The Heterogeneity of Adult Neural Stem Cells and the Emerging Complexity of Their Niche

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Neural stem cells persist in the adult mammalian brain in a neurogenic niche known as the subventricular zone (SVZ). SVZ neural stem cells (NSCs) can self-renew and are multipotent in culture. In rodents, adult NSCs correspond to SVZ astrocytes (type B cells) that are derived from radial glia, the NSCs of the embryonic and early postnatal brain. Type B cells generate transit-amplifying (type C) cells that give rise to young neurons (type A cells) and oligodendrocytes. Young neurons are born throughout the adult neurogenic niche and migrate tangentially through a complex network of chains that merge into the rostral migratory stream (RMS), a major pathway that leads into the olfactory bulb (OB). Within the OB, young neurons differentiate into multiple types of interneurons. The SVZ was thought to be limited to the lateral wall of the lateral ventricle, but recent work shows that the adult neurogenic niche is significantly more extensive and includes portions of the medial and dorsal walls of the lateral ventricle and the RMS itself. Furthermore, several recent studies explain why young OB neurons are generated in such an extensive region. Type B cells in different regions of the SVZ, although able to self-renew and generate both neurons and glial cells in vitro, are heterogeneous and committed to producing defined neuronal subtypes in vivo. The adult SVZ therefore provides a rich system to study not only neural replacement, but also the cellular and molecular mechanisms underlying regionalization and cell-fate specification.

The discovery of neural stem cells in the adult mammalian brain shattered the long-standing belief that neurogenesis is restricted to embryonic and early postnatal periods. The largest germinal region in the adult mammalian brain is the SVZ. The SVZ is classically described as a thin layer of proliferative cells lining the lateral wall of the lateral ventricle (LV) and separated from the ventricular lumen by a layer of ependymal cells (Smart and Leblond 1961; Altman 1969). The existence of NSCs in this region was first suggested by in vitro experiments in which SVZ cells were shown to self-renew and produce neurons, astrocytes, and oligodendrocytes (Reynolds and Weiss 1992; Morshed et al. 1994). In vivo, NSCs generate a large number of young neurons that migrate along the RMS to the OB where they replace multiple types of interneurons (Luskin 1993; Lois and Alvarez-Buylla 1994). NSCs in the SVZ also generate both parenchymal oligodendrocyte progenitors (OPCs) and myelinating oligodendrocytes, most of which migrate into the neighboring corpus callosum (Nait-Oumesmar et al. 1999; Picard-Riera et al. 2002; Menn et al. 2006).

Adult neurogenesis leads to the generation and replacement of specific types of neurons in restricted brain regions, including the OB and dentate gyrus of the hippocampus. Many processes of embryonic development are recapitulated during adult neurogenesis, such as neuronal differentiation, migration, maturation, and cell death. However, adult-born neurons confront an environment very different from those born in the developing brain. Adult-born neurons migrate through more complex and frequently extensive territories and must integrate into circuits that are already fully functional. Young neurons in the SVZ and RMS migrate along each other, forming long aggregates of cells called chains (Lois et al. 1996) and are able to migrate long distances in relatively short periods of time (Wichterle et al. 1997). Within 2–5 days from their time of birth in the SVZ, the majority of these young neurons have reached the OB. Once in the OB, young neurons move radially away from the RMS and begin their final differentiation and maturation, a process that takes 5–10 days (Petreraum and Alvarez-Buylla 2002). During this period, new neurons develop dendritic trees and synaptic spines and become functionally integrated into the OB circuitry (Carleton et al. 2003).

Initial studies in the neonatal rat brain suggested that new OB neurons originate from a restricted territory in the anterior SVZ, in a region close to the RMS (Luskin 1993; Lois and Alvarez-Buylla 1994). However, subsequent work uncovered an extensive network of chains of young neurons throughout most of the SVZ on the lateral wall of the LV (Doetsch and Alvarez-Buylla 1996), suggesting that migrating cells originate along the length of the lateral ventricular wall. Below, we review new experiments that suggest that the neurogenic SVZ covers regions of the lateral ventricular walls facing the pallium, subpallium, and septum, as well as the RMS. Furthermore, it was commonly assumed that adult NSCs would correspond to rare cells that divided infrequently but were highly plastic in their potential to generate multiple types of neurons and glial cells. We also review work that suggests that NSCs dedicated to producing specific cell types at specific developmental time points are restricted to different domains of the adult neurogenic niche. Thus, the adult neurogenic niche not only is more extensive than has been appreciated, but is also remarkably heterogeneous and complex. These insights are of great biological interest and could have important therapeutic implications. For example, if we were to learn how mature neural circuits eliminate some neurons and recruit new ones, we might be able to...
develop effective strategies to replace neurons that are lost due to disease or trauma (Nottebolun 2004). Furthermore, the newfound heterogeneity of the neurogenic niche highlights the adult SVZ as a rich and unique experimental system to investigate the origin of neuronal diversity and to develop new strategies for cell replacement therapy.

ADULT NSCs ARE ASTROGLIAL CELLS THAT ORIGINATE FROM RADIAL GLIA

The primary precursors in the SVZ correspond to type B cells, a subpopulation of slowly dividing astroglial cells adjacent to a layer of ependymal cells (Doetsch et al. 1999; Laywell et al. 2000; Imura et al. 2003). Type B cells produce type C cells, a type of transit-amplifying cell that divides rapidly to produce young neurons, also known as neuroblasts or type A cells. It was thought that cells in this lineage (B→C→A) were separated from the ventricle by a layer of ependymal cells; therefore, the adult neurogenic region was referred to as an SVZ. However, recent work suggests that most, if not all, B cells actually contact the ventricle through small, specialized apical processes (Mizadeh et al. 2008). These apical processes are tightly packed and surrounded by multiple ependymal cells, forming pinwheel-like structures that are only observed in the walls of the ventricle where neurogenesis continues throughout adult life. Therefore, it appears that the adult neurogenic niche is characterized by a proliferative ventricular zone (VZ) in addition to the SVZ. A similar neurogenic VZ has been described in the adult avian brain, where postmitotic ependymal cells are mixed with mitotic astroglial cells (Alvarez-Buylla et al. 1998).

This finding also illustrates the developmental history of adult NSCs. It is now clear that these astrocyte-like cells are derived from radial glial cells present in the embryonic and early postnatal brains. (for review, see Merkle and Alvarez-Buylla 2006). Radial glia are now appreciated as the principal NSCs of the developing brain (Anthony et al. 2004; Miyata et al. 2004; Noctor et al. 2004). The cell bodies of radial glia form a VZ around the ventricles of the developing brain, which they contact via an apical process, much like B cells in the adult brain. Radial glia also contact the pial surface of the brain via a long, radially projecting basal process. These basal processes can be infected by Cre-expressing adenovirus. When this virus is injected into the brains of neonatal Cre-reporter mice, these basal processes become infected, and radial glia are specifically labeled in the VZ. This technique was used to demonstrate that adult NSCs are derived from radial glia (Merkle et al. 2004) and that the neurogenic niche is not restricted to the lateral wall of the LV, as discussed below.

THE ADULT GERMINAL LAYER IS MORE EXTENSIVE THAN PREVIOUSLY THOUGHT

The adult SVZ neurogenic niche was initially thought to be limited to the anterior lateral wall of the LV (Luskin 1993), but subsequent work shows that OB interneurons are born throughout the lateral ventricular wall (Doetsch and Alvarez-Buylla 1996). This conclusion has been confirmed by the labeling of primary progenitors in posterior regions of the lateral wall of the LV in neonates and adults (Merkle et al. 2007). In vitro and in vivo studies in neonates and adults also show that progenitors in the RMS produce neurons (Gritti et al. 2002; Liu and Martin 2003; Hack et al. 2005). These progenitors continue to produce neurons for at least 4 weeks after being labeled with an adenovirus that expresses Cre under the GFAP (glial fibrillary acidic protein) promoter, suggesting that RMS progenitors are long-lived GFAP+ cells, much like SVZ stem cells (Merkle et al. 2007).

Perhaps more surprising is the recent demonstration that the dorsal wall of the LV facing the pallial VZ also generates interneurons destined for the OB (Kohwi et al. 2007; Merkle et al. 2007; Ventura and Goldman 2007). This pallial region extends laterally and caudally into the subcallosal zone (SCZ), a derivative of the pallial wall of the LV formed by the collapse of the LV due to an expanding hippocampus (Fig. 1). This region contains migratory neuroblasts (Seri et al. 2006) and produces neurons when its primary precursors are labeled in the neonate or adult (Merkle et al. 2007). Generation of OB interneurons from precursors of the dorsal wall of the LV is particularly unexpected given that during mouse development, the pallial VZ does not appear to generate its own inhibitory interneurons. Cortical interneurons are instead generated by the medial and caudal ganglionic eminences (Marin and Rubenstein 2003).

The adult germinal layer also includes regions of the anterior medial wall of the LV (SVZam). Embryonic and adult progenitors in this region can generate neurons in culture (Morhead et al. 1994) and produce OB interneurons after grafting (Kohwi et al. 2007) and labeling in vivo (Merkle et al. 2007). This medial neurogenic area appears to face a small region of the septum as well as the nucleus accumbens, a part of the ventral striatum. Therefore, the adult SVZ is a remarkably extensive germinai region covering most of the neuroepithelium-derived germinal compartments in the developing telencephalon (Fig. 1).

TYPE B CELLS GENERATE MULTIPLE TYPES OF INTERNEURONS IN THE OB

The rostral extension of the RMS forms the core of the OB. It is through this route that thousands of young neurons enter into the mouse OB and complete their tangential trajectory every day. The OB is a highly laminated structure; its neurons are organized into different layers based on their functions. Upon entering the bulb through its core, young neurons migrate radially into the granule and glomerular layers where they differentiate into granular and periglomerular local inhibitory interneurons (Kosaka et al. 1995; Carleton et al. 2003; Kohwi et al. 2005). These interneurons have important roles in modulating the activity of mitral and tufted cells, the primary projection neurons that relay sensory input from the olfactory epithelia directly to the cortex (Greer 1987; Shepherd and Greer 1998; Lledo et al. 2008).

Several subtypes of granular and periglomerular interneurons can be distinguished on the basis of morphology, connectivity, and expression of molecular markers. For example, within the granule cell layer, new neurons can be localized in the superficial or deep granule cell layers (Fig. 2) and can be further subdivided based on their expression...
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Figure 1. Spatial extent of the SVZ neurogenic niche in the adult mouse brain. The SVZ is traditionally thought to consist of a thin sheet of cells lining the lateral walls of the lateral ventricle, but recent work demonstrates that OB interneurons are born in a much more extensive neurogenic niche. To display its complex topography, the niche was digitally traced in serial sections of an adult mouse brain and combined to generate a three-dimensional model that is shown in dorsal (A), lateral (B), and oblique (C) views within a transparent model of the adult mouse brain surface that was reconstructed from serial magnetic resonance imaging (MRI) sections. The newly discovered neurogenic regions (red) include the rostral migratory stream (RMS), the anterior medial wall (SVZam) of the lateral ventricle (LV), and the dorsal (cortical) wall of the LV, which becomes the subcallosal zone (SCZ) in more posterior regions. Each region in the niche generates OB interneurons when its stem cells are targeted in the postnatal brain. For reference, the SVZ niche is shown relative to the ventricular system (blue) and the other major neurogenic region in the adult brain, the dentate gyrus (DG) of the hippocampus (purple). The borders of the niche are approximate and have been assembled from available stem-cell-targeting data.

Figure 2. Region-specific production of OB interneuron subtypes in the SVZ. Colored and labeled camera lucida traces of different OB interneuron subtypes are shown in the central panel. To show their relative positions in the OB, the tracings are superimposed over a photomicrograph showing a partial cross section of the OB where superficial is up and the OB core is down. The side panels show oblique views of the adult neurogenic niche, colored to show the region of origin of periglomerular cells (PGCs, left panel) and granule cells (GCs, right panel). For example, superficial GCs (green) are largely produced by neural stem cells in the dorsal part of the neurogenic niche, which comprises the pallial SVZ (SCZ) and dorsal wall of the lateral ventricle and the dorsal portion of the subpallial SVZ, whereas deep GCs (blue) are produced in the ventral subpallial SVZ. CalR+ GCs (yellow) are produced primarily in the medial wall of the anterior SVZ but also at low levels in the pallium. PGCs are also produced in a region-specific pattern in the anterior SVZ but are rarely produced in the posterior SVZ as indicated by a diminished intensity of coloring. It has been difficult to study regionalization within the RMS due to its small size, but because it is derived from both pallium and subpallium, it may give rise to different OB interneuron subtypes. The RMS is speckled with a mixture of colors to reflect this ambiguity.
of the calcium-binding protein calretinin (CalR). Superficial granule cells (GCs) primarily target the dendrites of tufted cells in the superficial external plexiform layer (EPL), whereas deep GCs primarily target the deep EPL and connect to mitral cells (Orona et al. 1983; Mori and Shepherd 1994). Because mitral and tufted cells project to different regions of the olfactory cortex, superficial and deep GCs likely subserve separate olfactory functions (Liu and Shipley 1994). Periglomerular cells (PGCs) can be subdivided into at least three different cell types: CalR+, dopaminergic (tyrosine hydroxylase expressing [TH+]), and calbindin (CalB+) (Parrish-Aungst et al. 2007). Although in the mouse, all three PGC subpopulations are GABAergic (Kohwi et al. 2007), there appears to be a species-specific difference in the neurotransmitter characteristics of these cells because only the TH+ PGCs are immunopositive for GABA among rat PGCs (Kosaka and Kosaka 2007).

Most, if not all, of these many cell types are continuously replaced throughout the life of the animal. We are now beginning to understand the origins of their heterogeneity and molecular factors involved in their specification, as described below.

**MIGRATING NEUROBLASTS ARE HETEROGENEOUS**

Many different types of OB interneurons are generated in the adult brain, but the origins of this diversity are not clear. One possibility is that neuroblasts migrating within the RMS are a homogeneous population of cells that differentiate into different subtypes only after reaching the OB, perhaps in response to local cues. Another possibility is that the migrating neuroblasts are already a heterogeneous group of cells destined to acquire specific fates. Staining for Pax6, a member of the paired-homeobox transcription factor family (Simpson and Price 2002), suggest that neuroblasts in the RMS are not homogeneous (Hack et al. 2005; Kohwi et al. 2005). Only a small subpopulation of neuroblasts expresses Pax6, whereas others are clearly negative. In the OB, Pax6 is expressed in a subpopulation of mature GCs and PGCs (Stoykova and Gruss 1994). Interestingly, Pax6 appears to be cell-autonomously required for the generation of dopaminergic PGCs and a subpopulation of superficial GCs (Hack et al. 2005; Kohwi et al. 2005). The deficit of even one copy of Pax6 results in profound reduction in TH+ PGC production (Dellovade et al. 1998; Kohwi et al. 2005), indicating that Pax6 expression is essential for the generation of specific subpopulations of adult OB neurons. These studies raised the question of how diverse neuroblasts are generated. In addition to Pax6, other transcription factors have been associated with particular subpopulations of interneurons in the adult OB. In particular, the transcription factor SP8, which is highly expressed by the developing dorsolateral ganglionic eminence, cortex, and septum (Long et al. 2007), has been associated with the formation of CalR+ interneurons in the OB (Waclaw et al. 2006). These studies demonstrate that neuroblasts are transcriptionally heterogeneous, suggesting that they are restricted to particular fates soon after they are born. Subsequent studies reveal that this heterogeneity arises in the SVZ and is influenced by both time and space.

**TEMPORAL SPECIFICATION OF SVZ PROGENITORS**

In the mouse, OB interneurons are first generated from embryonic day (E) 12–14 (Wichterle et al. 2001; Stenman et al. 2003; Tucker et al. 2006). However, the NSCs that produce these neurons in the embryo are morphologically very different from the NSCs that produce OB interneurons in the adult, likely due to the dramatic morphological changes that occur in the developing brain. A growing body of evidence suggests that in parallel with these morphological changes, NSCs produce different OB interneuron types at different times in development (De Marchis et al. 2007; Batista-Brito et al. 2008). This temporal code for cell-type production has been well described for cortical NSCs, which sequentially generate neurons that migrate to and occupy the deep to superficial layers of the cortex (for review, see McConnell 1992). Similarly, retinal progenitor cells sequentially generate different interneurons in a specific order (Cepko et al. 1996). Temporal specification of neural subtypes may be a theme that also applies to OB interneuron production as well. Earlier work suggests that early born neurons survive longer in the OB compared to those generated later in life (Kaplan et al. 1985). More recently, it has been shown that neurons born within the first week of life are more likely to differentiate into superficial GCs than cells born at later ages (Lemasson et al. 2005). Differences in the production of different types of PGCs have also been suggested on the basis of heterochronous and homeochronous transplantation experiments (De Marchis et al. 2007). Compared to adults, neonate grafts derive a larger proportion of CalB+ PGCs. Conversely, CalR+ and TH+ PGCs are more frequently observed from adult SVZ grafts, suggesting that progenitors at different developmental stages preferentially produce specific subtypes of OB interneurons. A more recent study used transgenic mice carrying a floxed reporter gene and Cre-ER under the control of the Dlx1/2 intragenic enhancer to label OB interneurons at different stages of development (Batista-Brito et al. 2008). This study confirms that CalB+ cells are more likely to be produced at early developmental stages, whereas most CalR+ cells are produced later, but in contrast to the earlier study, this genetic labeling technique suggests that TH+ cells are produced in larger numbers at earlier time points rather than later. These inconsistent observations may be explained by technical differences. For example, SVZ transplants taken at different stages may not represent truly homologous regions because the morphology of germinal zones and transcription factor expression patterns change dramatically from the embryo to the adult. However, it is also possible that the Dlx1/2 intragenic enhancer may be differentially active at different time points and therefore label different subsets of SVZ cells.

These studies suggest that different OB interneuron types are produced in different numbers at different developmental time points, which should result in constantly shifting ratios of different OB interneuron subtypes. This would raise important functional questions about the maturation of olfactory function. At least among PGCs, however, the ratios of CalB+, CalR+, and TH+ cells...
do not appear to change from P0 to adult (Kohwi et al. 2007), suggesting that factors besides temporal generation of these cells influence OB circuitry development and maintenance. For example, it is not clear that the rate of maturation of specific cell types remains constant from early development to adult. Indeed, many TH⁺, CalR⁺, and CalB⁺ cells can already be detected in the P0 OB, soon after the onset of OB interneuron production in the embryo. In contrast, adult-born TH⁺ neurons do not reach their peak of maturation until postnatal day 45 (Kohwi et al. 2007). Additionally, we do not yet know whether the turnover rate of different cell types changes over time. Half of adult-born OB interneurons undergo activity-dependent apoptosis between 15 and 45 days after birth (Petrerau and Alvarez-Buylla 2002), but it is not known whether this rate of apoptosis applies to all interneuron subtypes equally or whether this profile is consistent throughout early development to the adult. In the future, it will be important to understand the impact of development, maturation, and turnover of individual interneuron subtypes on OB neuronal circuitry. Because some markers such as TH are also modulated by activity, better cell-type-specific markers will help to clarify this issue.

**SPATIAL SPECIFICATION OF SVZ PROGENITORS**

Although it is clear that migrating neuroblasts are heterogeneous and that there is a temporal component to OB interneuron production, these studies do not address how multiple OB interneuron types are simultaneously produced in the developing and adult brain or why neurogenesis occurs in such an extensive niche. SVZ NSCs could be equivalent, each generating every different OB interneuron type, or they may be a heterogeneous population of progenitors, each restricted to producing just one cell type or a few cell types.

Regionalization of germinal zones is a general feature of neurogenesis in the developing brain where progenitors in different regions become specified for the production of different neuronal types. This parcellation has been extensively documented in the developing spinal cord where gradients of morphogens establish discrete dorsoventral territories of transcription factor expression, each associated—in time and space—with the production of different types of neurons and glial cells (Ericson et al. 1997; Hochstam et al. 2008). Similarly, segregation of progenitor zones based on distinct transcription factor expression profiles is also observed in the developing telencephalon (Fig. 3) (Campbell 2003; Puellas and Rubenstein 2003; Guilleminot 2005; Long et al. 2007); some of these regions are retained in the walls of the adult LV. The lateral wall of the adult SVZ expresses Dlx5/6 (Stuhmer et al. 2002), ER81 (Stemman et al. 2003), Mash1 (Parras et al. 2004), Pax6 (HACK et al. 2005; Kohwi et al. 2005), Sp8 (Waclaw et al. 2006), and Gsh2 (Young et al. 2007), a set of transcription factors that are also expressed in the lateral ganglionic eminence (LGE) during embryonic pallium development (Fig. 3). This common pattern of gene expression, together with transplantation experiments (Wichterle et al. 1999), led to the conclusion that the adult SVZ is largely equivalent to the developing LGE and is likely derived from this structure (Stemman et al. 2003; Waclaw et al. 2006).

The LGE is an important contributor to the adult SVZ as discussed above, but more recent work indicates that other domains of the developing telencephalon, including the pallium (cortex) and septum, contribute importantly to the adult germinal niche that produces OB interneurons (Fig. 3). Consistent with the view that a significant fraction of the adult SVZ is derived from the LGE, lineage-tracing experiments show that a large number of postnatally derived neurons are from progenitors that express Dlx5/6 (Kohwi et al. 2007) and Gsh2 (Young et al. 2007). However, several observations suggest that the embryonic pallium could contribute to OB interneurons as well. For example, lineage tracing of Emx1, a transcription factor highly expressed in the pallium during development, indicates that derived Emx1-expressing progenitor cells remain proliferative in the adult dorsal (cortical) SVZ and generate OB interneurons (Willaine-Morawek et al. 2006; Kohwi et al. 2007; Young et al. 2007). Interestingly, a significant subset of CalR⁺ cells is derived from Emx1-expressing progenitors, whereas very few CalR⁺ neurons are derived from the Gsh2 lineage (Fig. 3) (Kohwi et al. 2007; Young et al. 2007). Consistent with these observations, CalR⁺ cells are generated when embryonic pallium, but not embryonic LGE, was grafted to the adult SVZ (Kohwi et al. 2007). These results suggest that the embryonic pallium also contributes to the adult neurogenic niche and may constitute a population of progenitors different from those derived from the LGE. However, because a small subpopulation of Emx1-lineage cells is also detected in the subpallium in the developing telencephalon of Emx1-Cre mice (Gorski et al. 2002; Willaine-Morawek et al. 2006), it is possible that these cells, and not the pallial progenitors, give rise to the subpopulation of Emx1-derived OB interneurons.

Direct evidence for the spatial specification of SVZ progenitors came from a study that used a modification of the technique originally used to demonstrate that adult NSCs were derived from radial glia (Merkle et al. 2004). A small volume of adenosine-virus-expressing Cre was injected in the neonatal brain in a region traversed by the radial processes of radial glial cells, the precursors of adult SVZ type B cells. The adenosine virus was retrogradely transported up the radial process to the nucleus, where the virally encoded Cre recombines a conditional reporter gene, allowing the lineage of labeled NSCs to be traced. This procedure enables small groups of radial glia to be labeled in different discrete locations of the periventricular germinal niche (Merkle et al. 2007). This approach demonstrates that different types of OB interneurons are derived from unique locations of the SVZ (see Fig. 2). For example, neonatally targeted pallial radial glia, which project their radial processes through the corpus callosum into the cortex (Merkle et al. 2007; Ventura and Goldman 2007), generate many GCs and PGCs, confirming the conclusions of the Emx1 lineage-tracing studies. Interestingly, the majority of pallially derived PGCs expresses TH and a small subpopulation expresses CalR. In contrast, the majority of CalB⁺ cells is derived from ventrolateral SVZ.
Different types of GCs are also derived from different SVZ domains. Targeting of NSCs in the pallial and dorsal subpallial SVZ leads to primarily superficial GC production, whereas ventral SVZ targeting leads to mostly deep GC production (Merkle et al. 2007). However, CalR+ GCs are not produced when NSCs in the dorsal subpallium were targeted; rather, they are derived in small numbers from the pallium, and in larger numbers from the medial wall and RMS (Fig. 2). Similarly, a subpopulation of CalR+ PGCs is also derived from this medial germinal zone. Although the ventral portion of this medial region faces the nucleus accumbens (an LGE derivative), more anterior and dorsal regions appear to face the septum, which is not derived from the LGE, but expresses the transcription factors Gsh2 and Dlx1/2. Interestingly the RMS generates nearly all OB interneuron subtypes, including a large percentage of CalR+ GCs and PGCs (Merkle et al. 2007). The RMS forms along what earlier in development correspond to the olfactory ventricle. This ventricular wall is patterned and contains both pallial and subpallial components (Fig. 2) (Long et al. 2007).

The dendritic arbors of newborn OB interneurons also appear to be specified within different domains of the SVZ. A recent study used retroviruses to label proliferative cells in the anterior or posterior SVZ of neonatal rats (Kelsch et al. 2007). GCs with dendrites that branch into the superficial EPL are derived from cells labeled in the anterior SVZ of the neonatal rat, whereas GCs that branch deep within the EPL are derived from retroviral injections into the posterior SVZ. Because retroviruses can label all dividing cells in the SVZ, it is not clear whether this specification occurs in primary or secondary progenitors. Nonetheless, the work does suggest that differences in the rostrocaudal position of dividing SVZ progenitors affect the branching pattern of dendrites in young neurons that migrate to the OB.

To determine whether adult SVZ type B cells share the same regional specification as radial glial cells labeled in...
the neonatal brain, adult progenitors in different regions of the SVZ were targeted with an adenovirus-expressing Cre under the GFAP promoter. Type B cells labeled in this manner generate similar types of OB interneurons as radial glia targeted in the same region. This indicates that the spatial organization of SVZ progenitors is maintained during postnatal development (Merkle et al. 2007). Furthermore, it suggests that SVZ stem cells do not move tangentially during postnatal development. In summary, the growing body of evidence shows that the SVZ is organized into domains containing different types of progenitor cells. This conclusion parallels what has been described in the embryo, where the position of a progenitor in a germinal zone determines the types of neurons that it will generate.

When SVZ stem cells are cultured with high concentrations of growth factors, they can be passaged several times and generate neurons, astrocytes, and oligodendrocytes (Morshead et al. 1994), demonstrating self-renewal and multipotency under these conditions. To determine whether SVZ stem cells retain their regional identity in culture, they were cultured under adherent conditions developed by the Steindler laboratory (Scheffler et al. 2005). Under these conditions, SVZ stem cells produce OB interneuron types with regional specificity similar to that observed in vivo targeting. For example, cultures derived from the medial wall produce many more CalR^+ neurons than cultures derived from the lateral wall of the SVZ. Within the lateral wall, ventral cultures give rise to higher numbers of CalB^+ cells than dorsal cultures. Although under the above culture conditions SVZ progenitors appear to retain a remarkable level of specification, it remains to be determined whether different culture conditions might alter this program or allow adult progenitor cells to be respecified.

Positional specification in the neonatal mouse brain could be inherited from early development. Alternatively, positional cues in the postnatal brain may continually instruct stem cells to generate specific types of neurons. Grafting experiments, where progenitors from one region are transferred to another location of the postnatal SVZ, have failed to demonstrate plasticity indicative of environmental cues re instructing postnatal stem cells to acquire particular fates (Kelsch et al. 2007; Merkle et al. 2007). For example, radial glia in the ventral SVZ normally generate deep GCs continue to generate deep GCs when grafted into the dorsal SVZ, where superficial GCs are produced. This level of specification is maintained even after progenitors were cultured for multiple passages in vitro. This suggests that positional specification is an early event likely to occur before birth and is maintained postnatally. Although the data above strongly suggest that relocation within the SVZ is not sufficient to switch stem cell specification, this is not enough to conclude that cell fate cannot be modified. Environmental positional cues, which may have created positional patterns of transcription factor expression early in the embryo, may no longer be present in the postnatal brain. It will be interesting to determine whether some of the molecular mechanisms involved in the early specification remain operational in the adult.

CONCLUSION

The SVZ is the largest germinal region of the adult mammalian brain. Several studies suggest that the adult SVZ may be an important reservoir of precursor cells for brain repair (Lindvall and Kokaia 2006; Martino and Pluchino 2006). Although the extent to which the robust neurogenesis found in the rodent can be found in the adult human brain is unclear (Curtis et al. 2007; Sanai et al. 2007), NSCs and some level of SVZ proliferation has been described in autopsy material (Eriksson et al. 1998; Curtis et al. 2003; Sanai et al. 2005). Recent work in rodents indicates that this germinal region is much more extensive than previously thought and includes regions derived from all three main telencephalic walls in the embryo (Figs. 1–3). If a similar level of spatial specification exists in humans, different subtypes of neurons are likely to originate from SVZ regions that are separated from one another by long distances. This may be an important constraint in evolution. As brain size increases, there may be limits to the tangential migration required to transfer young neurons from unique sites of birth to their ultimate destination within the circuits where they will ultimately integrate. This is relevant not only to brain evolution, but also to its repair. The generation of specific neuronal subtypes for therapeutic applications may depend on whether the region-specific progenitors that give rise to them are still active in the adult. Stem cells in these different regions express unique sets of transcription factors likely derived from an early stage of embryonic development. An important challenge for future work is to decipher the mechanisms by which combinations of transcription factors expressed in different locations of the SVZ result in the generation of distinct subsets of olfactory interneurons. This new information will provide a fundamental basis for understanding how multiple variables, including time and space, regulate NSCs so that we can better harvest their therapeutic benefits.

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