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Molecular regulation of hypothalamic development and physiological functions

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Abstract

The hypothalamus is composed of many heterogeneous nuclei that control distinct physiological functions. Investigating molecular mechanisms that regulate the specification of these nuclei and specific neuronal subtypes, and their contribution to diverse hypothalamic functions, is an exciting research focus. Here, we begin by summarizing the hypothalamic functions of feeding regulation, sleep-wake cycles, stress responses, and circadian rhythm, and describing their anatomical bases. Next, we review the molecular regulation of formation of hypothalamic territories, specification of nuclei and subnuclei, and generation of specific neurons. Finally, we highlight physiological and behavioral consequences of altered hypothalamic development. Identifying molecules that regulate hypothalamic development and function will increase our understanding of hypothalamus-related disorders, such as obesity and diabetes, and aid in the development of therapies aimed specifically at their etiologies.

Keywords

Hypothalamus; Hypothalamic functions; Neurogenesis; Hypothalamic development; Hypothalamus-related disorders

Introduction

The hypothalamus can be rostrocaudally divided into four regions: preoptic, supraoptic, tuberal, and mammillary regions (Fig. 1). Each region consists of several nuclei, whose functions were defined mainly using lesions, stimulations, and genetic approaches. It has been found that besides the conventional neurotransmitters such as 5-hydroxytryptamine (5-HT), dopamine (DA), glutamate (Glu), and gamma-aminobutyric acid (GABA), the hypothalamus predominantly and selectively produces various restricted neuropeptides and hormones, which contribute to diverse basic physiological functions, such as feeding, sleep-wake cycles, body temperature, sex behaviors and reproduction, stress responses, and circadian rhythm [1]. In addition, these nuclei in the hypothalamus are frequently connected

by neuronal projections, which indicates neurobiological interactions between different physiological functions. In this review, we first summarize hypothalamic functions that are regulated by specific nuclei. We then highlight molecules such as mitogens, transcription factors, and microRNAs (miRNAs), which play crucial roles in the development of the hypothalamus. Finally, we elaborate pathophysiological functional consequences of abnormal development of the hypothalamus.

Functions of the hypothalamus

Feeding and energy expenditure

Roles of the hypothalamus in feeding and expenditure were recognized as early as 1940s, as some studies showed that ablation of the arcuate nucleus (ARC) and ventromedial nucleus (VMN) led to excessive eating and obesity, while ablation of the lateral hypothalamus (LH) caused aphagia and weight loss [2–4]. Later, the dorsomedial nucleus (DMN) and paraventricular nucleus (PVN) were also proven to regulate food intake and body weight, with aphagia and weight loss caused by lesions in the DMN, and overeating and obesity due to lesions in the PVN [5, 6]. Neurocircuits composed of these hypothalamic nuclei and other brain areas seem to play important roles in determining whether to eat, when to eat, what to eat, and how much to eat [7].

The ARC is a major site of feeding regulation, whose neurons mainly project to other nuclei of the hypothalamus, including the LH, PVN, and VMN [8, 9] (Fig. 2), and release the orexigenic neuropeptides (neuropeptide Y, NPY, and agouti-related protein, AgRP) to increase food intake, and the anorexigenic neuropeptides (proopiomelanocortin, POMC; and cocaine- and amphetamine-regulated transcript, CART) to decrease food intake [8, 10, 11]. Nutrient-related signals and hormones such as leptin [12], insulin [13], glucose [14], peptide YY3-36 (PYY3-36) [15], ghrelin [16], and cholecystikinin (CCK) [17] have been reported to repress these orexigenic neuropeptides and promote anorexigenic neuropeptides to decrease food intake. VMN neurons have been found to project to the ARC and activate anorexigenic pathways [18], as well as express abundant oxytocin receptor to mediate oxytocin action on food intake [19, 20]. In addition, LH and PVN neurons have been found to relay information from other food-related neurons, such as the parabrachial nucleus (PBN) in the midbrain and the nucleus of the solitary tract (NTS) in the hindbrain, which receive visceral inputs, to the ARC to influence satiety [7]. LH and PVN neurons also receive reward-related inputs from the nucleus accumbens (NAc) and amygdala (AMY), and project to the ARC to regulate appetite [7] (Fig. 2). Moreover, studies have shown that LH neurons produce orexigenic neuropeptides orexin, melanin concentrating hormone (MCH), and CART to promote food intake [21], while PVN neurons produce hormones such as the corticotropin-releasing hormone (CRH), the thyrotropin-releasing hormone (TRH), and oxytocin (OXT) to participate in the metabolic process to regulate food intake [20, 22]. The DMN has been shown to receive information from the suprachiasmatic nucleus (SCN) and export to the PVN and LH, and participate in regulating circadian rhythm of feeding [23–25] (Fig. 2).

Roles of specific neuronal circuits and substances involved in food intake and expenditure in the adult hypothalamus were well studied in the past. Not surprisingly, because maternal

obesity and diabetes increase risks for obesity and diabetes in the offspring, studies on correlations between hypothalamic development and feeding-related disorders are now emerging [26–28].

Sleep and wakefulness

Sleep and wakefulness are complex behaviors whose regulation is involved in coordination of neural networks, homeo-static drive, and circadian system, which are all associated with the hypothalamus [29, 30]. The ventrolateral preoptic nucleus (VLPO) and the median preoptic nucleus (MnPO) of the hypothalamus are two main sleep centers that promote sleep and repress wakefulness (Fig. 3). GABA and galanin neurons in the VLPO and MnPO are active during sleep and, in turn, repress the ascending arousal system that consists of the brain stem, hypothalamus, thalamus, basal forebrain, and cortex [31, 32]. On the other hand, as a part of the ascending arousal system, the LH and tuberomammillary nucleus (TMN) consist of arousal neurons that produce orexin and histamine/gamma-aminobutyric acid (His/GABA), respectively, which are all active during wakefulness [32–35] (Fig. 3). It has been found that orexin mutation in human or deletion in mice causes narcolepsy [36–38]. Moreover, the SCN, the subparaventricular zone (SPZ), and the dorsomedial nucleus (DMN) form a SCN-SPZ-DMN network, which is responsible for the regulation of the sleep-wake circadian rhythm [29, 39] (Fig. 3). Notably, diverse neuropeptides and hormones such as NPY, CRH, and Growth Hormone Releasing Hormone (GHRH) in the hypothalamus take part in the homeostatic regulation of sleep and wakefulness likely by the interaction with neuronal networks [31].

Dysregulation of hypothalamic development has been found to be associated with sleep disturbance, such as in Prader-Willi syndrome [40]. As a result, the hypothalamus is considered an important target for potential therapies for sleep disorders, for example, dexmedetomidine (a sleep-promoting drug) promotes sleep by activating the VLPO [41].

Responses to stress

When an organism confronts stress such as an adverse environment or threats, a series of physiological responses, also called stress responses, are triggered to adapt to these changes. Multiple nuclei and molecules of the hypothalamus participate in stress responses. The PVN, which interacts with the medial preoptic area (mPOA), DMN, LH, SCN, and some other non-hypothalamus nuclei, has been found to regulate stress by activating the hypothalamic–pituitary–adrenal (HPA) axis [42, 43]. Under stress, the PVN secretes CRH and arginine vasopressin (AVP) to stimulate release of the adrenocortico-tropic hormone (ACTH) in the pituitary, in turn to regulate the level and activity of the cortisol in the adrenal [44]. In addition, peptides such as orexin, NPY, and OXT participate in stress responses by regulating the HPA activity in a specific stress context, for example sleep loss and fear [45, 46].

Other functions of the hypothalamus

It has been shown that the SCN is a major circadian pacemaker in vertebrates. Lesions of the SCN lead to arrhythmic motor activity in rodents [47, 48]. The vasoactive intestinal peptide (Vip) or its receptor Vipr2, gastrin-releasing peptide (Grp), AVP, and prokineticin-2 have

been shown to be crucial for regulating rhythm of clock gene expression and neuronal firing in the SCN [49–53]. Moreover, lesion studies have shown that the mammillary body in the hypothalamus is related to memory, but its molecular mechanism is still unknown [54–57]. Thus, the hypothalamus regulates complex physiological behaviors related to feeding, sleep, stress responses, and rhythms. Investigation of molecular mechanisms that regulate formation of distinct nuclei and productions of specific neurons will shed light on understanding neurological bases of hypothalamus functions.

Molecular regulation of hypothalamic development and functions

The development of the hypothalamus, derived from prechordal mesoderm, is a complex series of events that includes establishment of the hypothalamic regional territory, specification of subdivision of regional territories, neuronal differentiation, neuronal migration, and formation of subnuclei. A gene expression profiling study has identified altered expression of over 1000 transcripts in the mouse hypothalamus during different developmental stages, suggesting that a large number of genes contribute to hypothalamic development and may play multiple roles in the process [58]. We here summarize major signaling pathways and molecules that regulate the process of hypothalamic development, and highlight their effects on behaviors.

Signaling pathways that determine hypothalamic regional territories

The Wnt and sonic hedgehog (Shh) signaling pathways play essential roles in neural development. They normally function as mitogens that control proliferation of progenitor cells and affect expression of downstream genes. Studies have shown that the Wnt and Shh pathways are also crucial for formation of hypothalamic regional territories (Fig. 4a).

Wnt signaling pathway

It has been shown that a gradient expression of the Wnt signaling contributes to the formation of the anterior-posterior (A-P) axis in the neural plate [59–61]. Wnt components are expressed in a distinct pattern to form a temporal local gradient Wnt activity and to participate in self-renewal and differentiation of prechordal mesoderm cells [62, 63]. For example, Wnt7b and Wnt8b are highly expressed in the central nervous system in mice between embryonic day 11 (E11) and E16 (<http://www.brain-map.org/>). Specifically, Wnt7 is expressed in the anterior hypothalamus and Lef1 is expressed in the pre-mammillary area [58] (Fig. 4a). In zebrafish embryos, Wnt8 is expressed in the posterior area, Lef1 in the mammillary area, and Axin1 and Tcf3 in the posterior area [64–67]. Specific expression of molecules in the Wnt signaling pathway implies their roles in regulating hypothalamic regional territories.

In the zebrafish, functional deletion of Wnt8b leads to loss of expression of the proneuron marker *Zash1a* and the neurogenesis marker *Sox3* [64]. Moreover, abrogation of Lef1, the essential mediator of the Wnt pathway, results in absence of expression of *Sox3*, *Zash1a*, and the postmitotic neuron marker *HuC/D* [64]. This suggests that the Wnt-Lef1 signaling is crucial for hypothalamic neurogenesis. Deletion of Axin1, a Wnt signaling inhibitor, leads to a reduced expression of the early posterior hypothalamic marker *Nkx2.1a*, and adding Axin1

to the mutant can restore its expression in the zebrafish [65]. Similarly, it has been found that Tcf3, another Wnt repressor, is expressed in the posterior hypothalamus of the zebrafish [67], and overexpression of Tcf3 results in an ectopic expression of Nkx2.1a [68]. This suggests that Axin1 and Tcf3 play an important role in specifying identity of the posterior hypothalamus (Fig. 4b).

Shh signaling pathway

Dynamic and transient expression of Shh in the prechordal plate has been shown to control the dorsal and ventral patterning of the developing brain [69, 70]. Sonic hedgehog (Shh) has a peak expression at E9.5 when it mainly plays a role in patterning the mouse hypothalamus. Later on, its expression becomes progressively reduced and does not reach another peak until E12.5 when it participates in hypothalamic glial differentiation [58, 71].

At the early stage of the hypothalamic development, Shh is specifically expressed in the ventral area [58] (Fig. 4a). Conditional knockout of *Shh* using the *Foxb1-Cre* line in the neural plate in mice disrupts the expression of *Dbx1* and *Dlx2*, and causes a reduction of the ventral domain and a reversion to dorsal identity in the hypothalamus [72]. Similarly, *Shh* deletion in the hypothalamic basal plate using the *Nkx2.1-Cre* line results in loss of the anterior hypothalamus including the ARC, PVN, and VMN, and a thinner posterior hypothalamus [58]. Moreover, the Shh signaling repressor Gli3 is expressed in presumptive hypothalamus region by E7.5 in mice and plays a role of specifying the hypothalamic subregions by balancing the Shh-Gli pathway [73] (Fig. 4). These studies suggest that Shh is required for formation of the ventral and anterior hypothalamus.

Genes interacting with the Wnt and Shh pathways

Previous work has shown that BMP7, expressed in the posteroventral hypothalamus, is crucial for repressing Shh expression and in turn contributes to the Shh dynamic expression [71, 74, 75]. Deletion of *Foxg1*, which marks the boundary between the telencephalon and basal diencephalon [58], results in expansion of the hypothalamus [76]. It has been found that *Foxg1* is induced by Shh to directly repress the expression of Wnt8b ligand [76], suggesting that *Foxg1* may regulate formation of the hypothalamic regional territory by integrating the Wnt and Shh signalings (Fig. 4b). Furthermore, *Vax1*, which marks the ventral anterior area, is induced by Shh to repress Wnt activity by activating the inhibitor Tcf4 (also called Tcf712) [77, 78] (Fig. 4b). *Six3* is expressed in the ventral area and may promote Shh expression by functioning as a remote enhancer [79].

Studies have shown that the combinatory expression of transcription factors such as *Nkx2.1*, *Emx2*, *Dlx2*, and growth factors such as fibroblast growth factors (Fgfs) also regulates hypothalamic borders [80]. For instance, *Nkx2.1* specifies the posteroventral hypothalamus [81], while *Rx3* is important for the anterior hypothalamus [82]. *Irx5* is expressed in the supramammillary nucleus, and *SF-1* and *Pomc* are expressed in the VMN and ARC, respectively [83, 84]. The combination of *Lhx6*, *Arx*, and *Nkx2.1* expression demarcates the tuberomammillary area, and positive expression of *Foxg1* and negative expression of *Sim1* and *Nkx6.2* define the preoptic area [58]. These transcription factors appear to be regulated

by the combinatory effect of the Wnt and Shh signalings directly or indirectly [80], suggesting downstream genes of the Wnt and Shh pathways (Fig. 4b).

Genes determining formation of hypothalamic nuclei and their functions

Formation of hypothalamic nuclei is organized by generation and migration of specific neurons. Neurogenesis in the developing hypothalamus occurs between E10 and E16 in the mouse. Genetic studies have shown that deletion of specific genes leads to neurogenesis defects and failure in organization of diverse nuclei, and in turn affects distinct hypothalamic functions, and results in physiological or behavioral changes (Table 1).

Genes regulating development and functions of the ARC and VMN

Studies have shown that Mash1 is expressed in the ARC and VMN of the hypothalamus and contributes to neurogenesis. Mash1 deletion results in loss of distinct neurons, including neurons secreting neuropeptides, GHRH, and dopamine neurons [85–87]. It has been shown that Mash1 is induced by the Shh signaling in vivo, suggesting that the Shh signaling controls the development of the ARC and VMN by regulating Mash1 expression [88] (Fig. 4b). Moreover, Ngn3 plays a role in mediating differentiation of the ARC and VMN. It has been found that Ngn3 promotes the development of neurons expressing POMC and SF-1 (a VMN marker), and inhibits the expansion of the NPY neuronal population [89]. Conditional deletion of *Ngn3* in the ventral hypothalamus causes hyper-phagia and reduces energy expenditure, and eventually results in obesity [90]. This indicates that Ngn3 may control energy balance by promoting differentiation of appetite-related neurons. Moreover, a recent work has found that Ngn3 is regulated by the sex-determining gene Sry on the Y chromosome and mediates sex-specific development in the hypothalamus [91].

In addition, NHLH2 is expressed in the hypothalamus, and *NHLH2* knockout mice present adult-onset obesity and infertility [92]. It appears that NHLH2 mediates infertility by regulating migration of neurons expressing GnRH, while it affects energy balance by mediating cleavage of the proneuropeptide POMC [93]. Furthermore, SF-1 is specifically expressed in the VMN, and *SF-1* deletion in the VMN makes cells more scattered, and in turn results in failure in VMN formation [94, 95]. The VMN-specific *SF-1* knockout mice are less sensitive to leptin and subsequently become obese in high fat diet (HFD), which suggests that the VMN regulates energy balance possibly by the leptin signal [96–98].

Moreover, VMN specific *SF-1* knockout mice display anxiety-like behaviors, which indicates that the VMN also participates in stress regulation [99].

SCN formation and rhythm

Studies have shown that Six3, Six6, and Lhx2 are required for SCN neuronal development, and deletion of any of them fails to specify the SCN [100–102]. Moreover, deletion of *Lhx1* specifically in the SCN selectively affects development of SCN neurons that produce neuropeptides AVP, Vip, and Grp, but has no effects on generation of neurons that secrete GABA, Gal, and SST [103]. *Lhx1*-deficient mice show disrupted SCN cellular rhythm and

circadian activity rhythm [103]. These studies indicate that normal development of SCN is also important for acquirement of its circadian properties.

Genes determining formation of nuclei related to the neuroendocrine system

The aPV, PVN, and SON consist of parvicellular neurons that secrete CRH, TRH, GHRH, and somatostatin (SST), and magnocellular neurons that produce AVP and OXT. These nuclei are the major developmental centers of the neuroendocrine system, where many endocrine progenitors are produced and then migrate to other brain sites [104–106].

The homeobox gene *Orthopedia* (*Otp*) is strongly expressed in the putative anterior paraventricular (aPV), PVN, and the supraoptic nucleus (SON) at the early stage, and deletion of *Otp* leads to failure in formation of the aPV/PVN/SON and causes loss of distinct neurons including neuroendocrine neurons secreting SST, AVP, OXT, CRH, TRH, and dopamine neurons [107–109]. *Otp* is found to be promoted by *Shh* and repressed by *Fgf8* in the zebrafish [110], which indicates that *Shh* may participate in specification of endocrine cells (Fig. 4b). While deletion of *Otp* leads to embryonic lethality in mice, deletion of *Otp* only affects stress adaptation in the zebrafish [111].

Sim1 is an important gene for organization and neuronal neurogenesis of the aPV/PVN/SON, and its expression is controlled by *Otp* [112, 113]. It has been shown that *Sim1* mutation causes decrease of almost all endocrine cells in mice. Together with *Arnt2*, *Sim1* regulates *Brn2* expression to promote differentiation of cells secreting AVP, CRH, and OXT in the PVN and SON, and also controls *Sim2* expression to determine differentiation of cells secreting SST and TRH in the aPV [114–116]. In humans, patients without *SIM1* show Prader-Willi-like symptoms [117–119]. Decreased *SIM1* expression in humans has been reported to cause obesity [120]. Similarly, *Sim1* haploinsufficient mice show hyperphagic obesity due to profound reduction of OXT [121–123]. In contrast, *Sim1* overexpressing mice show reduced diet-induced obesity [124]. This suggests that OXT is important for weight control and its function is regulated by *Sim1* expression.

Formation of neurocircuits and hypothalamic functions

Besides main outputs to pituitary and autonomic neurons in the midbrain and hindbrain, one of the most significant characterizations of the hypothalamus is that its efferent fibers reach the internal nuclei, especially the ARC. Neurons that produce NPY and POMC in the ARC send a bundle of projections to the VMN, LH, and PVN to regulate feeding and expenditure (Fig. 2). Interestingly, the number of these efferent fibers in the ARC influences energy metabolism. For example, decrease of outputs of ARC neurons due to the maternal HFD causes obesity and disorders of glucose metabolism in the offspring [125]. It has been found that *Fgf8* is expressed in the hypothalamus and contributes to growth of GnRH axons, and netrin-1 and its receptor DCC participate in orientation of retinal ganglion cell (RGC) axons in the ventral hypothalamus [126]. However, it is unclear what developmental genes play a role in formation of hypothalamic functional circuits.

Roles of miRNAs in the development and functions of hypothalamus

miRNAs are a large family of endogenous noncoding small RNAs. They are ~22 nucleotides long and function through degradation or posttranscriptional repression of target genes by imperfect complementary pairing. Studies have shown that miRNAs participate in neurogenesis, neuronal differentiation and migration, and regulate brain functions by silencing specific target molecules [127–129]. It has been found that some miRNAs are highly expressed in the hypothalamus, for example, miR-7, let-7, and miR-9 [130, 131]. Of these, let-7 has been shown to inhibit Mash1 to reduce neurogenesis in vitro [132]. Additionally, specific deletion of *Dicer*, an essential enzyme that processes miRNA precursors, in the POMC-expressing cells leads to failure in development of neurons secreting POMC, and in turn causes obesity and disrupted glucose metabolism [133]. However, what and how specific miRNAs affect development and functions of hypothalamic nuclei and its neuronal lineages are still unknown.

Perspectives

The hypothalamus is a brain region consisting of many nuclei that control a variety of endocrine, autonomic, and behavioral functions. Besides functions discussed above, the hypothalamus also participates in many other basic life functions including fluid and electrolyte balance, aggressive behaviors, and so on [1]. Molecular mechanisms that regulate hypothalamic development, especially specification of subnuclei and neuronal lineages, and formation of neurocircuits, and their consequences to hypothalamic physiological functions, need to be further studied. Genetic rescue will be helpful to further reveal the role of specific genes in regulating hypothalamic development and physiological functions. Roles of noncoding RNAs such as miRNAs in development of distinct hypothalamic subnuclei and functions are of great interests to be further explored. Revealing molecular mechanisms of genes that regulate hypothalamic development and physiological functions will provide significant insights into understanding the etiology of hypothalamus-associated diseases such as adolescent obesity, diabetes, and depression, and developing a means for treatment.

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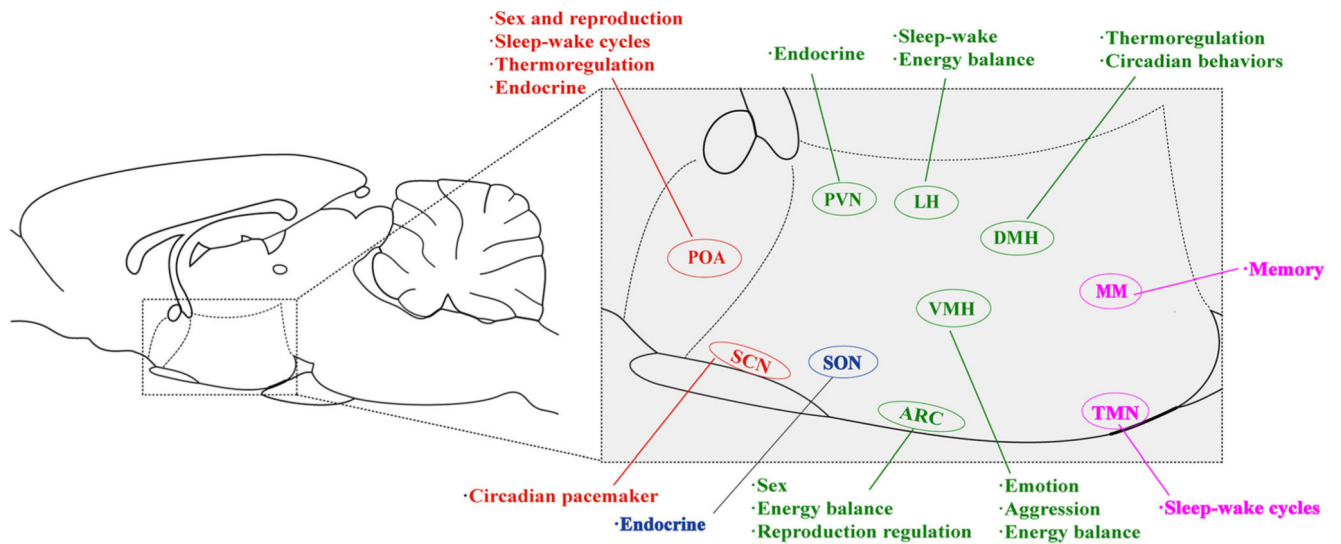


Fig. 1.

An overview of hypothalamic nuclei and functions. Nuclei and their functions in the preoptic region are denoted in *red*, including the preoptic area (POA) and the suprachiasmatic nucleus (SCN). Relative locations and functions of the supraoptic region are denoted in *blue*, including the supraoptic nucleus (SON). The tuberal region is denoted in *green*, including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamus (LH), the ventromedial hypothalamus (VMH), and the dorsomedial hypothalamus (DMH). The mammillary region, including the mammillary area (MM) and the tuberomammillary nucleus (TMN), is denoted in *purple*.

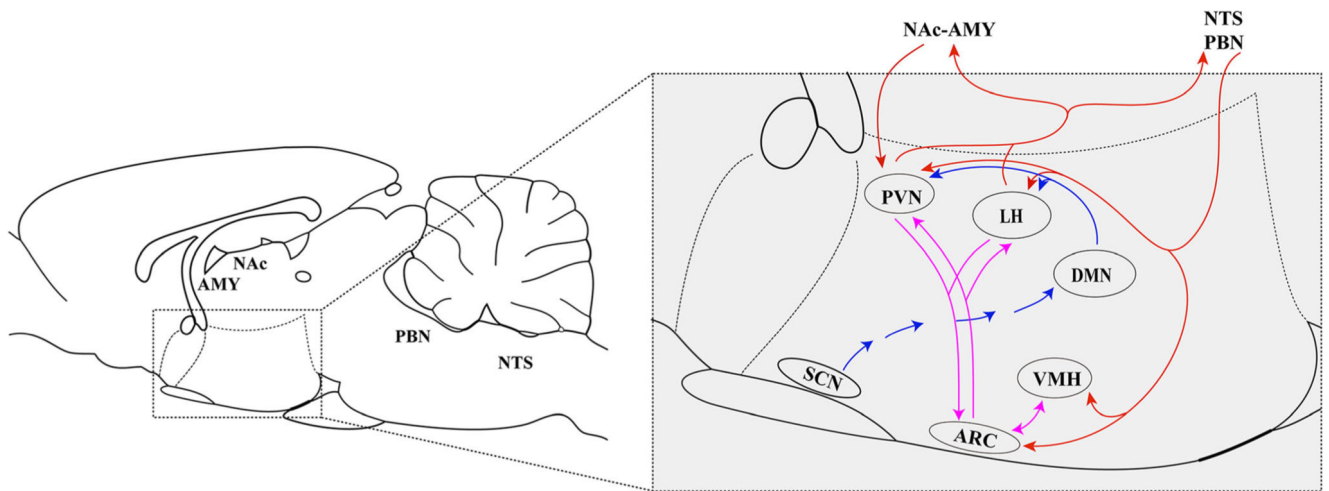


Fig. 2.

Hypothalamic neurocircuits involved in feeding and energy expenditure. The brain outline (*left*) shows relative locations of the hypothalamus and other related nuclei including the nucleus accumbens (NAc) and amygdala (AMY) in the forebrain, the parabrachial nucleus (PBN) in the midbrain, and the nucleus of the solitary tract (NTS) in the hindbrain. The highlighted image (*right*) shows nuclei and projections among nuclei and other brain sites in the hypothalamus. Projections between the arcuate nucleus (ARC) and other nuclei, which play important roles in mediating intrahypothalamic interactions, are denoted in *purple*. Pathways between hypothalamic subnuclei and other feeding-related (PBN and NTS) and reward-related (NAc and AMY) information are denoted in *red*. The paraventricular nucleus (PVN) and lateral hypothalamus (LH) seem to relay nuclei from feeding-related and reward-related sites to the ARC. Projections from the supraoptic nucleus, which are denoted in *blue*, indicate that circadian rhythm influences when to feed during the day. The *interrupted arrows* stand for indirect projections from suprachiasmatic nucleus (SCN) to the dorsomedial nucleus (DMN)

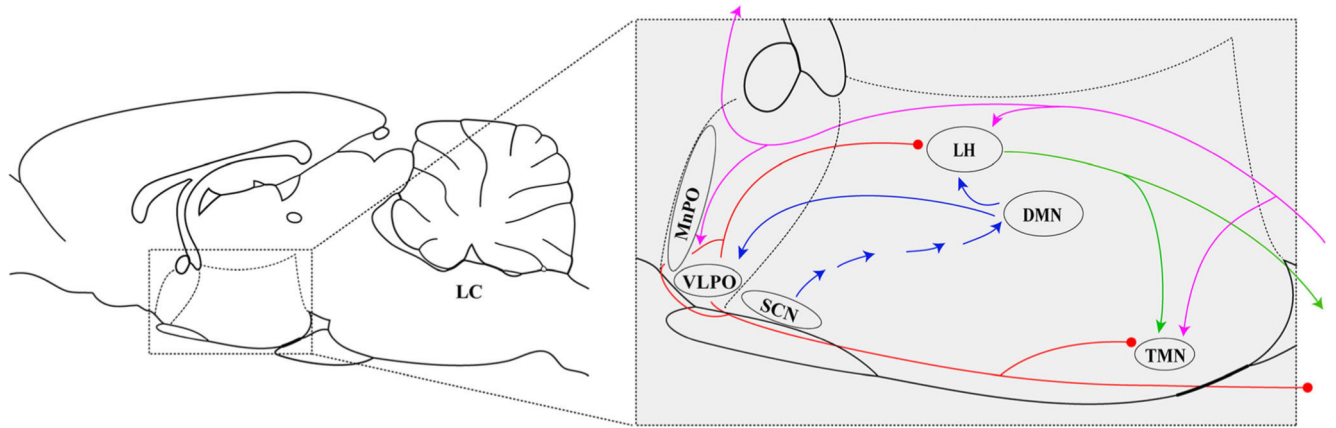


Fig. 3. Hypothalamic neurocircuits involved in sleep and wakefulness. The ascending arousal pathways, which originate from the locus coeruleus (LC) in the hindbrain and are all active during wakefulness, are denoted in *purple*. Sleep-promoting pathways, in which neurons in the lateral hypothalamus (LH) and tuberomammillary nucleus (TMN) are repressed by neurons producing GABA and galanin in the ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (MnPO) during sleep, are denoted in *red*. Outputs of orexin, which are important for stabilizing sleep, are shown in *green*, and pathways of circadian rhythm involved in sleep-wake cycles are shown in *blue*

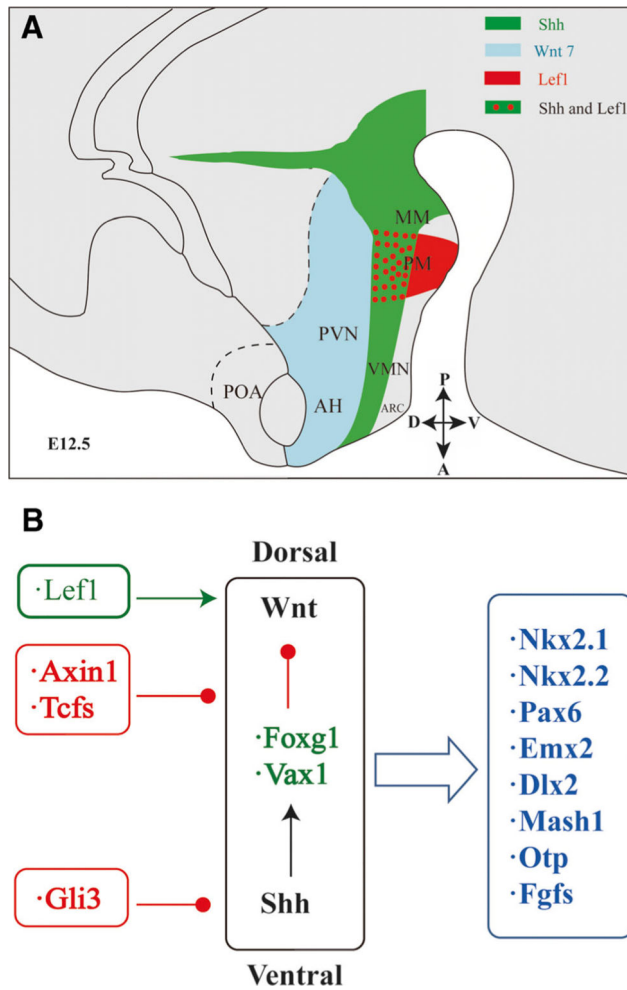


Fig. 4. The Wnt-Shh signaling network and hypothalamic development. **a** Schematic drawing of expression regions of Shh and Wnt components in the hypothalamus of the E12.5 mouse brain. The expression region of Shh is denoted in *green*; Lef1 is denoted in *red*; Wnt7 is denoted in *blue*. Both Shh and Lef1 are expressed in regions with *red dots* and *green background*. Other brain areas are shown in *gray*. AH anterior hypothalamus, ARC arcuate nucleus, MM mammillary area, PM pre-mammillary region, POA preoptic area, PVN paraventricular nucleus, VMN ventromedial nucleus. The anterior-posterior (A-P) and dorsal-ventral (D-V) directions of the hypothalamus in the E12.5 mouse brain are shown. **b** Genes of the Wnt-Shh signaling network in the developing hypothalamus. Shh signaling is mainly located in the ventral hypothalamus while Wnt signaling is in the dorsal area. The essential mediator of the Wnt pathway Lef1 and inhibitors of Wnt signaling Axin1 and Tcfs regulate Wnt activity. Shh components Gli3 represses Shh activity in the developing hypothalamus. Early developmental genes Vax1 and Foxg1 regulate interactions between Wnt and Shh pathways. The Wnt-Shh signaling network regulates expression of downstream genes related to neurogenesis such as Nkx2.1, Nkx2.2, Pax6, Emx2, and Dlx2, and molecules that specify subnuclei and neuronal lineage such as Mash1, Otp, and Fgfs

Table 1

Roles of developmental genes in hypothalamic formation and physiological functions

Gene mutations	Developmental phenotypes	Functional consequences	References
Mash1 deletion	Failure of neurogenesis in the ARC and VMN	Unknown	[85–87]
Otp deletion	Failure of formation of the aPV/PVN/SON	Disrupted stress responses	[107–109, 111]
Sim1 heterozygous deletion	Reduction of OXT	Obesity	[121–123]
Ngn3 deletion	Decrease in neurons expressing POMC and SF-1, and increase in NPY cells	Obesity	[89, 90]
NHLH2 deletion	Disrupted GnRH migration	Infertility	[92, 93]
SF-1 deletion	Failure of formation of VMN	Obesity and anxiety	[94–99]
Lhx1 deletion	Disrupted production of neuropeptides AVP, Vip, and Grp	Disrupted SCN rhythm	[103]

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