© 2021. Published by The Company of Biologists Ltd | Development (2021) 148, dev199778. doi:10.1242/dev.199778

REVIEW

In vivo live imaging of postnatal neural stem cells

Alina Marymonchyk[1,2], Sarah Malvaut[1,2] and Armen Saghatelyan[1,2,*]

ABSTRACT

Neural stem cells (NSCs) are maintained in specific regions of the postnatal brain and contribute to its structural and functional plasticity. However, the long-term renewal potential of NSCs and their mode of division remain elusive. The use of advanced *in vivo* live imaging approaches may expand our knowledge of NSC physiology and provide new information for cell replacement therapies. In this Review, we discuss the *in vivo* imaging methods used to study NSC dynamics and recent live-imaging results with respect to specific intracellular pathways that allow NSCs to integrate and decode different micro-environmental signals. Lastly, we discuss future directions that may provide answers to unresolved questions regarding NSC physiology.

KEY WORDS: Adult neural stem cells, *In vivo* imaging, Mini-endoscopes, Mouse, Two-photon imaging, Zebrafish

Introduction

The idea that the brain is deprived of regenerative ability was held as dogma for a long time. However, almost 60 years ago, the pioneering work of Altman et al. shook up this dogma by showing for the first time that new neurons constantly replenish the adult hippocampus and olfactory bulb (OB) (Altman, 1969; Altman and Das, 1965). These observations were later corroborated by other studies (Doetsch et al., 1999a,b; Kuhn et al., 1996; Lois and Alvarez-Buylla, 1994; Luskin, 1993) and neural stem cells (NSCs) have since been found in the brains of all mammals, including humans (Denoth-Lippuner and Jessberger, 2021; Ming and Song, 2005). Endogenous NSCs react to different pathological conditions (Goldman, 2016; Ohab and Carmichael, 2008) and, although their activation is insufficient for full structural and functional recovery (Arvidsson et al., 2002; Goldman, 2016), understanding the mechanisms that regulate NSC quiescence and activation as well as their mode of division may provide new information for cell replacement therapies to heal devastating neurodegenerative disorders and brain trauma. This knowledge is required to fine-tune NSC activity rather than to induce the uncontrolled transition of NSCs from one state to another.

In vivo live imaging methods may provide profound insights into NSC dynamics and the spatiotemporal regulation of their activation, mode of division and interaction with the constantly changing micro-environmental niche. In this Review, we discuss the state-of-the-art *in vivo* imaging methods used to investigate postnatal NSC physiology (Fig. 1, Table 1) and some insights gained from *in vitro* studies (Table 1, Box 1). We also discuss recent imaging data of specific intracellular pathways that allow NSCs to integrate and decode different micro-environmental signals. Lastly, we suggest future directions that may provide answers to unresolved questions regarding NSC physiology (Fig. 2).

NSCs in the postnatal brain: a brief summary

In adults, NSCs are located in only a few regions of the brain, with the dentate gyrus (DG) and subventricular zone (SVZ) harboring the most NSCs (Denoth-Lippuner and Jessberger, 2021; Obernier and Alvarez-Buylla, 2019). Adult neurogenesis has been also reported in other regions, such as the hypothalamus and spinal cord (Barnabé-Heider and Frisén, 2008; Kokoeva et al., 2007; Lee et al., 2012; Migaud et al., 2010). However, as no *in vivo* live imaging of NSCs has been performed in these regions to date, we will not discuss these neurogenic sites. Adult NSC populations in the adult DG and SVZ are radial glia-like and type B cells, respectively (Bonaguidi et al., 2011; Doetsch et al., 1999a; Seri et al., 2001). These cells display certain astrocytic characteristics such as an astrocyte-like morphology (Seri et al., 2001) and the expression of glial fibrillary acidic protein (GFAP), glutamate aspartate transporter (GLAST) and brain lipid binding-protein markers (Codega et al., 2014; DeCarolis et al., 2013; Fuentealba et al., 2015; Lugert et al., 2010; Moss et al., 2016). The SVZ is home to the majority of adult NSCs that give rise to neuronal precursors. These migrate along the rostral migratory stream (RMS) to reach the OB, where they differentiate into GABAergic or dopaminergic interneurons that play a role in olfactory processing (Malvaut and Saghatelyan, 2016). NSCs in the DG give rise to excitatory granule cells that are involved in several cognitive processes, including learning, memory and pattern separation (Denoth-Lippuner and Jessberger, 2021).

NSCs can be either in the quiescent or the active state. Upon activation in the SVZ, GFAP+ cells express epidermal growth factor receptor (EGFR) and give rise to EGFR+ transit-amplifying progenitor (type C) cells that in turn undergo several rounds of division to generate migrating neuroblasts (Pastrana et al., 2009). Cells that express both EGFR and GFAP are activated NSCs (Pastrana et al., 2009). In contrast, GFAP+ cells that do not express EGFR may be either quiescent stem cells or SVZ niche astrocytes. These cell populations can be further distinguished by CD133 (prominin) expression, a transmembrane glycoprotein on the primary cilia of NSCs (Beckervordersandforth et al., 2010; Codega et al., 2014; Mirzadeh et al., 2008). In the adult DG, NSCs display a radial glia-like morphology, with a long process that crosses the entire granule cell layer and small processes that are horizontally oriented along the subgranular zone (SGZ). These radial-glia like NSCs give rise to non-radial glia-like progenitors that have only short processes and do not express GFAP (Denoth-Lippuner and Jessberger, 2021). These cells in turn generate neuroblasts that mature and become newborn granule cells (Filippov et al., 2003; Fukuda et al., 2003). Sustained neurogenesis for extended periods of time requires the coordinated regulation of quiescence, self-renewal and differentiation (Ahn and Joyner, 2005; Garcia et al., 2004; Imayoshi et al., 2008; Kim et al., 2011; Ninkovic et al., 2007). These processes require that the cell division

[1]CERVO Brain Research Center, Quebec City, QC, Canada G1J 2G3. [2]Department of Psychiatry and Neuroscience, Université Laval, Quebec City, QC, Canada G1V 0A6.

*Author for correspondence (armen.saghatelyan@fmed.ulaval.ca)

A.S., 0000-0003-4962-0465

Development (2021) 148, dev199778. doi:10.1242/dev.199778

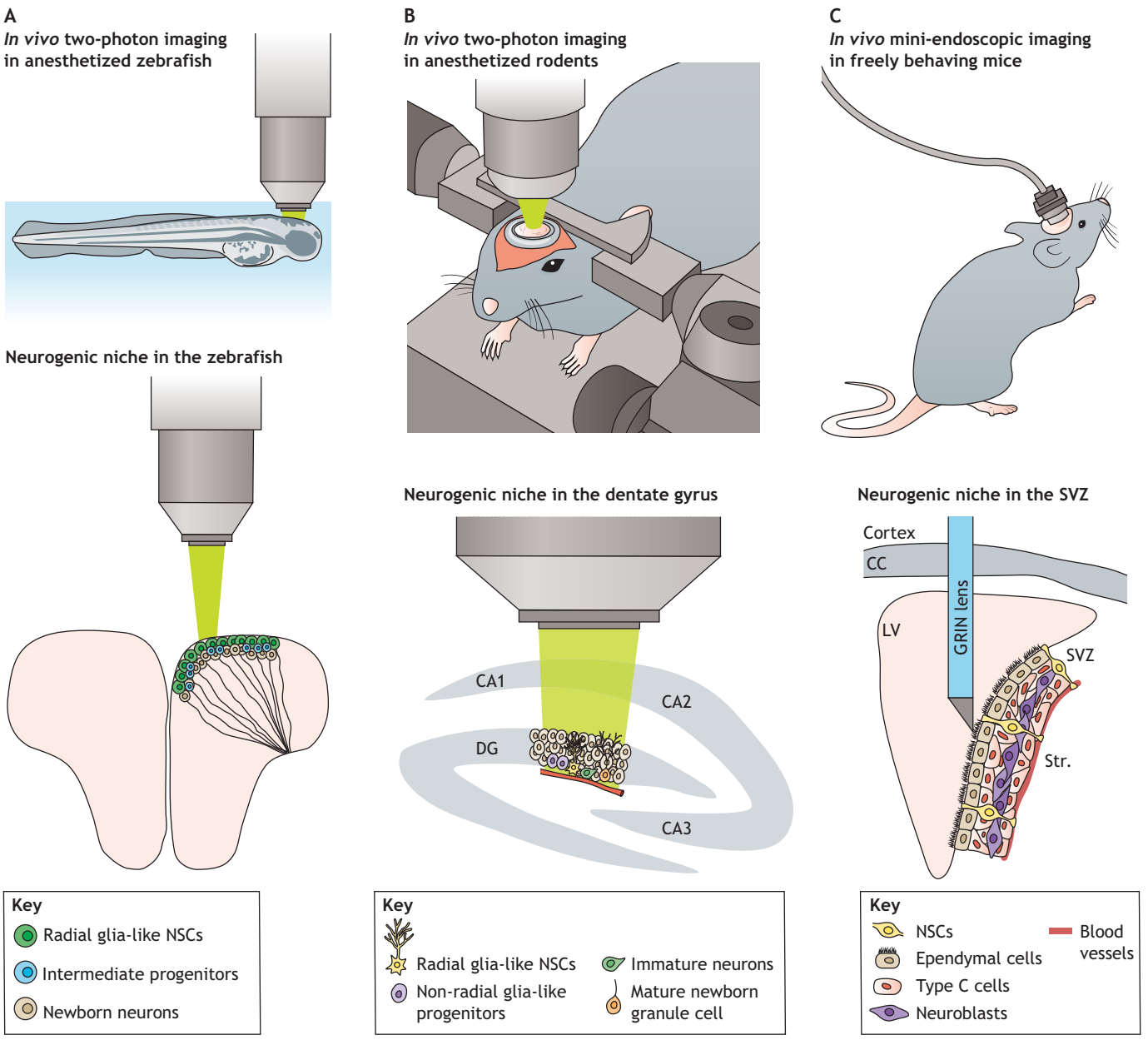

Fig. 1. Existing live-imaging approaches used to record and investigate adult NSC physiology. (A) Scheme of intravital *in vivo* imaging in anesthetized zebrafish. Thanks to the transparency of this animal model, NSC physiology can be accurately investigated through the skin and bone of the fish in the same region for extended periods of time. This approach allows imaging of the dorsal part of the zebrafish neurogenic niche (bottom panel). (B) Intravital imaging in the rodent dentate gyrus (DG). NSC imaging in the DG is performed following the surgical installation of a cranial window above the target region. With this approach, NSC activation and progeny can be tracked for extended periods of time in anaesthetized or awake head-restrained animals in the adult DG neurogenic niche, illustrated in the bottom panel. (C) *In vivo* mini-endoscopic imaging in freely behaving rodents. The installation of a GRIN lens in the neurogenic regions of rodents makes it possible to investigate NSC physiology while the animals are freely moving in an arena or are performing particular behavioral tasks. Placement of a 45° tip GRIN lens at the level of the lateral ventricles (LV) allows imaging of the intact subventricular zone (SVZ) neurogenic niche *in vivo* (bottom panel). NSC, neural stem cell; CA1, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3; CC, corpus callosum; Str., striatum; SVZ, subventricular zone.

mode is controlled, i.e. symmetric versus asymmetric cell division (Obernier and Alvarez-Buylla, 2019). Symmetric division of NSCs can result in either two stem cells (self-renewal) or two non-NSCs (stem cell exhaustion; also referred to as consuming division) and can thus affect the number of stem cells. On the other hand, asymmetric division of NSCs generates one stem cell and one progenitor (Silva-Vargas et al., 2018).

Adult NSCs in both the SVZ and DG are a heterogeneous population of cells (Obernier and Alvarez-Buylla, 2019). This heterogeneity may originate from the lineage, from differences in the embryonic precursors from which adult NSCs originate or from the developmental stage during which they were generated (Bonaguidi et al., 2016; Li et al., 2013). Lineage-tracing experiments performed at the level of the SVZ have revealed that, based on their location along the dorso-ventral and rostro-caudal axes, this region can be divided into different microdomains in which NSCs can express different combinatorial transcriptional or morphogen codes such as the Pax6, SP8, Sox2, Neurog2, Tbr2 (also known as Eomes), Emx1, Nkx2.1 and Gsh2 (Gsx2) transcription factors (Lledo et al., 2008; Merkle et al., 2014; Obernier and

Development (2021) 148, dev199778. doi:10.1242/dev.199778

Table 1. Principal live imaging studies of NSCs and the main findings

Study	Method	Animal model	Main findings
NSC imaging *in vitro*			
Costa et al., 2011	Live imaging of cultured neural stem cells (NSCs)	Mice, subventricular zone (SVZ) primary culture transduced with pseudotyped retroviruses that infect cells when they are undergoing cell division.	Cultured SVZ cells are primarily neurogenic and adult NSCs progress through stereotypic lineage trees. Enhanced frequency of symmetric self-renewing divisions was observed after application of EGF and FGF2. Cell growth correlated with the number of subsequent divisions of SVZ cells, with slow-dividing NSCs exhibiting the most substantial growth before division.
Ortega et al., 2013	Live imaging of cultured NSCs	Mice, SVZ primary culture from wild-type, hGFAP-RFP or GLAST::Cre^{ERT2}/GFP mice	NSCs exclusively generate oligodendroglia or neurons, but not in the single lineage. Wnt signaling selectively stimulates proliferation within the oligodendrogliogenic lineage.
Obernier et al., 2018	*Ex vivo* (whole-mount preparations) live imaging	Mice, hGFAP::Cre^{ERT2};Ai14 or hGFAP::Cre^{ERT2};Ai14;Fucci-S/G2/M	Adult NSCs mostly undergo symmetric divisions, both self-renewing and depleting. The prevailing symmetric NSC divisions generate transient-amplifying cells and deplete the niche.
NSC imaging *in vivo*			
Barbosa et al., 2015	Two-photon imaging+global population assessments	Brassy zebrafish line, crossed with the Tg(gfap:GFP)mi201 transgenic line	Direct conversion of NSCs to neurons along with asymmetric and symmetric self-renewing divisions, which results in the depletion of stem cells over time. After brain injury, neuronal progenitors are recruited to the injury site. However, they are generated by symmetric divisions that deplete the pool of stem cells.
Dray et al., 2015	Two-photon imaging	Zebrafish, gfap:GFP, her4:dRFP and mcm5:eGFP^{gy2} in transparent *Casper* double mutant background	Tracking of the entire niche. Measuring of the frequency, distribution and modes of NSC divisions.
Than-Trong et al., 2020	Two-photon imaging+global population assessments	Zebrafish, gfap:dTomato in transparent *Casper* double mutant background	Hierarchy-organized neurogenesis in which a subpopulation of a deeply quiescent NSC reservoir with long-term self-renewal potential generate, through asymmetric divisions, a pool of operational NSCs activating more frequently and taking stochastic fates biased toward neuronal differentiation.
Dray et al., 2021	Two-photon imaging+spatial statistic+modeling	Zebrafish, gfap:dTomato/mcm5:eGFP^{gy2} in *Casper* double mutant background	NSCs use spatiotemporally resolved local feedback to coordinate their activation. This mechanism involves a Notch-mediated inhibition from progenitors and previously divided NSCs.
Pilz et al., 2018	Two-photon imaging+modeling	Mice, NSCs in SGZ targeted by the endogenous Ascl1 promoter crossed with a tdTomato reporter line	Labeled NSCs undergo limited rounds of symmetric and asymmetric divisions, after which the pool is exhausted. Asymmetric divisions of non-radial glia-like progenitors that support life-long neurogenesis.
Bottes et al., 2021	Two-photon imaging+single-cell RNA-sequencing	Mice, NSCs in SGZ targeted in tamoxifen-inducible Gli1-Cre^{ERT2} and Ascl1-Cre^{ERT2} crossed with tdTomato reporter mice	Gli1-targeted NSCs showing long-term self-renewal in contrast to Ascl1-targeted NSCs, which are depleted after limited cycles of division, suggesting the existence of different subpopulations of adult NSCs.
Gengatharan et al., 2021	One and two-color mini-endoscopic imaging in freely behaving mice+*ex-vivo* imaging	Mice, NSCs in SVZ targeted by coincident activity of two promoters (GFAP and prominin)	NSC activation is regulated by the day/night cycle and darkness-induced melatonin signaling. NSCs in their activated and quiescent states are characterized by different Ca^{2+} dynamics.

Alvarez-Buylla, 2019) or the Shh and Wnt morphogens (Azim et al., 2014; Ihrie and Álvarez-Buylla, 2011; Tong et al., 2015). Lineage-tracing experiments performed at the level of the DG have also revealed heterogeneous NSC subsets (Berg et al., 2018). For example, GLI family zinc finger 1 (Gli1)-targeted NSCs show long-term self-renewal capacity, which is in contrast to achaete-scute homolog 1 (Ascl1)-targeted NSCs that are depleted after limited cycles of division (Bottes et al., 2021). It has been also shown that GLAST- and nestin-derived adult NSC populations react differently to environmental stimuli and that not all DG NSCs express nestin, which confirms that they are antigenically heterogeneous (DeCarolis et al., 2013). Some differences regarding the self-renewing capacity of nestin-derived NSCs in the DG have been also demonstrated, with data in favor of either a return to long-lasting quiescence or a disposable model of NSCs, in which they undergo a few rapid cycles of asymmetric divisions and then differentiate (Bonaguidi et al., 2011; Encinas et al., 2011).

Given the heterogeneity of adult NSCs, it is conceivable that the various subsets differ in their physiology, their mode of division, their regulation and even their function in the adult brain. Live-imaging data have already highlighted such differences and provided profound insights into NSC physiology in terms of their long-term self-renewal potential, their proliferation and differentiation rates and their interactions with various niche components (Bottes et al., 2021;

Development (2021) 148, dev199778. doi:10.1242/dev.199778

Box 1. Insights from in vitro imaging: imaging postnatal neural stem cells in a dish

In vitro live imaging studies are a useful approach for evaluating the types and number of neural stem cells (NSCs), their morphological features, self-renewal capacity and their proliferation and differentiation rates (Costa et al., 2011; Obernier et al., 2018; see Table 1). Adult SVZ NSCs *in vitro* are mostly neurogenic, suggesting that this lineage progression is, to a significant degree, cell-intrinsic (Costa et al., 2011). Neurogenic lineage trees in cell culture have been highly stereotyped and consist of asymmetric NSC divisions, symmetric transit-amplifying divisions and final symmetric neurogenic divisions (Costa et al., 2011). *In vitro* single-cell tracking of cultured SVZ cells has further shown that NSCs exclusively generate either oligodendroglia or neurons but never both within a single lineage (Costa et al., 2011). *In vivo*, oligodendrogliogenic NSCs are enriched in the dorsal aspect of the SVZ and are selectively responsive to canonical Wnt signaling, pointing to a regional specification of NSCs displaying distinct lineages (Azim et al., 2014; Ortega et al., 2013).

Although these *in vitro* data suggest that cell-intrinsic mechanisms regulate lineage progression, these mechanisms may be differently expressed and modulated *in vivo* by distinct extrinsic niche factors or cell-cell interactions. Live imaging in SVZ whole-mount preparations makes it possible to preserve the cellular organization of the tissue and to some degree also certain niche factors (Obernier et al., 2018). Whole-mount explants from postnatal mice with labeled cells expressing GFAP in combination with a cell cycle reporter have been used to show that the majority of GFAP+ NSCs divide symmetrically to either self-renew or to produce two Type C cells, accounting for ∼20% and ∼80% of all divisions, respectively (Obernier et al., 2018). Although self-renewing NSCs cells can reside in the V-SVZ for several months, they are more likely to undergo consuming divisions, leading to a decline in their numbers over time (Obernier et al., 2018). Interestingly, however, lineage-tracing experiments in the adult SVZ have shown that GLAST+ NSCs undergo a rapid sequence of asymmetric cell divisions before being consumed by a terminal symmetric division (Calzolari et al., 2015). Whether these distinct types of NSC divisions reflect differences in NSC subpopulations, as has been recently shown in the DG (Bottes et al., 2021), or result from a lack of some niche factors in explant cultures, such as those derived from vasculature or cerebrospinal fluid, remains to be examined.

Costa et al., 2011; Dray et al., 2021; Obernier et al., 2018). With the increasing panoply of labeling and imaging methods that makes it possible to target specific subtypes of NSCs and monitor their activation and lineage progression dynamics, it is expected that these approaches will further expand our knowledge of NSC functions under physiological and pathological conditions.

In vivo imaging of NSCs during adulthood

In vivo imaging makes it possible to overcome the potential pitfalls of studying NSCs *in vitro*, such as the absence of niche factors, implying that the mode and dynamics of NSC division can be altered due to the lack of micro-environmental cues from blood vessels, ependymal cells and NSC progeny. In this section, we discuss the insights obtained from *in vivo* imaging with regard to adult NSC physiology.

Imaging adult NSCs in anesthetized zebrafish

The zebrafish brain has a high regenerative capacity, with multiple neurogenic niches distributed along the entire rostro-caudal brain axis (Grandel et al., 2006). The most studied neurogenic niches are located in the telencephalon (pallium), where NSCs are harbored in three distinct domains: the anterior part of the pallium (Da), the dorsomedial pallium (Dm) and the lateral pallium (Dl) (Adolf et al., 2006; Grandel et al., 2006). The architecture of the zebrafish adult pallium, where the ventricular zone and NSCs lie as a superficial 100-150 µm thick layer, makes it possible to use non-invasive intravital two-photon imaging methods (Fig. 1A; Barbosa et al., 2016; Dray et al., 2015) that allow for imaging up to 500-800 µm deep into the tissue (Miller et al., 2017; Svoboda and Yasuda, 2006).

NSCs in individual fish can be reliably imaged for up to several weeks. The neurons delaminate and settle just below their mother NSC in a continuous process paralleled by pallial growth and ventricular zone expansion (Barbosa et al., 2016; Dray et al., 2015; Furlan et al., 2017). This architecture makes it possible to track individual NSC-derived clones and also to perform population analysis of up to 1000 NSCs, which can be imaged at the same time (Dray et al., 2015). In addition, harmonic multiphoton microscopy can be used to image skin and skull morphology, and the resulting information can be used to position the fish in an identical manner over different imaging sessions (Dray et al., 2015; Olivier et al., 2010).

NSCs in the Dm region are similar to those in mammals in terms of the expression of markers such as GFAP, Sox2, Nestin, enzyme glutamine synthetase (Gs) and Notch-target gene hairy-related 4 tandem duplicate 1 (her4.1; orthologous to mammalian Hes5) (März et al., 2010). Various labeling strategies based on the use of different transgenic reporter lines under the control of *GFAP* (Bernardos and Raymond, 2006) or *her4* (Yeo et al., 2007) promoters have been used, sometimes in combination with S/G2/M markers such as Mini-chromosome maintenance factor 5 (Mcm5) or Proliferating cell nuclear antigen (Pcna) for identifying activated NSCs (Dray et al., 2015). NSCs labeled with her4:RFP are randomly distributed within the large germinal zone. However, although the percentage of NSCs is higher in Da than in Dm and Dl, activation events are more frequent in Dm than in the other domains (Dray et al., 2015). Approximately 5% of NSCs in Dm are in the activated state, which lasts for a few days and is generally accompanied by cell division, after which most NSCs return to quiescence (Dray et al., 2021). Barbosa et al., who used another driver line that expresses GFP under the control of the *GFAP* promoter to repetitively image over 100 NSCs, observed that ∼13% of the NSCs are in the activated state (Barbosa et al., 2015). The authors also electroporated CMV-TdTomato to follow non-glial progeny and showed that NSCs mostly divide asymmetrically (93% of NSCs), which leads to the generation of another NSC and a neuronal progenitor or neuron (Barbosa et al., 2015). Less than 1% of NSCs undergo symmetric division, leading to the gradual depletion of NSCs (Barbosa et al., 2015). *In vivo* live imaging also revealed that NSCs are directly converted into neurons, which accounts for 17% of cases of NSC activation and which is otherwise impossible to observe using other approaches (Barbosa et al., 2015).

Interestingly, it has been also shown that NSCs have a hierarchical organization in the zebrafish telencephalon (Than-Trong et al., 2020). The authors identified a subpopulation of deeply quiescent NSCs with a long-term renewal potential that give rise to more frequently activated NSCs, called operational NSCs, through asymmetric division (Than-Trong et al., 2020). This functional heterogeneity in NSC behavior is consistent with recent *in vivo* live imaging in the mouse DG. The use of transgenic mouse lines with conditional recombination driven by the regulatory elements of the NSC-expressed genes has revealed that Gli1-targeted NSCs display long-term self-renewal potential and limited proliferative potential, whereas NSCs from the Ascl1 lineage divide frequently and are rapidly exhausted (Bottes et al., 2021).

Development (2021) 148, dev199778. doi:10.1242/dev.199778

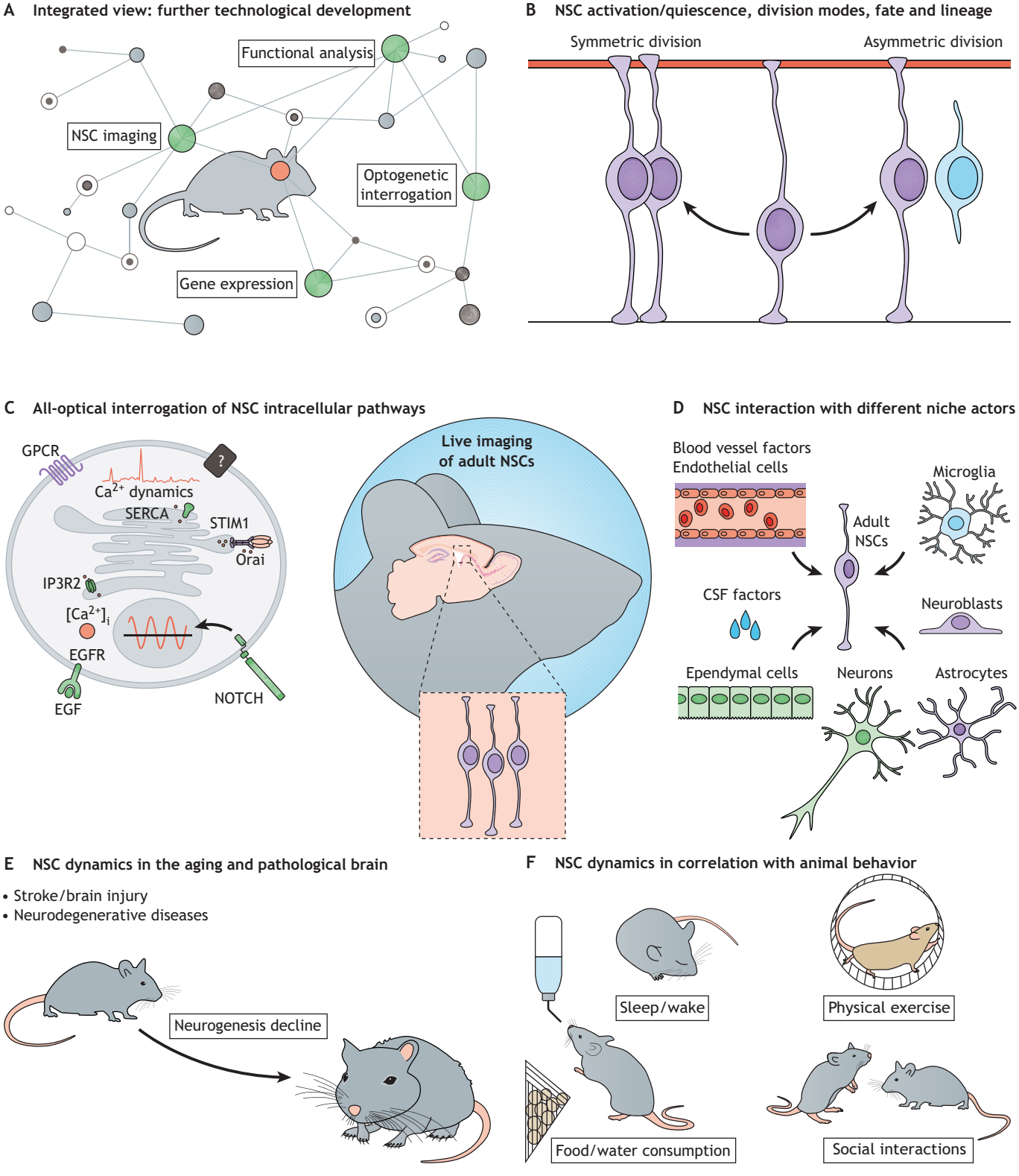

Fig. 2. See next page for legend.

Barbosa et al. also imaged NSCs after a stab injury in the telencephalon and showed that the proliferation of NSCs increases twofold, from 13% to 26% (Barbosa et al., 2015). This increased neurogenesis is due to higher recruitment of quiescent NSCs rather than multiple divisions of a single NSC (Barbosa et al., 2015). Asymmetric NSC divisions remain the predominant mode of division even after injury. In total, fewer NSCs remain quiescent, accompanied with symmetric divisions, which, unlike in the control brain, generated two cells that lost their radial glia morphology (Barbosa et al., 2015). How injury affects the activation and division

Development (2021) 148, dev199778. doi:10.1242/dev.199778

Fig. 2. Framework for future directions for *in vivo* live imaging of NSCs. (A) Further technical improvements of *in vivo* imaging tools may allow the use of multimodal analyses to investigate NSC physiology. A combination of NSC dynamics, functional and gene expression analyses as well as optogenetic interrogations may help expand our knowledge and gain an integrated view into NSC physiology. (B) Long-term *in vivo* imaging using various sensors and actuators for cell activation or differentiation may help further expand our knowledge with regard to NSC quiescence/activation, division modes, fate, differentiation and lineage. (C) All-optical live imaging of NSC intracellular pathways combined with the optogenetic, pharmacogenetic or genetic manipulation of these pathways may improve our understanding of the role of specific NSC intracellular cascades and how they are decoded and integrated. (D) *In vivo* NSC imaging in brain neurogenic niches combined with cell type-specific manipulations of different niche elements may make it possible to investigate intercellular communication and the feedback and feed-forward regulations of NSC physiology. (E) The use of *in vivo* imaging may also deepen our understanding of NSC physiology in some contexts previously shown to affect neurogenesis, such as normal aging or pathological conditions, including stroke/brain injury and some neurodegenerative diseases. (F) *In vivo* NSC dynamics can now be tracked and correlated with recordings of animal behavior during different tasks or during food or water consumption, social and parental interactions, and even during certain naturalistic behavioral states, such as sleep, arousal and anxiety.

of molecularly distinct NSC subpopulations and whether their hierarchical organization is modified remain to be elucidated.

Imaging adult NSCs in anesthetized mice

The use of experimental models such as zebrafish is a valuable advantage when imaging cellular processes without damaging the integrity of the brain. However, such approaches are more challenging to apply for investigating the *in vivo* dynamics of adult NSCs in rodents, mainly because of the light scattering properties of the tissues and deeper localization of NSCs in the rodent brain compared with zebrafish. Several imaging techniques have been used to overcome this limitation and to investigate adult NSC activation *in vivo* in the live rodent brain. Non-invasive imaging approaches such as magnetic resonance imaging (MRI), photon-emission tomography (PET) or bioluminescence imaging have been used to investigate neurogenesis in the adult rodent brain (Couillard-Despres and Aigner, 2011; Djuric et al., 2008; Friedman, 2008; Manganas et al., 2007; Ortega and Costa, 2016). However, these approaches lack cellular resolution and thus cannot be used to study the activation, fate and physiology of adult NSCs.

Multiphoton imaging has been a method of choice for investigating brain function, mostly because of the high spatial resolution, good optical sectioning and depth penetration it provides (up to 500-800 µm). However, despite the deeper penetration depth provided by two-photon excitation, the neurogenic regions in rodents are still too deep and cannot be imaged. NSC imaging can only be performed after the aspiration of the cortical regions just above the hippocampus and the installation of a cranial window (Fig. 1B) (Bottes et al., 2021; Pilz et al., 2018). This approach can be used to monitor the dynamics of NSCs and their progeny in the mouse DG for extended periods of time, up to several months, and has provided key insights into the types of NSC division and their hierarchy (Bottes et al., 2021; Pilz et al., 2018). Pilz et al. used this approach to monitor the dynamics of Ascl1+ NSCs in the DG in anesthetized mice and showed that adult radial glia-like NSCs undergo only limited rounds of cell division and are rapidly exhausted (Pilz et al., 2018). Interestingly, the authors also found that the progeny of these cells undergo several rounds of cell division with a substantial proportion of asymmetric self-renewing divisions, which may explain the life-long maintenance of neurogenesis in the adult DG (Pilz et al., 2018). In a subsequent study, Bottes et al. used a combination of intravital imaging and a single-cell RNA-sequencing strategy to shed light on the heterogeneity of the population of adult NSCs (Bottes et al., 2021). They showed that Gli1-targeted NSCs display long-term self-renewal potential, whereas Ascl1 NSCs tend to renew on a more short-term timescale and are depleted after a limited number of division cycles (Bottes et al., 2021). Interestingly, although these populations molecularly overlap, they are also distinct based on the expression of some factors such as Hopx and Mt3 (Bottes et al., 2021). Whether these different NSC dynamics reflect the presence of truly distinct NSCs types or a continuum where one population is upstream from another remains to be investigated. Self-renewing and consuming NSCs can be also linked to the degradation of the pro-activation factor Ascl1 in proliferating NSCs and their return to quiescence (Harris et al., 2021; Urbán et al., 2016). Thus, live imaging of molecularly and functionally distinct sub-populations of NSCs that can be labeled using different genetic strategies has made it possible to directly visualize different types of NSC division and to reconcile disparate data with regard to whether the activation of NSCs leads to their self-renewal or their exhaustion (Bonaguidi et al., 2011; Calzolari et al., 2015; Encinas et al., 2011; Obernier et al., 2018).

Imaging adult NSCs in freely behaving animals

Although the two-photon imaging approach mentioned above has provided key insights and a closer look at adult NSC physiology by preserving the integrity of the surrounding niche and by tracking changes for extended periods, intravital multiphoton imaging limits the use of this approach to anesthetized or head-restrained animals performing restricted behavioral tasks. In the last two decades, the advent of miniaturized fluorescent microscopes or mini-microscopes (also called mini-endoscopes, Fig. 1C) has made it possible to record brain activity and function in freely behaving subjects (Aharoni and Hoogland, 2019; Malvaut et al., 2020). Mini-endoscopy has already been used in the context of adult neurogenesis but has been restricted to the integration or activity of adult-born hippocampal granule cells (Kumar et al., 2020; Wang et al., 2019). Several studies have shown that the survival and function of new neurons is modulated by a palette of behavioral states and by stimuli such as physical exercise, stress, external environment, diet and social interactions (Obernier and Alvarez-Buylla, 2019; Paul et al., 2017; Urbán et al., 2019). It was unclear, however, whether NSC functions and dynamics are modulated by the different natural states or behaviors of freely displacing animals.

We recently used a customized mini-endoscopic imaging approach to monitor NSC dynamics in the intact SVZ (Gengatharan et al., 2021). This approach involved placing a 500 µm diameter gradient index (GRIN) lens coupled to a 45° prism in the lateral ventricle to image NSCs in the intact SVZ (Gengatharan et al., 2021). This allowed us to continuously image NSC activation and division for 2-3 consecutive days and to track the same NSCs for up to several months in freely behaving mice (Gengatharan et al., 2021). We showed that NSC activation is regulated by the day/night cycle and by darkness-induced melatonin signaling (Gengatharan et al., 2021). The use of a two-color mini-endoscope allowed us to ascertain the identity of NSCs based on the coincident activity of two different promoters (Gengatharan et al., 2021). This approach makes it now possible to track the dynamics of different NSC subtypes while animals are performing some naturalistic behaviors, such as food or water consumption, social interactions with

Development (2021) 148, dev199778. doi:10.1242/dev.199778

conspecifics and interactions between parents and pups, or when animals experience various behavioral states such as stress and anxiety.

Live imaging of intracellular pathways that orchestrate the fate and function of adult NSCs

NSCs receive numerous signals from their local environment, long-ranging neuronal projections and circulating factors. In the SVZ, for example, NSC basal processes come into contact with the endothelial cells of blood vessels and they extend apical processes toward the lateral ventricle in order to receive signals from the cerebrospinal fluid (Obernier and Alvarez-Buylla, 2019; Shen et al., 2008; Tavazoie et al., 2008). They are also interconnected via gap junctions (Lacar et al., 2011) and receive signals from local niche cells (Liu et al., 2005; Paez-Gonzalez et al., 2014) and long-ranging axonal projections (Höglinger et al., 2004; Paul et al., 2017; Tong et al., 2014). Interestingly, recent *in vitro* live imaging data suggest that these niche interactions are not static, as NSCs constantly remodel their processes to collect signals from various sources (Obernier et al., 2018). Circulating factors in the blood and cerebrospinal fluid may also influence NSC proliferation, making them responsive to systemic changes (Katsimpardi et al., 2014; Silva-Vargas et al., 2016). In keeping with this, it has recently been shown by combinations of *in vivo* end *ex vivo* live imaging that constant light conditions and pharmacological blocking of melatonin receptors during the night increase NSC activation and division, whereas constant darkness or stimulating melatonin receptors during the day decreases NSC activation, indicating that NSCs are regulated by the day/night cycle (Gengatharan et al., 2021).

How do NSCs decode and integrate a multitude of factors from their immediate surroundings and from distant sources? In this section we discuss the current state of knowledge and recent advances in our understanding of the intracellular pathways that operate in NSCs and that allow them to respond to micro-environmental cues by adjusting their quiescence/activation profile. We focus specifically on pathways that are dynamically modulated during the transition of NSCs from quiescence to proliferation, as revealed by live imaging and electrophysiology experiments.

Calcium as a cellular signature of adult NSC states

Calcium (Ca^{2+}) appears to serve as a unifying signal integrator in NSCs given that several pathways that are important for NSC physiology converge on it (Gengatharan et al., 2021; Lacar et al., 2011; Petrik et al., 2018). It has also been shown that quiescent and proliferative NSCs are characterized by very distinct Ca^{2+} dynamics and steady-state intracellular levels and that IP3-sensitive intracellular stores sustain Ca^{2+} signaling of the quiescent state (Gengatharan et al., 2021). Interestingly, the inhibition of anti-proliferative melatonin signaling or the activation of NSC division by the pro-proliferative factor EGF (Doetsch et al., 2002; Joppé et al., 2015) both activate NSCs by reducing the frequency of Ca^{2+} fluctuations and increasing intracellular Ca^{2+} levels (Gengatharan et al., 2021), which provides support for the concept that the dynamics of Ca^{2+} signals may act as an integrator and decoder of various factors that then mediate the transition of NSCs from the quiescent to the active state.

Consistent with these results are findings showing that fluid flow promotes the proliferation of NSCs in an epithelial sodium channel (ENaC)-dependent manner through store-operated Ca^{2+} release-activated Ca^{2+} (CRAC) channels (Petrik et al., 2018). The deletion of ENaC drastically decreases NSC and progenitor proliferation and increases the frequency of Ca^{2+} fluctuations in these cells (Petrik et al., 2018) that are associated with the NSC quiescent phenotype (Gengatharan et al., 2021). Interestingly, however, the modulation of ENaC activity in *in vitro* preparations still affects proliferation, suggesting that this channel may also be constitutively open (Petrik et al., 2018). It has previously been shown that ENaC may be constitutively open in other cell types (Boscardin et al., 2016), but its activity and opening probability may be modulated by fluid flow or other mechanical forces (Fronius et al., 2010). Whether constitutively active ENaC provides a basic neurochemical or mechanical tone to NSCs that allows them to sense their presence at the ventricle (Petrik et al., 2018) or to maintain them in 'pre-activation' mode by subthreshold voltage changes remains to be investigated.

Less is known about the role of Ca^{2+} signaling in DG NSCs. Comparative electrophysiological recordings of NSCs (radial glia-like cells) have shown, however, that glutamatergic receptors (AMPAR) are present in their radial processes but not in their soma (Renzel et al., 2013). Almost all NSCs express the GluA2 subunit, which confers the Ca^{2+} permeability of AMPA receptors (Renzel et al., 2013). In addition, immunogold labeling has revealed the presence of NMDA receptor NR1 subunits in the perisynaptic processes of NSCs in the inner molecular layer of the DG (Yeh et al., 2018). The radial processes of RG-like cells travel through the densely packed granule cells, and the Ca^{2+} permeable AMPA and NMDA receptors expressed along these processes appear to be perfectly located to synaptically sense released glutamate and to regulate NSC activity. These findings suggest a putative role for Ca^{2+} signaling in NSC processes in the DG by collecting and computing niche signals.

Although different Ca^{2+} dynamics specify the quiescent and activated states of NSCs, it has been unclear until recently as to whether changes in Ca^{2+} signaling by pro-proliferative and pro-quiescent signals are causative or merely reflect a consequence of altered NSC states. *In vivo* optogenetic modulation of Ca^{2+} fluxes specifically in NSCs, through the expression of the light-inducible actuator hBACCS2 (Ishii et al., 2015), has recently been used to mimic the temporal pattern of Ca^{2+} dynamics of the NSC quiescent state in mice that had higher level of NSC activation induced by constant 250-lux light conditions or by genetic alterations of signaling of IP3-sensitive intracellular Ca^{2+} stores (Gengatharan et al., 2021). These data revealed that, by mimicking the Ca^{2+} dynamics of the quiescent state, NSCs can be reverted from a higher activation state to a more quiescent state, indicating that Ca^{2+} events are the cause and not the consequence of distinct NSC states.

It remains to be determined how NSCs initiate gene expression and the transition from one state to another in response to both pro-proliferative and anti-proliferative Ca^{2+} signals of different cytosolic levels, amplitudes and frequencies. Distinct Ca^{2+} dynamics may activate distinct isoforms of nuclear factor of activated T cells (NFAT) in different ways. The calcineurin-NFAT pathway is required to initiate gene transcription in skin stem cells (Horsley et al., 2008), and Serrano-Pérez et al. have shown that NFATc3 is the predominant NFAT isoform in cultured NSCs from the postnatal SVZ and that the constitutively active form of NFATc3 reduces NSC proliferation (Serrano-Pérez et al., 2015). However, there are four isoforms of NFAT with distinct kinetics, and it is not known whether they respond differently to distinct Ca^{2+} patterns and contribute each in their own way to the proliferation, self-renewal and mode of division of NSCs.

It should be also mentioned that Ca^{2+} is not only an integrator and decoder of different signals that regulate NSC quiescence and activation, but it also plays a role in communication within the NSC

Development (2021) 148, dev199778. doi:10.1242/dev.199778

population and in the regulation of other cells in the niche. NSCs can regulate capillary blood flow by the Ca^{2+}-dependent release of ATP, which is sensed by purinergic receptors that are likely located in pericytes (Lacar et al., 2012). When the SVZ is electrically stimulated *ex vivo*, Ca^{2+} waves propagate across SVZ cells and reach cells or processes adjacent to capillaries, resulting in vasoconstriction (Lacar et al., 2012). The use of new *in vivo* live-imaging tools in combination with optogenetic approaches now makes it possible to probe the roles of the different intracellular pathways and niche signals involved in NSC activation *in vivo*.

Notch signaling pathway

The Notch signaling pathway is another intracellular cascade that is dynamically modulated in NSCs during quiescence and activation (Choe et al., 2015; Giachino and Taylor, 2014; Imayoshi et al., 2010), as it has been shown by live-imaging studies investigating oscillatory changes in Hes genes, which are Notch pathway effectors (Manning et al., 2019; Masamizu et al., 2006; Shimojo et al., 2008, 2011; Sueda et al., 2019). Hes1 is a transcriptional repressor that oscillates with a period of 2-3 h and in turn induces the oscillatory expression of the pro-neural gene *Neurogenin2* (*Ngn2*; *Neurog2*) and the Notch ligand gene *Delta-like1* (*Dll1*) (Shimojo et al., 2011). Interestingly, Hes1 oscillates in a different phase than Ngn2 and Dll1, such that when Hes1 expression is low, Ngn2 and Dll1 expressions are high (Shimojo et al., 2011). The oscillatory activity of Hes1 allows for proliferation and differentiation, whereas sustained expression leads to dormancy (Shimojo et al., 2008). When Hes1 expression is repressed, Ngn2 begins to be expressed in a sustained manner, promoting neuronal differentiation. Such reciprocal dynamic changes are required for the maintenance and differentiation of NSCs (Shimojo et al., 2008, 2011).

Hes1, as well as Hes5, also regulate the expression of *Ascl1*, another pro-neural gene (Shimojo et al., 2011). Inactivation of the Notch pathway and thus the activity of the Hes1 and Hes5 transcriptional repressors, upregulates *Ascl1* expression, which leads to a transient increase in neurogenesis by depleting NSC populations (Ables et al., 2010; Andersen et al., 2014; Ehm et al., 2010; Imayoshi et al., 2010). However, Ascl1 is associated not only with the regulation of proliferation (Sueda et al., 2019), but also with the determination of the fate and the differentiation of NSCs (Pilz et al., 2018). As is the case with Ngn2, such opposing functions are controlled by different expression patterns (Imayoshi et al., 2013; Sueda et al., 2019) and the oscillatory activity of the Hes1 transcriptional repressor. High Hes1 expression results in the inhibition of pro-neural Ascl1 and promotes NSC quiescence, whereas Hes1 repression greatly upregulates the expression of Ascl1, which promotes neuronal differentiation. Thus, cyclically active Ascl1, driven by oscillatory Hes1 expression, results in Notch pathway-dependent regulation of both the active and quiescent states of NSCs (Sueda et al., 2019).

The Notch pathway also plays a role in the lateral inhibition of NSC proliferation, as shown in zebrafish (Dray et al., 2021). Active NSCs tend to not localize near progeny and the transient pharmacological inhibition of the Notch pathway abrogates this spatial bias (Dray et al., 2021). Based on these results and longitudinal *in vivo* imaging, it has been proposed that lateral inhibition from progeny is sufficient to spatiotemporally homogenize neuronal production and the dispersion of NSC division events with an ∼12-day cycle, leading to the formation of so-called 'NSC memory' (Dray et al., 2021). This type of interaction remains to be investigated, but it is presumably short-ranging, within a diameter of one or two cells (Dray et al., 2021). In the mouse SVZ and SGZ, intermediate progenitors also express Dll1 and might exert a negative influence on NSC proliferation by Notch-dependent feedback (Aguirre et al., 2010; Engler et al., 2018; Kawaguchi et al., 2013; Nelson et al., 2020). It is conceivable, however, that due to differences in niche organization, the spatiotemporal dynamics of Notch-dependent NSC activation events may differ in a niche-dependent manner. Differences in regulation may result either from the composition of the niche, which can be distinct based on the immediate proximity of receptor- and ligand-expressing cells, or from the efficiency of inhibition, which influences the spatial distribution of activation events.

An interesting question is how different intracellular pathways that all regulate NSC function are integrated. For example, how are the Ca^{2+}-dependent signatures of distinct NSC states superimposed on the Notch-dependent regulation of the proliferation and fate of NSCs? Interesting insights have been provided by studies of *Drosophila* intestinal stem cells showing that Ca^{2+} signaling can compensate, to some extent, the disrupted Notch signaling and maintain the stem cell quiescence (Deng et al., 2015). These questions remain to be addressed in NSCs in order to get a more integrated view of how they cope with a multitude of different factors and intercellular interactions to decode and integrate all these signals.

Activity-dependent regulation and membrane potential changes in NSCs

The dynamics of membrane potential changes control cell behavior and proliferation in many cell types, including embryonic and adult NSCs (Aprea and Calegari, 2012; Levin, 2014; Yeh et al., 2018). Activated NSCs exhibit more positive membrane potentials than quiescent cells (Aprea and Calegari, 2012; Swayne and Wicki-Stordeur, 2012). In the SVZ, NSCs are characterized by a hyperpolarized resting membrane potential of −75 to −85 mV that is regulated by various ion channels (Liu et al., 2005; Yasuda and Adams, 2010). Pro-proliferative signals such as fluid flow depolarize NSCs by ENaC to promote NSC activation through changes in Ca^{2+} signaling and ERK phosphorylation (Petrik et al., 2018). In addition to fluid flow, the membrane potential of NSCs may be affected by the release of neurotransmitters from local sources (Liu et al., 2005; Paez-Gonzalez et al., 2014) and/or from long-ranging axonal projections (Höglinger et al., 2004; Paul et al., 2017; Tong et al., 2014). Electron microscopic results have shown that some of these projections, such as 5-hydroxytryptamine (5-HT; serotonin) axons originating from raphe nuclei, make direct contact with NSCs (Tong et al., 2014). The application of 5-HT receptor agonists induces an inward current and depolarization in NSCs, which triggers their proliferation (Tong et al., 2014). Pharmacological, genetic and optogenetic manipulations of several axonal projections affect the quiescence and activation of NSCs (Bao et al., 2017; Höglinger et al., 2004; Tong et al., 2014).

NSCs in the adult DG also receive massive excitatory and inhibitory inputs from local neurons (Asrican et al., 2020; Song et al., 2012) and from long-ranging projections (Bao et al., 2017; Yeh et al., 2018) that directly or indirectly affect the physiology and neurogenic output of NSCs. The resting membrane potential of quiescent NSCs is ∼−80 mV, which is in part maintained by tonic GABA inhibition (Yeh et al., 2018). The signals that either hyperpolarize or depolarize NSCs dynamically shift their equilibrium from quiescence to activation (Asrican and Song, 2021; Asrican et al., 2020; Song et al., 2012; Yeh et al., 2018). The application of muscimol, a $GABA_A$ receptor agonist (Yeh et al., 2018), or the optogenetic stimulation of GABAergic parvalbumin+ neurons in the DG (Song et al., 2012), hyperpolarize NSCs and hamper their activation, thus promoting quiescence. Conversely,

Development (2021) 148, dev199778. doi:10.1242/dev.199778

depolarizing glutamatergic signaling to NSCs, mediated by a chain of events starting from the release of the neuropeptide cholecystokinin (CCK) from CCK+ interneurons on astrocytes, promotes their activation (Asrican et al., 2020). The depolarization induced by glutamatergic signaling has been linked to the activation of NMDA and AMPA receptors, as well as metabotropic Glu receptors (mGluR1) expressed by NSCs, which in turn activate ERK/MAPK signaling pathways (Asrican et al., 2020).

The dynamic regulation of the membrane potential of NSCs by various factors may thus provide another level of signal integration in the neurogenic niche. However, the causal relationship between membrane potential changes and NSC activation remains to be shown. It may be possible to test this causal relationship by the optogenetic manipulation of the membrane potential of NSCs and the monitoring of their activation and quiescence dynamics. It is also unclear how these depolarizing and hyperpolarizing signals are translated into changes in the gene expression pattern that trigger NSC activation or quiescence and whether these signals are further amplified by downstream pathways such as Ca^{2+} signaling.

In sum, these imaging and electrophysiological results have shed new light on the dynamic features of several pathways that operate in NSCs and regulate quiescence and activation decisions by controlling transitions from one state to another. The increasing panoply of sensors and actuators that can be used to monitor the dynamics of distinct intracellular processes and interrogate their functions (Rost et al., 2017; Roth, 2016; Specht et al., 2017; Zhang and Cui, 2015) will likely provide more information on these processes in the near future. The signal transduction mechanisms at the level of NSC processes are an issue that also needs to be addressed. As discussed above, NSCs have apical and basal processes that contact and receive signals from different niche elements. It is important to determine how NSCs decode and integrate these signals locally at the level of their processes and which signals are transmitted to cell bodies to regulate the fate and function of NSCs.

Shining light on adult NSC physiology *in vivo*: future directions

The use of *in vivo* live imaging approaches to directly monitor NSC activation and function has already furthered our knowledge and will likely give new key insights in the near future. These tools may also be combined with other approaches that will make it possible to manipulate specific intracellular pathways or niche elements or to get a better understanding of the molecular diversity and hierarchy of distinct NSC types. This will provide a more integrated view of how these cells behave in their natural microenvironment and respond to a multitude of signals (Fig. 2). In the last section of this Review, we discuss future directions for the *in vivo* imaging of postnatal NSCs.

NSCs are present in a specialized microenvironment involving different actors that contribute in their own way to the physiology of NSCs (Bonafina et al., 2020; Lim and Alvarez-Buylla, 2016). These actors include other cell types such as non-neurogenic astrocytes, ependymal and endothelial cells, microglia, neuroblasts and neurons, as well as a broad array of local and systemic factors released from cerebrospinal fluid or blood vessels (Fig. 2). Although it is now clear that this microenvironment allows for the dynamic regulation of adult NSC physiology, the complexity of its architecture means that more studies are required to fully understand NSC-niche functioning. *In vivo* techniques such as two-photon imaging and mini-endoscopy now provide an unprecedented ability to track and visualize NSCs for extended periods of time in a context where the integrity of the neurogenic niche is preserved (Bottes et al., 2021; Gengatharan et al., 2021; Pilz et al., 2018). Moreover, combining these *in vivo* techniques with optogenetic tools provides us with the temporal control required to study the efficiency and the impact of different intracellular pathways operating intrinsically in NSCs (Gengatharan et al., 2021) or extrinsically in surrounding actuators, such as diverse cell types and systemic factors, on NSC activation and physiology. It is noteworthy that, at the moment when NSC imaging starts, the history of that NSC is unknown. Significant progress has been also made with labeling techniques and the genetic profiling of cells by single-cell RNA-sequencing and barcoding (Berg et al., 2019; Bottes et al., 2021; Fuentealba et al., 2015), which allows efficient tracking of individual cells both in time and space. These tools may facilitate the investigation of the diverse NSC subtypes, their lineage and hierarchical organization depending on the previous history of the cell, their cellular output and interactions between different cell types (Bottes et al., 2021; Clark et al., 2021; Than-Trong et al., 2020). Moreover, the use of these tools has already been combined with *in vivo* imaging to study NSCs (Bottes et al., 2021). A combination of all these approaches will make it possible to gain an integrated view of NSC physiology, understand how these cells are dynamically regulated in the niche and how they decode and integrate various signals.

The use of *in vivo* imaging may also provide key insights into how NSC functions are affected during brain aging and regeneration and in a context of neurodegenerative diseases or brain tumors (Fig. 2). All these conditions lead to some quantifiable changes in neurogenic niches (Arvidsson et al., 2002; Kalamakis et al., 2019; Llorens-Bobadilla et al., 2015). However, the specific changes and their temporal evolution remain to be studied. Similarly, the uncontrolled division of NSCs and progenitor cells in brain tumors can be now monitored to better understand the dynamics of these processes, determine how the fine-tuned equilibrium between quiescence and activation is hijacked and ascertain whether the acute manipulation of specific intracellular pathways or niche elements can stop this uncontrolled cellular behavior.

Lastly, it should be noted that expanding our knowledge of NSC physiology is closely linked to further improvements in imaging techniques in the future. Indeed, both two-photon and mini-endoscopic imaging have their share of advantages and disadvantages (Malvaut et al., 2020). Although two-photon microscopy can be used for high spatial resolution imaging of NSCs in order to study their fine processes and track their lineage, such imaging has been, to date, performed in anesthetized and head-restrained animals, which limits their behavioral repertoire (Barbosa et al., 2015; Bottes et al., 2021; Dray et al., 2015; Pilz et al., 2018). Two-photon excitation allows to image regions that are up to 500-800 µm deep from the surface of the brain (Miller et al., 2017; Svoboda and Yasuda, 2006; Pilz et al., 2018) and thus requires the removal of cortical layers in rodents when NSCs in the DG are imaged (Bottes et al., 2021; Pilz et al., 2018). Mini-endoscopic imaging provides excellent temporal resolution, making it possible to observe NSC activation and division as a continuum in naturally behaving subjects. However, the focus of the imaged field of view during imaging and in between imaging sessions cannot be adjusted, which is a major drawback. Given the low occurrence of NSC division *in vivo* (Gengatharan et al., 2021), it is possible that some divisions are missed with time and consecutive imaging sessions because daughter cells may arise in a different focal plan or migrate outside the field of view. It should also be noted that GRIN lenses of different length can be used to reach any deep brain region without removing the surrounding tissue (Malvaut et al., 2020), but

as these lenses are at least 500 µm in diameter, it takes several weeks post-implantation for the surrounding tissues to settle and recover (Malvaut et al., 2021). Similarly, it is important to ascertain that when *in vivo* two-photon or mini-endoscopic imaging in the DG and SVZ are performed, the installation of cranial window or GRIN lenses does not result in gliosis or damage-induced activation of NSCs when imaging starts (Gengatharan et al., 2021; Gu et al., 2014; Pilz et al., 2016). Recently, new approaches including sectioning, dual-modality recordings and wireless features for mini-endoscopic imaging (Aharoni and Hoogland, 2019; Malvaut et al., 2020), lightweight two-photon microscopy systems for imaging in freely behaving rodents (Zong et al., 2017) and three-photon imaging systems allowing the imaging of structures more than 1 mm deep in tissue without removing the overlying structure (Klioutchnikov et al., 2020) have been implemented. These technological advances may further improve our technical arsenal and help us uncover yet hidden features of NSC activation and function.

Conclusion

The advent of *in vivo* live imaging approaches has made it possible to directly monitor and decipher the dynamics of NSC activation, their hierarchical organization, their diversity and their lineage trees, providing key insights into NSC activation and function. Importantly, these approaches make it possible to observe NSCs in a complex, constantly changing microenvironment and to gain key insights into complex niche interactions. Combined with the use of genetic, pharmacogenetic and optogenetic approaches to selectively manipulate NSCs, their intracellular pathways and distinct cell types in the niche, live imaging will further deepen our understanding of the specific roles of various niche players and pathways underlying the transmission, reception and integration of signals. Future NSC live-imaging studies will not only expand our knowledge but also guide us to a more complete understanding of NSC physiology in their microenvironment. This knowledge can eventually be applied to cell replacement therapies or to abrogate abnormal functions of NSCs in some pathological conditions such as brain tumors.

Acknowledgements

We thank Mireille Massouh at MassouhBioMedia for the schematic drawings.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by a Canadian Institutes of Health Research (CIHR) grant to A.S.

References

Ables, J. L., Decarolis, N. A., Johnson, M. A., Rivera, P. D., Gao, Z., Cooper, D. C., Radtke, F., Hsieh, J. and Eisch, A. J. (2010). Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. *J. Neurosci.* **30**, 10484-10492. doi:10.1523/JNEUROSCI.4721-09.2010

Adolf, B., Chapouton, P., Lam, C. S., Topp, S., Tannhäuser, B., Strahle, U., Gotz, M. and Bally-Cuif, L. (2006). Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Dev. Biol.* **295**, 278-293. doi:10.1016/j.ydbio.2006.03.023

Aguirre, A., Rubio, M. E. and Gallo, V. (2010). Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. *Nature* **467**, 323-327. doi:10.1038/nature09347

Aharoni, D. and Hoogland, T. M. (2019). Circuit investigations with open-source miniaturized microscopes: past, present and future. *Front. Cell Neurosci.* **13**, 141. doi:10.3389/fncel.2019.00141

Ahn, S. and Joyner, A. L. (2005). In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437**, 894-897. doi:10.1038/nature03994

Altman, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137**, 433-457. doi:10.1002/cne.901370404

Altman, J. and Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319-335. doi:10.1002/cne.901240303

Andersen, J., Urbán, N., Achimastou, A., Ito, A., Simic, M., Ullom, K., Martynoga, B., Lebel, M., Göritz, C., Frisén, J. et al. (2014). A transcriptional mechanism integrating inputs from extracellular signals to activate hippocampal stem cells. *Neuron* **83**, 1085-1097. doi:10.1016/j.neuron.2014.08.004

Aprea, J. and Calegari, F. (2012). Bioelectric state and cell cycle control of Mammalian neural stem cells. *Stem Cells Int.* **2012**, 816049. doi:10.1155/2012/816049

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. and Lindvall, O. (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat. Med.* **8**, 963-970. doi:10.1038/nm747

Asrican, B. and Song, J. (2021). Recording membrane potential in adult neural stem cells as readout for stem cell activation following neural circuit stimulation in mouse hippocampal slices. *STAR Protoc.* **2**, 100335. doi:10.1016/j.xpro.2021.100335

Asrican, B., Wooten, J., Li, Y.-D., Quintanilla, L., Zhang, F., Wander, C., Bao, H., Yeh, C.-Y., Luo, Y.-J., Olsen, R. et al. (2020). Neuropeptides modulate local astrocytes to regulate adult hippocampal neural stem cells. *Neuron* **108**, 349-366.e346. doi:10.1016/j.neuron.2020.07.039

Azim, K., Fischer, B., Hurtado-Chong, A., Draganova, K., Cantù, C., Zemke, M., Sommer, L., Butt, A. and Raineteau, O. (2014). Persistent Wnt/beta-catenin signaling determines dorsalization of the postnatal subventricular zone and neural stem cell specification into oligodendrocytes and glutamatergic neurons. *Stem Cells* **32**, 1301-1312. doi:10.1002/stem.1639

Bao, H., Asrican, B., Li, W., Gu, B., Wen, Z., Lim, S. A., Haniff, I., Ramakrishnan, C., Deisseroth, K., Philpot, B. et al. (2017). Long-range GABAergic inputs regulate neural stem cell quiescence and control adult hippocampal neurogenesis. *Cell Stem Cell* **21**, 604-617.e605. doi:10.1016/j.stem.2017.10.003

Barbosa, J. S., Sanchez-Gonzalez, R., Di Giaimo, R., Baumgart, E. V., Theis, F. J., Gotz, M. and Ninkovic, J. (2015). Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. *Science* **348**, 789-793. doi:10.1126/science.aaa2729

Barbosa, J. S., Di Giaimo, R., Götz, M. and Ninkovic, J. (2016). Single-cell in vivo imaging of adult neural stem cells in the zebrafish telencephalon. *Nat. Protoc.* **11**, 1360-1370. doi:10.1038/nprot.2016.077

Barnabé-Heider, F. and Frisén, J. (2008). Stem cells for spinal cord repair. *Cell Stem Cell* **3**, 16-24. doi:10.1016/j.stem.2008.06.011

Beckervordersandforth, R., Tripathi, P., Ninkovic, J., Bayam, E., Lepier, A., Stempfhuber, B., Kirchhoff, F., Hirrlinger, J., Haslinger, A., Lie, D. C. et al. (2010). In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. *Cell Stem Cell* **7**, 744-758. doi:10.1016/j.stem.2010.11.017

Berg, D. A., Bond, A. M., Ming, G.-L. and Song, H. (2018). Radial glial cells in the adult dentate gyrus: what are they and where do they come from? *F1000Res* **7**, 277. doi:10.12688/f1000research.12684.1

Berg, D. A., Su, Y., Jimenez-Cyrus, D., Patel, A., Huang, N., Morizet, D., Lee, S., Shah, R., Ringeling, F. R., Jain, R. et al. (2019). A common embryonic origin of stem cells drives developmental and adult neurogenesis. *Cell* **177**, 654-668.e615. doi:10.1016/j.cell.2019.02.010

Bernardos, R. L. and Raymond, P. A. (2006). GFAP transgenic zebrafish. *Gene Expr. Patterns* **6**, 1007-1013. doi:10.1016/j.modgep.2006.04.006

Bonafina, A., Paratcha, G. and Ledda, F. (2020). Deciphering new players in the neurogenic adult hippocampal niche. *Front. Cell Dev. Biol.* **8**, 548. doi:10.3389/fcell.2020.00548

Bonaguidi, M. A., Wheeler, M. A., Shapiro, J. S., Stadel, R. P., Sun, G. J., Ming, G.-L. and Song, H. (2011). In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. *Cell* **145**, 1142-1155. doi:10.1016/j.cell.2011.05.024

Bonaguidi, M. A., Stadel, R. P., Berg, D. A., Sun, J., Ming, G.-L. and Song, H. (2016). Diversity of neural precursors in the adult mammalian brain. *Cold Spring Harb. Perspect. Biol.* **8**, a018838. doi:10.1101/cshperspect.a018838

Boscardin, E., Alijevic, O., Hummler, E., Frateschi, S. and Kellenberger, S. (2016). The function and regulation of acid-sensing ion channels (ASICs) and the epithelial Na(+) channel (ENaC): IUPHAR Review 19. *Br. J. Pharmacol.* **173**, 2671-2701. doi:10.1111/bph.13533

Bottes, S., Jaeger, B. N., Pilz, G.-A., Jörg, D. J., Cole, J. D., Kruse, M., Harris, L., Korobeynyk, V. I., Mallona, I., Helmchen, F. et al. (2021). Long-term self-renewing stem cells in the adult mouse hippocampus identified by intravital imaging. *Nat. Neurosci.* **24**, 225-233. doi:10.1038/s41593-020-00759-4

Calzolari, F., Michel, J., Baumgart, E. V., Theis, F., Götz, M. and Ninkovic, J. (2015). Fast clonal expansion and limited neural stem cell self-renewal in the adult subependymal zone. *Nat. Neurosci.* **18**, 490-492. doi:10.1038/nn.3963

Choe, Y., Pleasure, S. J. and Mira, H. (2015). Control of adult neurogenesis by short-range morphogenic-signaling molecules. *Cold Spring Harb. Perspect. Biol.* **8**, a018887. doi:10.1101/cshperspect.a018887

Clark, I. C., Gutiérrez-Vázquez, C., Wheeler, M. A., Li, Z., Rothhammer, V., Linnerbauer, M., Sanmarco, L. M., Guo, L., Blain, M., Zandee, S. E. J. et al. (2021). Barcoded viral tracing of single-cell interactions in central nervous system inflammation. *Science* **372**, eabf1230. doi:10.1126/science.abf1230

Codega, P., Silva-Vargas, V., Paul, A., Maldonado-Soto, A. R., Deleo, A. M., Pastrana, E. and Doetsch, F. (2014). Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. *Neuron* **82**, 545-559. doi:10.1016/j.neuron.2014.02.039

Costa, M. R., Ortega, F., Brill, M. S., Beckervordersandforth, R., Petrone, C., Schroeder, T., Götz, M. and Berninger, B. (2011). Continuous live imaging of adult neural stem cell division and lineage progression in vitro. *Development* **138**, 1057-1068. doi:10.1242/dev.061663

Couillard-Despres, S. and Aigner, L. (2011). In vivo imaging of adult neurogenesis. *Eur. J. Neurosci.* **33**, 1037-1044. doi:10.1111/j.1460-9568.2011.07601.x

DeCarolis, N. A., Mechanic, M., Petrik, D., Carlton, A., Ables, J. L., Malhotra, S., Bachoo, R., Götz, M., Lagace, D. C. and Eisch, A. J. (2013). In vivo contribution of nestin- and GLAST-lineage cells to adult hippocampal neurogenesis. *Hippocampus* **23**, 708-719. doi:10.1002/hipo.22130

Deng, H., Gerencser, A. A. and Jasper, H. (2015). Signal integration by Ca(2+) regulates intestinal stem-cell activity. *Nature* **528**, 212-217. doi:10.1038/nature16170

Denoth-Lippuner, A. and Jessberger, S. (2021). Formation and integration of new neurons in the adult hippocampus. *Nat. Rev. Neurosci.* **22**, 223-236. doi:10.1038/s41583-021-00433-z

Djuric, P. M., Benveniste, H., Wagshul, M. E., Henn, F., Enikolopov, G. and Maletic-Savatic, M. (2008). Response to comments on "magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain". *Science* **321**, 640. doi:10.1126/science.1156889

Doetsch, F., Caillé, I., Lim, D. A., García-Verdugo, J. M. and Alvarez-Buylla, A. (1999a). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703-716. doi:10.1016/S0092-8674(00)80783-7

Doetsch, F., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (1999b). Regeneration of a germinal layer in the adult mammalian brain. *Proc. Natl. Acad. Sci. USA* **96**, 11619-11624. doi:10.1073/pnas.96.20.11619

Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J.-M. and Alvarez-Buylla, A. (2002). EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* **36**, 1021-1034. doi:10.1016/S0896-6273(02)01133-9

Dray, N., Bedu, S., Vuillemin, N., Alunni, A., Coolen, M., Krecsmarik, M., Supatto, W., Beaurepaire, E. and Bally-Cuif, L. (2015). Large-scale live imaging of adult neural stem cells in their endogenous niche. *Development* **142**, 3592-3600. doi:10.1242/dev.123018

Dray, N., Mancini, L., Binshtok, U., Cheysson, F., Supatto, W., Mahou, P., Bedu, S., Ortica, S., Than-Trong, E., Krecsmarik, M. et al. (2021). Dynamic spatiotemporal coordination of neural stem cell fate decisions occurs through local feedback in the adult vertebrate brain. *Cell Stem Cell* **28**, 1-16. doi:10.1016/j.stem.2021.03.014

Ehm, O., Goritz, C., Covic, M., Schaffner, I., Schwarz, T. J., Karaca, E., Kempkes, B., Kremmer, E., Pfrieger, F. W., Espinosa, L. et al. (2010). RBPJkappa-dependent signaling is essential for long-term maintenance of neural stem cells in the adult hippocampus. *J. Neurosci.* **30**, 13794-13807. doi:10.1523/JNEUROSCI.1567-10.2010

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J.-H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A. and Enikolopov, G. (2011). Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. *Cell Stem Cell* **8**, 566-579. doi:10.1016/j.stem.2011.03.010

Engler, A., Rolando, C., Giachino, C., Saotome, I., Erni, A., Brien, C., Zhang, R., Zimber-Strobl, U., Radtke, F., Artavanis-Tsakonas, S. et al. (2018). Notch2 signaling maintains NSC quiescence in the murine ventricular-subventricular zone. *Cell Rep.* **22**, 992-1002. doi:10.1016/j.celrep.2017.12.094

Filippov, V., Kronenberg, G., Pivneva, T., Reuter, K., Steiner, B., Wang, L.-P., Yamaguchi, M., Kettenmann, H. and Kempermann, G. (2003). Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol. Cell. Neurosci.* **23**, 373-382. doi:10.1016/S1044-7431(03)00060-5

Friedman, S. D. (2008). Comment on "Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain". *Science* **321**, 640. doi:10.1126/science.1153484

Fronius, M., Bogdan, R., Althaus, M., Morty, R. E. and Clauss, W. G. (2010). Epithelial Na+ channels derived from human lung are activated by shear force. *Respir. Physiol. Neurobiol.* **170**, 113-119. doi:10.1016/j.resp.2009.11.004

Fuentealba, L. C., Rompani, S. B., Parraguez, J. I., Obernier, K., Romero, R., Cepko, C. L. and Alvarez-Buylla, A. (2015). Embryonic origin of postnatal neural stem cells. *Cell* **161**, 1644-1655. doi:10.1016/j.cell.2015.05.041

Fukuda, S., Kato, F., Tozuka, Y., Yamaguchi, M., Miyamoto, Y. and Hisatsune, T. (2003). Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *J. Neurosci.* **23**, 9357-9366. doi:10.1523/JNEUROSCI.23-28-09357.2003

Furlan, G., Cuccioli, V., Vuillemin, N., Dirian, L., Muntasell, A. J., Coolen, M., Dray, N., Bedu, S., Houart, C., Beaurepaire, E. et al. (2017). Life-long neurogenic activity of individual neural stem cells and continuous growth establish an outside-in architecture in the Teleost Pallium. *Curr. Biol.* **27**, 3288-3301.e3283. doi:10.1016/j.cub.2017.09.052

Garcia, A. D. R., Doan, N. B., Imura, T., Bush, T. G. and Sofroniew, M. V. (2004). GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat. Neurosci.* **7**, 1233-1241. doi:10.1038/nn1340

Gengatharan, A., Malvaut, S., Marymonchyk, A., Ghareghani, M., Snapyan, M., Fischer-Sternjak, J., Ninkovic, J., Götz, M. and Saghatelyan, A. (2021). Adult neural stem cell activation in mice is regulated by the day/night cycle and intracellular calcium dynamics. *Cell* **184**, 709-722.e713. doi:10.1016/j.cell.2020.12.026

Giachino, C. and Taylor, V. (2014). Notching up neural stem cell homogeneity in homeostasis and disease. *Front. Neurosci.* **8**, 32. doi:10.3389/fnins.2014.00032

Goldman, S. A. (2016). Stem and progenitor cell-based therapy of the central nervous system: hopes, hype, and wishful thinking. *Cell Stem Cell* **18**, 174-188. doi:10.1016/j.stem.2016.01.012

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I. and Brand, M. (2006). Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Dev. Biol.* **295**, 263-277. doi:10.1016/j.ydbio.2006.03.040

Gu, L., Kleiber, S., Schmid, L., Nebeling, F., Chamoun, M., Steffen, J., Wagner, J. and Fuhrmann, M. (2014). Long-term in vivo imaging of dendritic spines in the hippocampus reveals structural plasticity. *J. Neurosci.* **34**, 13948-13953. doi:10.1523/JNEUROSCI.1464-14.2014

Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. D. M., Edwards, A., Urbán, N., Marciniak-Czochra, A. and Guillemot, F. (2021). Coordinated changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem cell population. *Cell Stem Cell* **28**, 863-876.e866. doi:10.1016/j.stem.2021.01.003

Höglinger, G. U., Rizk, P., Muriel, M. P., Duyckaerts, C., Oertel, W. H., Caille, I. and Hirsch, E. C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat. Neurosci.* **7**, 726-735. doi:10.1038/nn1265

Horsley, V., Aliprantis, A. O., Polak, L., Glimcher, L. H. and Fuchs, E. (2008). NFATc1 balances quiescence and proliferation of skin stem cells. *Cell* **132**, 299-310. doi:10.1016/j.cell.2007.11.047

Ihrie, R. A. and Álvarez-Buylla, A. (2011). Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* **70**, 674-686. doi:10.1016/j.neuron.2011.05.004

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S. and Kageyama, R. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat. Neurosci.* **11**, 1153-1161. doi:10.1038/nn.2185

Imayoshi, I., Sakamoto, M., Yamaguchi, M., Mori, K. and Kageyama, R. (2010). Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. *J. Neurosci.* **30**, 3489-3498. doi:10.1523/JNEUROSCI.4987-09.2010

Imayoshi, I., Isomura, A., Harima, Y., Kawaguchi, K., Kori, H., Miyachi, H., Fujiwara, T., Ishidate, F. and Kageyama, R. (2013). Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. *Science* **342**, 1203-1208. doi:10.1126/science.1242366

Ishii, T., Sato, K., Kakumoto, T., Miura, S., Touhara, K., Takeuchi, S. and Nakata, T. (2015). Light generation of intracellular Ca(2+) signals by a genetically encoded protein BACCS. *Nat. Commun.* **6**, 8021. doi:10.1038/ncomms9021

Joppé, S. E., Hamilton, L. K., Cochard, L. M., Levros, L.-C., Aumont, A., Barnabé-Heider, F. and Fernandes, K. J. L. (2015). Bone morphogenetic protein dominantly suppresses epidermal growth factor-induced proliferative expansion of adult forebrain neural precursors. *Front. Neurosci.* **9**, 407. doi:10.3389/fnins.2015.00407

Kalamakis, G., Brüne, D., Ravichandran, S., Bolz, J., Fan, W., Ziebell, F., Stiehl, T., Catalá-Martinez, F., Kupke, J., Zhao, S. et al. (2019). Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. *Cell* **176**, 1407-1419.e1414. doi:10.1016/j.cell.2019.01.040

Katsimpardi, L., Litterman, N. K., Schein, P. A., Miller, C. M., Loffredo, F. S., Wojtkiewicz, G. R., Chen, J. W., Lee, R. T., Wagers, A. J. and Rubin, L. L. (2014). Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science* **344**, 630-634. doi:10.1126/science.1251141

Kawaguchi, D., Furutachi, S., Kawai, H., Hozumi, K. and Gotoh, Y. (2013). Dll1 maintains quiescence of adult neural stem cells and segregates asymmetrically during mitosis. *Nat. Commun.* **4**, 1880. doi:10.1038/ncomms2895

Kim, E. J., Ables, J. L., Dickel, L. K., Eisch, A. J. and Johnson, J. E. (2011). Ascl1 (Mash1) defines cells with long-term neurogenic potential in subgranular and subventricular zones in adult mouse brain. *PLoS ONE* **6**, e18472. doi:10.1371/journal.pone.0018472

Klioutchnikov, A., Wallace, D. J., Frosz, M. H., Zeltner, R., Sawinski, J., Pawlak, V., Voit, K.-M., Russell, P. S. J. and Kerr, J. N. D. (2020). Three-photon

head-mounted microscope for imaging deep cortical layers in freely moving rats. *Nat. Methods* **17**, 509-513. doi:10.1038/s41592-020-0817-9

Kokoeva, M. V., Yin, H. and Flier, J. S. (2007). Evidence for constitutive neural cell proliferation in the adult murine hypothalamus. *J. Comp. Neurol.* **505**, 209-220. doi:10.1002/cne.21492

Kuhn, H. G., Dickinson-Anson, H. and Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* **16**, 2027-2033. doi:10.1523/JNEUROSCI.16-06-02027.1996

Kumar, D., Koyanagi, I., Carrier-Ruiz, A., Vergara, P., Srinivasan, S., Sugaya, Y., Kasuya, M., Yu, T.-S., Vogt, K. E., Muratani, M. et al. (2020). Sparse activity of hippocampal adult-born neurons during REM sleep is necessary for memory consolidation. *Neuron* **107**, 552-565.e510. doi:10.1016/j.neuron.2020.05.008

Lacar, B., Young, S. Z., Platel, J.-C. and Bordey, A. (2011). Gap junction-mediated calcium waves define communication networks among murine postnatal neural progenitor cells. *Eur. J. Neurosci.* **34**, 1895-1905. doi:10.1111/j.1460-9568.2011.07901.x

Lacar, B., Herman, P., Platel, J.-C., Kubera, C., Hyder, F. and Bordey, A. (2012). Neural progenitor cells regulate capillary blood flow in the postnatal subventricular zone. *J. Neurosci.* **32**, 16435-16448. doi:10.1523/JNEUROSCI.1457-12.2012

Lee, D. A., Bedont, J. L., Pak, T., Wang, H., Song, J., Miranda-Angulo, A., Takiar, V., Charubhumi, V., Balordi, F., Takebayashi, H. et al. (2012). Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. *Nat. Neurosci.* **15**, 700-702. doi:10.1038/nn.3079

Levin, M. (2014). Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol. Biol. Cell* **25**, 3835-3850. doi:10.1091/mbc.e13-12-0708

Li, G., Fang, L., Fernández, G. and Pleasure, S. J. (2013). The ventral hippocampus is the embryonic origin for adult neural stem cells in the dentate gyrus. *Neuron* **78**, 658-672. doi:10.1016/j.neuron.2013.03.019

Lim, D. A. and Alvarez-Buylla, A. (2016). The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. *Cold Spring Harb. Perspect. Biol.* **8**, a018820. doi:10.1101/cshperspect.a018820

Liu, X., Wang, Q., Haydar, T. F. and Bordey, A. (2005). Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat. Neurosci.* **8**, 1179-1187. doi:10.1038/nn1522

Lledo, P.-M., Merkle, F. T. and Alvarez-Buylla, A. (2008). Origin and function of olfactory bulb interneuron diversity. *Trends Neurosci.* **31**, 392-400. doi:10.1016/j.tins.2008.05.006

Llorens-Bobadilla, E., Zhao, S., Baser, A., Saiz-Castro, G., Zwadlo, K. and Martin-Villalba, A. (2015). Single-cell transcriptomics reveals a population of dormant neural stem cells that become activated upon brain injury. *Cell Stem Cell* **17**, 329-340. doi:10.1016/j.stem.2015.07.002

Lois, C. and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145-1148. doi:10.1126/science.8178174

Lugert, S., Basak, O., Knuckles, P., Haussler, U., Fabel, K., Götz, M., Haas, C. A., Kempermann, G., Taylor, V. and Giachino, C. (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. *Cell Stem Cell* **6**, 445-456. doi:10.1016/j.stem.2010.03.017

Luskin, M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11**, 173-189. doi:10.1016/0896-6273(93)90281-U

Malvaut, S. and Saghatelyan, A. (2016). The role of adult-born neurons in the constantly changing olfactory bulb network. *Neural Plast.* **2016**, 1614329. doi:10.1155/2016/1614329

Malvaut, S., Constantinescu, V.-S., Dehez, H., Doric, S. and Saghatelyan, A. (2020). Deciphering brain function by miniaturized fluorescence microscopy in freely behaving animals. *Front. Neurosci.* **14**, 819. doi:10.3389/fnins.2020.00819

Malvaut, S., Marymonchyk, A., Gengatharan, A. and Saghatelyan, A. (2021). Live imaging of adult neural stem cells in freely behaving mice using mini-endoscopes. *STAR Protoc.* **2**, 100596. doi:10.1016/j.xpro.2021.100596

Manganas, L. N., Zhang, X., Li, Y., Hazel, R. D., Smith, S. D., Wagshul, M. E., Henn, F., Benveniste, H., Djuric, P. M., Enikolopov, G. et al. (2007). Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. *Science* **318**, 980-985. doi:10.1126/science.1147851

Manning, C. S., Biga, V., Boyd, J., Kursawe, J., Ymisson, B., Spiller, D. G., Sanderson, C. M., Galla, T., Rattray, M. and Papalopulu, N. (2019). Quantitative single-cell live imaging links HES5 dynamics with cell-state and fate in murine neurogenesis. *Nat. Commun.* **10**, 2835. doi:10.1038/s41467-019-10734-8

März, M., Chapouton, P., Diotel, N., Vaillant, C., Hesl, B., Takamiya, M., Lam, C. S., Kah, O., Bally-Cuif, L. and Strahle, U. (2010). Heterogeneity in progenitor cell subtypes in the ventricular zone of the zebrafish adult telencephalon. *Glia* **58**, 870-888. doi:10.1002/glia.20971

Masamizu, Y., Ohtsuka, T., Takashima, Y., Nagahara, H., Takenaka, Y., Yoshikawa, K., Okamura, H. and Kageyama, R. (2006). Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. *Proc. Natl. Acad. Sci. USA* **103**, 1313-1318. doi:10.1073/pnas.0508658103

Merkle, F. T., Fuentealba, L. C., Sanders, T. A., Magno, L., Kessaris, N. and Alvarez-Buylla, A. (2014). Adult neural stem cells in distinct microdomains generate previously unknown interneuron types. *Nat. Neurosci.* **17**, 207-214. doi:10.1038/nn.3610

Migaud, M., Batailler, M., Segura, S., Duittoz, A., Franceschini, I. and Pillon, D. (2010). Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones. *Eur. J. Neurosci.* **32**, 2042-2052. doi:10.1111/j.1460-9568.2010.07521.x

Miller, D. R., Jarrett, J. W., Hassan, A. M. and Dunn, A. K. (2017). Deep tissue imaging with multiphoton fluorescence microscopy. *Curr. Opin. Biomed. Eng.* **4**, 32-39. doi:10.1016/j.cobme.2017.09.004

Ming, G.-L. and Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223-250. doi:10.1146/annurev.neuro.28.051804.101459

Mirzadeh, Z., Merkle, F. T., Soriano-Navarro, M., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2008). Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* **3**, 265-278. doi:10.1016/j.stem.2008.07.004

Moss, J., Gebara, E., Bushong, E. A., Sánchez-Pascual, I., O'Laoi, R., El M'Ghari, I., Kocher-Braissant, J., Ellisman, M. H. and Toni, N. (2016). Fine processes of Nestin-GFP-positive radial glia-like stem cells in the adult dentate gyrus ensheathe local synapses and vasculature. *Proc. Natl. Acad. Sci. USA* **113**, E2536-E2545. doi:10.1073/pnas.1514652113

Nelson, B. R., Hodge, R. D., Daza, R. A. M., Tripathi, P. P., Arnold, S. J., Millen, K. J. and Hevner, R. F. (2020). Intermediate progenitors support migration of neural stem cells into dentate gyrus outer neurogenic niches. *eLife* **9**, e53777. doi:10.7554/eLife.53777

Ninkovic, J., Mori, T. and Gotz, M. (2007). Distinct modes of neuron addition in adult mouse neurogenesis. *J. Neurosci.* **27**, 10906-10911. doi:10.1523/JNEUROSCI.2572-07.2007

Obernier, K. and Alvarez-Buylla, A. (2019). Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. *Development* **146**, dev156059. doi:10.1242/dev.156059

Obernier, K., Cebrian-Silla, A., Thomson, M., Parraguez, J. I., Anderson, R., Guinto, C., Rodas Rodriguez, J., Garcia-Verdugo, J.-M. and Alvarez-Buylla, A. (2018). Adult neurogenesis is sustained by symmetric self-renewal and differentiation. *Cell Stem Cell* **22**, 221-234.e228. doi:10.1016/j.stem.2018.01.003

Ohab, J. J. and Carmichael, S. T. (2008). Poststroke neurogenesis: emerging principles of migration and localization of immature neurons. *Neuroscientist* **14**, 369-380. doi:10.1177/1073858407309545

Olivier, N., Luengo-Oroz, M. A., Duloquin, L., Faure, E., Savy, T., Veilleux, I., Solinas, X., Debarre, D., Bourgine, P., Santos, A. et al. (2010). Cell lineage reconstruction of early zebrafish embryos using label-free nonlinear microscopy. *Science* **329**, 967-971. doi:10.1126/science.1189428

Ortega, F. and Costa, M. R. (2016). Live imaging of adult neural stem cells in rodents. *Front. Neurosci.* **10**, 78. doi:10.3389/fnins.2016.00078

Ortega, F., Berninger, B. and Costa, M. R. (2013). Primary culture and live imaging of adult neural stem cells and their progeny. *Methods Mol. Biol.* **1052**, 1-11. doi:10.1007/7651_2013_22

Paez-Gonzalez, P., Asrican, B., Rodriguez, E. and Kuo, C. T. (2014). Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. *Nat. Neurosci.* **17**, 934-942. doi:10.1038/nn.3734

Pastrana, E., Cheng, L.-C. and Doetsch, F. (2009). Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. *Proc. Natl. Acad. Sci. USA* **106**, 6387-6392. doi:10.1073/pnas.0810407106

Paul, A., Chaker, Z. and Doetsch, F. (2017). Hypothalamic regulation of regionally distinct adult neural stem cells and neurogenesis. *Science* **356**, 1383-1386. doi:10.1126/science.aal3839

Petrik, D., Myoga, M. H., Grade, S., Gerkau, N. J., Pusch, M., Rose, C. R., Grothe, B. and Götz, M. (2018). Epithelial sodium channel regulates adult neural stem cell proliferation in a flow-dependent manner. *Cell Stem Cell* **22**, 865-878.e868. doi:10.1016/j.stem.2018.04.016

Pilz, G.-A., Carta, S., Stauble, A., Ayaz, A., Jessberger, S. and Helmchen, F. (2016). Functional imaging of dentate granule cells in the adult mouse hippocampus. *J. Neurosci.* **36**, 7407-7414. doi:10.1523/JNEUROSCI.3065-15.2016

Pilz, G.-A., Bottes, S., Betizeau, M., Jörg, D. J., Carta, S., Simons, B. D., Helmchen, F. and Jessberger, S. (2018). Live imaging of neurogenesis in the adult mouse hippocampus. *Science* **359**, 658-662. doi:10.1126/science.aao5056

Renzel, R., Sadek, A.-R., Chang, C.-H., Gray, W. P., Seifert, G. and Steinhäuser, C. (2013). Polarized distribution of AMPA, but not GABAA, receptors in radial glia-like cells of the adult dentate gyrus. *Glia* **61**, 1146-1154. doi:10.1002/glia.22505

Rost, B. R., Schneider-Warme, F., Schmitz, D. and Hegemann, P. (2017). Optogenetic Tools for Subcellular Applications in Neuroscience. *Neuron* **96**, 572-603. doi:10.1016/j.neuron.2017.09.047

Roth, B. L. (2016). DREADDs for Neuroscientists. *Neuron* **89**, 683-694. doi:10.1016/j.neuron.2016.01.040

Seri, B., García-Verdugo, J. M., McEwen, B. S. and Alvarez-Buylla, A. (2001). Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* **21**, 7153-7160. doi:10.1523/JNEUROSCI.21-18-07153.2001

Serrano-Pérez, M. C., Fernández, M., Neria, F., Berjón-Otero, M., Doncel-Pérez, E., Cano, E. and Tranque, P. (2015). NFAT transcription factors regulate survival, proliferation, migration, and differentiation of neural precursor cells. *Glia* **63**, 987-1004. doi:10.1002/glia.22797

Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.-M., Goderie, S. K., Roysam, B. and Temple, S. (2008). Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* **3**, 289-300. doi:10.1016/j.stem.2008.07.026

Shimojo, H., Ohtsuka, T. and Kageyama, R. (2008). Oscillations in notch signaling regulate maintenance of neural progenitors. *Neuron* **58**, 52-64. doi:10.1016/j.neuron.2008.02.014

Shimojo, H., Ohtsuka, T. and Kageyama, R. (2011). Dynamic expression of notch signaling genes in neural stem/progenitor cells. *Front Neurosci* **5**, 78. doi:10.3389/fnins.2011.00078

Silva-Vargas, V., Maldonado-Soto, A. R., Mizrak, D., Codega, P. and Doetsch, F. (2016). Age-dependent niche signals from the choroid plexus regulate adult neural stem cells. *Cell Stem Cell* **19**, 643-652. doi:10.1016/j.stem.2016.06.013

Silva-Vargas, V., Delgado, A. C. and Doetsch, F. (2018). Symmetric stem cell division at the heart of adult neurogenesis. *Neuron* **98**, 246-248. doi:10.1016/j.neuron.2018.04.005

Song, J., Zhong, C., Bonaguidi, M. A., Sun, G. J., Hsu, D., Gu, Y., Meletis, K., Huang, Z. J., Ge, S., Enikolopov, G. et al. (2012). Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature* **489**, 150-154. doi:10.1038/nature11306

Specht, E. A., Braselmann, E. and Palmer, A. E. (2017). A critical and comparative review of fluorescent tools for live-cell imaging. *Annu. Rev. Physiol.* **79**, 93-117. doi:10.1146/annurev-physiol-022516-034055

Sueda, R., Imayoshi, I., Harima, Y. and Kageyama, R. (2019). High Hes1 expression and resultant Ascl1 suppression regulate quiescent vs. active neural stem cells in the adult mouse brain. *Genes Dev.* **33**, 511-523. doi:10.1101/gad.323196.118

Svoboda, K. and Yasuda, R. (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. *Neuron* **50**, 823-839. doi:10.1016/j.neuron.2006.05.019

Swayne, L. A. and Wicki-Stordeur, L. (2012). Ion channels in postnatal neurogenesis: potential targets for brain repair. *Channels (Austin)* **6**, 69-74. doi:10.4161/chan.19721

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., Garcia-Verdugo, J. M. and Doetsch, F. (2008). A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* **3**, 279-288. doi:10.1016/j.stem.2008.07.025

Than-Trong, E., Kiani, B., Dray, N., Ortica, S., Simons, B., Rulands, S., Alunni, A. and Bally-Cuif, L. (2020). Lineage hierarchies and stochasticity ensure the long-term maintenance of adult neural stem cells. *Sci. Adv.* **6**, eaaz5424. doi:10.1126/sciadv.aaz5424

Tong, C. K., Chen, J., Cebrián-Silla, A., Mirzadeh, Z., Obernier, K., Guinto, C. D., Tecott, L. H., García-Verdugo, J. M., Kriegstein, A. and Alvarez-Buylla, A. (2014). Axonal control of the adult neural stem cell niche. *Cell Stem Cell* **14**, 500-511. doi:10.1016/j.stem.2014.01.014

Tong, C. K., Fuentealba, L. C., Shah, J. K., Lindquist, R. A., Ihrie, R. A., Guinto, C. D., Rodas-Rodriguez, J. L. and Alvarez-Buylla, A. (2015). A Dorsal SHH-dependent domain in the V-SVZ produces large numbers of oligodendroglial lineage cells in the postnatal brain. *Stem Cell Rep.* **5**, 461-470. doi:10.1016/j.stemcr.2015.08.013

Urbán, N., van den Berg, D. L. C., Forget, A., Andersen, J., Demmers, J. A. A., Hunt, C., Ayrault, O. and Guillemot, F. (2016). Return to quiescence of mouse neural stem cells by degradation of a proactivation protein. *Science* **353**, 292-295. doi:10.1126/science.aaf4802

Urbán, N., Blomfield, I. M. and Guillemot, F. (2019). Quiescence of adult mammalian neural stem cells: a highly regulated rest. *Neuron* **104**, 834-848. doi:10.1016/j.neuron.2019.09.026

Wang, J., Shen, J., Kirschen, G. W., Gu, Y., Jessberger, S. and Ge, S. (2019). Lateral dispersion is required for circuit integration of newly generated dentate granule cells. *Nat. Commun.* **10**, 3324. doi:10.1038/s41467-019-11206-9

Yasuda, T. and Adams, D. J. (2010). Physiological roles of ion channels in adult neural stem cells and their progeny. *J. Neurochem.* **114**, 946-959. doi:10.1111/j.1471-4159.2010.06822.x

Yeh, C.-Y., Asrican, B., Moss, J., Quintanilla, L. J., He, T., Mao, X., Cassé, F., Gebara, E., Bao, H., Lu, W. et al. (2018). Mossy cells control adult neural stem cell quiescence and maintenance through a dynamic balance between direct and indirect pathways. *Neuron* **99**, 493-510.e494. doi:10.1016/j.neuron.2018.07.010

Yeo, S.-Y., Kim, M., Kim, H.-S., Huh, T.-L. and Chitnis, A. B. (2007). Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos. *Dev. Biol.* **301**, 555-567. doi:10.1016/j.ydbio.2006.10.020

Zhang, K. and Cui, B. (2015). Optogenetic control of intracellular signaling pathways. *Trends Biotechnol.* **33**, 92-100. doi:10.1016/j.tibtech.2014.11.007

Zong, W., Wu, R., Li, M., Hu, Y., Li, Y., Li, J., Rong, H., Wu, H., Xu, Y., Lu, Y. et al. (2017). Fast high-resolution miniature two-photon microscopy for brain imaging in freely behaving mice. *Nat. Methods* **14**, 713-719. doi:10.1038/nmeth.4305