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REVIEW

Development of the hypothalamus: conservation, modification and innovation

Yuanyuan Xie and Richard I. Dorsky*

ABSTRACT

The hypothalamus, which regulates fundamental aspects of physiological homeostasis and behavior, is a brain region that exhibits highly conserved anatomy across vertebrate species. Its development involves conserved basic mechanisms of induction and patterning, combined with a more plastic process of neuronal fate specification, to produce brain circuits that mediate physiology and behavior according to the needs of each species. Here, we review the factors involved in the induction, patterning and neuronal differentiation of the hypothalamus, highlighting recent evidence that illustrates how changes in Wnt/β-catenin signaling during development may lead to species-specific form and function of this important brain structure.

KEY WORDS: Hypothalamus, Wnt, Evolution, Adult neurogenesis

Introduction

The hypothalamus is a brain structure with highly conserved anatomy throughout vertebrate species (Fig. 1) owing to its essential function in regulating fundamental aspects of physiological homeostasis and behavior. Located at the most ventral position in the forebrain (Fig. 1A), the hypothalamus has integral developmental and functional connections with the pituitary gland, through which it controls endocrine hormone release (Burbridge et al., 2016). Some of the basic body functions regulated by pituitary hormones and their targets in the adrenal glands, thyroid and gonads include fluid balance, stress response, reproduction and growth. In addition, the hypothalamus makes neural connections via the autonomic nervous system and other pathways to regulate sleep, body temperature and feeding (Elson and Simerly, 2015; Caron and Richard, 2016; Morrison, 2016; Herrera et al., 2017). Because it also receives neuronal and circulatory input from the entire body, the hypothalamus has been seen as a center for responding to the body's physiological status through these hormonal and neural outputs. Dysfunction of the hypothalamus can thus have a profound effect on diverse areas of health, including energy imbalance, diabetes insipidus and sleep disorders (Box 1) (Maghnie et al., 2000; Gao and Horvath, 2014; Shan et al., 2015).

Despite its relatively small size, many discrete cellular nuclei are found throughout the hypothalamus (Fig. 1B), expressing a large number of different neurotransmitters and peptide hormones. Yet the presence of individual neuronal subtypes, and even their general locations, can be very similar in species as diverse as fish and mammals (Löhr and Hammerschmidt, 2011). These similarities raise the question of whether conserved molecular mechanisms regulate hypothalamic induction, patterning and neurogenesis in all vertebrates. Many years of investigation using different organisms have lent strong support to such a model, finding strikingly similar roles for specific signaling pathways in hypothalamus development in each species. We therefore have a fairly complete picture of how basic hypothalamic anatomy is determined, as well as how relatively small changes in signaling can reshape this anatomy. However, other important aspects of hypothalamus development remain unaddressed, particularly those involving the building and remodeling of behavioral circuits that differ between vertebrate species. Indeed, by comparing individual vertebrate models it is possible to observe biologically relevant variations in hypothalamic anatomy and neurogenesis that clearly exist but are poorly understood. One example is the gain and/or loss of apparent 'modules' comprising anatomical structures, specific cell types and gene expression (Box 2) (Puelles et al., 2012). Another example is the specialization of neuronal populations into novel subtypes with different physiology, neurotransmitter expression or anatomical projections (Chometton et al., 2016). Both processes could lead to the control of specific behaviors that differ between species, and therefore represent potential mechanisms for modifying behavioral circuitry through evolution.

A separate but equally important process in circuit remodeling is the contribution of adult neurogenesis to specific hypothalamic populations. While neural progenitors have been identified in the postnatal mammalian hypothalamus (Kokoeva et al., 2005, 2007), it is less clear which specific populations function as true adult stem cells. Furthermore, study of the physiological function of postnatal neurogenesis within the hypothalamus has been focused mainly on feeding and energy balance (Xu et al., 2005; Perez-Martin et al., 2010; Haan et al., 2013), and it is not known whether neurons for other behavioral circuits are also generated postnatally. There is also wide variation in the scope of adult neurogenesis between species. In zebrafish, for example, the adult hypothalamus contains a high number of proliferating neural progenitors, which contribute to multiple lineages (Wang et al., 2012; Duncan et al., 2016). However, the role of these cells in physiology and behavior is poorly understood.

Although we have little knowledge of the molecular mechanisms that underlie these processes of circuit building and neurogenesis, recent studies have begun to identify candidate pathways that might be involved. Here, we provide an overview of the factors that control the early induction and patterning of the hypothalamus, as well as those that regulate neurogenesis in the embryonic and adult hypothalamus, topics that have been recently reviewed in detail (Bedont et al., 2015; Burbridge et al., 2016). Based on data from multiple model systems, we also speculate that changes in Wnt signaling may modify hypothalamic regionalization and alter the specification and differentiation of neural progenitors in the embryo and adult, and thereby give rise to species-specific hypothalamic circuitry.

Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84112, USA.

*Author for correspondence (richard.dorsky@neuro.utah.edu)

Y.X., 0000-0001-9796-7514; R.I.D., 0000-0002-8037-6694

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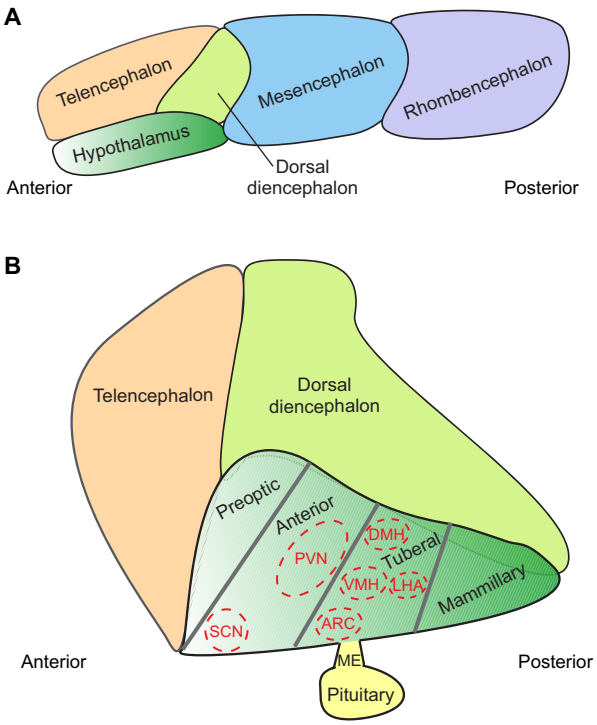

Fig. 1. Location and structure of the hypothalamus. The anatomical position and basic anatomy of the hypothalamus is conserved throughout vertebrates. (A) Lateral view of the position of the hypothalamus primordium in a prototypical vertebrate during early brain development. The presumptive hypothalamus arises from the ventral forebrain at the rostroventral limit of the neural plate. (B) Lateral view of the forebrain showing conserved hypothalamic divisions in a prototypical vertebrate following initial patterning. Four rostrocaudal regions are delineated by solid gray lines, and selected hypothalamic nuclei are outlined by dashed red lines. ARC, arcuate nucleus; DMH, dorsomedial hypothalamus; LHA, lateral hypothalamic area; ME, median eminence; PVN, paraventricular nucleus; SCN, superchiasmatic nucleus; VMH, ventromedial hypothalamus.

Hypothalamic anatomy

The general anatomy and functional organization of the hypothalamus are remarkably well conserved throughout vertebrate species (Fig. 1). In contrast to other regions of the central nervous system (CNS) such as the cortex and spinal cord, which are composed of columnar structures, the hypothalamus comprises various nuclei arrayed in a three-dimensional patchwork. The adult mammalian hypothalamus can be anatomically divided into four rostrocaudal levels and three mediolateral zones. From rostral to caudal, the four regions are preoptic, anterior, tuberal and mammillary hypothalamus (Fig. 1B), each of which has a lateral, medial and periventricular zone (Markakis, 2002). Each hypothalamic region has distinct patches of nuclei and associated functions (Burbridge et al., 2016). The preoptic area is known to control thermoregulation, reproduction and electrolyte balance. The anterior hypothalamus, including the supraoptic nucleus (SON), suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN) and anterior periventricular nucleus (aPV), regulates feeding, circadian rhythms and other homeostatic processes. The tuberal hypothalamus includes the arcuate nucleus (ARC), median eminence (ME), and ventromedial (VMH) and dorsomedial (DMH) hypothalamus, and plays a role in energy balance, stress response and aggression. The mammillary hypothalamus, which includes the mammillary bodies, is involved in arousal and stress response, as well as spatial and episodic memory (Vann and Nelson, 2015).

The developmental origin of distinct hypothalamic regions is currently a topic of considerable debate. A primary difference between existing models is the orientation of the alar/basal plate boundary. In the prosomeric model, this boundary separates the anterior and tuberal hypothalamus, whereas the columnar model places the boundary parallel to the anterior/posterior (A/P) axis, spanning all compartments (Puelles and Rubenstein, 2015). While the prosomeric model supports hypotheses relating hypothalamic and telencephalic co-evolution (Croizier et al., 2011), it has been challenged by experimental evidence from gene expression and function (Bedont et al., 2015). Additional comparative studies will be needed to shed further light on this fundamental anatomical question.

Hypothalamic control of pituitary hormone release is regulated by two types of neuroendocrine neurons. The magnocellular neurons are located in the PVN and SCN, where they project to the posterior lobe of the pituitary (neurohypophysis) to directly release oxytocin and arginine vasopressin. The parvocellular neurons are located in the tuberal nuclei, preoptic nuclei, ARC, aPV and PVN, and project to the ME where they secrete releasing or release-inhibiting hormones into a small portal blood system connected to the anterior pituitary (adenohypophysis). Most major anatomical and functional aspects of the neuroendocrine hypothalamus are conserved from zebrafish to mammals (Löhr and Hammerschmidt, 2011). Orthologs of mammalian neuropeptides and hormones are expressed in zebrafish by 5 days post-fertilization (dpf) (Löhr and Hammerschmidt, 2011). However, two gross anatomical differences have been documented: the ME is much more prominent (Löhr and Hammerschmidt, 2011) and the periventricular nuclei are greatly reduced (Puelles et al., 2012) in mammals compared with zebrafish.

Induction and patterning of the hypothalamus

In all vertebrates, a hypothalamic primordium is induced during neural plate formation, in the medial region of the prosencephalon (Woo and Fraser, 1995; Kobayashi et al., 2002). In line with general principles of brain development, hypothalamic induction requires signaling from morphogens secreted by surrounding tissues that create a map of positional identity in the neural plate. As the hypothalamus is induced and patterned, a set of transcription factors is then used to define specific regions of the tissue; while the relative positions of these regions are generally consistent throughout vertebrates, the proportional size of individual regions may vary between species. This variation could lead to changes in combinatorial factor expression and thus be a mechanism for the development of novel neuronal populations and circuits.

Box 1. Hypothalamic dysfunction

Genetic studies have indicated that developmental defects can lead to hypothalamic and pituitary dysfunction, and provide model systems to study the etiology of human disease. For example, in a hypothalamus-specific *Shh* knockout mouse, a defect in hypothalamic patterning also caused pituitary hypoplasia and absence of the optic disc, consistent with phenotypes in human patients with septo-optic dysplasia (SOD) (Jeong et al., 2006; Zhao et al., 2012). Other evidence shows that the Wnt/β-catenin pathway also plays a clinically relevant role in hypothalamic patterning, as SOD patients with variants in the Wnt effector *TCF7L1* have been identified (Gaston-Massuet et al., 2016). Multiple additional genes have been linked to developmental disorders of the hypothalamus that lead to hypopituitarism (Mehta and Dattani, 2008), including *SOX2* and *SOX3* (Woods et al., 2005; Macchiaroli et al., 2014).

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Hypothalamic induction

The initial induction of the hypothalamus relies on a combination of posteriorizing signals secreted from the caudal neurectoderm and paraxial mesoderm, and ventralizing signals arising from the axial mesoderm (Fig. 2A). During gastrulation, the dorsal axis organizer (dorsal lip of the blastopore in amphibians, shield in teleosts, and Hensen's node in birds and mammals) secretes Bmp antagonists to induce a neural plate (Stern, 2005). The presumptive hypothalamus (marked by *Nkx2-1* expression in all vertebrates) is first induced shortly after gastrulation when the neural plate has adopted a rostroventral fate.

The Wnt/β-catenin pathway plays an essential role in A/P axis formation. Wnts from the posterior neurectoderm and somites promote hindbrain fate (Erter et al., 2001; Lekven et al., 2001; Braun et al., 2003) and are antagonized by secreted and intracellular molecules expressed in the anterior neurectoderm (Kim et al., 2000; Shinya et al., 2000). Together, these factors create a gradient of A/P positional fate that ensures the proper position and size of the dorsal diencephalon (Dorsky et al., 2003) as well as the hypothalamus (Kapsimali et al., 2004). While Fgf and retinoic acid (RA) also posteriorize the CNS during neural patterning (Kudoh et al., 2004), their roles in hypothalamic induction are less clear, as most studies have only examined markers for dorsal neural fate. Bmp7 is secreted from the prechordal mesoderm and can promote a hypothalamic fate at the expense of floor plate based on experimental evidence from chick (Dale et al., 1997). However, it is still not clear whether this function is evolutionarily conserved, as in zebrafish genetic models Bmp signaling appears to play little role in A/P neural patterning (Barth et al., 1999; Kapsimali et al., 2004).

The axial mesoderm underlying the neural plate secretes Shh to ventralize the CNS (Lupo et al., 2006) (Fig. 2A). Both embryological evidence in chick and genetic evidence in mouse have shown that Shh is necessary and sufficient to induce the entire ventral CNS (Marti et al., 1995; Chiang et al., 1996), including the hypothalamus. The prechordal mesoderm, which lies ventral to the presumptive hypothalamus, produces Shh as well as the TGFβ ligand Nodal (Rohr et al., 2001) (Fig. 2A). Genetic work in zebrafish suggests a direct involvement of Nodal signaling in ventralizing the CNS by activating Shh expression (Hatta et al., 1991; Sampath et al., 1998). Although it has been shown that Nodal is essential for hypothalamic induction in zebrafish (Rohr et al., 2001), this role may be species-specific (Strähle et al., 2004) because Nodal appears to be dispensable in mouse (Camus et al., 2006), and acts together with Shh in chick (Patten et al., 2003).

Work from amphibians is consistent with two widely accepted models for neural induction and patterning: the 'default model' and the 'two-signal model'. In the default model, ectodermal cells will become neurons by default if they receive no signals (Hemmati-Brivanlou and Melton, 1997; Stern, 2006). In the two-signal model originally proposed by Nieuwkoop (Nieuwkoop, 1952), the ectoderm first differentiates into rostralmost neural tissues upon neural induction during the 'activation' step; caudal neural tissues are then induced during the 'transformation' step. As the hypothalamus is located in the most rostral part of the CNS, these two models raise the possibility of hypothalamic identity being a default fate. Indeed, cultured *Xenopus* gastrula dorsal ectoderm in the absence of any growth factors will express the hypothalamic marker *nkx2-1* at tailbud stage (Lupo et al., 2002). When exogenous signals are minimized, cultured mouse and human embryonic stem cells, and human induced pluripotent stem cells (hiPSCs) will also differentiate into hypothalamic neurons (Wataya et al., 2008; Merkle et al., 2015). Sasai and colleagues have since proposed an intriguing hypothesis that the hypothalamic anlage represents the origin of the Cartesian coordinates during neural patterning, which might be related to its neuroendocrine role as a homeostasis center during evolution (Wataya et al., 2008).

Box 2. Modular changes in hypothalamic anatomy: a means to evolve behavioral circuits

Comparative studies suggest that species-specific developmental programs may link anatomy, cellular differentiation and gene expression to create hypothalamic 'modules' that can be gained or lost through evolution. One example is the posterior ventricular recess (also referred to as the posterior paraventricular organ), which has a high level of continuous neurogenesis in zebrafish (Grandel et al., 2006; Wang et al., 2012). Although the posterior recess is absent in placental mammals, several neuronal subtypes that are specifically located in this region in non-mammalian vertebrates, such as histaminergic neurons (Eriksson et al., 1998), can be found in the premammillary hypothalamus of rodents (Steinbusch et al., 1986), suggesting possible homology at the anatomical and functional levels. However, a population of ciliated ependymal serotonergic cells has been well characterized in many non-mammalian vertebrates (Sano et al., 1983) but is absent in the mammalian hypothalamus. Likewise, dopaminergic neurons expressing the synthetic enzyme Tyrosine hydroxylase 2 (Th2) are present in the zebrafish posterior recess (McPherson et al., 2016), but the *Th2* gene itself has been lost in mammalian genomes (Yamamoto et al., 2010). These observations suggest that either the functional circuitry within the posterior recess has been replaced by other mammalian brain regions, or that these functions are unnecessary for mammalian behavior and have been eliminated due to a lack of selective pressure. Evidence for the first possibility will require a better understanding of functional circuits in specialized areas of the mammalian brain, such as the neocortex. The second possibility could be supported by examining the impact of mammalian-specific anatomy on behaviors such as nursing and weaning.

Early patterning of the hypothalamus

During hypothalamic patterning, local environmental signals subdivide the developing hypothalamus (Fig. 2B). These signals establish discrete A/P zones (Kapsimali et al., 2004) and eventually help to define the boundaries of hypothalamic subregions (Fig. 1B) (Muthu et al., 2016). The hypothalamus also has intrinsic dorsal/ventral (D/V) identity consistent with the alar, basal, and floor plate territories of the developing neural tube, and specific gene markers define these regions (Domínguez et al., 2011). Transcription factors then act downstream of secreted signals to further subdivide these territories into smaller divisions (Shimogori et al., 2010). The ultimate organization of the hypothalamus into functional nuclei depends on these divisions, as shown by the broad conservation of neuronal subtypes in each region, from fish to mammals (Domínguez et al., 2015; Ferran et al., 2015).

Upon hypothalamic induction, genes encoding secreted morphogens are expressed within restricted regions of the hypothalamus, such as *Bmp4*, *Fgf8*, *Fgf10* and *Wnt8b* in the caudal hypothalamus and *Shh* in the rostral hypothalamus (Lako et al., 1998; Orquera et al., 2016; Zhao et al., 2012), all of which may play a role in hypothalamic patterning. Indeed, a hypothalamic patterning defect was reported in mice lacking noggin, which encodes a Bmp antagonist (Davis and Camper, 2007). Fgf signaling has been shown to play an essential role in formation of the infundibulum, which arises from the floor plate region to produce the ME and posterior pituitary (Ohuchi et al., 2000).

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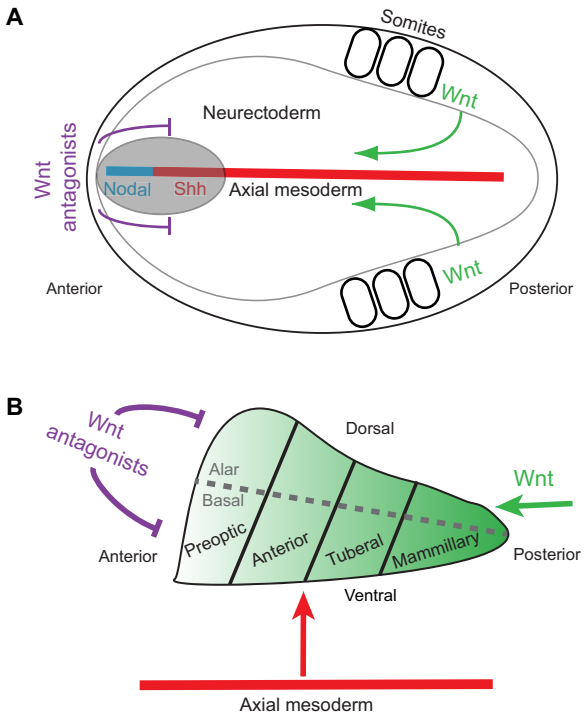

Fig. 2. Induction and patterning of the hypothalamus. Several morphogen signals induce and pattern the hypothalamus. (A) At neural plate stages, the hypothalamus primordium (gray oval) is induced by Wnt antagonists in the anterior neurectoderm that inhibit Wnt signals from the posterior mesoderm, and by Nodal and Shh from the prechordal axial mesoderm. Dorsal view is shown. (B) During hypothalamic patterning, a gradient of Wnt signaling signals creates discrete anterior/posterior regions of the hypothalamus (depicted by solid black lines). Hedgehog signaling provides dorsal/ventral polarity and establishes alar and basal zones within each region (delineated by dashed gray line), as well as floor plate (not shown). Orientation of boundaries is depicted according to current columnar models of hypothalamic anatomy. Lateral view is shown.

Shh is initially expressed throughout the presumptive hypothalamus, but its expression is later restricted to the rostral hypothalamus, where it promotes an anterior fate in zebrafish (Mathieu et al., 2002), chick (Manning et al., 2006) and mouse (Zhao et al., 2012). Additional work has identified upstream regulators of *Shh* during hypothalamic patterning. In mice, Sox2 and Sox3 directly activate *Shh* in the rostral hypothalamus through the SBE2 enhancer, whereas Tbx2 and Tbx3 repress *Shh* in the caudal hypothalamus by sequestering Sox2 away from SBE2 (Trowe et al., 2013; Zhao et al., 2012). A similar pathway was shown in chick, where Bmp2 and Bmp7 activate Tbx2, which represses *Shh* expression in the caudal hypothalamus (Manning et al., 2006). *Shh* expression may also be mediated by Rx3/Rax, as in both zebrafish *rx3* and mouse *Rax* mutants, *Shh* expression is lost accompanied by a hypothalamic patterning defect (Muthu et al., 2016; Orquera et al., 2016). Interestingly, this phenotype is only observed when *Rax* is knocked out prior to embryonic day (E) 8.5 in mouse embryos, whereas a more specific defect in specification and differentiation of hypothalamic neurons is revealed when *Rax* is knocked out later (Lu et al., 2013; Orquera et al., 2016).

In addition to a role for Wnt signaling in neural plate patterning, it also directly posteriorizes the hypothalamus, as revealed by genetic analysis of zebrafish mutants (Kapsimali et al., 2004). Given conserved *Wnt8b* expression in the caudal hypothalamus of all vertebrates tested (Lako et al., 1998; Garda et al., 2002; Lee et al., 2006), this posteriorizing function is likely to be conserved in other species. Indeed, when *Tcf7l1*, which encodes a transcriptional repressor of Wnt targets, is conditionally knocked out in the mouse hypothalamus and pituitary, the developing hypothalamus is posteriorized (Gaston-Massuet et al., 2016). Lhx2, a potential upstream inhibitor of Wnt signaling (Peukert et al., 2011), has also been shown to play a role in the patterning of the telencephalic-optic-hypothalamic field, and to specify SCN neurons at the expense of neuroendocrine fates (Roy et al., 2013).

The use of conserved morphogen signals to establish regional identity in the hypothalamus has two important implications for hypothalamic plasticity during evolution. First, it allows relatively small changes in pathway activity to reshape hypothalamic structure. For example, a shift in the Wnt gradient caused by alterations in the expression of ligands or secreted inhibitors could reposition downstream target genes and hence modulate regionalization of the hypothalamus. Second, the gain or loss of regulatory elements in these same target genes could change their responsiveness to signaling pathways, creating or eliminating specific expression domains. As discussed in the following section, these genes encode transcription factors that can also specify cell fate. Thus, their repositioning would not only alter the location of individual neuronal populations, but would also allow new combinations of genes to be co-expressed and thus create novel neuronal subtypes and circuits. In fact, such a mechanism has been demonstrated in recently diverged species of cichlid fish, where changes in Wnt regulation and activity can mimic changes in forebrain structure between species (Sylvester et al., 2010).

Embryonic neurogenesis and gliogenesis

Hypothalamic histogenesis follows the same general pattern as that observed in other neural tube-derived brain regions, with dividing progenitors residing in the ventricular zone and producing neuronal and glial precursors that migrate laterally into the parenchyma. However, as differentiating neurons move to the final location where they begin to make functional connections, some populations undergo a large tangential migration along the A/P or D/V axes (Puelles et al., 2016). An exceptional example of this process is illustrated by GnRH neurons, which originate outside the hypothalamus, migrate into the preoptic area, and send projections to the ME (Wierman et al., 2011). Through the processes of neuronal specification and migration, species-specific anatomical modules can be created that contain multiple cell types functioning together to regulate individual behaviors. Furthermore, the ultimate diversity of neuronal subtypes within the hypothalamus, with regard to both molecular and anatomical identity, provides a substrate for the development of species-specific populations and circuitry.

Basic mechanisms regulating hypothalamic neurogenesis

The molecular and cellular processes involved in hypothalamic neurogenesis are also similar to those used in other brain regions. Progenitor cells first undergo a rapid proliferative expansion, followed by a period of asymmetric divisions that generate neural precursors, ending in the terminal differentiation of neurons (Duncan et al., 2016). Evolutionary conservation again exists for the molecular pathways that regulate these processes. For example, as hypothalamic progenitors become specified to form neurons in different species, they express proneural genes that encode basic helix-loop-helix (bHLH) transcription factors in similar patterns (Osório et al., 2010; Ware et al., 2014). These proneural genes have been shown to play direct roles in the formation of specific

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neuroendocrine cell types, most prominently in the tuberal region (McNay et al., 2006; Pelling et al., 2011). In addition, and as seen throughout the nervous system, Notch/Delta signaling acts to restrict proneural gene expression and function, maintaining a pool of neural progenitors (Aujla et al., 2013).

Next, factors specifying general cell fates are expressed as neuronal precursors become committed to particular lineages. For example, a series of Dlx family transcription factors labels GABAergic precursors in the hypothalamus, just as in the basal telencephalon (Petryniak et al., 2007; Yee et al., 2009). Other transcription factors define and/or induce cells that express specific hypothalamic neuropeptides (Bedont et al., 2015; Burbridge et al., 2016), which have now been characterized at the single-cell level (Campbell et al., 2017; Romanov et al., 2016) (Fig. 3, Table 1). For instance, Isl1 directly activates *Pomc* and *Agrp* in ARC neurons, and is required for their expression as well as that of *Sst*, *Ghrh* and *Npy* (Nasif et al., 2015; Lee et al., 2016). In the anterior hypothalamus, Lhx1 and Otp have been shown to play an important role in the differentiation of neurons in the SCN and PVN/SON/aPV, respectively (Acampora et al., 1999; Wang and Lufkin, 2000; Bedont et al., 2014). The function of some factors appears to be evolutionarily conserved, as demonstrated by the role of Lhx9 in specifying Hcrt^{+} neurons in zebrafish and mice (Liu et al., 2015). Now that effective methods have been developed to generate hypothalamic neurons from hiPSCs *in vitro* (Merkle et al., 2015), it will be possible to rigorously test hypothesized transcriptional 'codes' for specific neuronal types, and potentially apply this knowledge to therapeutic uses.

The behavioral functions of neuroendocrine subpopulations may also differ depending on the developmental program from which they are derived. For example, Dbx1-dependent Agrp^{+}/Npy^{+} neurons in the ARC and Pmch^{+} neurons in the lateral hypothalamic area (LHA) play a specific role in innate stress responses rather than their well-known function in feeding (Sokolowski et al., 2015). Similarly, Nkx2-1-dependent estrogen receptor alpha (ERα)$^{+}$ neurons in the VMH promote female locomotion instead of reproduction (Correa et al., 2015). Therefore, further studies are needed to uncover a complete map of

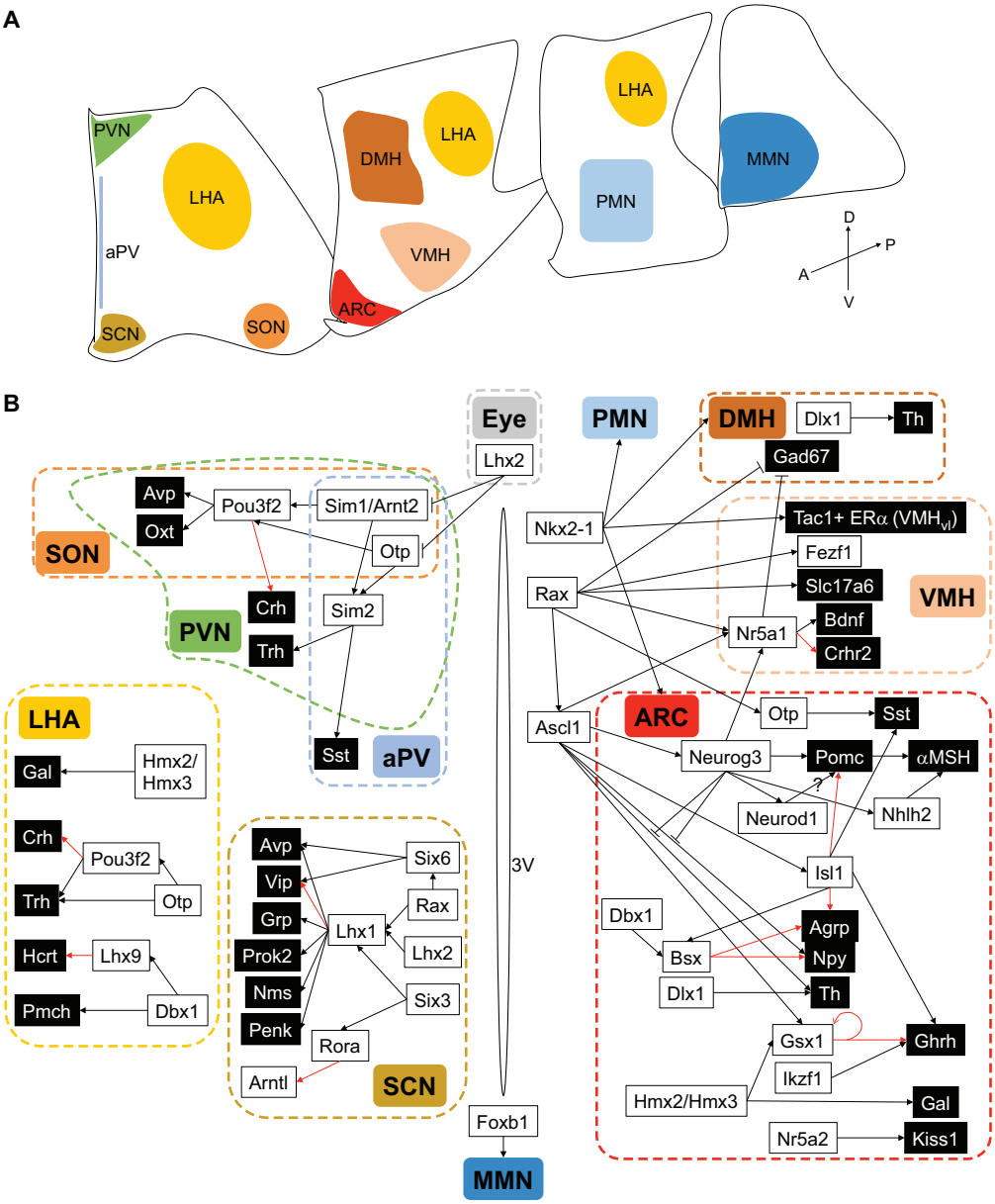

Fig. 3. Factors involved in hypothalamic differentiation. (A) Schematic of hypothalamic nuclei in coronal rodent brain sections. A, anterior; D, dorsal; P, posterior; V, ventral. (B) Known factors involved in hypothalamic specification and differentiation. Red arrows indicate direct transcriptional activation. See Table 1 for references. aPV, anterior periventricular nucleus; ARC, arcuate nucleus; DMH, dorsomedial hypothalamus; LHA, lateral hypothalamic area; MMN, medial mammillary nucleus; PMN, premammillary nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamus; 3V, third ventricle.

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Table 1. Factors involved in hypothalamic neurogenesis

Tools*	Phenotypes	References
DMH, VMH, ARC		
Nkx2-1$^{-/-}$	H&E staining: reduced and fused VMH and DMH; loss of ARC and PMN	Kimura et al. (1996)
Nkx2-1^{flox};*Nr5a1*^{Cre}	Loss of *Tac1*$^{+}$/*ER*α^{+} (*Esr1*) neurons in VMH$_{vl}$; hypoactivity and obesity	Correa et al. (2015)
Nr5a1$^{-/-}$	Increased *Gad67* (*Gad1*) and reduced *Bdnf* in VMH	Tran et al. (2003)
Nr5a1^{flox};*Nestin*^{Cre}	Loss of dense VMH identity by Nissl staining; redistributed *ER*α^{+} neurons; reduced *Bdnf* and *Crhr2* but normal *Ucn3* in VMH; increased anxiety and locomotion	Zhao et al. (2008)
Rax^{flox};*Shh*^{Cre}	Loss of *Fezf1*, *Nr5a1*, *Slc17a6*, *Nkx2-1* in VMH; ectopic expression of other markers *Gad67* (DMH marker), *Lhx1*, *Dlx2* in VMH; normal *Pomc* in ARC	Lu et al. (2013)
Rax^{flox};*Six3*^{Cre}	Loss of *Fezf1*, *Nkx2-1* in VMH; loss of *Nkx2-1*, *Pomc* in ARC; ectopic expression of *Gad67* in VMH	
Rax^{flox};*CAAG*^{CreERT}	TM at E8: loss of *Pomc*, *Th*, *Sst*, *Otp*, *Ascl1*, *Neurog3*, *Isl1*, *Six6*, *Lhx1*, *Nkx2-2*; normal *Nkx2-1*, *Sox2*	Orquera et al. (2016)
Zebrafish *rx3*$^{-/-}$	Loss of *pomca* but normal *sst3*, *oxt*, *crh*; abnormal circadian cell cycle rhythms	Dickmeis et al. (2007)
Zebrafish *rx3*$^{-/-}$	Loss of *avp*; normal or expanded *oxt*, *nkx2.4b*, *nkx2.1*, *otpb*, *gnrh3*, *gnrh2*, *tmtopsa*	Tessmar-Raible et al. (2007)
Zebrafish *rx3*$^{-/-}$	Loss of *pomca*, *nr5a1a*, *fezf1*, *otpb*, *th* in tuberal and anterior hypothalamus	Muthu et al. (2016)
Shh^{flox};*Shh*$^{Cre(SBE2)}$	Patterning defect (see main text)	Zhao et al. (2012)
Shh^{flox};*Foxb1*^{Cre}	Reduced *Nkx2-1*, *Dbx1*, *Pmch*, *Avp*; loss of *Dbx2*, *Hcrt*; maintained or expanded *Emx2*, *Lhx5*, *Wnt8b*	Szabo et al. (2009)
Shh^{flox};*Nkx2-1*^{Cre}	Loss of *Nkx2-1*, *Lhx9*, *Lhx1*, *Lhx6*, *Nr5a1*, *Pomc*, *Nkx6-2*; reduced *Lef1*; normal *Sim1*, *Irx5*, *Gsx2*; maintained *Foxb1*, *Arx*, *Rax*, *Foxg1*	Shimogori et al. (2010)
Ascl1$^{-/-}$	Delayed *Neurog3* expression; loss of *Gsx1*; normal *Hmx2*/*Hmx3*; reduced *Nr5a1*$^{+}$ neurons in VMH; reduced *Ghrh*$^{+}$, *Pomc*$^{+}$, *Th*$^{+}$ neurons and reduced *Pomc*, *Npy* expression in ARC	McNay et al. (2006)
Ascl1$^{Neurog2/Neurog2}$	Delayed *Neurog3* expression and loss of *Pomc*$^{+}$ neurons in ARC of *Ascl1*$^{-/-}$ were recovered	
Neurog3$^{-/-}$	Reduced *Nhlh2*, *Neurod1* but normal *Ascl1*; reduced *Pomc*$^{+}$ and *Nr5a1*$^{+}$ neurons, but increased *Th*$^{+}$ and *Npy*$^{+}$, and normal *Ghrh*$^{+}$ neurons in VMH and ARC	Pelling et al. (2011)
Ascl1$^{-/-}$;*Neurog3*$^{-/-}$	Reduced *Pomc* and *Isl1* in *Ascl1*$^{-/-}$;*Neurog3*$^{-/-}$ and *Ascl1*$^{-/-}$; reduced *Pomc* but normal *Isl1* in *Neurog3*$^{-/-}$	Nasif et al. (2015)
Isl1^{flox};*CAAG*^{CreERT}	TM at E9.5: Loss of *Pomc*$^{+}$ neurons, but normal *Ascl1* and *Neurog3*. TM at P21 or P90: reduced *Pomc*	
Isl1^{flox};*Pomc*^{Cre}	Reduced *Pomc*; hyperphagia and obesity	
Zebrafish *Isl1* MO	Reduced *pomca*	
Isl1^{flox};*Nkx2-1*^{Cre}	Loss of *Npy*, *Agrp*, *Pomc*, *Ghrh*, *Sst* in ARC at E16.5 and P21; reduced *Nr3c1* and *Bsx*; normal *Ascl1*, *Gsx1*, *Hmx2*, *Nr5a1*, *Crh* and *Trh*; increased or normal *Dlx1*, *Gad67*; postnatal dwarfism	Lee et al. (2016)
Neurog3^{flox};*Nkx2-1*^{Cre}	Reduced *Pomc*, but normal *Cartpt* expression in Pomc^{+} cells; normal *Npy*, *Th* and *ER*α; obesity, hyperphagia and reduced energy expenditure	Anthwal et al. (2013)
Bsx$^{-/-}$	Reduced *Agrp*/*Npy*, but normal *Pomc*, *Cartpt* and *Bsx*^{H2BeGFP} in ARC; hypophagia and hypoactivity	Sakkou et al. (2007)
Gsx1$^{-/-}$	Loss of *Ghrh*$^{+}$ neurons	Li et al. (1996)
Hmx2$^{-/-}$;*Hmx3*$^{-/-}$	Loss of *Gsx1* and *Gal* in ARC and LHA; loss of *Ghrh* in ARC; postnatal dwarfism	Wang et al. (2004)
Ikzf1$^{-/-}$	Loss of *Ghrh*$^{+}$ neurons in ARC; postnatal dwarfism	Ezzat et al. (2006)
Nr5a2^{flox};*Kiss1*^{Cre}	Reduced *Kiss1* in ARC; overexpressed Nr5a2 directly activated *Kiss1*; abnormal reproductive fitness	Atkin et al. (2013)
Dlx1$^{-/-}$	Reduced *Th*$^{+}$ neurons in ARC and DMH	Yee et al. (2009)
PVN, SON, aPV		
Pou3f2$^{-/-}$	Loss of dense PVN identity by nuclei staining; loss of *Avp* and *Oxt* in PVN and SON; loss of *Crh* in PVN; loss of *Pou3f4*; normal *Sst*, *Trh*, *Grh*; normal *Avp* in SCN	Schonemann et al. (1995)
Otp$^{-/-}$	Loss of *Avp*, *Oxt* in PVN and SON; loss of *Trh*, *Crh* in PVN; loss of *Sst* in aPV and ARC; normal *Ghrh* in ARC; normal *Avp* in SCN; reduced *Pou3f2* at E12.5 and *Sim1* at E13.5 in PVN and SON	Acampora et al. (1999)
Otp$^{-/-}$	*Otp*^{lacZ} was lost in aPV and PVN consistent with cell loss; normal *Otp*^{lacZ} in ARC; loss of *Avp*, *Oxt* in PVN and SON; loss of *Trh*, *Crh* in PVN and LHA; loss of *Sst* in aPV and ARC; progressive loss of *Pou3f4*; early loss of *Sim2* (only in PVN); redistributed *Pou3f2* and *Sim1*; ectopic *Six3*; normal *Hmx2*	Wang and Lufkin (2000)
Sim1$^{-/-}$	Loss of *Avp*, *Oxt* and *Pou3f2* in PVN and SON; loss of *Trh*, *Crh* in PVN; loss of *Sst* in aPV	Michaud et al. (1998)
Sim1$^{-/-}$	Loss of *Sim2* expression	Goshu et al. (2004)
Sim1$^{-/-}$	Redistributed *Sim1*^{lacZ}; normal *Otp*, *Arnt2*; increased *Plxnc1*; reduced *Plxna1*	Xu and Fan (2007)
Arnt2$^{-/-}$	Phenocopied *Sim1*$^{-/-}$, except redistributed *Sim1* and *Pou3f2* in *Arnt2*$^{-/-}$; Arnt2 dimerized with Sim1	Michaud et al. (2000)
Sim2$^{-/-}$	Reduced *Trh*$^{+}$ neurons in PVN and *Sst*$^{+}$ neurons in aPV; normal *Crh* and *Apv* in PVN; normal *Sim2*	Goshu et al. (2004)
SCN		
Lhx2$^{-/-}$	Ectopic *Otp* and *Sim1*; reduced *Lhx1*	Roy et al. (2013)
Six3^{flox};*Nestin*^{Cre}	Loss of dense SCN identity by nuclei staining; loss of *Lhx1*, *Avp*, *Rora* (only in SCN)	VanDunk et al. (2011)
Rora$^{-/-}$	Normal *Avp* and *Vip* in SCN	
Rora$^{-/-}$	Reduced *Arntl* (circadian clock gene); Rora directly activated *Arntl*; abnormal circadian rhythms	Sato et al. (2004)
Six6$^{-/-}$	Loss of dense SCN identity by Nissl staining; loss of *Avp* and *Vip*; abnormal circadian rhythms	Clark et al. (2013)
Lhx1^{flox};*Six3*^{Cre}	Loss of *Vip*, *Grp*, *Prok2*, *Nms*; reduced *Avp*, *Penk*; retained SCN identity; abnormal circadian rhythms	Bedont et al. (2014)
Lhx1^{flox};*Rora*^{Cre}	Retained SCN identity; Lhx1 directly activated *Vip*	Hatori et al. (2014)
MMN, LHA and others		
Foxb1$^{-/-}$	Loss of dense MMN identity by histology nuclei staining; postnatal dwarfism; nursing defect in female	Wehr et al. (1997)
Lhx9$^{-/-}$	Loss of 30% Hcrt^{+} neurons in LHA; Lhx9 did not directly activate *Hcrt*; hypersomnolence	Dalal et al. (2013)
Zebrafish *lhx9* MO and CRISPR	Loss of 40% and 90% Hcrt^{+} neurons in morphants and CRISPR-injected embryos, respectively; Lhx9 could induce ectopic Hcrt^{+} neurons in both zebrafish and mouse; Lhx9 could directly activate *hcrt*	Liu et al. (2015)
Dbx1^{flox};*Nkx2-1*^{Cre}	Reduced *Bsx*, *Npy* and *Agrp* in ARC; delayed *Lhx9*, *Hcrt* expression, reduced *Pmch*$^{+}$ neurons but expanded Wnt-responsive cells in LHA; normal *Pomc*, *Lef1*, *Avp*, *Sim1*, *Oxt*, *Fezf1*, *Nr5a1* and *Th*	Sokolowski et al. (2015)

*All are from mouse work, excepted as noted otherwise. aPV, anterior periventricular nucleus; ARC, arcuate nucleus; DMH, dorsomedial nucleus; H&E, Hematoxylin and Eosin; LHA, lateral hypothalamic area; MMN, medial mammillary nucleus; MO, morpholino oligonucleotide; PMN, premammillary nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; TM, tamoxifen [with age of administration indicated (E, embryonic day; P, postnatal day)]; VMH, ventromedial hypothalamus.

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developmental programs and the behavioral circuits that they regulate in the hypothalamus.

While individual genes define hypothalamic neuronal populations that are conserved throughout vertebrates (Bedont et al., 2015), there are also many examples of species-specific subtypes of cells within these populations. These subtypes can vary in terms of transmitter co-expression, anatomical localization of cell soma, and target projection. One of the best-characterized cases is that of Pmch^{+} neurons, for which the comparative anatomy has been studied in detail. In teleosts, this cell type regulates multiple physiological systems and behaviors associated with stress response (Baker et al., 1985), and the neurons are generally concentrated in the periventricular hypothalamus (Berman et al., 2009). However, some mammalian Pmch^{+} neurons can migrate extensively from the periventricular zone to the LHA, where they express additional markers and project to telencephalic targets (Chometton et al., 2016). Another example is *Otp*, where its zebrafish orthologs and upstream regulator *fezf2* are also required for hypothalamic dopaminergic neurogenesis, whereas mouse *Otp* and *Fezf2* are not (Levkowitz et al., 2003; Blechman et al., 2007; Ryu et al., 2007; Shimizu and Hibi, 2009). This species-specific circuitry has been proposed to represent a neofunctionalization of the ancestral cell type to allow more complex behavioral control that is coordinated with structural evolution of the brain (Croizier et al., 2011).

Gliogenesis in the hypothalamus

As well as giving rise to neurons, embryonic neural progenitors can generate radial glia, astrocytes and oligodendrocytes via the process of gliogenesis. In anamniotes, including fish and some amphibians, progenitors remaining after neurogenesis transform into radial glia that populate much of the mature CNS (Grupp et al., 2010). Radial glia also persist as a heterogeneous population throughout the entire zebrafish hypothalamus (Berberoglu et al., 2009), and include a subset of cells analogous to mammalian hypothalamic tanycytes (discussed in detail below). In amniotes, hypothalamic neural progenitors also produce astrocytes, which play important roles in synaptic homeostasis and trophic support of neurons (Haan et al., 2013; Robins et al., 2013). By contrast, the development of hypothalamic oligodendrocytes is not well characterized; although oligodendrocyte progenitors have been observed in the mammalian hypothalamus (Timsit et al., 1995), the mechanisms regulating their differentiation are unknown.

Wnt signaling as a regulator of hypothalamic progenitor differentiation

Wnt pathway ligands, intracellular signaling components and transcriptional reporters are expressed in caudal hypothalamic progenitors from the earliest stages of tissue patterning (Lee et al., 2006; Wang et al., 2012). In zebrafish, Wnt activity is present in neural progenitors throughout the posterior ventricular recess (Fig. 4A) (Wang et al., 2012). A similar pattern of Wnt activity and expression of pathway components has been observed in the caudal avian and mammalian hypothalamus (Lako et al., 1998; Garda et al., 2002; Liu et al., 2008; Wang et al., 2012; Gaston-Massuet et al., 2016), suggesting a conserved role throughout vertebrates. Functional manipulation of Wnt signaling in zebrafish can affect the number of differentiating neurons in the hypothalamus, suggesting that the pathway plays a crucial role in neurogenesis (Wang et al., 2012). Functional analyses have also revealed an inhibitory role for Wnt signaling in the formation and maintenance of radial glial progenitors. In zebrafish and mouse, ectopic Wnt activation leads to loss of hypothalamic radial glial cells (Wang et al., 2012; Duncan et al., 2016). These data are all consistent with a model in which Wnt activity promotes an active state in radial glia, from which they undergo neurogenesis (Fig. 4B).

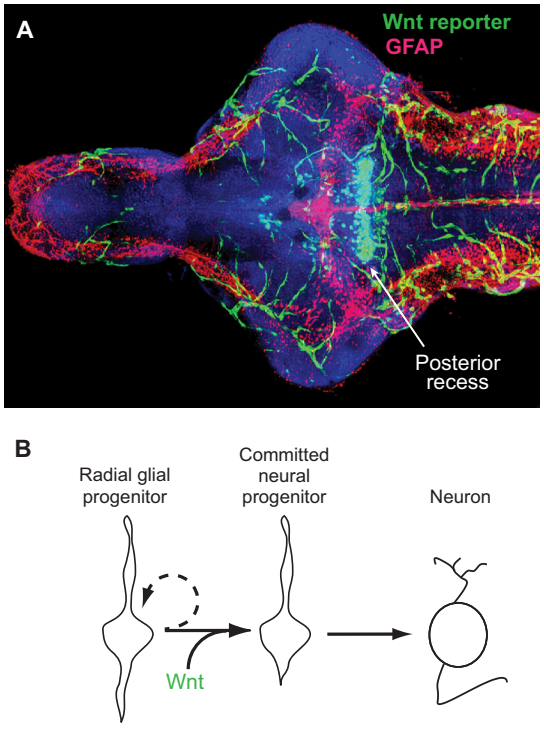

Fig. 4. Wnt signaling in the zebrafish posterior recess. (A) Ventral view of a whole larval zebrafish brain at 10 days post-fertilization stained for Wnt activity with the transgenic reporter *TCFSiam:GFP* (Wang et al., 2012) (green), Gfap^{+} radial glia (red) and cell nuclei (blue). Wnt signaling is present in the posterior ventricular recess of the hypothalamus (arrow) and is also observed in blood vessels throughout the brain. Anterior is to the left. (B) Schematic of Wnt function in hypothalamic progenitor differentiation. Wnt activity promotes neurogenesis by activating quiescent radial glia to produce committed neural progenitors.

Interestingly, most of the Wnt-responsive cells in the developing zebrafish hypothalamus are non-proliferative (Wang et al., 2009, 2012). There are two alternate interpretations of this finding: either Wnt-responsive cells are postmitotic committed progenitors poised for differentiation or, as in the cortex (Munji et al., 2011; Gan et al., 2014), they are progenitor cells that can re-enter the cell cycle and divide symmetrically or asymmetrically. The latter possibility offers a potentially novel mechanism for hypothalamic neurogenesis, and these scenarios can be resolved experimentally by performing lineage tracing, for instance by using inducible Cre recombination driven in Wnt-responsive cells (Bowman et al., 2013). Clones derived from committed progenitors would be expected to be relatively small and composed only of differentiating precursors and neuronal progeny. By contrast, clones derived from stem cells could be quite large and heterogeneous, representing the entire developmental process including self-renewal and multipotent differentiation.

Adult neurogenesis within the hypothalamus

The amount and distribution of neurogenesis within the adult brain varies widely among vertebrates. In mammals, adult neurogenesis is present at a high level in only a few brain regions, including the subgranular zone (SGZ) of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (Gonçalves et al., 2016; Lim and Alvarez-Buylla, 2016). By contrast, zebrafish

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have been shown to harbor actively proliferating and differentiating neural progenitor cells in the ventricular zone of almost every brain region, including the hypothalamus (Than-Trong and Bally-Cuif, 2015), and the neurons produced could integrate into existing circuitry. This increased level of adult hypothalamic neurogenesis in teleosts, reptiles and amphibians could be related to continuous brain growth, or could represent a by-product of the increased regenerative potential in these species (Kaslin et al., 2008).

Source of adult neurogenesis

With the growing understanding of the importance of SGZ and SVZ neural stem cells (NSCs) in circuit plasticity and behavior, it is worth taking a closer look at any potential contributions of adult neurogenesis in the hypothalamus. Studies using histological and genetic methods have identified low levels of hypothalamic neurogenesis in the postnatal mouse, where newborn cells have been reported primarily in the ARC and ME (Kokoeva et al., 2005, 2007). Genetic lineage tracing, via the expression of Cre recombinase in specific cell types, has similarly identified neurogenic progenitors in several hypothalamic regions (Lee et al., 2012; Haan et al., 2013; Robins et al., 2013). These studies all demonstrate self-renewal and multipotency within the labeled population, consistent with the presence of NSCs. Because many hypothalamic neuronal populations are relatively small, and a few hormone-secreting neurons may be sufficient to elicit specific behaviors (Aponte et al., 2011), even low-level neurogenesis could have a significant effect on circuit function.

Although population-level analyses can identify potential stem cell-like characteristics, rigorous long-term clonal analyses have only been performed on selected populations to discriminate between the presence of true stem cells versus committed progenitors (Robins et al., 2013). Most postnatal hypothalamic neural progenitors that have been observed experimentally are tanycytes – a heterogeneous population of specialized radial glia surrounding the third ventricle (Goodman and Hajihosseini, 2015). When explanted, these tanycytes have been shown to form neurospheres in culture (Xu et al., 2005; Robins et al., 2013), and *in vivo* studies have demonstrated their proliferative response to growth factors and neurogenic potential (Kokoeva et al., 2007; Perez-Martin et al., 2010; Robins et al., 2013). However, some tanycytes appear to only generate glia (Wang et al., 2012), and others shown to produce neurons have not been assayed for long-term self-renewal (Lee et al., 2012). Interestingly, postnatally born hypothalamic neurons in rodents appear to be localized throughout the parenchyma rather than just near the ventricular zone (Kokoeva et al., 2007). It is therefore possible that quiescent neural progenitors reside in the parenchyma as well (Djogo et al., 2016), and might represent a stem cell or progenitor pool that is distinct from tanycytes.

Function of adult neurogenesis

Adult-born neurons in the SGZ of the dentate gyrus and in the SVZ play a clear role in behavioral plasticity: the generation of new dentate gyrus neurons is required for spatial memory and pattern separation (Danielson et al., 2016), while the generation of new olfactory bulb neurons within the SVZ is required for novel odor discrimination (Livneh et al., 2014). Given the important functions of the hypothalamus in physiological homeostasis, it is reasonable to assume that adult neurogenesis there may regulate responses to environmental stimuli. The phenotype of postnatally born neurons within the mouse hypothalamus has mostly been associated with a function in feeding and energy balance (Kokoeva et al., 2005). Because of this, studies have focused on hypothalamic regions that are known to be involved in feeding (Xu et al., 2005; Perez-Martin et al., 2010; Haan et al., 2013). However, little is known about postembryonic proliferation or neurogenesis in other hypothalamic regions, or about other functionally defined neuronal populations. One clue for identifying hypothalamic neurons that are continuously generated throughout life might come from examining published gene expression patterns at different time points. Genes that exhibit additional domains of expression, or an increased density of discrete expression loci in progressively older postnatal animals, are good candidates for markers of continuous neurogenesis.

In order to analyze the functional contributions of postnatal hypothalamic neurogenesis, experimental methods must be employed to conditionally ablate or inactivate newly born neurons or their progenitors. This has proven to be difficult due to a lack of specific tools, and existing studies have not achieved a sufficient level of precision to rule out alternative interpretations of their data. General approaches, such as administration of AraC to block cell proliferation (Kokoeva et al., 2005), are not specifically targeted to neural progenitors and may miss slowly dividing stem cells. Radiation-induced DNA damage can provide spatial precision to target cells in a specific region (Lee et al., 2012), but also cannot specifically target neural progenitors. Genetic approaches are likely to be the optimal choice, but require specific alleles for targeted expression of Cre recombinase or other effector proteins. Although new genetic tools have been developed to selectively manipulate hypothalamic stem cells and progenitors (Lee et al., 2012; Haan et al., 2013; Robins et al., 2013; Pak et al., 2014), none is exclusively expressed in the hypothalamus. Therefore, a combinatorial approach will likely be necessary, using either a tissue-specific promoter or local virus injections to drive Cre or effector expression in a spatially restricted manner.

A role for Wnt signaling in regulating adult neurogenesis

In contrast to the widespread requirements for Wnt function in embryonic development, Wnt pathway activity in the postembryonic brain is limited to a few regions corresponding to known areas of continuous neurogenesis. High levels of Wnt reporter expression are present in the mammalian dentate gyrus and telencephalic SVZ (Bowman et al., 2013), where the pathway is known to regulate NSC self-renewal and differentiation (Lie et al.,

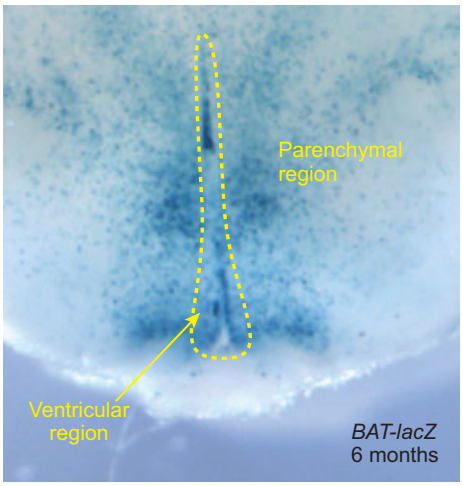

Fig. 5. Wnt activity in the adult hypothalamus. β-galactosidase staining in a coronal section (dorsal at the top) through the tuberal hypothalamus of a 6-month-old *BAT-lacZ* mouse (Wang et al., 2012). Wnt activity is present in ventricular and parenchymal regions.

2005). Within the postembryonic zebrafish and mouse hypothalamus, Wnt activity is present in the ventricular zones (Fig. 4A, Fig. 5) and correlates with progenitor differentiation (Wang et al., 2009, 2012). Together, these observations suggest that Wnt signaling might play a general role in promoting adult neurogenesis, rather than functioning to specify particular cell fates. In addition, numerous cells with Wnt activity can also be observed in the parenchyma of the adult mouse hypothalamus (Fig. 5) (Wang et al., 2012). These data are consistent with the possibility that some neurons may arise from quiescent progenitors local to this area, and suggest that Wnt signaling might regulate extraventricular neurogenesis similarly to its role in cortical intermediate progenitors (Munji et al., 2011). Further studies are needed to directly test the requirement for Wnt pathway activity in mammals and other vertebrate models, but a role in adult neurogenesis would be consistent with data from other tissues that exhibit continuous cell generation, such as hair follicles and intestinal crypts (Lien and Fuchs, 2014).

Conclusions

While the vertebrate forebrain generally exhibits the greatest evolutionary diversity in form and function of all the brain regions, the hypothalamus stands as an exception. In addition to highlighting anatomical similarity, the use of diverse model systems to study hypothalamus development has provided evidence that the molecular pathways regulating hypothalamic induction and patterning are generally conserved from fish to mammals. In addition, the basic hypothalamic cell types (Fig. 1B) and the codes of gene expression that specify them (Fig. 3) are also highly homologous throughout vertebrate species.

However, the differences that do exist represent opportunities that can be exploited to address unanswered questions regarding plasticity in the development of hypothalamic circuitry. First, physiological and behavioral functions can be assigned to structures, such as the posterior recess, in order to determine whether homologous systems exist in species where they are absent. This information will lead to novel models for circuit evolution, driven by either anatomical specialization or environment-based selective pressure. Second, the function of species-specific neuronal subtypes can be dissected using genetic manipulations. For example, by selectively targeting neurons based on combinatorial gene expression, birthdate or location, it will be possible to isolate their role in physiology and behavior and to interrogate the developmental mechanisms regulating their differentiation. Third, genetic targeting of adult-born neurons can be used to determine their contribution to behavioral plasticity. It is possible that multiple behaviors could be identified that are regulated by adult neurogenesis, with important implications for human health. Furthermore, uncovering the environmental stimuli that regulate this process could potentially reveal novel mechanisms mediating stem cell differentiation.

Until recently, the role of Wnt signaling in hypothalamus development was underappreciated. It is now clear that the Wnt pathway has important functions in processes from tissue induction to adult neurogenesis. Most importantly, Wnt activity regulates the same developmental steps that appear to underlie the evolutionary diversification of hypothalamic circuitry. Indeed, changes in Wnt signaling can modify the size of hypothalamic subregions, alter transcriptional codes in neural progenitors, and affect the timing and rate of neuronal and glial differentiation in the embryo and adult. Considering the importance of the hypothalamus in physiology and behavior, Wnt signaling may thus represent a crucial link between development and function, and further investigation into how this and other pathways shape the hypothalamus will hopefully provide insights into the neural basis of homeostasis and disease.

Competing interests

The authors declare no competing or financial interests.

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