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Supportive or information-processing functions of the mature protoplasmic astrocyte in the mammalian CNS? A critical appraisal

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Abstract

It has been proposed that astrocytes should no longer be viewed purely as support cells for neurons, such as providing a constant environment and metabolic substrates, but that they should also be viewed as being involved in affecting synaptic activity in an active way and, therefore, an integral part of the information-processing properties of the brain. This essay discusses the possible differences between a support and an instructive role, and concludes that any distinction has to be blurred. In view of this, and a brief overview of the nature of the data, the new evidence seems insufficient to conclude that the physiological roles of mature astrocytes go beyond a general support role. I propose a model of mature protoplasmic astrocyte function that is drawn from the most recent data on their structure, the domain concept and their syncytial characteristics, of an independent rather than integrative functioning of the ends of each process where the activities that affect synaptic activity and blood vessel diameter will be concentrated.

Keywords

Homeostasis; information processing; voltage clamp; support roles; vesicular release

Arguably the major problem for astrocyte research is to find a guiding function for these cells. This seems surprising considering that, as the neuroglia, their distinctive morphologies have been recognized for over 100 years (Kettenmann and Ransom, 2005). Neurons were also first accurately described at the same time using the same technique of Golgi's *reazione nera* by microscopists, such as the fabled Ramón y Cajal. However, although the general function of neurons is agreed on; namely that they underlie all the unique functions of the CNS based on neuronal excitability and organization of neurons into circuits, agreement on the functions of astrocytes remains clouded. This difference gives every study on neurons some type of hypothesis (i.e. question-to-be-answered), in terms of how a process influences synaptic transmission, neuronal excitability and its potentiation or inhibition, but presents a problem for focusing studies on astrocytes. Neurons were first found to be excitable based on pioneering electrophysiological work in 1902, although elucidation of the true nature of the action potential was determined during the period 1939–1955 (Katz, 1966). Starting in the 1960s sharp electrode recordings showed that the neuroglia are non-excitable (Nicholls *et al.*, 2000).

Application of modern techniques has expanded on the early microscopic studies by showing more clearly the extent of the complex morphology of mature mammalian protoplasmic astrocytes; namely a small cell body with 1000–10 000 processes that connect at their ends with synapses and blood vessels, and communicate with the process of other astrocytes through

gap junctions. Although this morphology is consistent with the role of maintaining the optimal functioning of the neuronal arrays by stabilizing the ECS (i.e. a support role), it has also been argued that the astrocytes might have an ancillary, instructive role in information-processing in the mammalian CNS and that the syncytium provides another information-processing pathway (Todd *et al.*, 2006). The purpose of this essay is to critically present these two general viewpoints with the conclusion that the majority of the data to date is most consistent with a support role.

Like all scientists, I have to assume that the results published in peer-reviewed journals are correct as far as they go, but that differences with the authors in interpretation are quite admissible. Why, you might ask, since the interpretations were also presumably reviewed, are these to be questioned. Well, interpretations are never exclusionary and the data might fit into other schemes of what the cells do. Also, further data can always make a current interpretation no longer valid because of new data or newer, more correct ways of looking at the same data. How one distinguishes the 'best' interpretation is a judgment based on consistency and reasonableness. The only formal principle that is sometimes expressed is that of 'Ockham's razor', which can be stated as 'all other things being equal the simplest explanation is to be favored' (Russell, 1989). This seems equivalent to saying that interpretations are often a best guess based on the evidence.

I recently wrote an article entitled 'The problem of astrocyte identity' (Kimelberg, 2004a) because the definition of what constitutes a mature astrocyte is also generally recognized as a major problem. In that article and the present one we are actually dealing with the same problem because understanding the functions of mature astrocytes will imply a classification. Here, I discuss only the mature protoplasmic astrocyte in gray matter (Fig. 1) and its physiological properties. Whether this belongs to a larger class of astrocytes and how they might differ within the class requires further study and discussion.

Evidence in support of supportive roles for the mature mammalian astrocyte

I now briefly summarize what I consider to be the properties that support the role of astrocytes as support cells.

1. Very dominant K^+ conductance that gives a negative resting membrane potential that is very close to the K^+ equilibrium potential and a linear I-V relationship. This, together with little or no anion conductance, led to a proposed role in the control of increased $[K^+]_o$ by a current loop that was carried into and within the cell by K^+ with the release of K^+ distal to the region of increased of ECS $[K^+]$ (Orkand *et al.*, 1966). An exclusive K^+ conductance also maintains the negative membrane potential with minimal energy expenditure.
2. pH control by different transporters leading to control of ECS $[H^+]$ (Bourke *et al.*, 1978; Kimelberg *et al.*, 1979; Chesler and Kraig, 1987; Newman, 1999; Deitmer and Schneider, 2000).
3. Transmitter uptake and metabolism (especially glutamate). Inactivation of glutamate by conversion to glutamine by the astrocyte-specific, intracellular enzyme glutamine synthetase, which consumes ATP and ammonia (Berl *et al.*, 1961; Martinez-Hernandez *et al.*, 1977).
4. Water transport and aquaporins (AQPs), particularly AQP4 located perivascularly (Amiry-Moghaddam and Ottersen, 2003). This seems to assume water transport is rate-limiting relative to the fast transport of substrates such as glucose and K^+ , and is, presumably, needed to avoid increases in osmotic pressure inside the cell limiting the further transport of these substances (Kimelberg, 2004b). The processes described

in (2)–(4) above can lead to swelling, and it has been proposed that astrocytes have volume-regulatory anion channels that are activated by such swelling to allow the efflux of Cl^- with K^+ efflux via the always-open K^+ channels, to regulate volume (Mongin and Kimelberg, 2005). It has been hypothesized that, because such channels are also permeable to glutamate and that astrocytes swell markedly in pathological states, efflux of glutamate from astrocytes adds to the excitotoxic glutamate burden (Kimelberg, 2005).

5. Regulation of blood flow. Astrocytic end-feet exclusively surround all blood vessels in the CNS, including arterioles. They might, thus, act as transducers of increased neuronal activity, and increased Ca^{2+} in these end-feet correlates with either vasoconstriction or vasodilatation *in vivo* (Zonta *et al.*, 2002; Mulligan and MacVicar, 2004; Takano *et al.*, 2005; Iadecola and Nedergaard, 2007). Vasodilatation is caused by the production of prostenoids such as prostaglandin E_2 (PGE_2) by astrocytes (Takano *et al.*, 2006). I interpret these effects on arteriolar diameter as a permissive/supportive role that provides neurons with adequate substrates for their increased energy metabolism.
6. Other supportive biochemical properties. Ammonia homeostasis, which is involved in glutamine synthesis has been mentioned in regard to (3). Astrocytes are a major site of antioxidants such as glutathione in the CNS (Aschner, 2000), and they supply substrate to neurons in the form of lactate as described in the lactate shuttle hypothesis (Magistretti *et al.*, 1999).

Evidence in support of an instructional, information-processing role

In the past 15 years several authors have proposed that astrocytes might have a direct role in information-processing (Araque *et al.*, 1999; Haydon, 2001; Volterra and Meldolesi, 2005; Perea and Araque, 2005a; Perea and Araque, 2006; Deitmer *et al.*, 2006). Although the experiments reported in these papers are elegant, it is my view that the most reasonable and simplest conclusion from what we know about astrocytes is that they perform a supportive role. It is not impossible that astrocytes have an active role in information-processing but, as the well-regarded physicist Richard Feynman pointed out, the role of science is to determine what is less or more likely and not what is possible or impossible (Feynman, 1965).

Astrocytic syncytium

The linking of mature astrocytes into a syncytium that consists of up to hundreds of cells is a distinctive feature of mature astrocytes that was seen first *in situ* using the electron microscope to identify gap junctions (Massa and Mugnaini, 1982), and then by transfer of electrode-injected dyes (e.g. Fig. 2). This has recently been proposed to form an astrocyte-based information processing system that is additional to neuronal circuits and/or to integrate separated neuronal circuits (Pascual *et al.*, 2005; Perea and Araque, 2005a; Volterra and Meldolesi, 2005; Todd *et al.*, 2006), based mainly on the Ca^{2+} increases and flow through the cell to the processes and then to neighboring gap-junction-linked other astrocytes. A proposed function of this Ca^{2+} increase in releasing neuroactive agents at the ends of the processes is discussed later.

However, there is an alternative, simpler interpretation. That the function of the syncytium is to provide a large reservoir that buffers substances such as K^+ and H^+ , which are taken up at particular sites where they locally increase because of increased neuronal activity. This was proposed initially for K^+ spatial buffering (Kuffler and Potter, 1964; Kuffler *et al.*, 1966). This intracellular K^+ current-loop mechanism is based on K^+ -channel activity and no detectable Cl^- conductances (Orkand *et al.*, 1966).

Several either electrically neutral or electrogenic K^+ or H^+ transporters were identified originally in primary astrocyte cultures (Kimelberg *et al.*, 1979) and then in astrocytes *in situ* (Deitmer and Schneider, 2000; Chesler, 2003). However, these transporters are essentially ubiquitous, so this is not definitive for astrocytes. It was hypothesized that they would also buffer K^+ (such as the $Na^+K^+2Cl^-$ co-transporter) and H^+ (via Na^+/H^+ and HCO_3^- transporting systems) (Kimelberg *et al.*, 1979; Chesler, 2003). Uptake by either transporters or coupled cation plus anion uptake on separate channels might then be followed by diffusion and dilution within the syncytium.

Electrophysiological consequences of the syncytium

The syncytium might also be responsible for the diagnostic very low membrane (input) resistances of mature astrocytes with a fixed density of K^+ channels because resistance is inversely proportional to area. The reversal potential in I–V plots, which is equivalent to the resting membrane potential, shows a close to K^+ selective conductance. This can be explained by the K^+ -selective R_m being in series with the access resistance of the electrode tip (R_a) (Fig. 3A). The low resistance creates serious technical problems when using the most common technique for measuring the electrophysiological properties of small-cell soma, the continuous single electrode voltage clamp (cSEVC) because R_a can be as great as or greater than R_m (Sontheimer, 1995). Thus, a major drop of the voltage command (say 50–80%) will be across R_a , which contributes to a linear I–V curve instead of an outwardly rectifying one as expected from the Goldman-Huxley-Katz (GHK) constant field equations for purely ohmic channels (Hille, 1992) with the physiological K^+ gradient as set up by the experimenter, as R_a is non-selective for ions. However, the relative contribution made by channels and/or the large increase in area due to the syncytium to the very low R_m resistance is unclear.

The latest pCLAMP 9 program used by a number of investigators for cSEVC has a membrane test feature that gives R_a from tau estimated for the small decline of the initial capacity transient corrected for the rapidly developing current flow from the amplifier through R_a+R_m (Fig. 3B). The total charging current not attributable to resistive current flow is shown as Q_t . Q_t gives the total capacitance from $C_m = Q_t/V_c$, and R_a can be obtained from the standard relation $\tau = R_a.C$. Finally, R_m can be obtained by subtraction from the total measured R_t , which is $R_m + R_a$. However, the values given by the program are of uncertain precision because of difficulties in accurately assessing tau, so we are left only with approximations.

With these caveats in mind, when we studied glial cells in hippocampal slices by cSEVC we got three different forms of I–V relations (Fig. 4). Other investigators have reported similar results (Steinhauser *et al.*, 1992; D'Ambrosio *et al.*, 1998). In a systematic developmental study we found progressive changes in the expression of these electrophysiological phenotypes, with the cells that give linear I–V curves representing ~90% of the recorded glia after 21 days (Zhou *et al.*, 2006). Such linear (passive) I–V curves are now being used as diagnostic of mature astrocytes before studying other aspects (D'Ascenzo *et al.*, 2007; Perea and Araque, 2007; Jourdain *et al.*, 2007). Post-recording staining of smaller representative groups showed that these cells were all GLAST(+) astrocytes, whereas the two nonlinear phenotypes shown in Fig. 4 represented a mixture of GLAST(+) and NG2(+) cells whose relative proportions varied at different ages (see Zhou *et al.*, 2006).

Vesicular transmitter release at synapses

An instructional role for astrocytes has been proposed, based on evidence indicating that the release of either glutamate or ATP from the ends of astrocytic processes at synapses occurs through exocytotic release from astrocytes and modulates synaptic activity. The original observations, which were interpreted as vesicular release, were made with primary astrocyte cultures (Parpura *et al.*, 1994; Araque *et al.*, 2000; Pasti *et al.*, 2001; Montana *et al.*, 2004).

Later work presented evidence for vesicular release from astrocytes *in situ* (Pasti *et al.*, 2001; Bezzi *et al.*, 2004; Zhang *et al.*, 2004; Jourdain *et al.*, 2007; Perea and Araque, 2007). Increases in intracellular Ca^{2+} in astrocytes in response to neuronal stimulation have several effects on synaptic activity (Perea and Araque, 2007), and increased sensory input also results in increased intracellular Ca^{2+} (Perea and Araque, 2005b; Wang *et al.*, 2006). The data to date provides convincing evidence that vesicular release of glutamate can be obtained under some conditions, leading to increased EPSC frequency in granule cell neurons in the dentate of the rat hippocampus (Jourdain *et al.*, 2007) and induction of a slow inward current (SIC) that increases action potential firing in the target neuron in the nucleus accumbens (D'Ascenzo *et al.*, 2007). The SIC has been attributed to glutamate acting on NR2B subunit-containing extrasynaptic NMDA receptors (Fellin *et al.*, 2004; D'Ascenzo *et al.*, 2007).

Recently, a genetic engineering approach has showed that inhibition of SNARE protein action that is considered targeted specifically to astrocytes via the GFAP promoter, alters long-term potentiation (LTP) (Pascual *et al.*, 2005). Because astrocytes and neurons have many Ca^{2+} -linked receptors in common, the other approach to ensure specific stimulation of Ca^{2+} responses in astrocytes is to uncage photosensitive Ca^{2+} complexes that have been injected into single astrocytes.

To prove this premise, however, studies must establish that the impaired fusion event only involves vesicles that release effective amounts of transmitters from astrocytes and not some other fusion-related event such as insertion of channels or other components into membranes that also leads to the release of transmitters or other substances that affect neurons. It must also be established that these vesicle fusion events occur physiologically to affect behavior. Vesicle density appears to be ten times less in astrocytes compared to presynaptic neuronal terminals (Jourdain *et al.*, 2007), so the magnitude of the response is likely to be much less. Also Fiacco *et al.* (2007) produced transgenic mice that express a Gq-linked receptor only in astrocytes. Activation of this receptor was assumed to produce a more physiological increase in astrocytic Ca^{2+} . This approach failed to produce SICs in neurons in the hippocampus, leading to the idea that changes seen using other means of increasing astrocytic Ca^{2+} are of a pathological rather than physiological nature. Also some of the astrocytic fusion events appear to involve lysosomes (Jaiswal *et al.*, 2007; Zhang *et al.*, 2007), which is unlikely to be physiological. The question also arises of what it means if there is *bona fide* vesicular release from astrocytes. By itself, this does not unambiguously indicate independent control of synaptic activity and a controlling role in information processing.

Are there widespread consequences of alterations in astrocyte support functions?

It is immensely difficult to distinguish between an essential supportive and a discretionary instructional role for astrocytes. For example, inhibiting a crucial SNARE fusion protein using genetic engineering has been shown to be associated with a change in the accepted information-processing linked property, LTP (Pascual *et al.*, 2005). One really cannot say, however, that the alteration in activity of the fusion protein in the astrocyte directly caused the change in LTP. Even assuming a direct effect, how can we distinguish between astrocytes that affect the circuits involved in LTP in a manner that initiates LTP, or that allows neuronal circuits to generate LTP behavior? In the latter case involvement of astrocytes would seem to be permissive. This is a difficult distinction to make and, in the latter case, I would take this to mean that the astrocyte allows the process to take place but does not initiate it. The current assumption seems to be that an effect of altering fusion proteins in astrocytes on neuron-based electrophysiological activities does indicate modulation of synaptic activity in an initiating manner, but this does not seem to follow. If it did then, logically, the same reasoning would apply to changes in, for example, H^+ -transporting systems such as $\text{Cl}^-/\text{HCO}_3^-$ and Na^+/H^+

exchangers that are involved in maintaining $[H^+]_o$ in the face of changes caused by neuronal activity (Newman, 1999; Chesler, 2003). Would we conclude from such observations that astrocytes have an instructional role in information processing via changes in $[H^+]_o$? Alternatively, astrocytes might release transmitter as a response to changed synaptic activity in order to produce a homeostatic response in the neuron and avoid some excessive change that might prevent the synapse doing what it had been instructed to do as part of a neuronal circuit.

The key point from the studies referred to above is that, when a transmitter is released from astrocytic processes by Ca^{2+} -dependent fusion of vesicles that store the transmitter, it indicates crucial control of synaptic events that is equivalent to the control of information flow. However, we then add to the unknown complexity of neuronal information-processing a need to understand what controls the astrocyte control, and so on.

There is also much evidence for alternative pathways of transmitter release from astrocytes, with the necessary caveat of the experimental astrocyte model in which the tests (experiments) are done. Primary cultures of astrocytes, which provide a seductive experimental simplicity, are not reliable indicators of the properties of astrocytes and how they behave *in situ* (Kimelberg, 1995; Kimelberg *et al.*, 1997; Kimelberg, 2001; Lovatt *et al.*, 2007; Cahoy *et al.*, 2008). These alternative paths are (1) release by reversal of excitatory amino acid (EAA) transporters, which is only likely to occur under pathological conditions, (2) swelling activated anion channels and also hemi-channels, and (3) P2X receptors for ATP. These routes, and others, are described in Deitmer *et al.* (2006). Clearly, these different pathways will have to be subject to the same criteria for establishing their functional relevance and, if astrocytes modulate synaptic activity in the normal course of events by transmitter release, why should these other routes not be involved to some degree? It would also seem that there should only be one main route, otherwise one might either expect a lot of noise in astrocytic control of synaptic activity or another level of regulation must be invoked, with no end in sight.

A general support role for astrocytes

What would a general support role for astrocytes look like? Bushong *et al.* (2002) emphasized in their combined immunohistochemical and dye-filling studies in the CA1 region of 1-month-old Sprague-Dawley rats, that the dye-filled astrocytes were morphologically homogenous and their highly bushy processes (1000–10 000 per cell body) occupied separate territories of $\sim 70\,000\,\mu m^3$. This is an important concept. Note they cut 100- μm sections from brains of paraformaldehyde-perfused rats, presumably to avoid rapid, post-mortem, prefixation changes, so they did not see cell–cell coupling. In a later study (Bushong *et al.*, 2004) the same group showed that these features develop with age and resemble the mature form at $\geq$ postnatal day 21 (PN21). This is after the glutamate synaptic system matures ($\sim$ PN14) and corresponds with the dominance of the purely electrophysiologically passive astrocyte in the CA1 (Fig. 5). Thus, their data are consistent with our finding that the electrophysiological diversity of astrocytes is a feature of the developing hippocampus, whereas in the mature brain a single astrocytic electrophysiological phenotype, characterized by a linear I–V relationship, is a characteristic of astrocyte maturation, be it the maturation of the syncytium or K^+ channels, or something else.

The large number of processes of mature astrocytes, that the processes of separate astrocytes overlap by only $\sim 10\%$, and that gap junctional, electrophysiological, synaptic and domain maturity all occur at around the same time in the hippocampus (Bushong *et al.*, 2002; Bushong *et al.*, 2004; Zhou *et al.*, 2006) can be incorporated into a general hypothesis of astrocyte function in the mature brain, or at least in the hippocampus (Fig. 6). This is that the vascular and synaptic end-feet function as autonomous units that respond to specific events at these loci

as independent entities driven by local feedback signals. A proposal for independent domains for stimulation-induced Ca^{2+} increases was proposed a few years ago, and supported experimentally for the Bergmann glia lamellae and filipodia that enwrap the synapses that the Purkinje dendritic spines make with the parallel fibers (Grosche *et al.*, 1999). In terms of either synchronizing or otherwise integrating neuronal activity, there has been no demonstration of any polarity in regard to these populous processes, so it would require a signal from one or several synapses to be transmitted randomly to all other synapses touched by processes of the same cell, limited by either the rate of diffusion of an intracellular messenger or the electrotonic decay of a membrane potential depolarization. However, it has also been suggested that each astrocytic domain integrates all the synaptic activity within it and integrates them with vascular activity (Nedergaard *et al.*, 2003). Tests to distinguish local autonomous control versus a more global integrative role are, unfortunately, difficult and as Galileo wrote in the Dialogue Concerning the Two Chief World Systems ‘where the senses fail us, reason must step in’.

The more limited support model of the mature astrocyte is well-grounded in cell and biological principles. Basic cell theory states that the autonomous ends of the many processes cannot exist independently of the cell nucleus, which is needed to replace proteins and synthesize and export to the processes either more of the same proteins or new ones induced by a change in conditions. Such transport needs a transporting system to carry proteins and remove them for degradation in soma lysosomes, or partly they might be degraded in lysosomes present in the process ends. Because the ends of the perisynaptic processes (fine lamellae and filipodia that arise from the processes emanating from the astrocytic cell body) are too small to contain mitochondria, as emphasized by (Hertz *et al.*, 2007), some of the ATP they need will diffuse from mitochondria in the soma and larger diameter processes that are more proximal to the soma. Otherwise the energy requirements at the process ends would have to be met by glycolysis, which is much less efficient. However, a recent paper in which GFAP promoter-driven GFP was used to identify small astrocyte perisynaptic end-feet found that the number of mitochondria in the end-feet was either as great or greater than other profiles in the surrounding neuropil (Lovatt *et al.*, 2007). However, Chao *et al.* (2002) using electron microscopy and S100-immunoreactivity to identify the processes have noted previously that the fine lamellae and filipodia are devoid of organelles, unlike the astrocytic cell body and processes. Therefore it remains an open question whether end-feet supply sufficient energy by oxidative metabolism to meet their needs, which would diminish the amount of lactate available for export to neurons, as envisaged in the neuronal–astroglial metabolic compartmentation theory of Magistretti and Pellerin (1999).

Another general biological principle is economy of space. The complex morphology of mature astrocytes, with their massive arborizations of processes, might have evolved, not because they participate actively in information-processing, but for the more prosaic task of conserving space in the fixed volume that complex brains occupy while still being able to carry out their supportive functions. 1000 to 10 000 synaptic targets and blood vessels can be served by one cell soma (Fig. 6), thus, conserving space, which is at a premium in mammalian brains. This constraint has also, for example, led to myelination to reduce axonal diameters to conserve space for a given action potential velocity (Katz, 1966; Hille, 1992; Chao *et al.*, 2002). Thus, the domain concept for populous, process-bearing mature astrocytes is consistent with autonomous homeostatic end-feet functions rather than integrative activity for neuronal networks.

The next question is to determine the nature and location of different transporters and channels in the membranes so that we can better infer the precise ‘support’ function. Numerous techniques would then need to be applied to test these inferences (hypotheses) of function, according to the criteria of the scientific method.

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Fig. 1. Astrocytes at PND 21 (A–C) and at 1 month (D–F) from CA1
Images are maximum projections of dye-filled astrocytes in optical slices through 1 μm of tissue. Scale bars, 10 μm . Reproduced, with permission, from Bushong *et al.* (2004).

Fig. 2. Cells stained for GLAST

Cells filled with biocytin through a patch pipette were immunohistochemically stained for GLAST and images encompassing the entire z dimension of the syncytium were acquired by confocal microscopy. (A) An example of a highly coupled GLAST(+), passive, recorded cell. This is a projection image acquired along the entire z axis of the biocytin-Cy2-Streptavidin channel. (B,C) The smaller panels on the right show portions of single z planes for Biocytin (B) and anti-GLAST (C) from the left image. Arrows point to the recorded and injected cell body. From a P7 rat. Scale bar, 20 μ m. Reproduced, with permission, from Schools *et al.* (2006).

Fig. 3. Membrane tests

(A) Circuit and (B) pCLAMP9 membrane test. R_a = access resistance of the pipette, R_m = effective membrane resistance and C_m the effective capacitance. Effective means what is measured by the electrode. V_c is the command potential. V_a is the voltage drop across R_a and V_m the voltage drop across R_m . Adapted from pCLAMP 9, 2003 Axon Instruments, Inc. See text for further details.

Fig. 4. Whole cell recordings from cells *in situ*

(a–c) Whole cell recordings in response to the voltage steps shown in d for the three types of electrophysiological phenotypes seen for cells *in situ*. The insert (a) shows Na^+ currents. Reproduced, with permission, from Zhou *et al.* (2006).

Fig. 5. Electrophysiological phenotypes of glial cells

The percentage of glial cells, as first recognized by their morphology in differential interference contrast optics, in a living hippocampal slice that correspond to the three electrophysiological phenotypes shown in Fig. 4. The numbers on the x axis show the postnatal age or age range and n = number of cells recorded in newborn, juvenile and adult stages. Reproduced, with permission, from Zhou *et al.* (2006).

Fig. 6. Schematic of astrocyte processes interacting with synapses and one blood vessel (red circle) The black circle in the center represents an astrocyte cell body whose processes extend to many synapses ($\leq 10\,000$). Shown are six synapses in two parallel neuronal circuits. Dashed black lines show members of part of the extensive astrocyte process domain in other planes. The postulated information pathway between astrocyte processes and some of the synapses of the same circuit, or between parallel synapses, is shown as a dashed red line. Astrocytes do not fire action potentials, so the signal is viewed as a spread of either intracellular Ca^{2+} or another intracellular messenger or messenger system. It is unlikely to be an electrotonic spread of a local depolarization based on calculations of the length constant of astrocytes *in situ* (Trachtenberg and Pollen, 1970). It is proposed that the perisynaptic astrocyte processes serve to maintain the local environment around the synapses for optimal functioning by localizing key proteins involved in uptake of, for example, glutamate and H^+ and channels or transporters for K^+ clearance released at the active synapse, and the activity of these fluxes is controlled by local feedback loops. These two possibilities (synaptic homeostasis based on feedback versus an instructional role based on initiating and integrative functions) are extremely difficult to distinguish either *in situ* or *in vivo* and, so far, there seem to be insufficient reasons to postulate the latter. See text for a fuller discussion.