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Developmental and functional relationships between hypothalamic tanycytes and embryonic radial glia

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The hypothalamus is a key regulator of several homeostatic processes, such as circadian rhythms, energy balance, thirst, and thermoregulation. Recently, the hypothalamic third ventricle has emerged as a site of postnatal neurogenesis and gliogenesis. This hypothalamic neural stem potential resides in a heterogeneous population of cells known as tanycytes, which, not unlike radial glia, line the floor and ventrolateral walls of the third ventricle and extend a long process into the hypothalamic parenchyma. Here, we will review historical and recent data regarding tanycyte biology across the lifespan, focusing on the developmental emergence of these diverse cells from embryonic radial glia and their eventual role contributing to a fascinating, but relatively poorly characterized, adult neural stem cell niche.

KEYWORDS

tanycyte, radial glia, neural stem cell, neurodevelopment, hypothalamus

Introduction

The hypothalamus is a small but powerful brain region that functions as the body's homeostatic control center, regulating fundamental processes such as feeding, reproduction, and sleep, as well as social behaviors including parenting and attachment (Saper and Lowell, 2014). Corresponding with its exquisite functional diversity, the hypothalamus contains a vast array of unique neurons and glia, which populate its anterior, tuberal, and mammillary regions along the rostrocaudal axis. Intriguingly, while the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampus are the best-known neural stem cell niches in the adult rodent brain, the hypothalamic ventricular zone is increasingly being recognized as a third site of postnatal neurogenesis and gliogenesis (Goodman and Hajihosseini, 2015; Yoo and Blackshaw, 2018). These newborn neurons and/or glia in the hypothalamus could facilitate plasticity in the circuits controlling various physiologies and behaviors.

Postnatal hypothalamic neural stem potential is largely thought to reside in tanycytes (coined from the Greek word *tanus*, meaning "elongated"), an apparently multifunctional cell population that can contribute to diverse physiological processes (Horstmann, 1954; Goodman and Hajihosseini, 2015; Prevot et al., 2018; Yoo and Blackshaw, 2018). In this review, we provide an overview of tanycyte biology from the developing to the mature hypothalamic niche.

Morphology, localization, and molecular features of tanycytes

In the adult brain, tanycytes resemble embryonic radial glia not only in function, but also in morphology, location, and molecular profile. Tanycytes position their cell bodies along the walls of the third ventricle and are characterized by a long basal process that projects into the hypothalamic parenchyma or toward portal vessels in the median eminence (Rodriguez et al., 2005). Together with multi-ciliated cuboidal ependymal cells, tanycytes form a tight boundary between the ventricular cerebrospinal fluid, which directly contacts the cells' uni- or bi-ciliated apical surface, and the flanking parenchyma (Mirzadeh et al., 2017). Along the rostrocaudal axis, tanycytes are abundant within the tuberal hypothalamus, particularly around the level of the median eminence (Millhouse, 1971; Rodriguez et al., 2005; Mathew, 2008). In addition, tanycytes are found along most of the dorsoventral length of the hypothalamic third ventricle, comprising the majority cell population of the floor and ventrolateral walls, then progressively decreasing in density dorsally; following a transition zone of interdigitating tanycytes and ependymal cells, the dorsal-most aspect of the third ventricle is lined almost exclusively by ependymal cells (Millhouse, 1971; Mathew, 2008). With respect to gene expression, tanycytes in seemingly all mammalian species broadly express numerous hypothalamic progenitor and/or adult neural stem cell markers (Pellegrino et al., 2018), including a variety of transcription factors (*Rax*, *Lhx2*, *Sox2*, *Sox9*), intermediate filaments (*Nestin*, *Vimentin*), and Notch signaling components (*Notch1*, *Hes5*), among others (Lee et al., 2012; Miranda-Angulo et al., 2014; Salvatierra et al., 2014).

Development of tanycytes

From a developmental view, the retention of radial glial morphology and at least some neurogenic and/or gliogenic function in tanycytes is particularly interesting, raising the question as to when and how tanycytes differentiate from embryonic radial glia. While the development of tanycytes is poorly characterized compared to that of other hypothalamic cell types, there is a growing body of data that refines the current understanding of tanycyte ontogeny.

Birthdating studies using ³H-thymidine autoradiography in the rat hypothalamus indicate that cells lining the third ventricle emerge during late embryonic development. Multi-ciliated ependymal cells, which are thought to possess minimal, if any, neural stem capacity in the mature hypothalamus (Robins et al., 2013), were reported to be generated before tanycytes, with the bulk of rat ependymogenesis occurring between embryonic day (E) 16 and E20 and peaking at approximately E18 (Altman and Bayer, 1978; Das, 1979). Tanycyte differentiation, on the other hand, was observed to start around E19 (equivalent to E17 in mice) and continued through the first and second postnatal weeks (Altman and Bayer, 1978; Das, 1979). These early investigations conclude that tanycytes terminally differentiate from the same radial glial cells that, just a few days earlier, gave rise to neurons of the ventromedial and dorsomedial hypothalamic nuclei. In contrast, however, a recent study employing single-cell RNA sequencing (scRNA-seq) of *Rax*⁺ lineage cells and EdU birthdating in mice suggests that a subset of radial glia exit the cell cycle to become primitive tanycytes as early as E13, with the majority of tanycytes born between E13 and E15 (Zhang et al., 2021). Species

differences and/or differences in the duration of the birthdating chase periods may account, at least in part, for these disparate results; however, it appears that tanycytes may be specified much earlier than previously thought and indicates that tanycytes are formed in parallel with—rather than after—neurons (Shimada and Nakamura, 1973) and other glia in the developing hypothalamus (Marsters et al., 2016). These findings also provide evidence for a “state-switching” model for embryonic hypothalamic progenitors (Zhang et al., 2021). Indeed, results from a genetic inducible fate mapping experiment in the developing mouse hypothalamus are consistent with this inference, with *Shh*-expressing progenitors labeled before E9.5 contributing neurons and astrocytes to the posterior tuberal and mammillary regions, in addition to generating tanycytes at the third ventricle floor, near the median eminence (Alvarez-Bolado et al., 2012).

Importantly, the developmental programs that govern the formation of tanycytes from embryonic hypothalamic progenitors are now being revealed. With respect to the intrinsic mechanisms that direct tanycyte development, recent transcriptional profiling of the hypothalamus across a comprehensive range of embryonic and postnatal timespoints provides valuable insights into the combinatorial code of regulons, or transcription factor-target gene regulatory networks, that shape hypothalamic cell type identity (Kim et al., 2020; Romanov et al., 2020; Zhang et al., 2021). Prospective glia in the hypothalamus show high activity of the *Hes5*, *Sox9*, and *Nfia* regulons, and within this lineage, tanycytes are further specified by *Nr1d1*; notably, *Nfia*-knockout mice exhibit impaired formation of tanycytes and astrocytes at E18.5 (Romanov et al., 2020). In addition, during the early stages of tanycyte and ependymal cell development, a variety of transcription factors such as *Nr2f1* and *Nfib* are differentially expressed between the two cell types, and are thus candidates to drive ventricular lineage specification (Kim et al., 2020). Consistent with an earlier time-of-origin for tanycytes, this developmental bifurcation between tanycytes and ependymal cells may occur as early as E13 in mice, the timespoints at which differential expression of *Rax* and *Foxj1*—well-established transcriptional regulators of tanycyte and ependymal cell fate, respectively—is first detected (Jacquet et al., 2009; Miranda-Angulo et al., 2014; Kim et al., 2020). In mice with *Rax* haploinsufficiency, ventral expansion of the ependymal cell marker *Rarres2* is observed, and selective knockout of *Rax* in early hypothalamic progenitors leads to a loss of tanycyte-specific gene expression in the third ventricle wall (Miranda-Angulo et al., 2014; Salvatierra et al., 2014). Both effects are phenocopied by the deletion of *Lhx2*, the upstream activator of *Rax*, from tuberal hypothalamic progenitors, with presumptive tanycytes along the ventral aspect of the third ventricle also exhibiting a hybrid tanycyte-ependymal cell identity (Salvatierra et al., 2014). Indeed, these hybrid cells maintain a radial glia-like morphology while becoming multi-ciliated, suggesting that *Lhx2* not only promotes tanycyte differentiation, but also represses ependymal cell fate in the developing hypothalamus (Salvatierra et al., 2014).

Much less is currently known about the extrinsic factors that influence the formation of tanycytes. scRNA-seq data suggests that various morphogenic and growth factor signaling components such as *Wnt7b*, *Ptch1*, and *Igf2bp2* are enriched in early stages of their developmental trajectory (Kim et al., 2020), and a knockout mouse model indicates that at later timespoints, the cell adhesion molecule *NrCAM* plays a role in regulating postnatal tanycyte number (Moore et al., 2022). In addition, microglia may modulate tanycyte development, as these phagocytic cells have emerged as key modulators of diverse neurodevelopmental processes in many areas

of the central nervous system, including the hypothalamus (Bilimoria and Stevens, 2015; Rosin and Kurrasch, 2021). In the developing hypothalamus, microglia influence gliogenesis and oligodendrocyte precursor cell migration from the ventricular zone (Marsters et al., 2020), and further, a subpopulation of stress-responsive microglia lie adjacent to and influence neural stem cells along the embryonic third ventricle (Rosin et al., 2021). These effects, likely mediated through secreted cytokines, together raise the possibility that similar extrinsic mechanisms contribute to the development of ventricle-residing tanyocytes (Marsters et al., 2020; Rosin et al., 2021).

Following their specification, tanyocytes are thought to mature over a protracted postnatal period, reaching terminal differentiation in rodents by approximately 4 weeks after birth according to cytological, histochemical, and ultrastructural criteria (Rodriguez et al., 2005). The apical profile of postnatal day (P) 0 mouse tanyocytes, for example, resembles that of embryonic radial glia, and maturation of the apical surface occurs over the first month of life (Mirzadeh et al., 2017). Furthermore, Golgi analyses of the rat hypothalamus reveal that P5 tanyocyte processes tend to be shorter and devoid of fine spines when compared to those at P60 (Altman and Bayer, 1978). Between P0 and P10 in mice, cells along the ventrolateral walls of the third ventricle demonstrate gradual downregulation of the radial glia marker RC2, and a concomitant upregulation of GFAP and GLUT1, consistent with a progressive generation of tanyocytes from radial glia (de Vitry et al., 1981; Silva-Alvarez et al., 2005; Mirzadeh et al., 2017). These histochemical changes also are accompanied at the ultrastructural level by increases in neuroglial contacts, organelle content, and the number and size of lipid bodies, as determined through electron microscopy of tanyocytes in the median eminence of E18 to P7 rats (Rutzel and Schiebler, 1980).

Interestingly, tanyocyte development across both embryonic and postnatal stages displays regional variation, suggesting unappreciated heterogeneity in this process. According to incidental observations from early birthdating studies, tanyocytes located at the floor of the hypothalamic third ventricle are generated before those occupying more dorsal regions (Altman and Bayer, 1978). Additionally, tanyocytes around the level of the arcuate nucleus acquire adult fine structure and potentially function as early as the first postnatal week, while those near the ventromedial nucleus need more time to mature and may not be operative in the early postnatal period (Walsh et al., 1978). Given these observations, it is likely that at the molecular level, tanyocyte developmental regulons are differentially activated as a function of dorsoventral positioning along the third ventricle, but this remains to be determined.

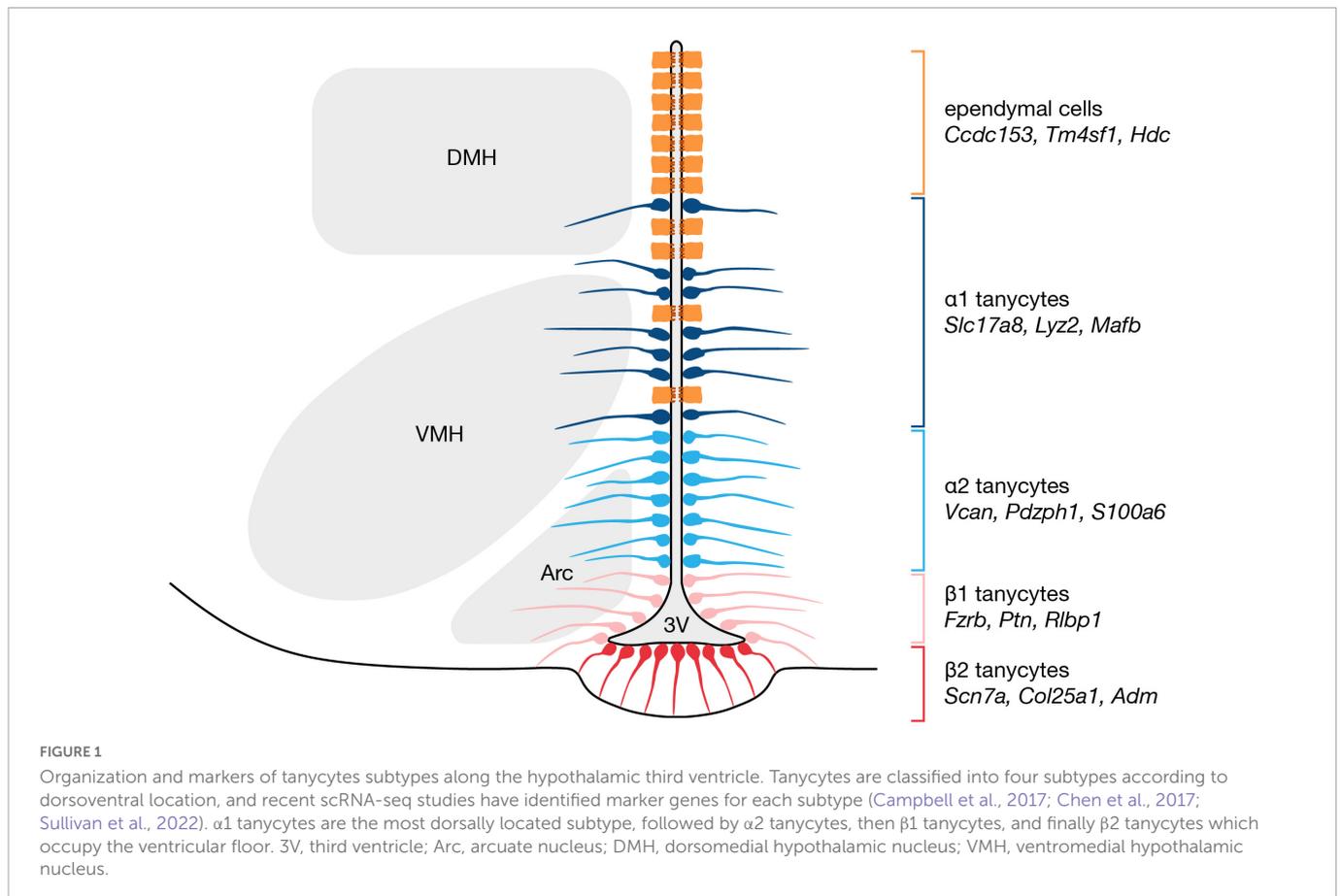
Tanyocyte subtypes and heterogeneity

Although the developmental timing and mechanisms of diversification are still unclear, tanyocytes in the mature hypothalamus are a distinctly heterogeneous population of cells. This heterogeneity was recognized even in initial characterizations of the cells using the Golgi method, which proposed that tanyocytes “may not be identical cytochemical units” (Millhouse, 1971). In the 1970s, a series of enzyme histochemical studies on rat tanyocytes was conducted under physiological and experimental conditions, and proved to be immensely influential, establishing a four subtype classification that prevails today. Both deafferentation of the medial basal hypothalamus and bilateral adrenalectomy led to changes

in tanyocyte metabolic activity that varied with localization along the third ventricle (Akmayev et al., 1973; Akmayev and Fidelina, 1974). Moreover, only a subpopulation of ventrally located tanyocytes showed sex differences in metabolic activity during the critical perinatal period of hypothalamic sexual differentiation (Akmayev and Fidelina, 1976). Based on these collective observations, tanyocytes were divided into four subtypes— α 1, α 2, β 1, and β 2—according to dorsoventral position (Akmayev et al., 1973; Akmayev and Fidelina, 1974, 1976). Subsequent ultrastructural analyses adopted and corroborated this nomenclature, describing differences in fine structural features such as lipid inclusions, endocytic machinery, and spines between the tanyocyte subtypes (Rodriguez et al., 1979, 2005, 2019).

In the tuberal hypothalamus, from dorsal to ventral, α 1 tanyocytes face the dorsomedial and ventromedial nuclei, α 2 tanyocytes border the arcuate nucleus, β 1 tanyocytes occupy the lateral extensions of the third ventricle, and β 2 tanyocytes reside at the third ventricle floor (Figure 1; Rodriguez et al., 2005). More recently, the four classical tanyocyte subtypes have been delineated based on gene expression profiles. From cross-referencing hypothalamic scRNA-seq data with *in situ* hybridization data from the Allen Mouse Brain Atlas, potential markers for α 1 tanyocytes include *Slc17a8* and *Lyz2*, and α 2 tanyocytes may be characterized by *Vcan* and *Pdzph1* expression (Campbell et al., 2017; Chen et al., 2017). Likewise, possible marker genes for β 1 and β 2 tanyocytes include *Frzb* and *Scn7a*, respectively (Campbell et al., 2017; Chen et al., 2017). Neural stem markers also are differentially expressed across tanyocyte subtypes, which may reflect diverse neurogenic and/or gliogenic potential, and intriguingly, some appear specific to small subpopulations of a given subtype. For example, while GLAST expression distinguishes α tanyocytes from β tanyocytes, the α tanyocyte population itself can be further subdivided into discrete GFAP-expressing dorsal and Prss56-expressing ventral subsets (Robins et al., 2013; Jourdon et al., 2016). In addition, the expression of many tanyocyte-enriched genes—neural stem-related or otherwise—is graded with dorsoventral positioning along the third ventricle (Goodman and Hajihosseini, 2015; Campbell et al., 2017; Chen et al., 2017), further complicating the assignment of distinct transcriptomic profiles to each subtype. These expression gradients may reflect the presence of transition zones between tanyocyte subtypes, or alternatively, might suggest that tanyocytes exist along a continuum of phenotypes (Mathew, 2008; Goodman and Hajihosseini, 2015).

Taken altogether, it is possible, and perhaps even likely, that the traditional four subtype classification does not fully capture the molecular heterogeneity displayed by tanyocytes. Sub-clustering of non-neuronal cells from an arcuate nucleus and median eminence scRNA-seq dataset, for instance, identified subclasses of α 1 and β 2 tanyocytes, yielding a total of six potential tanyocyte subtypes (Campbell et al., 2017). In addition, *Sprr1a* transcripts were detected in a small number of cells assigned to either the β 1 or β 2 tanyocyte subtypes, and *Sprr1a* immunoreactivity was precisely restricted to the lateral aspects of the third ventricle floor (Campbell et al., 2017). As such, *Sprr1a* expression may characterize an additional tanyocyte subtype occupying the border between the arcuate nucleus and the median eminence, a region that is traditionally regarded as part of the β 1 tanyocyte domain. Further molecular profiling is likely to uncover other new tanyocyte subtypes along the hypothalamic third ventricle, and may also provide insight into an apparently related cell type that resides in the median eminence proper; due to shared ultrastructure, gene expression, and elongated morphology with



ventricular tanyocytes, some have referred to these cells as γ tanyocytes (Wittmann et al., 2017; Wittmann and Lechan, 2018), though others designate them as pituicytes (Rodriguez et al., 2019).

Given the puzzling and seemingly increasing heterogeneity of tanyocytes, alternative criteria have been proposed to facilitate categorization of these cells. One recently advanced classification system groups tanyocytes according to the nature of the blood vessels and the neuroendocrine axons that their basal process are associated with, and distinguishes four subpopulations of tanyocytes: dorsomedial and ventromedial nuclei, dorsomedial arcuate nucleus, ventromedial arcuate nucleus, and median eminence tanyocytes (Prevot et al., 2018). These four classes are largely congruent with the classical $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$ subtypes, but this approach may provide a more robust framework for classifying any newly identified tanyocyte phenotypes. At variance, cataloging tanyocytes based on apical organization is somewhat inconsistent with the traditional tanyocyte classification (Mirzadeh et al., 2017). Morphological, ultrastructural, and immunohistochemical characterization of the third ventricle apical surface identified three cell types, termed E1, E2, and E3 cells, that correspond to ependymal cells, α tanyocytes, and β tanyocytes, respectively (Mirzadeh et al., 2017). In the caudal direction, the E3 domain expands dorsally at the expense of the E2 domain, but the strictly dorsoventral assignment of the classical subtypes implies that α and β tanyocytes maintain a uniform distribution along the rostrocaudal axis (Mirzadeh et al., 2017). Altogether, further refinements of tanyocyte classification are likely needed to better delineate the heterogeneity of these cells, and to more reliably identify tanyocyte subtypes that together comprise a complex neural stem cell niche.

Neural stem potential of postnatal tanyocytes

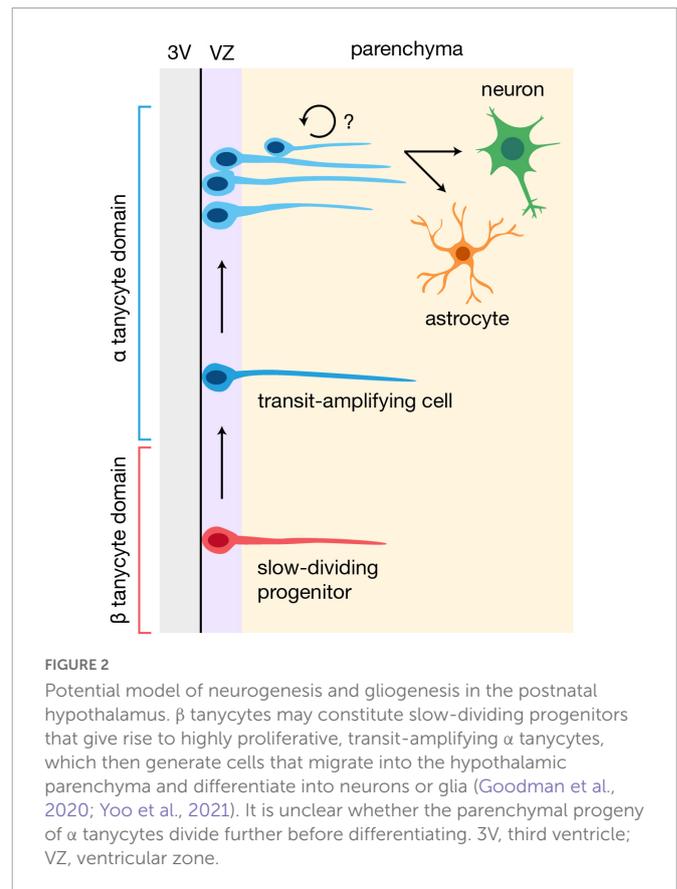
Apart from the fascinating range of homeostatic processes that tanyocytes have been implicated to participate in, which has been discussed in many excellent reviews (Rodriguez et al., 2005; Bolborea and Dale, 2013; Ebling, 2015; Goodman and Hajihosseini, 2015; Prevot et al., 2018; Bolborea and Langlet, 2021), perhaps one of the most perplexing functions of tanyocytes is their neurogenic and gliogenic potential in the postnatal hypothalamus. This has been explored in greater detail elsewhere (Yoo and Blackshaw, 2018; Sharif et al., 2021), but in brief, tanyocyte neural stem capacity appears heterogeneous with respect to time and the types of neural cells that are generated. Shortly after birth, Nestin-CreER^{T2} lineage-traced $\beta 2$ tanyocytes give rise almost exclusively to neurons in the median eminence in a diet-responsive manner (Lee et al., 2012). Later, during the early post-weaning period, Fgf10-expressing β tanyocytes primarily generate orexigenic neurons in the arcuate nucleus, but also contribute neurons to the ventromedial, dorsomedial, and lateral hypothalamic nuclei, as well as produce a small number of parenchymal astrocytes (Haan et al., 2013). Most studies on neural stem potential in the mature hypothalamus, on the other hand, suggest that this capability resides with α tanyocytes. *In vivo*, GLAST-expressing α tanyocytes can self-renew and give rise to $\beta 1$ tanyocytes, while most of their parenchymal progeny are astrocytes rather than neurons (Robins et al., 2013). Additionally, a subset of $\alpha 2$ tanyocytes characterized by *Prss56* expression are thought to generate neurons and astrocytes in the arcuate and dorsomedial nuclei, potentially

via a transitory population of parenchymal tanyocyte-like cells that translocate their soma from the third ventricle wall (Jourdon et al., 2016). The gliogenic potential of α tanyocytes appears to decrease over time, however, as fewer newborn glia than neurons—presumed to be α tanyocyte-derived based on proximity to the corresponding domain along the third ventricle—are observed in the mouse hypothalamus at 9 and 16 months of age (Chaker et al., 2016). Overall, diverse subpopulations of tanyocytes possess neural stem potential, which may suggest the presence of distinct neurogenic and/or gliogenic zones along the third ventricle, but their lineage relationships, if any, are still unclear.

At present, there is no consensus on the hierarchical organization of the hypothalamic niche. Some have speculated that α tanyocytes constitute the bona fide neural stem cells and β tanyocytes are more committed neuronal progenitors, given that α tanyocytes can generate β 1 tanyocytes, and further, that only α tanyocytes are neurospherogenic (Robins et al., 2013; Prevot et al., 2018). It is important to note, however, that β tanyocytes constitute a relatively dormant, slow dividing stem-population under physiological conditions (Mu et al., 2021), and that the neurosphere assay does not reliably identify quiescent neural stem cells (Codega et al., 2014). Indeed, Fgf10-expressing β tanyocytes are reported to incorporate BrdU at a slower rate than α tanyocytes, and to give rise to highly proliferative α tanyocytes (Goodman et al., 2020). These newly generated α tanyocytes, which resemble transit-amplifying cells, can subsequently divide to generate daughter cells that migrate laterally into the hypothalamic parenchyma (Goodman et al., 2020), which may be related to the Prss56⁺ lineage parenchymal tanyocyte-like cells noted above (Jourdon et al., 2016). While it is not yet clear whether these parenchymal descendants divide further before differentiating, or are at all related to the controversial parenchyma-residing progenitors that have been reported (Yoo and Blackshaw, 2018), this model thus places β tanyocytes at the top of the hypothalamic neural stem cell hierarchy, followed by their transit-amplifying α tanyocyte descendants (Figure 2; Goodman et al., 2020). Interestingly, RNA velocity analysis of a scRNA-seq dataset consisting of postnatal tanyocytes and tanyocyte-derived cells also indicates that neuronal precursors arise from α 2 tanyocytes that enter a proliferative state (Yoo et al., 2021). Finally, and perhaps unsurprisingly, ventricle-derived parenchymal progenitors have also been observed in both the developing mouse and human hypothalamus (Zhou et al., 2020), and regulators of postnatal tanyocyte neurogenesis, such as Fgf signaling (Goodman et al., 2020) and the NFI family of transcription factors (Yoo et al., 2021), also modulate hypothalamic progenitor cells during development (Burbridge et al., 2016; Romanov et al., 2020). Taken altogether, this suggests that while the constituents of the hypothalamic niche may evolve over time—including a varying combination of radial glial cells, tanyocytes, and potentially other neural stem populations (Son et al., 2021), at different developmental stages—the overall process of neurogenesis and gliogenesis in the region likely remains largely the same.

Discussion

Tanyocytes, which bear morphological, spatial, and molecular resemblance to embryonic radial glia, are unique in that they not only modulate and participate in various hypothalamic functions, but also remodel the corresponding neural circuits by supplying new neurons and glia. Notably, the developmental programs



controlling tanyocyte differentiation appear to be initiated relatively early in neurodevelopment, raising several interesting questions. For example, are tanyocytes functional neural stem cells in the embryonic hypothalamus, and if so, do they contribute to developmental neurogenesis and/or gliogenesis alongside classical radial glia, potentially in an environmentally responsive manner? In the mature hypothalamus, tanyocytes are a heterogeneous population of cells, and it is possible that subpopulations beyond the four classical subtypes—perhaps along the often-overlooked rostrocaudal axis—will be identified. This heterogeneity complicates efforts to understand the organization of the postnatal hypothalamic niche, but a growing body of evidence indicates that tanyocyte-derived neurons and glia populate diverse regions of the hypothalamus. Notably, given the low basal levels of tanyocyte-derived neurogenesis and gliogenesis, it may be necessary to challenge or perturb the system to determine the physiological relevance of this enigmatic niche across a range of developmental stages.

Author contributions

HF prepared the manuscript and the figures, with the assistance of the DMK who helped edit. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

DMK was the co-founder of Path Therapeutics, focused on the development of drugs for rare pediatric epilepsies.

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