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The multifaceted subventricular zone astrocyte: from a metabolic and pro-neurogenic role to acting as a neural stem cell

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Abstract

A few decades ago it was discovered that two regions of the adult brain retain the ability to generate new neurons. These regions include the subgranular zone of the hippocampal dentate gyrus and the ventricular-subventricular zone (V-SVZ) located at the border of the lateral ventricle. In the V-SVZ, it was discovered that neural progenitor cells share many features of mature astrocytes and are often referred as V-SVZ astrocytes. We will first describe the markers, the morphology, and the neurophysiological characteristics of V-SVZ astrocytes. We will then discuss the fact that V-SVZ astrocytes constitute a mixed population with respect to their neurogenic properties, e.g., quiescent versus activated state, neurogenic fate, and transcription factors expression. Finally, we will describe two functions of V-SVZ astrocytes, their metabolic coupling to blood vessels and their neurogenic supportive role consisting of providing guidance and survival cues to migrating newborn neurons.

Keywords

Subventricular zone; Adult neurogenesis; Neural stem cells; Astrocyte

Introduction

Until recently, it was thought that the generation of neurons in mammals occurred only during the embryonic period. By now it has been clearly demonstrated that new neurons continue to be generated in two regions of the adult brain, the subgranular zone (SGZ) of the dentate gyrus of the hippocampus and the ventricular-subventricular zone (V-SVZ) lining the lateral wall of the lateral ventricle. The neural progenitor cells in the V-SVZ give rise to transit amplifying precursors that themselves give birth to neuroblasts (Doetsch *et al.* 1999).

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These neuroblasts migrate tangentially to the olfactory bulb to become interneurons (Luskin 1993, Lois and Alvarez-Buylla 1994). The neural progenitor cells of the V-SVZ arise during embryonic development and persist into adulhood. In the embryonic brain, a very particular cell type, called radial glia was initially described with the classical Golgi silver impregnation method at the end of nineteenth century (Magini, 1888; Ramon y Cajal, 1890; Retzius, 1893; von Lenhossék, 1895), and found to provide a scaffolding for the migration and placement of newborn neurons (Rakic 1971). Radial glia were found to possess another critical function, first identified in songbirds, which is their ability to proliferate in the ventricular zone coinciding with sites of neurogenesis (Alvarez-Buylla *et al.* 1990). This finding was later confirmed and expanded in the embryonic mammalian brain and it is now well-accepted that radial glia act as neural progenitor cells (NPCs) and generate the majority of neurons in the embryonic brain (Miyata *et al.* 2001, Noctor *et al.* 2001 , Malatesta *et al.* 2003). After birth, most radial glia transform into parenchymal astrocytes throughout the central nervous system (Schmechel and Rakic 1979 , Voigt 1989 , Alves *et al.* 2002 , Merkle *et al.* 2004), except in the two postnatal neurogenic regions, where radial glia act as NPCs and generate the three main neural cell types, including neurons, oligodendrocytes, and astrocytes (Kriegstein and Alvarez-Buylla 2009). These NPCs persist throughout adult life in these two regions, in all mammalian species examined including humans (Bonfanti and Peretto 2011). The exact fate of NPCs is being more carefully examined using novel labeling methods and lines of transgenic mice based on the concept that not all NPCs are equal in terms of their fate. NPCs in the dorsal V-SVZ were shown to generate oligodendrocyte precursors (OPCs) that migrated radially into the white matter (Marshall and Goldman 2002, Marshall *et al.* 2003, Menn *et al.* 2006). Nevertheless, the generation of neurons predominates over that of oligodendrocytes (Menn et al. 2006) although OPC production is significantly increased following injury, in particular demyelination (Picard-Riera *et al.* 2002, Aguirre *et al.* 2007, El Waly *et al.* 2014)). Importantly, it was elegantly shown that a single NPC exclusively generates OPCs or immature neurons, but not both (Ortega *et al.* 2013). Here, we do not distinguish between OPC or neuroblast-fated NPCs with respect to their properties.

In the V-SVZ, It was found that NPCs share many features of mature astrocytes including morphological and biophysical characteristics as well as antigens such as glial fibrillary acidic protein (GFAP) (Doetsch *et al.* 1997, Doetsch et al. 1999, Liu *et al.* 2006). Here, we will focus on describing specific properties of the V-SVZ NPCs, referred to as V-SVZ astrocytes. We will first describe the unique set of markers, the morphology, and the neurophysiological characteristics of V-SVZ astrocytes. We will discuss the fact that although the population of V-SVZ astrocytes seems homogenous with respect to their neurophysiological properties (electrophysiological, coupling, neurotransmitter receptors, and transporters expression) they differ with respect to their neurogenic properties, e.g., stages of the cell cycle, quiescence versus activation state, neurogenic fate, and transcription factor expression. Finally, we will describe two functions of V-SVZ astrocytes, their coupling to blood vessels and their neurogenic supportive role consisting of providing guidance and survival cues to migrating newborn neurons.

1. Morphological and antigenic properties defining V-SVZ astrocytes

Two main populations of GFAP-positive cells and a third more discret population were observed in the V-SVZ (Jankovski and Sotelo 1996, Doetsch et al. 1997)(Fig. 1). The two main populations were called type B1 and B2 cells (Doetsch et al. 1997), both of them exhibiting characteristics of astrocytes. B1 cells were initially described morphologically with a simple fusiform cell body, only 2 to 3 processes with few branches (Jankovski and Sotelo 1996). These cells were identified as the NPCs of the V-SVZ (Doetsch et al. 1999). Type B1 slowly divide to give birth to highly proliferative transit amplifying precursors (Type C cells) that themselves give birth to neuroblasts (Type A cells), which migrate to the olfactory bulb to become interneurons (Doetsch et al. 1999). Both type B cells directly contact neuroblasts and form a glial sheath around chains of newborn neurons as they migrate toward the olfactory bulb (Lois *et al.* 1996 , Doetsch et al. 1997). Through improvement in labeling technique, the morphology of type B1 cells has been found to be more specialized and complex than initially described. In particular, the cell bodies of B1 cells are located beneath the ependymal layer and their apical process is frequently intercalated between ependymal cells (Doetsch *et al.* 1999, Mirzadeh *et al.* 2008) while their basal process projects across the V-SVZ thickness and terminates on blood vessels, in particular capillaries (Mirzadeh et al. 2008 , Shen *et al.* 2008, Tavazoie *et al.* 2008 , Lacar *et al.* 2011). The basal processes are of variable lengths depending on the proximity of the vessels to the ependyma and confer a radial glia-like morphology to NPCs (Mirzadeh et al. 2008 , Lacar et al. 2011)(Fig. 1). B1 cells have fewer processes than typical stellate astrocytes. Nevertheless by examining the very fine morphology of individually labeled B1 cells, it became apparent that the complexity of their morphology has been underestimated. They possess a high density of short and thin projections extending from their apical and basal processes (Fig. 1) (Mirzadeh et al. 2008 , Lacar *et al.* 2011, Lacar *et al.* 2012). The function, the molecular composition, and the presence of receptors on these thin processes are unknown, but they may allow B1 cells to better sense cues in their microenvironment.

The second population of astrocytes, the type B2 cells, also called niche astrocytes, displays a stellate appearance resembling that of parenchymal astrocytes (Mirzadeh et al. 2008, Lacar et al. 2011)(Fig. 1). They are mostly located at the periphery of the V-SVZ creating a physical boundary between the SVZ and the striatum (Liu et al., 2005) and are probably non-neurogenic. They do not contact the ventricle but interact with blood vessels (Mirzadeh et al. 2008, Lacar et al. 2011). A third very small population of astrocytes was observed in the V-SVZ of 4 weeks old animals and represent only very few cells (Platel *et al.* 2009). These astrocytes are S100B positive (Platel et al. 2009), but surprisingly have not been observed in *s100b*-GFP mice (Raponi *et al.* 2007). In human (h) *Gfap*-GFP mice, few S100B-positive cells are GFP-positive while the others are GFP-negative (Platel et al. 2009). Approximately half of these S100+ cells are also NG2 positive. These cells present a relatively immature stellate appearance and don’t have a specific location along the V-SVZ, but are never in contact with the ventricle (Platel et al. 2009). This population may represent astrocytes or NG2 cells born in the V-SVZ. Their function and potential migration pattern remain unknown.

Type B cells have been reported to express several markers associated with astrocytes or radial glia, like GFAP (Jankovski and Sotelo 1996, Doetsch et al. 1997), nestin, vimentin (Doetsch et al. 1997), the glutamate transporter GLAST (Braun *et al.* 2003 , Bolteus and Bordey 2004), and brain lipid binding protein (BLBP)(Platel et al. 2009, Giachino *et al.* 2014) (Table 1). Therefore, type B cells are also frequently referred to as V-SVZ astrocytes. Additional markers expressed in other cell types outside of the neurogenic region have been identified in V-SVZ astrocytes and further divide this population of cells, including CD133 (prominin-1), EGFR, and Sox2 (Komitova and Eriksson 2004, Codega *et al.* 2014). The different combination of markers is thought to reflect different activation states of V-SVZ astrocytes from quiescent to activated stages. CD133 has been shown to label V-SVZ cells with stem cell characteristics (Fischer *et al.* 2011) while EGFR+ cells label activated (i.e., proliferative) V-SVZ astrocytes as well as transit amplifying cells (Pastrana *et al.* 2009). It is thus important to use a combination of markers to identify the quiescent and activated V-SVZ NPCs with for example CD133+/EGFR-(quiescent) and CD133+/EGFR+ (proliferative, activated). It was also recently shown that BLBP is more restricted that initially thought and that it is expressed in mitotically activated and not in quiescent V-SVZ astrocytes (Giachino et al. 2014).

2. Neurophysiological characteristics of V-SVZ astrocytes

Studies during the last decade have characterized the neurophysiological properties of SVZ astrocytes. Because the V-SVZ is very densely populated by several cell types, including V-SVZ astrocytes, transit amplifying cells, neuroblasts, ependymal cells, and microglial cells, it is complicated to identify V-SVZ astrocytes from the other cell types in acute brain slices without a counter-staining. Thus, most neurophysiological studies took advantage of transgenic mice, such as the h*gfap*-GFP mice originally generated to study mature astrocytes (Liu et al. 2006) and h*gfap*-DsRed mouse line (Young *et al.* 2010). In these lines, V-SVZ astrocytes display GFP or DsRed expression that matches the expression of GFAP detected by immunostaining. However, the number of GFAP+ cells expressing DsRed is lower than in the GFP mouse line and DsRed persists in neuroblasts due to its long half-life although fainter than in V-SVZ astrocytes (Young et al. 2010). Therefore, in most studies using brain slices from these lines, B1 and B2 V-SVZ astrocytes were not distinguished. Electrophysiological recordings of GFP-positive cells have shown that V-SVZ astrocytes display K+ conductances at rest like mature astrocytes, but have a low level of expression of barium-sensitive inwardly rectifying K+-mediated current (Wang *et al.* 2003, Liu et al. 2006), which is a hallmark of astrocytic differentiation and cell cycle exit (Bordey and Sontheimer, 1997; Macfarlane and Sontheimer, 2000). In addition, V-SVZ astrocytes express several other functional ion channels, neurotransmitter receptors, and transporters detailed below.

GABA signaling has been the most studied neurotransmitter signaling in the V-SVZ. Functional $GABA_A$ receptors have been identified on V-SVZ astrocytes (Stewart *et al.* 2002, Nguyen *et al.* 2003, Wang *et al.* 2003, Gascon *et al.* 2006). The exact subunit composition remains to be examined. The expression of $GABA_B$ and $GABA_C$ receptors has not been explored. V-SVZ astrocytes also express the high affinity GABA transporter, GAT4 (Bolteus and Bordey 2004) while GAT1 is expressed by neuroblasts. It was demonstrated that GABA

controls the proliferation of V-SVZ astrocytes through $GABA_A$ receptor activation (Nguyen et al. 2003, Liu *et al.* 2005). Neuroblasts are a paracrine source of GABA. They synthesize and release GABA in a calcium-dependent but non-vesicular manner (Liu et al. 2005). Such a paracrine release of GABA increases V-SVZ astrocyte proliferation and was proposed to act as a negative proliferative cue from neuroblasts to V-SVZ astrocytes (Liu et al. 2005). However, GABA can also come from an external source. It was shown that striatal GABAergic neurons located at the border of the V-SVZ could regulate intracellular Ca2+ dynamics in V-SVZ astrocytes through $GABA_A$ receptors activation and depolarization of L- and T-type voltage-gated calcium channels (Young et al. 2010, Young *et al.* 2012).

Glutamate signaling is also present in the V-SVZ. V-SVZ astrocytes do not display any NMDA, kainate, or AMPA-induced currents or calcium increases in acute slices (Liu et al., 2006; Platel et al., 2010). In vitro, metabotropic glutamate receptor subtypes 3 and 5 are expressed by GFAP-positive cells isolated from the V-SVZ (Di Giorgi-Gerevini *et al.* 2005). Nevertheless, it is not clear whether these receptors are expressed in V-SVZ astrocytes *in vivo* especially since mGluR5-induced calcium currents were detected in neuroblasts but not in surrounding cells (Platel *et al.* 2008). As mentioned above, V-SVZ astrocytes express the glutamate transporters GLAST (EAAT1) and GLT-1 (EAAT2) (Braun et al. 2003 , Bolteus and Bordey 2004 , Liu et al. 2006). Although it seems that V-SVZ astrocytes do not express any glutamate receptors, we will further detail in part 5 their role as master regulators of glutamate signaling (Platel *et al.* 2008, Platel *et al.* 2010).

It was recently shown that V-SVZ astrocytes express α3 and α4 nicotinic and muscarinic receptors. Local subependymal choline acetyl transferase positive ($ChAT^+$) neurons can release acetylcholine into the V-SVZ niche in an activity-dependent manner (Paez-Gonzalez *et al.* 2014). Indeed, optogenetic stimulation of these subependymal ChAT+ neurons revealed that their activity could trigger release of acetylcholine that activates V-SVZ astrocytes and increase SVZ cell number (Paez-Gonzalez et al. 2014). It is nevertheless possible that this effect on proliferation is indirect through the release of other factors such as GABA from striatal neurons.

V-SVZ astrocytes express connexin 30 and 43, components of gap junctions in mature astrocytes that allow intercellular communication (Liu et al. 2006, Nomura *et al.* 2010, Lacar et al. 2011). Connexin can also form hemi-channels, which unlike gap junctional coupling allow direct communication between the intracellular and extracellular milieu (Bennett *et al.* 2003). Hemi-channels have been implicated in the initiation and propagation of calcium waves between radial glia during corticogenesis (Weissman *et al.* 2004). Similarly, it was reported that V-SVZ astrocytes display functional coupling involving 50–60 cells as well as intercellular calcium waves (Lacar et al. 2011, Lacar et al. 2012). These waves travelled bidirectionally between type B1 and B2 cells and propagated onto blood vessels. They were absent in the presence of a gap junction blocker, but persisted with purinergic receptor blockers (Lacar et al. 2011). Such functional coupling among V-SVZ astrocytes and between V-SVZ astrocytes and blood vessels are another evidence that V-SVZ astrocytes do not merely have a structural role, but may play an active role in coordinating intercellular communication and cell behavior in this neurogenic region.

3. Do all V-SVZ astrocytes possess the same neurophysiological properties?

The neurophysiological characteristics of V-SVZ astrocytes have been measured in h*gfap*-GFP-positive V-SVZ astrocytes without distinguishing the different subpopulations mentioned earlier leading to the conculsions that neurophysiological properties described in the previous chapter are homogenous among these cells. For example, all V-SVZ astrocytes were reported to express the neurotransmitter transporters GLAST, GLT-1, and GAT4 as well as connexin 43 resulting in functional coupling. All V-SVZ astrocytes were also reported to respond to focal applications of GABA, which induced inward currents (Liu et al. 2005).

These results are surprising since the major subpopulations of V-SVZ astrocytes, type B1 and B2 cells, are different with respect to their antigenic markers, morphology, and location in the V-SVZ. In addition, different subtypes of V-SVZ astrocytes were identified based on their location along the ventricle, their transcription factor expression, and the fate of their neuronal progeny (Kohwi *et al.* 2007, Merkle *et al.* 2007, Fiorelli *et al.* 2015). . For example, dorsal V-SVZ astrocytes express the transcription factor Emx1 and Pax6 and mainly generate periglomerular dopaminergic neurons and superficial granule cells. The ventral V-SVZ astrocytes are mainly Gli1-positive and produce deep granule cells and calbindin-positive periglomerular cells whereas the lateral V-SVZ is Gsx2- and Dlx5/6-positive and produce all periglomerular neurons subtypes (Kohwi et al. 2007).

These data lead to the following intriguing questions: are the neurophysiological characteristics of the different populations of V-SVZ astrocytes similar? Do these characteristics (e.g., expression of receptors) change depending on the phase of the cell cycle? Are these neurophysiological characteristics similar in different compartments of the V-SVZ, i.e. dorsal, lateral, ventral and medial?

It is possible that some of the neurophysiological characteristics of V-SVZ astrocytes described above represent only basic functions that are also present in parenchymal astrocytes (Wang and Bordey 2008). Nevertheless, it has been shown that several specific signaling are different between compartments. For example, the Wnt family of soluble ligands regulates the self-renewal of a small population of V-SVZ astrocytes, which are located in the dorsal compartment and generate glutamatergic olfactory bulb neurons (Azim *et al.* 2014). Another example is the sonic hedgehog (Shh) signaling. Shh is selectively produced by a small group of ventral forebrain neurons and its receptor gli1 is only expressed in the ventral V-SVZ astrocytes. This signaling regulates the formation of calbindin periglomerular neurons and deep granule cells (Ihrie *et al.* 2011).

Collectively, we hypothetize that some mechanisms are common in all the V-SVZ astrocytes while some differences and specificity appear in the different populations or during different phases of the cell cycle. New neurophysiological studies will need to take into account the different V-SVZ populations showing distinct expression of molecular determinants along the ventricle axes.

In the next two sections, we specifcally focus on two specific functions of V-SVZ astrocytes, the metabolic coupling and their supportive role during neuroblast migration.

4. Metabolic coupling in the V-SVZ niche

In the brain, neuronal activity dictates transfer of oxygen and nutrients from the blood stream into active neuronal assemblies through a local “neurovascular coupling” in part carried out by astrocytes (Giaume *et al.* 2010). Although SVZ cells do not generate action potentials, the V-SVZ contains cells undergoing proliferation, which is a metabolically demanding process (Bolanos *et al.* 2010). It is thus not completely surprising that the V-SVZ contains a large network of blood vessels and in particular capillaries (Mercier *et al.* 2002, Shen et al. 2008, Tavazoie et al. 2008, Snapyan *et al.* 2009, Lacar et al. 2012). A pioneering study reported that V-SVZ astrocytes closely ensheath blood vessels around the V-SVZ (Mercier et al. 2002). Additional studies showed that dividing V-SVZ astrocytes and type C cells are tightly apposed to V-SVZ blood vessels (Shen et al. 2008, Tavazoie et al. 2008). They frequently contact the vasculature at specific sites that lack astrocyte endfeet and pericyte coverage, and display a more permeable blood brain barrier allowing small diffusible molecules to enter the neurogenic zone (Tavazoie et al. 2008). As such, type B and C cells are uniquely poised to receive signals from the vasculature, like hormones and growth factors. In addition, both hormones and growth factors can control cell proliferation in the V-SVZ under normal condition and following injury (for review (Silva-Vargas *et al.* 2013)).

Reciprocally, it was demonstrated that V-SVZ cells have the ability to modify blood flow, which is expected to alter metabolite supply into this region. It has been shown that a 30 minutes injection of epidermal and basic fibroblast growth factor (EGF and bFGF) into the lateral ventricular significantly increased the number of cells entering S-phase of the cell cycle (i.e., analyzed with BrdU uptake) in the V-SVZ. In addition, EGF + bFGF injection led to a sustained rise in blood flow in the V-SVZ. Because a similar injection in the cortex, where there is no proliferation, did not lead to an increase in blood flow, the authors suggested that the increase in the number of cycling cells was the biological process leading to an increase in blood flow.

Based on the anatomical arrangement of astrocytes-capillaries in the V-SVZ, these same authors examined whether V-SVZ astrocytes could regulate capillary diameter in acute slices and blood flow in vivo. A series of elegant studies have been performed on mature, parenchymal astrocytes suggesting that calcium signaling in astrocytes leads to the release of vasoconstricting or dilating factors. Similarly, in the V-SVZ, astrocytes display spontaneous calcium waves and stimulation-induced (electrical and GABA application) calcium increases (Lacar et al. 2011 , Lacar et al. 2012). To selectively increase calcium in V-SVZ astrocytes and not in surrounding V-SVZ cells, Lacar et al. used transgenic mice, in which GFAP+ cells express a Gq-protein-coupled receptor (called Mas-related gene A1, MrgA1) that is not expressed in the brain and has no endogenous ligands (Fiacco *et al.* 2007). These mice were initially generated to study astrocytic functions at synapses (Fiacco et al. 2007). Application of the MrgA1-selective peptide agonist FLRFα resulted in calcium increases in V-SVZ astrocytes. Using these mice, they then showed that intracellular calcium increases in V-SVZ astrocytes induces ATP release followed by purinergic ($P2Y_{2/4}$ receptor activation on pericytes and dilation (Lacar et al. 2012). Perhaps the most elegant and challenging experiment was to selectively express MrgA1 receptors in V-SVZ astrocytes in vivo using

neonatal electroporation and show that ventricular injection of the ligand led to increase in blood flow in the V-SVZ monitored using laser Doppler flowmetry. Using neonatal electroporation allows to express a plasmid of interest in V-SVZ cells which are radial glila cells in neonates. Over time, the fast cycling cells dilute the plasmid and the neuroblasts migrate away resulting in plasmid expression selectively in ependymal cells and quiescent or slow-cycling NPCs (Lacar *et al.* 2010).

This coupling underscores the intimate reciprocal interaction of V-SVZ astrocytes and niche cells. Considering that V-SVZ astrocytes receive signals from other V-SVZ cells such as GABA-induced depolarizing signal leading to calcium increases, these findings further suggest that V-SVZ astrocytes may act as transducers of neurometabolic demand and neural-vascular coupling in the V-SVZ.

5. V-SVZ astrocytes support neuroblast survival during migration by releasing glutamate

Type B1 cells generate neuroblasts that migrate a long distance to the olfactory bulb through the rostral migratory stream (RMS). While V-SVZ astrocytes don't express any ionotropic glutamate receptors, neuroblasts express functional AMPA (Platel *et al.* 2007), kainate (Platel et al. 2008), NMDA receptors (Platel et al. 2010), and mGluR5 (Di Giorgi Gerevini *et al.* 2004, Platel et al. 2008) shown using neurophysiological recordings in acute slices and immunohistochmistry in fixed sections. Each of these receptor subtypes has a different function in neuroblasts. Kainate receptors control migration (Platel et al. 2008), mGluR5 regulate proliferation (Di Giorgi Gerevini et al. 2004) while NMDARs are important for neuroblast survival (Platel et al. 2010). Electrophysiological recordings showed that these receptors were activated by endogenous levels of glutamate (Platel et al. 2008, Platel et al. 2010) suggesting a source of glutamate onto neuroblasts. Projection of glutamergic terminals had not been observed in the V-SVZ, but given the role of astrocytes in glutamate homeostasis during synaptic transmission, it was speculated that V-SVZ astrocytes may be a local source of glutamate (Bordey 2006). Although there are no synaptic contacts between V-SVZ astrocytes and neuroblasts, V-SVZ astrocytes closely ensheath them (Doetsch et al. 1997). In addition, V-SVZ astrocytes contain high level of glutamate (Platel et al. 2007) and express glutamate transporters GLAST and GLT-1 conferring them the ability to regulate ambient glutamate levels (Bolteus and Bordey 2004, Liu et al. 2006). In addition, vesicular glutamate transporter 1 (VGLUT1) was found in V-SVZ astrocytes, located mainly in the rostral V-SVZ (Platel et al. 2007) using immunostaining as well as post-embedding immunogold labeling (Platel et al. 2010). In order to demonstrate a potential calcium-dependent glutamate release from V-SVZ astrocytes, transgenic mice, in which V-SVZ astrocytes express MrgA1 receptors were used. The MrgA1-selective peptide agonist, FLRFα, which increased calcium in V-SVZ astrocytes, led to an increase in the frequency of NMDA receptor-mediated channel activity in neuroblasts recorded with the patch clamp technique in acute slices (Platel et al. 2010). It was concluded that V-SVZ astrocytes release glutamate in a calcium-dependent manner onto neuroblasts. It is noticeable that in acute hippocampal slices from MrgA1 mice, FLRFa application did not affect synaptic transmission (Fiacco et al. 2007). This emphasizes the importance of the glutamatergic signal from V-SVZ astrocytes to neuroblasts in the neurogenic zone. These findings raise additional questions; it was shown that V-SVZ astrocytes tonically release glutamate in a

calcium-dependent manner. However, the signal(s) leading to intracellular calcium increases in V-SVZ astrocytes that could trigger the release of glutamate remain unclear. One possibility is the neurotransmitter GABA released from neuroblasts (Liu et al., 2005).

Conclusion

The subventricular zone is a more complex region than previously appreciated in terms of its cellular diversity among a similar group of cells, like NPCs. The neurogenesis community is progressively finding new markers and generating new genetic tools such as new lines of transgenic mice to better characterize the morphological and neurophysiological properties of NSCs. In addition, additional work is needed to better identify the similarities and the differences between the neurogenic domains in the V-SVZ. This is particularly important given the regional specification of NPCs in terms of their fate, neuronal vs oligodendroglial or the different types of neurons generated. Moreover, while we have gained insights into how V-SVZ astrocytes can control blood flow and perhaps address the metabolic demand of proliferating and migrating cells in the niche, it is important to determine how this metabolic coupling is modulated by physiological states and how this will impact the neurogenic niche. It is also important to better understand the interactions between neurotransmitters and intracellular rresponses, and how this impact NPC and neuroblast behavior.

In conclusion, one of the most exciting findings in neurogenesis and the astrocyte field is the discovery that a specialized type of astrocytes acts as NPCs in the adult neurogenic zone. Future studies will uncover the mechanisms that allow these specialized neurogenic region and cells to retain their proliferative capacity in the adult brain. As a correlate of this discovery is a question related to parenchymal astrocytes and in particular addressing whether parenchymal astrocytes could regain their ability to generate neurons given the right environment and set of neurogenic factors. Ultimately, understanding the biology of the V-SVZ astrocytes has important therapeutic potential in treating brain injuries and neurological disorders.

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Abbreviations

RMS	rostral migratory stream
GFAP	glial fibrillary acidic protein
V-SVZ	ventricular-subventricular zone

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- Neurogenesis persist in the brain in the ventricular-subventricular zone (V-SVZ)
- Neural progenitor cells in the V-SVZ have characteristics of astrocytes
- Do all V-SVZ astrocytes possess the same features?
- V-SVZ astrocytes act as transducers of neurometabolic coupling in the SVZ
- V-SVZ astrocytes support neuroblast survival during migration

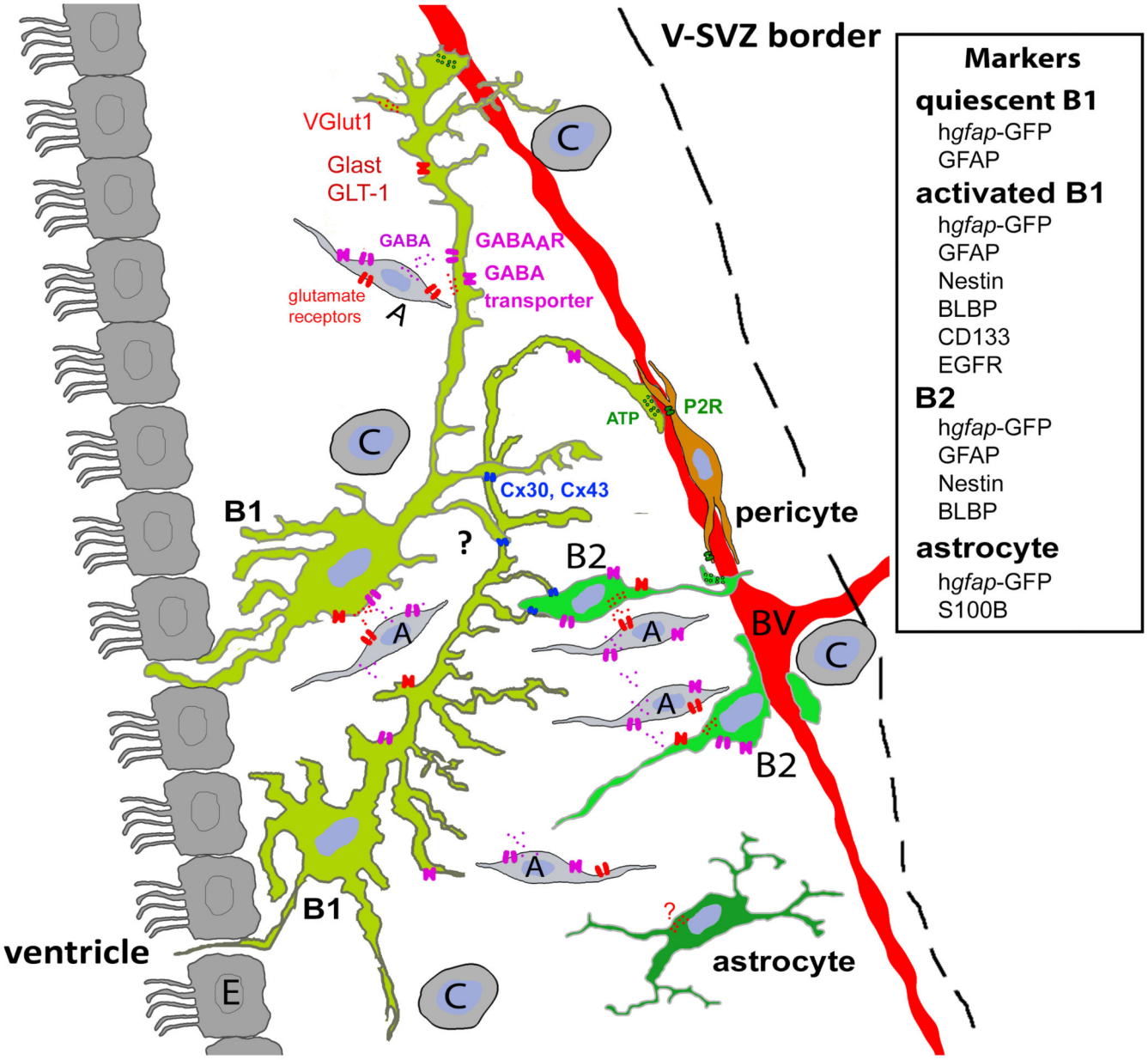

Figure 1.

The different populations of astrocyte like cells and their markers in the adult V-SVZ drawn from cells expressing GFP after electroporation.

Ependymal cells (E) are multiciliated located at the border of the lateral ventricle. Activated and quiescent type B1 cells extend an apical process intercalated between ependymal cells while their basal process contact the blood vessels. Type B2 cells, also called niche astrocytes, display an intermediate fusiform/stellate appearance and are located mostly at the periphery of the V-SVZ. They never contact the ventricle but interact with blood vessels. A third very discreet population of astrocyte was observed in the V-SVZ and is S100B positive. These cells present a stellate appearance (Platel *et al.* 2009) but their arborisation is not well developped. It is unknown if they connect to blood vessels. Type A cell (neuroblasts) have a

migratory morphology and are located throughout the V-SVZ. Type C cells (transit amplifying progenitors) are located throughout the V-SVZ and apposed to blood vessels. Pericytes are located on capillaries and present several processes that run on the blood vessel.

Table 1

Anatomical, antigenic, and neurophysiological properties of V-SVZ cells.

Type	Subtype		Morphology	Location	Markers	Glutamate related marker	GABA related marker
V-SVZ astrocytes	Type B1	Overall Type B1	fusiform	Attached to the ventricle and blood vessels Cell body in V-SVZ	GFAP + CD133 +/− Nestin +/− C×43 ; C×30 h*gfap*-GFP BLBP +/−	GLAST GLT-1 VGlut1 Glutamate mGluR1-5	GAT4 $GABA_AR$
		quiescent	"	"	GFAP +; CD133 +/−	?	?
		activated	"	"	GFAP +; Nestin + EGFR +; CD133 + h*gfap*-GFP BLBP	?	?
	Type B2		Intermediate fusiform /stellate	Periphery of the V-SVZ, not in contact with the ependymal layer	GFAP +; BLBP + Nestin +; C×43 +; Cx30? h*gfap*-GFP	GLAST; GLT-1 VGlut1 ? Glutamate mGluR1-5 ?	GAT4 $GABA_AR$
	Other astrocytes		Stellate	no specific location	h*gfap*-GFP +/− S100B +; GFAP? C×43 ? ; C×30?	?	?
Type C			Larger, more spherical than type B	Core SVZ	Mash1 +; EGFR + Lex +; Dl×2 + BLBP+/−	?	?
Type A			bipolar elongated	Core SVZ	DCX +; Tuj 1 + PSA-NCAM +	NMDA-R Kainate-R AMPA-R mGluR	GAT1 VGAT $GABA_AR$ GABA
Ependymal cells			cubic	border of the ventricle	S100B +; CD24 + Nestin +; C×43+ CD133 (cilia) +	-	-

+/− indicates that the marker is not expressed in all the population