Role of hypothalamic neurogenesis in feeding regulation

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The recently described generation of new neurons in the adult hypothalamus, the center for energy regulation, suggests that hypothalamic neurogenesis is a crucial part of the mechanisms that regulate food intake. Accordingly, neurogenesis in both the adult and embryonic hypothalamus is affected by nutritional cues and metabolic disorders such as obesity, with consequent effects on energy-balance. This review critically discusses recent findings on the contribution of adult hypothalamic neurogenesis to feeding regulation, the impact of energy-balance disorders on adult hypothalamic neurogenesis, and the influence of embryonic hypothalamic neurogenesis upon feeding regulation in the adult. Understanding how hypothalamic neurogenesis contributes to food intake control will change the paradigm on how we perceive energy-balance regulation.

Neurogenesis in the adult brain

Neurogenesis is a complex and highly regulated process that results in the production of new neurons (see Glossary). Neurogenesis occurs at high rates during the embryonic period when substantial quantities of new cells are generated by the proliferation of neuroprogenitor cells (NPCs), and subsequently migrate to the developing tissue [1]. In the adult, neurogenesis occurs at low rates in discrete regions of the brain such as the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus [2]. NPCs from these regions proliferate and migrate to their final destination, the olfactory bulb and the granule cell layer of the dentate gyrus, respectively, where they differentiate to form new neurons and integrate into pre-existing circuits [3]. Importantly, neurogenesis is not simply a restorative mechanism; it represents a functional response of the organism to daily challenges imposed by environmental and internal states [2]. Moreover, the new neurons produced through the adult life seem to contribute to behavioral functions such as learning, memory consolidation, and mood regulation [3].

A new neural stem/progenitor cell niche has recently been described to reside in the adult hypothalamus, a brain region that functions as the central regulator of homeostasis by controlling food intake, metabolism, and

Glossary

Arcuate nucleus (ARC): a region of the hypothalamus where the nutrient-sensing neurons are located.
Ependymal cells: cubic ciliated cells that line the ventricles (cavities) of the brain. In addition to barrier functions, ependymal cells are involved in the production and circulation of the CSF. In the hypothalamus, ependymal cells are the interface between the third ventricle (3V) and hypothalamic parenchyma.
Feeding regulation: a complex mechanism that includes central processes that take place in the hypothalamus. In brief, ARC neurons receive peripheral signals regarding the energy status of the organism and synthesize neuropeptides in response to those signals. The neuropeptides help in integrating and translating those signals into motivated behavior. Usually, hypothalamic responses counteract the nutritional signals to re-establish energy homeostasis.
Growth factors: molecules that increase the generation and/or survival of progenitor neuronal cells in neurogenic areas of the adult brain.
Hypothalamus: a brain area responsible for feeding regulation and metabolism. It consists of several regions (nuclei) with different neuronal populations that express specific neuropeptides. The hypothalamus is located in close proximity to the 3V.
Hypothalamic neurogenesis: the generation of new neurons in the adult or embryonic hypothalamus that arise from the proliferation and differentiation of hypothalamic NPCs.
Leptin: an anorexigenic hormone released from adipocytes into the circulation as a function of body fat content. High levels of fat storage raise the concentrations of leptin, and leptin suppresses food intake by inhibiting NPY/AgRP neurons and activating POMC neurons.
Neurogenesis: the set of events leading to the production of new neurons from NPCs. This process includes division (proliferation) of NPCs, migration, maturation (differentiation to a specific neuronal type), and functional integration of the new neurons into existing neuronal circuits.
Neuronal niche: the zone(s) in the adult brain where NPCs are retained after embryonic development. The microenvironment in the neurogenic niche, including cell-cell interactions, can retain the NPCs in the undifferentiated state and regulate the proliferation and differentiation of NPCs [66].
Neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons: a group of neurons located in the ARC that express NPY and AgRP; these neuropeptides have orexigenic actions (increase food intake) and are activated during energy deficiency.
Neuroprogenitor cells (NPC): the population of cells with proliferative and self-renewal capacity that can differentiate to different neural phenotypes.
Paraventricular nucleus (PVN): region of the hypothalamus that receives most of the neuronal projections from the ARC neurons.
Proopiomelanocortin (POMC) neurons: a group of neurons located in the ARC that express POMC; neuropeptides resulting from processing and cleavage of the POMC gene product have anorexigenic actions (decrease food intake) and are increased in situations of energy excess.
Subependymal astrocytes: glial cells located beneath the ependymal layer of the 3V wall that present features of neural stem cells, including expression of glial fibrillary acidic protein (GFAP) and proliferation upon growth factor stimulation. Therefore, subependymal astrocytes are identified as adult hypothalamic NPCs.
Tanyocytes: specialized non-ciliated ependymal cells characterized by a long basal process that penetrates into the hypothalamic parenchyma. Tanyocytes are considered to be adult hypothalamic NPCs because they are proliferative in basal conditions and upon stimulation with growth factors, and express the neuroprogenitor markers vimentin and nestin.
body temperature, among other functions. Emerging evidence discussed in detail below suggests that these adult-borne hypothalamic neurons play a crucial role in feeding regulation, underscoring their significance in the central control of energy-balance.

**Hypothalamus and feeding regulation**

Feeding behavior and energy-balance are regulated centrally by the hypothalamus. Distinct nuclei within the hypothalamus, including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial (VMH) and dorsomedial (DMH) hypothalamus, and the lateral hypothalamic area, share neuronal interconnections and together they maintain body homeostasis [4]. ARC neurons produce the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (AgRP) that act to increase food intake, and the anorexigenic neuropeptide proopiomelanocortin (POMC), the product of which (α-MSH) acts to decrease food intake [5]. The activity of ARC neurons is regulated by metabolic peripheral signals including hormones and gastrointestinal peptides [4]. For example, the adipose-derived hormone leptin, which is produced in proportion to body fat content, can activate the leptin receptors present in ARC neurons [4] to increase the expression and release of POMC and reduce the expression and release of NPY, resulting in a decrease in food intake [5]. More recently, neurogenesis has been described in the hypothalamus and has been shown to participate in the response of hypothalamic neuronal circuits to metabolic signals [6–9].

**Embryonic hypothalamic neurogenesis influences adult feeding regulation**

**Cell populations during embryonic hypothalamic neurogenesis**

The development of hypothalamic feeding circuits starts during the embryonic period and, in rodents, continues through the first 2 weeks of postnatal life [10]. Recent studies show that hypothalamic NPCs are present in the embryonic hypothalamus; for example, in rodents, POMC and NPY neuroprecursors are identified as early as embryonic (E) days E10.5 and E14.5, respectively [11,12]. In agreement, NPCs isolated from fetal rat hypothalamus express neuropeptides NPY, AgRP, and POMC [13]. Importantly, the number of immature POMC neurons in the ARC triples and reaches its adult number during the fetal period [11]. Surprisingly, some POMC neuronal progenitors adopt a distinct fate, giving rise to antagonistic neuronal populations expressing NPY [11]. Therefore, NPY and POMC, which are expressed in mutually exclusive cell populations in the adult hypothalamus, colocalize in a subset of embryonic neuronal precursors [11].

Other hypothalamic cell populations are generated during the embryonic period including ependymal cells, cuboidal ciliated cells that line the third ventricle (3V) wall of the hypothalamus, formed at E16–E18 [14]. Tanyocytes are specialized ependymal cells that are located in the floor of the 3V. In rodents, a subpopulation of radial glial cells (NPCs of the embryonic brain) initiate their differentiation into tanyocytes between E18 and the first postnatal days, and this process is completed by the first month of life [14]. Tanyocyte functions are not clearly understood, but they seem to be involved in feeding behavior as chemosensory cells and adult NPCs that respond to diet alterations [14,15].

**Impact of early nutritional and neurotrophic environment**

The nutritional and neurotrophic environment during the embryonic period, when hypothalamic NPCs are developing, impacts upon the formation of an adequate NPC population [16–18]. For example, offspring of dams with higher body weight have a lower number of immature neurons in the ARC [19]. Accordingly, proliferation of hypothalamic NPCs in rodents during the perinatal period is influenced by maternal diet manipulation and hormone availability [16–18]. Specifically, offspring subjected to nutritional restriction during the gestational period present a reduced NPC population with decreased proliferative capacity [16]; this results in body weight and feeding deficits in newborns that persist after weaning [18,20]. By contrast, a maternal high-fat diet (HFD) regime promotes the proliferation, differentiation, and migration of orexigenic neuronal precursors in the hypothalamic PVN of rats, increasing the density of orexigenic neurons in newborns and leading to a higher risk of obesity [18].

A hypothesis has emerged from these findings that hypothalamic neurogenesis during the neurodevelopment period is crucial, and that conditions affecting neurogenesis during this period can modify the number of hypothalamic NPCs and thus affect satiety pathways. As a consequence, persistent changes on newborn homeostasis and, possibly, reduced capacity to adapt to metabolic challenges during adulthood may be observed. In accordance, this has been proposed as one possible mechanism responsible for the high incidence of obesity in our society [21].

**Transcriptional regulation of embryonic hypothalamic neurogenesis**

Several transcription factors have been identified as key regulators of hypothalamic neurogenesis during the embryonic period [22–29]. For example, the proneural transcription factor Mash1 (mammalian achaete-scute homolog 1) is required for the generation of hypothalamic neurons, and Mash1 null mice present severe hypoplasia of hypothalamic nuclei including the ARC [26]. In addition, Mash1 and neurogenin 3 (Ngn3) are involved in subtype specification of neurons that are important for feeding regulation [26,27]. Mash1 is required for the differentiation of NPY-expressing neurons and for the normal development of POMC neurons [26]. By contrast, Ngn3 inhibits the expansion of the NPY neuronal population and promotes the development of POMC-expressing neurons [27].

The transcription factors single-minded homolog 1 (Sim1), aryl hydrocarbon receptor nuclear translocator 2 (ARNT2), and orthopedia (Otp) control the terminal differentiation of neuroendocrine lineages within the PVN, including the specification of corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) neurons [22–25]. It has been reported that in mice null for either of these genes, TRH and CRH neuroprecursors fail to differentiate and produce hormones [22,24,25]. In addition, Sim1 heterozygous mice have a reduced total number of PVN...
neurons, including oxytocin neurons [30,31]. This developmental defect impacts upon food intake regulation because Sim1 heterozygous mice are hyperphagic and obese, a phenotype that can be reversed by oxytocin administration [30,32].

Nescent helix-loop-helix (NHLH/NSCL) proteins 1 and 2 are neuronal transcription factors that control the development of gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus [28,29]. NHLH2 and double NHLH1/2 knockout mice showed dramatic loss of GnRH neurons, most likely due to deficient migration of neuroprogenitor cells to the corresponding part of the hypothalamus [28,29].

**Adult hypothalamic neurogenesis**

Emerging evidence suggests that, in addition to the SVZ and SGZ zones, active neurogenesis takes place in other regions of the adult rodent brain [33–36]. These regions include the hypothalamus where a potential neurogenic niche has been characterized [8,37–40]. Some reports suggest that the basal (unstimulated) levels of neurogenesis in the hypothalamus are low [37,38,40], and are probably less than those seen in well-established neurogenic zones [35]. However, recent papers have reported the contrary, and have shown robust neurogenesis within the hypothalamus of mice under normal conditions [12,39]. These differences may be related to the experimental approaches used to estimate the number of new neurons in the hypothalamus and probably to the identification of different NPC subsets by genetic and immunolabeling techniques (Box 1). This is a crucial point, and reconciling these differences will need further investigation.

Nevertheless, studies in support of the existence of basal hypothalamic neurogenesis indicate that the adult rodent

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**Box 1. Difficulties in the identification, localization, and determination of the proliferative capacity of hypothalamic NPCs**

| Endogenous and technical factors may contribute to conflicting data on hypothalamic neurogenesis, and alternative approaches to overcome these difficulties are needed. |

**Endogenous factors**

**Heterogeneous NPC population.** Hypothalamic NPCs are a heterogeneous cellular population that includes α- and β-tanycytes [9,39,40], subependymal astrocytes [42], and parenchymal progenitor cells [46]; these may represent subsets of NPCs in sequential differentiation stages [39,40]. The existence of NPC subsets within the hypothalamic neurogenic niche may be a major internal factor underlying difficulties in reaching a consensus about the origin and localization of these cells. Genetic models allowing tracing of all neuroprogenitor cells (and not only of specific cell types) may help to clarify this question.

**Unknown NPC cell cycle.** Putative hypothalamic NPC subsets may have different cell cycle durations, as reported for other niches [67], but this point needs investigation. Cell cycle length may determine the number and subpopulation of cells labeled with BrdU. For example, when BrdU is administered for only a brief period of time, NPCs with a shorter cell cycle have a higher probability of incorporating BrdU than other cells.

**Technical factors**

**BrdU administration.** Most proliferation studies evaluating hypothalamic neurogenesis are based on the delivery of BrdU. However, BrdU administration through specific routes (peripheral or intraventricular) results in distinct labeling patterns, and these seem to reflect differing BrdU availability to different types of cells [38]. Peripheral administration of BrdU preferentially labels proliferative tanycytes in the median eminence which are in close proximity to the peripheral blood supply [9,39], whereas intracerebroventricular (i.c.v.) BrdU is preferentially incorporated into proliferative cells in the 3V lateral wall and hypothalamic parenchyma, possibly because these cells only have access to BrdU in the CSF following i.c.v. administration [7,42]. Length and dosages of BrdU administration may interfere with experimental observations [9,39]. For example, peripheral delivery of BrdU for 15 days labels tanycytes of the median eminence and cells in the hypothalamic parenchyma [39], whereas shorter periods of administration failed to show parenchymal staining [9]. These important factors vary considerably between studies and should be considered when comparing experimental results.

**BrdU labeling of non-proliferative cells.** BrdU may be incorporated in apoptotic or DNA-repairing cells [68–71], leading to possible overestimation of proliferation rates in the hypothalamus (e.g., BrdU-positive neurons may be neurons undergoing apoptosis). This is a crucial issue for models where hypothalamic neuronal degeneration may be present, such as obesity [54,65]. To confirm that BrdU incorporation is due to cell proliferation, double-labeling protocols with BrdU and proliferation markers such as Ki-67 or PCNA can be employed [33,71]. Another option is to confirm that cells marked with BrdU are true newborn neurons by colabeling with doublecortin, a transient marker of immature neurons that, in the SGZ cells, disappears few weeks after birth [51].

**Type of stimulus.** Various stimuli have been used to induce hypothalamic neurogenesis, including growth factors [7,37,40–42], dietary manipulation [12,46], and injurious conditions [8,46]. It is reasonable to assume that specific factors activate different NPC subsets and, therefore, different cell types are eventually detected in these studies.

**Time-point of analysis.** Analysis of neurogenesis using BrdU incorporation or other methods is often performed at a single time-point, which varies between studies. This is an important factor because BrdU-labeled cells can be found in distinct hypothalamic localizations depending on the length of time elapsed between BrdU delivery and neurogenesis assessment [40,46]. In accordance, brain examination immediately after BrdU infusion shows that most positive cells are located in the ventricular wall [40], whereas long-term analysis reveals an additional population of labeled cells in subventricular layers [40] and an enriched number in the ARC [46]. Analyses of two time-points within each experiment can provide crucial information about the localization of hypothalamic NPCs as well as the neurogenesis process. For example, short-term BrdU analyses should label immature precursor cells whereas later time-points may determine the localization of newly generated neurons [9,40,71].

**Age of rodents.** Postnatal neurogenesis studies have been performed in rodents of different ages [8,9,37,40–42]. This is a crucial aspect that may influence the data because the proliferative capacity of hypothalamic NPCs decreases with age [12,39,53].

**Technical artifacts.** The experimental methods used to label NPCs can introduce artifacts and make the interpretation of neurogenesis studies difficult. For example, BrdU labeling is the most commonly employed technique for assessing hypothalamic neurogenesis [7–9,12,19,37–40,42,46], but the presence of incorporated BrdU molecules within the cells may induce aberrant cellular proliferation, migration, and differentiation [72,73]. In addition, genetic models including viral vectors, Cre recombinaison, and inducible Cre-recombinase systems have been used for long-term lineage tracing of NPCs in the hypothalamus [9,19,39–41,46,50]. In general, genetic technologies employ an endogenous promoter to drive the expression of a reporter gene, such as GFP or β-galactosidase, and mimic the physiological expression patterns of a relevant gene. However, discrepant expression patterns between reporter and endogenous proteins [74,75] or incomplete labeling [19] have been described in several models, raising the question on whether genetic models can feasibly recapitulate endogenous neurogenesis processes. Nevertheless, genetic labeling of NPCs represents a valuable and useful strategy, and the technical limitations can be minimized by careful characterization of every model. For a detailed critical review about the technical limitations of methods used to study neurogenesis see [71].
hypothalamus contains a resident population of NPCs capable of generating new neurons [9,39–42]. In addition, proliferative NPCs have also been observed in the adult hypothalamus of other species including sheep [43], hamster [44], and zebrafish [45].

Importantly, recent findings indicate that the adult hypothalamic neurogenesis contributes to the regulation of food intake [7–9]. For example, most of the newborn hypothalamic progenitor cells follow a neuronal fate and differentiate to phenotypes important for the regulation of appetite, including NPY/AgRP- and POMC-expressing neurons [7,8,19,37–39]. These new neurons are also responsive to fasting and leptin, characteristics of functional hypothalamic neurons [7–9,19,39].

Cellular remodeling of feeding circuits, including replacement of mature POMC and NPY neurons, occurs continuously during adulthood [12,46]. In young mice, half of the NPY and POMC neurons are substituted by neurons born postnatally within an 8 week period [12]. However, neuronal turnover seems to slow down in adulthood [12,46]. Noteworthy, this adult neurogenesis is important because it is involved in the response of energy-balance circuits to environmental and physiological challenges, such as diet alterations, and possibly in replacing degenerative cells during adulthood [8,12,46].

The hypothalamic neurogenic niche
Two distinct ventricular proliferative zones in the hypothalamus have been described (Figure 1) – the medial part of the 3V wall, with proliferative tanyocytes and proliferative subependymal astrocytes [40,42], and the ventral part of the 3V wall (median eminence), which includes proliferative tanyocytes [9,39]. Moreover, evidence suggests that newborn neurons originating from the ventricular proliferative cells migrate to the hypothalamic parenchyma where they are incorporated into the appetite circuits [19,39,41] (Figure 1).

Tanyocytes are considered to be adult hypothalamic NPCs in view of their proliferative capacity both in basal and stimulated conditions [9,39,40]. They express the neuroprogenitor markers vimentin and nestin, and are classified in two major subpopulations: α-tanyocytes (in the lateral 3V wall) and β-tanyocytes (in the median eminence) [14,40]. Astrocytes in the subependymal layers are also regarded as possible adult hypothalamic NPCs because they share common features with neural stem cells from the SVZ [47], including subependymal localization, staining for glia-specific markers, and increased proliferation upon growth factor stimulation [42].

The shape and location of proliferative hypothalamic NPCs (tanyocytes and subependymal astrocytes) places them in a strategic position to sense and integrate peripheral metabolic alterations (Figure 1). For example, tanyocytes lying on the median eminence have privileged access, via fenestrated capillaries, to nutritional signals carried by the bloodstream such as glucose and hormones [14]. Moreover, tanyocyte body cells that line the lateral wall of the 3V and subependymal astrocytes, via a cellular process that protrude the ependymal cell layer, are in contact with the cerebrospinal fluid (CSF) and molecules present in this fluid [14,42]. In addition, tanyocytes have a long basal process that penetrates into the hypothalamic parenchyma and allows close proximity to energy-sensing neurons [14].

The proximity of hypothalamic NPCs to nutritional cues is an important factor supporting the putative role of neurogenesis in feeding regulation because NPC proliferation and differentiation to neuronal phenotypes could then be controlled by cues that signal the metabolic needs of the organism. Indeed, several lines of evidence indicate that hypothalamic NPCs can sense and respond to peripheral

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**Figure 1.** Schematic representation of the hypothalamic neurogenic niche in the adult rodent brain. The third ventricle (3V) wall is constituted by ependymal cells (light grey), tanyocytes (blue), and subependymal cells (dark brown). There are different proliferative zones on the 3V wall: (A) the medial part, containing proliferative tanyocytes and proliferative subependymal astrocytes (light and dark green, respectively); and (B) the ventral part (median eminence), containing proliferative tanyocytes (light green) [9,42]. The ventricular neuroprogenitor cells migrate to the hypothalamic parenchyma (green arrow) and are incorporated in the appetite circuits (red arrows) [41]. (C) Proliferative cells are also present in the hypothalamic parenchyma (green) [46]; these cells may represent a cellular subtype generated from ventricular progenitor cells [50]. Hypothalamic neurons (red) express different neuropeptides important for the regulation of feeding and are located in hypothalamic nuclei (grey). Cellular processes are not represented. Abbreviations: AgRP, agouti-related protein; ARC, arcuate nucleus; CRH, corticotropin-releasing hormone; LH, lateral hypothalamus; MCH, melanin concentrating hormone; NPY, neuropeptide Y; ORX, orexin; POMC, proopiomelanocortin; PVN, paraventricular nucleus; TRH, thyrotropin-releasing hormone; 3V, third ventricle.
nutritional signals [9,12,46,48,49]. For example, tanycytes have chemosensory properties for glucose, hormones, and neurotrophic factors that activate intracellular pathways [48,49]. In addition, energy-balance alterations, such as HFD regime and caloric restriction, can modify the proliferation rates and differentiation of hypothalamic NPCs [9,12,46]. However, the cellular mechanisms that can stimulate or inhibit the division and differentiation of hypothalamic NPCs are unknown; this hypothesis is therefore not fully validated but represents an emerging area of investigation.

There is no consensus about the identity of adult hypothalamic NPCs. For example, a recent report identifies the ventral β-tanycytes as hypothalamic NPCs because they proliferate in basal conditions and their neuronal descendants populate the hypothalamus of young adult mice [9,39]. However, another study showed that lateral α-tanycytes are the key components of this neurogenic niche [40]. Moreover, each of these two reports excluded the other tanycyte subtype as potential hypothalamic NPCs [39,40]. Interestingly, although both studies are based on mouse models with tanycyte-specific Cre recombinase expression, the different promoters driving Cre expression were specific for different tanycyte subpopulations, and this may account for the conflicting results [39,40]. Another possibility is that α- and β-tanycytes represent functionally distinct NPC subsets, or possibly NPCs in sequential differentiation states, because α-tanycytes can give rise to more ventrally located β-tanycytes [40].

The hypothalamic parenchyma may also harbor dividing NPCs because proliferating cell ‘pairs’ were observed interiorly to the ventricular zones [8,37–39] (Figure 1). Furthermore, studies based on the stem cell marker SOX2 revealed a significant number of NPCs within the mouse hypothalamic parenchyma under normal conditions [39,46]. Parenchymal proliferative cells may arise from the cellular division of ventricular progenitors that migrate inwardly during the differentiation process [50] (Figure 1). In agreement, descendents of ventricular β-tanycytes can remain undifferentiated and continue to divide within the hypothalamic parenchyma [39]. However, because the cellular origin of most parenchymal proliferative cells is still unknown this hypothesis requires further investigation. In addition, BrdU (bromodeoxyuridine, a thymidine analog that is selectively incorporated by dividing cells) injected into the 3 V labels a greater number of cells in the hypothalamic parenchyma than in the ventricular zone [7,37,39,46], suggesting that parenchymal dividing cells may not originate from the proliferation of ventricular NPCs. Collectively, these data suggest that hypothalamic parenchymal NPCs have a shorter cell cycle and incorporate BrdU faster than ventricular cells. This raises the hypothesis that parenchymal NPCs constitute a specific cell subset (which may or may not be derived from the ventricular zone) which, because of their location, can respond more rapidly to metabolic signals by proliferating and differentiating to new neurons within the hypothalamus.

In summary, although several studies have reported on the identity, location, and proliferative capacity of hypothalamic NPCs [9,38–40,42,46], the often conflicting findings warrant further investigation.

**Adult hypothalamic neurogenesis and the role of neural growth factors**

In rodents, neurogenesis in the adult hypothalamus is enhanced by intraventricular administration of neural growth factors (Table 1); these factors drive a higher percentage of cells towards the neuronal phenotype compared to endogenous neurogenesis [7,37,42]. Moreover, the identity of proliferative hypothalamic cells as newborn neurons was confirmed by colabeling with BrdU and doublecortin (transient marker of immature neurons [51]) [7].

More importantly, ciliary neurotrophic factor (CNTF) promotes reductions in food intake and weight gain in diet-induced obese mice that persist after treatment withdrawal and are reversed upon inhibition of cell proliferation [7], suggesting that a mechanism by which CNTF promotes long-term weight loss is by enhancing hypothalamic neurogenesis [7]. Inhibition of cell proliferation in the ventral regions of the hypothalamus (ARC and median eminence) also results in weight loss and food intake deficits in mice [8,9]. These data suggest that neurogenesis is part of the hypothalamic mechanisms that regulate energy-balance.

Endogenous growth factors may also be important mediators of hypothalamic neurogenesis [52]. For example, intraventricular infusion of brain-derived neurotrophic factor (BDNF) or insulin-like growth factor 1 (IGF-1) (growth factors that are also expressed endogenously in the hypothalamus, as well as their receptors), activates the production of new neurons in this region [7,37,42]. Moreover, GnRH, which is expressed by hypothalamic neurons, can promote the proliferation and survival of NPCs in the hypothalamus of aged mice [53]. Thus, neurogenesis activation by endogenous molecules may constitute a physiological mechanism to respond to metabolic challenges, helping to modulate feeding circuits appropriately throughout adult life. However, it remains unclear if metabolic cues, such as moderate disturbances in energy-balance, can trigger a compensatory upregulation of neurotrophic factors (or other molecules) in the hypothalamus.

Interestingly, growth factors induce different levels of neurogenesis in different hypothalamic nuclei [8,37,42]. For example, IGF-1 administration induces neurogenesis in the medial periventricular and parenchymal zones [42] and BDNF infusion leads to high density of new cells in the PVN [37]. Finally, degeneration of AgRP neurons increases cell proliferation exclusively in the ARC [8]. Collectively, these observations highlight a pattern of region-specific activation of hypothalamic neurogenesis which might be influenced by the energy status of the organism.

**Impact of energy-balance disorders on adult hypothalamic neurogenesis**

Neurogenesis in the adult hypothalamus is modulated by cellular dysfunction such as neuronal cell loss, obesity-induced inflammation, and neurodegeneration [6,8,12,46]. Obesity and HFD induce hypothalamic injuries in rodents and humans, including activation of inflammatory and endoplasmic reticulum stress pathways, microglia and
Table 1. Activation of adult hypothalamic neurogenesis by growth factors and correlation with receptors in rodents

<table>
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<tr>
<th>Stimulus</th>
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<tr>
<td>BDNF</td>
<td>I.c.v. infusion for 12 days</td>
<td>Increases cell proliferation (BrdU* cells) A significant percentage of new cells are neurons Some new cells are oligodendrocytes</td>
<td>Diffusely in the hypothalamus parenchyma High density in the PVN</td>
<td>Newborn cells do not express BDNF receptors Localization of newborn cells and BDNF receptors correlate strongly</td>
<td>[37]</td>
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<td>CNTF</td>
<td>I.c.v. infusion for 7 days</td>
<td>Increases cell proliferation (BrdU* cells) A significant percentage of new cells are neurons (including NPY and POMC neurons) Some new cells are oligodendrocytes</td>
<td>Diffusely in the hypothalamus parenchyma High density in the ARC and median eminence</td>
<td>Some newborn cells express CNTF receptor Localization of newborn cells and CNTF receptors correlate strongly</td>
<td>[7]</td>
</tr>
<tr>
<td>IGF-1</td>
<td>I.c.v. infusion for 7 days</td>
<td>Increases cell proliferation (BrdU* cells) A significant percentage of new cells are neurons</td>
<td>High density in the hypothalamus parenchyma and in the caudal periventricular zone</td>
<td>Some newborn cells express IGF-1 receptor in the periventricular zone Localization of newborn cells and IGF-1 receptors correlate strongly</td>
<td>[42]</td>
</tr>
<tr>
<td>FGF, EGF, and FGF+EGF</td>
<td>Single injection directly into the 3V</td>
<td>Increases cell proliferation (BrdU* and nestin* cells) FGF is more potent in inducing hypothalamic neurogenesis Newborn cells are astrocytes and neurons (including orexin-A neurons)</td>
<td>3V wall (2–3 cell layers in the ependymal layer)</td>
<td>FGF receptors are expressed in the cells of the 3V wall</td>
<td>[41]</td>
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<tr>
<td>EGF+FGF</td>
<td>Subcutaneous injection for 10 days</td>
<td>Increases cell proliferation (BrdU* cells) Promotes hypothalamic neuronal repopulation upon acute toxic effect of glutamate</td>
<td>ARC parenchyma</td>
<td>N/A</td>
<td>[60]</td>
</tr>
<tr>
<td>FGF</td>
<td>I.c.v. infusion for 7 days</td>
<td>Increases cell proliferation (BrdU* cells) Newborn cells are vimentin* tanycytes (in the 3V wall)</td>
<td>In the medial part of the 3V wall Sub/periventricular zone</td>
<td>FGF-10 and FGF-18 are expressed within tanycyte-containing regions</td>
<td>[40]</td>
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</table>

*Abbreviations: ARC, arcuate nucleus; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; CNTF, ciliary neurotrophic factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; I.c.v., intracerebroventricular; IGF-1, insulin-like growth factor 1; N/A, not studied; PVN, paraventricular nucleus; 3V, third ventricle.

Astrocyte activation, and neuronal cell death [54–56]. It was recently shown that obesity also inhibits adult hypothalamic neurogenesis (Figure 2), resulting in reduced generation of new neurons, including NPY and POMC neurons, in addition to decreased proliferative capacity of hypothalamic NPCs [12,46]. Further, a HFD regime in mice restrains the constitutive self-renewal capacity of NPY and POMC neurons by promoting the retention of old neurons and the apoptosis of newborn neurons [12]. Conversely caloric restriction has the opposite effect and is able to reverse the reduction in hypothalamic neurogenesis in obese mice [12].

Figure 2. Endogenous hypothalamic neurogenesis contributes to the regulation of energy-balance. (A) Hypothalamic neurogenesis is stimulated by growth factors or mild neuronal loss to re-establish homeostasis as a physiological response [7,8]. (B) Hypothalamic neurogenesis is inhibited by severe neuronal loss or obesity-induced inflammation, contributing to a defective energy-balance in pathological conditions [12,46].
In addition to hypothalamic neuronal cell loss [54,55], obesity may further exacerbate this damage by promoting a reduction in the levels of neuron replacement. Indeed, adult hypothalamic NPCs are vulnerable to HFD-induced inflammation, including IKKB/NF-κB upregulation [46]. These inflammatory mediators are responsible for inhibiting the survival and proliferation of adult hypothalamic NPCs and inducing neuronal differentiation deficits in these cells [46]. It should be noted that selective overexpression of IKKB in hypothalamic NPCs is sufficient to impair neurogenesis and induce obesity in adult mice on normal chow diet [46].

Genetic obesity is also associated with defective remodeling of feeding circuits, and obese leptin-deficient (ob/ob) mice reveal decreased hypothalamic neurogenesis [12]. However, it is possible that ob/ob mice exhibit fewer NPCs as a result of the absence of leptin, which is known to stimulate the proliferation of hypothalamic NPCs both in vivo and in vitro [12,16].

It was recently reported that hypothalamic neurogenesis is reduced in aged mice [53], which is of interest because aging is associated with increased risk of obesity and neuroinflammation [57,58]. This putative link between obesity, aging, and hypothalamic neurogenesis warrants further investigation.

The effects of obesity on hypothalamic neurogenesis may be more complex because a recent investigation shows that HFD consumption increases the neurogenic rates in the hypothalamus of adult mice, whereas selective loss of the median eminence neurogenic niche protects from excessive weight-gain upon HFD feeding [9]. In particular, hypothalamic neurogenesis was increased in mice under a shorter HFD period (1 month) [9]. Therefore, a possible explanation for these contradictory findings is that alterations in neurogenesis follow an activation–inhibition pattern, because this is known to take place for other hypothalamic mechanisms following HFD consumption [54] (Figure 2). Thus, this latter study may be reporting an early compensatory activation of hypothalamic neurogenesis promoted by mild neuronal loss – which is known to occur upon HFD consumption [55]. This event can stimulate neurogenesis in the hypothalamus, as reported in mouse models of neurodegeneration [8].

Accordingly, HFD has a transient effect in hypothalamic cell renewal, with a proliferation peak occurring 3 days after the introduction of HFD, followed by a gradual reduction over time [6]. A possible reason for this rapid effect includes direct activation of NPC division by nutritional cues or inflammatory mediators, which are elevated within 1–3 days of HFD onset [54].

Several lines of evidence suggest that adult hypothalamic neurogenesis can constitute an early protective mechanism to preserve homeostasis under detrimental nutritional conditions (Figure 2) [6,8]. For example, blocking cerebral cell proliferation during the initial period of HFD-stimulated neurogenesis accelerates weight gain and obesity onset in mice [6]. Moreover, HFD regime increases the percentage of neurons adopting the anorexigenic POMC phenotype, suggesting that the maturation of neurons in feeding circuits is nutritionally regulated to prevent further weight gain [6]. Finally, another study showed that progressive neurodegeneration of NPY/AgRP neurons in mutant mice leads to increased hypothalamic cell proliferation [8] in an effort to maintain energy-balance. If hypothalamic cell proliferation is blocked, the decrease in food intake and body adiposity in these mice can be detrimental [8].

In summary, several stimuli promoting hypothalamic cell proliferation scenarios have been identified, and include mild neuronal cell loss and upregulation of neurotrophic factors [7,8] (Figure 2). Notably, energy-balance can be re-established upon activation of hypothalamic neurogenesis by these stimuli [7,8]. Therefore, we suggest that modulation of hypothalamic neurogenesis, and stimulation of a self-repair system driven by resident NPCs, may provide potential therapies for disorders affecting the hypothalamus. This strategy is already being investigated for other neurogenic regions [59]. Endogenous molecules that can activate the hypothalamic neurogenic niche are evident candidates for therapeutic objectives. This seems to be a promising strategy because peripheral administration of growth factor has been shown to repopulate the hypothalamic ARC upon acute loss of hypothalamic neurons induced by glutamate insult [60]. Nevertheless, more research is needed to fine-tune the induction of cell proliferation (and subsequent cell differentiation) and selective modulation of food intake and body weight.

Concluding remarks
The concept of adult hypothalamic neurogenesis has changed the paradigm of how we understand energy-balance regulation. It is now widely accepted that neurogenic processes occurring in the adult rodent hypothalamus contribute to the physiological regulation of food intake and body weight. Mechanistically, this neuronal plasticity offers the organism flexibility to respond/adapt to daily metabolic challenges. However, the exact role of adult hypothalamic neurogenesis in feeding regulation can only be clearly demonstrated after specific ablation of newly formed neurons in this region. This may be possible by using genetic strategies similar to those that revealed the functional/structural importance of hippocampal neurogenesis [61,62].

There are other relevant topics that need further investigation before the basis of hypothalamic neurogenesis can be unraveled. These include the need to clarify the origin of putative NPC subsets, to identify the endogenous factors that promote NPC proliferation/differentiation, and to determine the basal rates of neurogenesis in the hypothalamus.

Drawing from previous work on the SVZ and SGZ [61,63], another key experiment would be to follow genetically labeled hypothalamic NPCs from early postnatal days until adulthood, and to elucidate the proliferation, migration, and differentiation processes of these cells. However, the challenge is to find a promoter to drive transgene expression that is widely and actively expressed in hypothalamic NPCs and that can be used as a marker in genetic strategies. One candidate for such a marker is nestin, an intermediate filament protein often used to label neural stem cells [64]; apparently, nestin is expressed in hypothalamic NPCs including tanycyes (α and β) and
SOX2-positive parenchymal cells [39,40,46,65]. Unfortunately, the existing transgenic mouse models revealed a discordant pattern of nestin expression and variable numbers of nestin-positive cells within the hypothalamus [9,46]. It is crucial to recognize that NPCs are a heterogeneous cell population and that different proliferative zones may exist within the hypothalamus; careful design of future studies will be necessary to obtain new reliable information about the hypothalamic neurogenic niche and to reconcile existing contradictory findings.

Another important consideration is that the current studies were based solely on rodent models, and the relevance of key observations for human physiology needs to be established. Although many crucial questions remain unanswered (Box 2), the idea of exploiting hypothalamic neurogenesis therapeutically for diseases such as obesity is innovative and exciting.

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