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Neural Stem Cells in the Adult Brain

Implications of Their Glial Characteristics

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INTRODUCTION

It is now widely accepted that new neurons are added continuously to some regions of the adult mammalian brain. More than 30 yr of reports describing neurogenesis in the adult brains of fish, frogs, reptiles, birds, and rodents *(1–6)* have recently culminated in studies demonstrating the birth of new central nervous system neurons in both primates *(7)* and humans *(8,9)*. Hence, the century-old, dogmatic proposition of a fixed, ended, immutable adult brain has been refuted, spurring new investigations into the regenerative capacity of the central nervous system.

The dentate gyrus of the hippocampus *(10)* and the lateral ventricle subventricular zone (SVZ) *(11)* are two brain regions in which neurons are born in the adult. The SVZ is the larger of these two germinal zones, and consists of a layer of cells adjacent to the ependyma along the entire length of the lateral ventricular wall. In postnatal *(12)* and adult rodents *(13)*, cells born in the SVZ migrate from the ventricular wall into the olfactory bulb (OB) where they differentiate into interneurons. In the monkey brain, the SVZ also generates neurons for the OB *(14,15)*; interestingly, it has been suggested that adult monkey SVZ cells also generate new neurons for the prefrontal, inferior temporal, and posterior parietal cortex *(16)*. These latter observations are a matter of debate *(17,18)*. The adult human SVZ also contains proliferating cells *(8,19)*, and, recently, astrocyte-like cells close to the walls of the human lateral ventricle have been shown to behave as stem cells in vitro *(9)*.

The proliferation of SVZ cells continues throughout life *(20,21)*. It has been estimated that at least 30,000 new OB neurons are born in the mouse every day to replace those that are dying *(13)*. This profound level of continuous neurogenesis argues for the presence of a self-renewing primary progenitor, or stem cell, within the SVZ. Self-renewing cells from the SVZ have been propagated in vitro in both adherent and nonadherent cultures, and these cells can differentiate into neurons, astrocytes, and oligodendrocytes *(22–24)*. Self-renewal and multilineage differentiation are two generic attributes of stem cells. A population of cells in the SVZ satisfies these two criteria and can thus be described as a neural stem cell. However, the precise definition of stem cells is a matter of debate *(25–27)*. The SVZ stem cell is perhaps most analogous

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to stem cells found in the skin, intestine, and blood. Stem cells of the SVZ and these other regions generate new cells for their respective organ systems throughout the life of the animal. The constant production of new cells complements normal cell turnover, maintaining the tissue cell population. It is not clear how similar adult brain stem cells are to those of the embryo. We define the adult mouse SVZ stem cell as the self-renewing cell type responsible for maintaining the constant production of OB neurons in vivo.

Perhaps the most misleading notion about stem cells is that they should be undifferentiated or primitive, lacking expression of markers attributed to more mature cells. This perception has led many researchers to ignore the "mature-looking" cell as potential stem cells. However, it is becoming increasingly clear that stem cells can bear what were thought to be the biochemical and structural hallmarks of differentiated cells. For instance, skin stem cells express intermediate filament keratins found in mature keratinocytes *(28,29)*. Intestinal crypt stem cells, which continuously replace the epithelial lining of the digestive tract, have been described as being more epithelial than primitive *(30)*. Hematopoietic stem cells (HSCs), perhaps the best studied of all stem cells, express what have been considered to be lineage-restricted factors *(31)*.

In this chapter, we incorporate our understanding of the cellular composition of the SVZ with recent experimental results that identify the neural stem cell. Surprisingly, the stem cell candidate possesses attributes of mature glial cells. Given the prevailing view that glial cells represent an end point in neural development, a glial-like stem cell seems extraordinary. We therefore review the data concerning the SVZ stem cell identity. We then discuss the possibility that glial-like cells might be stem cells at other developmental times. Considering the accumulating evidence of glial-like stem cells, we propose a revision to our current understanding of developmental neural cell lineages.

CELLULAR COMPOSITION AND ORGANIZATION OF THE ADULT MOUSE SVZ

In the adult mouse, neuroblasts born along the entire length of the SVZ migrate anteriorly to the OB. The migratory neuroblasts (type A cells) move along each other forming elongated clusters of young neurons called chains *(32,33)*. The SVZ is organized as a network of interconnecting paths for chain migration widely distributed throughout the lateral ventricle wall *(34)*. These paths converge at the anterior SVZ, where the confluence of chains of type A cells continues along the rostral migratory stream (RMS), a restricted path that leads into the core of OB. In the OB, new neurons differentiate into local interneurons that become incorporated into local circuits *(35,36)*. It has been suggested that in neonatal rat brain, SVZ neuroblasts originate exclusively in the anterior SVZ, the so-called SVZa *(37)*. This is not observed in the adult *(34)*. The high concentration of neuroblasts that converge at the anterior SVZ may give the impression that this is the site of origin of these cells. Further work is required to describe potential differences between neonatal and adult SVZ and to determine the nature of cells in the neonatal caudal SVZ.

Chains of migrating type A cells in the adult mouse brain are ensheathed by the processes of slowly dividing SVZ astrocytes (type B cells) *(32,38)*. Scattered along the type A cell chains are clusters of rapidly dividing immature cells (type C cells). Type C cell clusters are often interposed between type B and A cells *(38)*. See Fig. 1 for a schematic cross section of the SVZ.

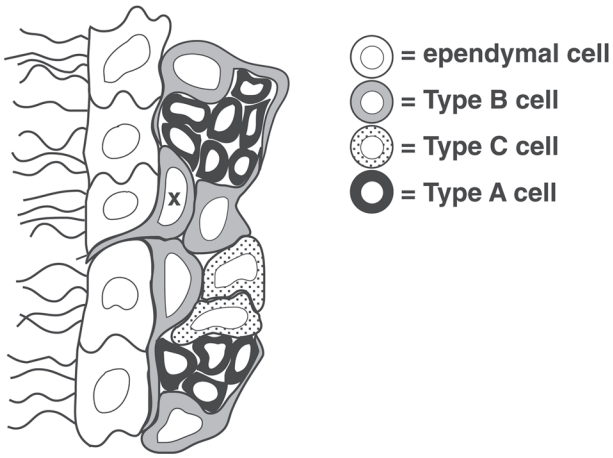

Fig. 1. Schematic cross section of the adult SVZ. Ependymal cells (*white*) are multiciliated and are closely apposed to the underlying SVZ cells. The ventricular lumen is to the left. Type B cells (*gray*) are slowly dividing astrocyte-like cells that ensheath chains of migrating type A cells (*black*). In this cross section, type A cells would be migrating out of the plane of the paper. Type C cells (*stippled*) are highly mitotic and found as clusters along the chains of type A cells. See "Cellular Composition and Organization of the Adult Mouse SVZ" for details. Some type B cells (marked with "x") extend a cellular process between ependymal cells and contact the ventricle. Many of these ventricle-contacting type B cells have a short, single cilium lacking the central pair of microtubules (9+0 arrangement). Ventricle-contacting type B cells may be an actively dividing SVZ stem cell. See "The Ventricle-Contacting Type B Cell: Interkinetic Nuclear Movement?" for details.

SVZ cell types were identified based on morphological, immunocytochemical, and ultrastructural characteristics *(38)*. Type A cells are immunopositive for a neuron-specific β-tubulin revealed by monoclonal antibody Tuj1 and express a polysialylated form of neural cell adhesion molecule (PSA-NCAM). Type B cells contain intermediate filament bundles containing glial fibrillary acidic protein (GFAP), a marker assigned to mature astrocytes. Type C cells are ultrastructurally immature and do not stain for markers of mature brain cells. Both type A and C cells express the transcription factor Dlx2 *(39)*, which in development is involved in the production of cortical interneurons which migrate from the medial ganglionic eminences *(40,41)*. Adjacent to the SVZ is the layer of multiciliated ependymal cells. Interestingly, all SVZ cell types and the ependyma express nestin *(38)*, an intermediate filament protein found in neuroepithelial stem cells *(42)*.

Ependymal cells line the luminal surface of the brain ventricle and appear highly differentiated, bearing multiple beating cilia that move cerebrospinal fluid through the ventricular system. Ependymal cells express high levels of the cell surface marker CD24 (type A cells express lower levels of this antigen) *(43–45)*. The lateral ventricle ependyma is generally described as a layer of multiciliated epithelial cells that separate the SVZ from the ventricular lumen. However, on closer examination using electron microscopy (EM), the ependymal layer does not appear entirely contiguous. In normal mice, a small number of type B cells make direct contact with the ventricle *(39,46)*. Some of these type B cells contact the ventricle by extending a thin cellular process

between ependymal cells while a few have a larger luminal surface (see "x"-marked cell in Fig. 1). Thus, the boundary between the ependymal layer and the SVZ is somewhat blurred by the small number of type B cells that are interdigitated with the ependymal cells. In addition to their unusual cellular location, some of the ventricle-contacting type B cells possess a single, thin cilium lacking the central pair of microtubules. Similar single cilia with this 9+0 microtubule arrangement have been described in embryonic neuroepithelial cells *(47,48)* and adult avian brain neuronal precursors *(49)*. As we discuss later, this cilium may be an indicator of stem cell progression through the cell cycle.

WHICH ARE THE SVZ STEM CELLS?

As mentioned in the introduction, stem cells can express markers of differentiated cells. Perhaps, then, it should not be surprising that candidates for the adult SVZ stem cell have been found to possess attributes of mature glia. Here, we review the recent accumulation of data indicating that the SVZ stem cell is an astrocyte-like, GFAP-positive cell.

The Label-Retaining Cell of the SVZ

A traditional view of adult stem cells is that they divide very slowly. For instance, to maintain hematopoiesis, HSCs enter the cell cycle every 1–3 mo *(50,51)*, and the slowest cycling cell in the skin has stem cell behavior *(52)*. Accordingly, data from two studies suggest that SVZ stem cells are the most slowly dividing cell of this region *(46,53)*. Owing to their slow cell cycle, stem cells are labeled infrequently by a single pulse of a nucleotide analog such as [^{3}H]thymidine or bromodeoxyuridine (BrdU). Efficient labeling of stem cells requires continuous or repeated administration of [^{3}H]thymidine or BrdU for a prolonged duration. Once having incorporated the label, the stem cells retain the mitotic marker for an extended period of time and can thus be identified as label-retaining cells (LRCs). Rapidly dividing progenitor cells dilute out the label and/or migrate from the region.

The label-retaining experiment has been performed in the SVZ *(44,54)*. BrdU was administered to animals for 2 wk in the drinking water, and 1 wk after the end of the BrdU administration, brain sections were processed with BrdU immunohistochemistry. Although the BrdU-positive nuclei are very clearly labeled, the resolution of the light microscope is not sufficient to distinguish ependymal cells from the closely apposed SVZ cells. Type B cells sometimes have their nuclei separated from the ventricular lumen by only a thin process of an adjacent ependymal cell, and such a nuclei could be easily mistaken as belonging to the ependymal layer *(38,39,46)*. In addition, some type B cells are interposed between ependymal cells and actually contact the ventricle. Double-immunohistochemistry for BrdU and the ependymal markers CD24 or S100 as well as EM analysis of [^{3}H]thymidine-treated brains did not reveal evidence of ependymal cell division.

Do ependymal cells ever divide in vivo? Previous reports are also conflicting. A survey of the literature reveals reports concluding that the lateral ventricle ependyma do not divide and others that claim to have evidence of ependymal proliferation in the same area *(55)*. It is difficult to come to any conclusion from the earlier reports as EM

was not used to confirm ependymal cell identity in the lateral ventricle wall. There are also reports of ependymal cell proliferation in the fourth ventricle and central canal of the spinal cord *(56,57)*. However, more detailed EM analysis is required to confirm that the proliferating cells in these caudal regions correspond to multiciliated ependymal cells. Johannson et al. show by EM a ventricle-contacting cell in mitosis in the central canal; however, this cell appears unciliated. Perhaps the ependymal cells along the neuraxis are not all equivalent. In children, ependymal tumors occur most frequently in the fourth ventricle, and this may reflect such intrinsic differences *(58)*.

Epithelial layers with a stem cell component often display a remarkable regenerative capacity in pathological conditions. If the lateral ventricle ependyma contains a stem cell, then one might expect this epithelium to regenerate after injury. However, there is at present no convincing evidence that the lateral ventricle ependyma regenerates after injury. Interestingly, injury to the ependymal cells stimulates the subependymal astrocytes to proliferate and form a gliotic scar, which appears to substitute for the missing ependyma *(55,59)*. Again, ependyma of more caudal regions may behave differently and have some capacity to proliferate, and comparisons of the molecular characteristics of the ependyma throughout the neuraxis would be revealing.

Hence, the majority of SVZ LRCs are type B cells. LRCs, of course, are not necessarily stem cells. Labeled type B cells might simply represent endogenous local glial cell turnover. Furthermore, the SVZ stem cells may enter the cell cycle so rarely that a 2-wk period of labeling would not identify them.

Regeneration of SVZ From Slowly Dividing Type B Cells

Because they are believed to divide more slowly than other cell types, adult stem cells should be more resistant to antimitotic agents. Thus, treatment with certain types of antimitotic drugs should be able to eliminate rapidly dividing progenitor cells while sparing a population of stem cells capable of regenerating the killed cells. Infusion of the antimitotic cytosine-β-D-arabinofuranoside (Ara-C) into the SVZ for 6 d eliminates all type A and C cells *(46)*. The only cell types remaining are type B and ependyma. At the end of Ara-C treatment, no BrdU or [^{3}H]thymidine incorporation is observed in the SVZ. However, 12 h after Ara-C removal, type B cells begin incorporating BrdU. No ependymal cells incorporate the mitotic marker. Two days later, the first type C cells appear, and by 14 d, the entire cellular and architectural composition of the SVZ is regenerated. The appearance of type C cells followed by type A cells suggests a developmental lineage of B to C to A. See Fig. 2 for a lineage schematic.

Type B cells incorporate BrdU almost immediately after Ara-C removal. This rapid appearance of mitotic cells suggests that stem cells are recruited into cell division by the absence of progenitor cells (negative feedback loop). If this notion of stem cell induction were true, then one would predict the stem cells themselves to be slowly killed off with continued Ara-C administration. This appears to be the case. Increasing the duration of Ara-C treatment decreases the number of type B cells remaining in the SVZ (Doetsch and Alvarez-Buylla, *unpublished observations*). With continued Ara-C treatment or local irradiation *(60)*, it may be possible to deplete completely the SVZ of stem cells.

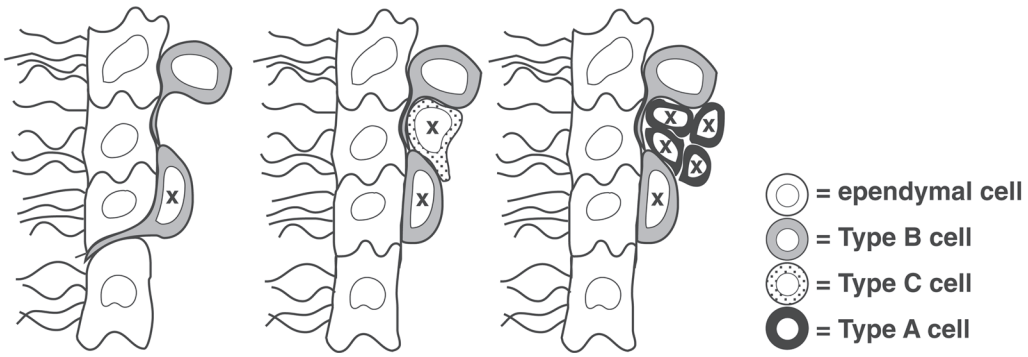

Fig. 2. Proposed SVZ cell lineage. In this model, a type B cell (*left*, marked with "x") divides asymmetrically to produce another type B cell and a type C cell (middle, marked with "x"). Type C cells are transit-amplifying cells that divide before generating type A cells (*right*, *black* cells marked with "x"). The remaining type B cell can later reenter the cell cycle to produce more neurons. Type B cells may also divide symmetrically.

Lineage Analysis of SVZ Stem Cells

To follow specifically the fate of type B cells, Doetsch et al. *(44)* injected an avian leukosis retroviral vector (RCAS) encoding alkaline phosphatase (AP) into the SVZ of transgenic mice. The transgene in the recipient mice directs expression of the avian retrovirus receptor to GFAP-positive cells *(61)*. Hence, the RCAS vector labels only mitotic type B cells. One day after injection, only type B cells express the RCAS marker AP gene, confirming the specificity of the initial infection. Three and one half days later, AP-positive cells are found en route to the OB, and by 14 d, many AP-positive neurons integrate into the OB. Although this experiment clearly demonstrates that type B cells can produce OB neurons, it does not exclude ependymal cells from this lineage. One could argue that ependymal cells produce OB neurons directly or through a type B cell intermediate. Nor do the data demonstrate that type B cells self-renew in vivo. However, the presence of AP-positive type B cells in the SVZ 14 d after infection suggests that stem cells were originally infected.

Tracing the Fate of Ependymal Cells In Vivo?

To determine if ependymal cells can give rise to OB neurons, Johannsen et al. performed experiments that were designed to follow the fate of ependymal cells in vivo. To label ependymal cells, they injected either the fluorescent lipophilic label DiI or adenovirus carrying the β-galactosidase marker into the lateral ventricular lumen. One day after injection, labeled cells appeared restricted to the ependymal layer. Ten days after injection, a large number of labeled cells were found either en route to the OB or in the OB. The interpretation of these data is difficult. Intraventricular injections of tracer substances label all cells that contact the ventricle. Because some type B cells contact the ventricle, it is possible that the DiI injection labeled B cells as well as the ciliated ependyma. Transfer of DiI from the ependyma to other cell types is also difficult to rule out. Johannsen et al. employed the adenoviral vector in an attempt to exclude these possibilities. They found that only ependymal cells express the adenovirus receptor CXADR and thus assumed that this would restrict adenoviral infection to

the ependyma. This assumption is, however, not correct as adenoviral vectors have been reported to infect multiple brain cell types *(62)* including type B astrocytes and other SVZ cells *(63)*. Furthermore, the fact that type B cells contact the ventricle complicates the adenovirus result in the same way it renders the DiI experiment difficult to interpret. The present data support a more general conclusion that cells closely associated with the ependymal layer can generate OB neurons.

Neural Stem Cells In Vitro

Neural stem cells isolated from the adult brain SVZ can be propagated as nonadherent clusters of cells called neurospheres *(53,64,65)*. Cell proliferation is maintained by high concentrations of epidermal growth factor (EGF). On removal of EGF, these cultured cells are capable of differentiating into neurons, astrocytes, and oligodendrocytes.

Neurospheres can be grown from type B and C cells, making them both candidates for neural stem cells *(39)*. Given this finding, it seems that "stemness"—at least in vitro—may be more related to competence of a group of precursors early within a lineage rather than to a specific cell type. This notion is also supported by the finding that oligodendrocyte progenitors also show a similar potential to form neural stem cells in vitro when exposed to growth factors *(66,67)*. However, the aforementioned lineage tracing experiments as well as the AraC regeneration data indicate that type B cells are the primary precursors of the SVZ in vivo.

Two other groups have found that GFAP-positive astrocytes can behave as neural stem cells. Laywell et al. *(68)* found that postnatal GFAP-expressing cells from the multiple brain regions of the mouse can behave as neural stem cells in vitro. However, after 14 d, these astrocytes from the cortex, cerebellum, and spinal cord no longer display stem cell characteristics. SVZ GFAP-positive type B cells maintain stem cell behavior into adulthood *(44)*, and this finding was supported by data from Imura et al. *(69)*. In that study, the authors employed a mouse expressing the thymidine kinase gene under the control of the GFAP promoter. Gancyclovir selectively kills dividing cells expressing the thymidine kinase gene, and so by delivering gancyclovir to this GFAP-TK transgenic mouse, Imura et al. were able to eliminate GFAP-positive cells at various stages of development including adulthood. Very few neurospheres could be made from adult GFAP-TK animals treated with GCV, which can be explained by the selective loss of GFAP-positive type B cells as well as their type C cell descendants. Interestingly, neurospheres can be isolated from GCV-treated GFAP-TK embryos, indicating that neural stem cells earlier in development do not express GFAP.

It has been suggested that ependymal cells lining the lateral wall of the lateral ventricles can also give rise to neurospheres in cultures. However, recent data do not support this contention. Johanssen et al. grew neurospheres from putative ependymal cells based on their multiciliated morphologies or by injecting DiI into the lateral ventricles and selecting labeled cells. Rietze et al. *(70)* similarly labeled cells lining the lateral ventricle with DiI and used fluorescence-activated cell sorting (FACS) to identify the cell population capable of giving rise to neurospheres; the authors found that the neural stem cell-enriched population did not include ependymal cells but did include DiI-labeled cells. What is the explanation for these differences in experimental results? Some astrocytes make contact with the ventricle lumen, and, as mentioned earlier, DiI ventricular injections may label ependymal cells as well as this subpopulation of

astrocytes; these astrocytes may be the cell type that gives rise to neurospheres. Other data from Chiasson et al. *(71)* and Laywell et al. *(68)* demonstrate that ependymal cells isolated from early postnatal or adult mouse brain are able to form neurosphere-like cell clusters; however, these cells are neither self-renewing nor multipotent. More recently, Capela and Temple *(45)* used the Lewis X cell surface marker, a carbohydrate in embryonic pluripotent stem cells as well as the adult SVZ, to separate SVZ cells by FACS for in vitro neurosphere assay analysis. The LeX-positive fraction was enriched for neurosphere-generating cells as compared to the LeX-negative fraction, which includes ependymal cells. Capela and Temple also isolated ependymal cells with the marker CD24 and negligible numbers of these cells produce neurospheres, leading to their conclusion that ependymal cells are not stem cells.

Although it is tempting to relate EGF-responsive cells in vitro to the in vivo stem cell behavior in vivo, a more conservative viewpoint is that the neurosphere assay reveals cell types that can self-renew and become multipotent in response to EGF signaling. The caveats of in vitro stem cell study are perhaps obvious but should be reiterated. Stem cells in vivo reside in niches that provide these primary progenitors with a microenvironment critical for their behavior. Cultured stem cells are removed from their normal cellular context. Furthermore, stem cells in culture are exposed to nonphysiological concentrations of mitogenic factors, which may alter their "normal" developmental potential. For example, hippocampal precursors grown in the presence of fibroblast growth factor-2 (FGF-2) can differentiate into neurons phenotypically distinct from those of the hippocampus *(72)*. In vitro cultures might remove transcriptional silencing and in such a way "deprogram" a cell, making the transcriptional profile more "generic," allowing a wider diversity of final cell fates.

Thus, any demonstration of stem cell behavior in vitro must be interpreted cautiously. In vitro manipulations may be necessary for stem cell behavior to be unveiled in a particular cell. While ciliated ependymal cells may divide in vitro in response to EGF, the evidence for ependymal cell division in vivo is not conclusive. Likewise, it is not clear that EGF is the primary mitogen for type B cells in vivo *(73)*, and so the multipotentiality of neurospheres may be a consequence of high levels of EGF signaling. Discovering the molecular signals present in the SVZ is critical for future in vitro studies.

Clues about the molecular signals critical for stem cell biology may come from the intercellular interactions observed in vivo. For instance, hematopoietic stem cells are best maintained in vitro on cultures of bone marrow stromal cells monolayers *(74)*. Skin stem cells are similarly clonogenic when cultured in contact with fibroblasts, their in vivo cellular neighbors *(75)*. In the SVZ, all cell types are in contact with astrocytes. Reconstituting the interaction between astrocytes and SVZ stem cells in vitro recapitulates the extensive production of young neurons observed in vivo *(76,77)*. Neurogenesis in these cultures is not dependent on exogenously added growth factors or serum. Understanding the molecular nature of the astrocyte–stem cell interaction may allow for the design of culture assays that fully reproduce in vivo stem cell behavior. Furthermore, the coculture in vitro assay may prove to more faithfully recapitulate the biology of SVZ stem cells than high concentrations of EGF or FGF-2.

The SVZ Stem Cell: A Particular Subtype of Astrocyte?

Type B cells produce OB neurons and can form multipotent neurospheres in vitro. Type B cells are also sufficient to regenerate the SVZ after the elimination of rapidly dividing cells. This body of evidence demonstrating stem cell behavior of type B cells should alter our perception of cells with glial characteristics in the brain. The expression of GFAP can no longer be ascribed only to cells committed to a glial lineage. Cells with morphological, ultrastructural, and antigenic features of astrocytes may very well have the ability to serve as stem cells. It remains to be determined if all brain astrocytes retain the ability to become stem cells. In the SVZ, only a small fraction of the GFAP-positive type B cells can form multipotent neurospheres. Given these data, it is likely that at any one time, only a subset of type B cells can serve as stem cells. If only a subset of type B cells are stem cells, markers specific to those cells would be useful for identification and isolation. Alternatively, a wider population of astrocytes in the adult brain may have stem cell potential, but at any one time only a small subpopulation may be competent to express this potential.

NEURAL STEM CELLS: FROM THE EMBRYO TO THE ADULT

The Ventricle-Contacting Type B Cell: Interkinetic Nuclear Movement?

As described and reviewed earlier, some type B cells contact the ventricle. In some of these ventricle-contacting type B cells the centriole projects a single 9+0 cilium similar to those on neuroepithelial cells and neuronal precursors of the avian brain *(49)*. In cultured cells, the appearance of a 9+0 cilium has been correlated with cell cycle progression *(78)*. About 6 h before S-phase, one of the interphase centrioles of fibroblasts become ciliated, differentiating them from quiescent cells. Perhaps, then, the 9+0 cilium on type B cells indicates their progression through the cell cycle. If this is correct, the ventricle-contacting type B cells could be activated SVZ stem cells just hours away from DNA replication.

Could it be that all dividing type B cells transiently contact the ventricle at some point during the cell cycle? Such a mechanism would be reminiscent of the interkinetic nuclear movement observed in the ventricular zone of embryos *(79,80)* and the adult avian brain *(49)*. In the ventricular zone, the nucleus of an actively dividing cell migrates to and from the ventricular lumen at different points in the cell cycle with mitosis occurring at the ventricular wall. Similarly, a dividing type B cell may actually push aside neighboring ependymal cells and contact the ventricle as it progresses through the cell cycle.

Nomenclature: Is There an Adult Ventricular Zone?

It is perhaps appropriate at this point to raise the problem of nomenclature and its inherent conceptual influences. First, the SVZ, sometimes called the subependymal layer (SEL) or zone (SEZ), suggests that this germinal center functions underneath the ependymal covering without contacting ventricular fluid. In fact, it is widely accepted that the so-called ventricular zone (VZ) *(81)* disappears during development and is no longer present in the adult. Notice that these influential statements are purely based on gross anatomical observations and have not been based on testing whether a VZ-like cell is present in the adult. Clearly, the recent finding that some type B cells may tran-

siently come into contact with the cerebrospinal fluid both challenges this view and contradicts the prefix of "sub-" in SVZ, SEL, or SEZ. Second, using the terms SEL or SEZ may not only be inaccurate in terms of the localization of the germinal cells, but would also suggest that the adult cell genesis in this region is fundamentally different than that of the embryo. When is the SVZ is no longer a SVZ and become a SEZ? Based on the anatomical localization of the embryonic SVZ, and recent transplantation experiments *(82)*, it is evident that the SVZ of the lateral ganglionic eminence (LGE) has cells of similar properties to those present in the adult SVZ. Until an updated terminology becomes available, it is perhaps best to keep one term, SVZ, to describe both the developing and adult germinal zone that underlies the VZ and ependyma, respectively.

Do the Lineages of Neurons and Glia Separate Early in Development?

The recent identification of glial-like neural stem cells raises an important long-standing controversy concerning the developmental origin of neurons and glia in general. Do neurons and glia arise from a multipotential cell type, or are there specific types of cells devoted to one lineage or the other?

The controversy of multipotent stem cells vs lineage-restricted precursors can be traced back to the earliest studies of the neural tube *(83)*. The central nervous system arises from a sheet of cells called the neural epithelium. Early in development, the neural epithelium invaginates from the rest of the embryo, forming the neural tube. In 1887, Wilhelm His founded the concept of subclasses of neuroepithelial cells that are consigned to becoming either neurons or glia. His described two neural tube cell types based on their appearance. Germinal cells were the rounded cells near the lumen, and he proposed these to be precursors of neurons. He also described a columnar matrix of cells, known at that time as spongioblasts (today referred as radial glia), and proposed these to be committed to giving rise to glial cells. His was probably misled by artifacts of histology as he improperly described spongioblasts as a syncitium rather than as separate cells. The theories of His were countered by Schaper in 1894 and 1897. Schaper concluded that the germinal cells and spongioblasts are essentially the same cell type at different stages of cell cycle. However, it was not until 1935 that F. C. Sauer produced new evidence in favor of Schaper's theory. Later, more cell cycle studies confirmed that all neuroepithelial cells are the same, and that the differences in their location and appearance simply represent a different stage of the cell cycle. Nevertheless, the concept of a different origin for glial and neuronal cell types in the brain remained heavily ingrained in the neurosciences. The preceding description of a cell with glial characteristics challenges this view. Other recent work also suggests that the developmental predecessor of astrocytes, the radial glia, are also not committed glial progenitors, but the stem cells of the developing nervous system.

Radial Glial Cells as Neural Stem Cells

Radial glial cells arise during VZ development and are unique in that they extend processes from the ventricular lumen to the pial surface. Radial glial processes are commonly thought to serve as guides that neuroblasts migrate upon to reach their final destination *(84)*. After neuronal production ceases, radial glial are believed to retract from the ventricular and pial surfaces and differentiate into brain astrocytes *(85–88)*.

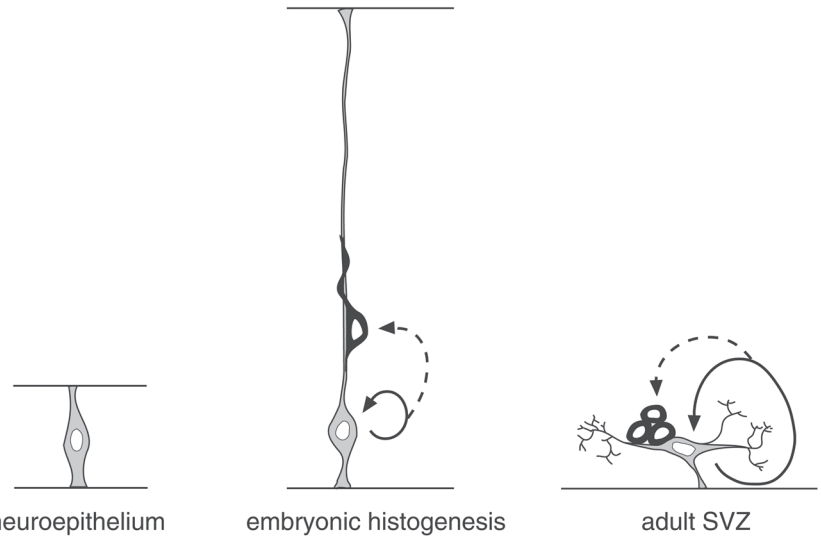

Fig. 3. Hypothetical relationship of neural stem cells from the early embryo to the adult SVZ. (**Left**) Neural stem cells (*gray*) in the early neuroepithelium extend from the ventricular lumen (*bottom*) to the pial (*top*) surfaces. (**Middle**) Like neuroepithelial stem cells, many radial glial cells (*gray*) also contact both the ventricular and pial surfaces. Radial glia behave as neural stem cells, perhaps an elongated form of the stem cell of the early neuroepithelium. Radial glia are known to divide and may self-renew (*solid arrow*) and produce neurons (*black*), possibly through intermediate cell types (*dotted arrow*). (**Right**) Radial glial give rise to astrocytes later in development. Cells derived from radial glial may come to reside in the adult SVZ where they are identified as type B cells (*gray*). Like radial glia and neuroepithelial cells, some SVZ type B cells contact the ventricle. These astrocyte-like cells behave as stem cells in that they self-renew (*solid arrow*) and produce neurons (*black*), possibly through intermediate cell types (*dotted arrow*).

It has previously been suggested that radial glia do not divide during the period of neurogenesis *(89)*.

It is interesting to compare the morphology of the neuroepithelial stem cell with that of radial glial cells. In the primitive neural tube, the neuroepithelial stem cells contact both the ventricular and pial surfaces (Fig. 3, left). As development progresses, the wall of the neural tube thickens as layers of cells are added. If multipotent neuroepithelial cells are to maintain their ventricular and pial contacts, then they must elongate to accommodate the thickening of the neural tube wall. Radial glial cells are elongated cells, many extending processes to both the ventricular and pial surfaces (Fig. 3, middle). Is it possible, then, that what we call radial glial cells are really just neuroepithelial stem cells with an elongated morphology?

There has been a gradual accumulation of data leading to a recent spurt of studies supporting the idea that radial glia are neural stem cells. Mammalian radial glial cells express nestin *(42,90)*, an intermediate filament that is found in neuroepithelial cells *(91)* as well as in cultured neural stem cells *(24)*. Radial glial cells are also found to be mitotic in vivo *(92,93)*, suggesting that radial glial cells have neuroepithelial characteristics *(94)*. In the avian brain, radial glia persist into adult life *(95)*. These cells continue

to divide in the adult avian brain and their division correlates spatially and temporally with the appearance of new neurons, leading to the proposition that these radial cells are neuronal precursors *(96)*. Furthermore, the primary precursors in the adult avian brain undergo interkinetic nuclear migration *(49)*, a phenomenon typified by neuroepithelial cells. Earlier retroviral lineage analysis and in vitro studies demonstrate that at least some VZ cells are capable of producing both neurons and glia *(97–101)*. These earlier studies have been further bolstered by more recent studies *(102–106)*. Noctor et al. *(103)* showed in vivo with a green fluorescent protein (GFP)-retrovirus that the progeny of radial glia include neurons. Malatesta et al. *(106)* used a Cre-based fate map analysis to demonstrate that the majority of cortical projection neurons are derived from radial glia. Taken together, the data challenge the historical notions that neuroglia develop from a lineage separate from that of neurons and that radial glia are committed progenitors to astrocytes.

What Is the Origin and Nature of SVZ Type B Cells?

There is good evidence that radial glia become astrocytes late in development. Transitional forms between radial glia and astrocytes have been observed in vivo *(85–88)* and in vitro *(107)*. Furthermore, radial glial vitally labeled by injections of tracers onto the surface of the brain can differentiate into astrocytes in vitro *(108)*. It is interesting to consider that SVZ type B cells might be derived from radial glial cells and retain some neuroepithelial stem cell characteristics into adulthood (Fig. 3, right). There are, in fact, data supporting this consideration: Gaiano et al. *(109)* found that activated Notch signaling in embryonic neural progenitors instructed a radial glial fate, and later, these cells became astrocytes, many of which ended up in the SVZ. The microenvironment of the SVZ might provide signals that program type B cells for continuous OB neuron production. Astrocytes throughout the brain are also thought to be derived from radial glia, raising the intriguing possibility that some of these cells may also behave as neural stem cells under appropriate conditions. Compelling evidence in support of this idea comes from the demonstrations that in vitro stem cells can be propagated from regions other than the SVZ. Neurosphere-generating cells are found all along the entire ventricular neuraxis *(110)*. In addition, multipotent stem cells can also be isolated in vitro from the cortex, septum, hippocampus, and SVZ *(111,112)*. It will be interesting to determine if precursors similar to type B cells exist in these diverse brain regions. In the dentate gyrus of the hippocampus, astrocytes with characteristics similar to the SVZ type B cells have been found to function as primary progenitors in the generation of new neurons *(113–115)*. The origin of adult neural stem cells with glial characteristics in the SVZ and dentate gyrus is not known. The preceding arguments suggest that these adult stem cells may be derived from radial glia, but this hypothesis remains to be demonstrated.

Are Glial Tumors Neural Stem Cell Tumors?

Most brain tumors are glial. Based on the series of findings discussed earlier suggesting that adult neural stem cells have glial characteristics, we should consider the possibility that tumors may arise from stem cells. In fact, EGF receptor overexpression in nestin-positive postnatal brain cells lacking the INK4a-ARF locus leads to a high incidence of gliomas *(116)*, suggesting that a genetic alterations in a stem-like cell can

generate a tumor that would be histologically classified as a glioma. Interestingly, the SVZ in some mammals is the most common site of gliomas induced by chemical carcinogens *(117)*. Although it is not a frequent site of tumors in humans, the SVZ is perhaps a site where stem cells acquire the initial genetic alterations in tumor cell progression. Certain mutations may enhance the migration of stem cells, leading to the subsequent formation of tumors at a distance from the SVZ. In fact, the overexpression of FGF2 in glial cells stimulates their migration *(61)*. Also, infusion of FGF2 or EGF into the lateral ventricle causes SVZ cells to migrate deeper into the striatum *(39,118,119)*.

THE PROBLEM WITH CALLING A CELL A GLIAL CELL

Some of the unavoidable, historical misconceptions about glial cells appear to have persisted to the present, and it is now important to reexamine the cells that we call glial in a new light. The concept of neuroglia, meaning "nerve glue," was originally based on an assumption by Rudolf Virchow in the mid-1800s that there must be a mesoderm-derived connective tissue-like component to the nervous system *(83)*. Virchow's theory has since been refuted; however, his ideas about the derivation and nature of glial cells seemingly instilled the field with the concept that glial cells should be distantly related to neurons. Perhaps the shadow of Virchow's conjectures extends to the present day, making it difficult to consider the possibility that glial-like cells are neural stem cells. As proposed in an earlier study, perhaps the radial glial cell should be called simply a radial cell to remove the influential connotation of the word "glial" *(96)*. Alternatively, some of the historical weight of the word "glial" needs to be lightened. In the brain, the term "glia" may be taken to encompass both fully differentiated supportive cells and others that are capable of behaving as neural stem cells. Neural stem cells may have roles often assigned to glia. The cellular anatomy of both the adult SVZ and developing VZ suggest that stem cells play important structural roles as the scaffold upon which neurogenesis and neuronal migration occur. Hence, it appears that in some cases, glial cells and stem cells are one in the same.

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