

REVIEW

Adding a spatial dimension to postnatal ventricular-subventricular zone neurogenesis

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ABSTRACT

Neurogenesis does not stop abruptly at birth, but persists in specific brain regions throughout life. The neural stem cells (NSCs) located in the largest germinal region of the forebrain, the ventricular-subventricular zone (V-SVZ), replenish olfactory neurons throughout life. However, V-SVZ NSCs are heterogeneous: they have different embryonic origins and give rise to distinct neuronal subtypes depending on their location. In this Review, we discuss how this spatial heterogeneity arises, how it affects NSC biology, and why its consideration in future studies is crucial for understanding general principles guiding NSC self-renewal, differentiation and specification.

KEY WORDS: Neurogenesis, Patterning, Specification, Subventricular zone, Transcription factors

Introduction

Although most regions of the postnatal central nervous system have lost their capacity to produce new neurons, neurogenesis persists in two main regions of the forebrain, namely the subgranular zone of the dentate gyrus in the hippocampal formation and the ventricular-subventricular zone (V-SVZ, also referred to as the subventricular zone or subependymal zone) located around fluid-filled cavities termed lateral ventricles (LVs) (Mirzadeh et al., 2008). These two regions contain a high density of stem and progenitor cells and are therefore defined as germinal regions. The largest of these two germinal regions is the V-SVZ (Fig. 1; supplementary material Movie 1). In young adult rodents, thousands of neuroblasts are generated in the V-SVZ every day. They migrate via the rostral migratory stream (RMS) to the olfactory bulb (OB), where they terminally differentiate into subpopulations of GABAergic interneurons: granule cells (GCs), which are located deep in the OB, and periglomerular cells (PGCs), which populate the superficial olfactory layers (Lois and Alvarez-Buylla, 1994; Lledo et al., 2008) (Fig. 1). In addition, a small population of juxtglomerular glutamatergic neurons continues to be produced during the first postnatal months (Brill et al., 2009; Winpenny et al., 2011).

The postnatal germinal V-SVZ exhibits a three-dimensional (3D) organization at different scales. At the macroscopic level, the V-SVZ exhibits a highly distinctive spatial organization: distinct V-SVZ microdomains generate defined neuronal subtypes, the diversity of which has only begun to be unveiled (Weinandy et al., 2011). At the cellular level, the neurogenic niche is a highly specialized cellular compartment that, with its particular 3D architecture, allows neural stem cells (NSCs) to survive, proliferate and generate new neurons.

In this Review, we summarize the 3D properties of the V-SVZ, discuss the importance of integrating these properties when studying this germinal region and propose new avenues to address questions that remain unanswered. Finally, we detail the challenges that remain to fully integrate spatial information in the study of postnatal neurogenesis using current databases.

Understanding V-SVZ structure: adding volume to the mix

In the mouse forebrain, the V-SVZ is located around a spatially complex ventricular system: a network of cavities composed of two LVs (one in each hemisphere) that join caudally to form the third ventricle where the cerebrospinal fluid (CSF) circulates. Serial sectioning and 3D rendering are necessary to appreciate its complexity (Box 1; supplementary material Movie 2). The shape of the LV changes considerably along the anteroposterior axis. Coronal sections reveal a narrow, crescent-shaped fissure in the rostral forebrain, which widens into its characteristic triangular shape at the mediostriatal level and narrows again in the temporal horns (Box 1A). The LVs of opposite hemispheres join into a third ventricle at the fimbrial level. The third ventricle is composed of a dorsal cavity and a narrow ventral cavity that runs into the hypothalamic area. A specialized monolayer of ciliated ependymal cells lines the walls of these spatially complex ducts.

The CSF is produced in the choroid plexi, which are predominantly found in the mediadorsal and caudal aspects of the LV; from there, it first flows rostrally along the dorsal wall of the LV (Sawamoto et al., 2006), being then conveyed into the third ventricle (both ventral and dorsal) to reach the caudal fourth ventricle via the aqueduct of Sylvius (Fig. 2). This macro-scale flow of CSF, which has been mainly studied in humans, is believed to be generated by our breathing and by the pulsations of the choroid plexi and ventricles, while it is fine-tuned at the level of the ventricular walls by the rhythmic beating of ependymal cilia (Siyahhan et al., 2014; Dreha-Kulaczewski et al., 2015).

The neurogenic V-SVZ, which is the main topic of this Review, defines the highly heterogeneous cell layer that surrounds the open LV. However, it must be noted that populations of progenitors are also found in the RMS and subcallosal zone, which respectively represent rostral and caudal extensions of the V-SVZ that are no longer associated with an open ventricle (Gritti et al., 2002; Seri et al., 2006; Kim et al., 2011), as well as in the ventral third ventricle (Robins et al., 2013).

The density and fate of NSCs are heterogeneous across the ventricular surface

Even though all the NSCs contained in the V-SVZ are geared towards the life-long replenishment of olfactory neurons, NSCs across the ventricular system differ in many aspects. For example, although NSCs are sessile, their number and density vary markedly along the dorsoventral and rostrocaudal axes, with the presence of neurogenic hotspots (Mirzadeh et al., 2008; Azim

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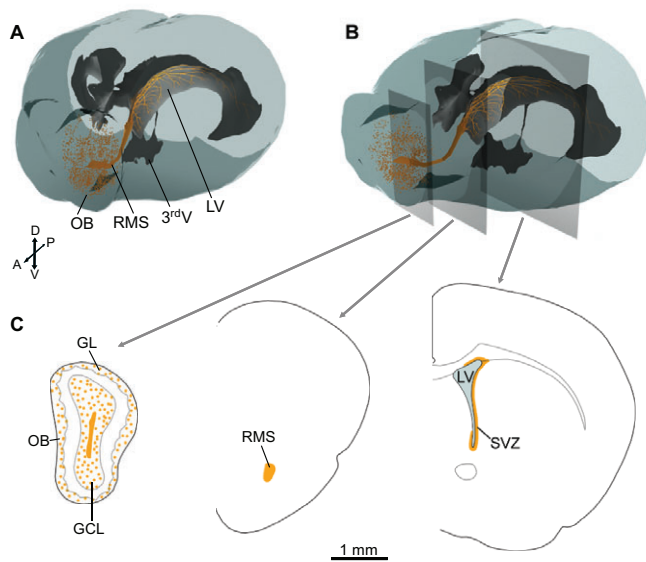


Fig. 1. The V-SVZ replenishes olfactory neurons throughout life.

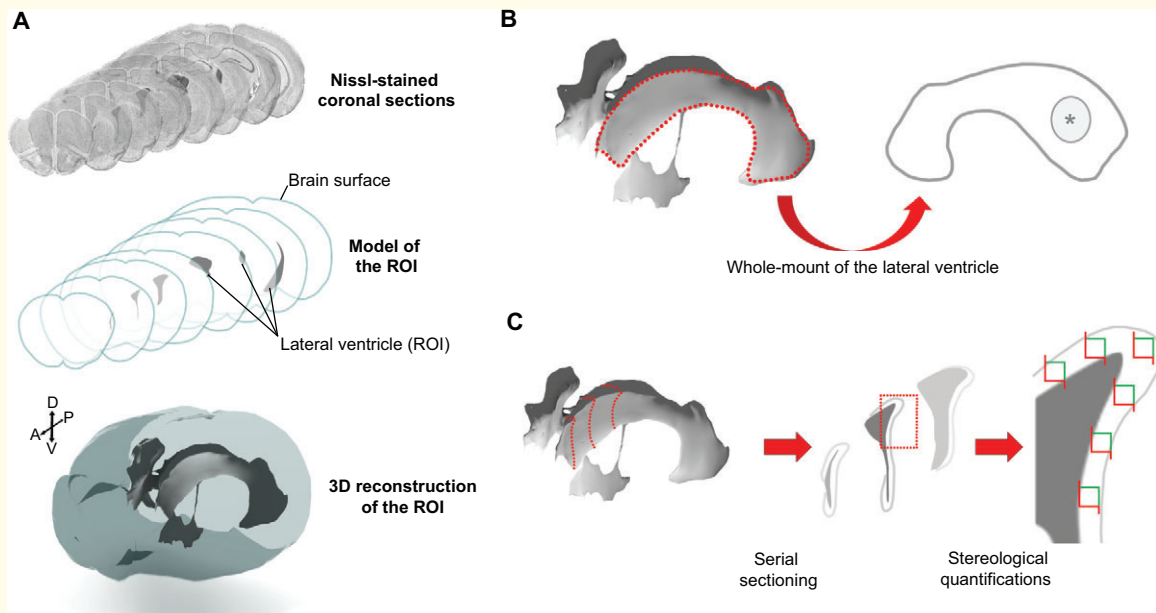
Schematic depiction of the neurogenic process along the entire extent of the ventricular system of the neonatal/adult rodent forebrain. (A) 3D representation of V-SVZ neurogenesis. Neural stem cells (NSCs) are located in the walls of the lateral ventricle (LV) and generate an irregular network of migratory neuroblasts (orange) that converge into the rostral migratory stream (RMS). Upon arrival at the olfactory bulb (OB), the neuroblasts migrate radially to reach their final destination and mature into GABAergic neurons (granule cells and periglomerular neurons) and a minor population of glutamatergic neurons in the granule cell layer (GCL) or the glomerular layer (GL). (B) Sectioning planes through the OB, RMS and LV show the distribution of newly generated neuroblasts in these three forebrain compartments. 3rdV, third ventricle; SVZ, subventricular zone. See also supplementary material Movie 1.

et al., 2012c; Falcao et al., 2012) (Fig. 3A). In addition, NSCs reside in all three walls of the V-SVZ (dorsal, medial and lateral; Fig. 3B), but are biased to generate specific subtypes of neurons depending on their exact location. The first evidence for such regional heterogeneity was revealed by retroviral lineage-tracing studies, which showed that the position of NSCs along the rostrocaudal axis had major consequences for the location, and thus the subtype, of newborn neurons in the OB. The V-SVZ itself is therefore regionalized along the anteroposterior axis: rostrally located NSCs give rise to GCs that are more superficial in the GC layer than those generated by more caudally located NSCs (Kelsch et al., 2007). Furthermore, the V-SVZ is also patterned along the dorsoventral axis: selective electroporation of postnatal V-SVZ subregions (Fernandez et al., 2011; de Chevigny et al., 2012) showed that NSCs from the medial wall are biased to generate PGCs (>85%), whereas those from the lateral wall primarily adopt a GC fate (>90%). In an intermediate fashion, NSCs located in the dorsal wall generate PGCs and GCs in a 1 to 2 proportion (Fernandez et al., 2011). Thus, a first general principle of the spatial coding of OB neuron diversity applies as follows: a rostral and medial/dorsal localization primes NSCs to generate neurons that populate more superficial layers of the OB, whereas a caudal and lateral origin correlates with deeper fates (Fig. 3C). This statement is in agreement with the regional changes seen in the density of the different progenitor pools. Thus, at the population level, whereas medial and dorsal progenitor pools are concentrated in the rostralmost V-SVZ and accordingly generate a greater proportion of superficial neurons, the lateral pool occupies a much larger area that extends caudally and therefore generates a greater proportion of deeper neurons (see Fig. 3A,C).

Embryonic patterning dictates V-SVZ regionalization

Fate-mapping experiments in which Cre recombinase expression was driven by promoters of pallial and subpallial transcription factors (TFs) have shown that each of the walls of the postnatal LV derives from its earlier embryonic counterparts (Willaime-Morawek et al., 2006; Kohwi et al., 2007; Ventura and Goldman, 2007; Young et al., 2007; Willaime-Morawek and van der Kooy, 2008). The septum, the medial ganglionic eminence, the lateral ganglionic eminence and the embryonic cortex generate NSCs that populate the medial, ventral, lateral and dorsal aspects of the adult V-SVZ, respectively (Fig. 4A). Between these regions, mixing of NSCs occurs, defining zones of transition as observed earlier during development (Long et al., 2007). For example, NSCs of pallial origin were shown to migrate into the lateral V-SVZ around birth, where they acquire the expression of ventral markers (Willaime-Morawek et al., 2006). These migratory events are likely to explain the discrepancies that exist between different studies. For example, although the lateral V-SVZ derives from the lateral ganglionic eminence, which produces no interneurons expressing calretinin [CR; also known as calbindin 2 (Calb2)], some CR⁺ neurons are reported to originate from this wall at postnatal time points (De Marchis et al., 2007; Kohwi et al., 2007). In agreement with their developmental origin, pallial markers [Emx1, Pax6, Tbr2 (Eomes), Tbr1, Ngn2 (Neurog2)] are expressed in the dorsalmost regions of the neonatal and adult LV, whereas subpallial [Dlx1/2/5, Gsx1/2 (Gsh1/2), Mash1 (Ascl1), Nkx2.1, Nkx6.2] and septal (Zic1/3) markers are expressed in the lateral and medial walls of the LV, respectively (Fig. 4B) (Kohwi et al., 2007; Winpenny et al., 2011; Azim et al., 2012c; Lopez-Juarez et al., 2013; Merkle et al., 2014). These probably represent a mere fraction of the TFs that are regionally expressed in the postnatal V-SVZ. Indeed, a strikingly larger number of region-specific TFs can be observed earlier during development (Flames et al., 2007), suggesting that a greater number of developmental TFs might persist at postnatal ages. The expression pattern of these TFs can also vary significantly along the rostrocaudal extent of the V-SVZ, a characteristic recently described for Zic1/3, Nkx6.2 and Nkx2.1 (Merkle et al., 2014) (Fig. 4B).

Additionally, accumulating evidence underlines a relationship between the embryonic origin of the NSCs, their localization within the SVZ and the neuronal subtypes that they produce (Fig. 4C,D). In the early postnatal V-SVZ, NSCs still harbor a radial glial cell (RGC) morphology, including a basal process that extends to the pial surface of the brain. Viral transduction through this basal process targets NSCs in smaller V-SVZ areas, allowing investigators to describe postnatal V-SVZ regionalization in exquisite detail (Merkle et al., 2007, 2014). Results obtained by viral infection, genetic fate-mapping or targeted electroporation experiments revealed that CR⁺ PGCs are preferentially generated in the medial V-SVZ (Merkle et al., 2007, 2014; Fernandez et al., 2011), whereas superficial GCs, tyrosine hydroxylase (TH)-expressing PGCs and a small number of glutamatergic juxtglomerular cells are preferentially generated in the dorsal V-SVZ (Merkle et al., 2007; Young et al., 2007; Brill et al., 2009; Fernandez et al., 2011; de Chevigny et al., 2012), and deep GCs as well as CB⁺ [expressing calbindin; also known as calbindin 1 (Calb1)] PGCs in the lateral aspect of the V-SVZ (Merkle et al., 2007; Young et al., 2007; Fernandez et al., 2011). A closer analysis of NSCs located in the most anterior ventral regions of the LV revealed the existence of additional microdomains. There, the rostroventral region of the medial wall contributes to the generation of two subtypes of superficial GCs, namely the deep branching GCs and perimitral GCs. A lateral region of the very

Box 1. Methodological aspects of 3D analysis of the V-SVZ

(A) Serial sectioning (here Nissl-stained coronal sections) allows the complexity of the ventricular system to be visualized in one single series. The anatomical features of the region of interest (ROI) – here the brain surface (light blue) and the LV (dark gray) – can be traced over individual sections to generate a 3D mesh onto which textures, such as the brain surface (blue, semi-transparent) and ventricular system (dark gray), can be applied using appropriate modeling software (Blender 2.8). See also supplementary material Movie 2. (B) Whole-mount preparations (whole-mount microdissection and staining) have been used to study the cytoarchitecture of the SVZ [e.g. migratory neuroblast chains (Doetsch and Alvarez-Buylla, 1996) or precise cellular organization (Spassky et al., 2005; Sawamoto et al., 2006; Mirzadeh et al., 2008)]. However, this preparation does not enable all walls of the ventricle to be analyzed concomitantly, and parts of the walls (e.g. the site of adhesion, indicated by the asterisk) can be lost or damaged during the microdissection procedure (Mirzadeh et al., 2010). (C) Stereological sampling based on the systematic quantification of cells, with or without the aid of the dissector (red/green probes) depending on their density, is the classic method for 3D estimation of cell numbers in tissues, with minimal workload (Schmitz and Hof, 2005). The frequency of counts needed for extrapolating total cell numbers greatly depends on the overall cell density and the cell distribution. Thus, when cells are sparse and/or distributed in clusters, such as in the SVZ, a greater number of serial sections must be quantified in order to minimize variance.

ventral tip of the lateral wall generates shrub GCs and satellite cells of the plexiform layer (Merkle et al., 2014).

By examining the embryonic origin of NSCs, their perinatal migration and TF expression, recent studies have begun to unravel an intrinsic code that generates OB neuron diversity. This is best illustrated by TH⁺ PGCs, which are generated from NSCs of both pallial (*Emx1*) and subpallial (*Gsx2*) origin (Young et al., 2007), relying on the expression of *Pax6*, *Dlx2* and *Meis2* (Brill et al., 2008; Agoston et al., 2014). These experiments further suggest that CB⁺ PGCs derive from *Dlx2*⁺, *Pax6*⁻ progenitors, whereas the acquisition of a CR⁺ periglomerular phenotype is observed when both *Dlx2* and *Pax6* are silenced (Brill et al., 2008). Other TFs have been suggested to be regionally expressed in the V-SVZ, including *Er81* (*Etv1*), *Nurr1* (*Nr4a2*), *Sall3*, *Zic1/3* and *Sp8*, and might be associated with the acquisition of defined neuronal fates, although this remains to be directly investigated in the postnatal forebrain (Diaz-Guerra et al., 2013).

The V-SVZ forms a complex 3D environment at the cellular level

During prenatal life, RGCs, which are embryonic NSCs, reside in the immediate vicinity of the ventricular system and their cellular processes contact both the ventricular (apical) and the pial (basal) surface of the neuroepithelium (basal) (Fig. 5A). After birth, this ventricular zone undergoes major cytoarchitectural modifications whereby RGCs adopt an astrocytic morphology and retract their

basal process from the pial surface to contact blood vessels (Fig. 5B). A fraction of these cells give rise to type B1 astrocytes, whereas the rest acquire an ependymal or glial fate (Tramontin et al., 2003; Merkle et al., 2004). During the first postnatal weeks, the ependymal cells generated by RGCs earlier during development [mostly between embryonic day (E) 14 and E16] gradually mature following a caudorostral and a ventrodorsal gradient to form a thin epithelium lining the ventricle, separating the V-SVZ from the CSF (Spassky et al., 2005; Mirzadeh et al., 2008).

Although variations exist across species, in particular in humans in which a hypocellular gap forms between the ependymal cell and a putative NSC layer (Sanai et al., 2004; Quinones-Hinojosa et al., 2006), some basic principles of the V-SVZ cellular organization can be determined and a specific nomenclature is now widely used (Doetsch et al., 1997). This has been extensively reviewed elsewhere (Ihrle and Alvarez-Buylla, 2011; Mamber et al., 2013) and so we provide just a brief overview.

The first layer of cells contacting the ventricular surface is mostly composed of multiciliated ependymal cells (called type E cells) with some intercalated type B1 cells. Indeed, the analysis of whole-mount preparations of the LV walls (see Box 1B) revealed that apical processes from multiple B1 astrocytes merge together and are radially surrounded by ependymal cells, forming a characteristic pinwheel structure. These apical processes resemble the end-feet of RGCs and possess single cilia that enable adult NSCs to remain in

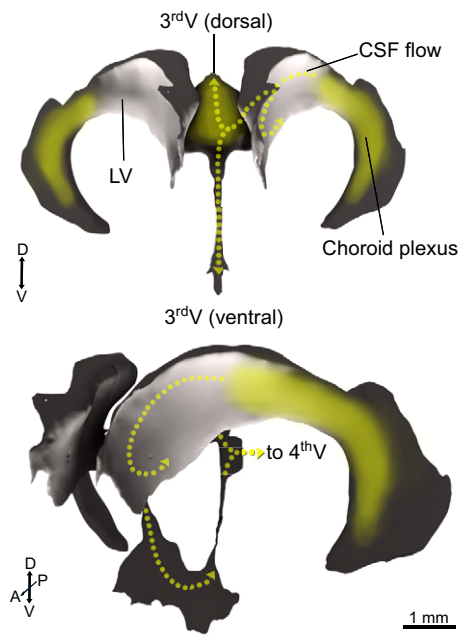


Fig. 2. The spatially complex ventricular system. The cerebrospinal fluid (CSF), which is the source of many of the essential factors regulating NSC maintenance and proliferation, is produced in the choroid plexus (yellow), an extraversion of ependymal/vascular tissue found in the caudal LV and the dorsal third ventricle. Rhythmic pulsations of the choroid plexi and the rhythmic movement of ependymal cilia (not shown) push the newly produced CSF rostrally, before being conveyed into the third ventricle and reaching the caudal central nervous system (dotted arrows). 4^{th} V, fourth ventricle.

contact with the CSF and sense potential circulating cues (Mirzadeh et al., 2008). B1 astrocytes represent the bona fide NSCs of the mature SVZ (Doetsch et al., 1999) and can be further classified into quiescent (B1) and active (B1a) (Fig. 5B) based on their expression of the RGC markers BLBP (Fabp7), nestin and EGF receptor, which are absent during the quiescent phase and expressed following activation (Codega et al., 2014; Giachino et al., 2014). Clonal analysis suggests that active NSCs generate several rounds of actively proliferating type C cells [also referred to as cycling progenitors or transit amplifying progenitors (TAPs)] before becoming exhausted after a few weeks (Calzolari et al., 2015), while other NSCs become active. Type C cells can be seen as small clusters associated with streams of elongated neuroblasts (type A cells) that undergo chain migration towards the OB (Doetsch et al., 1997). B2 astrocytes (niche astrocytes) exist in close proximity to the type A cells, on the striatal side of the SVZ, and have been proposed to isolate the neurogenic niche from surrounding mature tissue. Lastly, on the striatal end of the V-SVZ, the basal processes of type B1 cells contact blood vessels at sites that lack unsheathing astrocytes or pericytes, facilitating direct access to circulating trophic factors (Tavazoie et al., 2008).

This intricate constant cross-talk between the vasculature and the SVZ has been further highlighted by coupling whole-mount preparation with automated 3D analysis of confocal images. Immediately underneath the ependymal cell layer the blood vessels form a dense plexus that is spatially associated with proliferating NSCs and type C progenitors (Shen et al., 2008; Tavazoie et al., 2008). An important component of blood vessels is the associated extracellular matrix, which forms extravascular structures named fractones. These fractones engulf astrocytic processes, as well as ependymal, microglial and progenitor cells, underlying their intimate

association with the neurogenic niche and adding to the complexity of the NSC environment (Mercier et al., 2002; Shen et al., 2008). This specialized cytoarchitecture results in a unique microenvironment in which multiple signals act to maintain germinal activity in the adult brain (Ihrie and Alvarez-Buylla, 2011).

Altogether, these studies demonstrate the complex 3D organization of the V-SVZ, which can be appreciated at the cellular (i.e. the neurogenic niche) as well as the regional (i.e. a V-SVZ patterning established during development and preserved postnatally) level. Although the organization of the neurogenic niche has long been studied, the description of V-SVZ patterning is more recent and remains to be fully explored. The small size of some of the recently described V-SVZ microdomains – 100-300 μm along the dorsoventral axis and 400-800 μm along the rostrocaudal axis (Merkle et al., 2014) – suggests that further complexity exists and will require the use of systematic, near-clonal, lineage fate-mapping methods to be fully unraveled. By providing spatial maps of progenitor pools that give rise to specific subtypes of OB neurons, this research allows the classification of OB neurons to be appreciated with an ontogenic perspective. Such a classification is complementary to conventional classifications that are currently based on the layers in which neuronal cell bodies lie, and might help in categorizing the rapidly increasing diversity of OB neuron subtypes (Nagayama et al., 2014). Documenting these progenitor pools will further facilitate the identification of regionally expressed TFs or signaling pathways, and allow their roles during lineage specification to be studied.

Why examine the postnatal V-SVZ in 3D?

The concepts discussed above have profound implications for neurogenesis research. Heterogeneity in density and distribution across the ventricular surface has to be taken into consideration when quantifying NSCs and their progeny. Furthermore, sampling the heterogeneity of NSCs might provide insights into potential region-specific mechanisms or cues that guide NSC self-renewal or differentiation as well as their specification into defined cell types. The discovery of these cues might offer new methods for manipulating V-SVZ NSCs in health or disease.

Taking regionalization into account to improve cell type-specific quantifications in the V-SVZ

Accurate quantification of NSCs and their progeny is essential to detect changes in their numbers, proliferation or specification, which may occur following experimental manipulations. Several attempts have been made to quantify these cell types in the postnatal V-SVZ (Encinas and Enikolopov, 2008; Azim et al., 2012c; Falcao et al., 2012; Ponti et al., 2013). However, owing to its thinness, irregular shape and, most importantly, the dynamic nature and heterogeneous distribution of NSCs and their progeny, the V-SVZ is a complex structure, and performing such quantitative studies is challenging. Whole-mount preparations represent the method of choice when dynamic processes are being investigated (Sawamoto et al., 2006) or to reduce errors in analysis due to cell motility, which can bias the number of cells present in a restricted area at a given time (Box 1B) (Ponti et al., 2013). However, it should be noted that this preparation does not enable all walls of the ventricle to be analyzed simultaneously, and that parts of the walls can be lost or damaged during the microdissection procedure (Mirzadeh et al., 2010). Alternative methods consist of serial sectioning of the region of interest (ROI) to perform stereological estimates of cell numbers (Box 1C) (Schmitz and Hof, 2005). Coronal sections of the forebrain are often preferred, as they allow optimal visualization and stereological assessment of all the ventricular walls at once. Precise

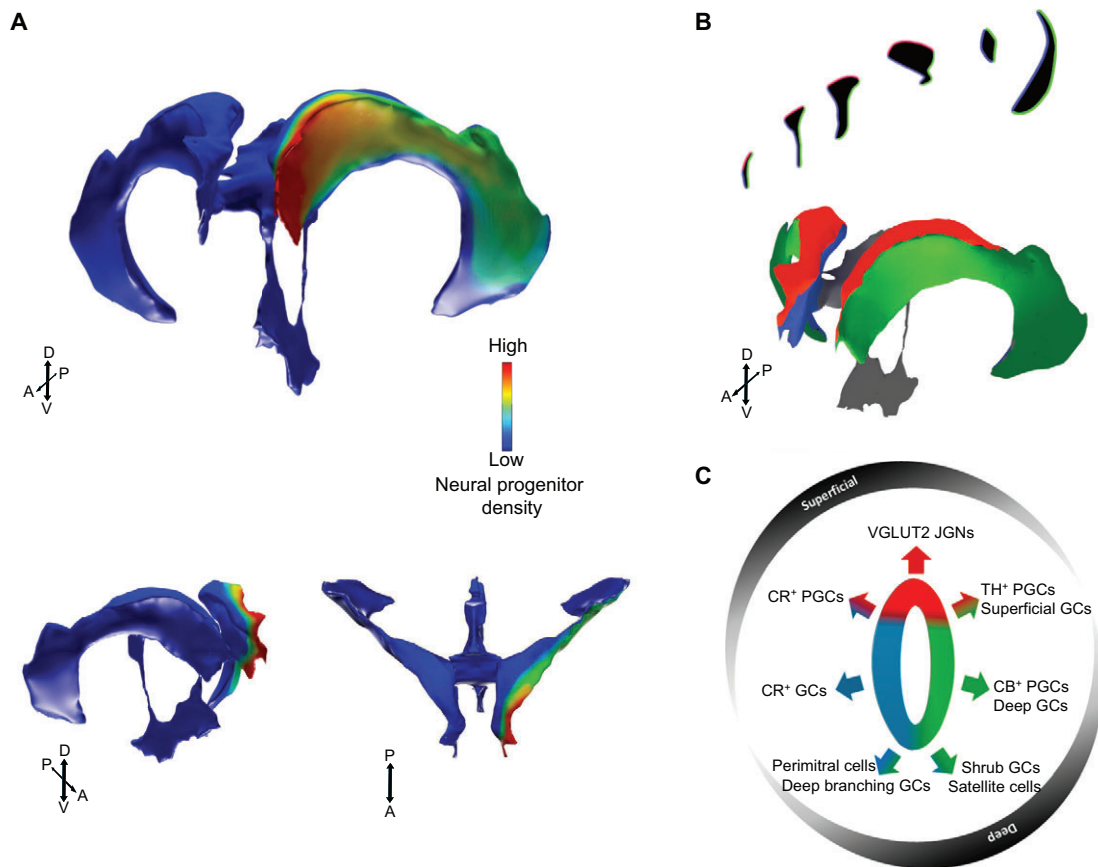


Fig. 3. NSC fate and density are heterogeneous across the ventricular surface. (A) The density of NSCs, and therefore germinal activity, varies across the ventricular surface. The top scheme is a heat map of neural progenitor density in the medial, dorsal and lateral walls of young adult mouse [postnatal day (P) 60] LVs. The bottom schemes are 3D representations of the LVs in different orientations and highlight that only portions of the dorsal, lateral and medial walls (mostly rostral regions) remain germinal. For clarity, only the left ventricle is colored. (B) The three walls of the LVs can be individually identified: red, dorsal; green, lateral; blue, medial. (C) The spatial location of NSCs dictates neuronal subtype production, as summarized in a schematic representation of a coronal section of the SVZ. In general, a dorsal or medial localization results in newly born neurons acquiring a more superficial fate in the OB, whereas those neurons originating from the lateral wall (where neurogenic activity is also observed in considerably more caudal areas) adopt a deeper location. CR, calretinin; CB, calbindin; TH, tyrosine hydroxylase; VGLUT2, vesicular glutamate transporter 2 (Slc17a6); GCs, granule cells; PGCs, periglomerular cells; JGNs, juxtglomerular neurons.

quantification of the number of NSCs and their progeny, as well as their behavior, are influenced by two main factors: (1) the biological variability between individual animals; and (2) the methodological variability, reflecting the bias introduced by the researcher's method of quantification.

The biological variability observed between individual animals is an important factor that has to be considered when comparing absolute numbers of cells in the brain across different studies. Despite being extremely time consuming, systematic quantifications of several individuals should not be forgone and the numbers obtained should be considered with caution. The methodological biological variability is particularly relevant when quantifying the V-SVZ cellular content, since the precision of cell number estimations is highly sensitive to regional fluctuations in cell density within a single animal, as well as to cell motility, a characteristic of germinal regions such as the V-SVZ. As mentioned above, the number and the density of NSCs exhibit significant variations along the dorsoventral and rostrocaudal axis, with the presence of neurogenic hotspots (Mirzadeh et al., 2008; Azim et al., 2012c; Falcao et al., 2012) (Fig. 2B). Stereological estimates of active NSCs in the young adult mouse V-SVZ range between 600 and 750 cells (Azim et al., 2012c), a number that is in line with recent quantifications performed using V-SVZ whole-mount preparations (Ponti et al., 2013). Quantification of other proliferative cell populations, i.e. TAPS and neuroblasts, that are respectively sessile

and highly motile and represent >90% of all cycling cells in the V-SVZ, is subject to more caution. The presence of highly motile cells introduces a bias in the distribution of cycling cells enriched in the rostralmost regions and the dorsolateral corner of the LV in rodents, where neuroblasts converge before advancing to the RMS to begin their rostral-bound migration (Azim et al., 2012c; Falcao et al., 2012). These studies have consistently shown a drop of ~50% in the number of proliferating cells between the anterior and mediocaudal V-SVZ.

Cell cycle parameters also vary within cell types (Ponti et al., 2013) and/or cell location (Falcao et al., 2012). TAPs are heterogeneous in terms of the duration of their cell cycle (18-25 h), which tends to lengthen after each cell division (Ponti et al., 2013). A significant proportion of migrating neuroblasts also cycle, with a study reporting a significantly longer cell cycle (~25%) for ventrolateral neuroblasts as compared with those in the dorsolateral V-SVZ in adult rats (Falcao et al., 2012). These variations influence the precision of stereological appraisals of the number of proliferating cells in the V-SVZ, which range between 10,000 and 17,000 cells depending on the detection method used, the region sampled (V-SVZ only or including the RMS) and the age of the animals (Lois and Alvarez-Buylla, 1994; Encinas and Enikolopov, 2008; Azim et al., 2012c). Finally, the size of the progenitor subpopulations, which reside in different SVZ microdomains and express distinct TFs, also varies considerably.

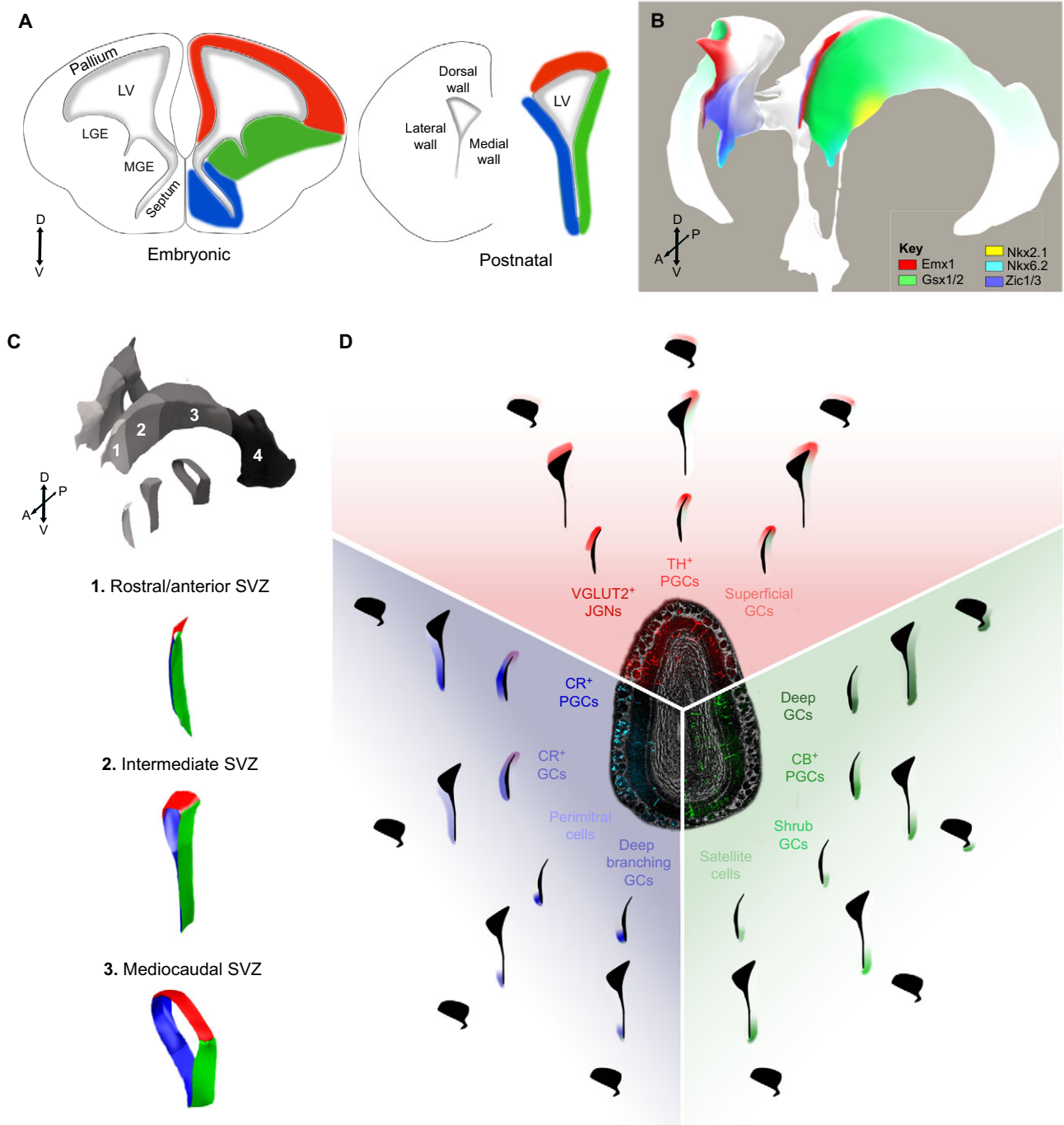


Fig. 4. V-SVZ heterogeneity stems from its embryonic origin. (A) The V-SVZ walls (right) derive from their embryonic counterparts (left): the pallium, lateral and medial ganglionic eminences (LGE and MGE) and septum. Color-coding: red, pallium/dorsal wall; blue, septal/medial wall; green, ganglionic eminences/lateral wall. (B) Fate-mapping studies have shown that fate specification programs are conserved from development to adulthood in the NSCs of the different walls. Dorsal NSCs derive from Pax6⁺, Emx1/2⁺ pallial progenitors (red domain). Medial wall NSCs derive from ventral septal progenitors expressing Zic1/3 and Nkx6.2 (blue and cyan domains). Lateral wall NSCs, the most abundant population in the adult, derive from ventral subpallial Gsx2⁺, Nkx2.1⁺ progenitors (green and yellow domains). (C) A volumetric 3D reconstruction of the LVs is shown (top). Four anteroposterior subdivisions are illustrated by different shades of gray. Transverse sections of subdivisions 1, 2 and 3 with color-coded walls (red, dorsal; green, lateral; blue, medial) are shown beneath. Overall, these three regions are the source of the vast majority of OB neurons produced after birth. (D) The color-coding in A-C is conserved here to illustrate the SVZ microdomains of origin of specific OB neuron subtypes. The colored shading around each LV delineates known TF gradients (Emx1, red; Gsx1/2, green; Zic1/3, blue), as illustrated in B, with the color intensity distinguishing densities of TF/marker expression. The DAPI-counterstained OB in the center shows neurons in their final destination in specific OB layers following fate mapping of their region-specific NSC pools (modified from Fernandez et al., 2011). Neurons are colored according to their origin: red, green and blue define neurons that have originated from NSCs of the dorsal, ventral and medial microdomains of the SVZ, respectively. Labels as in Fig. 3.

For example, in the adult V-SVZ, the number of progenitors expressing Dlx2 is 40-fold greater than dorsally located Tbr2⁺ progenitors (Azim et al., 2012c). These marked differences directly

impact the precision of cell number estimates, imposing a higher sampling rate for stereological quantifications of rare progenitor populations (Azim et al., 2012c).

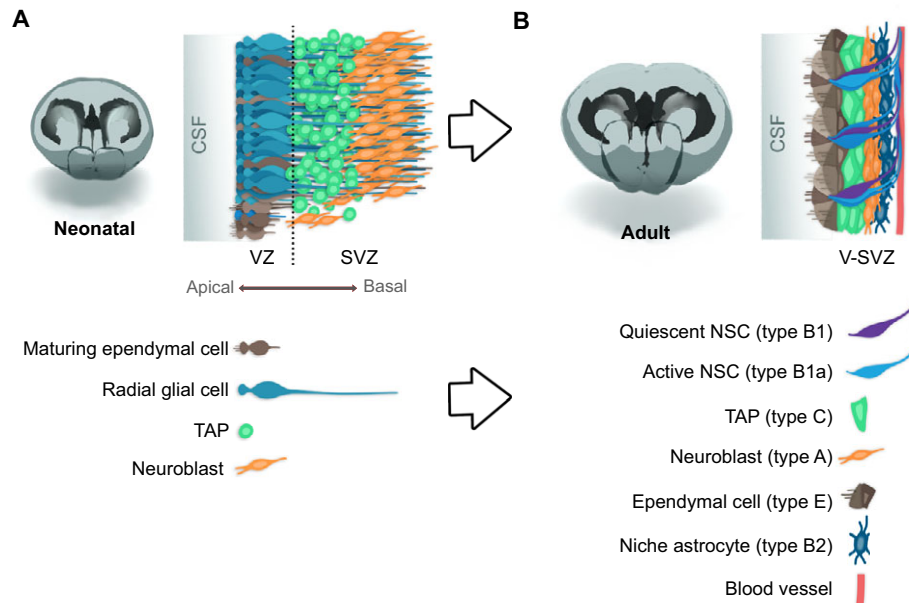


Fig. 5. Cellular organization of the postnatal and adult V-SVZ. (A) In the first days after birth, the lateral wall is layered in a ventricular zone (VZ), which is composed of three layers of radial glial cells (RGCs), and a subventricular zone (SVZ), which is populated by proliferating transit amplifying progenitors (TAPs) and migrating neuroblasts. In the lateral wall, RGCs begin to retract their long basal processes following a ventrodorsal gradient. Some RGCs begin to differentiate into ciliated ependymal cells. (B) Following postnatal maturation, the V-SVZ becomes considerably thinner and clearly structured (from ~300 μm at birth to <100 μm in the adult mouse). Adult quiescent and actively proliferating NSCs contact the CSF with an apical process that is surrounded by ciliated ependymal cells arranged in stereotypical pinwheel structures, although the density and organization of these pinwheels vary throughout walls and rostrocaudal coordinates. TAPs and neuroblasts are also more tightly organized compared with the neonatal V-SVZ – respectively in clusters (green) and dorsally migrating chains (orange) in close proximity to the NSCs. On the parenchymal side of the V-SVZ, the NSC basal end-feet contact the blood vessels. Note that the pattern of the pinwheel structures is shown as being very regular for simplification purposes; it is actually more complex and irregular *in vivo*.

Spatial patterns in the V-SVZ provide insights into the mechanisms that control postnatal neurogenesis

The identification of non-random patterns in the cytoarchitecture of neurogenic niches has guided research to elucidate general mechanisms that regulate neurogenesis, allowing researchers to identify cues from the CSF (Sawamoto et al., 2006), ependymal cells (Lim et al., 2000; Gajera et al., 2010) and the vascular/extracellular matrix (Kerever et al., 2007; Shen et al., 2008; Ottone et al., 2014) that play important roles in maintaining V-SVZ neurogenesis.

Similarly, the description of regional differences in V-SVZ NSC specification has guided recent efforts to identify TFs that are regionally expressed in the postnatal V-SVZ (Merkle et al., 2014). Some of these TFs are direct gene targets of specific morphogens recently shown to be active in the postnatal V-SVZ, including sonic hedgehog (Shh), wingless (Wnt), bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs) and insulin growth factors (IGFs). In the adult V-SVZ, these morphogens are required for NSC maintenance (Zheng et al., 2004; Balordi and Fishell, 2007a,b; Han et al., 2008), proliferation (Kosaka et al., 2006; Adachi et al., 2007) and glial versus neuronal differentiation (Colak et al., 2008; Azim et al., 2012a; Marinaro et al., 2012).

In addition to their generic role in NSC proliferation and differentiation, these morphogens are likely to impact V-SVZ regionalization directly by influencing NSC specification. Indeed, important regional differences were recently described in the activity of Shh and canonical Wnt signaling pathways. For instance, in accordance with its ventralizing role during early forebrain development (Hoch et al., 2009), Shh signaling is mainly active in the ventral V-SVZ at postnatal ages, where it is associated with the generation of specific neuronal subtypes, namely CB^+ PGCs and, to a lesser extent, deep GCs (Ihrle et al., 2011)

(Fig. 6A–C). Similarly, canonical Wnt signaling is active exclusively in the dorsal V-SVZ and within dorsally derived lineages (Ortega et al., 2013; Azim et al., 2014b), in agreement with its described role in dorsalization and caudalization of the developing telencephalon (Lee et al., 2000; Muzio et al., 2005; Hoch et al., 2009) (Fig. 6A–C).

Several factors are likely to underlie the regional differences in morphogen activity observed in the postnatal V-SVZ. First, morphogen sources and modes of diffusion differ greatly from those at earlier developmental stages. During development, morphogens are expressed by signaling centers in and around the cortical neuroepithelium and diffuse in a tissue-wide pattern (Hoch et al., 2009). By contrast, after birth, morphogens are secreted in small amounts by discrete cell populations and show a more limited diffusion in the parenchyma. For example, in the adult brain, Shh is expressed by a subset of ventrally located neurons and is locally released by neuronal processes (Ihrle et al., 2011) or diffusely released in the ventricle but ‘sensed’ by the primary cilium of unique ventrally located subpopulations of NSCs (Tong et al., 2014). Likewise, oligodendrocyte progenitors that populate the corpus callosum are the main source of canonical Wnt ligands in the postnatal V-SVZ (Ortega et al., 2013).

Second, numerous patterning molecules are carried away from their source by the CSF (Thouvenot et al., 2006; Marques et al., 2011), creating signaling gradients (Fig. 2A) and thus affecting V-SVZ microdomains differently (Sawamoto et al., 2006).

Third, the expression of receptors of patterning molecules, their downstream intracellular machinery, as well as the accessibility of downstream target genes also differ significantly between spatially distinct regions, as demonstrated for FGF and IGF receptor transcripts (Azim et al., 2012a; Ziegler et al., 2012) and the Shh

signaling pathway (Tong et al., 2014). Differences in these receptors and downstream effectors are likely to underlie the reported regional variability in the capacity of morphogens to induce NSC respecification in response to an exogenous source of morphogens. For example, the NSCs of the lateral wall are not permissive to ectopic Wnt signaling (Azim et al., 2014a,b), contrasting with the capacity of Shh signaling to respecify dorsal NSCs towards the GABAergic lineage even in adulthood (Ihrie et al., 2011). Regional differences in the density and maturation of cell types are also likely to actively influence regional signaling. For example, ependymal cells, which are known to modulate BMP signaling and fate choice (Colak et al., 2008), are denser and mature more rapidly in the medial and dorsal V-SVZ (Spassky et al., 2005).

Finally, the combined action of these diverse signaling molecules on V-SVZ NSCs adds a supplementary layer of complexity to their involvement in coding V-SVZ regionalization. Some of these interactions are likely to be antagonistic in nature. For example, the infusion of exogenous BMP4 or EGF into the LV represses downstream Wnt signaling in the dorsal V-SVZ (Azim et al., 2014a). Other interactions are likely to be synergistic. For example, FGF2, which promotes NSC self-renewal, amplification of TAPs and, subsequently, OB neuron generation (Kuhn et al., 1997; Zheng et al., 2004; Azim et al., 2012a), cross-reacts with canonical Wnt signaling to inhibit GSK3 β and increase the nuclear translocation of β -catenin in the postnatal dorsal V-SVZ (Azim et al., 2014a).

Thus, identifying the signaling pathways that are active in the different regions of the postnatal V-SVZ and studying their integration and role during cell renewal and lineage specification represents an important step in developing innovative pharmacological approaches to manipulate germinal regions in health and disease.

Future challenges in 3D analysis in the postnatal subventricular zone

As discussed above, taking spatial information into account when studying the V-SVZ is of fundamental importance in the context of NSC biology, and goes well beyond illustration purposes. Experimentally, it allows researchers to establish appropriate sampling schemes to quantify cells, but also to measure and compare gene expression and signaling activity between different regions of the V-SVZ. Additionally, a better appreciation and appropriate annotation of V-SVZ subregions is crucial when searching datasets, including publicly accessible databases that allow the spatiotemporal expression pattern of genes and proteins to be queried (e.g. the Allen Brain Atlas, <http://www.brain-map.org/>). Adequate reporting of V-SVZ subregions on these publicly accessible platforms would further stimulate the formulation of broader questions and working hypotheses that can be experimentally addressed.

Defining the V-SVZ as an ROI when querying existing datasets

Germinal regions present unique challenges with regards to annotation in atlases owing to their dynamic nature. During postnatal stages, the anterior extremity of the V-SVZ is in continuity with the RMS, which forms around birth upon closure of the embryonic olfactory ventricles (Pencea and Luskin, 2003; Peretto et al., 2005). As a consequence, new neurons are also generated from progenitors located in the OB and RMS (Gritti et al., 2002; Golmohammadi et al., 2008; Giachino and Taylor, 2009). Similarly, the caudal V-SVZ boundaries are not well defined. Although it was previously thought that the neurogenic region of the LV was confined to the anterior V-SVZ, retroviral injections

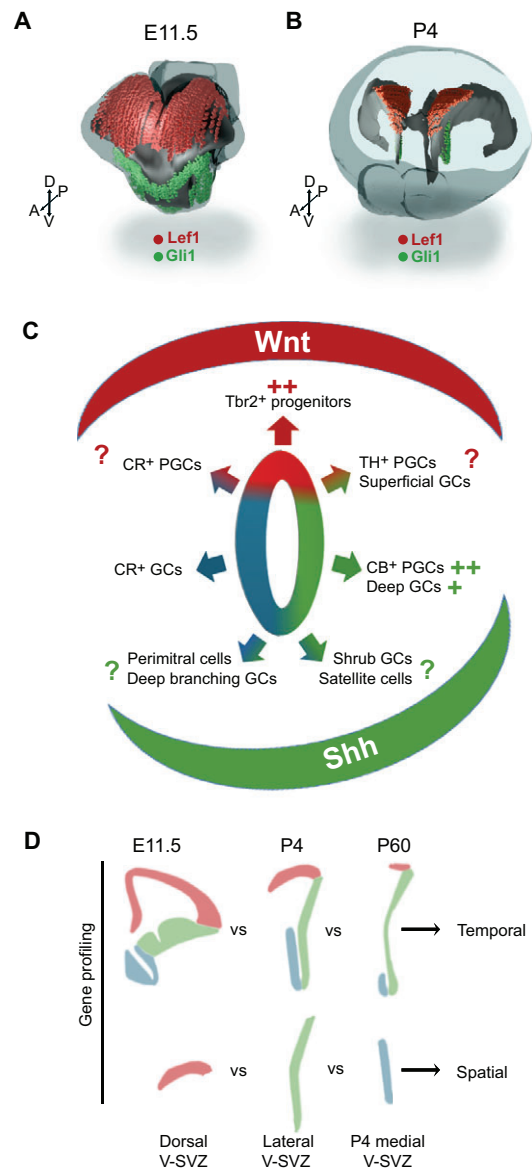


Fig. 6. The regionalization of signaling contributes to the heterogeneity of NSCs. (A,B) Volumetric reconstructions of E11.5 and P4 mouse forebrains depicting the brain surface (blue, semi-transparent) and ventricular system (dark gray). Note that most of the brain volume is occupied by the ventricular system at E11.5. The embryonic expression of specific signaling pathway downstream target genes (red, *Lef1* or *BatGal* for canonical Wnt signaling; green, *Gli1* for Shh signaling) persists after birth into postnatal life. Images used for generating reconstructions were obtained from the Allen Brain Atlas or collected in the authors' laboratory (*BatGal* reporter mouse; Azim et al., 2014a,b). (C) Schematic representation of a coronal section of the SVZ showing the microdomains and the neuronal subtypes that they generate (see Fig. 3). The approximate location of Wnt and Shh signaling activity is shown, as well as their known (++, +) and putative (?) effects on the specification of progenitor types and neuronal subtypes. (D) Analysis of gene expression at a given time in different subregions and/or at different times in a given region might allow developmentally important genes involved in key temporal or spatial developmental events occurring in the forebrain germinal zones to be identified.

revealed that the caudalmost region of the LV also contributes to OB neurogenesis by generating GCs (Kelsch et al., 2007; Merkle et al., 2007). Between these ill-defined rostral and caudal boundaries, the V-SVZ of the LV can be subdivided into four major rostrocaudal areas based on LV morphology (Falcao et al., 2012, 2013)

(Fig. 4A). As discussed above, this rostrocaudal heterogeneity is accompanied by a dorsoventral heterogeneity, with all walls giving rise to new neurons in the rostralmost regions, whereas only the lateral wall remains neurogenic in caudal areas (Fig. 3A).

Appropriate annotation of the V-SVZ across species is also relevant, as important regional differences have also been identified in non-rodent species. Examination of the primate forebrain revealed that neurogenesis occurs mainly in a rostral subregion representing less than one-fifth of the entire LV (Azim et al., 2012b), further illustrating the need for homogeneous sampling of the LV and appropriate annotation of its germinal regions. Similarly, although contrasting results were obtained at first regarding the persistence of neurogenesis in the human forebrain (Sanai et al., 2004; Curtis et al., 2007), systematic sampling of individuals of different ages settled this controversy by providing a convincing spatiotemporal overview of the early decline of OB neurogenesis in humans (Sanai et al., 2011).

Integrating the V-SVZ into current ontology models in conformity with earlier developmental stages (Puelles et al., 2013) would facilitate the automatic annotation of this germinal region. Introducing this germinal region and progenitor domains into current brain development databases (Thompson et al., 2014), together with the homogenization of the ontological nomenclature across species (Haudry et al., 2008), represent important steps for screening databases or sampling subregions of the postnatal V-SVZ to identify regionally enriched genes.

Beyond space: adding the temporal dimension

In addition to the spatial variability observed in the V-SVZ, this region is also subject to key developmental changes in cellular organization, in the distribution, number and activity of the NSCs, as well as in the fate of their progeny throughout postnatal life (Fig. 5A,B and Fig. 6D). Indeed, during the first three postnatal weeks, as RGCs lose their basal processes and differentiate into ependymal cells, B1 and B2 cells (see above), neuroblasts migrate tangentially and form longitudinally oriented tubes that isolate the SVZ from the surrounding mature tissue (Fig. 5A,B). Simultaneously, the open olfactory ventricle closes and gives rise to the RMS (Peretto et al., 2005), and cycling cells, which are initially distributed along the entire V-SVZ-RMS-OB axis, are gradually confined to the RMS and LV walls (Lemasson et al., 2005). Gradual changes in the specification of newborn PGCs also occur in the first postnatal weeks, when CR⁺ periglomerular cells become progressively predominant while the proportion of CB⁺ and TH⁺ neurons declines (Batista-Brito et al., 2008). These changes are accompanied by an altered migration of newborn neurons to specific regions of the GC layer in the first months of postnatal life (Lemasson et al., 2005).

Aging further restricts the activity of NSCs. A dramatic decrease in neurogenesis occurs by ~6-12 months of age in rodents (Enwere et al., 2004; Molofsky et al., 2006; Shook et al., 2012). Accordingly, the total number of proliferating cells in the niche is reduced by 50-75% at 24 months of age (Tropepe et al., 1997; Maslov et al., 2004; Luo et al., 2006; Capilla-Gonzalez et al., 2014), while the cell cycle is concomitantly lengthened (Luo et al., 2006). Interestingly, the ratio of newly formed periglomerular interneuron subtypes (TH⁺, CB⁺ or CR⁺) does not change further between 3 months and 2 years, suggesting that the loss of NSC activity over time is lineage independent (Shook et al., 2012).

Thus, two key developmental events occur in the postnatal V-SVZ: (1) a rapid regional decline in neuronal subtypes produced in the first weeks after birth; and (2) a general decline in the number

of NSCs and in the neurogenic potential of the V-SVZ. The genes involved in these developmental events can be identified by gene profile analysis performed at a given time in different V-SVZ subregions and/or at different times throughout the entire V-SVZ (Fig. 6D). In the hippocampal subgranular zone, where the majority of temporal gene expression profiling studies have been performed, gene expression signatures were shown to be upregulated during aging. For example, antagonists of the Wnt signaling pathway are highly upregulated with aging and their ablation reverses the age-related decline in neurogenesis (Seib et al., 2013). In the case of the V-SVZ, microdissection of defined microdomains followed by RNA or protein extraction can be performed to study the differential expression of TFs and/or the activity of signaling pathways (Azim et al., 2014b, 2015) (Fig. 6D).

Using these approaches, regional variations have been uncovered for signaling pathways throughout postnatal life, and these are likely to shape the observed changes in neuronal subtypes generated by the V-SVZ and the cell proliferation patterns after birth. For example, a rapid decline in FGF2 and Wnt signaling can be observed in the dorsal V-SVZ in the first postnatal weeks, correlating with the gradual decline of germinal activity observed in this region (Zheng et al., 2004; Azim et al., 2012a). Combining this approach with magnetic or fluorescence-activated cell sorting of stem or progenitor cells (Azim et al., 2012a) will prove to be useful in deciphering the transcriptome and proteome of spatially restricted V-SVZ subpopulations. This, together with the emergence and sharing of gene expression datasets for stem/progenitor cells isolated from different subregions of prenatal and postnatal germinal zones (Pinto et al., 2008; Beckervordersandforth et al., 2010; Azim et al., 2015) will enable regional/temporal comparison on a larger scale. Such meta-analysis of gene expression profiling will facilitate the identification of novel regional and temporal regulators of postnatal neurogenesis.

Conclusions

We have discussed how integrating space when studying the V-SVZ has already, and will continue to, shed light on the fundamental principles that govern neurogenesis in the postnatal forebrain. Acknowledging V-SVZ regionalization allows appropriate sampling schemes to quantify NSCs and their progeny to be established. It also allows the transcriptional differences that define V-SVZ microdomains to be identified. Gaining knowledge of the transcriptional and signaling specificities of these microdomains is leading to the identification of region-specific cues that can be used to promote the generation of defined lineages. Ultimately, this might allow the design of new approaches to promote the generation of defined cell types in regenerative contexts, when they need to be replaced.

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Competing interests

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Supplementary material

Supplementary material available online at <http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.119966/-/DC1>

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