New roles for astrocytes: Redefining the functional architecture of the brain

Maiken Nedergaard[1], Bruce Ransom[2] and Steven A. Goldman[3]

[1]Department of Cell Biology, New York Medical College, Valhalla, NY 10595, USA
[2]Department of Neurology, University of Washington School of Medicine, Seattle, WA 98195-6465, USA
[3]Department of Neurology and Neuroscience, Cornell University Medical Center, NY 10021, USA

Astrocytes have traditionally been considered ancillary, satellite cells of the nervous system. However, work over the past decade has revealed that they interact with the vasculature to form a gliovascular network that might organize not only the structural architecture of the brain but also its communication pathways, activation, thresholds and plasticity. The net effect is that astroglia demarcate gray matter regions, both cortical and subcortical, into functional compartments whose internal activation thresholds and external outputs are regulated by single glial cells. The array of these astrocyte-delimited microdomains along the capillary microvasculature allows the formation of higher-order gliovascular units, which serve to match local neural activity and blood flow while regulating neuronal firing thresholds through coordinative glial signaling. By these means, astrocytes might establish the functional as well as the structural architecture of the adult brain.

To classical histologists, glial cells – including astrocytes and oligodendrocytes – were after-thoughts, given the overt complexity and beauty of the neuronal elements of the brain. The very word glia evinces the relative disdain with which early microscopists must have viewed this small stubby cell, whose silver impregnation profile was so much less impressive than that of its neuronal neighbors [1]. The word glia is derived from the Greek word gliok which, although commonly translated as glue, also means slime. But astrocytes are highly fibrous cells of great structural complexity, uniquely characterized by a dense array of processes interposed between neuronal elements, some of which contact and envelop local vascular walls. The traditional concept of astrocytes has been one of a phenotype intended for service to others – to regulate and optimize the environment within which neurons function. As such, astrocytes maintain tight control of local ion and pH homeostasis, deliver glucose and provide metabolic substrates. Astrocytes also clear neuronal waste, including not only metabolic byproducts but also neurotransmitters released during synaptic transmission, which are sequestered through active uptake. In short, astrocytes are multifunctional housekeeping cells that, by their functions, have allowed neurons to become progressively specialized for the tasks of information processing, encoding and transfer.

In this review, these traditional conceptions of astrocytic form and function will be reconsidered, to make the point that astrocytes provide not so much the glue of the neuronal network of the brain as its dynamic, self-organizing and auto-regenerative scaffold. We can now consider astrocytes as dynamic regulators of neuronal production, network insertion, phenotype and functional activity. As such, astrocytes might serve neurons not so much as servants but as parents – both literally, as a developmental source of new neurons, and figuratively, as the regulators and judges of neuronal behavior and activities. Simply stated, astrocytes tell neurons what to do, besides just cleaning up their mess. The addition of these functions to the known repertoire of glial activities over the past decade has expanded our conception of this phenotype and our appreciation for its seminal importance in normal functioning of the adult brain.

The phylogenetic advance of the astrocyte

The relative number of astrocytes, expressed both as a proportion of total brain cell number and as a ratio to neuronal number, increases with dramatically with phylogeny and brain complexity. In the leech, a typical ganglion is composed of 25–30 neurons and only one astrocyte (Figure 1). In *Caenorhabditis elegans*, neurons outnumber glia by 6:1 [2], whereas astrocytes and neurons are represented in a ratio of 1:3 in the cortex of lower mammals such as rats and mice. In the human cortex, there are 1.4 astrocytes for every neuron [3]. The relative expansion of astrocytes compared to neurons with evolution and, more specifically, the relative predominance of astrocytes in the human brain cannot be readily explained on the basis of differences in glial metabolic support, which appear little different across species and are certainly similar among higher vertebrates.

What evolutionary pressure (or pressures) led to this expansion of astrocytes relative to neurons? Setting aside the 'how' question to focus on 'why', it is plausible that the greater abundance of astrocytes with evolution could be a function of network complexity: increasingly dense and sophisticated synaptic networks require greater degrees of local modulation and control. In fact, astrocytes are beginning to seem very well suited for such duties. Compelling recent evidence supports the concept that

Corresponding author: Maiken Nedergaard (maiken_nedergaard@nymc.edu).

http://tins.trends.com 0166-2236/$ - see front matter © 2003 Elsevier Ltd. All rights reserved. doi:10.1016/j.tins.2003.08.008

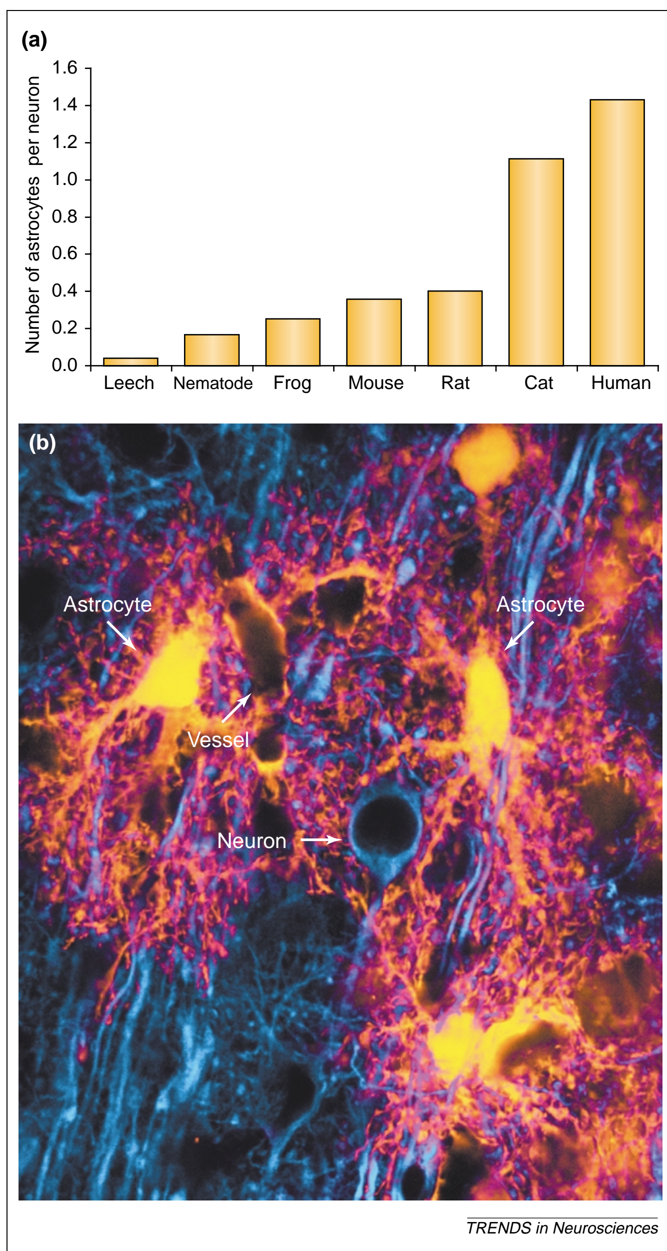

Figure 1. Relative ratios of astrocytes to neurons. **(a)** Ratios of astrocytes to neurons for different species, showing an increase in the number of astrocytes per neuron with increasing brain complexity and size. The ratios for mice, rats, cats and humans are representative for cortex only [2,3]. **(b)** Complex spatial organization of astrocytes and neurons in rat cortex. Neurons are labeled with antibody to microtubule-associated protein 2 (MAP-2; blue), whereas astrocytes are expressing enhanced green-fluorescent protein (eGFP; yellow). A small vessel is outlined by eGFP-positive astrocytic endfeet (X. Wang and M. Nedergaard, unpublished).

astrocytes transmit signals to neurons that directly modulate synaptic strength in the CNS [4–6]. The first step in this novel signaling mechanism is a receptor-induced increase in astrocyte intracellular Ca^{2+} concentration ($[Ca^{2+}]$). Astrocytes might also regulate synaptogenesis [7]. Neurons co-cultured with astrocytes developed approximately sevenfold more synapses, and a sevenfold increase in synaptic efficacy, compared with neurons raised in the absence of astrocytes [7,8]. As if that were not enough, recent studies in the adult rat hippocampus indicate that astrocytes might regulate the appearance of new neurons, even before they exercise modulatory control of neuronal function [9,10] (Figure 2). Thus, astrocytes might directly regulate the mitotic generation of new neurons, their capacity to form (and the strength of) synaptic networks, and their moment-to-moment synaptic instructions. These heretical ideas, based on in-depth studies, drastically reshape how one thinks about brain function. And of course, they have implications for how function could go awry. Let us be clear about one thing: we are still in the early stages of understanding how these findings fit together with conventional neuron-oriented doctrine. Other facts about astrocyte behavior and organization might help in thinking about possible ways forward.

Astrocytes establish non-overlapping territories that define functional domains

Astrocytes typically extend between five and eight major processes, each of which ramifies into fine and essentially uniformly distributed leaflet-like appendages [11]. Unexpectedly, the elaborate and dense processes of each hippocampal astrocyte define a 3D space that is free of processes from any other astrocytes. In this way, the astrocyte defines its own anatomical domain. Only the most peripheral processes interdigitate with one another, doing so along a narrow interface within which $<5\%$ of the volumes of adjacent astrocytic domains overlap [12]. The cell body and the major processes of astrocytes are enriched with glial fibrillary acidic protein (GFAP)-defined intermediate filaments, recognition of which by either Golgi staining or immunolabeling is the basis for the classically defined star-shaped appearance of astrocytes. However, GFAP does not give a true representation of astrocytes. The finer processes, which fill the territory of an astrocyte, are GFAP-negative, and the overall outlines of astrocytes is far from star-like –rather, they are cubic or rounded [11,12]. Astrocytes, therefore, fail to live up to the morphological expectation of their name. Their star-like appearance is only apparent with GFAP staining and the resulting picture has little to do with their true form. In fact, their extraordinarily profuse processes fill a volume that is best defined as a polyhedron, not a star [11,12].

As a result of this geometry, and contrary to prior conceptions of glial architecture, the distribution of astrocytes throughout the adult CNS is far from random; rather, it is highly organized. The distribution of astrocytes is dictated primarily by contact spacing, such that astrocytes are evenly separated and their cell bodies and the larger processes are not in contact with each other [13] (Figure 3). This orderly pattern of organization, which parcellates the entire neuropil into small astrocyte-delimited domains, appears to be manifested throughout the gray matter of the entire brain and spinal cord. The functional significance of these non-overlapping astrocytic domains is unknown, but one remarkable consequence of this structural organization is that all synapses lying within a given volumetrically defined compartment might be under the sole influence of a single astrocyte.

The stacked and repeated polyhedral astrocytic domains suggest an order of primary structure almost crystalline in nature. Lending an order of secondary structure to this basic repeated motif, the capillary

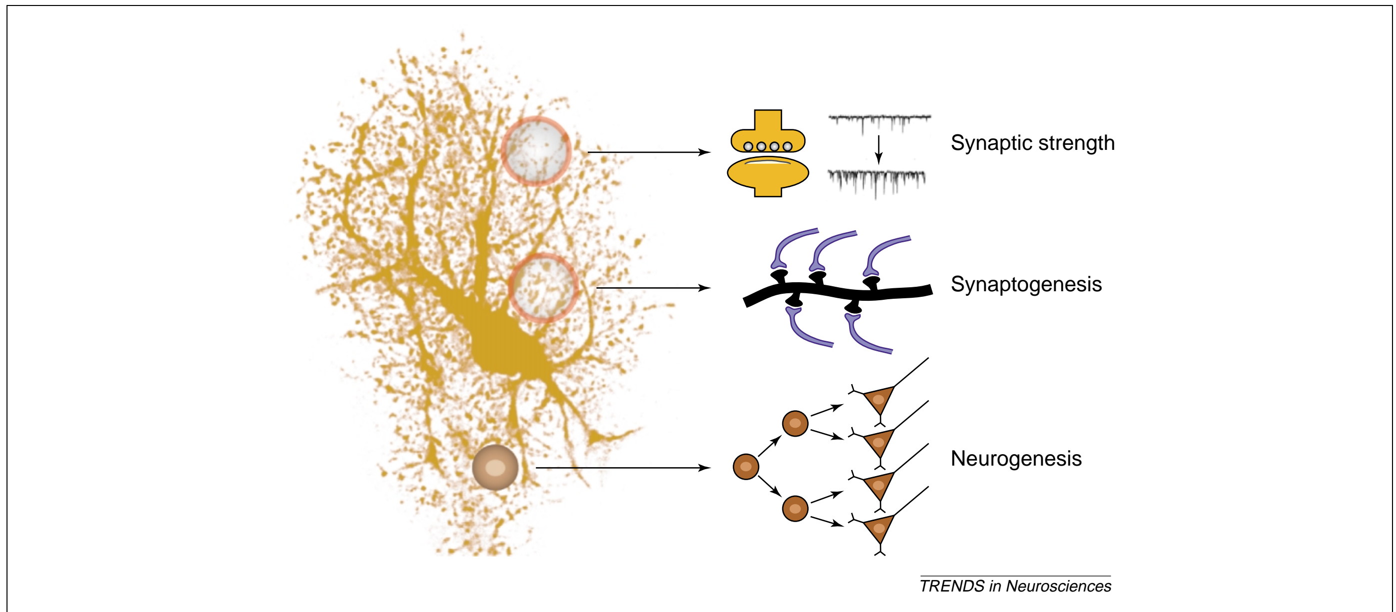

Figure 2. Astrocytes as regulators of synaptic activity, synaptogenesis and neurogenesis. Astrocytes can modulate synaptic strength (illustrated here by an increase in spontaneous excitatory postsynaptic currents) [6]. Several mediators of astrocyte–neuron signaling have been identified, including glutamate, ATP, adenosine and gap-junction signaling. Moreover, astrocytes are potent inducers of synaptogenesis, although the means by which this is accomplished remain unclear [57]. In addition, astrocytes can regulate neurogenesis from endogenous progenitor populations, at least in the dentate gyrus of adult rats [10].

microvasculature wends its way through the brain in a similarly organized fashion, with microvessels typically positioned along the interfaces between adjacent astrocytic domains and the endfeet of adjacent astrocytes, providing a contiguous but non-overlapping sheath around the capillaries bordering its domain [14]. Remarkably, the linear organization of parenchymal astrocytes along cerebral microvessels might not differ conceptually

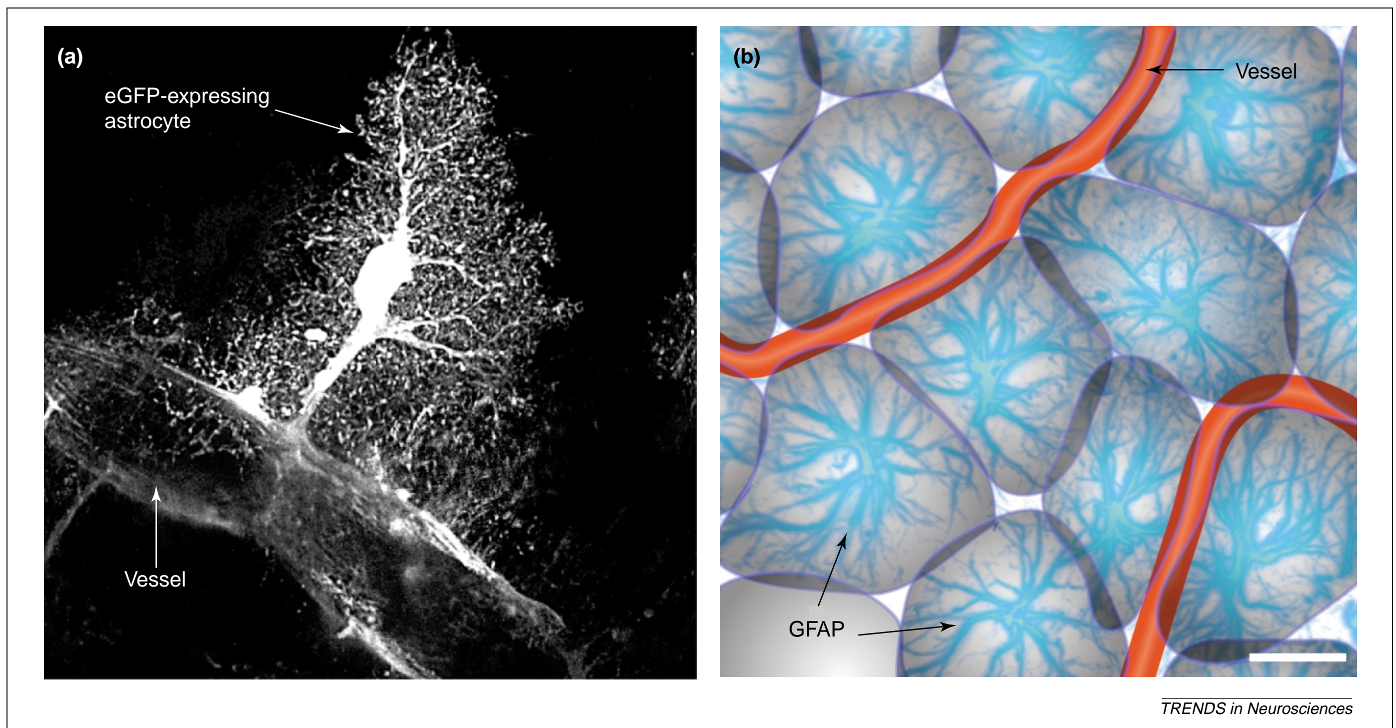

Figure 3. Contact spacing dictates the spatial organization of astrocytes. Astrocytes are homogeneously distributed within the gray matter [11,12]. Their processes are not merely tubular but veil-like and extremely complex. The processes of a single astrocyte densely invest a 3D space of polyhedral shape. **(a)** Two-photon confocal imaging of an enhanced green-fluorescent protein (eGFP)-expressing astrocyte in a cortical slice, illustrating the dense array of processes from a single cell. The misconception that astrocytes are simple star-shaped cells stems from traditional studies using silver impregnation techniques that stained intermediate filaments, and from later studies using antibodies directed against the principal glial filament, glial fibrillary acidic protein (GFAP). GFAP is densely expressed within the cell body and in the primary and secondary processes of astrocytes; these filaments fill a small fraction of the cell and extend into only the larger processes. The ‘true’ morphology of gray matter astrocytes is revealed with single-cell dye fills or eGFP expression, as shown here. The fine GFAP-negative processes might include the majority of the total volume of the astrocyte, and define in their extent its territorial domain (T. Takano and M. Nedergaard, unpublished). **(b)** Astrocytes are organized in rows along the vessels, which are typically positioned in the narrow interface between astrocytes. This schematic is based on a cortical rat section immunolabeled for GFAP (blue). The astrocytic territories are arbitrarily outlined, demonstrating that the GFAP-positive processes fill only the center of each territory. Vessels are indicated in red.

Box 1. Mechanism of astrocytic Ca^{2+} waves

Our understanding of how astrocytes propagate Ca^{2+} waves has undergone substantial revision during the past few years (Figure I). Originally, it was believed that Ca^{2+} or inositol (1,4,5)-trisphosphate diffused through gap junction and, in a regenerative fashion involving phospholipase C (PLC), increased Ca^{2+} levels in neighboring cells (Model 1). This model was based on the observation that connexin (Cx)-deficient cell lines, which were otherwise unable to propagate Ca^{2+} waves, achieved the ability to do so after transfection with Cx43 or Cx32 [58]. However, the role of gap junctions in Ca^{2+}-wave propagation was then questioned because of the observation that astrocytes lacking physical contact with other astrocytes also engaged in Ca^{2+} waves; indeed, Ca^{2+} waves appeared to leap over areas of non-contiguity [59]. This suggested that an extracellular component might mediate intercellular Ca^{2+} signaling. ATP was subsequently identified as the diffusible messenger of Ca^{2+} signaling in astrocytes [60], just as it has been shown to be in bronchial epithelial cells, hepatocytes, cardiomyocytes and osteoblasts [61] (Model 2). Astrocytes express the purine receptors P2Y1, P2Y2, P2Y4 and P2X7 [62–65], and one or more of these appear to participate in the mobilization of intracellular Ca^{2+} stores during wave propagation. But gap-junction proteins are indeed part of the story: compelling evidence now indicates that cell lines expressing Cx43, Cx32 or Cx26 release 5–20-fold more ATP than Cx-deficient controls [66,67]. These observations suggest a model whereby Cx-expression potentiates Ca^{2+} wave propagation by enhancing ATP release, rather than by providing an intercellular gap-junction pathway for signal diffusion, as had been proposed in model 1. Gap junction channels, which are composed of the two apposed hemichannels of adjacent cells, have an inner pore diameter of 10–12 Å and are freely permeable to large anions, including ADP and ATP. Thus, ATP efflux could be carried through open hemichannels [68].

The newest development has suggested that ATP is not released in a regenerative fashion, as was suggested in Model 2, resulting in a revision of our concept of astrocytic Ca^{2+} signaling (Model 3). In short, bioluminescence imaging of extracellular ATP has revealed that ATP is released by a single cell, from a single point source, so that the propagating wave front reflects the diffusion of ATP following a single burst event, rather than the sequential release of ATP [60]. According to this scheme, neighboring cells increase intracellular Ca^{2+} levels in response to P2Y-receptor activation but do not further amplify wave propagation by release of additional ATP. This model accounts the observation that Ca^{2+} waves rarely expand beyond 300–400 μm from their point of origin, and typically act in a non-regenerative fashion (Model 3).

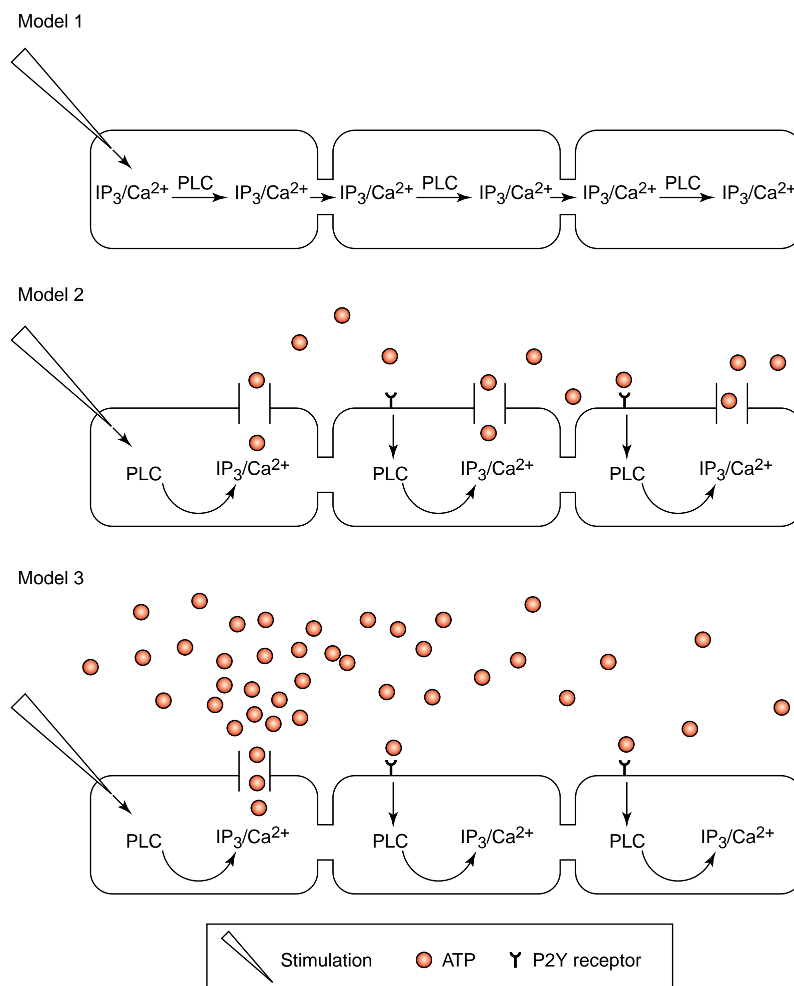

Figure I. Models of the mechanism of astrocytic Ca^{2+} signaling. The mechanism of astrocytic Ca^{2+} signaling has been revised during the past few years; development of the concept is illustrated. Abbreviations: Ca^{2+}/IP_3, Ca^{2+} and/or inositol (1,4,5)-trisphosphate; PLC, phospholipase C.

from that exhibited in exocrine tissues, in which cells are typically arrayed lined in rows along the vasculature. Unlike exocrine tissues, however, another order of structural complexity has been imposed upon the glial syncytium, in that neurons are dispersed among the astrocytic domains, with an almost unfathomable number of fine neurites penetrating each astrocytic domain and being surrounded by its glial processes.

Communication within this multicellular syncytium is rapid and coordinated, despite the electrical inexcitability of the cells. Gap junctions are evenly distributed along the astrocytic processes, often interconnecting adjacent astrocytic processes derived from the same cell. Only in the narrow interface of adjacent cells do gap junctions appear to couple processes from different cells [15]. The function of gap junctions, which interconnect astrocytic processes widely, is to minimize the differences between individual processes by mediating the indiscriminate sharing of all molecules with molecular weights <1000 kDa [16]. The significance of this arrangement is unknown but gap junctions are permeable to glucose and, among other functions, might facilitate the diffusion of energy substrates from the nutritive vessels to metabolically active neurons embedded within the astrocytic matrix. Thin processes of astrocytes are probably are still able to operate in a manner that is independent of their neighbors. Ca^{2+}-imaging experiments have revealed that Bergman glial cells displayed Ca^{2+} increments limited to 'microdomains' in response to stimulation of parallel fibers, and that these local increases in $[Ca^{2+}]$ remain localized with no propensity for spread to surrounding processes or the cell body [17].

Signal propagation among astrocytes

Having learned from neurons by classical electrophysiological methods, these same methods were eagerly applied to astrocytes. The results were somewhat boring. Astrocytes are electrically non-excitable and they respond to current injection with only passive changes in membrane potential. Their resting membrane potential is maintained at ~ -85 mV and displays little fluctuation in response to a wide variety of stimuli [4,18]. This stability and their low input resistance is likely to reflect the fact that gap junctions effectively couple many of these cells into an electrical syncytium. The predominance of K^+ channels over other ion channels in these cells also contributes to their inexcitability [19]. Having determined that astrocytes were electrically non-excitable, they were immediately presumed to have no involvement in signal transmission. Although this view is no longer tenable, it must also be said that we are still struggling to understand the 'rules' of glia–neuron communication.

Cornell-Bell and colleagues reported in 1990 that astrocytes reacted to glutamate with transient increases in cytosolic $[Ca^{2+}]$, followed by inter-astrocytic propagation of a wave of increased $[Ca^{2+}]$ [20]. Further analyses revealed that this process of astrocytic 'Ca^{2+} signaling' can be divided into two separate forms: (i) Ca^{2+} oscillations, defined as repetitive increases in $[Ca^{2+}]$ within single cells, and (ii) Ca^{2+} waves, defined as radially propagating increases in cytosolic $[Ca^{2+}]$ that originate from a single cell and sequentially engage neighboring cells [21]. The primary mechanism underlying both types of signaling is the mobilization of intracellular Ca^{2+} stores. Receptor activation is coupled to a membrane-associated G protein, which stimulates the release of Ca^{2+} from the endoplasmic reticulum via the activation of phospholipase C and consequent production of inositol (1,4,5)-trisphosphate. Although the presence of both L-type and T-type voltage-gated Ca^{2+} channels has been demonstrated *in vitro* and by immunolabeling of brain sections [22], astrocytes *in situ* do not appear to use this mechanism as a pathway for Ca^{2+} entry [23]. Likewise, ion channels gated by glutamate and ACh receptors, which cause Ca^{2+} influx in cultured astrocytes, seem to play at best a minor role in astrocyte Ca^{2+} signaling *in situ* [23,24] – although Bergmann glia do exhibit a response to glutamate that is modified by AMPA or the AMPA-receptor antagonist 6-nitro-7-sulfamoyl-benz(*f*)quinoxaline-2,3-dione (NBQX) [23].

Astrocytic Ca^{2+} signaling was originally described in cultured monolayers, in which Ca^{2+} increments can be propagated from a point source to extend throughout an astrocytic syncytium [25]. Astrocytes in freshly prepared brain slices also support propagation of Ca^{2+} waves [26] (Box 1). Ca^{2+} oscillations of astrocytes *in situ* are triggered by mechanical stimulation or exposure to neurotransmitters, including glutamate, GABA and ATP [27]. Spontaneous Ca^{2+} oscillations in astrocytes *in situ* are seen in both the thalamus [28] and hippocampus [29]. A subpopulation of astrocytes in these two regions displayed spontaneous Ca^{2+} oscillation in the absence of neuronal activity. The oscillations were not blocked by antagonists of metabotropic glutamate receptors or purine receptors, suggesting that astrocytes *in situ* might exhibit intrinsic signaling.

The functional significance of these spontaneous, neuron-independent astrocytic Ca^{2+} signals is unclear. Ca^{2+} oscillations in immature neurons have been associated with neurotransmitter differentiation, axonal growth and establishment of neuronal networks [30,31]. Similarly, different frequencies of Ca^{2+} activity are associated with acute and systematic differences in patterns of gene expression by lymphoid cells [32]. Another important aspect of frequency-coded information is the requirement for specific cellular 'decoders' that can translate Ca^{2+} signaling into a cellular response. The two most studied Ca^{2+}-sensitive proteins that are involved in decoding Ca^{2+} signals are Ca^{2+}/calmodulin-dependent (CaM) kinase and Ca^{2+}/phosphlipid-dependent protein kinase (PKC), although only the latter has been evaluated in astrocytes. Glutamate-induced astrocytic Ca^{2+} oscillations appear to depend on the periodic translocation and activation of PKC which, in turn, responds to the oscillating concentrations of diacylglycerol and Ca^{2+} [33].

Both modalities of astrocytic signaling, Ca^{2+} oscillations and Ca^{2+} waves, are readily transmitted to surrounding neurons, which display prolonged increases in intracellular $[Ca^{2+}]$. The discovery that astrocytes actively signal to each other and surrounding neurons has led to the realization that astrocytes are likely to play a more active role in information processing than previously recognized [34,35]. The key observation that certifies this

position is that astrocyte Ca^{2+} waves can either enhance or diminish nearby neuronal activity. Astrocytic Ca^{2+} signaling has been associated with brief (seconds to minutes) modulation of synaptic strength [4]. Less commonly, activation of Ca^{2+} signaling in hippocampal astrocytes has exerted longer-lasting potentiation of inhibitory synaptic transmission, which was sustained for >20 min beyond the cessation of glial Ca^{2+} signaling [4].

Normal and pathological manifestations of glial Ca^{2+} signaling *in vivo*

Astrocytic Ca^{2+} waves are mediated primarily by release of ATP and activation of purine receptors (Box 1). In cultured astrocytes, Ca^{2+} waves are routinely generated by electrical or mechanical stimulation, but they can also be initiated by exposure to transmitters or by removal of extracellular Ca^{2+}. In addition, recent studies have used confocal imaging to demonstrate that astrocytes *in situ* can propagate long-distance Ca^{2+} waves. However, Ca^{2+} waves in slices can be generated only by intense electrical stimulation (≥ 100 Hz) and waves do not appear to be elicited by neuronal activity or transmitter challenge [26]. Thus, it remains possible that long-distance intercellular Ca^{2+} waves represent a pathophysiological manifestation of astrocytic Ca^{2+} signaling that might not contribute to normal physiological signaling events.

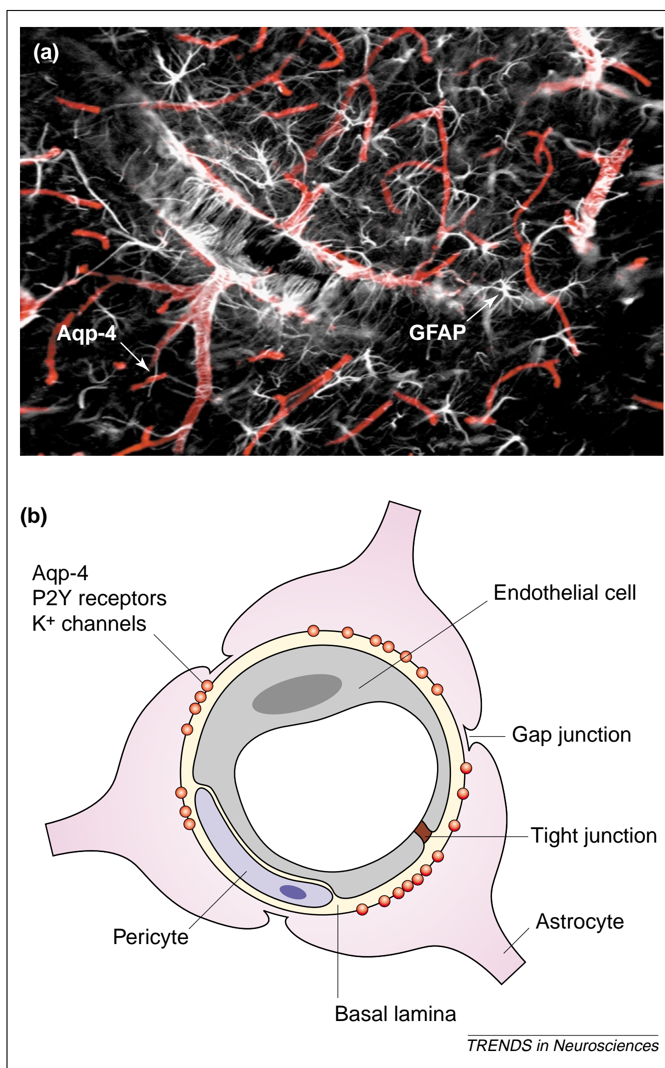

Figure 4. Functional anatomy of the gliovascular interface. Many channels and receptors essential for astrocyte function are densely localized to the vascular endfeet facing the vessel wall. **(a)** Within the brain, the water channel protein aquaporin-4 (Aqp-4; red) is expressed by astrocytes exclusively. Aqp-4 is concentrated at the luminal surfaces of the astrocytic endfeet, which clearly outline the vascular bed to which they adhere. Glial fibrillary acidic protein (GFAP; white) is present in the cell bodies and large processes of astrocytes. GFAP is also expressed by those astrocytic processes that make contact with larger vessels. As a result, vascular endfeet plastered along the walls of larger vessels typically coexpress Aqp-4 and GFAP [14]. By contrast, astrocytic endfeet in contact with capillaries are Aqp-4-positive but only occasionally GFAP-positive. Thus, astrocyte endfeet in contact with capillaries are detectable only by labeling of Aqp-4. **(b)** A schematic cross section of the blood–brain barrier. Receptors (e.g. P2Y) and channels (e.g. K^+ channels and Aqp-4) are concentrated at the luminal surface of the astrocytic endfeet, apposing the perivascular basal lamina.

Indeed, astrocytic Ca^{2+} waves share several properties with the phenomenon of spreading depression, a pathological suppression of cortical neuronal excitability that has been implicated in the propagation of ischemic injury [36]. Pathologically, spreading depression is a generalized response of gray matter to a variety of noxious influences [37], and has been associated not only with secondary injury after stroke and trauma but also with migraine and post-seizure depression. Mechanistically, spreading depression is best described as a slowly moving wave of tissue depolarization; experimentally, it can be evoked either by local electrical stimulation or by application of K^+ or glutamate. Spontaneous waves of spreading depression are generated in the setting of cerebral ischemia and trauma, and it has been estimated that the infarct volume increases by 23% for each wave of spreading depression that is generated [38]. It now appears likely that astrocytic Ca^{2+} waves underlie spreading depression. Ca^{2+} imaging experiments have shown that astrocytic Ca^{2+} waves constitute the leading edge of a propagating spreading depression wave [39,40]. That said, surprisingly little information exists with regard to astrocytic Ca^{2+} signaling in the setting of brain trauma or ischemia. Yet this is a field in marked flux: new imaging techniques, based upon multiphoton imaging of live animals, can now visualize astrocytic Ca^{2+} signaling in response to pathological insults and are expected to increase rapidly our understanding of the extent to which astrocytes contribute to secondary injury.

Transmitter release by astrocytes

One of the principal functions of astrocytes is uptake of neurotransmitters released from nerve terminals [41]. But astrocytes can also release neuroactive agents, including transmitters, eicosanoids, steroids, neuropeptides and growth factors [42]. The regulation and mechanism (or mechanisms) of astrocyte-mediated release of neuroactive compounds are for most agents poorly defined. They are also hotly debated, given their theoretical importance in brain function. Release of glutamate appears to be the primary mechanism by which astrocytes modulate synaptic transmission [4]. Astrocytic glutamate release in response to synaptic activity appears strictly dependent on mobilization of intracellular Ca^{2+} stores and is attenuated, as expected, when intracellular $[Ca^{2+}]$ is stabilized by preloading with either the Ca^{2+} chelator BAPTA or the Ca^{2+}-ATPase inhibitor thapsigargin. Despite intense effort, the pathway of glutamate release

in this context is not understood. One line of work has suggested that glutamate is released by regulated exocytosis [43], whereas others have provided evidence for channel-mediated release of glutamate [44]. The primary arguments for vesicular release are the requirement for increases in cytosolic Ca^{2+} and for sensitivity to tetanus neurotoxin and bafilomycin [45]. The major arguments for channel-mediated release are that several Cl^- channel blockers reversibly inhibit release and that glutamate is not released in isolation but, rather, together with other amino acids present in high concentration in astrocytes, including taurine and aspartate [46]. Recently, unopposed gap junctions, called hemichannels, have been shown to release large amounts of glutamate and other amino acids in response to manipulation of extracellular $[Ca^{2+}]$ [47]. Likewise, activation of the P2X7 purine receptor results in glutamate release from cultured astrocytes [48]. Both of these pathways are insensitive to preloading with BAPTA or thapsigargin, and might thereby not play a role in Ca^{2+}-dependent glutamate release [46,47]. The possibility that reversal of glutamate transport has a significant role in this has been eliminated: glutamate transport inhibitors do not affect Ca^{2+}-dependent astrocytic glutamate release [45].

Interactions among astrocytes, neurons and endothelial cells define the gliovascular unit

The blood–brain barrier is a diffusion barrier that impedes exchange of molecules between the two tissues. The primary seal of the blood–brain barrier is formed by endothelial tight junctions. Astrocytes enwrap the vasculature with a large number of endfeet plastered at the vessel wall (Figure 4), although their role in the blood–brain barrier is poorly defined and they are not believed to have a barrier function in the mammalian brain [49]. Several factors released by astrocytes might be important for the induction and maintenance of the blood–brain barrier, as manifested by the appearance of endothelial tight junctions in cells ensheathed by astrocytic endfeet. Several investigators have studied this issue and have identified agents released by astrocytes, including transforming growth factor α (TGFα) and glial-derived neurotrophic factor (GDNF), that support the formation of tight junctions in cultured endothelial cells [50]. An inherent problem in the field is that the cellular interactions of the immature blood–brain barrier have thus far been studied in 2D culture models lacking the structural complexity of the cerebral microvasculature. As a result, although these studies have shown that astrocytes promote maturation of brain endothelial cells *in vitro*, it has been difficult to determine definitively the role of astrocytes in the initiation and the consolidation of the tight-junction seals of the blood–brain barrier.

The tight organization of astrocytes around the vasculature provides anatomical evidence for the necessity for glucose to enter astrocytes on its way to neurons. Astrocytes might not impede glucose uptake because they express a large number of glucose transporters. A variation on this theme is that astrocytes take up the glucose by transport mechanisms, convert it to lactate, and then deliver lactate to neurons on demand [51,52]. Although there might be proof for this mechanism under severe hypoglycemic conditions necessitating the breakdown of astrocytic glycogen [52,53], it remains controversial in the context of normal brain function [54]. To the extent that this does occur, the limited interdigitation of astrocytic processes suggests that individual astrocytes would be responsible for glucose delivery to neurons within their own territory – in essence, parceling the neuropil into small metabolically independent domains (Figure 3).

New lines of work have shown that receptors and channels essential for the function of astrocytes are densely concentrated at their vascular endfeet. Especially intriguing is the observation that the water channel aquaporin-4 and purine receptors – mediators of astrocytic Ca^{2+} signaling (Box 1) – are expressed primarily at the gliovascular interface [14] (Figure 4). A recent study has shown that astrocytes can convey signals from neurons to the vasculature and that astrocytes thereby might play a central role in functional hyperemia. The model predicts that glutamate released during synaptic transmission, through activation of metabotropic glutamate receptors, triggers astrocytic Ca^{2+} signaling. In turn, astrocytic Ca^{2+} signaling is associated with release of a vasoactive cyclooxygenase product resulting in arteriolar dilation and thereby in activity dependent increases in local blood flow [55,56].

Concluding remarks

Ideas about glial function originally sprang from the anatomy of these cells. Modern researchers have gained knowledge about glial cells by studying their physiology and biochemistry in isolation from their normal cellular partners. This was necessary to avoid the confounding complexity of the intact CNS. The risk, however, is that we lose sight of the *in vivo* anatomical relationships of these cells, which define what their sphere of influence can be. Current evidence indicates that anatomy and function converge in practical ways around the concept of a gliovascular unit. In fact, it is appealing to think of this as a basic unit of functional organization, with great utility in moving forward from our current state of understanding. It also highlights the important limits of proceeding to study astrocytes in the absence of their natural partners.

Acknowledgements

We thank Marisa Cotrina and Greg Arcuino for discussions, and Marie Simard, Takahiro Takano and Xiaohai Wang for the data illustrated in Figures 1–4. Our studies are supported by NINDS/NIH, the Brain Tumor Association, and New York State Spinal Cord Injury Research Program.

References

1 Virchow, R. (1846) Uber das granulierte Anschen der Waudungen der Gerhirnventrikel. *Allg. Z. Psychiatr.* 3, 242
2 Sulston, J.E. *et al.* (1983) The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100, 64–119
3 Bass, N.H. *et al.* (1971) Quantitative cytoarchitectonic distribution of neurons, glia, and DNA in rat cerebral cortex. *J. Comp. Neurol.* 143, 481–490
4 Kang, J. *et al.* (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat. Neurosci.* 1, 683–692
5 Haydon, P.G. (2001) Glia: listening and talking to the synapse. *Nat. Rev. Neurosci.* 2, 185–193
6 Newman, E.A. New roles for astrocytes: regulation of synaptic function. *Trends Neurosci.* (in press)

7 Pfrieger, F.W. and Barres, B.A. (1997) Synaptic efficacy enhanced by glial cells *in vitro*. *Science* 277, 1684–1687
8 Ullian, E.M. *et al.* (2001) Control of synapse number by glia. *Science* 291, 657–661
9 Song, H. *et al.* (2002) Astroglia induce neurogenesis from adult neural stem cells. *Nature* 417, 39–44
10 Horner, P. and Palmer, T. New roles for astrocytes: La vida loca! The nightlife of an astrocyte. *Trends Neurosci.* (in press)
11 Bushong, E.A. *et al.* (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* 22, 183–192
12 Ogata, K. and Kosaka, T. (2002) Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* 113, 221–233
13 Chan-Ling, T. and Stone, J. (1991) Factors determining the shape, spacing and distribution of astrocytes in the cat retina: The contact-spacing model of astrocyte interaction. *J. Comp. Neurol.* 303, 387–399
14 Simard, M. *et al.* Signaling at the gliovascular interface. *J. Neurosci.* (in press)
15 Rohlmann, A. and Wolff, J. (1996) *Subcellular Topography and Plasticity of Gap Junction Distribution on Astrocytes*, R.G. Landes Company
16 Rose, C. and Ransom, B.R. (1997) Gap junctions equalize intracellular Na^+ concentrations in astrocytes. *Glia* 20, 299–307
17 Grosche, J. *et al.* (1999) Microdomains for neuron–glia interaction: parallel fiber signaling to Bergmann glial cells. *Nat. Neurosci.* 2, 139–143
18 Kang, J. and Nedergaard, M. (1999) *Imaging Astrocytes in Acute Brain Slices*, Cold Spring Harbor Laboratory Press
19 Ransom, B.R. and Sontheimer, H. (1992) The neurophysiology of glial cells. *J. Clin. Neurophysiol.* 9, 224–251
20 Cornell-Bell, A.H. *et al.* (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247, 470–473
21 Berridge, M.J. *et al.* (1998) Calcium – a life and death signal. *Nature* 395, 645–648
22 Westenbroek, R.E. *et al.* (1998) Upregulation of L-type Ca^{2+} channels in reactive astrocytes after brain injury, hypomyelination, and ischemia. *J. Neurosci.* 18, 2321–2334
23 Verkhratsky, A. *et al.* (1998) Glial calcium: homeostasis and signaling function. *Physiol. Rev.* 78, 99–141
24 Bergles, D.E. and Jahr, C.E. (1998) Glial contribution to glutamate uptake at Schaffer collateral–commissural synapses in the hippocampus. *J. Neurosci.* 18, 7709–7716
25 Cornell-Bell, A.H. *et al.* (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247, 470–473
26 Schipke, C.G. *et al.* (2002) Astrocyte Ca^{2+} waves trigger responses in microglial cells in brain slices. *FASEB J.* 16, 255–257
27 Dani, J.W. and Smith, S.J. (1995) The triggering of astrocytic calcium waves by NMDA-induced neuronal activation. *Ciba Found. Symp.* 188, 195–205
28 Parri, H.R. *et al.* (2001) Spontaneous astrocytic Ca^{2+} oscillations *in situ* drive NMDAR-mediated neuronal excitation. *Nat. Neurosci.* 4, 803–812
29 Nett, W.J. *et al.* (2002) Hippocampal astrocytes *in situ* exhibit calcium oscillations that occur independent of neuronal activity. *J. Neurophysiol.* 87, 528–537
30 Yuste, R. *et al.* (1992) Neuronal domains in developing neocortex. *Science* 257, 665–669
31 Gu, X. and Spitzer, N.C. (1995) Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients. *Nature* 375, 784–787
32 Li, W. *et al.* (1998) Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* 392, 936–941
33 Codazzi, F. *et al.* (2001) Control of astrocyte Ca^{2+} oscillations and waves by oscillating translocation and activation of protein kinase C. *Curr. Biol.* 11, 1089–1097
34 Nedergaard, M. (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263, 1768–1771
35 Parpura, V. *et al.* (1994) Glutamate-mediated astrocyte-neuron signalling. *Nature* 369, 744–747
36 Nedergaard, M. (1996) Spreading depression as a contributor to ischemic brain damage. *Adv. Neurol.* 71, 75–83
37 Hansen, A.J. and Nedergaard, M. (1988) Brain ion homeostasis in cerebral ischemia. *Neurochem. Pathol.* 9, 195–209
38 Mies, G. *et al.* (1993) Correlation between peri-infarct DC shifts and ischaemic neuronal damage in rat. *NeuroReport* 4, 709–711
39 Basarsky, T.A. *et al.* (1998) Imaging spreading depression and associated intracellular calcium waves in brain slices. *J. Neurosci.* 18, 7189–7199
40 Kunkler, P.E. and Kraig, R.P. (1998) Calcium waves precede electrophysiological changes of spreading depression in hippocampal organ cultures. *J. Neurosci.* 18, 3416–3425
41 Anderson, C.M. and Swanson, R.A. (2000) Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 32, 1–14
42 Martin, D.L. (1992) Synthesis and release of neuroactive substances by glial cells. *Glia* 5, 81–94
43 Araque, A. *et al.* (2000) SNARE protein-dependent glutamate release from astrocytes. *J. Neurosci.* 20, 666–673
44 Jeremic, A. *et al.* (2001) ATP stimulates calcium-dependent glutamate release from cultured astrocytes. *J. Neurochem.* 77, 664–675
45 Bezzi, P. *et al.* (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 391, 281–285
46 Nedergaard, M. *et al.* (2002) Beyond the role of glutamate as a neurotransmitter. *Nat. Rev. Neurosci.* 3, 748–755
47 Ye, Z.C. *et al.* (2003) Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J. Neurosci.* 23, 3588–3596
48 Duan, S. *et al.* (2003) P2X7 receptor-mediated release of excitatory amino acids from astrocytes. *J. Neurosci.* 23, 1320–1328
49 del Zoppo, G.J. and Hallenbeck, J.M. (2000) Advances in the vascular pathophysiology of ischemic stroke. *Thromb. Res.* 98, 73–81
50 Abbott, N.J. (2002) Astrocyte–endothelial interactions and blood–brain barrier permeability. *J. Anat.* 200, 629–638
51 Tsacopoulos, M. and Magistretti, P.J. (1996) Metabolic coupling between glia and neurons. *J. Neurosci.* 16, 877–885
52 Wender, R. *et al.* (2000) Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J. Neurosci.* 20, 6804–6810
53 Hertz, L. and Dienel, G.A. (2002) Energy metabolism in the brain. *Int. Rev. Neurobiol.* 51, 1–102
54 Chih, C.P. *et al.* (2001) Do active cerebral neurons really use lactate rather than glucose? *Trends Neurosci.* 24, 573–578
55 Zonta, M. *et al.* (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* 6, 43–50
56 Anderson, C.M. and Nedergaard, M. (2003) Astrocyte-mediated control of cerebral microcirculation. *Trends Neurosci.* 26, 340–344
57 Pfrieger, F. New roles for astrocytes: regulation of synaptogenesis. Trends Neurosci. (in press)
58 Charles, A.C. *et al.* (1992) Intercellular calcium signaling via gap junctions in glioma cells. *J. Cell Biol.* 118, 195–201
59 Hassinger, T.D. *et al.* (1996) An extracellular signaling component in propagation of astrocytic calcium waves. *Proc. Natl. Acad. Sci. U. S. A.* 93, 13268–13273
60 Arcuino, G. *et al.* (2002) Intercellular calcium signaling mediated by point-source burst release of ATP. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9840–9845
61 Burnstock, G. (2002) Potential therapeutic targets in the rapidly expanding field of purinergic signalling. *Clin. Med.* 2, 45–53
62 Franke, H. *et al.* (2001) P2X receptor expression on astrocytes in the nucleus accumbens of rats. *Neuroscience* 108, 421–429
63 John, G.R. *et al.* (2001) Extracellular nucleotides differentially regulate interleukin-1β signaling in primary human astrocytes: implications for inflammatory gene expression. *J. Neurosci.* 21, 4134–4142
64 Moran-Jimenez, M.J. and Matute, C. (2000) Immunohistochemical localization of the P2Y(1) purinergic receptor in neurons and glial cells of the central nervous system. *Brain Res. Mol. Brain Res.* 78, 50–58
65 Zhu, Y. and Kimelberg, H.K. (2001) Developmental expression of metabotropic P2Y(1) and P2Y(2) receptors in freshly isolated astrocytes from rat hippocampus. *J. Neurochem.* 77, 530–541
66 Cotrina, M.L. *et al.* (1998) Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15735–15740
67 Cotrina, M.L. *et al.* (2000) ATP-mediated glia signaling. *J. Neurosci.* 20, 2835–2844
68 Bennett, M.V. New roles for astrocytes: release of neuroactive substances by Cx-hemichannels. *Trends Neurosci.* (in press)