Hypothalamic regulation of regionally distinct adult neural stem cells and neurogenesis

Alex Paul,1,2 Zayna Chaker,3 Fiona Doetsch2,3*

Neural stem cells (NSCs) in specialized niches in the adult mammalian brain generate neurons throughout life. NSCs in the adult mouse ventricular-subventricular zone (V-SVZ) exhibit a regional identity and, depending on their location, generate distinct olfactory bulb interneuron subtypes. Here, we show that the hypothalamus, a brain area regulating physiological states, provides long-range regionalized input to the V-SVZ niche and can regulate specific NSC subpopulations. Hypothalamic proopiomelanocortin neurons selectively innervate the anterior ventral V-SVZ and promote the proliferation of Nkx2.1+ NSCs and the generation of deep granule neurons. Accordingly, hunger and satiety regulate adult neurogenesis by modulating the activity of this hypothalamic–V-SVZ connection. Our findings reveal that neural circuitry, via mosaic innervation of the V-SVZ, can recruit distinct NSC pools, allowing on-demand neurogenesis in response to physiology and environmental signals.

*Corresponding author. Email: fiona.doetsch@unibas.ch

1Department of Genetics and Development, Columbia University, New York, NY 10032, USA.
2Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA.
3Biorcentrum, University of Basel, CH 4056 Basel, Switzerland.

Neural stem cells dynamically sense and integrate diverse signals in the microenvironment to modulate adult neurogenesis. The adult ventricular-subventricular zone (V-SVZ) stem cell niche adjacent to the lateral ventricles in the brain generates thousands of olfactory bulb (OB) interneurons each day (fig. S1A) (1, 2). The extensive heterogeneity of adult neural stem cells is just emerging (3). Neural stem cells (NSCs) residing in different regions of the V-SVZ have an intrinsic regional identity that determines the subtype of OB interneuron they produce (3). However, whether V-SVZ niche components and signals are regionalized and differentially regulate distinct pools of adult NSCs is largely unknown.

Adult V-SVZ NSCs are radial cells that express glial fibrillary acidic protein (GFAP) (4, 5), V-SVZ NSCs are mostly quiescent but upon activation divide to generate transit-amplifying cells and in turn neuroblasts that migrate to the OB (4, 6). Extrinsic cues are thought to regulate the transition from the quiescent- to activated-NSC state, but the specific signals that trigger activation of quiescent NSCs (qNSCs) are poorly understood.

To identify activators of qNSCs, we tested the Tocriscreen Mini Library (Tocris) for up-regulation of Nestin and MCM2 in prospectively purified qNSCs (6) and chose ICI 204448 (ICI), an agonist of the kappa-opioid receptor (Oprk1), for further analysis. ICI significantly increased Nestin+ MCM2+ qNSC-derived clones, as did the endogenous opioid ligands b-endorphin (Fig. 1A to C, and fig. S1D) and DIAP1. In vivo, acute intraventricular administration of ICI or DIAP1 (fig. S1E) selectively affected proliferation in the anterior ventral (AV) V-SVZ, a domain containing Nkx2.1+ NSCs (7, 8). ICI increased, and DIAP1 decreased, proliferation of AV V-SVZ NSCs (GFAP+ Ki67+DCX+ (fig. S1, F to H)) and neuroblasts (DCX+) (fig. S1Q) but had no effect on transit-amplifying cells (GFAP+ Ki67+DCX+) and neuroblasts (DCX+) (fig. S1Q) in anterior dorsal (AD) V-SVZ NSCs (fig. S1, I to L). Moreover, only proliferation of Nkx2.1+ NSCs was affected (Fig. 1D and fig. S1, I to L), with no change in total number of GFAP+Nkx2.1+ NSCs (Fig. 1E). Oprk1 immunostaining was also enriched in the AV V-SVZ as compared with AD V-SVZ (fig. S1, M to P) and predominantly expressed in NSCs (fig. S1T).

To identify an in vivo source of b-endorphin, we immunostained coronal brain sections from adult GFAP:GFAP mice (GFAP, green fluorescent protein) (9). b-endorphin expression was regionalized, labeling the AV—but not AD—V-SVZ and encompassing the entire Nkx2.1+ domain (Fig. 1, F and G, and fig. S2, A to E). Moreover, b-endorphin fibers occasionally directly contacted AV NSCs and colocalized with the presynaptic marker VAMP2 (fig. S3). No b-endorphin+ somas were detected in the vicinity of the AV V-SVZ, suggesting a long-range, neuronal source (Fig. 1G and fig. S2B). Because b-endorphin is a posttranslational cleavage product of proopiomelanocortin (POMC) (10), we investigated whether POMC-expressing neurons in the arcuate nucleus of the hypothalamus innervated the V-SVZ using viral approaches. An adeno-associated virus (AAV) expressing yellow fluorescent protein (YFP) (AAV-Flex-YFP) (II, 12) in a cre-dependent manner was injected into the hypothalamus of Pomc-cre mice (Fig. 2, A and C) (13) to selectively transduce POMC+ neurons (fig. S4A). Hypothalamic POMC+ neurons (YFP+) sent long-distance projections to the V-SVZ, innervating the AV but not other V-SVZ regions (Fig. 2, B and D, and fig. S4, B and C).

To determine whether hypothalamic POMC+ neurons regulate adult NSC proliferation in vivo, we used viral approaches to selectively ablate POMC+ neurons or acutely manipulate their activity. To ablate POMC neurons, an AAV that expresses cleaved caspase-3 in a cre-dependent manner (AAV-Flex-Casp3) (14), or control AAV-Flex-YFP, was unilaterally injected into the hypothalamus of Pomc-cre mice (Fig. 2A and fig. S3A). Seven weeks after AAV-Flex-Casp3 injection, the number of POMC+ hypothalamic neurons was...
Hypothalamic POMC+ neurons innervate the AV V-SVZ and promote the proliferation of Nkx2.1+ NSCs. (A) AAV-Flex-YFP, AAV-Flex-Casp3, or AAV-Flex-hM3DGq-mCherry were injected unilaterally into the hypothalamus of Pomc-cre mice. (B) Confocal projection of V-SVZ from AAV-Flex-YFP–injected Pomc-cre mouse immunostained for YFP, Ki67, and DAPI. YFP+ fibers are present in the septum and target ventral V-SVZ, but not other V-SVZ regions. Iv, lateral ventricle. (C) Confocal image showing unilateral YFP expression in the hypothalamic arcuate nucleus. (D) Confocal projection showing YFP+ fibers in the AV V-SVZ in the Nkx2.1 NSC domain. (E and F) Quantification of Nkx2.1+ and Nkx2.1+ proliferating NSCs (Nkx2.1+GFAP::MCM2+/Nkx2.1+GFAP+ and Nkx2.1+GFAP+MCM2+/Nkx2.1+GFAP+ cells) in AV V-SVZ after ablating (E) or activating POMC neurons with DREADDs (F). (G to J) Confocal images of NSCs in AV V-SVZ after POMC neuron ablation [(G) and (J)] or activation [(H) and (I)]. Arrowheads indicate dividing Nkx2.1+GFAP+MCM2+ NSCs. Scale bars, 200 μm (C), 100 μm (B), and 20 μm [(D), (I), and (J)].

Reduced by 66% (fig. S5, B and C). This led to a decrease in NSC proliferation by 61% specifically in the AV V-SVZ of AAV-Flex-Casp3–injected animals (fig. S5, E to H), with only Nkx2.1+ NSCs affected (fig. 2, E, G, and I), and a concomitant decrease in transit-amplifying cells (fig. S5H). The total number of GFAP+ Nkx2.1+ V-SVZ cells was unchanged between AAV-Flex-Casp3 and control animals (fig. S5D), indicating that the decrease in proliferating Nkx2.1+ NSCs is not due to cell death. The effects were restricted to the AV V-SVZ ipsilateral to the ablation, suggesting that they are not due to systemic changes (fig. 2E and fig. S5H).

We next acutely increased the activity of POMC neurons using activating DREADDs (designer receptors exclusively activated by designer drugs) (15). AAV-Flex-hM3DGq-mCherry (16) was unilaterally injected into the hypothalamus of Pomc-cre mice. Three weeks later, upon administration of clozapine N-oxide (CNO), the number of active (c-fos‘mCherry’) hypothalamic POMC neurons doubled (fig. S5, I to M). In the V-SVZ, Nkx2.1+ NSC proliferation was specifically increased on the ipsilateral side (fig. 2, F, H, and J), with no change in total GFAP+Nkx2.1+ NSCs (fig. S5N). Virally transduced POMC neurons directly contacted NSCs as well as niche cells (fig. S4, D to L). As such, the effect on NSC proliferation caused by modulating hypothalamic POMC neuron activity could be due to direct stimulation of NSCs, volume transmission, or via niche cells.

Hypothalamic POMC neurons are a key component of a feedback circuit that regulates feeding behavior during hunger and satiety (17, 18), suggesting that these physiological states may affect regional NSC proliferation. POMC neuron activity is increased upon feeding and decreased during fasting periods (fig. S6, A and B). To test whether hunger and satiety states regulate NSC proliferation in the AV V-SVZ, we quantified NSC proliferation in GFAP::GFAP+ mice that were either ad libitum fed, fasted, or fasted then refeed (fig. S6C). Hypothalamic POMC neuron activity (β-endorphin c-fos’ cells) decreased by 56% in fasted animals and returned to baseline upon refeeding (fig. S6, D to G). In the AV V-SVZ, NSC proliferation (GFAP::GFAP+MCM2+) decreased by 68% in fasted animals and recovered to control levels upon refeeding (fig. S6, L to O). NSC proliferation in the AV V-SVZ (fig. S6, H to K), total numbers of GFAP::GFAP+ cells, transit-amplifying cells, or neural stem cells in the AV V-SVZ were not affected (fig. S6P). The decrease in NSC proliferation in fasted animals was specific to Nkx2.1+ NSCs (fig. 3, A, C, and D), whereas the total proportion of Nkx2.1+ NSCs in the AV V-SVZ of fed and fasted GFAP::GFAP+ animals. Arrowheads indicate dividing Nkx2.1+GFAP+MCM2+ NSCs. Scale bars, 20 μm.

AD V-SVZ (fig. S6, H to K), total numbers of GFAP::GFAP+ cells, transit-amplifying cells, or neural stem cells in the AV V-SVZ were not affected (fig. S6P). The decrease in NSC proliferation in fasted animals was specific to Nkx2.1+ NSCs (fig. 3, A, C, and D), whereas the total proportion of Nkx2.1+ NSCs in the AV V-SVZ was unchanged (fig. S6Q). This decrease was fully rescued by activating POMC neurons during fasting with DREADDs (fig. 3, B, E, and F, and fig. S7) only on the ipsilateral side. Therefore, hunger and satiety states specifically regulate Nkx2.1+ NSC proliferation.
in the AV V-SVZ via modulating POMC neuron activity.

NSCs residing in different regions of the V-SVZ give rise to distinct OB interneuron subtypes (3). Adult-born OB interneurons can be classified by their position within the granule cell layer (GCL) or glomerular layer (GL) and expression by specific molecular markers (3). Adult Nkx2.1+ NSCs in the AV V-SVZ generate deep GCL interneurons (7,8) that express Neurogranin (Nrgn) (20). To test whether hypothalamic POMC+ neurons regulate the production of Nkx2.1+ deep GCL interneurons, we examined the generation of new neurons either after ablation of POMC+ neurons, or during fasting. Mice were pulsed with BrdU 3 weeks after ablation of POMC neurons (fig. S8A) or during fasting (fig. S8A), and the proportions of BrdU+ OB interneuron subtypes were quantified 30 days later. In both ablation and fasting paradigms, BrdU+ Nrgn+ DCX+ deep GCL interneurons decreased 32 and 33%, respectively (Fig. 4, A to F, and figs. S8, L and M, and S9, L and M), but adult-generated Nrgn+ and CalR+ superficial GCL—and CalR−, TH−, or CalB+ periglomerular interneurons—did not change (figs. S8, B to K, and S9, B to K). In the POMC ablation paradigm, these effects were specific to the ipsilateral side (Fig. 4E). Taken together, our results reveal that hypothalamic POMC+ neurons provide long-range regionalized innervation to the AV V-SVZ and regulate the proliferation of Nkx2.1+ NSCs during hunger and satiety states, in an activity-dependent manner, regulating the production of deep GCL interneurons (red dots) in the OB. The exact trajectory of POMC+ neuron projections to the V-SVZ is not known and is therefore shown as dashed lines. Scale bars, 10 μm.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/356/6345/1383/suppl/DC1
Materials and Methods
Figs. S1 to S9
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Specialization in brain neurogenic niche
The adult mammalian brain generates neurons from the subventricular zone (SVZ). In mice, Paul et al. were able to link environmental signals with the type of neurons that are generated and showed that anatomical subspecialization occurs in the SVZ. Neural circuits that respond to hunger or satiety enervate a subregion of the SVZ and retune the production of new olfactory neurons just from that portion of the subventricular niche.

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