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The Astrocyte Odyssey

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Abstract

Neurons have long held the spotlight as the central players of the nervous system, but we must remember that we have equal numbers of astrocytes and neurons in the brain. Are these cells only filling up the space and passively nurturing the neurons, or do they also contribute to information transfer and processing? After several years of intense research since the pioneer discovery of astrocytic calcium waves and glutamate release onto neurons *in vitro*, the neuronal-glial studies have answered many questions thanks to technological advances. However, the definitive *in vivo* role of astrocytes remains to be addressed. In addition, it is becoming clear that diverse populations of astrocytes coexist with different molecular identities and specialized functions adjusted to their microenvironment, but do they all belong to the umbrella family of astrocytes? One population of astrocytes takes on a new function by displaying both support cell and stem cell characteristics in the neurogenic niches. Here, we define characteristics that classify a cell as an astrocyte under physiological conditions. We will also discuss the well-established and emerging functions of astrocytes with an emphasis on their roles on neuronal activity and as neural stem cells in adult neurogenic zones.

1. Introduction

In the late 1800's, neuroglia were recognized as distinct cellular elements that included all supporting cells in the central nervous system (CNS). Neuroglial cells are subdivided into different classes: astrocytes, oligodendrocytes, and, more recently, NG2 cells (i.e. oligodendrocyte precursor cells). Today, the term glia is commonly used to refer to neuroglia, Schwann cells, and microglia. Occasionally, ependymal cells (also called ependymoglia) are included in the term glia since they are derived from radial glia (Spassky *et al.*, 2005) and share astrocytic properties (Reichenbach and Robinson, 1995; Liu *et al.*, 2006). This review focuses on astrocytes under physiological conditions and will not discuss the reactive astrocytes that contribute to gliosis under pathological conditions.

We divided the review into four main sections encompassing three themes: 1) the definition of an astrocyte, 2) the functions of astrocytes sub-divided into two groups: their well-established and emerging functions, and 3) the novel progenitor function of a sub-group of astrocytes in neurogenic zones. We encourage readers to refer to two recent reviews by Dr.

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Kimelberg that discusses the identity of astrocytes as well as their supportive and instructional functions (Kimelberg, 2004; 2007). Constructive criticisms of the recent literature as well as historical perspectives are provided in these reviews. Regarding the first theme, we propose that the term astrocyte encompasses a family of cells with shared properties and functions that nevertheless exhibit heterogeneity as a result of their different microenvironments. We discuss the anatomical, antigenic, and electrophysiological features that help define a cell as an astrocyte, as well as recent advances in identifying new astrocyte markers using new transcriptome analysis (Cahoy *et al.*, 2008). Second, we discuss many of the well-established and emerging functions of astrocytes with a special emphasis on their roles on neuronal activity.

One of the accepted roles for astrocytes is their house-keeping functions maintaining a viable nervous system environment for neurons. This includes buffering excess potassium and neurotransmitters, providing nutrients and structural support around synapses, and contributing to the integrity of the blood brain barrier (BBB). Astrocytes are also known to release molecules important for neuronal survival and neurite formation. Some of the emerging functions of astrocytes have been clearly demonstrated, others remain speculative and controversial, as will be discussed in this review. Changes in intracellular calcium (Ca^{2+}) dynamics upon neuronal activity provide a mode of excitability to astrocytes. One recent study reported that Ca^{2+} transients in individual astrocytes are functionally coupled to neuronal activity with remarkable spatial specificity in the ferret visual cortex *in vivo* (Schummers *et al.*, 2008). In addition, they showed an unambiguous coupling between the astrocyte response to visual stimuli and local blood flow. However, intercellar Ca^{2+} waves allowing astrocyte-to-astrocyte communication have not been observed in acute slices or *in vivo*. The occurrence of intercellular Ca^{2+} waves may be more expected in pathological situations as proposed in a recent review (Scemes and Giaume, 2006). Novel time-lapse imaging studies clearly revealed that astrocytes in acute slices shape the structural plasticity of synapses. However, their instructive role at synapses, in particular their fast release of gliotransmitters controlling synaptic activity, remains controversial. In particular, issues will be raised regarding the methodologies used to stimulate astrocytes.

Finally, cells expressing the astrocytic marker glial fibrillary acidic protein (GFAP) (Eng *et al.*, 1971; Eng, 1985) display neural stem cell characteristics in the adult neurogenic zones, the subventricular zone (SVZ), and subgranular zone (SGZ) of the hippocampal dentate gyrus. This finding triggered a lot of confusion regarding the identity of GFAP-expressing cells and whether these neural stem cells should be considered astrocytes. This finding also questioned the ability of mature astrocytes to revert into a more immature phenotype to regain their stem cell characteristics. We discuss evidence here that these GFAP-expressing stem cells display characteristics of astrocytes and thus may be part of the astrocyte family. We will also discuss the function of these SVZ astrocytes as neuronal stem cells and critical elements of the stem cell niche that are necessary for maintaining neurogenesis.

2. Defining an astrocyte

The term neuroglia or Nervenkitt (i.e. nerve-putty) was first introduced by Rudolf Virchow, a celebrated pathologist in the 1850's (please see the review by Somjen (1988) for further details and references). Virchow pictured neuroglia as small round-shaped cells that filled up the extracellular space and were part of the connective tissue. Although the term neuroglia survives, our knowledge on the diversity and properties of neuroglial cells, and in particular astrocytes, has dramatically changed. Astrocytes have been viewed as a homogeneous cell population that have a star-shaped morphology, extend numerous processes surrounding neighboring neurons and blood vessels, and contain intermediate filaments (glial fibrils). While astrocytes are classically defined by their morphology and expression of glial fibrils, defining a cell as an astrocyte is not a simple task as discussed in a recent review (Kimelberg, 2004).

With the development of electrophysiological, molecular, and genetic tools, it is now well-accepted that astrocytes represent a diverse population of cells with numerous functions. In addition, the finding that a subpopulation of GFAP-expressing cells displays neural progenitor or stem cell features and that astrocytes possess neuronal properties (e.g. glutamate vesicular release) further confuses the definition of an astrocyte. Below we summarize the characteristics that collectively would help define a cell as an astrocyte (see also Kimerlberg (2004) for additional comments).

2.1. Lineages of astrocytes

In mammals, gliogenesis, which corresponds to the generation of astrocytes and oligodendrocytes, begins late in embryonic development and continues during the neonatal and postnatal period (Fig. 1). In the cerebral cortex, one of the best-studied regions for gliogenesis, astrocytes are generated from three different sources (Goldman, 2007): radial glia residing in the embryonic ventricular zone (VZ), progenitors in the postnatal SVZ, and a possible third lineage coming from glial-restricted precursors as illustrated in Figure 1. Radial glia, which may also be referred to as radial neuroglia, originate from the early transformation of neuroepithelial cells in the VZ and behave as neural progenitors for both neurons and astrocytes during development (Malatesta *et al.*, 2000;Noctor *et al.*, 2001). After the period of neuronal migration along their radial fibers, radial glia in most regions of the CNS retract their processes and transform into star-shaped astrocytes during the perinatal period (Schmechel and Rakic, 1979). They can also transform into specialized astrocytes such as Bergmann glia in the cerebellum (for reviews see Rakic, 2003;Fishell and Kriegstein, 2003). In the neonatal SVZ, progenitors that display radial glia characteristics before transforming into SVZ astrocytes (see section 2.4) can generate intermediate progenitors. These glial intermediate progenitors migrate into the cortex where they differentiate and become mature astrocytes and oligodendrocytes (Levison and Goldman, 1993;Ganat *et al.*, 2006). Pioneer studies by Dr. James Goldman's group in the 1990's using retroviral-mediated gene transfer *in vivo* selectively infected SVZ cells at postnatal (P) days 1-3. By staining infected cells for glial markers several days post-infection, they found that SVZ cells generate both gray and white matter astrocytes as well as oligodendrocytes (Levison *et al.*, 1993;Levison and Goldman, 1993;Levison and Goldman, 1997). More recent studies suggest the presence of multipotent, bipotential progenitors (Levison and Goldman, 1997;Aguirre *et al.*, 2004;Aguirre and Gallo, 2004), and perhaps astrocyte-restricted progenitors in the neonatal SVZ (Levison and Goldman, 1997). In contrast to the embryonic VZ and neonatal SVZ glial progenitors, the identity and genesis of the glial-restricted precursors is not as clear-cut. These progenitors include bipotential glial progenitors (e.g. O2A progenitors) and progenitors restricted to an astrocytic fate that are thought to exist during embryonic development (Fig. 1, for review see Liu and Rao, 2004;Carmen *et al.*, 2007). These progenitors are hypothesized to be generated directly from neuroepithelial cells and thus bypass the radial glia stage (Liu *et al.*, 2002;Cai *et al.*, 2007). The identity of the different classes of intermediate progenitors needs to be further elucidated to obtain a clear antigenic signature of the lineage (Bryder *et al.*, 2006).

These different astrocyte lineages suggest that astrocytes are not created in the same manner, which may provide a developmental explanation for astrocyte diversity in the same brain region. For example, gray matter astrocytes, also called protoplasmic astrocytes (based on their morphology, see below), are generated from embryonic radial glia and, to a lesser extent, intermediate progenitors migrating from the neonatal SVZ. Clearly, these two waves of astrocytic development will generate astrocytes with different patterns of gene expression and possibly functions. This also holds true for white matter astrocytes, called fibrous astrocytes, which are predominantly generated from neonatal SVZ progenitors. Astrocyte diversity can also be obtained from regional differences in radial glia fate (Malatesta *et al.*, 2003). These progressive changes in the glial progenitor environment (e.g. growth factors levels) lead to

differential regulation of transcription factors and gene expression (for review see Sauvageot and Stiles, 2002). An example of the genetic diversity of astrocytes is provided by an elegant study on the transcription factor Olig2 using transgenic approaches (Cai *et al.*, 2007). Briefly, in the absence of Olig2, astrocyte formation is severely compromised in the white matter, whereas astrocytes in the cortical gray matter displayed up-regulation of GFAP (Cai *et al.*, 2007). These data strongly suggest that gray and white matter astrocytes differ not only in their spatial locations and morphologies, but also in their transcriptional regulation of gene expression. Another recent study reported a remarkable diversity of astrocytes in the spinal cord, which is likely applicable to other brain regions (Hochstim *et al.*, 2008). The authors identified several positionally distinct subtypes of astrocytes that can be distinguished by their expression of different axon guidance molecules. The positional identity of these astrocytes was found to be specified by a combinatorial homeodomain transcriptional code, resembling the one used to specify neuronal subtypes during development.

2.2 Anatomical, Molecular, and Electrophysiological Characteristics

Technological advances over the past decades have given us many novel tools to study astrocytes. From the early Golgi stains to immunostaining for glial fibrils, dye-filling techniques (e.g. sharp electrode, patch clamp recordings, and now single cell electroporation), and transgenic approaches to visualize fluorescent astrocytes, our understanding of astrocyte characteristics has dramatically evolved over the past decades.

2.2.1. Anatomy—Astrocytes have a highly complex and heterogeneous morphology, as well as an unexpected non-overlapping spatial distribution. First, astrocytes are and remain defined as process-bearing cells distributed throughout the nervous system that lack axons and dendrites. They were originally named for their stellate or star-shaped morphology as shown in Figure 2A. However, they are heterogeneous in their morphologies as illustrated by the following examples: the cerebellar Bergmann glia (previously called Golgi epithelial cells) (Palay and Chan-Palay, 1974), the retinal Müller cells (Newman and Reichenbach, 1996), the pituitary astrocytes pituicyte (Hatton, 1988), those forming the blood-brain-barrier conferring some polarity in their morphology (Fig. 2B), and the SVZ astrocytes (Liu *et al.*, 2005; Liu *et al.*, 2006) (see Fig. 5).

The notion of diversity was previously established by dividing astrocytes into two subpopulations based on their location and morphology: the fibrous and protoplasmic astrocytes in the white and gray matter, respectively (Miller and Raff, 1984; Privat and Rataboul, 2007). Protoplasmic astrocytes have many branching processes, which envelop synapses and whose endfeet cover blood vessels. Fibrous astrocytes have long, thin, unbranched processes whose endfeet envelop nodes of Ranvier. This nomenclature (fibrous and protoplasmic) is outdated in light of the great anatomical diversity. Astrocytic plasma membrane is thrown into folds in the form of lamellae and fibers, which infiltrate among the intricate network of neural processes including synaptic terminals, dendrites, and dendritic spines. The degree of synaptic ensheathment by astrocytic processes displays regional variability. In the hippocampus, only 57% of the synapses have astrocytic processes apposed to them. Of these, the astrocytic processes surround less than half (around 43%) of the synaptic interface (Ventura and Harris, 1999). This arrangement may favor neurotransmitter spillover (i.e. diffusion) between synapses. In the cerebellum, Purkinje cells receive two types of excitatory inputs, parallel and climbing fibers, which exhibit differences in their degree of astrocytic ensheathment (67% versus 94%, respectively) (Spacek, 1985; Xu-Friedman *et al.*, 2001). The astrocytic membrane is so convoluted that glial microdomains, i.e. spatially exclusive regions through the fine astrocytic processes, have been observed in Bergmann glia and hippocampal astrocytes using electron microscopy (Grosche *et al.*, 1999; Bushong et al., 2002; Hama *et al.*, 2004). These microdomains can limit the spread of astrocytic Ca^{2+} increases

in response to neuronal stimulation (Grosche *et al*, 1999). These subcellular compartments have a complex surface consisting of thin membrane sheets and mitochondria. This suggests that an astrocyte may consist of hundreds of independent compartments, each capable of autonomously interacting with the synapses that it ensheathes. Interestingly, recent studies show that adult astrocytes are organized in non-overlapping domains (Bushong *et al.*, 2002; Halassa *et al.*, 2007) and that one hippocampal astrocyte can contact in excess of 100,000 synapses (Bushong *et al.*, 2002). In addition, this compartmentalization of labor by individual astrocytes allows a one-to-one astrocyte-to-synaptic unit communication without information (or noise) mismatch from a second astrocyte receiving different inputs and in a different state of activity. The work of Bushong et al (2002) also led to a re-evaluation of the morphological definition of an astrocyte as being more spongiform rather than star-shaped. The term spongiform indicates that astrocytes possess very dense ramifications of fine processes extending 2-10 μm from the main branches. These anatomical features suggest that one astrocyte can modulate neurotransmission across many synapses (see section 4.5).

2.2.2. Antigenic markers and fluorescent labeling in transgenic mice—Astrocytes are commonly identified by the presence of intermediate filaments (glial fibrils), which are more prominent in white matter than gray matter astrocytes (Privat and Rataboul, 2007). The major component of glial fibrils, glial fibrillary acidic protein (GFAP), is thought to be specific for astrocytes in the CNS (Eng *et al.*, 1971; Bignami *et al.*, 1972). However, the low expression of GFAP may not be readily detected by immunohistochemistry, leading to confusing results regarding the identity of astrocytes. As an example, the cell filled with the fluorescent dye lucifer yellow in Figure 2B was GFAP-immunonegative, but had endfeet covering a blood vessel, suggesting that it may be an astrocyte. It is possible that whole cell patch clamp recordings render the GFAP antigen inaccessible for the anti-GFAP antibody. Other defining characteristics or ways to visualize filaments (such as by electron microscopy) are thus required to define a cell as an astrocyte. In addition, GFAP is expressed by other cell types in the CNS (i.e. ependymal cells, which are also derived from radial glia) that share similar properties with astrocytes (Liu *et al.*, 2006), but are not normally part of the astrocyte family (Spassky *et al.*, 2005). Ependymal cells form an epithelial layer lining the walls of the cerebral ventricles and display morphological features different from astrocytes such as motile cilia. They function as a barrier between the brain parenchyma and cerebrospinal fluid (CSF) and play a role in cerebral fluid balance, toxin metabolism and secretion into the CSF. They thus have specialized functions that distinguish them from astrocytes (Del Bigio, 1995). Amazingly, GFAP has also been located in rat kidney glomeruli and peritubular fibroblasts (Buniatian *et al.*, 2002), leydig cells of the testis (Davidoff *et al.*, 2002), skin keratinocytes (Danielyan *et al.*, 2007), osteocytes of bones, chondrocytes of epiglottis, bronchus (Kasantikul and Shuangshoti, 1989), and stellate-shaped cells of the pancreas and liver (Apte *et al.*, 1998). Another commonly used astrocytic marker is S100B, which belongs to the S100 family of EF-band calcium binding proteins (Baudier *et al.*, 1986). However, S100B is only expressed by a subtype of mature astrocytes that ensheath blood vessels and by NG2-expressing cells (Deloulme *et al.*, 2004; Hachem *et al.*, 2005). NG2-expressing cells are found in both the developing and adult mammalian CNS, and until recently, were referred to as smooth protoplasmic astrocytes because of their astrocytic appearance with less branched processes and a paucity of intracellular filaments (Levine and Card, 1987). However, they are GFAP-immunonegative and have been placed in the oligodendrocyte lineage, and now they are commonly referred to as oligodendrocyte precursor cells (OPCs) or NG2 cells (Nishiyama *et al.*, 1999). Other markers thought to be exclusive to astrocytes include, but are not limited to, the glutamate transporters GLT-1 (or human EAAT2, Rothstein *et al.*, 1994; Danbolt *et al.*, 1998b), glycogen granules, and glutamine synthase (GS), which is an enzyme that catalyzes the conversion of ammonia and glutamate to glutamine (Schousboe *et al.*, 1977; Martinez-Hernandez *et al.*, 1977). However, GS is also found in white and gray matter oligodendrocytes (Cammer,

1990; D'Amelio *et al.,* 1990). Other markers include inwardly rectifying K^+ channels like Kir4.1 (Takumi *et al.,* 1995; Higashi *et al.,* 2001) and aquaeporin 4 channels (Nielsen *et al.,* 1997). Additional markers have been found through microarray analysis of astrocytes *in vitro* (Bachoo *et al.,* 2004). While some of these molecules may be expressed by all astrocytes (e.g., GLT-1), others (e.g. Kir4.1) are only expressed by a subset of astrocytes (Higashi *et al.,* 2001). In addition, some markers provide only punctuate staining (GLT-1) or localize to only some parts of the astrocytic processes (i.e. aquaeporin) rendering identification of the whole cell difficult to interpret. Despite these limitations, new technology can expand our repertoire of astrocytic markers. In a new study, Cahoy and colleagues used a transcriptome database approach from FACS-sorted S100B-GFP-expressing P30 astrocytes and identified a new astrocyte-specific marker, AldhL1 (aldehyde dehydrogenase 1 family, member L1) (Cahoy *et al.,* 2008). This useful genomic approach can provide additional clues for astrocyte development and function.

Investigators have thus taken advantage of transgenic mice where a fluorescent reporter protein is expressed under promoters expressed in astrocytes, including GFAP (Zhuo *et al.,* 1997; Nolte *et al.,* 2001), GLT-1 and GLAST (Regan *et al.,* 2007), S100B (Vives *et al.,* 2003), and BLBP (Schmid *et al.,* 2006). The most commonly used transgenic mice for studying astrocytes are mice expressing green fluorescent protein (GFP) or enhanced GFP under the human GFAP promoter (hGFAP-GFP mice). These mice as well as GLT-1-GFP and BLBP-dsRed2 mice may confer the best selectivity to identify astrocytes compared to the other transgenic mice because they were reported to be specific for astrocytes. However, these transgenic lines may label non-astrocytes as well. GLAST-GFP is essentially expressed in radial glia and immature astrocytes, but is also expressed in oligodendrocytes (Regan *et al.,* 2007). S100B-GFP is expressed in both neurons and astrocytes (Vives *et al.,* 2003). It is also important to acknowledge that not all astrocytes may carry the reporter proteins. This was shown for different lines of GFAP-LacZ mice generated using different elements of the GFAP promoter. LacZ expression showed different distributions, and some were found in neurons (Lee *et al.,* 2008) because the element of the promoter used did not include the sequence necessary to silence GFAP expression in neurons. Despite these common problems with transgenic animals, the hGFAP-GFP mice have allowed investigations of astrocytes' biophysical properties, behavior, responses to injury, and intracellular Ca^{2+} activity in acute slices and *in vivo*. However, one elegant study in particular has raised controversy regarding the specificity of this transgenic line because EGFP expression was found in cells displaying characteristics of NG2 cells (i.e. oligodendrocytes precursor cells) and expressing NG2 transcripts (Matthias *et al.,* 2003). However, because the studies were performed in relatively young mice (P6-P20), some of these cells may have been immature astrocytes generated during the neonatal period via NG2-expressing intermediate progenitor cells as previously addressed (Nolte *et al.,* 2001; Houades *et al.,* 2006). Collectively, transgenic mice containing fluorescent proteins in astrocytes are invaluable and necessary tools, but it is nevertheless important to verify that fluorescept reporter expression coincides with GFAP expression using immunohistochemistry or electron microscopy.

2.2.3. Biophysical properties—Despite the diverse anatomical and antigenic characteristics of astrocytes, astrocytes do share a common set of biophysical properties. We can safely say that in acute slices, astrocytes do not generate action potentials under physiological conditions, have hyperpolarized resting membrane potentials (around -80 or -90 mV), and display large voltage-independent K^+ currents, which does not preclude the presence of voltage-dependent K^+ currents (Bordey and Sontheimer, 2000). The presence of these biophysical characteristics led investigators to call astrocytes as "passive" cells (Steinhauser *et al.,* 1992), a term that is not used anymore because of its negative connotation. The molecular nature of the channels responsible for large voltage-independent K^+ currents remains to be determined. Despite this, the background K^+ channels (e.g. TASK-1, TASK-3 and TREK-2),

which contain four transmembrane segments and two pore-forming domains, were found to be expressed in cultured astrocytes and may be good candidates for generating voltage-independent K^+ currents (Gnatenco *et al.*, 2002). These channels may also underline another degree of variability among populations of astrocytes. It is also well-known that astrocytes in cultures and in acute slices are also coupled by gap junctions, creating a large syncitium of connected cells. However, the current generated through electrical coupling does not appear to significantly contribute to the whole-cell currents in mature astrocytes as tested by acutely dissociating astrocytes (Schools *et al.*, 2006) (an exception are the SVZ astrocytes, Liu *et al.*, 2006). A controversy persists regarding the biophysical properties of astrocytes (Walz, 2000). It remains unclear whether astrocytes with primarily voltage-dependent currents exist or whether they represent a distinct population of glial cells, including NG2 cells (Zhou and Kimelberg, 2001; Matthias *et al.*, 2003; Zhou *et al.*, 2006). However, the age of the mice studied may explain some discrepancies among the studies. Indeed, some recorded glial cells in acute slices may be immature or in an intermediate stage between NG2 cells and fully differentiated GFAP-expressing astrocytes (Zhou *et al.*, 2006). Therefore, membrane physiology in combination with the expression of GFAP and other markers should determine whether these cells belong to the family of astrocytes.

2.2.4. Receptor expression—Astrocytes express a large repertoire of receptors, including G-protein coupled receptors and ionotropic receptors that have been extensively reviewed (for review see Salm and McCarthy, 1992; Hosli and Hosli, 1993; Magistretti *et al.*, 1993; Whitaker-Azmitia and Azmitia, 1994; Kimelberg, 1995; Vernadakis, 1996; Fraser *et al.*, 1997; Porter and McCarthy, 1997; Baba, 1998; Nedergaard *et al.*, 2002; Neary *et al.*, 2004; Abbracchio and Verderio, 2006; Furuta *et al.*, 2007; Fernandez-Ruiz *et al.*, 2007). Astrocytes also express receptors for growth factors, chemokines, steroids, and receptors involved in innate immunity (e.g. Toll-like receptors) that participate in regulating astrocyte development and response to neurons and injury (Owens *et al.*, 2005; Mong and Blutstein, 2006; Vaccarino *et al.*, 2007; Farina *et al.*, 2007; Liu and Neufeld, 2007a). Here, we make three remarks. First, cultured astrocytes express almost every receptor present in neurons while astrocytes in acute slices or *in vivo* lack some “neuronal” receptors. Typically, mature astrocytes do not express AMPA-type and NMDA-type glutamate receptors. Nevertheless, there are a few exceptions. For example, in acute slices Bergmann glia express calcium-permeable AMPA receptors (Geiger *et al.*, 1995) and cortical astrocytes were recently shown to express NMDA-type glutamate receptors (Lalo *et al.*, 2006). However, these latter receptors in astrocytes are not typical because they are insensitive to magnesium block. Second, astrocytes display heterogeneity in their pattern of receptor expression, as shown by the examples above. In addition, astrocytes will adjust their receptor expression according to their surrounding environment. For instance, in response to brain injury, astrocytes up-regulate the expression of epidermal growth factor receptors (EGFRs), which trigger quiescent astrocytes to become reactive astrocytes (Liu and Neufeld, 2007b). Third, CA1 hippocampal astrocytes do not express P2X7-type ionotropic ATP receptors (Jabs *et al.*, 2007). Although this recent finding needs to be tested in other brain regions, it questions the existing dogma, which originally postulated that astrocytes expressed P2X7, though these data were not convincing due to the lack of selective pharmacology and poor antibodies.

2.3. Functional definitions of astrocytes

All of these diverse structural, biochemical, and biophysical characteristics of astrocytes are tightly related to their functions summarized and discussed in the next sections. The functions of astrocytes can be divided into three groups: those that provide housekeeping functions necessary to maintain neuronal function, those that actively shape synaptic function, and those that act as neural precursors in adult neurogenic regions. Functionally, astrocytes are still defined as cells that do not generate action potentials and do not myelinate axons, but clean up

the extracellular space and provide substrates necessary for neuronal function. However, more recent evidence shows that astrocytes can actively contribute to synaptic plasticity and activity by releasing neurotransmitters and affecting blood flow (see below). These emerging functions suggest that astrocytes are active participants in brain activity rather than passive elements in maintaining the extracellular space.

One important note is that neurons and astrocytes share a lot of similar functions, e.g. buffering K^+, neurotransmitter, and glucose in the extracellular space. However, astrocytes are often better positioned and possess more efficient mechanisms to perform these functions. For example, some but not all astrocytes contact blood vessels and are thus well-poised to take up glucose and other nutrients. In addition, astrocytes may have specialized functions based on their microenvironment. A clear example is the difference between gray and white matter astrocytes. White matter astrocytes contact the node of Ranvier where they may regulate spike propagation while gray matter astrocytes ensheath synaptic terminals where they influence synaptic transmission.

2.4. The astrocyte-like neural progenitors of the neurogenic zones

A fascinating characteristic of astrocytes that came into light in the last decade is that GFAP-expressing cells can contribute to cell genesis both as stem cells and as important cellular elements of the neurogenic microenvironment (also called niche). In the adult SVZ and subgranular zone (SGZ), the multipotent neural stem cells (interchangeably called neural progenitors) express GFAP (Cameron *et al.*, 1993; Doetsch *et al.*, 1999; Seri *et al.*, 2001). These GFAP-expressing cells in the SVZ give rise to neuroblasts that migrate to the olfactory bulb where they become synaptically integrated olfactory interneurons (Altman, 1969; Luskin, 1993; Doetsch *et al.*, 1999). Another population of neurogenic GFAP-expressing cells has been found in the SGZ, where GFAP-expressing cells can generate newborn granule neurons (Altman and Das, 1965; Kaplan and Hinds, 1977; Cameron *et al.*, 1993).

While these adult stem cells express GFAP, other cell types also express GFAP (see section 2.2.2.), but are not considered astrocytes. It is questionable whether these adult stem cells belong to the astrocyte family. First of all, these GFAP-expressing stem cells express nestin, an intermediate filament marker for embryonic precursor cells that is not present in mature astrocytes (Hockfield and McKay, 1985). In the adult SVZ, nestin is also expressed in neuroblasts and intermediate progenitors (Doetsch *et al.*, 1997). Interestingly, nestin is not expressed in the neonatal SVZ, but is present in immature cortical astrocytes at an early stage of differentiation (Zerlin *et al.*, 1995) and in reactive astrocytes (Clarke *et al.*, 1994), suggesting that nestin expression is not mutually exclusive with an astrocytic identity. GFAP-expressing stem cells do not express S100B, which is expressed in a minority of cells in the SVZ (Platel *et al.*, 2008). Considering that not all mature astrocytes express S100B, this finding again does not contradict with an astrocytic identity.

The following properties of GFAP-expressing stem cells suggest that they belong to the astrocyte family. In the SVZ, GFAP-expressing stem cells are derived from radial glia (Merkle *et al.*, 2004), which are neural progenitors during embryonic development (Malatesta *et al.*, 2000; Noctor *et al.*, 2001), for review see (Campbell and Gotz, 2002; Rakic, 2003; Fishell and Kriegstein, 2003). GFAP-expressing cells in the SVZ and SGZ have anatomical features in common with astrocytes. For instance, they have long processes that envelop and contact blood vessels and neuroblasts (Doetsch *et al.*, 1997; Seri *et al.*, 2004). In addition, just like mature astrocytes, they contain glycogen granules (Sturrock and Smart, 1980; Peretto *et al.*, 1999) and express astrocytic glutamate and GABA transporters (i.e. GLAST and GLT-1, and GAT-3, respectively) (Braun *et al.*, 2003; Bolteus and Bordey, 2004; Liu *et al.*, 2006). Functionally, GFAP-expressing cells of the SVZ studied in acute slices share properties with radial glia and astrocytes. They have K^+ conductance at rest, express connexin 43 gap junctions and

hemichannels, have functional glutamate transporters and $GABA_A$ receptors, but lack AMPA-type glutamate receptors (Filippov *et al.*, 2003; Wang *et al.*, 2005; Liu *et al.*, 2006), which are absent in most mature astrocytes (Matthias *et al.*, 2003) (with the exception of the specialized Bergmann glia) (Muller *et al.*, 1996). Nevertheless, GFAP-expressing cells of the SVZ lack barium-sensitive inwardly rectifying K^+ currents (K_{IR}), a hallmark of astrocytic differentiation and cell cycle exit (Bordey and Sontheimer, 1997; Macfarlane and Sontheimer, 2000). Together, these studies suggest that the GFAP-expressing stem cells have characteristics of embryonic radial glia and mature astrocytes, but display subtle differences and retain properties of neural progenitors. Perhaps these cells are retained in a transitional stage between radial glia and astrocytes, due to the persistence of embryonic extracellular matrix molecules. This permissive environment in the neurogenic niche allows the retention of intrinsic genetic programs to maintain "stemness" (Gates *et al.*, 1995) (see additional discussion in section 5.3). In light of these data, GFAP-expressing cells of the SVZ have been termed SVZ astrocytes or astrocyte-like cells. The previous findings strongly suggest that SVZ astrocytes belong to the large family of astrocytes, but they require a sub-branch to distinguish them from mature astrocytes.

Inside the neurogenic niche, it remains unclear whether every GFAP-expressing cell has the potential to behave as a neural stem cell. Alternatively, it is conceivable that two types of GFAP-expressing cells, those that are stem cells or those that play instructive roles on neurogenesis (see section 5), co-exist in addition to the S100B-expressing cells that do not co-express GFAP (Platel *et al.*, 2008). Such a finding would further complicate the nomenclature to properly distinguish these two types of cells. Considering that astrocytes display an incredible degree of plasticity, it is likely that the same cell can both behave as a stem cell and still direct neurogenesis. One option to address this issue would be to determine the genetic profile of proliferative versus non-proliferative SVZ astrocytes. Such an approach could also help to determine the differences between SVZ and mature astrocytes. Future transplant studies into the SVZ of genetically modified mature astrocytes could determine whether they can be reverted to a stem cell phenotype.

3. The well-accepted functions

Because astrocytes lack axons and the ability to form action potentials, astrocytes were traditionally thought to be mere "brain glue" that support neuronal activity. However, astrocytes have several critical functions including promoting neuronal maturation, synapse formation, neuronal survival during development, regulating angiogenesis, and maintaining a viable microenvironment for neurons. Although these functions are well-accepted, they should not be overlooked because much remains to be learned. This is especially true for astrocytes' contribution to synaptogenesis and angiogenesis for which trophic molecules and their mechanisms of action remain to be evaluated. In addition, a lot can be learned as the contribution of molecularly distinct channels (e.g. Kir4.1) for K^+ buffering and neuronal excitability have just begun to be explored. Furthermore, dysfunction in astrocytes can lead to disease progression such as in familial forms of Amyotrophic Lateral Sclerosis (which will not be discussed in this review). Below is a brief description of established astrocytic functions summarized in Figure 3 (for further information see Kimelberg, 2007) that summarizes the supportive functions of astrocytes.

3.1. Synthesis of extracellular matrix proteins, adhesion molecules, and trophic factors controlling neuronal maturation and synaptogenesis

Astrocytes are the major source of extracellular matrix (ECM) proteins and adhesion molecules in the CNS. Astrocytes in cultures can either promote or inhibit neurite outgrowth depending on the balance of ECM and adhesion molecules with guidance cues navigating neurites during development or in response to injury. Growth-promoting molecules include (but are not limited

to) laminin (Liesi *et al.,* 1983; Liesi, 1985; Liesi and Silver, 1988; Chiu *et al.,* 1991; Shea *et al.,* 1992), N-cadherin (Neugebauer *et al.,* 1988; Tomaselli *et al.,* 1988), neural cell adhesion molecule (NCAM) (Neugebauer *et al.,* 1988; Smith *et al.,* 1990), and fibronectin (Price and Hynes, 1985; Liesi *et al.,* 1986; Matthiessen *et al.,* 1989). More recently, spontaneous calcium oscillations in cultured astrocytes have been shown to regulate neurite growth by maintaining the expression of specific growth-enhancing proteins on astrocytic surface, such as N-cadherin (Kanemaru *et al.,* 2007). On the contrary, inhibitory proteoglycans associated with glial boundaries during development provide guidance cues (Snow *et al.,* 1990; Gonzalez *et al.,* 1993; Steindler, 1993). Astrocytes also synthesize and secrete proteolytic enzymes, in particular the matrix metalloproteinases (MMPs, Wells *et al.,* 1996; Muir *et al.,* 2002), which play a pivotal role in ECM degradation and remodeling (for review see (Shapiro, 1998; Yong *et al.,* 1998). For example astrocytes synthesize MMPs of the gelatinase subfamily, MMP-2 and -9, which contribute to extracellular amyloid-β peptide degradation and clearance (Yin *et al.,* 2006).

Astrocytes are well-known to release growth factors *in vitro*, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) (Rudge *et al.,* 1992), and fibroblast growth factor (FGF) (Vaca and Wendt, 1992). These molecules control neuronal maturation and survival (for review see Ojeda *et al.,* 2000). Astrocytes have also been shown to control neuronal differentiation *in vitro* via activity-dependent neurotrophic factor release (Blondel *et al.,* 2000). More specifically, ciliary neurotrophic factor (CNTF) and FGF can be released from cultured astrocytes possibly via a Ca^{2+}-dependent pathway and enhance neuronal survival and induce neuronal growth as well as differentiation (Vaca and Wendt, 1992). Other molecules such as S100B, which stimulates neurite outgrowth as well as astrocytic glutamate uptake, can be released by astrocytes to protect neurons against glutamate excitoxicity (for review see (Donato, 2001). These examples offer only a snapshot of the large repertoire of astrocytic molecules that regulate neuronal maturation and survival under physiological conditions and following injury (see reviews by Emsley *et al.,* 2004; Pehar *et al.,* 2005; Endo, 2005; Mahesh *et al.,* 2006 for additional examples).

More recently, astrocytes were shown to promote synaptogenesis between CNS neurons *in vitro* and *in vivo* during development. Interestingly, synaptogenesis coincides with the generation of astrocytes. Retinal ganglion cells cultured in the absence of astrocytes exhibited very little spontaneous synaptic activity but displayed robust postsynaptic excitatory activity when grown on a layer of feeder astrocytes (Pfrieger and Barres, 1997). In fact, astrocytes were found to induce a 7-fold increase in synapse number between retinal ganglionic cells and a nearly 100-fold increase in synaptic activity (Rudge *et al.,* 1992; Seil *et al.,* 1992). Some of the trophic factors secreted by astrocytes include cholesterol (Mauch *et al.,* 2001) and thrombospondins (Christopherson *et al.,* 2005). The latter has been shown to be secreted by immature astrocytes *in vivo* to induce the formation of ultrastructurally normal synapses (Christopherson *et al.,* 2005). It is likely that future studies will reveal many other molecules that can contribute to synaptogenesis in a temporal and regional-specific manner. It will be exciting to watch the expansion of this emerging field.

3.2. Angiogenesis, Blood-Brain Barrier (BBB) induction and maintenance

Glial-vascular interactions serve a number of important functions that span multiple time scales. In this section, we will discuss long lasting (hours to days) interactions. Later we will elaborate on the fast (seconds to minutes) interactions in the vascular unit, such as transient opening of the blood-brain barrier (BBB) and blood flow regulation. A better understanding of angiogenesis as well as the dynamic interactions between astrocytes and endothelial cells to regulate BBB stability and permeability is critical for understanding the process of tumorigenesis (Stiver, 2004) and neurogenesis. Indeed, adult neural stem cells, i.e. a specialized

astrocyte, preferentially reside close to blood vessels and send processes around endothelial cells (Palmer *et al.*, 2000 and see section 5.2). It is unclear which interactions are taking place between endothelial cells and neural stem cells, but there is likely a crosstalk between the cells types to create a neurogenic niche that coordinates growth and response to injury.

3.2.1. Angiogenesis—Angiogenesis, the formation of blood vessels, involves several steps including basement membrane degradation, endothelial cell proliferation and recruitment, tube formation, and maturation including reconstitution of the basement membrane. When co-cultured with astrocytes, endothelial cells form capillary-like structures (Laterra *et al.*, 1990; Laterra and Goldstein, 1991; Jiang *et al.*, 1995). Epoxyeicosatrienoic acid (EET), which is the product of cytochrome P450 epoxygenation of arachidonic acid (Zhang and Harder, 2002), is one of the molecules released from astrocytes that can act as a mitogen and morphogen for endothelial cells. In addition, astrocytes synthesize laminin to form the astrocyte-endothelial cell interface resembling the basement membrane (Laterra *et al.*, 1990). An optimal system to study angiogenesis is the retina, which contains regular stellate astrocytes and a specialized astrocytic type, called the Muller cell (Newman and Reichenbach, 1996). In the retina, angiogenesis is controlled by a tight cooperation between retinal neurons, astrocytes, and endothelial cells. In particular, retinal neurons release platelet-derived growth factor (PDGF) to stimulate proliferation of astrocytes, which in turn stimulate blood vessel growth by secreting vascular endothelial cell growth factor (VEGF) (Stone *et al.*, 1995; Fruttiger *et al.*, 1996; Provis *et al.*, 1997). In addition, developing vessels provide feedback signals that trigger astrocyte differentiation, including cessation of cell division and upregulation of GFAP (West *et al.*, 2005). In addition, the final arrangement of retinal blood vessels depends critically on the pre-patterning of astrocytes that directs the extension of filopodia budding off the vascular plexus via VEGF receptor activation (Jiang *et al.*, 1995; Fruttiger *et al.*, 1996; Zhang and Stone, 1997; Gerhardt *et al.*, 2003). Such cooperation may also occur in the CNS, but this remains to be examined.

3.2.2. BBB induction and maintenance—The BBB is the specialized system of brain microvascular endothelial cells that protects the brain from toxic substances in the blood, supplies the CNS with nutrients, and filters excess and toxic molecules from the brain to the bloodstream (for excellent reviews on BBB see Pardridge, 1999; Engelhardt, 2003; Hawkins and Davis, 2005; Zlokovic, 2008). The brain capillaries are 50–100 times tighter than peripheral microvessels as a result of complex tight junctions (zonula occludens) between endothelial cells that limit paracellular diffusion of hydrophilic solutes. As a result, entry across the brain endothelium is effectively confined to transcellular mechanisms using various transport mechanisms (e.g. GLUT1 glucose transporters, L-system carrier L1 for neutral amino acids, glutamate transporters, the efflux carrier P-glycoprotein, for review see (Zlokovic, 2008) and for the original idea see (Davson and Oldendorf, 1967)). Early studies with markers injected into the cerebral spinal fluid and electron microscopy demonstrate that astrocytes do not structurally contribute to the BBB (Brightman and Reese, 1969). Nevertheless, astrocytes send specialized processes, called endfeet, which ensheath the brain vasculature onto which they form rosette-like structures (Kacem *et al.*, 1998). In addition, astrocytes are beleived to regulate the induction of the BBB, i.e. tight junction formation and expression of transport systems (see (Haseloff *et al.*, 2005; Abbott *et al.*, 2006) for review).

During development, astrocytes are thought to contribute to BBB tightening and up-regulation of the different transport mechanisms. Freshly isolated brain endothelial cells in culture retain certain aspects of a BBB phenotype, but with some loss of full barrier characteristics (e.g. leakier tight junctions, down-regulation of enzyme and transport systems) (for review on culture model see Reichel *et al.*, 2003). However, tight junction proteins in the BBB are up-regulated by co-culturing with astrocytes (Rubin *et al.*, 1991; Dehouck *et al.*, 1994; Rist *et al.*, 1997; Sobue *et al.*, 1999). Some of the specific transport systems up-regulated in brain

endothelial cells exposed to astrocytes include GLUT1, the L-system and A-system amino acid carriers, and P-glycoprotein (El Hafny *et al.*, 1997) (for review see Bauer and Bauer, 2000). The regulations exerted by cultured astrocytes on BBB induction depend both on cell-cell contact and the diffusion of molecules. Several astrocytic signals regulating different aspects of BBB properties have been identified and include TGF-β (Tran *et al.*, 1999), GDNF (Igarashi *et al.*, 1999), basic FGF (Sobue *et al.*, 1999), IL-6 and hydrocortisone (Hoheisel *et al.*, 1998). The src-suppressed C-kinase substrate (SSeCKS) in astrocytes is responsible for the decreased expression of VEGF and increased release of the anti-permeability factor angiopotein-1 (Lee *et al.*, 2003). Conditioned media from astrocytes over-expressing SSeCKS blocked angiogenesis *in vivo* and *in vitro*. In addition, the conditioned media increased tight junction proteins in endothelial cells, consequently decreasing sucrose permeability. It is thought that astrocytes also maintain tight junction and microvascular permeability in the adult brain. However, short-term regulation of permeability *in vivo* has been difficult to assess and will be discussed later in the emerging functions section.

Finally, it is interesting to point out that endothelial cells have a reciprocal influence on cultured astrocytes by regulating their growth (Estrada *et al.*, 1990), glutamate synthetase activity (Spoerri *et al.*, 1997), laminin production (Wagner and Gardner, 2000), and up-regulation of antioxidant enzymes (Schroeter *et al.*, 1999). One such signaling molecule is the leukaemia inhibitory factor (LIF), released by endothelial cells of the optic nerve, which induces astrocytic differentiation *in vitro* (Mi *et al.*, 2001).

3.3. Extracellular ion buffering: a focus on K^+ buffering

The idea that astrocytes regulate the ionic content of the extracellular space was first proposed by Gerschenfeld et al. in 1959 (Gerschenfeld *et al.*, 1959) (see for review Kimelberg, 2007). During normal neuronal activity, neurotransmission leads to the build up of K^+ into the extracellular space, and if not corrected, results in neuronal depolarization, hyperexcitability, and seizures. The pioneering work by Kuffler's group showed that nerve impulses cause slow depolarization of glia attributable to K^+ influx in the amphibian optic nerve (Orkand *et al.*, 1966). This group then proposed the K^+ spatial buffering hypothesis, which states that astrocytes take up excess extracellular K^+ ions, distribute them through the gap junction-coupled astrocytic syncytium, and extrude the ions at sites of low extracellular K^+ level (Kuffler and Nicholls, 1966). This homeostatic function has been confirmed in astrocytes in rat neonortical slices (Holthoff and Witte, 2000). Astrocytes also release K^+ directly into the blood stream by direct discharge into capillaries by their end-feet connections. This process, termed spatial siphoning, was first shown in the retina (Newman, 1986).

To achieve spatial K^+ buffering, astrocytes are poised with passive uptake (via channels and cotransporters) and active uptake (via Na^+/K^+ ATPases) capabilities. Passive transport of K^+ into astrocytes is achieved predominantly by inwardly rectifying K^+ channels (Kir) and to a lesser extent, Na^+/K^+ or K^+/Cl^- antiporters, based on pharmacological studies (Ballanyi *et al.*, 1987; Karwoski *et al.*, 1989). In addition, Kir channels are distributed in a highly non-uniform manner, exhibiting high expressions in endfeet (Newman, 1986; Poopalasundaram *et al.*, 2000; Higashi *et al.*, 2001).

Although understanding the mechanisms of astrocytic K^+ buffering has been extensively studied over the years, here are two limitations to our knowledge. First, the consequences of impaired astrocytic K^+ uptake have remained unclear. Studies using extracellular cesium to block Kir channels induced epileptiform, interictal-like bursting and prevention of long-term depression in the hippocampus (Janigro *et al.*, 1997). However, cesium can have effects independent of Kir channels. To circumvent this problem, Ken McCarthy's group generated a conditional astrocyte-specific knock-out of Kir4.1 via the human *GFAP* promoter (Djukic *et al.*, 2007). Kir4.1 was the first astrocytic Kir channels identified (Takumi *et al.*, 1995) and was

thought to play an important role in K^+ buffering. Conditional knock-out of Kir4.1 resulted in severe depolarization of astrocytes, impairment of astrocyte K^+ and glutamate uptake, enhanced short-term synaptic potentiation, and pronounced behavioral abnormalities, including ataxia and seizures. Another limitation with studying astrocytic K^+ buffering owes to the fact that astrocytes are heterogeneous and all may not buffer extracellular K^+ using the same mechanism. For example, only 50% of astrocytes express Kir4.1 (Higashi *et al.*, 2001), and, therefore, those that lack expression must use alternate processes for K^+ buffering. Finally, some of the functions of K^+ buffering in astrocytes have gained widespread recognition without being tested. As an example, it was thought that K^+ siphoning resulting in increased extracellular K^+ around vessels resulting in localized changes in blood flow, a process called neurovascular coupling. However, in the retina, glial K^+ siphoning does not seem to contribute to neurovascular coupling (Metea *et al.*, 2007).

3.4. Glutamate and GABA uptake

Astrocytes have been long known to take up and metabolize GABA and glutamate (Schousboe *et al.*, 1992) as well as other transmitters not discussed here (but see (Schousboe and Westergaard, 1995) for review). Among the cloned GABA transporters (GAT1 to GAT4 in mice, GAT-1, BGT-1, GAT-2, and GAT-3 in rats for review see Borden, 1996), astrocytes express a high density of high affinity GABA transporters (GAT-1 and GAT-3, i.e. GAT1 and GAT4) (Brecha and Weigmann, 1994; Morara *et al.*, 1996; Yan and Ribak, 1998; Conti *et al.*, 1999; Barakat and Bordey, 2002) depending on the region, for review see Jursky *et al.*, 1994; Borden, 1996; Palacin *et al.*, 1998). It was reported that GABA transporters in neocortical astrocytes from acute slices are activated by synaptically-released GABA (Kinney and Spain, 2002), suggesting that astrocytes may limit spillover and extrasynaptic GABA receptor activation. Pharmacological blockade of GAT-2/3 in neocortical slices altered synaptic inhibition, suggesting that there is a tonic GAT-3-mediated GABA release (Kinney, 2005). However, another study suggested that synaptic GABA levels in neocortical neurons are controlled primarily by GAT-1, and that GAT-1 and GAT-2/3 work together extrasynaptically to limit tonic currents (for review on tonic current see Farrant and Nusser, 2005). That astrocytic GABA transporters are located near synaptic clefts suggests that astrocytes likely control GABA spillover from the cleft either alone or in cooperation with neuronal transporters. Considering that astrocytes catabolize GABA very quickly, it remains unclear whether GABA transporters directly contribute to synaptic transmission by mediating GABA release (for references see Barakat and Bordey, 2002).

Among the five cloned high affinity glutamate transporters (excitatory amino acid transporter EAAT1 to 5, for review see Palacin *et al.*, 1998; Torres and Amara, 2007), astrocytes express EAAT1 and EAAT2 (also called GLAST and GLT-1, respectively) (Lehre *et al.*, 1995; Chaudhry *et al.*, 1995; Ullensvang *et al.*, 1997; Schmitt *et al.*, 1997; Conti *et al.*, 1998; Minelli *et al.*, 2001). Although astrocytes were thought to express both GLT-1 and GLAST, a recent study using transgenic mice carrying GFP or DsRed under the promoters of GLT-1 and GLAST, respectively, show that GLT-1 promoter activity is almost completely restricted to astrocytes, often in a non-overlapping pattern with GLAST (Regan *et al.*, 2007). The GLAST promoter is active in both radial glia and many astrocytes in the developing CNS but is down-regulated in most astrocytes as the mice mature. Nonetheless, GLAST expression persists in radial-like astrocytes of the SGZ, SVZ, and cerebellum (i.e. Bergmann glia) (Schmitt *et al.*, 1997; Yamada *et al.*, 2000), and is only observed in oligodendrocytes of the white matter. The exciting demonstrations showing that astrocytic glutamate transporter GLT-1 was critical for preventing glutamate build-up during neurotransmission leading to excitotoxicity (Rothstein *et al.*, 1996; Tanaka *et al.*, 1997) led to an avalanche of studies on astrocytic glutamate uptake in both health and disease (for reviews see Gegelashvili and Schousboe, 1997; Furuta *et al.*, 1997; Billups *et al.*, 1998; Danbolt *et al.*, 1998a; Anderson and Swanson, 2000; Danbolt,

2001; Gegelashvili *et al.*, 2001; Gadea and Lopez-Colome, 2001). In particular, a series of elegant studies showed that astrocyte glutamate transporters are activated by synaptically released glutamate in acute slices (Bergles and Jahr, 1997; Bergles *et al.*, 1997; Clark and Barbour, 1997; Bergles and Jahr, 1998; Bordey and Sontheimer, 2003). These findings had important implications for future work as glutamate transporters were then used as sensors to determine the time course of the transient glutamate concentration at synapses (Bergles and Jahr, 1998; Linden, 1998; Mennerick *et al.*, 1999). Thus, transporters were shown to be critical for limiting glutamate spillover from the synaptic cleft (Marcaggi *et al.*, 2003; Huang and Bordey, 2004; Huang *et al.*, 2004). As a result, they may serve to decrease synaptic noise and improve the reliability of synaptic transmission.

3.5. Metabolic support

Before the cloning of the different glutamate transporters, it was well established that glutamate uptake into astrocytes was critical for the glutamate-glutamine cycle (Westergaard *et al.*, 1995; Sonnewald *et al.*, 1997). Briefly, astrocytes take up and metabolize glutamate into glutamine via the glutamine synthetase. Glutamine is then redistributed to neurons for *de-novo* synthesis of glutamate. Importantly, glutamate transporters are not limited to buffering ambient glutamate; they bridge neuronal activity and astrocytes' ability to meet this metabolic need (as discussed below).

Astrocytes are uniquely poised to provide a nurturing environment for neurons, a role suggested by Golgi more than 100 years ago. As mentioned previously, astrocyte processes project toward blood vessels that terminate into structures called endfeet, which almost entirely cover the blood vessel walls (Abbott *et al.*, 2006). On the membrane of endfeet facing blood vessels, astrocytes express a specific form of glucose transporters, GLUT1 (Morgello *et al.*, 1995; Yu and Ding, 1998) (for review see Vannucci *et al.*, 1997). It was originally thought that astrocytes take up glucose from the blood making it available to neurons. However, research over the last two decades has generated much controversy over the precise mechanims for how astrocytes metabolically support for neurons. We will review recent evidence regarding two other substrates by which astrocytes can regulate neuronal metabolic responses to activity: glycogen and lactate.

Glycogen serves as the short-term repository for glucose and is found primarily in astrocytes in the brain (Cataldo and Broadwell, 1986; Wender *et al.*, 2000; Kong *et al.*, 2002). Along with the presence of glycogen in astrocytes are the expressions of glycogen synthase and glycogen phosphorylase, enzymes that synthesize and metabolize glycogen, respectively (Pellegri *et al.*, 1996; Pfeiffer-Guglielmi *et al.*, 2003). A possible role for brain glycogen stores has been dismissed due to the negligible concentration of brain glycogen (6-12 μmol) compared with those in the liver (100-500 μmol) and skeletal muscle (300-350 μmol) (see review by (Brown and Ransom, 2007). However, recent evidence has shown that under hypoglycemic conditions and periods of increased tissue energy demand, astrocytic glycogen provides the energy substrate for the brain. Studies in rodent optic nerve (a central white matter tract that is devoid of synapses or neuronal cell bodies) demonstrated that increasing glycogen content by pre-incubation in elevated glucose could prolong axonal function following glucose withdrawal. By contrast, down-regulating glycogen content or inhibiting glycogen metabolism by blocking glycogen phosphorylase function prevented the neuroprotective effects of elevated brain glycogen content (Brown *et al.*, 2003; Brown *et al.*, 2005; Tekkok *et al.*, 2005). The role of glycogen in hypoglycemia has been demonstrated *in vivo* using a glycogen phosphorylase inhibitor, which increased brain glycogen stores and was able to preserve brain function for up to 90 min during profound systemic hypoglycemia (Suh *et al.*, 2007). Another proposed role for astrocyte glycogen occurs during increased brain activation, when the immediate supply of neuronal glucose is exhausted and unable to meet the high metabolic demand of the

tissue. Here, glycogen metabolism can provide a rapid energy source that bypasses the rate-limiting step in glucose metabolism (glucose phosphorylation via hexokinase) (see (Brown and Ransom, 2007) for review). Indeed, in the optic nerve preparation, increased neuronal energy demand induced by high frequency stimulus resulted in a rapid decrease in glycogen content, even with normoglycemic concentrations of bath glucose (Brown *et al.*, 2003). These lines of evidence strongly suggest that astrocyte glycogen provides an important energy source to the brain under physiological conditions of high neuronal activity.

Another important, though controversial, astrocyte metabolic pathway that has emerged in the last two decades is the role of lactate as an oxidative substrate for energy metabolism. It was observed that during the astrocyte glutamate-glutamine cycle for neurotransmitter recycling, astrocytic aerobic glycolysis provides the ATP required for glutamate amidation, which results in lactate production (Martinez-Hernandez *et al.*, 1977; Pellerin and Magistretti, 1994). On the basis of this result, a mechanism of coupling between neuronal activation and glucose utilization was proposed, a model known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH) (Pellerin and Magistretti, 1994). In this model, glucose taken up by astrocytic GLUT1 can be processed in part oxidatively via the tricarboxylic acid (TCA) cycle pathway, while the remaining glucose is converted to lactate and released into the extracellular space via monocarboxylate transporters (MCT1 and MCT4). Lactate released by astrocytes is then transported into neurons via MCT2 and converted to pyruvate for oxidative use in the TCA cycle (for review see Pellerin *et al.*, 2007). A plethora of *in vitro* and *in vivo* studies have demonstrated evidence for lactate as a preferential oxidative substrate for neurons (for review see Pellerin, 2003; Pellerin *et al.*, 2007). Here, we will only mention a few key studies. Two independent studies using different quantitative measurements showed that lactate is the predominant oxidative substrate over glucose in cultured neurons (Bouzier-Sore *et al.*, 2003; Itoh *et al.*, 2003; Bouzier-Sore *et al.*, 2006). In addition, it was found that glutamate stimulation caused a reduction of glucose transport in neurons but an increase in astrocytes, implying that under neuronal glutamatergic activation, lactate utilization by neurons is favored (Porras *et al.*, 2004). Finally, i*n vivo* evidence demonstrated that sustained activation of the perforant pathway in the hippocampus led first to a decrease in extracellular lactate concentration, followed by a massive increase, which seem to support the ANLSH (Hu and Wilson, 1997).

There are some arguments against the use of lactate by neurons. First, both glucose and lactate metabolisms require nicotinamide adenine dinucleotide (NAD^+), a competition that might favor glycolysis (Cruz *et al.*, 2001) (for review see Chih and Roberts Jr, 2003; Cerdan *et al.*, 2006). Second, the evidence for a net lactate transfer between astrocytes and neurons seems uncertain (Gjedde and Marrett, 2001; Hertz, 2004). Using magnetic resonance spectroscopy, it was shown *in vivo* that increased neuronal metabolism with higher levels of brain activity is supported by lactate generated within the brain from a nonneuronal source (Serres *et al.*, 2003; Serres *et al.*, 2004; Serres *et al.*, 2005).

Despite all the controversies regarding the brain metabolic pathways, it is important to recognize that these ideas are not mutually exclusive and that under different conditions, neurons may rely upon different sources of substrate (glucose, glycogen, or lactate) to support their function. Based on the emerging role of astrocytes-neuron interactions, it is clear that alternative approaches to improving energy metabolism in neurons (such as enhancing glucose uptake in astrocytes or lactate uptake in neurons) may provide valuable neuroprotection strategies for therapy.

3.6. Others: detoxification and immune functions

One of the most important roles of astrocytes is to protect neurons against excitotoxicity by capturing excess ammonia and glutamate and converting them into glutamine. In addition, astrocytes may also participate in the uptake of some heavy metals, such as lead (Struzynska

et al., 2001). Astrocytes contain metal binding proteins such as metallothioneins that endow astrocytes with both neuroprotective and neuroregenerative properties following injury or exposure to toxic metals (Aschner, 1997; Chung *et al.*, 2008).

Astrocytes can serve as a bridge between the CNS and immune system. In particular, astrocytes can phagocytose cells and act as antigen-presenting cells (for references prior 1994 see Montgomery, 1994). For example, cultured astrocytes were shown to present antigens to T lymphocytes in a specific manner which is restricted by the major histocompatibility complex, and in particular they could activate myelin basic protein -specific encephalitogenic T-cell lines (Fontana *et al.*, 1984). Astrocytes can also express class II major histocompatibility complex antigens and costimulatory molecules (B7 and CD40) that are critical for antigen presentation and T-cell activation (for review see Dong and Benveniste, 2001; Farina *et al.*, 2007). Furthermore, astrocytes were found to express receptors involved in innate immunity, including Toll-like receptors, nucleotide-binding oligomerization domains, double-stranded RNA-dependent protein kinase, scavenger receptors, mannose receptor and components of the complement system (Owens, 2005; Farina *et al.*, 2007). Finally, astrocytes produce a wide array of chemokines and cytokines that act as immune mediators in cooperation with those produced by microglia. One intriguing idea is that molecules involved in immune responses may serve additional roles as adhesion molecules between astrocytes and neuronal elements such as dendritic spines. However, this proposed function has not been explored and remains to be elucidated.

4. The emerging and controversial functions

Presumed to be passive elements at synapses, astrocytes were once considered to be mere structural elements that provide anchoring for synapses. However, several recent studies have elucidated the plasticity of astrocytic processes and their participation in synaptic transmission. Like the mobile dendritic spines that respond to changes in activity by altering their structure, astrocytic processes dynamically alter their coupling to neurons in response to environmental cues. Pioneering work performed in the supraoptic nucleus of the rat hypothalamus show that astrocytes are capable of retracting from synapses in a reversible manner during maternal lactation. This retraction reduces coverage of the excitatory synapses and permits greater neurotransmitter diffusion beyond the synapse (Oliet *et al.*, 2001a). These studies open up many avenues for future investigation on the role of astrocytes on neurotransmission. Before discussing the emerging functions of astrocytes, we will discuss how astrocytes sense neuronal activity and their mode of excitability, particularly intracellular calcium dynamics.

4.1. Sensing neuronal activity

It is well established that astrocytes sense neuronal activity through activation of ion channels, transporters, and receptors resulting in fast depolarization and/or intracellular calcium increases (for reviews see Cornell-Bell and Finkbeiner, 1991; Finkbeiner, 1992; Vernadakis, 1996; Parri and Crunelli, 2002; Schipke and Kettenmann, 2004, and more recently (Fiacco and McCarthy, 2006). Astrocytes are endowed with K^+ channels and since the 1960's it has been known that astrocytes are depolarized by neuronal spiking (Kuffler and Nicholls, 1966; Kuffler, 1967). Pioneering work in 1990 showed that glutamate triggers intercellular calcium waves in cultured astrocytes (see next section discussing their existence *in vivo*) (Cornell-Bell *et al.*, 1990b). Later, others showed that neuronal stimulation triggered calcium waves in hippocampal astrocytes from cultured slices (Dani *et al.*, 1992) and calcium transients in astrocytes in acute slices via metabotropic glutamate receptor activation (Porter and McCarthy, 1996). Calcium transients in astrocytes can be induced following activation of different types of metabotropic receptors (for review see Fiacco and McCarthy, 2006). A series of elegant studies also demonstrated that stimulation of glutamatergic fibers in neuron-astrocyte microislands *in vitro* trigger glutamate transporter-mediated currents in astrocytes (Mennerick

and Zorumski, 1994; Mennerick *et al.*, 1996) and acute slices (Bergles and Jahr, 1997; Clark and Barbour, 1997; Bergles and Jahr, 1998; Bordey and Sontheimer, 2003). Transporter currents provide a sensitive probe to determine the time course and concentration of the neurotransmitter transients at synapses (Luscher *et al.*, 1998; Mennerick *et al.*, 1999). Calcium increases from neuron-to-astrocyte signaling can be confined to subcellular compartments and microdomains, or sometimes the entire cell, as elegantly shown in Bergmann glia in acute slices (Grosche *et al.*, 1999). Interestingly, astrocytic depolarizations following neuronal stimulation display selectivity and short-term plasticity (Linden, 1997; Pasti *et al.*, 1997; Perea and Araque, 2005). These findings suggest that astrocytes have the capacity to process and integrate selective information in response to neuronal activity. Finally, with the development of novel tools, spontaneous Ca^{2+} transients in astrocytes have been detected *in vivo* and are regulated by neuronal activity (Hirase *et al.*, 2004; Wang *et al.*, 2006; Winship *et al.*, 2007). In particular, it was shown that whisker stimulation or contralateral hindlimb mechanical stimulation induced a short-latency calcium signal in astrocytes that operated on a time scale similar to neuronal activity (Winship *et al.*, 2007). More recently, an elegant study reported that astrocytes (like neurons) in the ferret visual cortex respond to visual stimuli *in vivo* and display distinct spatial receptive fields and sharp tuning to visual stimulus features including orientation and spatial frequency (Schummers *et al.*, 2008). Collectively, evoked or spontaneous intracellular calcium dynamics in astrocytes demonstrates a mode of astrocytic excitability since changes in intracellular calcium convey a powerful signal that can travel throughout a cell and activate protein kinases, ion channels, and vesicular release (see section 4.5). However, as discussed below, it is unclear whether intercellular calcium waves can spontaneously arise in normal conditions or following a physiological stimulation *in vivo*.

Astrocytes express many types of receptors commonly found in neurons. In addition, there is evidence of receptor clustering in astrocytic somata. Indeed, in a recent study, quantal events due to ectopic vesicular release of glutamate was reported in Bergmann glia (Matsui and Jahr, 2003). In a subsequent study, the authors found that the density of functional AMPA-type glutamate receptors at Bergmann glia somata is ~17-fold higher than that at the Purkinje neuron somata (the principal neuronal type of the cerebellum) (Matsui *et al.*, 2005). However, compared with the neuronal postsynaptic site, the clustering of receptors in astrocytes is much lower than that of the neuronal postsynaptic site. For example, the Purkinje neuron's postsynaptic density (1000 receptors/μm^2) is much greater than the AMPA receptor density in Bergmann glia (Momiyama *et al.*, 2003; Tanaka *et al.*, 2005). Nevertheless, these studies provide evidence that astrocytes are poised to sense neuronal activity by expressing the machinery to respond to neurons.

4.2. Calcium dynamics: do intercellular calcium waves exit?

1990 was the year when a paradigm shift took place in the astrocytic field. The existence of glutamate-induced intercellular calcium waves provided a mode of excitability and long distance communication between astrocytes that received much attention (Cornell-Bell *et al.*, 1990b) (for review see Cornell-Bell and Finkbeiner, 1991; Parri and Crunelli, 2002; Fiacco and McCarthy, 2006, and for mechanism based on ATP release and receptor activation see Finkbeiner, 1992; Dupont *et al.*, 2007). This led to several elegant hypotheses related to the role of long-range astrocytic calcium signals on neuronal transmission (Smith, 1992). However, it still remains unclear whether calcium waves between mature astrocytes occur in acute slices or *in vivo*. It is clear that intracellular calcium transient increases and calcium waves *within* a cell occur in response to neuronal stimulation both in acute slices (as supposed to cultured slices (Dani *et al.*, 1992)} and *in vivo* (Hirase *et al.*, 2004; Wang *et al.*, 2006; Winship *et al.*, 2007; Bekar *et al.*, 2008) (for an excellent recent review see Fiacco and McCarthy, 2006). *In vivo* calcium transients were found to be coordinated (Hirase *et al.*, 2004), but no intercellular waves have been observed in a mature astrocytic network besides calcium waves

in retinal Muller cells (Newman and Zahs, 1998). Nevertheless, intercellular calcium waves have been observed in young astrocytes in acute slices from 5-14 days old mice and 5-17-days old rats (Parri *et al.,* 2001; Schipke *et al.,* 2002; Haas *et al.,* 2006). In neonatal thalamic slices, calcium waves occurred spontaneously and resulted in NMDA-type glutamate receptor-activation in neighboring neurons (Parri *et al.,* 2001). In addition, Ca^{2+} waves could be induced in cortical astrocytes *in vivo* upon pressure application of a noradrenergic agonist (Bekar *et al.,* 2008). In the recent study in the visual cortext of ferret, it was reported that *in vivo*, astrocytes behave relatively independent of each other (Schummers *et al.,* 2008). Each astrocyte was reported to be sharply tuned for orientation, and two astrocytes separated by only tens of microns can respond to different, orthogonal stimulus orientations.

Intracellular calcium dynamics represent the mode of excitability in astrocytes and some of its functions on the vasculature and synaptic activity are illustrated in the next sections. Intercellular calcium waves remain to be shown in acute slices and *in vivo*. Alternatively, other ions may provide long-range communication, as sodium waves have been shown in cultured astrocytes (Bernardinelli *et al.,* 2004). Future studies on intercellular calcium and sodium waves *in vivo* would elucidate mechanisms of intercellular communication in astrocytes.

4.3. The neurovascular unit: control of BBB permeability, metabolism and vascular tone

4.3.1. BBB permeability—A number of molecules modulate BBB permeability, including neurotransmitters, neuromodulators, arachidonic acid and prostanoids, cytokines, macrophage inflammatory proteins (MIP) and nitric oxide (for reviews see Abbott and Revest, 1991; Abbott, 2000; Hawkins and Davis, 2005; Persidsky *et al.,* 2006). Some of these molecules, including ATP, endothelin-1, glutamate, IL-6 and TNF-a, MIP-2 and nitric oxide, are released from astrocytes. These molecules result in opening of the paracellular pathway by increasing permeability at the tight junctions. However, no study *in vivo* or in acute slices has directly reported a function of astrocytes on BBB permeability on a second-to-minute time-scale. Nevertheless, astrocyte-endothelial cell interactions may depend on calcium and ATP-receptor-mediated signaling from astrocytes to endothelial cells (Paemeleire and Leybaert, 2000a). Considering that endothelial cytoplasmic calcium is an important factor in the regulation of blood-brain barrier permeability, astrocytes may dynamically regulate BBB permeability. Such a dynamic regulation and transient increase in BBB permeability in the adult brain could be beneficial for allowing plasma molecules (e.g. growth factors) to enter the CNS and promote brain repair.

4.3.2. Metabolism—According to a newly revised model of energy metabolism in the brain, 75% of the glucose that enters the parenchyma may be phosphorylated by astrocytes (Hyder *et al.,* 2006). Glucose enters astrocytes through GLUT1, the major isoform of the facilitative glucose transporter, which is strategically concentrated in astrocyte endfeet, the thin processes that surround capillaries (Morgello *et al.,* 1995; Yu and Ding, 1998; Kacem *et al.,* 1998). It was recently shown in cultured astrocytes that coordinated Na^+ and Ca^{2+} increases in astrocytic endfeet induced dynamic changes in glucose uptake (Porras *et al.,* 2008). Ca^{2+} or Na^+ increases alone were not sufficient to modulate GLUT1 transport activity, but changes in both intracellular ions were required for an increase in glucose uptake. Na^+ increases were due to influx through the Na^+-glutamate cotransporter and the Na^+/K^+-ATPase pump. This remarkable finding illustrates a critical function of coordinated Na^+ and Ca^{2+} movement in astrocytes to stimulate glucose uptake. Coordinated Na^+ and Ca^{2+} waves in astrocytes that drive glucose uptake (Mahesh *et al.,* 2006) have also been reported *in vitro* but not in slices (Paemeleire and Leybaert, 2000b). Manipulations that block Ca^{2+} waves also inhibited Na^+ waves. However, inhibition of glutamate uptake or enzymatic degradation of extracellular glutamate selectively inhibited Na^+ waves. These data suggest that glutamate released by a Ca^{2+}-dependent mechanism was taken up by the Na^+/glutamate cotransporters into cultured

astrocytes, resulting in a regenerative propagation of cytosolic Na^+ increases (Bernardinelli *et al.*, 2004). Intracellular Na^+ in astrocytes may thus be a powerful intracellular messenger, which couples neuronal activity to glucose uptake and glycolysis (see section 3.5) in astrocytes, thus matching the excitability of a neuroglial network to the metabolic supply.

4.3.3. Control of vascular tone—It has been known for more than a century than brain activity can induce localized changes in cerebral blood flow (CBF) (Mosso, 1880). More specifically, when the activity of a brain region increases, blood flow to that region also increases to provide adequate oxygenation, energy supply, and removal of toxic byproducts of metabolism (for review see Iadecola, 2004). This mechanism, termed functional hyperaemia, is thought to be the basis for functional brain imaging, which maps changes in CBF induced by neural activity (Raichle and Mintun, 2006). Astrocytes, due to their proximity and extensive ensheathment of blood vessels, as well as their ability to sense neuronal activity (e.g. via metabotropic glutamate receptor activation), were suspected to be part of CBF regulation (Nedergaard *et al.*, 2003). However, it was only in the early 2000's that two research groups discovered that astrocytes participated in neurovascular coupling by matching neuronal activity to vascular tone in acute slices (Zonta *et al.*, 2003; Mulligan and MacVicar, 2004) (see summary diagram in Fig. 4). Stimulation of astrocytes led to intracellular calcium increases and wave propagation to endfeet, which resulted in either vasodilation or vasoconstriction. In one study, uncaging Ca^{2+} in astrocytes or applying the vasoactive molecule noradrenaline induced Ca^{2+} rise in endfeet and vasoconstriction of vessels (Mulligan and MacVicar, 2004). The study also found that vasoconstriction was due to activation of the phospholipase A2-arachidonic acid pathway and 20-hydroxyeicosatetraenoic acid production from astrocytic endfeet. Another study showed in rat cortical slices that neuronal activity triggered Ca^{2+} oscillations in astrocytes resulting in vasodilation (Zonta *et al.*, 2003). Selective activation of single astrocytes (via a patch pipette) contacting an arteriole also caused vessel relaxation. Importantly, the authors showed that *in vivo* blockade of glutamate-mediated Ca^{2+} increases in astrocytes reduced the blood flow increase in the somatosensory cortex during contralateral forepaw stimulation. A very recent study also showed the loss of blood flow regulation following visual stimulation *in vivo* when astrocytic responses to visual stimulation were attenuated (Schummers *et al.*, 2008). The mechanisms of vascular tone regulation is not detailed here, but has been the subject of several excellent and recent reviews (Anderson and Nedergaard, 2003; Ballabh *et al.*, 2004; Straub and Nelson, 2007; Gordon *et al.*, 2007; Iadecola and Nedergaard, 2007).

These exciting findings were followed by a series of elegant studies showing similar coupling between astrocyte activation and blood-flow regulation in the retina (Metea and Newman, 2006) and in the cortex *in vivo* (Takano *et al.*, 2006; Winship *et al.*, 2007). In the retina, both light and Muller cell stimulation evoked dilation or constriction of arterioles. Vasodilation was blocked by inhibitors of cytochrome P450 epoxygenase, the synthetic enzyme for epoxyeicosatrienoic acids, while vasoconstriction was blocked by an inhibitor of ω-hydroxylase, which synthesizes 20-hydroxyeicosatetraenoicacid. Nitric oxide levels were key players in determining whether vasodilations or vasoconstrictions occur in response to light and astrocyte stimulation. It was also thought that another astrocyte function, i.e. K^+ siphoning, contributed to neurovascular coupling. However, a recent study by Metea and colleagues (2007) convincingly showed that although retinal glial cells undoubtedly control neurovascular coupling, the glial K^+ siphoning does not appear to be a major contributor (Metea *et al.*, 2007).

It is clear that *in vivo* studies are more accurate than slice preparations to study blood flow regulation by astrocytes and neurons, as well as blood flow autoregulation in response to physiological fluctuations in blood pressure (for review see Iadecola, 2004). It is also possible to monitor Ca^{2+} activity in astrocytes and the hemodynamic response using intrinsic signal

imaging *in vivo* (Winship *et al.,* 2007). However, the slice preparation has the advantage of allowing for greater spatial resolution and improved access for pharmacological manipulations and cell stimulation. All of the above studies were performed on arterioles, which are surrounded by a layer of smooth muscle cells that either constrict or dilate upon astrocytic activation. Arterioles branch into capillaries that do not have smooth muscle cells, but these capillaries are lined by pericytes at discrete locations. Pericytes were recently shown to induce the constriction of capillaries, serving an analogous function to arteriolar smooth muscle cells (Peppiatt *et al.,* 2006). Considering that astrocytic endfeet terminate on pericytes as well as smooth muscle cells, it is possible that astrocytes contribute to regulation of capillary tone via pericyte stimulation. Although the concept of astrocytes providing neurons–to-blood vessels coupling and blood flow regulation seems universal, some neurons bypass astrocytes by directly projecting onto blood vessels (for review see Iadecola, 2004). These neurons, some of them being nitridergic, have the ability to directly regulate changes in vascular tone. Finally, most of the research on neurovascular coupling has been unidirectional, i.e. studying signals from neurons to astrocytes and smooth muscle cells. Interestingly, there is an emerging concept of signaling from the blood vessel to astrocytes and then neurons via stretch-activated channels (for hypothesis see Moore and Cao, 2008). Together, the signals among these key players may provide the crosstalk to match neuronal activity and metabolic supply.

4.4. Dynamic control of synaptic structure: from spine to synapse

At the electron microscopy level, 57% of synapses in the mature hippocampus are in direct contact with astrocytes (Ventura and Harris, 1999). But is this ensheathment static or dynamic at the second to minute time-scale? In cultures, it was shown that glutamate induces the formation of filopodia in hippcampal astrocytes (Cornell-Bell *et al.,* 1990a). It was also recently shown that astrocytic processes contacting neuronal somata and wrapping active synaptic terminals (visualized with FM1-43 staining) display a high degree of dynamic morphological changes in acute slices from GFAP-eGFP mice (Hirrlinger *et al.,* 2004). There are two defined modes of spontaneous motility: thin lamellipodia-like membrane protrusions gliding along neuronal surfaces and transient extensions of filopodia-like processes (Hirrlinger *et al.,* 2004). This high motility was proposed to be important for correct placement of gliotransmitter release sites and shaping the neuroblast environment in addition to properly positioning processes containing glutamate transporters. This study also highlighted the need to generate novel transgenic mouse lines with fluorescently tagged signaling molecules on astrocytic processes as well as on neuronal terminals for examining plasticity at synapses. A more recent study analyzed the coordinated motility of both astrocytic processes and spines (Haber *et al.,* 2006). Using time-lapse confocal imaging in acute slices, they found that astrocytes could rapidly extend and retract fine processes to engage and disengage from motile postsynaptic dendritic spines. Surprisingly, astrocytic motility is, on average, higher than its dendritic spine motility. Collectively, these findings and the examples presented below suggest that such a motility of astrocytic processes around synapses is accompanied by changes in synaptic activity.

4.4.1. Structural synapse plasticity—Astrocytes have long been thought to be highly plastic. GFAP expression is itself very dynamic and can be up-regulated by physiological conditions (i.e. salt loading; Gary and Chronwall, 1995) and second messengers within minutes (Neary *et al.,* 1994; Hunter and Hatten, 1995; Kahn *et al.,* 1997; Rajan and McKay, 1998). One striking example of anatomical remodeling in the adult brain resulting in changes in synaptic activity is in the supraoptic nucleus of the hypothalamus (for review see Theodosis *et al.,* 2004; Oliet and Piet, 2004; Oliet *et al.,* 2006). During lactation or dehydration, astrocytes modify their coverage of magnocellular neurons and their synaptic afferent inputs. These changes occur within a few hours and are completely reversible upon the cessation of stimulation. By comparing synaptic transmission and diffusion properties before and during

this neuroglial remodeling, it was found that the astrocytic environment surrounding neurons contributes to the regulation of synaptic and extrasynaptic transmission (Piet *et al.*, 2004). In particular, astrocytes control the level of activation of presynaptic metabotropic glutamate receptors on glutamatergic terminals, thereby regulating synaptic strength at excitatory synapses (Oliet *et al.*, 2001b). Astrocytes also constitute a physical barrier to diffusion, spatially and temporally limiting spillover of neurotransmitters, and thus limit extrasynaptic transmission. Another example of anatomical plasticity is in the Bergmann glia of the cerebellum. In a study by Iino *et al* (2001), Ca^{2+}-permeable AMPA-type glutamate receptors in Bergmann glia were converted into Ca^{2+}-impermeable receptors by adenoviral-mediated delivery of the GluR2 gene *in vivo*. This conversion retracted the Bergmann glial processes ensheathing synapses on Purkinje neuron dendritic spines and retarded the removal of synaptically released glutamate. In addition, it caused multiple innervations of Purkinje neurons by the climbing fibers where each Purkinje neuron normally receives only one climbing fiber in adult tissue. These findings showed that Ca^{2+}-permeable AMPA receptors in Bergmann glial processes are critical for proper structural and functional interactions between Bergmann glia and glutamatergic synapses. These studies support the idea that astrocytic processes are highly plastic and can remodel upon synaptic receptor activation. It remains to be shown whether the anatomical remodeling and changes in synaptic transmission can occur on a timescale of seconds following electrical stimulation in acute slices or *in vivo*. Future studies in this area would highlight the physiological importance and implications of this dynamic interaction between neurons and astrocytes at the synapse.

4.4.2. Spine formation—As mentioned in section 3.1, astrocytes promote synaptogenesis during development by releasing several diffusible molecules. In 1992, it was shown that astrocyte-conditioned medium promotes the proliferation of spines prior to the appearance of axons (Seil *et al.*, 1992), an event that precedes synapse formation. Protrusive dendritic activity is prominent in developing neurons (Dailey and Smith, 1996; Ziv and Smith, 1996; Portera-Cailliau *et al.*, 2003). It is thought that a subset of these motile protrusions are stabilized and transformed into stable spines with synaptic contacts (for review see Yuste and Bonhoeffer, 2004). A recent study using two-photon time-lapse imaging in cultured slices showed that astrocytic motility was essential for stabilization of individual dendritic protrusions and their subsequent maturation into spines (Nishida and Okabe, 2007) (Fig. 5A). In this study, manipulating Rac1-dependent signaling in astrocytes resulted in induction of longer, filopodia-like dendritic protrusions. Rac1 belongs to the family of small GTPases of the Rho family that are important regulators of the actin cytoskeleton (for review see de Curtis, 2008). In addition, in the same study, manipulation of ephrin/Eph-dependent neuron-astrocyte signaling suggested the involvement of this signaling pathway in astrocyte-dependent stabilization of newly generated dendritic protrusions. An earlier study showed that the membrane-bound ligand, ephrin-A3, on astrocytes dynamically regulates spine morphology in the hippocampus via local activation of EphA receptors on spines in acute slices (Murai *et al.*, 2003). Improvements in imaging resolution and molecular tools to label different cell types and manipulate their properties would improve our understanding of the function of astrocytes on spine formation and stability.

4.5. Fast modulation of synaptic transmission

There has been an explosion of papers on this topic both at the level of regular manuscript and review publications. We apologize for not crediting all of the reviews on this topic (Volknandt, 2002; Schousboe, 2003; Newman, 2003b; Hertz and Zielke, 2004; Allen and Barres, 2005; Mongin and Kimelberg, 2005; Benarroch, 2005; Haydon and Carmignoto, 2006; Verkhratsky and Toescu, 2006; Parri and Crunelli, 2007; Bains and Oliet, 2007). In an effort to not repeat these reviews, we will essentially highlight some of the pioneer findings and the recent controversies. We also refer to Dr. Kimelberg's recent review (2007) that provides a critical

assessment of the studies on the instructive role of astrocytes at synapses and discuss the significance of the findings. The overall concept is that neurotransmitters released from presynaptic terminals of neurons can activate receptors on neighboring astrocytes, leading to calcium increases, and perhaps wave propagation in young tissue. These changes in astrocytes, along with the release of glial-derived factors, can signal back to the neurons and modulate synaptic transmission in a millisecond-to-second time-scale. Two pioneering studies in 1994 showed that cultured astrocytes can release glutamate or a diffusible messenger resulting in either neuronal NMDA-type glutamate receptor activation or calcium increases in neurons (Parpura *et al.*, 1994; Nedergaard, 1994). In one of these studies, communication between astrocytes and neurons was proposed to be based on a gap junction-mediated process (Nedergaard, 1994). The elevation of astrocyte intracellular calcium was sufficient to induce the release of glutamate (Parpura *et al.*, 1994) and another unknown diffusible molecule (Nedergaard, 1994). Cultured astrocytes can release ATP and glutamate through various means, including volume-sensitive anion channels, calcium-sensitive anion channels, gap junction hemichannels, and fusion of secretory vesicles (Mongin and Kimelberg, 2005) (for review see Evanko *et al.*, 2004). Astrocytic Ca^{2+} transients induced by metabotropic agonists, brief touch, or photolysis of caged Ca^{2+} caused the appearance of an NMDA receptor-dependent slow inward current (SIC) in nearby neurons and an increase in the frequency of synaptic currents in the absence of neuronal spiking (Araque *et al.*, 1998). SIC in neurons is thought to result from glutamate-containing vesicle secretion from astrocytes, as it can be prevented by toxins that cleave SNARE proteins or by overexpression of dominant-negative SNAREs in astrocytes (Araque *et al.*, 2000). Ca^{2+}-dependent exocytosis has been confirmed in astrocytes by monitoring whole-cell capacitance and destaining of FM dyes using total internal reflection fluorescence microscopy (TIRFM, Bezzi *et al.*, 2004). It was also shown that cultured astrocytes release glutamate via two modes of secretion from large vesicles (310 nm in diameter) in response to different types of stimulation (Chen *et al.*, 2005). Interestingly, in response to a physiological stimulation, cultured astrocytes release their vesicular contents via a kiss-and-run mechanism. However, it was recently reported that extracellular ATP or glutamate can induce calcium-dependent exocytosis of lysosomes resulting in ATP release (Zhang *et al.*, 2007; Jaiswal *et al.*, 2007). These studies used time-lapse confocal imaging of FM dye-labeled fluorescent puncta or acridine orange loaded puncta, together with extracellular quenching and TIRFM in cultured astrocytes. The functional significance of lysosomal release, if it holds true in acute slices, is unknown. An interesting study also showed that receptor-induced Ca^{2+} increase is associated with an increase in astrocytic cell volume, which leads to the activation of volume-sensitive channels mediating glutamate release *in vitro* and in acute slices (Takano *et al.*, 2005). Thus, the relative contribution of each pathway for astrocytic glutamate and ATP release at synapses remains unclear. In addition, cultured astrocytes may not represent astrocytes in physiological conditions; it is thus important to validate these findings in acute slices and *in vivo*.

In acute brain slices, Ca^{2+} increases in astrocytes via stimulation of Gq-coupled metabotropic receptors, photolysis of caged IP3 or caged Ca^{2+}, direct mechanical stimulation, or repetitive depolarization of the astrocyte membrane result in activation of presynaptic (Kang *et al.*, 1998; Brockhaus and Deitmer, 2002; Bordey and Sontheimer, 2003; Fiacco and McCarthy, 2004; Liu *et al.*, 2004; Jourdain *et al.*, 2007) and postsynaptic ionotropic receptors in neurons (Newman and Zahs, 1998; Fellin *et al.*, 2004; Lee *et al.*, 2007). Similarly, astrocyte-derived ATP has been shown to alter synaptic efficacy and neuronal excitability (Zhang *et al.*, 2003; Newman, 2003a; Pascual *et al.*, 2005; Gordon *et al.*, 2005). However, the manipulations used to stimulate astrocytes were not physiological. A recent study also reported that vesicular glutamate release from astrocytes resulted in extrasynaptic receptor activation in neighboring neurons (Jourdain *et al.*, 2007). They provided structural evidence of glutamate-containing synaptic-like microvesicles in the astrocytic processes facing NMDA receptors in neurons. As previously performed by others (Kang *et al.*, 1998), the authors intracellularly perfused

astrocytes with a calcium chelator (BAPTA) while recording spontaneous synaptic currents in a neighboring neuron using paired patch clamp recording. Using this approach, Jourdain and colleagues (2007) found that the frequency of spontaneous excitatory postsynaptic currents in the recorded neuron was reduced following calcium buffering in surrounding astrocytes. This latter approach suggests that calcium-dependent release of a transmitter from astrocytes tonically modulates spontaneous synaptic activity in hippocampal neurons. However, as recently pointed out in an earlier review, it is difficult to differentiate between a supportive role and an instructive role, i.e. an active release of a transmitter with this experiment (Kimelberg, 2007). To test for a vesicular release mechanism, they intracellularly perfused astrocytes with the active light-chain of tetanus neurotoxin ($TeNT_{LC}$) while simultaneously recording a neighboring neuron using the double patch clamp approach. $TeNT_{LC}$ selectively cleaves and inactivates the vesicle-associated membrane protein VAMP2 (also called synaptobrevin 2), which is an indispensable component of the SNARE fusion complex. While depolarization of naïve astrocytes induced an increase in synaptic current frequency, depolarization of $TeNT_{LC}$-loaded astrocytes did not. One caveat of this experiment is that the astrocytic depolarization was not a physiological stimulus. However, in transgenic mice expressing an inducible dominant negative for VAMP2 in astrocytes, astrocytes were shown to tonically suppress synaptic transmission by releasing ATP *in vivo* (Pascual *et al.,* 2005). As mentioned above, it is important to acknowledge that other mechanisms could account for calcium-dependent glutamate release, such as through lysosome release (shown *in vitro*) and increase in astrocytic cell volume leading to the activation of volume sensitive channels (Takano *et al.,* 2005). The impact on synaptic activity of each mode of calcium-dependent transmitter release from astrocytes remains to be elucidated.

While these studies offer exciting new ideas into the role of astrocyte-neuron signaling on synaptic transmission, they remain very controversial. In particular, a recent study by Fiacco and colleagues questions the validity of some of these findings (Fiacco *et al.,* 2007). Utilizing the Gq-coupled receptor MrgA1, which is only expressed in nociceptive peripheral sensory neurons (Dong *et al.,* 2001) and has no known endogenous ligand, Fiacco *et al.* generated a line of mice in which MrgA1 is selectively expressed in astrocytes of the CNS using a portion of the *hGFAP* promoter. By crossing hGFAP-tTA mice with tetOMrgA1-GFP mice they selectively expressed MrgA1 in astrocytes (~80% of hippocampal astrocytes). The MrgA1 receptor can be activated by the phe-leu-arg-phe amide (FLRFa) peptide (Han *et al.,* 2002), whose application increased Ca^{2+} in a majority of astrocytes in acute hippocampal slices. Unexpectedly, application of FLRFa did not induce SICs in CA1 pyramidal neurons in hippocampal slices from $MrgA1^{+}$ mice, nor did it affect the amplitude or kinetics of miniature excitatory postsynaptic currents (mEPSCs) or induce Ca^{2+} transients in the dendrites of these neurons. However, the authors were able to replicate their previous findings (Fiacco and McCarthy, 2004) by showing that uncaging of IP3 in astrocytes induced a small increase in the frequency of spontaneous excitatory postsynaptic currents in $MrgA1^{+}$ mice. Very puzzling was the fact that SICs could not be induced in hippocampal pyramidal neurons of wild-type mice following endothelin application or Ca^{2+} uncaging in astrocytes, manipulations that produced reliable Ca^{2+} transients in astrocytes. However, NMDA-dependent SIC-like events were observed in CA1 pyramidal neurons when $MrgA1^{+}$ hippocampal slices were exposed to hypotonic solution. These events were independent of glutamate-loaded vesicle fusions. Cell swelling has previously been shown to contribute to calcium-dependent glutamate release (Takano *et al.,* 2005). Collectively these findings suggest that some of the previously used stimulation methods to induce glutamate release may not be physiological, such as uncaging of IP3 or calcium, and thus questions the validity of previous findings based on these methods. However, we cannot ignore that expression of a foreign receptor in astrocytes may affect their biology considering that an endogenous ligand may exist. In addition, McCarthy and colleagues reported recently that constitutive expression of a different foreign receptor, one coupled to Gi, led to hydrocephalus, despite the fact that the transgenic animals were never exposed to an

agonist for this receptor (Sweger *et al.,* 2007). Given these caveats, it will be important to validate previous findings in newly generated mice with a dominant-negative SNARE in astrocytes (Pascual *et al.,* 2005) or in other new lines of mice where vesicular release or calcium handling can be selectively manipulated.

4.6. Astrocytes and Synaptic plasticity

Astrocytes release many diffusible factors other than ATP or glutamate. In particular, the release of tumor necrosis factor-α (TNFα) from astrocytes contributes to the preservation of synaptic strength at excitatory synapses (Beattie *et al.,* 2002). TNFα was shown to enhance synaptic efficacy by increasing surface expression of AMPA receptors, while preventing the actions of endogenous TNFα had the opposite effects. Thus, the continual presence of TNFα is required for preservation of synaptic strength at excitatory synapses. Another attractive astrocytic molecule is D-serine, an allosteric agonist of NMDA-type glutamate receptors (Mustafa *et al.,* 2004). Interestingly, astrocytes were shown to contribute to hippocampal long-term potentiation through release of D-serine in mixed neuron-glia cultures and acute slices (Yang *et al.,* 2003). A recent study also convincingly demonstrated that astrocytes in the supraoptic nucleus can orchestrate synaptic transmission and plasticity by releasing D-serine (Panatier *et al.,* 2006).

5. The astrocyte of the neurogenic zone

With the discovery of neurogenesis in the adult brain, astrocytes stepped into the spotlight as a source of stem cells in discrete regions of the adult brain. The adult CNS has long been regarded as a dormant environment for neuronal regeneration. It was traditionally believed that the mature brain loses its regenerative capacities, lending the brain vulnerable to injury and degeneration. However, specialized neural stem cells reside in the SVZ of the lateral ventricle and the SGZ of the hippocampal dentate gyrus throughout adulthood (Altman and Das, 1965; Altman, 1969; Reynolds and Weiss, 1992; Richards *et al.,* 1992; Eriksson *et al.,* 1998). The specialized neural stem cells in the SVZ and SGZ exhibit the two fundamental properties: the ability to self-renew and generate multiple cells types of the CNS, including neurons, astrocytes, and oligodendrocytes.

Interestingly, as mentioned in section 2.4, a subset of SVZ astrocytes display stem cell characteristics (i.e. multipotency and self-renewal) in rodents and humans (Doetsch *et al.,* 1999; Sanai *et al.,* 2004). It is thought that SVZ astrocytes generate transit-amplifying progenitors that generate neuroblasts. Neuroblasts migrate either to the olfactory bulb or, to a smaller extent, to the piriform cortex where they differentiate into interneurons (Luskin, 1993; Lois and Alvarez-Buylla, 1994) (for review see Bordey, 2006; Lledo *et al.,* 2006). This finding has challenged the definition of an astrocyte and also its functions. Nevertheless, we believe that these cells belong to the family of astrocytes as discussed in section 2.4. What remains unclear is whether each SVZ astrocyte can at some point in time behave as a neural stem cell or whether only a subset of them has this ability. Similarly, can all SVZ astrocytes instruct neurogenesis? We will present data and arguments suggesting that all SVZ astrocytes including those with stem cell ability act as instructors of neurogenesis. Another issue that has recently been raised is whether every astrocyte in the CNS could regain stem cell features, and this will be briefly discussed.

5.1 Are there different types of SVZ astrocytes?

Doetsch and colleagues suggested the existence of two types of SVZ astrocytes (called type B cells) and tanycytes using electron microscopy and proliferative marker thymidine (Doetsch *et al.,* 1997). Thymidine is incorporated into the DNA during the S phase of the cell cycle. Type B cells had irregular contours that filled the spaces between neighboring cells, irregular

nuclei with invaginations, and light cytoplasm with few free ribosomes. They also expressed abundant intermediate filaments. Type B cells were divided into type B1 and B2. Type B1 cells were larger than type B2 cells; the chromatin of type B2 cells was clumped as opposed to dispersed in type B1 cells. Type B1 cells were adjacent to ependymal cells, separating neuroblasts from contacting the ependymal. Type B2 cells are mostly located at the interface with the striatal parenchyma. Tanycytes were infrequent and located between ependymal cells, contacted the ventricle, and contained microvilli on their luminal surface. Their nuclei were irregularly shaped and contained dark chromatin aggregates. The cytoplasm of these cells was electron-dense and rich in organelles containing many mitochondria and lysosomes. Interestingly, 10% of type B cells incorporated thymidine and corresponded to type B2 cells; none of the type B1 cells or tanycytes showed any thymidine incorporation, suggesting that they are good candidates for the quiescent SVZ stem cells. Because type B cells and tanycytes stain strongly for GFAP, studies on GFAP-expressing cells do not distinguish tanycytes from type B cells, and both are included in the term SVZ astrocytes. Collectively, type B1 astrocytes, i.e. SVZ astrocytes wedged between or along ependymal cells, may be the SVZ stem cells. It was also suggested in a review on stem cell niches that ependymal cells are the stem cell partners, analogous to the fly stem cell niche (Ohlstein *et al.*, 2004). Using GFAP immunostaining or hGFAP-GFP mice, SVZ astrocytes resembling type B1 or tanycytes and B2 cells are visible based on their differential location in the lateral SVZ (Fig. 6A and B). However, GFAP-GFP cells can also be found at the border of the SVZ or RMS and send processes into the surrounding parenchyma. It is unclear whether these border astrocytes are part of the type B2 cells or a distinct type of B cells. Finally, very few cells expressing S100B have been identified in the SVZ (1-3 per section) (Platel *et al.*, 2008). Surprisingly, S100B-positive cells do not co-express GFAP (Raponi *et al.*, 2007), but a minority co-express NG2 (Platel *et al.*, 2008). The NG2-negative S100B-positive cells are nevertheless thought to represent a different population of fully differentiated astrocytes (Raponi *et al.*, 2007).

The search for a neural stem cell marker has been fierce, but unfortunately, an unambiguous marker has not been identified. This is mostly due to limitations in isolating cells and testing self-renewal, which have been based on flow cytometry and neurosphere assays, respectively. Neurospheres may essentially isolate fast cycling cells and thus analyze the behavior of transit amplifiers that retain self-renewal ability although more limited than stem cells. CD15, also called Lewis X antigen (Lex), has been used to isolate cells showing stem cell characteristics, and some of these cells co-express GFAP (Capela and Temple, 2002). Mcm2 has also been used to label SVZ stem cells (Maslov *et al.*, 2004), but it also labels other cycling cells (Platel *et al.*, 2008). It is likely that SVZ neural stem cells will be identified based on staining with several antigenic markers as described for hematopoietic stem cells (Bryder *et al.*, 2006).

Collectively, there are at least two types of astrocytes, S100B-positive/GFAP-negative and GFAP-positive/S100-negative. The GFAP-expressing astrocytes of the SVZ may as well contain two populations although additional antigenic distinctions are required.

5.2. The SVZ astrocyte: neural stem cell, instructor for neurogenesis, or both

It is now well-accepted that SVZ stem cells are GFAP-expressing cells of the SVZ, called here SVZ astrocytes (Fig. 6). However, it remains unclear whether every SVZ astrocyte has the ability to behave as a stem cell or act as an instructor of neurogenesis.

SVZ astrocytes ensheathe migrating neuroblasts and are in close proximity to transit amplifiers (Fig. 6A, B and D). They are thus in a perfect location to control the development of these two cell types. Based on immunostaining data, every SVZ astrocyte expresses glutamate and GABA transporters and has the ability to buffer ambient GABA and glutamate levels (Bolteus and Bordey, 2004; Liu *et al.*, 2006) (Fig. 6D). With the recent findings that GABA exerts a strong influence on both neuroblast and SVZ astrocyte production (Nguyen *et al.*, 2003; Liu

et al., 2005), neuroblast migration (Bolteus and Bordey, 2004) as well as neuroblast differentiation (Gascon *et al.*, 2006) (for review see Bordey, 2007), every SVZ astrocyte may thus have the ability to control neurogenesis by regulating GABA levels. Similarly, neuroblasts express AMPA/kainate-type glutamate receptors whose activation leads to intracellular Ca^{2+} increases (Platel *et al.*, 2007). Thus, regulation of extracellular glutamate levels by uptake into SVZ astrocytes may have considerable influence on neuroblast development. Furthermore, given the novel role of astrocytes in synaptic transmission and control of blow flow, it is appropriate to speculate that SVZ astrocytes have similar functions with regard to neuroblasts and blood vessels (for review see Bordey, 2006). It was shown that neural progenitors preferentially settle close to blood vessels in the SGZ (Palmer *et al.*, 2000), and SVZ astrocytes closely ensheath blood vessels around the SVZ (Mercier *et al.*, 2002). SVZ astrocytes may promote angiogenesis when proliferation is up-regulated or take up glucose necessary for glycolysis to provide metabolic support for proliferating progenitors and neuroblasts. SVZ astrocytes may also guide or stimulate directional neurite outgrowth of migrating neuroblasts as shown for neonatal astrocytes *in vitro* (Deumens *et al.*, 2004). A highly controlled communication has also been reported from neuroblasts to SVZ astrocytes. For instance, neuroblasts in the SVZ can synthesize and release GABA via a nonsynaptic mechanism, and GABA in turn tonically activates $GABA_A$ receptors on the SVZ astrocytes, reducing their proliferation (Liu *et al.*, 2005). This signaling provides a mechanism for the daughter cells to limit stem cell proliferation and thus daughter cell production. This communication is likely bidirectional as SVZ astrocytes, like mature astrocytes, contain glutamate that may signal to neuroblasts via glutamate receptor activation (Platel *et al.*, 2007). In mature astrocytes, Ca^{2+} and perhaps Na^+ dynamics are key to control astrocytes' functions including glycolysis, neurite outgrowth, and release of glutamate or other diffusible molecules. However, it remains unclear whether SVZ astrocytes display coordinated Ca^{2+} increases or waves.

Finally, whether each SVZ astrocyte can behave as stem cell at a certain time point during their life is unclear. One possibility to address this question may be to use the recently generated Brainbow mice by Livet and colleagues (Livet *et al.*, 2007). Fluorescent proteins have half-life of 1 to 4 days and persist in the first or second generation of daughter cells. Assuming that each SVZ astrocyte has different color, it may be possible to determine from which SVZ astrocytes lineage using color-coding.

5.3. Implications for parenchymal astrocytes

The presence of these neurogenic astrocytes challenges the traditional definition of astrocytes as described in section 2.4, which are viewed as mature, fully-differentiated cells. In addition, this raises some interesting questions regarding astrocytes: Can all astrocytes act as stem cells if placed in the right environment? Or do mature astrocytes undergo an irreversible genetic change which seals their fate as postmitotic cells? Can astrocytes in the adult brain be reactivated to regain stem cell features and help repair the damaged brain (see (Gotz and Steindler, 2003) for additional discussion).

These questions will likely lead to mixed answers. First, transplantation experiments have underscored the importance of the niche in regulating cell potential. For instance, cultured SGZ progenitors generate granule neurons when transplanted into another SGZ, but not to nonneurogenic brain regions (Gage *et al.*, 1995). The instructive role of the niche is also demonstrated when SGZ progenitors grafted into the rostral migratory stream can adopt the fate of SVZ neural precursors to produce olfactory bulb interneurons (Suhonen *et al.*, 1996). Based on this evidence, we can speculate that the radial glial-derived astrocytic stem cells need the unique components of the niche in order to retain their neurogenic potential. Second, it was recently shown that postnatal astrocytes from P5-7 mice can be reverted into a stem cell when reactivating the proper genetic program *in vitro* (Berninger *et al.*, 2007). In particular, the

proneural genes *neurogenin-2* and *Mash1* possess the ability to reprogram these young astrocytes to stem cells that can generate neurons. These astrocyte-derived neurons display neuronal excitability and also receive synaptic inputs when co-cultured with embryonic cortical neurons, although they fail to generate a functional presynaptic output within the culturing period. These findings are very exciting and raise hopes that reactive astrocytes that have been known to revert to an immature phenotype could be reprogrammed into neural stem cells and provide newborn neurons for repairing the damaged brain.

6. Conclusion

It is quite remarkable the wealth of information that has been discovered about astrocytes in the last century. The recent advances in our knowledge of astrocytes have raised more questions about their identity and functions. From the evidence discussed in this review, we can appreciate that the morphological and molecular heterogeneity, as well as the diverse origins of astrocytes rival the diversification of neuronal subtypes. However, we can still find a common set of characteristics to define an astrocyte. An astrocyte lacks axons and dendrites, does not spike, expresses GFAP, and has a highly branched and convoluted morphology leading to the ensheathment of neuronal elements (e.g. spines) and encapsulation of blood vessels. While all may be called under the umbrella term astrocytes, more specialized terminology should be applied with regional specification as in the case of SVZ astrocytes. Future studies aimed at better understanding the lineage of astrocytes and their repertoire of transcriptional regulation and molecular markers may be helpful in defining astrocytic populations and functions such as in the study by Cahoy and colleagues (2008).

In defining astrocytes, we should not overlook the well-accepted functions of astrocytes in providing homeostasis for neural networks. Astrocytes synthesize a plethora of extracellular matrix proteins, adhesion molecules, and trophic factors that regulate neuronal maturation and synapse formation. In addition, they form the BBB, regulate extracellular ionic buffering, control neurotransmitter uptake, and provide basic metabolic support for the brain. However, pioneering studies regarding the above trophic and homeostatic functions of astrocytes necessary for proper neural activity were performed *in vitro*. A major challenge has been to move these findings into an *in vivo* setting. Advances in molecular biology and virology for gene delivery now provide the means to address astrocytic functions in a physiological setting. Another challenge is mapping the diversity of the astrocyte-associated molecules that are expressed with temporal and spatial specificity. Additional proteomic and gene array analysis of astrocytes acutely isolated from the brain (e.g. with flow cytometry) will help identify new astrocytic molecules and their functions in maintaining normal neuronal functions.

The exciting newly discovered functions of astrocytes have catapulted interest in astrocyte research over the past few decades. The key discovery of calcium waves in astrocytes and the concept of gliotransmission (i.e. neurotransmitter release from astrocytes) brought these cells from the shadows and put them in the spotlight as active participants and modulators of neurotransmission. Intracellular calcium dynamics represent the mode of excitability in astrocytes and some of its *in vivo* functions on the vasculature and synaptic activity still remain to be elucidated. Another emerging field is the dynamic interaction between astrocytes and synapses. The surprisingly motile astrocytic processes surrounding synapses suggest that they may cause changes in synaptic activity. In addition, the discovery of the fast (millisecond to second time scale) communication between neuronal activity and astrocyte excitability strongly suggests that astrocytes play an important role in modulating synaptic transmission. As we understand more about the mechanisms for these emerging functions of astrocytes, we can eventually lay to rest the controversies surrounding the *in vivo* roles of astrocytes in neuronal and vascular crosstalk. With the advancements in high-resolution imaging and molecular tools, we may yet uncover new functions of astrocytes.

Perhaps one of the most exciting findings that has hit the astrocyte field is the discovery that astrocytes are the stem cells in the adult neurogenic zone. This finding fundamentally challenges the traditional definition of astrocytes. While some new antigenic markers and physiological properties have been used to define these astrocytic stem cells, we can better define this subclass of astrocytes as we learn about their molecular profile. As discussed above, the stem cells act in concert with resident astrocytes to maintain the neurogenic niche. Future studies will uncover the molecules and mechanisms that allow these specialized germinal zones to retain their regenerative and proliferative capacity in the adult brain. Another key question that has emerged is whether every astrocyte in the CNS has the ability to revert to stem cells given the right environment. Understanding the biology of these adult stem cells has enormous therapeutic potential in treating the diseased and injured brain.

In conclusion, we have just begun to uncover the true nature of astrocytes. With all the enthusiasm surrounding the astrocyte field, this star will undoubtedly create many new odysseys to come.

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Abbreviations

AMPA

alpha-Amino-3-hydroxy-5-Methyl-4-isoxazolePropionic Acid

ATP
Adenosine Triphosphate

BBB
Blood Brain Barrier

BDNF
Brain-Derived Neurotrophic Factor

BLBP
Brain Lipid Basic Protein

cKO
conditional Knock-Out

CNS
Central Nervous System

CNTF
Ciliary Neurotrophic Factor

EAAT
Excitatory Amino Acid Transporter

ECM
ExtraCellular Matrix

EET
Epoxyeicosatrienoic acids

FGF
Fibroblast Growth Factor

GABA
γ Amino-Butyric Acid

GAT
GABA Transporter

GFAP
Glial Fibrillary Acidic Protein

GFP
Green Fluorescent Protein

GLAST
Glutamate-Aspartate Transporter

GLUT1
Glucose Transporter 1

GS
Glutamine Synthetase

hGFAP
human GFAP

IL-6
Interleukin-6

Kir
Inwardly rectifying K^+ channels

LIF
Leukaemia Inhibitory Factor

MCT1
Monocarboxylate Transporters 1

MIP
Macrophage Inflammatory Protein

MMP
Matrix Metalloproteinase

NCAM
Neural Cell Adhesion Molecule

NT-3
Neurotrophin-3

NG2
Neuro-Glial proteoglycan

NGF
Nerve Growth Factor

NMDA
N-methyl-D-aspartic acid

OPC
Oligodendrocyte Progenitor Cells

P
Postnatal

PDGF
Platelet-Derived Growth Factor

SGZ
Subgranular zone

SIC
Slow Inward Current

SSeCKS
Src-Suppressed C-Kinase Substrate

SVZ
Subventricular Zone

$TeNT_{LC}$
active Light-Chain of Tetanus Neurotoxin

TGF-β
Transforming Growth Factor

TIRFM
Total Internal Reflection Fluorescence Microscopy

TNFα
Tumor Necrosis Factor-α

VEGF
Vascular Endothelial Growth Factor

VZ
Ventricular Zone

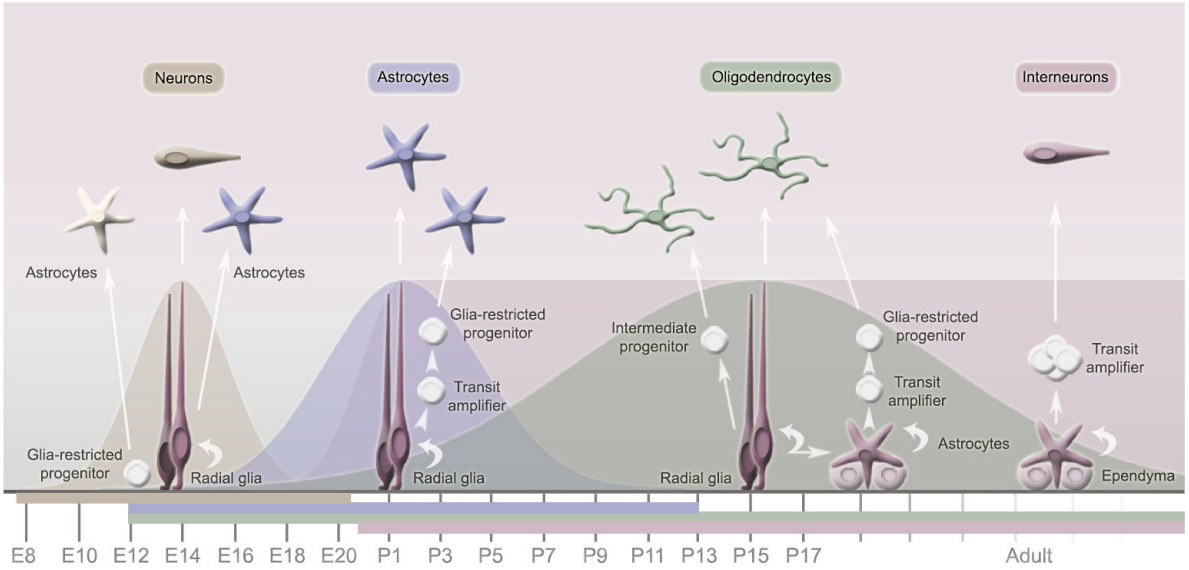

Figure 1. Diagram summarizing the sequence of neuron and glia development

The generation of the different neuronal and glial cell occurs in a temporally distinct yet overlapping pattern. In rodents, neurogenesis (e.g. generation of projection neurons) peaks at embryonic day 14, astrocytogenesis at postnatal day (P) 2, and oligodendrocytogenesis at P14. The generation of interneurons starts during embryonic life and continues postnatally. However, postnatal interneuron generation is essentially restricted to the olfactory bulb and the dentate gyrus. Astrocytes can be generated from several sources: radial glia during the perinatal life, glia restricted progenitors in the ventricular zone during embryonic life, and from glia restricted progenitors generated from transit amplifier during postnatal and adult life.

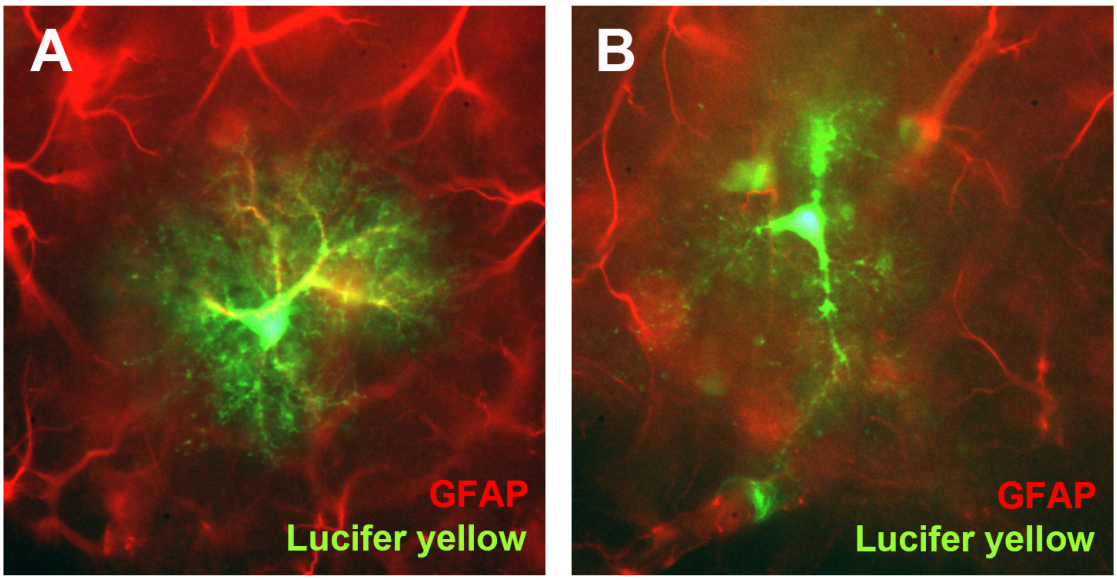

Figure 2. Morphology of astrocytes

(A and B) Photographs of astrocytes recorded in the hippocampus and filled with lucifer yellow during patch clamp recording. Immunostaining for GFAP was overlaid with the lucifer yellow fill. The cell in (A) displays a typical stellate morphology and strongly stains for GFAP, but does not display dye coupling. The cell in (B) displays dye coupling to other cells and send a process ensheathing a blood vessel typical of an astrocyte, but does not stain for GFAP.

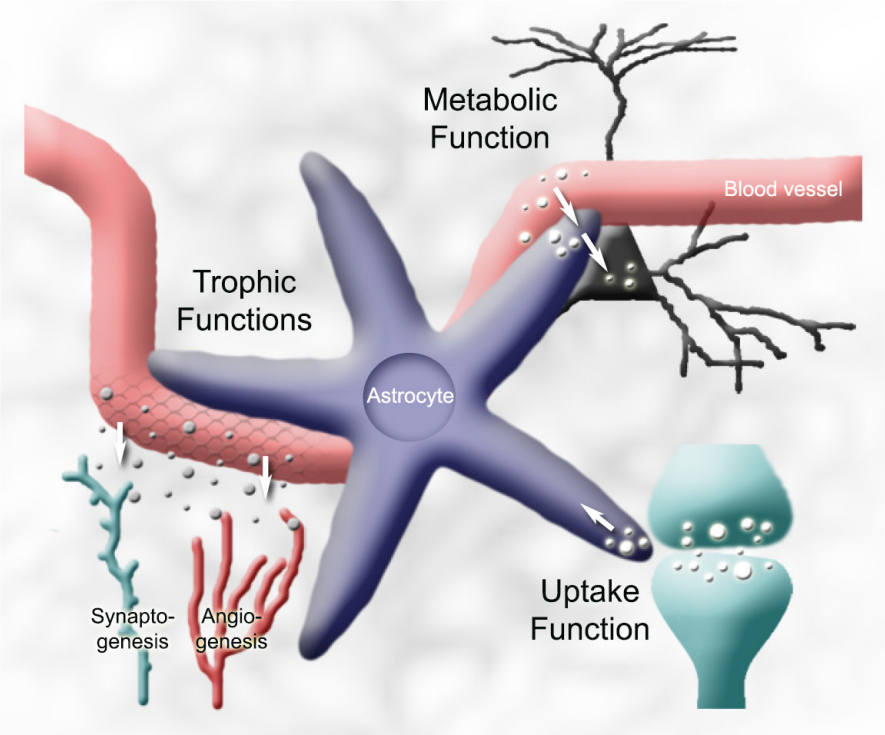

Figure 3. The well-established functions

Astrocytes have several homeostatic functions maintaining a viable nervous system environment for neurons. These functions include: (1) providing metabolic support for neurons (section 3.5), (2) taking up K^+ and neurotransmitters (sections 3.3 and 3.4, respectively), (3) Synaptogenesis (section 3.1), angiogenesis (sections 3.2.1), and BBB maintenance (section 3.3.2).

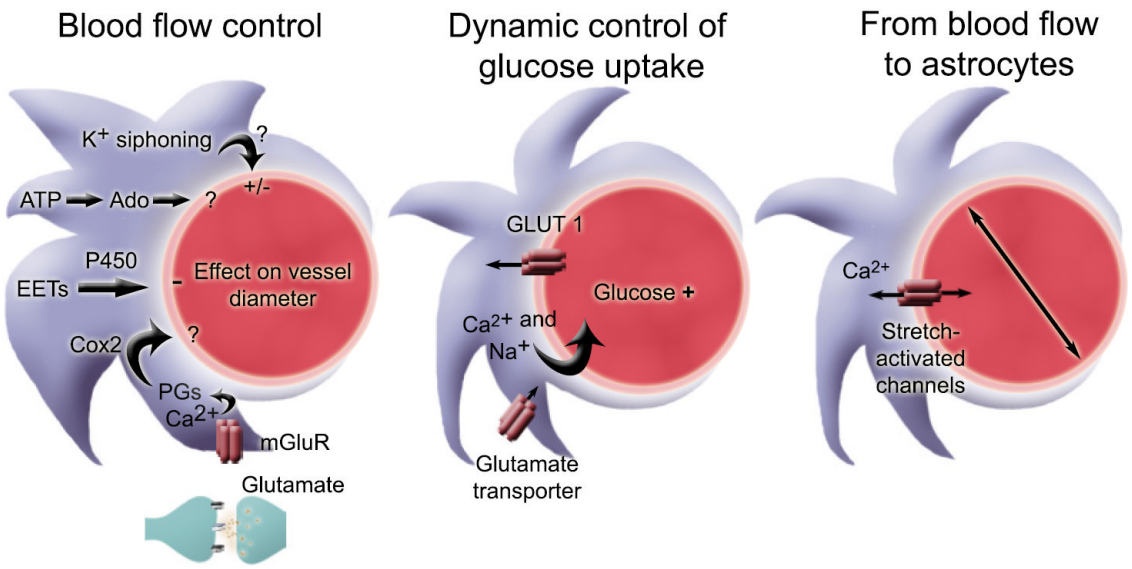

Figure 4. Astrocyte interactions with the vasculature

(A) Astrocytic processes ensheath blood vessels, including arterioles, which are composed of endothelial cells and smooth muscles cells (note shown on the diagram). The smooth muscle cells allow the vessels to contract or dilate. Neuronal activity induces mGluR activation in astrocytes leading to the synthesis of arachidonic acid (via PLA_2, not shown) and the formation of downstream messengers, including prostaglandins (PGs) and epoxyeicosatrienoic acid (EETs) via Cox2 and P450, respectively. PGs (in particular PGE2) and EETs induce vessel dilation. AA can also pass from astrocytes to smooth muscle cells where its downstream product 20-hydroxyeicosatetraenoic acid (20-HETE) induces vasoconstriction. In addition the activation of Ca^{2+} activated K^+ channels in astrocyte endfeet and the efflux of K^+ from astrocytes and subsequently from smooth muscle cells has been suggested to modify vascular tone by hyperpolarization and relaxation of smooth muscle cells, but this does not occur in the retina (See Metea *et al*, 2007). **(B)** Glutamate uptake into astrocytes is accompanied by Na^+ entry leading to Ca^{2+} elevation as a result of Na^+/Ca^{2+} exchange. Together Na^+ and Ca^{2+} can stimulate glucose uptake from the blood into astrocytes via GLUT1. **(C)** One newer hypothesis is whether changes in blood vessel diameter can affect the biology of astrocytes via activation of stretch-activated channels that can be permeable to Ca^{2+} or other ions.

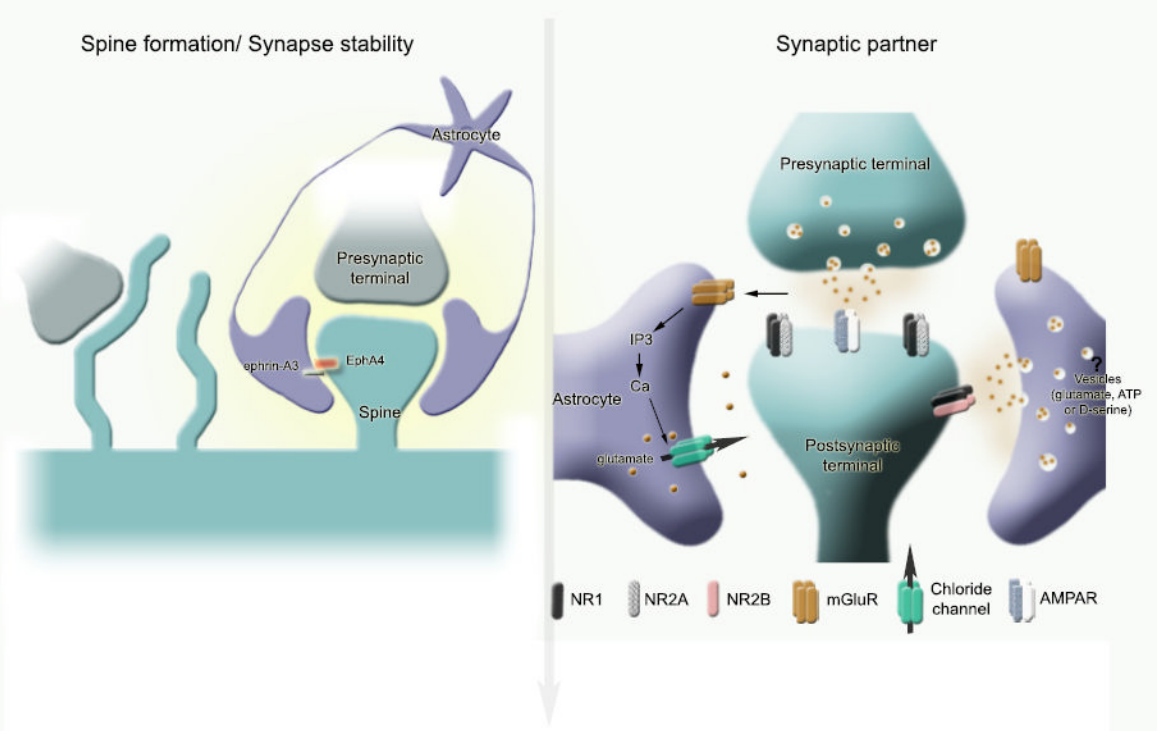

Figure 5. Astrocyte interactions with synapses

(A) Astrocytes encapsulate synapses including spines. This ensheathment allows spines to remain stable. At the molecular level, the ephrin-A3/EphA4 receptor signaling between astrocytic processes and spines has been shown to regulate the morphology of dendritic spines. In the absence of astrocytic processes, motile filopodia extend from dendrites. The astrocytic contact allows the filopodia to transform into a mature spine. (B) Astrocytes are an active synaptic partner. Not only they take up glutamate via high affinity transporters (not shown here), but they sense glutamate escaping synaptic clefts and release neuroactive substances upon glutamate receptor activation and intracellular Ca^{2+} elevation. Neuroactive substances include glutamate that is released via either vesicles or via Ca^{2+}-dependent chloride channels, or both. Glial glutamate activates extrasynaptic receptors, including NR2B, on neurons leading to changes in synaptic integration.

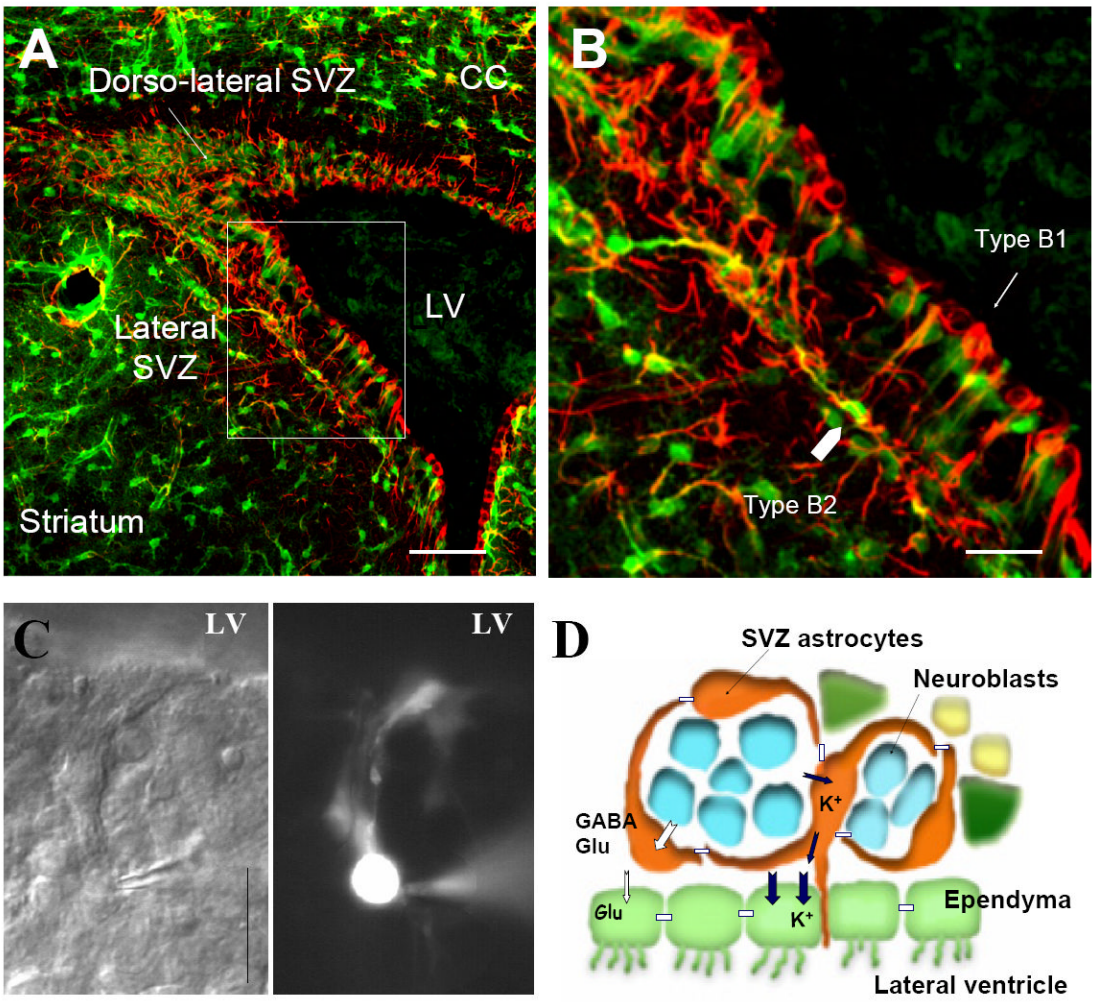

Figure 6. The SVZ astrocyte

(A and B) Photographs of GFP fluorescence (green) with immunostaining for GFAP (red). The image in the white squares in (A) is shown at higher magnification in (B). Astrocytes in the lateral SVZ have there cell bodies either touching the ventricle or touching the striatum. **(C)** Photographs of a DIC image and corresponding image of a lucifer yellow-filled astrocyte. The recorded astrocyte has a process projecting toward the lateral ventricle and displays dye coupling. Scale bar: 20 μm. **(D)** Scheme illustrating the arrangements of the different cell types in the SVZ. SVZ astrocytes (orange) ensheath neuroblasts (blue) and transit amplifying progenitors (green, also called transit amplifiers) are scattered among SVZ cells. Ependymal cells line the lateral ventricle. SVZ astrocytes and ependymal cells display uptake mechanisms for K^+, GABA and glutamate.