin-1 in the regeneration of primary olfactory axons. J Neurosci 1998;18:9962–9976.
34 Cariboni A, Hickok J, Rakic S, Andrews W, Maggi R, Tischkau S, Parnavelas JG: Neuropilins and their ligands are important in the migration of gonadotropin-releasing hormone neurons. J Neurosci 2007;27:2387–2395.
35 Walz A, Rodriguez I, Mombaerts P: Aberrant sensory innervation of the olfactory bulb in neuropilin-2 mutant mice. J Neurosci 2002;22:4025–4035.
36 Imai T, Yamazaki T, Kobayakawa R, Kobayakawa K, Abe T, Suzuki M, Sakano H: Pretarget axon sorting establishes the neural map topography. Science 2009;325:585–590.
37 Cummings DM, Brunjes PC: Migrating luteinizing hormone-releasing hormone (LHRH) neurons and processes are associated with a substrate that expresses S100. Brain Res Dev Brain Res 1995;88:148–157.
38 Geller S, Kolasa E, Tillet Y, Duittoz A, Vaudin P: Olfactory ensheathing cells form the microenvironment of migrating GnRH-1 neurons during mouse development. Glia 2013;61:550–566.
39 Miller AM, Treloar HB, Greer CA: Composition of the migratory mass during development of the olfactory nerve. J Comp Neurol 2010;518:4825–4841.
40 Forni PE, Taylor-Burds C, Melvin VS, Williams T, Wray S: Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons, and olfactory ensheathing cells. J Neurosci 2011;31:6915–6927.
41 Barraud P, Seferiadis AA, Tyson LD, Zwart MF, Szabo-Rogers HL, Ruhrberg C, Liu KJ, Baker CV: Neural crest origin of olfactory ensheathing glia. Proc Natl Acad Sci USA 2010;107:21040–21045.
42 Katoh H, Shibata S, Fukuda K, Sato M, Satoh E, Nagoshi N, Minematsu T, Matsuzaki Y, Akazawa C, Toyama Y, Nakamura M, Okano H: The dual origin of the peripheral olfactory system: placode and neural crest. Mol Brain 2011;4:34.

43 Palevitch O, Abraham E, Borodovsky N, Levkowitz G, Zohar Y, Gothilf Y: Cxcl12a-Cxcr4b signaling is important for proper development of the forebrain GnRH system in zebrafish. Gen Comp Endocrinol 2010;165:262–268.
44 Kramer PR, Wray S: Nasal embryonic LHRH factor (NELF) expression within the CNS and PNS of the rodent. Gene Expr Patterns 2001;1:23–26.
45 Toba Y, Tiong JD, Ma Q, Wray S: CXCR4/SDF-1 system modulates development of GnRH-1 neurons and the olfactory system. Dev Neurobiol 2008;68:487–503.
46 Shyu WC, Liu DD, Lin SZ, Li WW, Su CY, Chang YC, Wang HJ, Wang HW, Tsai CH, Li H: Implantation of olfactory ensheathing cells promotes neuroplasticity in murine models of stroke. J Clin Invest 2008;118:2482–2495.
47 Tham TN, Lazarini F, Franceschini IA, Lachapelle F, Amara A, Dubois-Dalcq M: Developmental pattern of expression of the alpha chemokine stromal cell-derived factor 1 in the rat central nervous system. Eur J Neurosci 2001;13:845–856.
48 Cariboni A, Davidson K, Rakic S, Maggi R, Parnavelas JG, Ruhrberg C: Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NRP1 and NRP2: Implications for the aetiology of hypogonadotropic hypogonadism. Hum Mol Genet 2011;20:336–344.
49 Cariboni A, Davidson K, Dozio E, Memi F, Schwarz Q, Stossi F, Parnavelas JG, Ruhrberg C: VEGF signalling controls GnRH neuron survival via NRP1 independently of KDR and blood vessels. Development 2011;138:3723–3733.
50 Hanchate NK, Giacobini P, Lhuillier P, et al: SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. PLoS Genet 2012;8:e1002896.
51 Schwanzel-Fukuda M, Bick D, Pfaff DW: Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. Brain Res Mol Brain Res 1989;6:311–326.
52 Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M: Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92:735–745.
53 Gluzman-Poltorak Z, Cohen T, Herzog Y, Neufeld G: Neuropilin-2 is a receptor for the vascular endothelial growth factor (VEGF) forms VEGF-145 and VEGF-165. J Biol Chem 2000;275:29922.
54 Karpanen T, Heckman CA, Keskitalo S, Jeltsch M, Ollila H, Neufeld G, Tamagnone L, Alitalo K: Functional interaction of VEGF-C and VEGF-D with neuropilin receptors. FASEB J 2006;20:1462–1472.
55 Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983–985.
56 Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306–1309.
57 Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A: Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435–439.
58 Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW: Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996;380:439–442.
59 Fantin A, Maden CH, Ruhrberg C: Neuropilin ligands in vascular and neuronal patterning. Biochem Soc Trans 2009;37:1228–1232.
60 Ogunshola OO, Antic A, Donoghue MJ, Fan SY, Kim H, Stewart WB, Madri JA, Ment LR: Paracrine and autocrine functions of neuronal vascular endothelial growth factor (VEGF) in the central nervous system. J Biol Chem 2002;277:11410–11415.
61 Oosthuyse B, Moons L, Storkebaum E, et al: Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet 2001;28:131–138.
62 Sondell M, Lundborg G, Kanje M: Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. J Neurosci 1999;19:5731–5740.
63 Ohoka Y, Hirotani M, Sugimoto H, Fujioka S, Furuyama T, Inagaki S: Semaphorin 4C, a transmembrane semaphorin, [corrected] associates with a neurite-outgrowth-related protein, SFAP75. Biochem Biophys Res Commun 2001;280:237–243.
64 Inagaki S, Ohoka Y, Sugimoto H, Fujioka S, Amazaki M, Kurinami H, Miyazaki N, Tohyama M, Furuyama T: Sema4c, a transmembrane semaphorin, interacts with a post-synaptic density protein, PSD-95. J Biol Chem 2001;276:9174–9181.
65 Elhabazi A, Delaire S, Bensussan A, Boumsell L, Bismuth G: Biological activity of soluble CD100. I. The extracellular region of CD100 is released from the surface of T lymphocytes by regulated proteolysis. J Immunol 2001;166:4341–4347.
66 Wang X, Kumanogoh A, Watanabe C, Shi W, Yoshida K, Kikutani H: Functional soluble CD100/Sema4D released from activated lymphocytes: possible role in normal and pathologic immune responses. Blood 2001;97:3498–3504.
67 Swiercz JM, Kuner R, Behrens J, Offermanns S: Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. Neuron 2002;35:51–63.
68 Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, Tamagnone L, Comoglio PM: The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat Cell Biol 2002;4:720–724.
69 Conrotto P, Corso S, Gamberini S, Comoglio PM, Giordano S: Interplay between scatter factor receptors and B plexins controls invasive growth. Oncogene 2004;23:5131–5137.
70 Conrotto P, Valdembri D, Corso S, Serini G, Tamagnone L, Comoglio PM, Bussolino F, Giordano S: Sema4D induces angiogenesis through Met recruitment by Plexin B1. Blood 2005;105:4321–4329.
71 Dacquin R, Domenget C, Kumanogoh A, Kikutani H, Jurdic P, Machuca-Gayet I: Control of bone resorption by semaphorin 4D is dependent on ovarian function. PLoS One 2011;6:e26627.
72 Giacobini P, Messina A, Wray S, Giampietro C, Crepaldi T, Carmeliet P, Fasolo A: Hepatocyte growth factor acts as a motogen and guidance signal for gonadotropin hormone-releasing hormone-1 neuronal migration. J Neurosci 2007;27:431–445.
73 Jongbloets BC, Ramakers GM, Pasterkamp RJ: Semaphorin7a and its receptors: pleiotropic regulators of immune cell function, bone homeostasis, and neural development. Semin Cell Dev Biol 2013;24:129–138.
74 Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, Kitao T, Takagi J, Rennert PD, Kolodkin AL, Kumanogoh A, Kikutani H: Semaphorin 7a initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. Nature 2007;446:680–684.
75 Lazova R, Gould Rothberg BE, Rimm D, Scott G: The semaphorin 7a receptor Plexin C1 is lost during melanoma metastasis. Am J Dermatopathol 2009;31:177–181.
76 Scott GA, McClelland LA, Fricke AF: Semaphorin 7a promotes spreading and dendricity in human melanocytes through beta1-integrins. J Invest Dermatol 2008;128:151–161.
77 Scott GA, McClelland LA, Fricke AF, Fender A: Plexin C1, a receptor for semaphorin 7a, inactivates cofilin and is a potential tumor suppressor for melanoma progression. J Invest Dermatol 2009;129:954–963.
78 Ohsawa S, Hamada S, Asou H, Kuida K, Uchiyama Y, Yoshida H, Miura M: Caspase-9 activation revealed by semaphorin 7a cleavage is independent of apoptosis in the aged olfactory bulb. J Neurosci 2009;29:11385–11392.
79 Ohsawa S, Hamada S, Kuida K, Yoshida H, Igaki T, Miura M: Maturation of the olfactory sensory neurons by Apaf-1/caspase-9-mediated caspase activity. Proc Natl Acad Sci USA 2010;107:13366–13371.
80 Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL: Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. Nature 2003;424:398–405.
81 Fukunishi A, Maruyama T, Zhao H, Tiwari M, Kang S, Kumanogoh A, Yamamoto N: The action of Semaphorin7A on thalamocortical axon branching. J Neurochem 2011;118:1008–1015.

82 Carcea I, Patil SB, Robison AJ, Mesias R, Huntsman MM, Froemke RC, Buxbaum JD, Huntley GW, Benson DL: Maturation of cortical circuits requires Semaphorin 7A. Proc Natl Acad Sci USA 2014;111:13978–13983.

83 Parkash J, Cimino I, Ferraris N, Casoni F, Wray S, Cappy H, Prevot V, Giacobini P: Suppression of beta1-integrin in gonadotropin-releasing hormone cells disrupts migration and axonal extension resulting in severe reproductive alterations. J Neurosci 2012;32:16992–17002.

84 de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E: A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 1997;337:1597–1602.

85 Mitchell AL, Dwyer A, Pitteloud N, Quinton R: Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. Trends Endocrinol Metab 2011;22:249–258.

86 Bonomi M, Libri DV, Guizzardi F, Guarducci E, Maiolo E, Pignatti E, Asci R, Persani L; Idiopathic Central Hypogonadism Study Group of the Italian Societies of Endocrinology and Pediatric Endocrinology and Diabetes: New understandings of the genetic basis of isolated idiopathic central hypogonadism. Asian J Androl 2012;14:49–56.

87 Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D, et al: The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. Cell 1991;67:423–435.

88 Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, et al: A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 1991;353:529–536.

89 Dode C, Levilliers J, Dupont JM, et al: Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet 2003;33:463–465.

90 Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N: Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest 2008;118:2822–2831.

91 Miraoui H, Dwyer AA, Sykiotis GP, et al: Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. Am J Hum Genet 2013;92:725–743.

92 Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J: Isolated familial hypogonadotropic hypogonadism and a GnRH1 mutation. N Engl J Med 2009;360:2742–2748.

93 Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF Jr, Amory JK, Pitteloud N, Seminara SB: GnRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci USA 2009;106:11703–11708.

94 Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD: Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet 1998;18:14–15.

95 Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B: Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 2012;366:629–635.

96 Seminara SB, Messager S, Chatzidaki EE, et al: The GPR54 gene as a regulator of puberty. N Engl J Med 2003;349:1614–1627.

97 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 USA 2003;100:10972–10976.

98 Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK: TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 2009;41:354–358.

99 Trarbach EB, Baptista MT, Garmes HM, Hackel C: Molecular analysis of KAL-1, GnRH-R, NELF and EBF2 genes in a series of Kallmann syndrome and normosmic hypogonadotropic hypogonadism patients. J Endocrinol 2005;187:361–368.

100 Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP: Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet 2006;2:e175.

101 Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, Cole LW, Plummer L, Jacobson-Dickman EE, Mellon PL, Zhou QY, Crowley WF Jr: Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci USA 2007;104:17447–17452.

102 Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, Kang GB, Rosenberger G, Tekin M, Ozata M, Bick DP, Sherins RJ, Walker SL, Shi Y, Gusella JF, Layman LC: Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet 2008;83:511–519.

103 Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, Quinton R, Seminara SB, Hughes V, Van Vliet G, Van Uum S, Crowley WF, Habuchi H, Kimata K, Pitteloud N, Bulow HE: Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. Proc Natl Acad Sci USA 2011;108:11524–11529.

104 Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, Ha KS, Itokawa Y, Meliciani I, Wenzel W, Lee D, Rosenberger G, Ozata M, Bick DP, Sherins RJ, Nagase T, Tekin M, Kim SH, Kim CH, Ropers HH, Gusella JF, Kalscheuer V, Choi CY, Layman LC: WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet 2010;87:465–479.

105 Kotan LD, Hutchins BI, Ozkan Y, Demirel F, Stoner H, Cheng PJ, Esen I, Gurbuz F, Bicakci YK, Mengen E, Yuksel B, Wray S, Topaloglu AK: Mutations in FEZF1 cause Kallmann syndrome. Am J Hum Genet 2014;95:326–331.

106 Pingault V, Bodereau V, Baral V, Marcos S, Watanabe Y, Chaoui A, Fouveaut C, Leroy C, Verier-Mine O, Francannet C, Dupin-Deguine D, Archambeaud F, Kurtz FJ, Young J, Bertherat J, Marlin S, Goossens M, Hardelin JP, Dode C, Bondurand N: Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. Am J Hum Genet 2013;92:707–724.

107 Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L, Tosca L, Sarfati J, Brioude F, Esteva B, Briand-Suleau A, Brisset S, Goossens M, Tachdjian G, Guiochon-Mantel A: SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. Hum Reprod 2012;27:1460–1465.

108 Kansakoski J, Fagerholm R, Laitinen EM, Vaaralahti K, Hackman P, Pitteloud N, Raivio T, Tommiska J: Mutation screening of SEMA3A and SEMA7A in patients with congenital hypogonadotropic hypogonadism. Pediatr Res 2014;75:641–644.

109 Balasubramanian R, Crowley WF Jr: Isolated GnRH deficiency: a disease model serving as a unique prism into the systems biology of the GnRH neuronal network. Mol Cell Endocrinol 2011;346:4–12.

110 Dode C, Hardelin JP: Kallmann syndrome. Eur J Hum Genet 2009;17:139–146.

111 Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, Dwyer AA, Quinton R, Hall JE, Gusella JF, Seminara SB, Crowley WF Jr, Pitteloud N: Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proc Natl Acad Sci USA 2010;107:15140–15144.

112 Schulz Y, Wehner P, Opitz L, Salinas-Riester G, Bongers EM, van Ravenswaaij-Arts CM, Wincent J, Schoumans J, Kohlhase J, Borchers A, Pauli S: CHD7, the gene mutated in charge syndrome, regulates genes involved in neural crest cell guidance. Hum Genet 2014;133:997–1009.

113 Kim HG, Layman LC: The role of CHD7 and the newly identified WDR11 gene in patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Mol Cell Endocrinol 2011;346:74–83.

114 Janssen N, Bergman JE, Swertz MA, Tranebjaerg L, Lodahl M, Schoots J, Hofstra RM, van Ravenswaaij-Arts CM, Hoefsloot LH: Mutation update on the CHD7 gene involved in CHARGE syndrome. Hum Mutat 2012;33:1149–1160.

115 Lalani SR, Safiullah AM, Molinari LM, Fernbach SD, Martin DM, Belmont JW: SEMA3E mutation in a patient with CHARGE syndrome. J Med Genet 2004;41:e94.

116 King JC, Letourneau RJ: Luteinizing hormone-releasing hormone terminals in the median eminence of rats undergo dramatic changes after gonadectomy, as revealed by electron microscopic image analysis. Endocrinology 1994;134:1340–1351.

117 Prevot V, Croix D, Bouret S, Dutoit S, Tramu G, Stefano GB, Beauvillain JC: Definitive evidence for the existence of morphological plasticity in the external zone of the median eminence during the rat estrous cycle: implication of neuro-glio-endothelial interactions in gonadotropin-releasing hormone release. Neuroscience 1999;94:809–819.

118 Herbison AE: Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocr Rev 1998;19:302–330.

119 Moenter SM, Chu Z, Christian CA: Neurobiological mechanisms underlying oestradiol negative and positive feedback regulation of gonadotrophin-releasing hormone neurones. J Neuroendocrinol 2009;21:327–333.

120 Ronnekleiv OK, Kelly MJ: Diversity of ovarian steroid signaling in the hypothalamus. Front Neuroendocrinol 2005;26:65–84.

121 Piet R, Boehm U, Herbison AE: Estrous cycle plasticity in the hyperpolarization-activated current IH is mediated by circulating 17beta-estradiol in preoptic area kisspeptin neurons. J Neurosci 2013;33:10828–10839.

122 Prevot V, Bellefontaine N, Baroncini M, Sharif A, Hanchate NK, Parkash J, Campagne C, de Seranno S: Gonadotrophin-releasing hormone nerve terminals, tanycytes and neurohaemal junction remodelling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. J Neuroendocrinol 2010;22:639–649.

123 Garcia-Segura LM, Lorenz B, DonCarlos LL: The role of glia in the hypothalamus: implications for gonadal steroid feedback and reproductive neuroendocrine output. Reproduction 2008;135:419–429.

124 Barres BA: A role for glia in LHRH release. Curr Biol 1992;2:645–647.

125 Ojeda SR, Lomniczi A, Sandau U: Contribution of glial-neuronal interactions to the neuroendocrine control of female puberty. Eur J Neurosci 2010;32:2003–2010.

126 Sharif A, Baroncini M, Prevot V: Role of glia in the regulation of gonadotropin-releasing hormone neuronal activity and secretion. Neuroendocrinology 2013;98:1–15.

127 Carmeliet P, Tessier-Lavigne M: Common mechanisms of nerve and blood vessel wiring. Nature 2005;436:193–200.

128 Larrivee B, Freitas C, Suchting S, Brunet I, Eichmann A: Guidance of vascular development: lessons from the nervous system. Circ Res 2009;104:428–441.

129 Makita T, Sucov HM, Gariepy CE, Yanagisawa M, Ginty DD: Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. Nature 2008;452:759–763.

130 Serini G, Valdembri D, Zanivan S, Morterra G, Burkhardt C, Caccavari F, Zammataro L, Primo L, Tamagnone L, Logan M, Tessier-Lavigne M, Taniguchi M, Puschel AW, Bussolino F: Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 2003;424:391–397.

131 Valdembri D, Caswell PT, Anderson KI, Schwarz JP, Konig I, Astanina E, Caccavari F, Norman JC, Humphries MJ, Bussolino F, Serini G: Neuropilin-1/GIPC1 signaling regulates alpha5beta1 integrin traffic and function in endothelial cells. PLoS Biol 2009;7:e25.

132 Giacobini P, Parkash J, Campagne C, Messina A, Casoni F, Vanacker C, Langlet F, Hobo B, Cagnoni G, Gallet S, Hanchate NK, Mazur D, Taniguchi M, Mazzone M, Verhaagen J, Ciofi P, Bouret SG, Tamagnone L, Prevot V: Brain endothelial cells control fertility through ovarian-steroid-dependent release of semaphorin 3A. PLoS Biol 2014;12:e1001808.

133 De Seranno S, Estrella C, Loyens A, Cornea A, Ojeda SR, Beauvillain JC, Prevot V: Vascular endothelial cells promote acute plasticity in ependymoglial cells of the neuroendocrine brain. J Neurosci 2004;24:10353–10363.

134 Bouret S, De Seranno S, Beauvillain JC, Prevot V: Transforming growth factor beta1 may directly influence gonadotropin-releasing hormone gene expression in the rat hypothalamus. Endocrinology 2004;145:1794–1801.

135 Givalois L, Arancibia S, Alonso G, Tapia-Arancibia L: Expression of brain-derived neurotrophic factor and its receptors in the median eminence cells with sensitivity to stress. Endocrinology 2004;145:4737–4747.

136 Song H, Ming G, He Z, Lehmann M, McKerracher L, Tessier-Lavigne M, Poo M: Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. Science 1998;281:1515–1518.

137 Ikegami R, Zheng H, Ong SH, Culotti J: Integration of semaphorin-2A/MAB-20, ephrin-4, and UNC-129 TGF-beta signaling pathways regulates sorting of distinct sensory rays in *C. elegans*. Dev Cell 2004;6:383–395.

138 Kettunen P, Loes S, Furmanek T, Fjeld K, Kvinnsland IH, Behar O, Yagi T, Fujisawa H, Vainio S, Taniguchi M, Luukko K: Coordination of trigeminal axon navigation and patterning with tooth organ formation: epithelial-mesenchymal interactions, and epithelial Wnt4 and Tgfbeta1 regulate semaphorin 3a expression in the dental mesenchyme. Development 2005;132:323–334.

139 Parkash J, Messina A, Langlet F, Cimino I, Loyens A, Mazur D, Gallet S, Balland E, Malone S, Pralong FP, Cagnoni G, Schellino R, De Marchi S, Mazzone M, Pasterkamp RJ, Tamagnone L, Prevot V, Giacobini P: Semaphorin7A regulates neuroglial plasticity at the adult hypothalamic median eminence. Nat Commun 2015;6:6385.

140 Prevot V, Dutoit S, Croix D, Tramu G, Beauvillain JC: Semi-quantitative ultrastructural analysis of the localization and neuropeptide content of gonadotropin releasing hormone nerve terminals in the median eminence throughout the estrous cycle of the rat. Neuroscience 1998;84:177–191.

141 Smith MS, Freeman ME, Neill JD: The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 1975;96:219–226.

142 Dafopoulos K, Mademtzis I, Vanakara P, Kallitsaris A, Stamatiou G, Kotsovassilis C, Messinis IE: Evidence that termination of the estradiol-induced luteinizing hormone surge in women is regulated by ovarian factors. J Clin Endocrinol Metab 2006;91:641–645.

143 Kasa-Vubu JZ, Dahl GE, Evans NP, Thrun LA, Moenter SM, Padmanabhan V, Karsch FJ: Progesterone blocks the estradiol-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 1992;131:208–212.

144 Baroncini M, Jissendi P, Catteau-Jonard S, Dewailly D, Pruvo JP, Francke JP, Prevot V: Sex steroid hormones-related structural plasticity in the human hypothalamus. Neuroimage 2010;50:428–433.

145 Messina A, Giacobini P: Semaphorin signaling in the development and function of the gonadotropin hormone-releasing hormone system. Front Endocrinol 2013;4:133.