The Postnatal Subventricular Zone: A Source of New Cells in This Old Brain

Angélique Bordey, PhD
Departments of Neurosurgery,
and Cellular & Molecular Physiology
Yale University School of Medicine
New Haven, Conneticut
USA

Address for correspondence:
Angélique Bordey, PhD
Department of Neurosurgery
Yale University School of Medicine
333 Cedar Street, FMB 422
New Haven, Conneticut 06520-8082
USA
Email: angelique.bordey@yale.edu

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Findings over the past decades demonstrating persistent neurogenesis in the adult brain have challenged the view of a fixed circuitry and raise hopes for self-renewal following brain injury. The subventricular zone (SVZ, also called subependymal layer, SEL) lining the lateral wall of the lateral ventricle is the largest germinal center where stem cells displaying astrocytic traits have been identified. These astrocyte-like cells ensheath neuroblasts, which migrate throughout the SVZ and along the rostral migratory stream to the olfactory bulb where they differentiate into interneurons. The cellular architecture of the SVZ has been essential for the development of hypotheses to explain how intercellular signaling and non-synaptic communication could regulate neurogenesis. An array of signaling molecules have recently been identified that may offer future strategies to promote neurogenesis and reroute neuroblasts to higher cognitive centers.

Key Words: brain, neurogenesis, subventricular zone

The existence of cells with stem cell attributes in two regions of the adult central nervous system (CNS) has overturned the long-held dogma that neurons are formed exclusively before birth and has raised hopes that self-renewal leading to repair may be possible in the mature CNS. These two regions include the SVZ also called SEL[24] and the subgranular zone of the hippocampal dentate gyrus. This review focuses on the SVZ, which contains the largest pool of dividing neural precursors in the adult brain.[4,26] Early in the past century Allen in 1912 identified mitotic cells in the SVZ of the lateral wall of the lateral ventricle in adult rats.[1] Later in the 1960s, Altman proposed that immature SVZ cells migrate to the olfactory bulb where they differentiate into mature neurons and glia.[4] However, according to Smart (1960), cell migration was thought to be minimal in adults.[105] It is only in the 1990s that focal labeling of the SVZ in the neonate,[69] and adult[37,66] indicated that proliferating SVZ cells migrate through the SVZ and along the rostral migratory stream (RMS, also called SVZ rostral extension[91]) into the olfactory bulb where they differentiate into interneurons. At almost the same time, the presence of cells with stem cell attributes (i.e. self-renewal and multipotency) in the SVZ was identified.[80,97] These findings opened a new field of investigation that triggered a rapidly increasing number of studies performed by research groups throughout the world. The present review gives a brief synopsis (mostly from studies in rodents) on the architecture of the SVZ, the identity of the stem cells in the SVZ,[7,36], the signaling molecules modulating neurogenesis,[35,104] and finally the potential strategies to promote neurogenesis.[49,92] The present review is focused on the postnatal and adult SVZ but not on the neonatal SVZ where neurogenesis and extensive gliogenesis coexist.[62, 63, 73]

In Search of the Neural Stem Cells

First, here is a brief description of the lineage and nomenclature. Stem cells are defined as undifferentiated cells capable of proliferation, self-renewal and multipotency (i.e. they can give rise to multiple differentiated cell types). **Figure 1** defines the different terms used to describe immature cells that are called precursors or progenitors.

Description of the Cellular Architecture of the SVZ

The architecture of the SVZ has been the subject of several reviews,[45,91] and will be briefly summarized here. It is important to point out that most studies referred to the mid-SVZ or striatal SVZ along the lateral ventricle but not the SVZ in the dorso-lateral portion or the ventral part of the ventricle (**Figure 2A &B**). It is likely that the dorsolateral and ventral parts of the SVZ display a different cellular architecture and cells with different properties than those in the mid-SVZ. For example, cells with a radial glial morphology remain in the ventral part of the SVZ while they disappear from other SVZ parts.[110] At the light microscopic level, the SVZ appears as a rather homogeneous tissue, formed by small, tightly packed cells, easily distinguishable from the adjacent ependyma and mature nervous tissue (**Figure 2C**). [2,58,95,105]

Earlier studies based on Nissl staining or other histological methods described the presence of two major cell types that were collectively called subependymal cells.[2,17,105] Early electron microscopic studies[17] showed that numerous subependymal cells displayed the features of highly undifferentiated cells while the remaining cells were considered predominantly as astrocytes with a few as

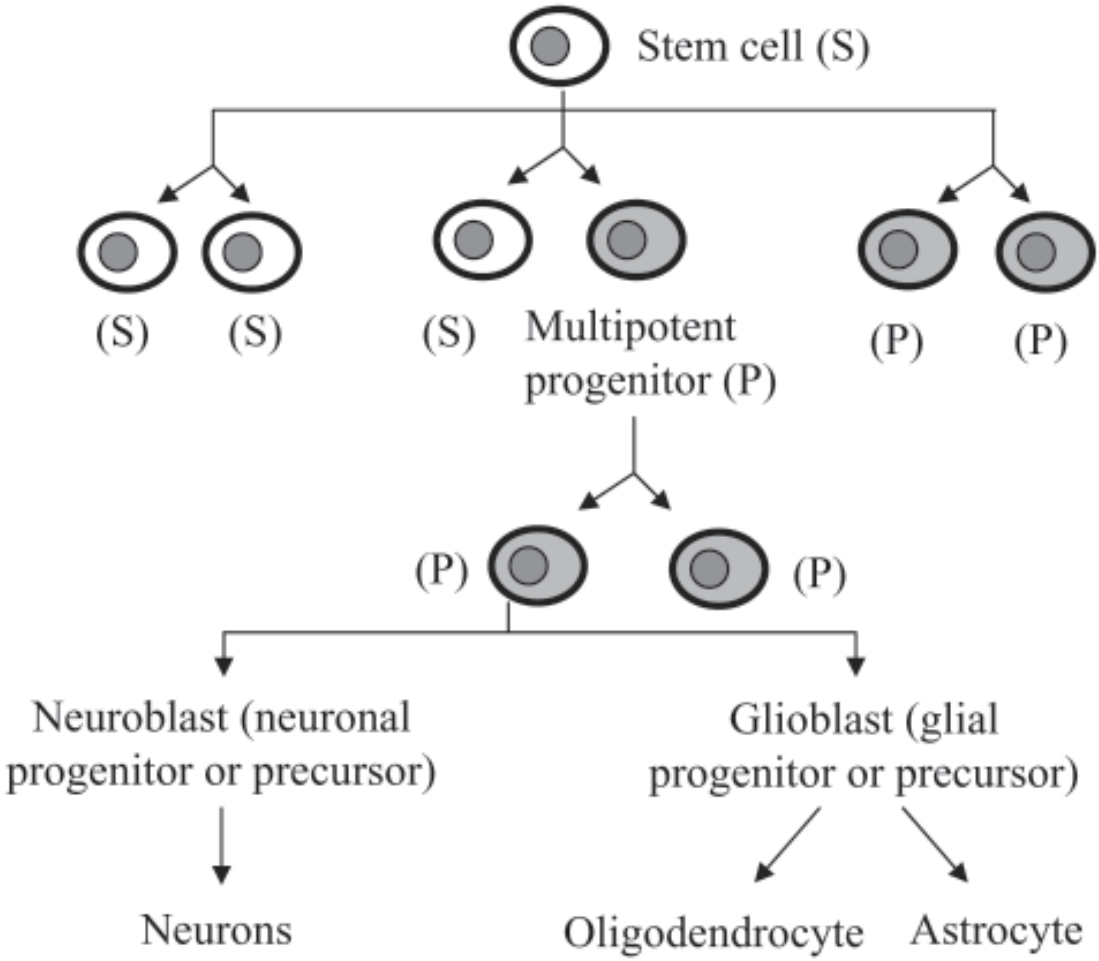

Figure 1. Definition and nomenclature of neural stem cell progeny. Neural stem cells are indefinitely self-renewing cells that can generate any cell type in the CNS. Stem cells (S) can divide and give rise to two identical daughter cells, or to one cell identical to itself and a more developmentally differentiated multipotent progenitor (P), or to two multipotent progenitors. These progenitors are capable of limited self-renewal before differentiating into lineage-committed offsprings termed neuroblasts and glioblasts (or neuronal and glial progenitors, respectively). These two cell populations can still replicate and generate progeny that remain within the committed lineage. The lineage-restricted progenitors then differentiate to give rise to mature neurons and glial cells.

microglia. More recent studies[54,67,90] confirmed that the SVZ of adult rodents contains two main cell types. The first cell type, referred to as type A[54] or class 1[54] or subependymal cell,[90] is very electron dense and shows the ultrastructural features of undifferentiated, migrating neuroblasts. These cells have an elongated, bipolar shape with one or two main processes emerging from the opposite poles. The second cell type, referred to as type B[67] or class 2,[54] is a particular type of protoplasmic astrocyte, which is less abundant than the undifferentiated cells (about a 1:4 ratio).

A recent ultrastructural analysis confirmed that undifferentiated precursors and the so-called astrocytes (type B cell) are the two main cell types lying between the ependymal cells and the striatal parenchyma in the adult SVZ.[39] The type B cells will be referred to as astrocyte-like cells here because they share some but not all of the properties of astrocytes (vide infra). This study further suggested that type A cells were the neuroblasts migrating to the olfactory bulb. However, it is worth mentioning that the authors identified a third cell type, called type C cells, which are scattered throughout the SVZ and incorporate tritiated thymidine, suggesting that they might correspond to constitutively proliferating precursors or presumably bipotential progenitors. Second, the same authors distinguished two types of astrocyte-like cells, indicated as type B1 and type B2 (smaller and more electron dense than type B1) that were adjacent to ependymal cells and located at the interface with the striatal parenchyma, respectively.

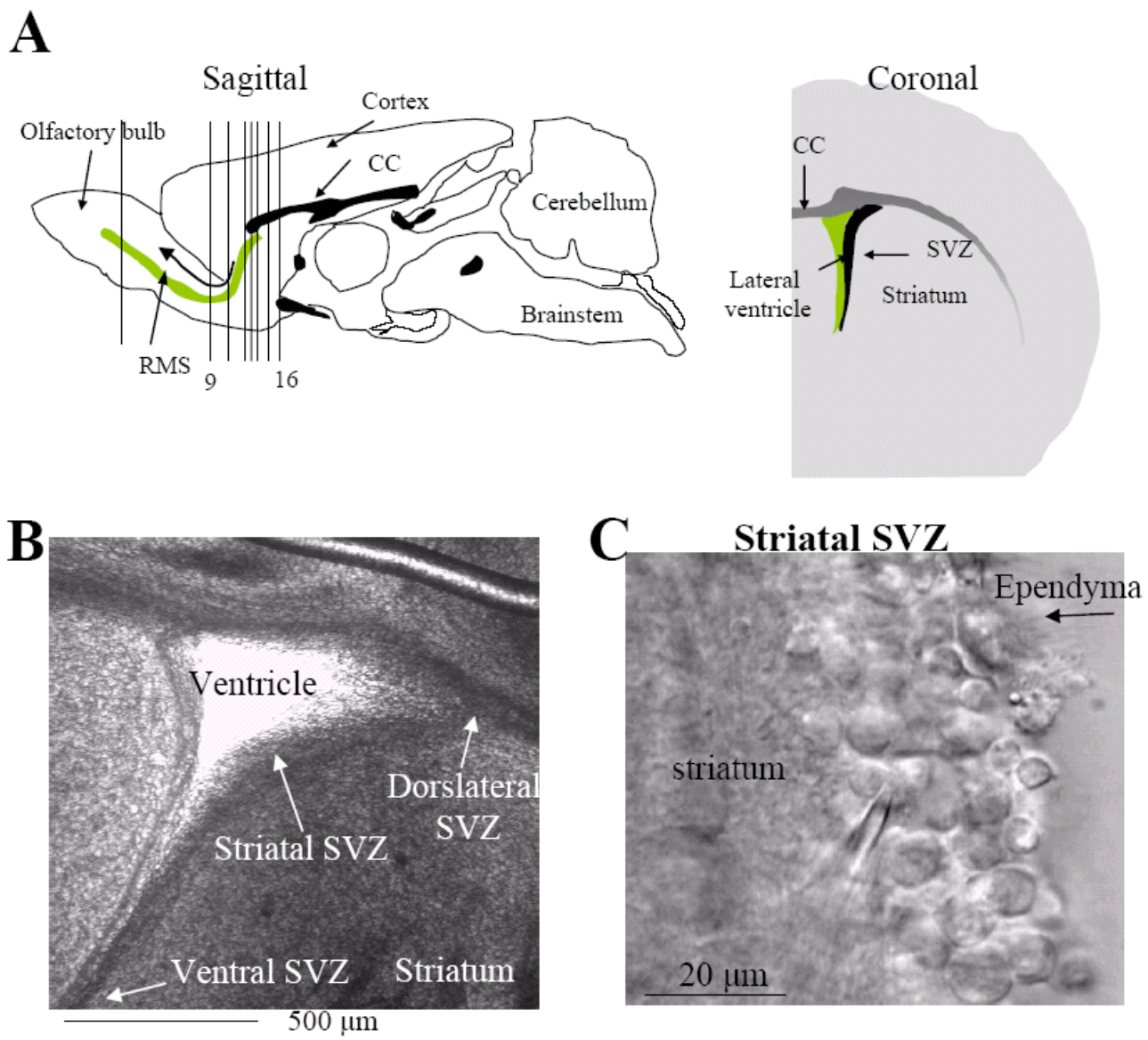

Figure 2. Location and architecture of the SVZ. A) Schematic view of a sagittal (left) and a coronal (right) section of the adult mouse brain. The lateral ventricle is located under the corpus callosum (CC). The rostral migratory stream (RMS) is highlighted in green in the sagittal section. B) Photographs (4x magnification) of a coronal section of the SVZ taken using infrared DIC optics. The SVZ lines the lateral side of the lateral ventricle and extends between the CC and striatum. The ventral, striatal and dorso-lateral parts of the SVZ are indicated on the photograph. C) High magnification photograph of precursors in the striatal SVZ.

Some type B2 cells were also observed to incorporate tritiated thymidine. Finally, the proportion of type A (neuroblast), B (astrocyte-like cells) and C (bipotential progenitors) cells differ along the rostro-caudal axis of the brain.

Cells in the SVZ and in the RMS display a specific arrangement defining two cellular compartments. Neuroblasts can be selectively detected by antibodies raised against the poly-sialylated form of the neural cell adhesion molecule (PSA-NCAM),[20,39,100] class III á-tubulin (TuJ1)[39]and doublecortin,[116] while astrocyte-like cells can be marked with antibodies against glial fibrillary acidic protein (GFAP)[39] and the astrocytic glutamate transporter GLAST (**Figure 3A**).[19,25] These antibodies revealed that the migrating cells form longitudinal chains (in sagittal sections) or clusters (in coronal sections) ensheathed by the processes of astrocyte-like cells **(Figure 3B)** that separate the migrating cells from the mature tissue.[19,67,90] While the chains are highly organized in the RMS, they appear looser in the SVZ where they form a three-dimensional network .[37]

Summary of organization: The SVZ-ependymal region contains at least four different cell types defined by their morphology, ultrastructure, and molecular markers. The migrating neuroblasts (type A cells) form chains ensheathed by astrocyte-like cells (type B cells). More spherical and highly proliferative precursors (type C cells) form clusters next to the chains of migrating neuroblasts. The SVZ is largely separated from the ventricle cavity by a layer of ependymal cells (previously called type E cells). Astrocyte-like cells interact closely with ependymal cells and occasionally contact the ventricle lumen.

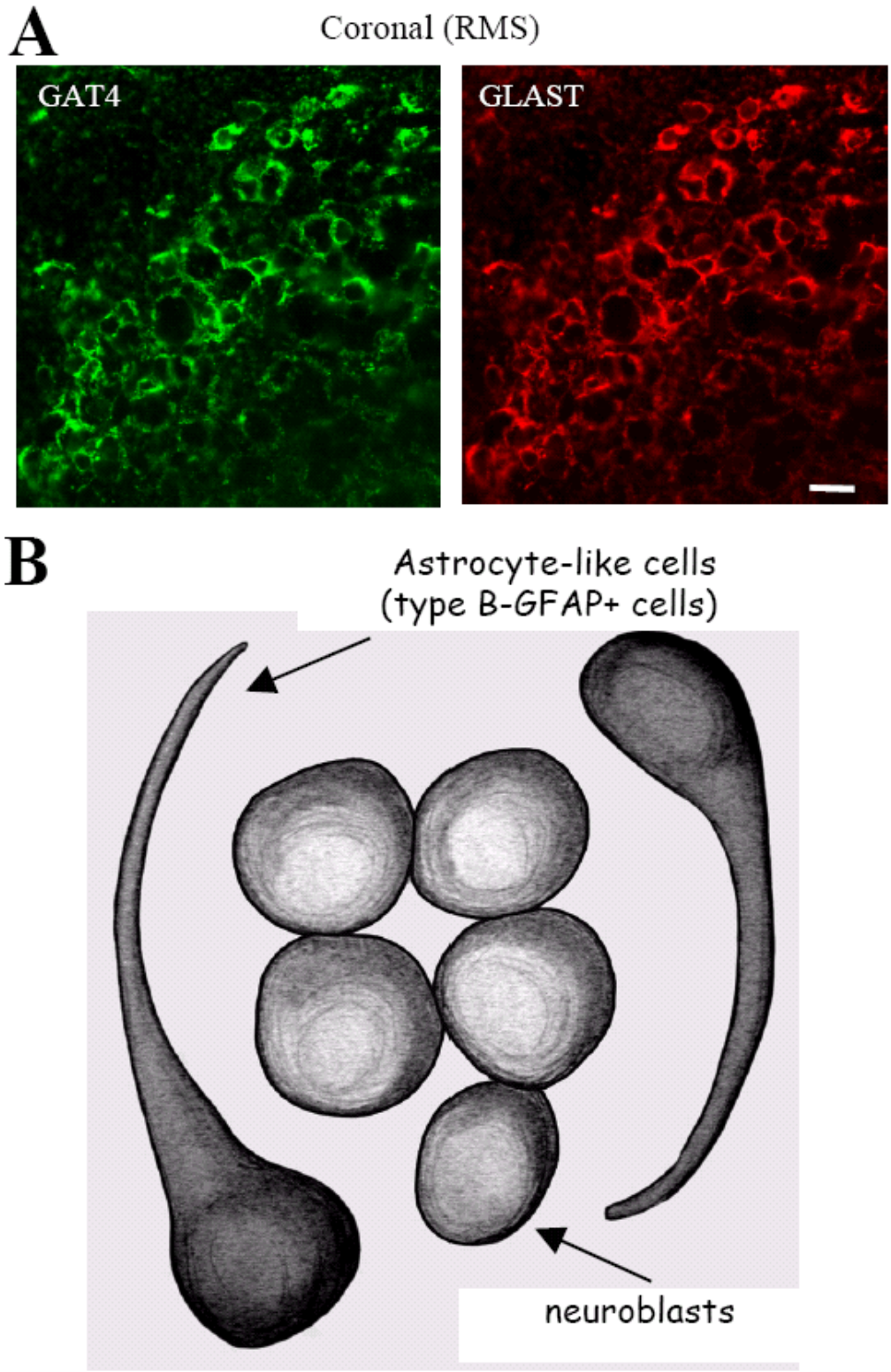

Figure 3. Specific cellular compartments in the SVZ. A.) Superimposition of the immunostainings for GAT4 (left) and GLAST (right) in a coronal section of the proximal RMS. Scale bar: 20 µm. B) A schematic diagram illustrating the ensheathment of neuroblasts by astrocyte-like cells.

Which Cells are the Stem Cells?

Uncovering the identity of neural stem cells has been the subject of intense research and debate for the past decade. It is now well accepted that GFAP-positive cells and not ependymal cells have stem cell attributes.[27,32,38,41,61] This finding was at first surprising, however, as the stem cell nature of cells with astrocytic attributes appears universal.[5,7] For example, early work in adult birds reported that neural stem cells had characteristics of radial glia.[8] Furthermore, in the developing rodent brain radial glia have long been known to produce cortical astrocytes, but recent data indicate that radial glia might also divide asymmetrically to produce cortical neurons..[50,71,84,85]

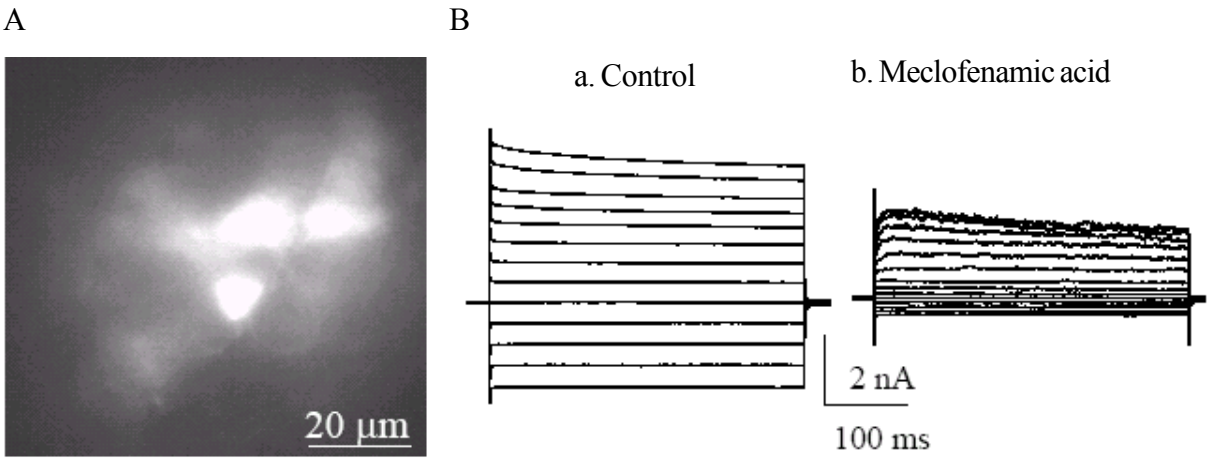

Figure 4. Astrocyte-like cells are dye-coupled. A) Photograph of lucifer yellow coupled astrocyte-like cells recorded in an acute coronal slice from a transgenic mouse expressing GFP under the GFAP promoter. B) Traces of currents in response to depolarizing voltage steps from -160 to +80 mV under control condition and following application of 100 ìM meclofenamic acid. The cells were held at -70 mV.

Although GFAP-immunopositive (GFAP+) cells in the SVZ were called astrocytes, it is important to determine whether they express features in common with astrocytes beyond GFAP expression. The definition of an astrocyte has raised concern over the past few years but it is safe to assume that mature astrocytes share (but are not limited to) the following features: expression of certain GABA and glutamate transporters, presence of specialized contacts onto blood vessels, absence of axons, presence of glycogen granules, close contact with neuronal elements, and typical biophysical features characterized by the lack of action potential and expression of large resting K^+ conductance.[77,114] Among these properties, GFAP+ cells of the SVZ immunostain positive for the glutamate transporters GLAST,[19,25] which is exclusively expressed in astrocytes.[29,99,104] GFAP+ cells of the SVZ also express functional GABA transporters[19] and ensheath neuronal precursors along their route to the olfactory bulb (vide supra). They display glycogen granules[91,109] and close contacts with blood vessels (**Figure 4**).[91] Their biophysical properties have, however, not been reported. Unpublished observations from Liu and Bordey suggest that GFAP+ cells in the striatal SVZ recorded in transgenic mice expressing GFP under the GFAP promoter display electrophysiological properties between those of radial glia and astrocytes. In particular, they display dye coupling to 3 or 4 surrounding GFAP+ cells (**Figure 4A**), have hyperpolarized resting membrane potentials (mean of -85 mV), do not generate action potentials upon current injection, and express large passive currents as observed in mature astrocytes (**Figure 4Ba**).[23,59] These passive currents are significantly reduced by gap junction channel blockers, meclofenamic acid (**Figure 4Bb**) or carbenoxolone, and are thus mostly due to electrical coupling (i.e. gap junction conductance) while passive currents in astrocytes are independent of electrical coupling and are presumably due to a large background K^+ conductance.[114] Upon blockade of gap junction coupling, GFAP+ cells of the SVZ display a small background K^+ conductance and an outwardly rectifying current profile, resembling that of radial glia in acute slices from mice.[9] Because radial glia give rise to GFAP+ cells in the SVZ, it is tempting to hypothesize that these GFAP+ cells remain in an intermediate state of transformation from radial glia possibly due to the persistent expression of immature environmental cues in the SVZ.[46] These findings and hypothesis further challenge the definition of astrocytes and its appellation, and question whether mature astrocytes derived from radial glial cell transformation could regain neural stem cell potential if placed in an appropriate neurogenic environment.

Why does Neurogenesis Persist?

Although this question has not been definitely answered, Altman in 1967 suggested that the newly generated interneurons may be responsible for neural plasticity or may be the substrate of memory.[3] In 1966, he refined his hypothesis and proposed that the plasticity or memory mediated by the interneurons formed postnatally in the cerebellum, dentate gyrus or olfactory bulb is associated with a circumscribed class of functions, namely the acquisition of locomotor memory (cerebellum) and the fixation of behavior patterns relating to affective, need-catering functions (olfactory bulb, hippocampus) and not with memory processes related to cognitive instrumental functions.[4] This concept has been further extended and supported by the work of Nottebohm and colleagues in the adult avian forebrain, in particular songbirds. They suggested that birth of projection neurons in the adult avian brain was related to the acquisition of perceptual or motor memories.[6] Nottebohm[86] proposed a model unifying the combined role of synaptic plasticity and neuronal replacement in promoting long-term memory at least for some kinds of neurons such as projection neurons in the avian brain and olfactory bulb neurons in the rodent brain. This model still needs to be validated.

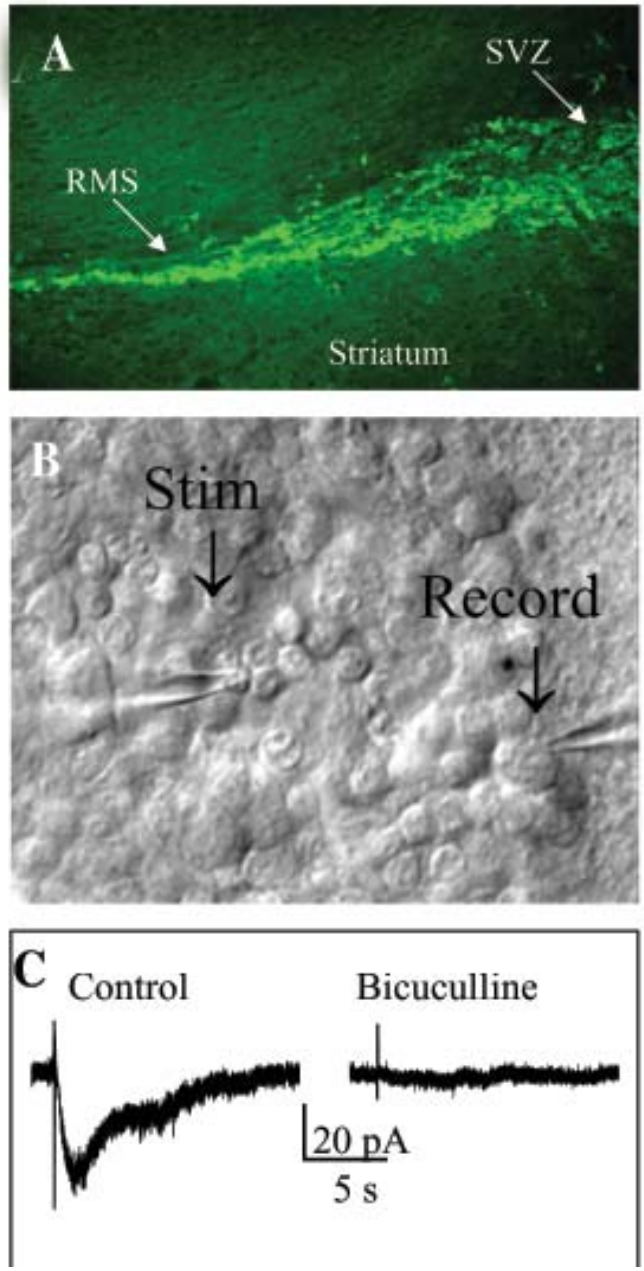

Figure 5. GABAergic signaling between SVZ precursors. A. Photograph of GABA immunostaining in a sagittal section including the anterior SVZ and proximal RMS. B) Photographs illustrating a representative position of the stimulating electrode (stim) with respect to the recorded cell (record). C) Repetitive focal electrical stimulation in the SVZ (5 pulses of 200 μs at 50 Hz) induced inward currents in astrocyte-like cells that are blocked with bicuculline (100 μM).

Non-synaptic Communication in Control of Neurogenesis in the SVZ

This paragraph argues for the view that intercellular signaling between neural stem cells and neuroblasts in the SVZ is critical for controlling postnatal neurogenesis (including proliferation, migration, survival/apoptosis, differentiation), and that non-synaptic communication between SVZ precursors displays characteristics of that between cells of neuron-glial networks elsewhere in the brain. Intercellular signaling can be mediated via diffusible molecules or via extracellular matrix protein and cell-cell contact. [35] Extracellular matrix proteins in the SVZ are thought to maintain an immature environment that promotes neurogenesis. The SVZ is thus considered to be a special neurogenic niche while the mature brain lacks immature cues or even expresses inhibitory molecules for neurogenesis. Diffusible molecules can promote interactions between SVZ precursors, between ependymal cells and precursors, and between mature cells in the surrounding parenchyma and SVZ precursors. This paragraph will be focused on diffusible molecules with a special emphasis on the neurotransmitter GABA that provides signals between astrocyte-like cells and neuroblasts, and nitric oxide (NO) that provides communication signals between neurons and

neuronal precursors. It is worth mentioning an important intercellular interaction between ependymal cells and astrocyte-like cells. Indeed, ependymal cells produce noggin, an antagonist of BMP signaling, which prevents glial differentiation of SVZ cells induced by BMPs,[64] further emphasizing this notion of a special neurogenic niche. Several other factors including Eph/ephrin signaling,[33] Sonic hedgehog,[60] prolactin,[103] adrenal hormones[92] affect some aspect of neurogenesis in particular proliferation and are not all listed in this review. The targeted cells of these molecules remain largely unknown.

GABAergic Signaling Controls Astrocyte-like Cell Proliferation

GABA plays an important signaling role in developmental processes, such as embryonic cell proliferation,[51,68] migration,[13,14,15,16,43] and differentiation.[10,31,72,74,75,81,88,106,107] The recent finding of GABA and its synthetic enzyme (glutamic acid decarboxylase 67, GAD-67) in neuroblasts[18,81,108,115](**Figure 5A**) but not in astrocyte-like cells[65] suggested the existence of a GABAergic signaling between SVZ cells. To consider GABA as an intercellular signaling molecule, GABA needs to be released, GABA receptors activated and GABA removed from the extracellular space since GABA is not degraded.

The action of GABA can be mediated by at least three types of GABA receptors called $GABA_A$, $GABA_B$ and $GABA_C$ receptors.[30] $GABA_B$ and $GABA_C$ receptors have not been detected in neuronal progenitors and agonists of $GABA_B$ receptors do not affect the migration or proliferation of neuroblasts.[18,81,115] The expression of $GABA_A$ receptor subunit transcripts and proteins were analyzed using polymerase chain reaction (PCR) and immunocytochemistry in neuroblasts in isolated cultures[108] and neurospheres.[81] Furthermore, using electrophysiological techniques, $GABA_A$ receptors ($GABA_A$Rs) were identified in both neuroblasts[115] and astrocyte-like cells.[65] Furthermore, $GABA_A$Rs in SVZ cells are tonically activated by endogenous GABA,[18,81] suggesting that there is enough ambient GABA to activate $GABA_A$Rs in SVZ precursors. Using mass spectrometry and patch-clamp recordings, it was reported that SVZ precursors spontaneously release GABA.[18,65,81] Liu ,et al., in 2004, also reported that non-synaptic GABA released from SVZ precursors following extracellular KCl application or tetanic stimulation activates $GABA_A$Rs in astrocyte-like cells (**Figure 5B**).[65] Regarding GABA uptake, pharmacological inhibition of GABA transporters in particular GAT4 [22] altered the kinetics of evoked $GABA_A$ currents in astrocyte-like cells[65] and reduced the speed of neuroblast migration via $GABA_A$R activation.[18] In addition, strong GAT4 immunostaining was observed in astrocyte-like cells but not in neuroblasts **(Figure 3A)**.[18] Therefore, in the SVZ GABA transporters play an important role in clearing GABA released from neuroblasts thereby controlling the degree of $GABA_A$R activation in astrocyte-like cells. The SVZ can thus be viewed as a local GABAergic network where GABA provides communication signals between precursors despite the absence of conventional synapses. While the role of synaptically released GABA on the biology of astrocytes remains unclear, tonic $GABA_A$R activation in astrocyte-like cells and neuroblasts was found to reduce their proliferation.[65,81]

Summary and model of GABAergic signaling between SVZ cells: As more neuroblasts are generated, it is expected that more GABA is released in the extracellular space resulting in increased ambient GABA levels and $GABA_A$R activation in SVZ precursors. Since astrocyte-like cells generate neuroblasts,[38,40a] an increase in the number of neuroblasts seems to serve as a negative feedback to decrease astrocyte-like cell proliferation and neuroblast production by activating $GABA_A$Rs. This negative feedback fits well with the constant migration of neuroblasts away from the SVZ to the olfactory bulb,[28,66,70] which limits ambient GABA accumulation, and with the increased proliferation of astrocyte-like cells following elimination of neuroblasts.[40] A similar feedback exerted on precursor proliferation by GABA may also occur in the embryonic ventricular zone where tonic $GABA_A$R activation by ambient GABA limits the proliferation of precursors.[51,68] Interestingly, the proliferative precursors in the ventricular zone, which are thought to be radial glial cells,[84,85] transform into astrocyte-like cells in the postnatal SVZ.[76,111] The postnatal SVZ can thus be viewed as an interface between the embryonic ventricular zone and the adult brain, sharing properties of both systems.

It is interesting to draw parallels between the neuroblast and astrocyte-like cell communication, and that between neurons and astrocytes. At GABAergic synapses, synaptically released GABA diffuses outside the synaptic cleft and activates GABA transporters in astrocytes.[56] Furthermore, inhibition of GABA transporters affects the degree of postsynaptic $GABA_A$ receptor activation and thus synaptic transmission.[34,48,96,98,113] Astrocytic processes encapsulate either one synapse or a group of GABAergic synapses like the cerebellar glomeruli.[89] This latter configuration resembles that of the SVZ where processes of astrocyte-like cells encapsulate clusters of migrating neuroblasts (**Figure 3**).

Influence from Surrounding Mature Nervous Tissue

Thus far, we described the SVZ as an independent network from surrounding brain regions. However, SVZ cells are adjacent to the striatum, which is rich in GABAergic cells and terminals. Furthermore, although glial processes ensheath migrating neuroblasts, migrating neuroblasts can be in contact with the surrounding neuropil (**Figure 5B**).[91] However, it remains unknown whether synaptically released GABA from GABAergic striatal terminals could diffuse from the striatum to the SVZ and activate $GABA_A$ receptors in neuroblasts and/or astrocyte-like cells. However, here are two examples to illustrate that mature cells adjacent to the SVZ can influence neurogenesis. The diffusible factor Slit, which is secreted from the septum, is a chemorepellent that directs the migration of SVZ neuroblasts toward the olfactory bulb.[52,53,83] Moreno-Lopez, et al., recently reported that in the adult mouse brain, the SVZ is surrounded by differentiated neurons expressing neuronal nitric oxide

synthase (nNOS).[78] These neurons project rich axonal networks in which SVZ neuroblasts are immersed. The spatial proximity between precursor cells and potential NO sources raised the question of whether NO may be one of the factors controlling neurogenesis in the adult brain. Systemic administration of NOS inhibitors to adult mice produced an increase in the number of mitotic cells in the SVZ, RMS, and olfactory bulb without affecting apoptosis.[78] In the SVZ, this effect was exerted selectively on a precursor subpopulation expressing nestin, presumably the type C cells but not on the neuroblasts or astrocyte-like cells. In addition, in the olfactory bulb, chronic NOS inhibition caused a delay in neuronal differentiation. Postmitotic cell survival and migration were not affected when NO production was impaired. All together these data suggested that NO, produced by nitrergic neurons in the adult SVZ and olfactory bulb, exerts a negative control on the size of the undifferentiated precursor pool and promotes neuronal differentiation.

How to Promote Neurogenesis in the Adult Brain

Does the SVZ Exist in Humans?

Neural progenitors have been isolated from regions along the lateral ventricular wall of humans with intractable temporal lobe epilepsy.[47,57,93,94] More recently Sanai,et al., described the presence of GFAP-positive cells lining the lateral ventricles of humans with stem cell attributes (i.e. they proliferate *in vivo* and behave as multipotent progenitor cells *in vitro*).[101] However, the organization of the human SVZ differs from that of rodent or monkey in that it is essentially formed by a ribbon of GFAP+ cells with no or very few neuroblasts. Furthermore, Sanai, et al., [101]did not observe chains of migrating neuroblasts in the SVZ or in the pathway to the olfactory bulb. However, newly generated neurons have been observed in the human olfactory bulb suggesting that either neuroblasts migrate from the SVZ to the olfactory bulb or persistent progenitors populate the olfactory bulb.[12] All of these studies were performed on tissue from adult humans. It remains to be determined whether the SVZ in infants or young adults is different from that in adults since neurogenesis decreases during aging.[42]

Challenges for Studying and Promoting Neurogenesis

Recruitment of new neurons is affected by environmental variables. It is known that captivity,[11] environmental simplicity as opposed to an enriched environment,[55] and physical inactivity,[112] significantly reduce neurogenesis in the adult brain. This has been essentially studied in birds and in the dentate gyrus of mammals but is likely to be true in the SVZ of mammals. It is thus critical to study neurogenesis and its regulation in animals as previously performed in their normal environment as performed.[11,21]

It is critical to define appropriate methods to deliver drugs and to identify the appropriate drugs to be delivered.[49,92] Our present knowledge suggests two possible strategies for promoting repair using neural stem cells. One approach is a cell replacement strategy to achieve structural brain repair, where neural stem cells are isolated from CNS tissue, expanded *in vitro* and grafted into the brain. The second, more elegant but more difficult approach is to recruit endogenous neural stem cells from the SVZ to proliferate, migrate, differentiate into functional glia or neurons, and properly integrate and survive to achieve self-repair. Both the grafting and recruitment approaches will require a thorough understanding of the factors and signals regulating neurogenesis. Furthermore, therapeutic progress will depend on the ability to deliver the appropriate factors or to regulate their endogenous expression, which requires close work with bioengineering research laboratories.

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