Neural stem cell heterogeneity through time and space in the ventricular-subventricular zone

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Abstract

BACKGROUND—The origin and classification of neural stem cells (NSCs) has been a subject of intense investigation for the past two decades. Efforts to categorize NSCs based on their location, function and expression have established that these cells are a heterogeneous pool in both the embryonic and adult brain. The discovery and additional characterization of adult NSCs has introduced the possibility of using these cells as a source for neuronal and glial replacement following injury or disease. To understand how one could manipulate NSC developmental programs for therapeutic use, additional work is needed to elucidate how NSCs are programmed and how signals during development are interpreted to determine cell fate.

OBJECTIVE—This review describes the identification, classification and characterization of NSCs within the large neurogenic niche of the ventricular-subventricular zone (V-SVZ).

METHODS—A literature search was conducted using Pubmed including the keywords "ventricular-subventricular zone," "neural stem cell," "heterogeneity," "identity" and/or "single cell" to find relevant manuscripts to include within the review. A special focus was placed on more recent findings using single-cell level analyses on neural stem cells within their niche(s).

RESULTS—This review discusses over 20 research articles detailing findings on V-SVZ NSC heterogeneity, over 25 articles describing fate determinants of NSCs, and focuses on 8 recent publications using distinct single-cell analyses of neural stem cells including flow cytometry and RNA-seq. Additionally, over 60 manuscripts highlighting the markers expressed on cells within the NSC lineage are included in a chart divided by cell type.

CONCLUSIONS—Investigation of NSC heterogeneity and fate decisions is ongoing. Thus far, much research has been conducted in mice however, findings in human and other mammalian species are also discussed here. Implications of NSC heterogeneity established in the embryo for the properties of NSCs in the adult brain are explored, including how these cells may be redirected after injury or genetic manipulation.

Keywords

Introduction

In the developing murine brain, multipotent neural stem cells (NSCs) are present in the germinal layers lining the lateral ventricles (Walsh and Cepko, 1992; Davis and Temple, 1994; Johe et al., 1996; Weiss et al., 1996; Qian et al., 1998), and proliferation extends through a ventricular (VZ) and subventricular zone (SVZ) as the generation of neurons and glia takes place. In the early 20th century, Wilhelm His determined that these germinal cells rapidly divide within the VZ, producing neuroblasts that migrate outwards from the inner VZ (His, 1904) to give rise to the cells of many brain structures, including the cortex. Originally, His hypothesized that multipotent NSCs existed only during this period of early development, producing separate progenitor pools for neurons and glia (Levitt et al., 1981). The research of Giuseppe Magini, Santiago Ramón y Cajal and Camillo Golgi then expanded the concept of two distinct cell types existing within the VZ, consisting of separate progenitor pools that only gave rise to neurons or glia, respectively (Bentivoglio and Mazzarello, 1999). In the late 1950s, [^{3}H]-thymidine incorporation studies illustrated that cell proliferation continued in distinct regions of the adult rodent brain, however, it was suggested that the labeled cells were glial progenitors (Sidman et al., 1959; Altman, 1962; Altman and Das, 1965; Kaplan and Hinds, 1977). Subsequent electron microscopy studies illustrated that newborn cells within the rat olfactory bulb and hippocampus were of the neuronal lineage (Kaplan and Hinds, 1977) and culture conditions were developed which propagated multipotent NSCs from the adult murine brain (Reynolds and Weiss, 1992). Much subsequent research has since been conducted indicating persistent neurogenesis within the adult brain (Goldman and Nottebohm, 1983; Galileo et al., 1990; Alvarez-Buylla et al., 2001; Temple, 2001; Gage, 2002; Merkle et al., 2004). The germinal regions subsequently identified within the adult mammalian brain were the ventricular-subventricular zone (V-SVZ) lining the lateral ventricles and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus (Lois and Alvarez-Buylla, 1993; Morshead et al., 1994; Eriksson et al., 1998). In this review, we focus on the larger of these two niches, the V-SVZ, including recent work highlighting the heterogeneity of NSCs within this niche and the restriction of their potential over time. V-SVZ NSCs are temporally and spatially specified in the mouse during embryonic development and this established specificity persists in the adult brain. The presence of a heterogeneous lineage-restricted pool of stem cells highlights a potential limiting factor when considering NSCs as a hypothetical avenue for therapeutics or postnatal brain repair. Here we discuss the development of adult mouse V-SVZ NSCs as well as their comparison across mammalian species, focus on how and when NSC heterogeneity within the murine V-SVZ is established and address the potential for NSC identity manipulation.

The neural stem cell continuum: Neuroepithelium to radial glia to V-SVZ astrocytes

The brain develops from a sheet of primary ectoderm-derived progenitors collectively termed the neuroepithelium. Neuroepithelial cells (NECs) fold in to form the neural plate, which later invaginates to form the neural tube (Purves, 2012). This transition polarizes the NECs such that the basal side is positioned outward, contacting the pial (outer) surface of the brain, and the apical face is oriented inward, later becoming the ventricular zone (VZ) (Fig. 1). Division occurs at the apical surface, forming the VZ. Following neural tube closure, neuroepithelial cells produce specialized bipolar glial cells called radial glia (RG) that serve dual roles: guiding migrating young neurons, which move long distances along the pial fibers of the RG from the ventricle to the pia (Rakic, 2006) and serving as the progenitors of both neurons and glia until shortly after birth (Anthony et al., 2004; Noctor et al., 2007; Kriegstein and Alvarez-Buylla, 2009). RG cells were first identified as precursor cells when Noctor and colleagues showed that almost all dividing VZ cells during neurogenesis have RG morphology and express markers of RG (Noctor et al., 2002). The transition from NECs to RG includes the acquisition of differentiated glial characteristics including the expression of brain lipid binding protein (BLBP) and glutamate aspartate transporter (GLAST) by neuroepithelial cells (Doetsch, 2003; Kriegstein and Götz, 2003) as well as the loss of tight junctions and gain of adherens junctions (Kriegstein and Götz, 2003). RG also express many intermediate filament (IF) proteins including Nestin, Vimentin and RC2, which have been attributed to NSCs in perinatal or adult brain (Mori et al., 2005) (Fig. 2, see Table 1 for marker expression references). In primates, RG express glial fibrillary acidic protein (GFAP), a marker of astrocytes, but this is not true in the rodent brain (Bignami and Dahl, 1974; Dahl et al., 1981; Pixley and de Vellis, 1984; Cameron and Rakic, 1991).

Like their neuroepithelial precursors, RG retain basal contact with the pial surface of the brain and apical contact with the ventricle. During early cortical development, RG divide symmetrically to expand the stem cell population but in later embryonic stages, they divide asymmetrically (Haubensak et al., 2004; Noctor et al., 2004) to generate an NSC that persists in the VZ and a daughter intermediate progenitor cell (IPC) that migrates outward to form the subventricular zone (SVZ) (Noctor et al., 2008). IPCs then divide in the SVZ without connection to the ventricular or pial surfaces (Noctor et al., 2004). The mammalian cortex consists of layers of projection neurons that are born in an "inside-out" manner (the outer-most neurons are born last) and this patterning has allowed the study of cell fate restriction over time (Molyneaux et al., 2007). VZ NSCs isolated from older ferret embryos (during the generation of superficial layers) can be transplanted to younger embryos (during the generation of deep layers) and only produce neurons in the outer cortical layers (Frantz and McConnell, 1996), suggesting that the cellular competence for specific neuronal production is partially cell-intrinsic. Intriguingly, recent work has shown that prolonging mitosis is sufficient to alter the fate of radial glial cell progeny (Pilaz et al., 2016). Cortical progenitors in mice with altered cell cycle kinetics showed distinct patterns of divisions, suggesting that the timing of division is also a key component of cell fate determination.

Several groups have investigated migration patterns of clonally related cortical cells using replication-incompetent retroviral vectors encoding histochemical reporter genes (Walsh and Cepko, 1988, 1992, 1993; Reid et al., 1995; Ware et al., 1999; McCarthy et al., 2001). As cortical layers are born "inside-out," it was once thought that radially oriented columns existed, with each column surrounding a single RG cell fiber and the same VZ precursor cell contributing to each cortical layer in sequence (Rakic, 1988). Subsequent work illustrated that clonally related cortical cells displayed several migration patterns including non-radial movements and thus, it was proposed that clonally related neurons can migrate along several RG cell fibers (Walsh and Cepko, 1988). Further work by this group suggested that cortical neurons are not fate-specified by lineage (i.e. where the precursor cell is located) but rather, specification occurs post-neurogenesis (Walsh and Cepko, 1992), in contrast with what was later observed with OB progeny derived from V-SVZ progenitors in the mouse (Merkle et al., 2007). However, these cortical lineage-tracing experiments were initiated mid-neurogenesis and may not represent the earliest post-mitotic cells within the developing cortex. Subsequent experiments have suggested that telencephalic neural progenitors' position at the onset of neurogenesis is highly predictive of the dispersal patterns of their cortical progeny (McCarthy et al., 2001). This work suggests that separate progenitor populations have intrinsic factors affecting their interpretation of specific guidance cues, resulting in migration and terminal differentiation close to related cells. Similar work has also been conducted analyzing the fates of cortical interneurons derived from the VZ however, the results are controversial. Some studies found that inhibitory interneurons are produced as spatially organized clonal clusters without random dispersion (Brown et al., 2011) suggesting that lineage relationships are essential for interneuron organization within the cortex. Other studies find the opposite- wide dispersion of clonally related forebrain interneurons and closely clustered unrelated neurons (Harwell et al., 2015; Mayer et al., 2015). Whether lineage is an essential feature in predicting an interneuron's final location or properties within the developing telencephalon is still a possibility that warrants further exploration.

Origin of adult NSCs

Early evidence of RG transforming into astrocytes came from experiments using subpial fluorescent dye injections in newborn ferret brains, when no GFAP$^+$ astrocytes are present. The dye stained RG cell somas in the VZ and over time, as the RG progressively disappeared, dye was found in newly created parenchymal GFAP$^+$ astrocytes (Voigt, 1989). This transformation was also observed using similar methods in rhesus monkeys (Schmechel and Rakic, 1979) and in the murine brain using conditional-reporter mice and neonatal (P0) injections of adenovirus carrying Cre recombinase (Merkle et al., 2004; Spassky et al., 2005). Work in the mouse determined that RG cells give rise to ependymal cells (Spassky et al., 2005) as well as neurons, oligodendrocytes, astrocytes and the adult neural stem cells (NSCs) of the V-SVZ (Merkle et al., 2004). Additional experiments using time-lapse microscopy and *in vivo* cell fate analysis have confirmed this finding (Noctor et al., 2008). Furthermore, transplantation experiments in which RG cells from the embryonic lateral ganglionic eminence (LGE) were placed into the adult V-SVZ showed that these cells were able to efficiently migrate to the OB but cells from the medial ganglionic eminence (MGE) migrated extensively toward the cortex (Wichterle et al., 1999). Although these cells

exhibited unique migratory potentials when transplanted into adult brain, subsequent Cre-lox fate mapping of the embryonic telencephalic neuroepithelium has since determined that the MGE, LGE and the embryonic cortex all generate NSCs that inhabit different parts of the adult V-SVZ (Young et al., 2007). Interestingly, in the postnatal brain most of the VZ compartment is replaced by the ependymal epithelium (Mirzadeh et al., 2008), thus displacing the primary progenitors in the adult brain from the ventricular surface into the SVZ, although the stem cells maintain a specialized apical contact described in the following section. Therefore, the adult germinal niche includes a subventricular compartment as well as a VZ, resulting in the descriptive term V-SVZ (Fuentealba et al., 2012).

Identification of neural stem cells as astrocytes

The V-SVZ surrounds the lateral ventricles (LVs) and is composed of four primary cell types: ependymal cells (E cells), infrequently dividing astrocytes (B1 cells), transit amplifying cells (C cells) and neuroblasts (A cells) (Doetsch et al., 1997). Efforts to elucidate which of these cell types served as the adult NSC included infusions of the antimitotic drug cytosine-β-D-arabinofuranoside (Ara-C) into the LVs of mice, resulting in the elimination of all A and C cells, but the survival of B1 and E cells (Doetsch et al., 1999b). [^{3}H]-thymidine injections followed by electron microscopy (EM) analysis showed that most labeled cells following Ara-C cessation corresponded to type B1 cells. Critically, no ependymal cells were labeled, thus identifying B1 cells as the primary precursors for new neurons generated in the adult murine brain. This work was further supported by Cre-lox fate mapping of RG cells in combination with BrdU labeling which showed that E cells are born between E14–E16 and are derived from RG cells (Spassky et al., 2005). Furthermore, E cells mature and form cilia within the first postnatal week and no evidence of E cell proliferation in adult brain was observed. After cessation of Ara-C treatment, the V-SVZ regenerated from B1 cells within 14 days (Doetsch et al., 1999b). Interestingly, B1 cells resemble astrocytes in both their structural and biologic features, including thick bundles of intermediate filaments positive for GFAP, a light cytoplasm, glycogen granules, gap junctions and dense bodies (Doetsch et al., 1999a; Doetsch et al., 1997) (see Fig. 3 for marker expression profiles). Experimental ablation of GFAP$^+$ cells resulted in reduced numbers of BrdU$^+$ cells within the V-SVZ, diminished neuroblast generation and neuronal loss in the OB (Garcia et al., 2004; Imura et al., 2003; Morshead et al., 2003). Long-term ablation prevented the production of new neurons, highlighting that the removal of GFAP$^+$ cells destroys the ability of the germinal niche to regenerate. Thus, adult V-SVZ NSCs are of glial origin and can be described as being "disguised as astrocytes" (for review, see (Ihrie, 2009)). More recent studies employing flow cytometry and single-cell sequencing have begun to further subdivide this lineage spatially and temporally, identifying both quiescent and activated B1 cells (qNSCs and aNSCs) and developing strategies for the prospective isolation of these cells using cell surface markers.

Cytoarchitecture of the primary germinal zone

The organization of the V-SVZ has been explored at the ultrastructural and microscopic levels using multiple approaches (for recent review of this topic, see (Lim and Alvarez-Buylla, 2016)). The stem cells within this region (type B1 cells) divide infrequently. The ventricular surface neighboring the V-SVZ is lined with multiciliated E cells, which create

an epithelial-like monolayer and maintain the flow of cerebrospinal fluid (CSF) through the ventricles using motile cilia (Sawamoto et al., 2006). Assembly of the V-SVZ cytoarchitecture is dependent on the upregulation of Ankyrin-3 (Ank3) for lateral adhesion in RG cells destined to become ependymal cells (Paez-Gonzalez et al., 2011). When Ank3 is depleted, there is a reduction in neurogenesis, illustrating the importance of intact ependymal layer organization. While the ependymal lining separates most of the niche from the lateral ventricles, type B1 cells contact the ventricle with an extended process that intercalates between the E cells and contains a primary cilium (Doetsch et al., 1999a, 1999b). Their retained ventricular contact exposes B1 cells to many soluble factors that have the ability to modulate NSC activity (Zappaterra et al., 2007; Lehtinen et al., 2011). Critically, B1 apical surface contact is a marker of neurogenic ventricular walls in the adult, as they are absent from the non-neurogenic third ventricle. Interestingly, apical contacts are concentrated in "hot spots" in the anterior-ventral and posterior-dorsal regions of the lateral wall of the LV, as well as in the most anterior portion of the medial wall (Mirzadeh et al., 2008). B1 cells also contact blood vessels (BVs) through a basal process that terminates in a specialized endfoot (Doetsch et al., 1999a). This close interaction with endothelial cells supports the proliferation and self-renewal of V-SVZ progenitor cells (NPCs) (Shen et al., 2004). A second subset of B cells referred to as Type B2 cells exist within the V-SVZ; however, they have a multipolar morphology (Doetsch et al., 1997), are located close to the brain parenchyma, and do not have ventricular contact (Mirzadeh et al., 2008). The role of these cells has yet to be elucidated but, it is possible that B2 cells serve as support cells which promote neurogenesis (Lim and Alvarez-Buylla, 1999). When activated to divide, B1 cells give rise to Type C cells (transit amplifying progenitors), which are thought to divide symmetrically three times before generating migratory neuroblasts (A cells) (Ponti et al., 2013). Subsequently, the A cells may divide one or two more times on the way to the olfactory bulb (OB). Neuroblasts migrate anteriorly as a network of tangentially oriented chains that converge at the anterior dorsal subregion of the V-SVZ to form the rostral migratory stream (RMS) (Doetsch and Alvarez-Buylla, 1996; Lois et al., 1996). This chain migration has also been observed in young primates and in early postnatal human brain (Kornack and Rakic, 2001b; Pencea et al., 2001; Sanai et al., 2011).

Postnatal V-SVZ neural stem and progenitor cells

What are the postnatal destinations of newborn cells?

In rodents, NSCs in the V-SVZ generate large numbers of neuroblasts that migrate along the RMS into the olfactory bulb (OB) where they differentiate into local interneurons. The function of the OB requires a constant influx of newborn neurons that provide plasticity to the processing of olfactory information (Lepousez et al., 2013; Sakamoto et al., 2014a, 2014b). Shortly after new neurons differentiate and integrate to form synapses within OB circuitry, they exhibit long-term synaptic plasticity that is gradually lost with time (Nissant et al., 2009). Newborn neurons in the OB facilitate this plasticity through the formation (and elimination over time) of dendrodendritic synapses with mitral and tufted cells (projection neurons which project their axons to the olfactory cortex to communicate odor information to higher order areas in the forebrain), contributing to flexible odor-associated learning processes (Sakamoto et al., 2014a). The large number of inhibitory synapses on the mitral/

tufted cells has been suggested to permit inhibitory circuits to refine odor representations (Isaacson and Strowbridge, 1998; Egger and Urban, 2006; Lepousez et al., 2013). Neuroblasts from the V-SVZ primarily mature into granule cells (GCs) or periglomerular cells (PGCs) in the olfactory bulb (OB). GCs are GABAergic interneurons and can be subdivided into types based on the location of their cell bodies after integration: the deep, intermediate or superficial layers of the granule cell layer (GCL). Superficial GCs can be further categorized by the presence or absence of calretinin (CalR$^+$) (Price and Powell, 1970). The PGCs also correspond to different subtypes of GABAergic interneurons and can be subdivided into three main non-overlapping subtypes based on marker expression: CalR$^+$, calbindin (CalB$^+$) and tyrosine hydroxylase (TH$^+$) (Kosaka et al., 1995; Kohwi et al., 2007), though the functional roles of these distinct cell types have been less well characterized (see (Kosaka and Kosaka, 2005) for a more detailed discussion). The V-SVZ also generates rare additional cell types, which populate the mitral and plexiform layers and have distinct morphological characteristics (Merkle et al., 2014).

In the adult mouse V-SVZ, it has been estimated that ~10 000 new neurons are generated daily (Alvarez-Buylla et al., 2002). While a great number of newborn neurons reach the OB, roughly half of them are integrated into pre-existing neural circuits while the remaining neurons are eliminated via apoptosis (Lledo et al., 2006). Whether or not a neuron survives appears to be dependent on olfactory sensory experience: sensory deprivation leads to a decrease in new GC neuron survival whereas olfactory learning enhances survival (Petreanu and Alvarez-Buylla, 2002; Rochefort et al., 2002). It is hypothesized that this turnover also contributes to the reorganization of local OB circuits (Sakamoto et al., 2014b), permitting long-term plasticity (Livneh et al., 2014). This is supported by studies showing that blockade of neurogenesis by administration of an anti-mitotic drug results in a variety of olfaction-related behavioral deficits (Breton-Provencher et al., 2009; Lazarini et al., 2009; Moreno et al., 2009; Sultan et al., 2010). Thus, a continuous supply of newborn OB interneurons is critical for the plasticity observed in the mouse olfactory system. Interestingly, upon removal of the OB, V-SVZ proliferation is not immediately affected; at three months post OB bulbectomy, migration continues in the RMS but the number of proliferating cells decreases by half and the number of dying cells doubles (Kirschenbaum et al., 1999). Thus, some intrinsic instructions within V-SVZ NSCs persist for some time regardless of whether their final destination exists. Furthermore, newborn neurons are integrated and replaced at different rates. Prior to postnatal day 35, newborn neurons are integrated by an addition mode to establish the OB circuit, and then new GC integration continues as turnover of old GCs begins (Sakamoto et al., 2014a). Interestingly, there is a preferential integration of newborn neurons into the deep layer of the GCL whereas in the superficial layer, approximately half of all GCs are derived embryonically and are maintained without replacement. Integration and elimination of PGCs also varies by subtype. For example, newborn PGCs are more likely to express CalR than CalB (Sakamoto et al., 2014a) suggesting a unique replacement and/or addition mode for each subtype. The functional roles and physiologic characteristics of each of these subtypes have yet to be determined. Further evidence suggests that this heterogeneity within the OB serves as a continuous source of neurons for serving experience-based circuit demands – that is, newborn neurons have an adaptive functional fate rather than a preset fate based on their final location within

the OB circuit (Livneh et al., 2014). Future work is necessary to investigate cell-type specific contributions to the functional output of the OB, and to explore how patterning specifying the neural stem cell of origin directs the ultimate specification of each interneuron.

During aging, there is a decrease in the number of multipotent progenitors, the frequency of progenitor proliferation, the self-renewal ability of neural stem cells, and the production of olfactory bulb progeny (Molofsky et al., 2006). Furthermore, mice 2 years and older exhibit a large reduction (50%–75%) in the total number of proliferating cells within the V-SVZ (Luo et al., 2006) and a major reduction (75%) in neuroblasts migrating to the olfactory bulb, leading to deficits in fine odor discrimination (Enwere et al., 2004). The contribution of V-SVZ NSCs to this age-related proliferative decline has recently been assessed; while the overall number of NSCs declines in aged mice (2 years) when compared with juvenile mice (3 months), the decline appears spatially consistent such that the olfactory bulb progeny subtypes are observed at the same ratios over time (Luo et al., 2006).

How is NSC activity regulated?

It is thought that a feedback mechanism exists to maintain a balance between NSC proliferation and the number of progeny already generated. As the intermediate domain of B1 cells is in close contact with type C and A cells, this permits direct feedback mechanisms from progeny to NSCs. Expression of Notch ligands has been observed throughout the V-SVZ; specifically, Delta1 and Jagged1 have been found on C and A cells (Irvin et al., 2004; Aguirre et al., 2010). Canonical Notch signaling induces the expression of transcriptional repressor genes such as *Hes1*, which represses the expression of proneural genes and promotes the maintenance of the NSC phenotype (Imayoshi et al., 2010). *Hes1* expression has been shown to repress levels of *Ascl1* (*Mash1*) expression, which is highly expressed in C cells. Conversely, *Ascl1* has been shown to promote the expression of Notch ligands (Kopan and Ilagan, 2009), suggesting a putative feedback mechanism via lateral inhibition between C and B1 cells by direct cell-cell contact. Interestingly, conditional deletion of a downstream Notch effector (*Rbpj*) results in premature differentiation into neurons and depletion of the NSC pool in both embryonic and adult brain, further supporting the contribution of Notch signaling to NSC maintenance. Single-cell RNASeq has confirmed a role for Notch signaling in regulating NSC quiescence (Llorens-Bobadilla et al., 2015). Transcripts for the *Notch2* receptor and the Notch target gene *Lfng* were enriched in qNSCs whereas the Notch ligands *Dll1* and *Dll3* were highly expressed in aNSCs. To further investigate the role of Notch, investigators treated qNSCs (GLAST$^+$/CD133$^+$/EGFR$^-$) with the γ-secretase inhibitor DAPT, resulting in an increase in protein translation and an increase in the proportion of activated cells. These data illustrate that Notch signaling is a key regulator of NSC quiescence and inhibition of Notch is sufficient to drive qNSCs into activation. It is not clear whether localized differences in activation of this pathway exist within subregions of the V-SVZ.

The V-SVZ across species

In addition to studies in rodents, several other mammalian species with varying lifespans and cortical complexity have been studied, including cow (Pérez-Martín et al., 2003; Rodriguez-

Pérez et al., 2003), rabbit (Luzzati et al., 2003), sheep (Brus et al., 2013; Low et al., 2013) and primate (McDermott and Lantos, 1989, 1990; Kornack and Rakic, 2001b; Sawamoto et al., 2011). Evidence for proliferative cell types within primate brain came from work in marmoset V-SVZ (McDermott and Lantos, 1989, 1990), including the finding that the number of proliferating cells decreases with time. In marmosets, the posterior V-SVZ is organized as three layers: an ependymal layer, a hypocellular gap and a ribbon of astrocytes (Sawamoto et al., 2011), similar to what has been observed in human brain (Sanai et al., 2004). Similar to the rodent V-SVZ, PSA-NCAM+ neuroblasts are present along the lateral wall of the ventricles, forming chains in the adult marmoset V-SVZ. Studies conducted in macaque brain have shown structures homologous to the V-SVZ and RMS in marmoset and human with an ependymal layer, a hypocellular gap layer and an astrocyte ribbon (Gil-Perotín et al., 2009). Proliferating cells from the macaque V-SVZ are contained within a RMS (i.e. not entering the nearby parenchyma) but their progeny do not differentiate until 11–14 weeks after birth (Kornack and Rakic, 2001b), contrasting to 4 weeks in mice (Petreanu and Alvarez-Buylla, 2002). These systems may more closely resemble the human brain, enabling studies of neural regeneration.

The human V-SVZ

The developing human SVZ has an expanded area basal to the SVZ, referred to as the outer SVZ (OSVZ), which is thought to contribute to the increased size and complexity of the human brain. The cells within the OSVZ are termed basal or outer RG (oRG) because of their resemblance to RG cells (Hansen et al., 2010). Recent work comparing the transcriptomes of human and mouse RG cells discovered a number of genes that are preferentially expressed in human oRG (basal) or apical RG and do not have mouse orthologs (Florio et al., 2015). Inducing expression of one of these genes, ARHGAPIIB, in mouse embryonic neocortex led to an increase in oRG progenitor proliferation, cortical plate area, and some gyrification (a feature not observed in the normally lissencephalic mouse brain). This work suggests that expression of ARHGAPIIB may have contributed to the evolutionary expansion of the human neocortex. Expansion of the progenitor pool across species is a potential mechanism for generating a more heterogeneous NSC pool, and should be considered when examining heterogeneity in other species.

In rodents and primates, neuroblasts migrate from the V-SVZ in chains along the RMS (Lois et al., 1996; Peretto et al., 1997; Kornack and Rakic, 2001b; Pencea et al., 2001) however, a similar process has not been observed in adult human brain (Sanai et al., 2004), suggesting that adult human neuronal precursors may migrate as individual cells or migration from the V-SVZ to the OB does not occur in adult humans. Moreover, the extent of neurogenesis in adult human brain remains a topic of debate (Curtis, 2007; Sanai et al., 2007).

Studies on the infant human V-SVZ found that the structure of V-SVZ differs over time. The infant V-SVZ lacks an astrocyte ribbon and a gap layer, but has cells with processes expressing GFAP and vimentin lining the lateral ventricular wall, similar to fetal brain (Zecevic et al., 2005; Guerrero-Cázares et al., 2011; Sanai et al., 2011). A dense network of elongated putative migratory neurons was discovered in the infant V-SVZ. These cells possessed ultrastructural characteristics of young migrating neurons (free ribosomes,

microtubule networks and leading processes containing growth cones) (Lois et al., 1996) and expressed the immature neuronal markers DCX, TuJ1 and PSA-NCAM. Between 6 and 18 months of age, the V-SVZ progressively loses this network of putative migratory neurons and forms structural characteristics observed in adult human V-SVZ, including an astrocyte ribbon and hypocellular gap layer (Sanai et al., 2011). Specifically, a robust stream of tangentially migrating immature neurons (DCX[+] chains) populates the space that precedes the postnatal human gap layer, but these chains gradually decrease during the first 6 months of life suggesting that human V-SVZ proliferation decreases rapidly after birth. Only a small number of proliferating cells were observed in adolescent and adult specimens included in these studies, contrasting with previous reports (Curtis et al., 2007). A third study identified an RMS in human fetal brain specimens, but found few DCX[+] neuroblasts in the same region when analyzing adult human brain specimens (Wang et al., 2011). These studies suggest a robust period of neurogenesis and neuroblast migration that persists into postnatal human life, but is restricted to early infancy. Another intriguing discovery was the presence of an additional migratory stream of DCX^{+} cells that branched off of the proximal limb of the RMS and ended in the ventromedial prefrontal cortex (VMPFC), termed the medial migratory stream (MMS). The MMS was observed in 4–6 month specimens, but not in specimens between 8 and 18 months of age. The MMS appears to be a unique feature of humans which has not to date been reported in other vertebrate species. The function of the VMPFC in children has not been elucidated but in the adult human brain, it is activated during certain cognitive tasks including spatial conceptualization and the emotional processing of visual cues (Szatkowska et al., 2004; Longe et al., 2009). Of note, this region is functionally inactivated in patients with advanced Alzheimer's disease (Herholz et al., 2002), suggesting important implications involving processes that change with age. Thus, the MMS is hypothesized to serve as a mechanism of delayed postnatal plasticity (Sanai et al., 2011).

Positional identity and heterogeneity of NSCs in the V-SVZ

Recent work has illustrated that subdomains of NSCs within the postnatal V-SVZ generate specific progeny in the olfactory bulb (Merkle et al., 2007; Ventura and Goldman, 2007; Young et al., 2007; Merkle et al., 2014). The ability of an NSC to create progeny subtypes correlated with its location within the V-SVZ is termed a cell's positional identity (Ihrie et al., 2011). This identity is based on transcription factor expression, the final location and morphology of progeny interneurons within the olfactory bulb, and their marker expression upon terminal differentiation. For example, ventral NSCs produce mainly deep granule cells (GCs) and CalB^{+} periglomerular cells (PGCs), while dorsal NSCS mainly produce superficial GCs and TH[+] PGCs. Thus, NSCs are not a homogeneous mix of equivalently plastic cells, but rather a spatially organized set of restricted, diverse populations (Lledo et al., 2006; Merkle et al., 2007; Young et al., 2007; Ihrie et al., 2011; Merkle et al., 2014). To date, it is less clear if we can ascribe a specific functional identity within the concept of positional identity. Importantly, heterotopic transplantation experiments have shown that these cells maintain their positional identity and continue to produce the types of progeny expected from their original location (i.e. cells derived from the ventral V-SVZ transplanted to the dorsal V-SVZ still produced deep GCs and CalB[+] PGCs, and not superficial GCs nor

TH[+] PGCs) (Merkle et al., 2007). This suggests that identity is mostly a cell-intrinsic feature and that cells "remember" positional cues experienced during the establishment of patterns in the developing brain or that some of these cues persist. There is also a temporal component to V-SVZ NSC fate: it has been determined that in the early postnatal mouse brain, there is a shift from PGC production to primarily GC production (De Marchis et al., 2007) and PGC subtypes are generated and eliminated at different rates (Kohwi et al., 2007). Furthermore, specific neurochemical PGCs within the OB were found to be preferentially produced at different ages: for example, CB^{+} cells are mainly produced in juvenile animals, CR^{+} cells are more frequently produced in adults, and TH^{+} cells are generated at a low level in neonatal brain but increase in the adult (De Marchis et al., 2007). This age-dependent change in PGC production from V-SVZ progenitors is a cell-intrinsic feature as determined by heterochronic transplantation studies (De Marchis et al., 2007). Given these spatial and temporal transplantation data, it is likely that there is a progressive restriction of developmental potential similar to what has been observed during cortical development. While positional identity has been most extensively characterized in mice, it has also been explored in the marmoset brain using IF analyses of transcription factor (TF) expression. Primarily, these data suggest that there is a spatial heterogeneity, similar to that observed in the murine brain, but that it is only observed in the early postnatal period and the diversity of V-SVZ NSCs may decline with age (Azim et al., 2013). It remains to be determined whether positional identity exists in the human V-SVZ and whether primate and human-specific structures like the MMS are derived from specific progenitors.

How is NSC fate encoded?

Recent work in mouse utilizing time lapse microscopy on the developing cortex has illustrated that the multipotent state of NSCs is correlated with oscillatory expression of several fate-determination factors including the basic helix–loop–helix TFs Ascl1/Mash1 (neurons), Hes1 (astrocytes), and Olig2 (oligodendrocytes), while the differentiated (committed) state correlates with sustained expression of a single factor (Imayoshi et al., 2013). This work warrants further exploration into what controls the expression of these factors over a fine time scale and how signaling inputs are integrated to determine the fate of a cell. There is evolving evidence to suggest that epigenetic mechanisms, specifically chromatin-based transcriptional regulation, are critical to maintaining NSC function and potential over time (Lim et al., 2009; Gonzales-Roybal and Lim, 2013). For example, the developmental switch from neurogenic divisions to gliogenic divisions is regulated in part by DNA methylation: the promoters of astrocytic genes (GFAP, S100β) possess STAT responsive elements that remain methylated (inactive) until ~E17 when the switch occurs (He et al., 2005). As time progresses during development, methylation on astrocyte promoters is lost and allows for STAT3 binding and astrocyte production to begin, though the exact mechanism is not well understood (Fan et al., 2005; He et al., 2005). A recently discovered epigenetic modulator of NSCs is that of mixed-lineage leukemia 1 (Mll1), a chromatin-modifying factor of the trxG family that activates gene expression (Lim et al., 2009). Mll1 is expressed throughout the V-SVZ; when it is deleted, NSCs remain proliferative and are efficient at gliogenesis but the production of neurons is severely impaired and the neurogenic TF Dlx2 is not expressed. Thus, fate-specifying TFs can be direct targets of chromatin-modifying factors and affect NSC cell fate decisions.

Additionally, the long non-coding RNA Pnky has been shown to regulate neurogenesis (Ramos et al., 2015). Depletion of Pnky results in an increase in commitment to neuronal fates apparently by increasing the transit amplifying pool and subsequently causing a depletion of V-SVZ NSCs. Still, further investigation is needed to elucidate how a cell's identity is progressively restricted and how these regulatory elements might affect positional identity in the adult.

How is neural stem cell heterogeneity established?

The regionalization of stem cell niches is a common developmental feature that allows progenitor cells in different regions to become different subtypes of cells, as in the developing spinal cord, where distinct dorsal-ventral boundaries of TF expression are generated by gradients of morphogens (Ericson et al., 1997a; Ericson et al., 1997b; Briscoe et al., 1999; Hochstim et al., 2008). Regionalization also occurs in the developing telencephalon (Campbell, 2003; Puelles and Rubenstein, 2003; Guillemot, 2005; Long et al., 2007), which later contributes to the V-SVZ. The lateral ganglionic eminence (LGE) in the developing telencephalon expresses a set of TFs including Dlx1/2 (Stühmer et al., 2002), Mash1 (Parras et al., 2004), Gsh2 (Young et al., 2007), Pax6 (Hack et al., 2005; Kohwi et al., 2005) and Sp8 (Waclaw et al., 2006), which are also expressed in the lateral wall of the adult V-SVZ.

Recent evidence has suggested that region-specific TF expression underlies the generation of distinct populations of OB interneurons. For example, a microdomain of anterior ventral V-SVZ cells that express Nkx6.2 have been shown to generate novel OB interneuron subtypes including deep-projecting GCs, shrub GCs, perimitral cells and satellite cells (Merkle et al., 2014). Also within the ventral V-SVZ, the TF Nkx2.1 is expressed and its progeny give rise to GCs located close to the OB core (deep layer) (Merkle et al., 2014; Delgado and Lim, 2015). It has been experimentally shown that Nkx2.1^{+} cells within the embryonic medial ganglionic eminence give rise to Nkx2.1[+] postnatal/adult NSCs within the ventral V-SVZ, contributing to regional heterogeneity (Delgado and Lim, 2015). Specific regional TF expression may be partly due to signaling pathways active during development, such as the Shh pathway, a pathway that is essential for the developmental patterning of the nervous system (McMahon et al., 2003; Fuccillo et al., 2006). Shh signaling is highly active within the ventral V-SVZ, resulting in high expression of the downstream transcriptional activator Gli1 and subsequently, progeny located deep within the OB (Ihrie et al., 2011). The homeobox gene Emx1 is expressed primarily in the developing pallium and in dorsal V-SVZ NPCs postnatally (Willaime-Morawek et al., 2006; Kohwi et al., 2007; Young et al., 2007). Emx1^{+} NPCs generate CalR[+] superficial GCs and PGC interneurons (Kohwi et al., 2007). Another TF of note is SP8, which is expressed in the developing dorsolateral ganglionic eminence, cortex and septum (Waclaw et al., 2006; Li et al., 2011). SP8^{+} NPCs mostly produce CalR[+] interneurons in the OB. Pax6, a TF expressed in the developing pallium, has been shown to be essential for the production of dopaminergic (TH[+]) PGCs and superficial GCs within the OB (Kohwi et al., 2005). These findings emphasize the link between TF patterning in the developing brain and cell-type specification postnatally. The transcriptional heterogeneity of these NPCs suggests that they are restricted to specific fates early in embryonic development. Recent single cell RNASeq analyses on adult V-SVZ NSCs

likewise indicate that NSCs may be "primed for lineage differentiation" with specific TFs exhibiting inverse correlations in expression levels between NSC subgroups (Llorens-Bobadilla et al., 2015). These findings highlight the heterogeneity of V-SVZ NSCs and the fate-restricted nature of these NSCs under normal conditions within the adult brain.

When is neural stem cell heterogeneity determined?

Recently, Fuentealba and colleagues determined that postnatal neural stem cells are generated during embryonic development, sharing common progenitors with cells of the cortex, striatum and septum (Fuentealba et al., 2015). Using a combination of retroviral "barcoding" and nucleoside analogs in reporter mice, they revealed that embryonic cells dividing between E13.5 to E15.5 give rise to B1 cells that remain mainly quiescent until they are reactivated postnatally. Surprisingly, the authors did not find many clones of B1 cells and OB interneurons that had overlapping tags, suggesting that B1 cells that become activated postnatally are eventually exhausted and new neurons then arise from distinct B1 cell pools. The regional specification of these progenitors is established in embryonic development and can be detected as early as E11.5 in the mouse. For instance, deep GCs, CalB$^+$ PGCs and Type 2 GCs produced postnatally were found to be clonally related to striatal cells generated during embryonic development. Thus, fate restriction of adult NSCs occurs when they are generated in the embryo and common TF patterning is likely due to sharing common progenitors with forebrain neurons. Evidence utilizing the 'Confetti' multicolor Cre reporter crossed to the Glast-Cre^{ERT2} mouse line supports these findings (Calzolari et al., 2015). While adult NSCs rapidly produced multiple clones, after 4–6 months only a few inactive mature clones remained, providing evidence for exhaustion of individual NSCs. Interestingly, this group also found that the majority of newborn neurons presented in the deep layer of the GCL in the OB. Considering these new data, it is likely that full plasticity of NSCs is restricted to a short window of time during embryonic development.

Can NSC fate be re-specified?

As previously mentioned, positional identity is partially cell-intrinsic (Merkle et al., 2007) but *in vivo* re-specification of cell fate has been observed in adult NSCs. One example is the manipulation of Gli1, which is expressed in approximately 25% of V-SVZ NSCs (Ahn and Joyner, 2005). NSCs within the dorsal V-SVZ could be re-programmed to resemble a ventral cell fate through ectopic modulation of the Sonic Hedgehog (Shh) pathway and forced Gli1 expression in the dorsal region (Ihrie et al., 2011). This has implications for therapeutic applications in humans; if the same regionalization occurs in human brain, the generation of specific neuronal subtypes following injury may be contingent on whether the region-specific NSCs that produce them are still present in the adult. NSC regionalization could also change after injury or could be manipulated to improve treatment. In one recent example following experimental demyelination in mice, Gli1$^+$ NSCs were recruited to re-populate lesions in the corpus callosum and generate differentiated glial progeny (Samanta et al., 2015). However, unlike the parent NSCs, the recruited progeny are not Gli1$^+$ once they enter the corpus callosum. Inhibiting Gli1 genetically or pharmacologically led to an increase in the recruitment of V-SVZ NSCs to demyelinated corpus callosum, but not healthy corpus callosum. These data implicate Gli1 as a potentially useful target for re-myelination therapies and highlight that NSC fate can be manipulated. However, another

group recently determined that within the subcallosal dorsal V-SVZ, there is a temporally transient period of high Gli1 expression that coincides with the production of a large number of oligodendrocytes (Tong et al., 2015). V-SVZ NSCs may respond differently to extrinsic signals in an injury setting (Samanta et al., 2015) vs. the healthy niche (Tong et al., 2015). Alternatively, injury may drive reversion to a cell state resembling that seen in early postnatal brain, thereby reactivating a program that is transient in uninjured brain.

Single cell analyses of NSC heterogeneity

Within adult mammalian stem cell niches, both quiescent (qNSCs) and activated NSCs (aNSCs) exist (Li and Clevers, 2010). Previously, distinguishing between these cells was difficult due to a lack of markers identifying different stages in the NSC lineage. Specifically, distinguishing between adult NSC astrocytes and niche astrocytes has been a major limitation as both express GFAP (Doetsch et al., 1997; Doetsch et al., 1999a). Until recent studies utilizing fluorescent flow cytometry techniques, attempts to sort cells of the V-SVZ NSC lineage utilized markers that are common to several stages within the lineage such as Nestin (Kawaguchi et al., 2001), Sox1 (Barraud et al., 2005), LeX (CD15) (Capela and Temple, 2002), Prominin1 (CD133) (Corti et al., 2007) and EGFR (Doetsch et al., 2002) without combining multiple markers, complicating the isolation of specific NSC populations. Recently, using fluorescence activated cell-sorting (FACs) methodologies, Pastrana and colleagues prospectively isolated adult neural stem cells and their progeny via the combination of transgenic mice, cell surface markers and fluorescent ligands (Pastrana et al., 2009). Researchers were able to sort V-SVZ populations of niche astrocytes (GFAP$^+$EGFR$^-$CD24$^-$), activated stem cell astrocytes (GFAP$^+$EGFR$^+$CD24$^-$), transit amplifying (Type C) cells (GFAP$^-$EGFR$^+$CD24$^-$) and neuroblasts (GFAP$^-$EGFR$^-$CD24^{low}). More recent work by this group suggests that B2 cells (parenchymal astrocytes) lack CD133 expression (Codega et al., 2014). To further assess the stem-like properties of purified populations (i.e. self-renewal and multipotency), the sorted populations were cultured as neurospheres. Only activated NSC astrocytes (GFAP$^+$EGFR$^+$) and transit amplifying cells (GFAP$^-$EGFR$^+$CD24$^-$) were able to form neurospheres *in vitro*, indicating that EGFR$^+$ expression correlates with the ability to form neurospheres in culture (Pastrana et al., 2009). In vivo, the aNSC population (GFAP$^+$EGFR$^+$CD24$^-$) was eliminated by antimitotic treatment (AraC) and reappeared with the first set of dividing cells that regenerated the V-SVZ, approximately 12 h post cessation of anti-mitotic infusion into the brain (Pastrana et al., 2009). The *in vivo* data corroborate the *in vitro* analysis in that GFAP$^+$EGFR$^+$CD24$^-$ cells are activated B1 NSCs that are capable of self-renewal, are multipotent and are able to re-populate the niche post-injury.

Codega et al. expanded on this work and explored the properties of qNSCs within the niche (Codega et al., 2014). The authors determined that qNSCs are CD133$^+$ and Nestin$^-$, but upregulate both Nestin and EGFR when activated. Interestingly, during embryonic development Nestin expression is regulated in a cell-cycle-dependent manner (Sunabori et al., 2008). While Nestin expression has been considered a marker of NSCs in both embryonic and adult brain (Lendahl et al., 1990; Imayoshi et al., 2011), these findings highlight that Nestin expression is dynamically regulated and may not be reliable as a single marker of all NSCs. Further, Nestin-CreER mice, frequently used to target the V-SVZ

broadly, also cause recombination in ependymal cells (Walker et al., 2010). qNSCs also appear to be largely dormant *in vivo*, generating olfactory bulb progeny with slower kinetics than aNSCs and appearing negative for the proliferation markers Ki67 and MCM2 (Maslov et al., 2004). In contrast, aNSCs (GFAP$^+$CD133$^+$EGFR$^+$) have a fast cell cycle and are highly neurogenic *in vivo* (Ponti et al., 2013). A similar study conducted by Mich and colleagues utilized flow cytometry techniques and cell surface markers to identify qNSCs and neurosphere-initiating cells (NICs) in the adult mouse V-SVZ (Mich et al., 2014). These NICs were highly mitotic but short-lived *in vivo* and can be characterized as Glast^{mid}EGFR^{high}PlexinB2^{high}CD24$^{-/low}$O4/PSA-NCAM$^{-/low}$Ter119/CD45$^-$ (GEPCOT) cells. In contrast, qNSCs (termed pre-GEPCOT cells) could not form neurospheres and were characterized by expressing higher GLAST, lower EGFR and PlexinB2 than GEPCOT cells and co-expressing Slc1a3, GFAP, Sox2 and Gli1. The authors used an alkylating agent, temozolomide, rather than AraC to eliminate dividing cells within the V-SVZ and found that GEPCOT cells were ablated while pre-GEPCOT cells survived treatment to re-populate the V-SVZ. These studies introduced the utility of flow cytometric assays for V-SVZ NSC cell sorting by lineage and advanced our knowledge both of cell surface markers for cell isolation and of properties differentiating aNSCs and qNSCs.

Although the above mentioned cell sorting studies prospectively isolate quiescent and activated NSCs from the V-SVZ, they did not permit the study of cell cycle phases at the single cell level. Use of a DNA dye (Vybrant Dye Cycle) to isolate cells within each purified population (i.e. whether a cell was in G_0/G_1 or in S/G_2-M) did reveal that EGFR$^+$ populations were more frequently in S/G_2-M phases than EGFR$^-$ populations (Pastrana et al., 2009). Recently, a protocol utilizing FUCCI (fluorescence ubiquitination cell cycle inhibitor) mice tested whether V-SVZ cells were in G_1 phase of the cell cycle vs. S/G_2/M (Daynac et al., 2015). This sorting strategy has potential applications which include the detection of NSC division, as the fluorescence intensity in cells from FUCCI mice increases when a cell starts to divide (Roccio et al., 2013). Single cell transcriptomic methodologies have also recently confirmed several molecular hallmarks of NSCs and have contributed to the discovery of a marker that separates parenchymal astrocytes from qNSCs (Llorens-Bobadilla et al., 2015). The tetraspanins CD9 and CD81 are more highly expressed in qNSCs than in in parenchymal astrocytes in the adult murine brain. Additionally, single cell RNA-seq on adult V-SVZ NSCs has identified a dormant NSC subpopulation that expresses lineage-specific TFs during homeostasis and suggested that these NSCs enter a 'primed-quiescent' state prior to activation (termed qNSC2). Key genes separating qNSCs and qNSC2s were involved in protein synthesis, implying that translational activation is an event that marks NSCs exiting the inactive state.

Recently, single cell RNA-seq combined with weighted gene co-expression network analysis (WGCNA) has identified potential signals to activate dormant NSCs within the V-SVZ (Luo et al., 2015). Although controversy remains regarding the stemness of ependymal cells, single cell RNA-seq discovered that ependymal cells express receptors for vascular endothelial growth factor (VEGF) 2015 (Luo et al., 2015). Administration of VEGF into the lateral ventricles seems to activate quiescent CD133$^+$ cells within the lateral ventricles of the adult brain. Additionally, administration of both VEGF and basic fibroblast growth factor (bFGF) at postnatal day 7 seemed to activate quiescent CD133$^+$ cells in the non-neurogenic

fourth ventricle. From these and other data (Coskun et al., 2008), it has recently been hypothesized that B1 cells are derived from E cells however, given the recent findings that the embryonic origins of adult B1 NSCs are RG cells (Fuentealba et al., 2015), it is unlikely that E cells serve as precursors to postnatal B1 cells. The limited number of cells sampled in these RNA-seq experiments (8 E cells, 2 B cells) and the different ages of mice used for the experiments potentially confound the interpretations of the data. The inclusion of more cells along with the use of bioinformatics tools developed to statistically quantify single-cell gene expression along continuous developmental trajectories will likely assist in resolving these questions (Bendall et al., 2014; Levine et al., 2015; Shin et al., 2015). Use of one such tool, Waterfall, uncovered molecular cascades underlying adult neurogenesis processes within the SGZ of the hippocampus, including indications that qNSC activation is associated with the downregulation of genes involved in transducing local environmental cues and the upregulation of genes encoding ribosomal subunits (priming of protein synthesis machinery) (Shin et al., 2015) Going forward, this method could easily be applied to transcriptomic studies of neurogenesis within the V-SVZ.

Single-cell analyses including flow cytometry techniques and RNA-seq have permitted high-throughput data collection and information that otherwise would not be available with standard methodologies and thus, will serve as an effective new tool for the identification of factors involved in specifying specific neuronal or glial fates of NSCs or triggering the activation of quiescent NSC populations within the V-SVZ. Challenges going forward include the generation of tools for the automatic discovery of cell populations and methods to determine what constitutes a population of cells vs. varying cellular transition states (i.e. activation vs. quiescence).

V-SVZ and other sources of new neurons and glia: Potential for brain repair?

Are other regions neurogenic or gliogenic?

In addition to the V-SVZ, the subgranular zone (SGZ) of the dentate gyrus of the hippocampus is a major neurogenic niche. Adult hippocampal neurogenesis generates granule cells in the dentate gyrus (DG), which are the excitatory principal neurons receiving input from the entorhinal cortex and giving output to pyramidal cells in area CA3 of the hippocampus. It appears that adult SGZ NSCs come from the ventral hippocampus during late gestation and subsequently re-locate to the dorsal hippocampus (Bannerman et al., 2004; Li et al., 2013). In young adult rats, approximately 9000 new neurons are born daily in the DG representing 6% of the total granule cell population each month, suggesting a critical role for newborn cells in hippocampal function (Cameron and McKay, 2001). In mice, it has not yet been shown that newborn GCs in the hippocampus have a positional identity. Adult hippocampal NSCs (type-1 cells or radial glia-like cells) produce granule neurons which then migrate a short distance to their final destination. These cells appear uniform, and there does not appear to be transcriptional heterogeneity within SGZ NSCs, but they do respond to diverse and distinct signals (see (Christian et al., 2014) for review) suggesting the possibility of functional heterogeneity. Support for this concept includes a functional separation of the dorsal and ventral hippocampus where the dorsal portion is responsible for cognitive

functions (declarative memories) while the ventral portion is primarily involved in stress, emotion and affect (for a detailed review, see (Fanselow and Dong, 2010)). Moreover, hippocampal neurogenesis is thought to be important in many correlates of cognition including pattern separation, facilitating recall of information, avoiding interference of old and new memories as well as mood regulation (see (Zhao et al., 2008) and (Deng et al., 2010) for detailed reviews).

Substantial neurogenesis has been detected within the dentate gyrus in adult human brain (Eriksson et al., 1998; Spalding et al., 2013) whereas, determining whether neurogenesis within the adult human V-SVZ exists and where postnatal V-SVZ progeny integrate has been challenging (discussed below). ^{14}C-dating strategies have estimated that 700 new neurons are produced in each hippocampus per day in adult humans, yielding an annual turnover of 1.75% of the renewing neuron population (Spalding et al., 2013), a much smaller turnover than that in rodent brain but important when considering the potential for brain repair (discussed below). This technique was also used to analyze post mortem olfactory bulb genomic DNA from adult humans and suggests a complete lack of new neurons being added to the olfactory bulb in adults (Bergmann et al., 2012). However, it appears that adult-born neurons generated from the V-SVZ may integrate within the striatum (adjacent to the lateral face of the V-SVZ) (Ernst et al., 2014), suggesting that humans have a unique location in the brain where new neurons integrate throughout life. Given the probable decreased reliance on olfaction in humans and the numerous ascribed functions of hippocampal neurogenesis, it is not surprising to think that human OB neurogenesis in the adult brain is minimal or absent whereas hippocampal neurogenesis remains abundant postnatally. Although the existence of extensive human OB neurogenesis is debated, the data indicating a contribution to the striatum present an exciting putative unique pattern of neurogenesis from the V-SVZ in the adult human brain.

Postnatal gliogenesis has also been demonstrated. Recent work has shown that Nestin$^+$ NSCs in the V-SVZ continue to produce astrocytes in the corpus callosum and RMS in adult mice (Sohn et al., 2015). In contrast, debates regarding the existence of cortical neurogenesis in the adult brain are ongoing. In vitro experiments suggest that astrocytes have a "global" ability to form neurospheres when cultured with epidermal growth factor and fibroblast growth factor (Laywell et al., 2000). Specifically, cortical astrocytes in the early postnatal period (< P11 in mouse) have demonstrated multipotency in culture conditions, suggesting a transient period of astrocyte multipotency until the cells acquire the genetic program of mature astrocytes (Cameron and Rakic, 1991). Many groups have not found supporting evidence for this feature (Kornack and Rakic, 2001a; Ehninger and Kempermann, 2003; Bhardwaj et al., 2006), while others have determined that cortical neuron production occurs in adult animals but at an extremely low rate (Huang et al., 1998; Gould et al., 2001; Bernier et al., 2002; Luzzati et al., 2003; Dayer et al., 2005; Inta et al., 2008;). Studies reporting ongoing cortical neurogenesis conveyed the presence of adult-born cortical neurons using BrdU incorporation and neuronal markers, labeling techniques with some key limitations. For example, BrdU primarily labels cells in S-phase, possibly underestimating the number of newborn cells. However, BrdU can also be taken up by neurons undergoing DNA repair (Burns et al., 2007). Another important consideration is overlapping marker expression, necessitating staining with multiple markers (reviewed in (Feliciano and Bordey, 2013)).

Many markers used to identify cells within the NSC lineage are neither static nor unique to progenitor populations- expression patterns often overlap during transition within the lineage. Thus, it is difficult to assess exclusive markers of stem cell populations and experiments require the use of multiple markers to differentiate between cell types.

Can these cells be used in brain repair?

NSC positional identity indicates that under normal conditions, NSCs produce specific progeny that will integrate into defined circuits within the olfactory bulb (Merkle et al., 2007). This poses a challenge when considering the concept of utilizing NSCs for regenerative therapies- if NSCs are intrinsically programmed to create specific progeny cells, their use may be limited, and therapeutics would be reliant on the NSCs that exist within the brain at a given time. Furthermore, the addition of new neurons in the postnatal mouse brain is limited to the OB and the dentate gyrus of the hippocampus. NSC grafts into non-neurogenic brain regions such as the cortex and striatum do not result in neurogenesis under normal physiologic conditions (Herrera et al., 1999). In some injury models, the intrinsic lineage specification of NSCs is not altered even when recruited to the site of damage, suggesting that NSCs could be misallocated in an injured state (Liu, 2009). It has been demonstrated that injury can induce the V-SVZ to produce astrocytes that migrate to the site of damage (Benner et al., 2013). Thus, certain local or environmental cues can trigger an increased production of cells from the postnatal V-SVZ and this may be critical to recovery from injury. Oligodendrocyte production has been observed in the adult corpus callosum and striatum, but these newborn cells are much fewer in number than neuroblasts (Menn et al., 2006; Jackson and Alvarez-Buylla, 2008; Gonzalez-Perez and Quiñones-Hinojosa, 2010; Gonzalez-Perez and Alvarez-Buylla, 2011). Recent findings demonstrating a wave of early postnatal oligodendrogenesis in the murine dorsal V-SVZ suggest that this capacity may be present, but decline in the early postnatal period (Tong et al., 2015). The production of oligodendrocytes is an important process to investigate, as these cells have the potential to remyelinate neurons within the mature CNS in response to demyelinating lesions (Picard-Riera et al., 2002; Menn et al., 2006; Samanta et al., 2015).

The restriction of an NSC's intrinsic potential over developmental time may not be as limiting as was once understood- recent work has demonstrated that forced expression of TFs can re-specify cells to an altered fate (Chen et al., 2012; Niu et al., 2013). For example, the expression of the TF Sox2 is sufficient to reprogram striatal astrocytes into proliferative neuroblasts in the adult mouse brain, and mature integrated neurons can be generated when appropriate factors permit the trans-differentiated cells to survive (Niu et al., 2013). Additionally, forced expression of the gene Neurogenin2 within V-SVZ NSCs cultured from neonatal rat brain and subsequent transplantation into the adult dentate gyrus allows their maturation into glutamatergic neurons that resemble mature dentate gyrus granule neurons (Chen et al., 2012). Furthermore, early postnatal forebrain V-SVZ progenitors have been shown to adapt to new environments upon heterotypic transplantation in rats without forced expression of a TF⁻ when transplanted into the cerebellum, approximately 78% of V-SVZ progenitors differentiated into oligodendrocytes, interneurons and cerebellar-specific velate astrocytes and Bergmann glia with the remaining 22% retaining a progenitor-like or unspecific morphology (Milosevic et al., 2008). It is likely that the transplant host

environment plays a key role in the ability of a transplanted progenitor to adapt and differentiate into region-specific cells. Given that the cerebellum is both a neurogenic and gliogenic region during the early postnatal period in rodents, it is possible that the host environment provided cues to the transplanted cells as to their terminal position and differentiation status. When considering the potential for brain repair, the hippocampus is also highly relevant as it remains as a source of newborn neurons in the adult human brain (Eriksson et al., 1998; Spalding et al., 2013). While these findings suggest the potential for re-specification and the use of NSCs for brain repair, differences across species must be taken into careful consideration. While both the murine V-SVZ and SGZ retain relatively robust proliferative activity in the mature mouse, proliferation in the postnatal human SGZ occurs to a much lesser extent than in mice (Spalding et al., 2013) and robust proliferation in the V-SVZ appears limited to early postnatal life which may further limit the potential for repair (Sanai et al., 2004; Sanai et al., 2011). This decline in proliferative activity, and the low levels of postnatal neurogenesis observed in the adult human V-SVZ, may in part be a consequence of increased complexity and obstructions to migration in the human brain (discussed in (Paredes et al., 2016)). Critically, it remains to be determined if human V-SVZ NSCs have a positional identity as is observed in rodents.

Conclusions

Ongoing adult neurogenesis in the mammalian brain has been a subject of widespread discussion from the initial identification of postnatal proliferation to the cell types responsible and the extent to which it occurs across different species. Characterizing NSC functions and features holds promise in revealing mechanisms of cellular programming and determinants of cell fate from a developmental biology perspective as well as in guiding therapies for many diseases with neurological phenotypes. Although proliferative potential is restricted in the mature brain, the NSCs are part of complex niches under the control of a variety of cell-intrinsic and extrinsic features that may reveal avenues for therapeutic intervention and thus, further examination of their regulation and potential is warranted.

Acknowledgments

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Persisting Questions

- Is regional transcription factor expression controlled in the same manner throughout development and in the postnatal brain?

- Which transcription factors are permissive for multiple fates vs. instructive for a specific one?

- How is regional identity maintained postnatally?

- Is there a signaling or transcriptional threshold to induce plasticity of NSCs?

- Is there a 'gradient of identity" or sharp cutoffs within the V-SVZ?

- How do signals within the developing V-SVZ affect specific TFs to determine the ultimate fate of an NSC?

- Can we mathematically model the input of signals experienced by NSCs that drive fate determination?

- Do SGZ NSCs have a positional identity?
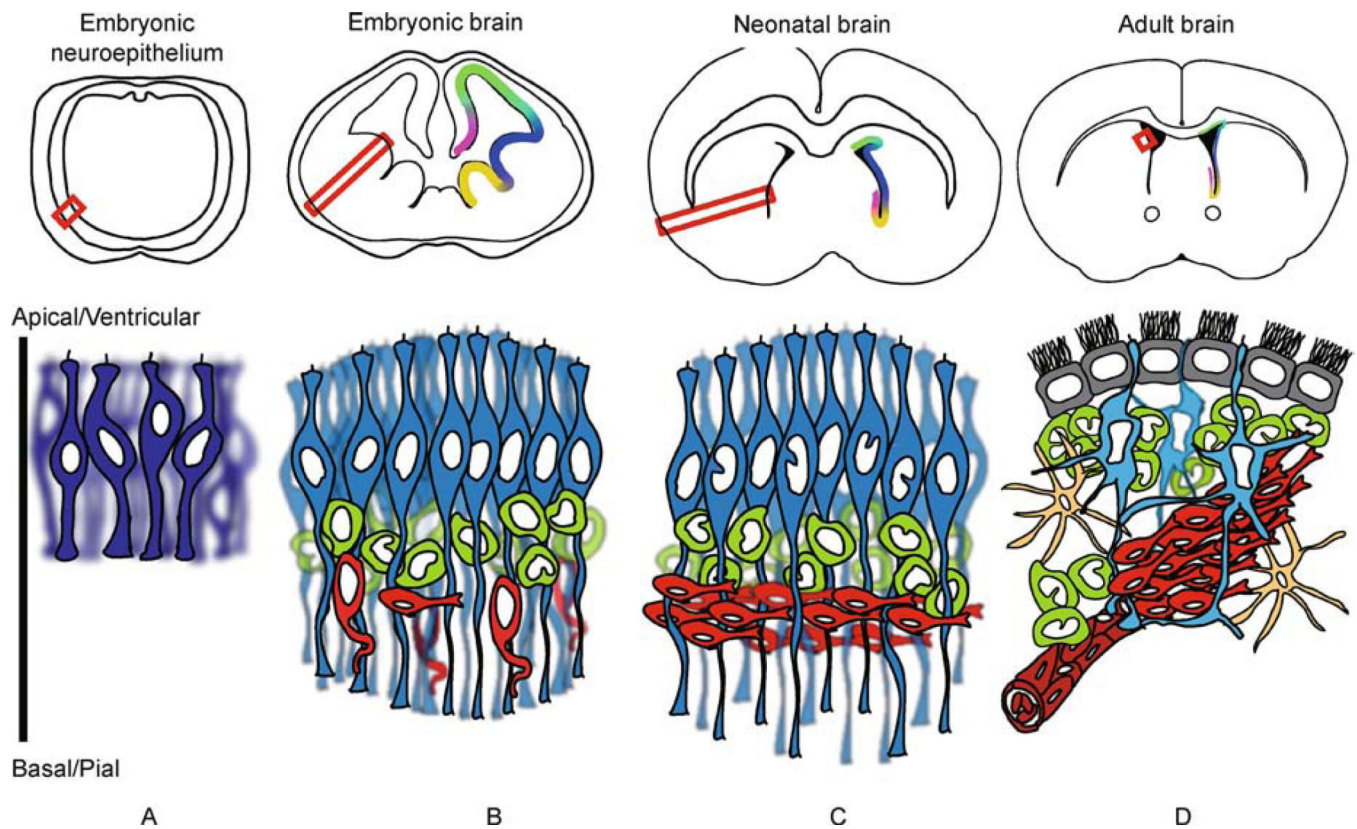
- Does positional identity exist in the human brain?

Figure 1.

Development of the mouse ventricular-subventricular zone. Representative coronal sections of mouse brain at indicated developmental times are shown at top. Colors in the coronal sections represent domains of transcription factor expression within the developing V-SVZ. At bottom, representative schematics of developing V-SVZ corresponding to red box within the coronal section above. Note that the size of coronal sections and corresponding representative images of cell types are not to scale. (A) Neuroepithelial cells (NECs; dark blue) fold in to form the neural tube. These cells contact both the pial and ventricular surfaces of the developing brain (below) and divide to form a densely packed VZ. (B) In developing telencephalon, NECs give rise to radial glia (RG; light blue), which retain properties of NECs (see text), including contact with the ventricular and pial surfaces. At this stage, the RG divide asymmetrically, producing a daughter RG and a daughter intermediate progenitor cell (IPC; green) located away from the ventricular surface in a subventricular zone (SVZ). Newborn neurons (red) use the RG processes as a scaffold for migration to their final destinations. (C) In the neonatal brain, the RG are retained until approximately postnatal day 7. After postnatal day 2, they begin to retract their basal (pial) processes and will give rise to ependymal (E) cells (grey; shown in (D)), B1 cells (teal; shown in (D)) and B2 cells (yellow; shown in (D)) in the mature brain. (D) In the adult brain, B1 cells are the NSCs. They have basal processes that wrap around blood vessels (dark red) and a single primary cilium that extends between the tightly connected E cells. The multiple motile cilia of E cells push CSF through the ventricles. Note the presence of transit-amplifying C cells (green), migrating neuroblasts (A cells, red) and parenchymal

astrocytes (B2 cells, orange). This structure in the adult is termed the ventricular-subventricular zone (V-SVZ). Note that other cell types exist in the region that are not discussed within this review including microglia and local and distant innervating neurons.
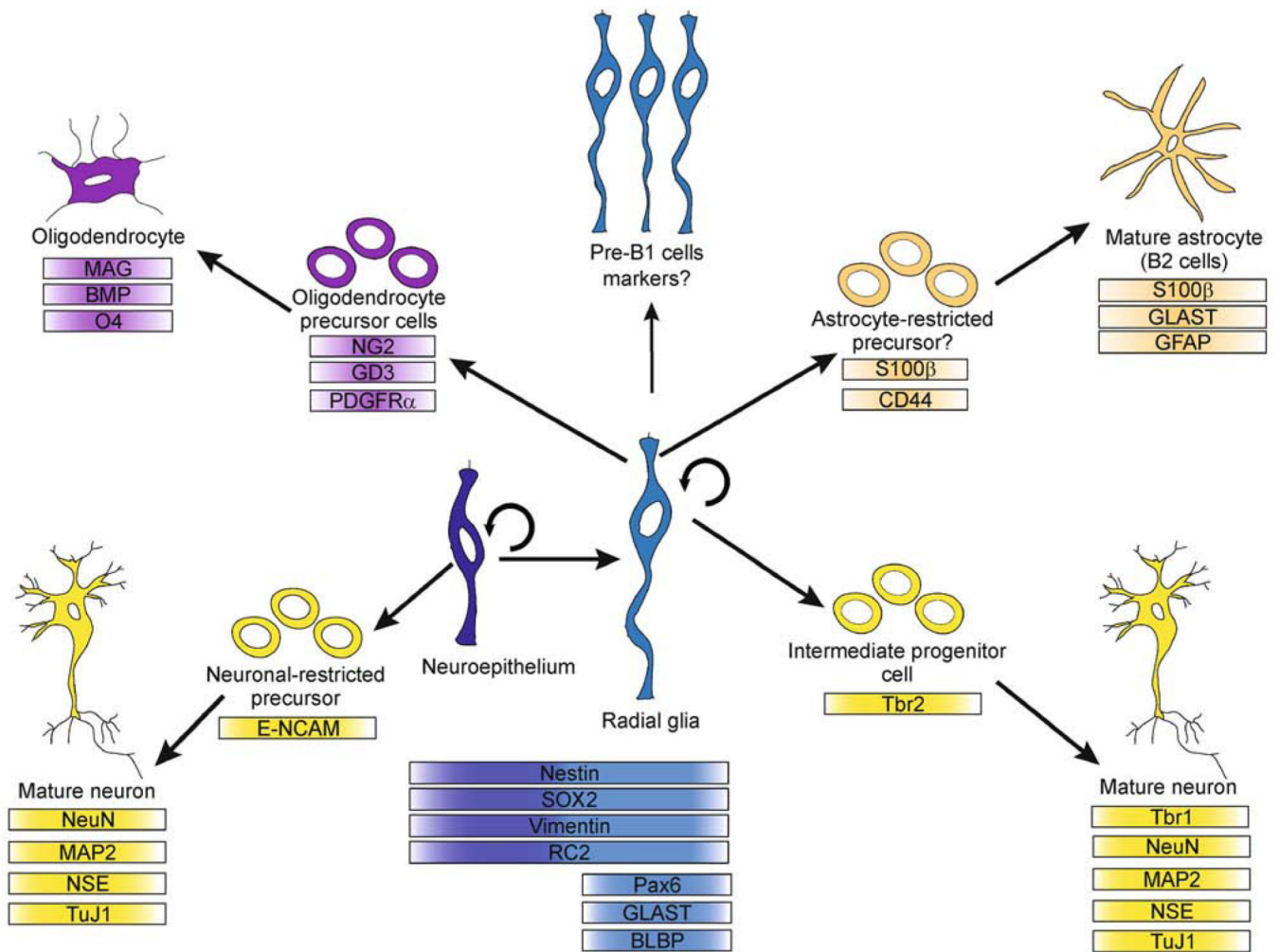
Figure 2.
Embryonic neural cell lineage and marker expression profiles. Neuroepithelial cells (NECs) are the earliest neural progenitors discussed here. These cells produce neurons but also give rise to radial glia cells (RG), which in turn act as the primary progenitors during cortical development. These cells can produce oligodendrocytes, astrocytes and neurons. While precursors for oligodendrocytes and neurons have been characterized, it is still debated whether an astrocyte-restricted precursor cell exists. RG cells also produce pre-B1 cells between E13.5–15.5 that remain relatively quiescent until postnatal reactivation (see text).
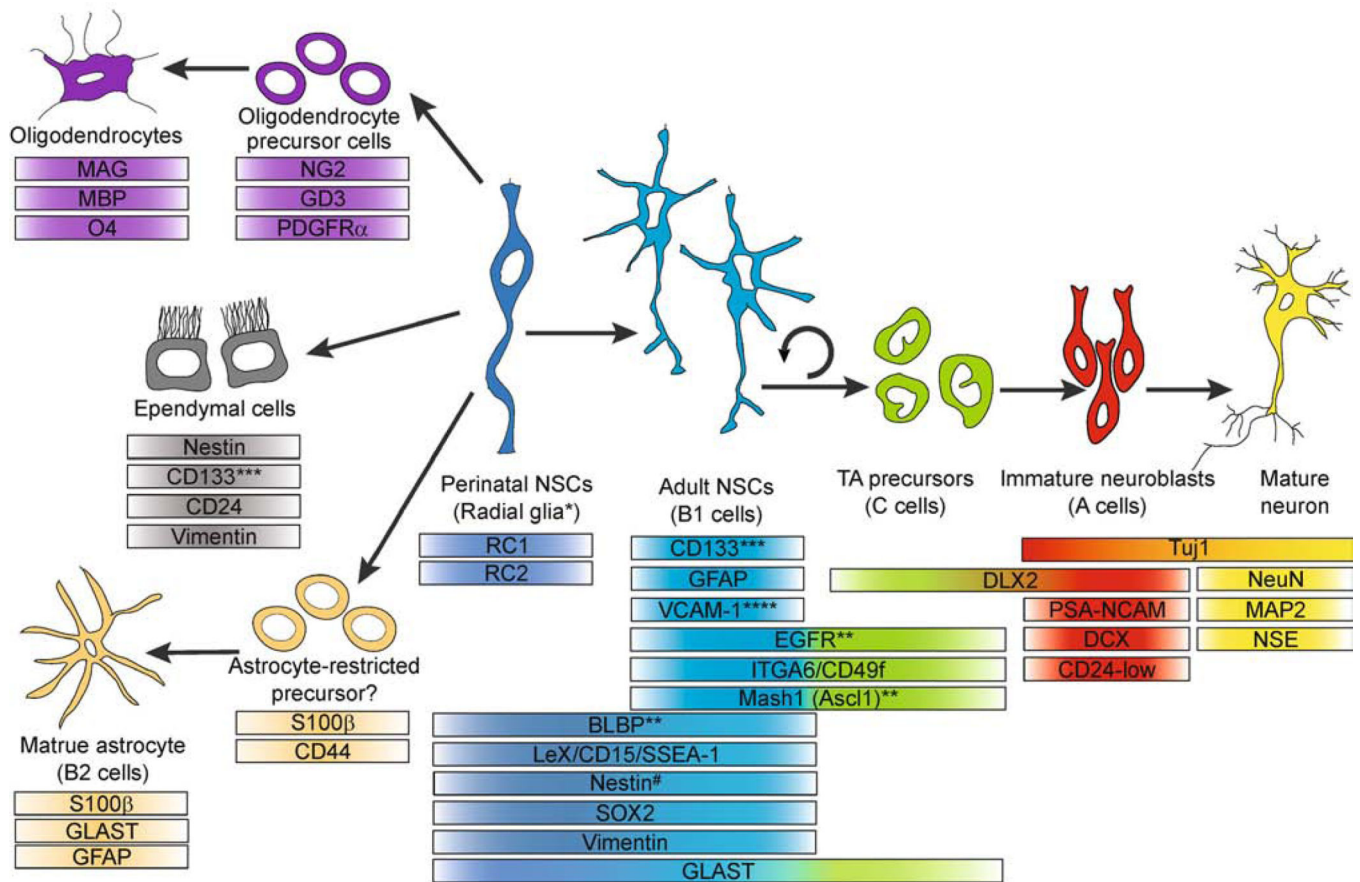
Figure 3.
Postnatal neural cell lineage and marker expression profiles. *Radial glia persist only during the first postnatal week and are non-self-renewing during this time. They retract their processes after postnatal day 2 in the mouse and give rise to parenchymal astrocytes (orange), ependymal cells (grey), oligodendrocytes (purple) and astrocyte-like adult neural stem cells (B1 cells, teal). B1 cells are self-renewing and also give rise to transit amplifying progenitors (green), which in turn produce neuroblasts (red) that will mature into neurons (yellow). **These markers are primarily expressed by activated B1 cells. ***CD133 is present on the primary cilia of B1 cells, as well as ependymal cells. ****VCAM-1 is expressed on quiescent B1 cells. # Note that Nestin is not expressed on all RG and B1 cells but rather, is dynamically regulated (see text).

Table 1

Marker expression on cell types within the V-SVZ and its precursor regions

Marker	Cell type labeled	Source
Alpha 6 integrin	Activated B1 cells C cells	Ramalho-Santos et al., 2002; Kokovay et al., 2010
Brain lipid binding protein (BLBP)	Radial glia Activated B1 cells	Feng et al., 1994; Doetsch, 2003; Kriegstein and Gotz, 2003 Giachino et al., 2014
CD44	Astrocyte-restricted precursor cells	Liu et al., 2004
Dlx2	C cells A cells	Doetsch et al., 2002
Doublecortin (DCX)	A cells	Doetsch et al., 1999a; Gleeson et al., 1999
E-NCAM	Neuronal-restricted precursors	Chuong and Edelman, 1984
Epidermal growth factor receptor (EGFR)	Activated B1 cells C cells	Doetsch et al., 2002; Pastrana et al., 2009; Codega et al., 2014
GD3	Oligodendrocyte precursor cells	LeVine and Goldman, 1988a, 1988b
Glial fibrillary acidic protein (GFAP)	Neuroepithelial cells Radial glia B1 cells Mature astrocytes	Bignami et al., 1972
Glutamate aspartate transporter (GLAST)	Radial glia	Shibata et al., 1997; Hartfuss et al., 2001; Doetsch, 2003; Kriegstein and Gotz, 2003
	Mature astrocytes	Shibata et al., 1997; Ullensvang et al., 1997
	Activated B1 cells C cells	Pastrana et al., 2009
ITGA6/CD49f	B1 cells C cells	Ramalho-Santos et al., 2002; Shen et al., 2008; Kokovay et al., 2010
LeX (CD15)	B1 cells	Capela and Temple, 2002
MAG	Mature oligodendrocytes	Quarles and Trapp, 1984
MAP2	Mature neurons	Garner et al., 1988
Mash1 (Ascl1)	Activated B cells	Pastrana et al., 2009
	C cells	Parras et al., 2004; Pastrana et al., 2009
mCD24 (aka heat-stable antigen, HSA)	E cells	Calaora et al., 1996
	A cells (transiently)	Rougon et al., 1991; Nedelec et al., 1992; Calaora et al., 1996
Myelin basic protein (MBP)	Mature oligodendrocytes	Poduslo and Braun, 1975; Golds and Braun, 1976; Omlin et al., 1982
Nestin	Neuroepithelial cells	Lendahl et al., 1990
	Ependymal cells	Lendahl et al., 1990; Doetsch et al., 1997
	Radial glia	Hockfield and McKay, 1985
	Activated B1 cells	Codega et al., 2014
Neuronal nuclear antigen (NeuN)	Mature neurons	Mullen et al., 1992
Neuron-specific enolase (NSE)	Mature neurons	Kirino et al., 1983
NG2	Oligodendrocyte precursor cells	Stallcup and Beasley, 1987; Nishiyama et al., 1996; Ong and Levine, 1999
O4	Mature oligodendrocytes	Sommer and Schachner, 1981
Pax6	Cortical radial glia	Gotz et al., 1998; Englund et al., 2005
Polysialylated neural cell adhesion molecule (PSA-NCAM)	A cells	Doetsch et al., 1997

Marker	Cell type labeled	Source
Prominin-1 (CD133)	Ependymal cells	Coskun et al., 2008
	Primary cilia of B1 cells	Uchida et al., 2000; Marzesco et al., 2005; Pinto et al., 2008; Beckervordersandforth et al., 2010
Platelet-derived growth factor receptor α (PDGFRα)	Oligodendrocyte precursor cells	Hart et al., 1989; Pringle et al., 1992; Hall et al., 1996
RC1	Neuroepithelial cells Radial glia	Edwards et al., 1990
RC2	Neuroepithelial cells Radial glia	Misson et al., 1988; Chanas-Sacre et al., 2000; Hartfuss et al., 2001
SOX2 (SRY-Box 2)	Embryonic NSCs (radial glia)	Zappone et al., 2000
	B1 cells	Ellis et al., 2004; Ferri et al., 2004
S100-β	Mature astrocytes (Not all astrocytes express it)	Wang and Bordey, 2008
	Ependymal cells	Didier et al., 1986
Tbr1	Intermediate progenitor cells	Englund et al., 2005; Hevner, 2006; Hevner et al., 2006
Tbr2	Mature neurons (cortex)	Englund et al., 2005; Hevner, 2006; Hevner et al., 2006
Tuj1 (βIII Tubulin)	A cells	Doetsch et al., 1997; Pastrana et al., 2009
VCAM-1	Quiescent B1 cells	Kokovay et al., 2012; Codega et al., 2014
Vimentin	Radial glia	Schnitzer and Schachner, 1981; Zecevic, 2004
	Ependymal cells	