Astrocytic processes

DOI:
10.1016/j.tins.2021.07.006

Document Version
Accepted author manuscript

Astrocytic processes: from tripartite synapse to the active milieu

Alexey Semyanov[1,2,3*] and Alexei Verkhratsky[3,4,5*]

[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, 117997, Russia; [2]Faculty of Biology, Moscow State University, Moscow, Russia; [3]Sechenov First Moscow State Medical University, Moscow, Russia [4]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK; [5]Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain & Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED, Leioa, Spain.

*Correspondence: semyanov@ibch.ru (A. Semyanov) and Alexej.Verkhratsky@manchester.ac.uk (A. Verkhratsky)

Keywords: extrasynaptic signalling, astrocytic branch, astrocytic leaflet

Abstract

We define a new concept of 'active milieu' that unifies all components of nervous tissue (neuronal and glial compartments, extracellular space, extracellular matrix and vasculature) into a dynamic information processing system. Within this framework, we focus on the role of astrocytic processes, classified into organelle-containing branches and organelle-free leaflets. Astrocytic branches with emanating leaflets are homologous to dendritic shafts with spines. Within the active milieu, astrocytic processes are engaged in reciprocal interactions with neuronal compartments and communication with other cellular and non-cellular elements of the nervous tissue.

Main text

The concept of active milieu

Axons of neurons provide the input and output of the central nervous system (CNS), whereas the intricate web of neurons, glia and vasculature underlies information processing and defines the CNS function as an organ. The concept of tripartite synapse [1] has been instrumental in rethinking the role of astrocytes in synaptic transmission. The subsequent morphological analysis identified more components of the synaptic structure. These components include microglial processes and extracellular matrix (ECM), thus upgrading the tripartite paradigm to tetra- or pentapartite synapse [2, 3]. These convolutions reflect a high degree of complexity of the perisynaptic microenvironment, a subject of remarkable morphological plasticity. In particular, interactions of synapses with their microenvironment are influenced by the interposition of individual synapses and nearby compartments of non-neuronal cells and extracellular space (ECS). Thus, an increase in our knowledge on physiological interactions of cellular and non-cellular components of nervous tissue necessitates advancing the concept of 'tri-(multi)-partite synapse' to the concept of 'active milieu', which is based on the dynamic interposition and interaction among compartments of neurons, astrocytes, oligodendrocytes, microglia, blood vessels, ECS, and ECM (Box 1, Fig. 1). In the framework of this concept, a neuronal activity not only propagates from one neuron to another but also signals to other cellular and non-cellular elements, which respond to this signal and affect all components of nervous tissue. Under the idea of the multipartite synapse, the synaptic microenvironment serves the needs of the synapse by providing nutrients, clearing neurotransmitters, maintaining ionic concentrations, and thus assisting synaptic transmission and plasticity. We propose to break away from synaptocentricity by postulating that synapses themselves are part of the active milieu; they influence their neighbours such as astrocytic and microglial processes, cells of oligodendroglial lineage, components on the neurovascular unit, ECS, and ECM. Synaptic activity signals to these components and triggers or modifies their activity (e.g. voltage, ionic or metabolic responses) and instigates plasticity (for example, morphological remodelling), which, in turn, influences synaptic connectivity, efficacy and plasticity. Hence, the active milieu is a morphofunctional concept that is based on the topological organization of the nervous tissue. This concept unifies several previous notions, such as multipartite synapse, neurovascular unit, synaptic and extrasynaptic signalling, and volume transmission employed to describe the functional organisation of the brain. The morphological organization and functional properties of the active milieu are specific to different brain regions (with significant differences between cortex and cerebellum or white and grey matter) being defined by local cellular complement and cellular characteristics. Here, we specifically focus on the astrocytic component of the active milieu in the grey matter, with emphasis on the morphological and functional classification of astrocytic processes.

Structural organization and classification of astrocytic processes

Astrocytes belong to a class of astroglia, which includes protoplasmic astrocytes, fibrous astrocytes, radial astrocytes, Bergmann and Műller glia, pituicytes, tanycytes, ependymocytes, and choroid plexus cells [4]. Protoplasmic astrocytes, which populate the grey matter of the cortex and hippocampus, are characterized by complex morphology.

Protoplasmic astrocytes occupy individual territorial domains [5], thus parcellating the grey matter into relatively independent neuro(glio)vascular units [6], although territorial organisation might be somewhat different in humans [7]. Protoplasmic astrocytes have a relatively small soma and elaborated arborization represented by several primary processes, from which secondary, tertiary and higher-order processes emanate. The terminal processes, also referred to as 'peripheral astrocytic processes' or PAPs [8], arguably account for 70 - 80% of astrocyte surface area [9] while occupying a small fraction (~10%) of the cell volume [10, 11] which is reflected by their high surface-to-volume (SVR) ratio (~25 μm^{-1}) [8, 12]). Often, however, the term PAP is used to denote perisynaptic astrocytic processes [13-15], which adds terminological confusion. Synapses can contact not only peripheral astrocytic processes but also soma and primary processes [16-18]. Hence, the term 'perisynaptic astrocytic processes' is not equivalent to the definition of 'peripheral astrocytic processes'.

Heterogeneity of peripheral astrocytic processes has been recently confirmed by super-resolution optical and electron microscopy [16, 18, 19]. Morphological differences parallel distinct mechanisms of ionic signalling in different processes. The arborization of protoplasmic astrocytes presents distinct morphology with shaft-like primary and flat terminal processes. The common classification of these processes based on specific morphological parameters has not been hitherto adopted. Terminal astrocytic processes appear in the literature under many names. Sometimes they are defined as lamellae, lamellipodia, and filopodia [20]. Astrocytic lamellae were defined as organelle-free laminar expansions of the larger astrocyte processes [21]. The motile astrocytic processes observed in cell culture and in acutely isolated brain slices have been described as filopodia [22, 23]. The concept of the functional tripartite synapse [1] posits that the astrocytic processes cover individual synapses, thus forming a single functional unit for neuron-glial interaction. As alluded to before, perisynaptic astrocytic processes are generally considered to be a homogenous entity. The synapses, however, contact with different parts of astrocytic arborization formed by a heterogeneous group of astrocytic processes [16-18, 24]. Processes with distinct morphology and complement of organelles may generate distinct functional (for example, ionic signals) and morphological responses to synaptic activity. Induction of long-term potentiation triggers rapid movement of astrocytic processes [13, 25]; arguably leaflets move readily, whereas motility of branches (and especially primary processes) seems to be less likely, or is much slower.

Recently, a classification based on morphological properties of astrocytic processes has been suggested [26]. According to this classification, arborization of protoplasmic astrocyte is classified into (i) branches and branchlets as main (primary, secondary, etc.) astrocytic processes, which can be visualized with diffraction-limited optical microscopy (ii) leaflets that are below the resolution of diffraction-limited optical microscopy and (iii) endfeet which are specialized extensions of astrocytic branches contacting and plastering blood vessel. The difference between branches and branchlets is only in their order, branches being the primary processes emanating from the soma, while branchlets are processes of secondary, tertiary, and higher orders. Since no other morphological or functional difference has been identified, we propose to combine these processes into a

single class of branches. By analogy, no separate names (for example, 'dendritlets') have been assigned to dendrites of higher orders in neurons.

Astrocytic endfeet are highly plastic structures forming well-defined rosette-like structures plastering brain vessels [27]. The smooth and rough endoplasmic reticulum, as well as the Golgi apparatus, are present in astrocyte perivascular processes and endfeet, suggesting local biosynthesis contributing to the maturation of membranes and protein secretion [28]. Endfeet Ca^{2+} signalling involves G-protein coupled receptors (GPCRs)-mediated Ca^{2+} release from endoplasmic reticulum (ER) [29]. The three-dimensional reconstruction of serial section electron microscopy images revealed large bundles of mitochondria in close apposition to the perivascular endfoot membrane, which can contribute to Ca^{2+} signalling and communication with blood vessels [30]. Endfeet Ca^{2+} dynamics is modulated by changes in osmotic pressure and cerebral perfusion associated with bidirectional signaling between astrocytes and blood vessels [31, 32].

Recent research has provided insights into the morphological properties of branches and leaflets (Fig. 2). Local SVR of these two types of processes shows a remarkable difference in hippocampal protoplasmic astrocytes [16, 33]. The SVR of rodlike branches is 2 - 3 times lower than the SVR of flat leaflets. Branches contain organelles such as mitochondria and ER, whereas the tiny volume of leaflets does not provide enough space for them [18, 33]. These two criteria (SVR and presence of organelles) unequivocally distinguish between branches and leaflets and can be used for their identification. Additionally, leaflets differ from branches in the expression of immunocytochemical markers [23]. Leaflets are devoid of GFAP, which labels somata and branches in a subpopulation of astrocytes [34]. Plasmalemmal actin linkers, ezrin, and radixin (members of the ezrin-radixin-moesin family), on the contrary, have been predominantly found in leaflets [23, 35, 36]. Nonetheless, specific antibodies for ezrin label not only leaflets but also branches [35]. Similarly, glycogen granules were reported to be preferentially localized in leaflets, although immunostaining revealed them also in branches [37, 38]. In addition, glutamate transporters, Na^+/Ca^{2+} exchanger - NCX and Na^+/K^+ ATPase - NKA seem to co-localize in the distal processes [15, 39-41]. Similarly, gap junctions formed by connexin 43 were reported to concentrate primarily in leaflets [36] and so are Best1 Cl^- channels [42]. Because a unified classification of astrocytic processes has not been adopted hitherto, these studies did not identify leaflets or branches based on specific criteria. Along with morphological differences, specific protein localization further points to the functional distinction between these two astrocytic compartments. At the same time, the degree of segregation of these potential markers between branches and leaflets needs precise quantification.

Two recent studies with super-resolution optical microscopy revealed that astrocytic processes form loop-like structures in organotypic slices [19, 43]. Subsequently, a serial block-face scanning EM demonstrated that these loop-like structures emerge from astrocytic processes forming recurrent gap junctions [17]. These structures allow astrocytes to encircle dendrites and axons like a belt with a buckle. Such loops are formed by both branches and leaflets. However, it remains unclear if these loops are formed by chance or carry a specific function, such as bundling neighbouring neurites together [17].

Ionic signalling in branches and leaflets

The SVR of astrocytic processes determines idiosyncrasies of intracellular ion signals generated by ionic fluxes through the plasma membrane [44]. Astrocytes accumulate K^+ released during synaptic transmission [45]. Small volumes of leaflets are likely to be loaded with K^+ rapidly, which may affect the time course of K^+ clearance. Astrocytic intracellular signalling mediated by Na^+ and Ca^{2+} [46, 47] can be similarly affected by the SVR of astrocytic leaflets. Indeed, Ca^{2+} entering the astrocytic compartment with higher SVR produces a larger increase in $[Ca^{2+}]_i$ [44, 48]. The same applies to fluctuations in $[Na^+]_i$. A major source of physiological Na^+ influx is the operation of excitatory amino acid transporters (EAATs) co-transporting glutamate and Na^+ [49]. The corresponding rise in $[Na^+]_i$ in the astrocytic leaflet is, arguably, larger than in the astrocytic branch with lower SVR; thus, $[Na^+]_i$-associated reversal of the Na^+/Ca^{2+} exchanger triggering Ca^{2+} signal [50] may specifically occur in the leaflets. Therefore, the SVR is a key parameter defining the physiological properties of astrocytic compartments.

Lower SVR, characteristic for branches [16, 33], is limiting the relative contribution of plasmalemmal ion influx to cytosolic ion signals [44]. Instead, branches contain Ca^{2+} stores (ER and mitochondria) that can trigger and amplify Ca^{2+} signals by inositol-1,4,5-trisphosphate ($InsP_3$)- and Ca^{2+}-dependent Ca^{2+} release. The Ca^{2+} release occurs mainly through $InsP_3$ receptors synergistically regulated by $InsP_3$ and $[Ca^{2+}]_i$; ryanodine receptors (regulated by $[Ca^{2+}]_i$ and possibly by cyclic ADP ribose) may also contribute [46, 48, 51]. Ca^{2+} release from the endoplasmic reticulum (ER) is partially regenerative, i.e. intracellular Ca^{2+} diffusion between clusters of $InsP_3$ receptors can initiate their opening along with the ER, thus creating propagating intracellular Ca^{2+} wave. This mechanism underlies the generation of spreading Ca^{2+} events in astrocytic branches. Initiation of spreading Ca^{2+} event requires that $[Ca^{2+}]_i$ in cytosol reaches the threshold for activation of $InsP_3$ receptor in the presence of $InsP_3$ controlled by plasmalemmal G-protein coupled receptors (GPCR). In addition, an increase in $[Ca^{2+}]_i$ in the branch can be mediated by Ca^{2+} diffusion to the branch from daughter leaflets. Converging signals (e.g. $InsP_3$, Ca^{2+}) from multiple daughter leaflets can provide the parent branch with a readout of local network activity. Such integrative function of astrocytic processes is reminiscent of the integrative function of neuronal dendrites, where excitatory postsynaptic potentials (EPSPs) at individual dendritic spines are integrated by the dendritic shaft where dendritic spike may occur (Box 2, Fig. 3).

The threshold for the dendritic spike is regulated by local membrane conductances, and it is linked to dendritic plasticity [52]. The threshold for propagating Ca^{2+} events in astrocytes depends on the Ca^{2+}-sensitivity of the $InsP_3$ receptor, which in turn depends on cytosolic $InsP_3$ [53]. Activation of GPCRs that stimulates phospholipase C-dependent production of $InsP_3$ serves both as a source of signalling and an enhancer of the integrative potential of astrocytic branches. In addition, Ca^{2+} release from ER can be mediated by $InsP_3$ receptor-independent mechanisms, or Ca^{2+} may be released from mitochondria in astrocytic branches [54, 55]. In summary, there is a fundamental difference in Ca^{2+} signal generation between leaflets and branches: in leaflets, it involves plasmalemmal Ca^{2+} entry, whereas, in branches, Ca^{2+} signals are generated by ER/mitochondria Ca^{2+} release.

Astrocytes as a component of the active milieu

Although astrocytes and neurons share a degree of homology, these cells contribute differently to the active milieu. Electrically excitable neurons are capable of rapid long-range propagating signalling and conveying excitation and inhibition to postsynaptic counterparts through chemical synapses. Synapses also communicate to astrocytic processes. In the *stratum radiatum* of hippocampal CA1 area, about 80% of synapses contact leaflets, while about 20% contact branches [16]. In the cortex, 60% of synapses are apposed by leaflets and 40% by branches [18]. A single astrocytic leaflet may be surrounded and contacted by several synapses, and *vice versa* several astrocytic leaflets may contact an individual synapse. Leaflets sense and integrate the activity of neighbouring synapses, which is subsequently converted into astrocytic responses, such as Ca^{2+}-dependent morphofunctional remodelling [56, 57]. Thus, astrocytic leaflets and branches contacting synapses, dendrites, and axons contribute to the morphological organization and functional activity of the active milieu. In addition, microglia and microglial processes dynamically interact with all components of the active milieu through various patrolling modes influencing synaptic structures and contributing to neurochemical signalling [58]. In the white matter, astrocytes and microglia communicate with oligodendrocytes and provide for the regulation of myelination and remyelination [59], whereas in the grey matter, oligodendrocytic precursors may also engage in the operation of active milieu [60]. Finally, the active milieu includes neurogliovascular units connecting nervous tissue to the brain vasculature [6].

High SVR of astrocytic leaflets maximizes the amount of astrocytic membrane carrying transporters, ion channels, and receptors facing ECS [11, 61]. Multiple leaflets create a tortuous microenvironment around synapses that extends the diffusion pathway in the extracellular space for neurotransmitters and ions released during synaptic transmission [10]. Neurotransmitter transporters along with this pathway buffer neurotransmitters and ultimately move them across the astrocytic membrane, whereas astrocytic Na^+/K^+ ATPase and K^+ channels regulate extracellular K^+ spread. High SVR of astrocytic leaflets allows efficient delivery of energy substrates (e.g., lactate), antioxidants (e.g., glutathione), and glutamate-glutamine shuttling [62]. The ultrastructural analysis shows that glycogen, the primary source of astrocytic lactate, is strategically localized in astrocytic leaflets [62, 63]. Lack of organelles in the leaflets only permits utilization of glycogen through glycolysis, which leads to lactate production. In contrast, the presence of mitochondria in branches allows oxidative phosphorylation to fulfill the energy needs of the cell.

According to the paradigm of the active milieu, neuronal activity and synaptic signalling reciprocally interact with surrounding elements. Changes in the leaflet SVR may occur under both physiological and pathological conditions that are associated with active milieu remodelling [13, 25, 64-66] and result in multiple changes in astrocyte Ca^{2+} and Na^+ dynamics, diffusion pathways of the ECS, glutamate uptake and K^+ clearance. In particular, rapid SVR changes accompany astrocytic volume transients triggered by neuronal activity [67]. The SVR may be transiently modulated because of water influx through aquaporins associated with synaptic activity-dependent changes in osmotic pressure [68]. More persistent changes in the SVR linked for example, to cytoskeleton

remodelling [69], may represent an astrocytic mechanism for learning and memory complementary to and supportive of the classic synaptic plasticity such as LTP.

Concluding remarks

We propose the concept of the active milieu as integration of previous concepts of synaptic and extrasynaptic signalling, multipartite synapse, neurovascular unit, and volume transmission. We suggest instead that all elements are key players in the active milieu. The synapses interact with astrocytic processes and affect their Ca^{2+} activity, trigger morphological remodelling and *vice versa* - astrocytic processes can affect multiple synapses. In addition, active media is formed by other non-neuronal cells such as microglia and oligodendrocytes [59, 70] although reports addressing interactions between non-neuronal cells and astrocytic branches and leaflets are limited. This warrants further studies (Box 3). It is known that astrocytes secrete components of ECM and are involved in shaping ECS [71, 72] and yet research on ECM and ECS effects on astrocytic (in contrast to neuronal) function remains limited [73, 74]. Thus we introduce a morphofunctional concept of active milieu, which integrates all types of complex interactions among cellular and non-cellular elements forming the brain, morphological and functional plasticity of these elements, their involvement in information processing and storage. Such functional aspect makes this concept distinct from largely morphological terms like ‘neuropil’, ‘nervous tissue’, or ‘brain microenvironment’.

We present the unifying classification of astrocytic processes into branches, leaflets, and endfeet based on their morphological and functional properties. We suggest refuting the commonly used term PAPs as confusing because it refers to two separate entities and is incorrect from both morphological and physiological points of view. We discuss the homology in the organization of dendritic shaft and astrocytic branches and dendritic spines and astrocytic leaflets. Finally, we also discuss the place and role of astrocytic leaflets in the local microcircuitry. The leaflets do not belong to specific tripartite synapses; rather, few local synapses may signal to and be taken care of by individual astrocytic leaflet and vice versa.

Morphological plasticity of leaflets dynamically changes the active milieu and modulates synaptic transmission by modifying diffusion pathways for neurotransmitters and localization of transporters. Such a view on neuron-astrocyte interactions and underlying ideology raises numerous questions related to the contribution of astrocytic morphological plasticity to the higher brain functions such as learning and memory, including cellular and network mechanisms of such plasticity (Box 3). The future holds the key to these questions.

Acknowledgements

We are grateful to Prof. Maiken Nedergaard and Prof. Alfonso Araque for their critical comments on the manuscript.

Box 1. Components of the active milieu.

1. Neuronal components.
Neurons form chemical synapses and are capable of long-range signalling through axonal propagation of action potentials. Neuronal activity affects the microenvironment through the release of neurotransmitters and modulators, local electrical fields [75], and ionic fluctuations such as, for example, K^+ rise or Ca^{2+} depletion [45, 76].

2. Glial components.
Astrocytic processes are distributed between synapses. An individual synapse can reside next to the astrocytic branch, contact single or multiple leaflets. An individual leaflet is typically contacted by multiple synapses (for a definition of leaflets and branches, see section 2). Astrocytic processes can also form 'loops' that bundle together neuronal compartments such as axons [17]. Astrocytes regulate synaptic transmission and plasticity, extrasynaptic signalling and ionostasis. Microglial processes patrol active milieu and contribute to shaping synaptic connectivity. Oligodendrocytes form myelin sheaths and support axons.

3. Extracellular space (ECS) and extracellular matrix (ECM).
The ECS, filled with interstitial fluid and ECM, separates brain cells and occupies about 20% of the total brain volume [77]. The ECS is a part of the active milieu shaped by all cellular components of the nervous tissue: the complexity of cellular elements defines the high tortuosity of the ECS [78]. Tortuosity of ECS determines the diffusion path of extrasynaptic signalling molecules (including neurotransmitters and neuromodulators) [79]. The ECM is present throughout the ECS, where it limits rearrangements of cellular compartments (such as the formation of new synapses [80]) and interacts with receptors and channels, regulating their function [81]. The ECS is highly dynamic [82] and changes upon osmotic challenge [43], in the sleep-wake cycle [83] and in ageing [84]. These changes in ECS significantly affect all the processes in the active milieu that rely on the local diffusion of signalling molecules and their concentrations.

4. Brain vasculature and neurovascular unit.
The brain vessels, which, at the capillary level, constitute an integral part of the neurovascular unit, are composed of endothelial cells, pericytes, smooth muscle cells (in arteries, veins, arterioles and venules) and basement membranes; from the parenchymal site, all brain vessels are covered by glia limitans formed by astrocytic endfeet [6].

Box 2. Homology between dendrites and astrocytic processes.

Astrocytes and neurons are close relatives being scions of neuroepithelial cells [85], and hence two cell types develop a certain degree of homology in their morphological organization, which is highlighted in this box.

Structure. The structural organization of astrocytic branches hosting leaflets resembles dendritic shafts with spines. The dendritic spine consists of the head and the neck, the latter connecting the head to the dendritic shaft. The head bears postsynaptic density (PSD), opposing the presynaptic axonal varicosity and hosting neurotransmitter receptors. The astrocytic leaflet is located on the parent branch and extends into the space between brain cell compartments, including synapses. Sizes of dendritic spines in cortical neurons *in vivo* follow log-normal distribution [86], for review see [87]. The size of the spine changes over time, and these changes are proportional to the spine size, suggesting multiplicative dynamics. Long-tail distribution of spine sizes has been observed in hippocampal pyramidal neurons [16, 88]. A very similar long-tail distribution of leaflet SVRs was also found in hippocampal astrocytes (Fig. 2, [16]).

Compartmentalization and morphological plasticity. The spine neck defines electrical and chemical compartmentalization of the spine [89, 90]. The size of the spine head, the length, and the width of the neck are highly plastic and can change transiently or permanently depending on the previous activity and local Ca^{2+} signalling [91]. Monte-Carlo simulation of a point source of Ca^{2+} has demonstrated that the flattened structure of leaflets provides optimal conditions for chemical compartmentalization [92]. Similarly to the dendritic spine, the structure of astrocytic leaflets changes in response to nearby synaptic activity [13, 25] in a Ca^{2+}-dependent manner [57].

Function. Spines concentrate neurotransmitter receptors that are activated during synaptic transmission to enable signal propagation into postsynaptic neurons. Leaflets concentrate glutamate transporters [41] which not only provide glutamate uptake but also translate synaptic activity into astrocyte response through Na^+ entry and subsequent reversal of Na^+/Ca^{2+} exchanger and generation of Ca^{2+} signal [50]. Excitatory postsynaptic potentials (EPSPs) generated in dendritic spines linked to the same dendritic shaft when reaching the threshold generate local regenerative events, known as dendritic spikes [93]. Local Ca^{2+} elevations in astrocytic leaflets similarly (when reaching the threshold) may generate propagating (that is regenerative) Ca^{2+} signal in the astrocytic branch through the opening of ER Ca^{2+} release channels (Fig. 3).

References

1 Araque, A.*, et al.* (1999) Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 22, 208-215

2 Rusakov, D.A. and Dityatev, A.E. (2014) Brain circuitry outside the synaptic cleft. *Philos Trans R Soc Lond B Biol Sci* 369, 20130591

3 De Luca, C.*, et al.* (2020) Neurons, Glia, Extracellular Matrix and Neurovascular Unit: A Systems Biology Approach to the Complexity of Synaptic Plasticity in Health and Disease. *Int J Mol Sci* 21

4 Verkhratsky, A. and Nedergaard, M. (2018) Physiology of Astroglia. *Physiol Rev* 98, 239-389

5 Bushong, E.A.*, et al.* (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 22, 183-192

6 Iadecola, C. (2017) The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. *Neuron* 96, 17-42

7 Shapson-Coe, A.*, et al.* (2021) A connectomic study of a petascale fragment ofhuman cerebral cortex. *BioRxiv* Preprint

8 Reichenbach, A.*, et al.* (2010) Morphology and dynamics of perisynaptic glia. *Brain Res Rev* 63, 11-25

9 Chao, T.I.*, et al.* (2002) The synapse-astrocyte boundary: anatomical basis for an integrative role of glia in synaptic transmission
In *The Tripartite Synapses: Glia in Synaptic Transmission* (Volterra, A.*, et al.*, eds), pp. 3-23, OUP

10 Ventura, R. and Harris, K.M. (1999) Three-dimensional relationships between hippocampal synapses and astrocytes. *J Neurosci* 19, 6897-6906

11 Lehre, K.P. and Rusakov, D.A. (2002) Asymmetry of glia near central synapses favors presynaptically directed glutamate escape. *Biophys J* 83, 125-134

12 Grosche, J.*, et al.* (2002) Bergmann glial cells form distinct morphological structures to interact with cerebellar neurons. *J Neurosci Res* 68, 138-149

13 Bernardinelli, Y.*, et al.* (2014) Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability. *Curr Biol* 24, 1679-1688

14 Mazare, N.*, et al.* (2020) Local Translation in Perisynaptic Astrocytic Processes Is Specific and Changes after Fear Conditioning. *Cell Rep* 32, 108076

15 Melone, M.*, et al.* (2019) Heterogeneity of Astrocytic and Neuronal GLT-1 at Cortical Excitatory Synapses, as Revealed by its Colocalization With Na+/K+-ATPase alpha Isoforms. *Cereb Cortex* 29, 3331-3350

16 Gavrilov, N.*, et al.* (2018) Astrocytic Coverage of Dendritic Spines, Dendritic Shafts, and Axonal Boutons in Hippocampal Neuropil. *Front Cell Neurosci* 12, 248

17 Kiyoshi, C.M.*, et al.* (2020) Ultrastructural view of astrocyte-astrocyte and astrocyte-synapse contacts within the hippocampus. *BioRxiv* Preprint

18 El Alaoui, A.A.*, et al.* (2021) Characterization of Subcellular Organelles in Cortical Perisynaptic Astrocytes. *Front. Cell. Neurosci* 14, a573944

19 Arizono, M.*, et al.* (2020) Structural basis of astrocytic Ca^{2+} signals at tripartite synapses. *Nat Commun* 11, 1906

20 Kimelberg, H.K. and Nedergaard, M. (2010) Functions of astrocytes and their potential as therapeutic targets. *Neurotherapeutics* 7, 338-353

21 Fernandez, B.*, et al.* (1984) Topographical distribution of the astrocytic lamellae in the hypothalamus. *Anat Anz* 156, 31-37
22 Hirrlinger, J.*, et al.* (2004) Astroglial processes show spontaneous motility at active synaptic terminals in situ. *Eur J Neurosci* 20, 2235-2239
23 Lavialle, M.*, et al.* (2011) Structural plasticity of perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors. *Proc Natl Acad Sci U S A* 108, 12915-12919
24 Witcher, M.R.*, et al.* (2007) Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia* 55, 13-23
25 Henneberger, C.*, et al.* (2020) LTP Induction Boosts Glutamate Spillover by Driving Withdrawal of Perisynaptic Astroglia. *Neuron* 108, 919-936
26 Khakh, B.S. and Sofroniew, M.V. (2015) Diversity of astrocyte functions and phenotypes in neural circuits. *Nat Neurosci* 18, 942-952
27 Kacem, K.*, et al.* (1998) Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 23, 1-10
28 Boulay, A.C.*, et al.* (2017) Translation in astrocyte distal processes sets molecular heterogeneity at the gliovascular interface. *Cell Discov* 3, 17005
29 Mulligan, S.J. and MacVicar, B.A. (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* 431, 195-199
30 Mathiisen, T.M.*, et al.* (2010) The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia* 58, 1094-1103
31 Eilert-Olsen, M.*, et al.* (2019) Astroglial endfeet exhibit distinct Ca^{2+} signals during hypoosmotic conditions. *Glia* 67, 2399-2409
32 Marina, N.*, et al.* (2020) Astrocytes monitor cerebral perfusion and control systemic circulation to maintain brain blood flow. *Nat Commun* 11, 131
33 Patrushev, I.*, et al.* (2013) Subcellular location of astrocytic calcium stores favors extrasynaptic neuron-astrocyte communication. *Cell Calcium* 54, 343-349
34 Hol, E.M. and Pekny, M. (2015) Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol* 32, 121-130
35 Derouiche, A. and Geiger, K.D. (2019) Perspectives for Ezrin and Radixin in Astrocytes: Kinases, Functions and Pathology. *Int J Mol Sci* 20
36 Haseleu, J.*, et al.* (2013) Studying subcellular detail in fixed astrocytes: dissociation of morphologically intact glial cells (DIMIGs). *Front Cell Neurosci* 7, 54
37 Oe, Y.*, et al.* (2016) Glycogen distribution in the microwave-fixed mouse brain reveals heterogeneous astrocytic patterns. *Glia* 64, 1532-1545
38 Cali, C.*, et al.* (2016) Three-dimensional immersive virtual reality for studying cellular compartments in 3D models from EM preparations of neural tissues. *J Comp Neurol* 524, 23-38
39 Minelli, A.*, et al.* (2007) Cellular and subcellular localization of Na^{+}-Ca^{2+} exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat. *Cell Calcium* 41, 221-234
40 Arizono, M.*, et al.* (2012) Receptor-selective diffusion barrier enhances sensitivity of astrocytic processes to metabotropic glutamate receptor stimulation. *Sci Signal* 5, ra27

41 Murphy-Royal, C., *et al.* (2015) Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. *Nat Neurosci* 18, 219-226
42 Woo, D.H., *et al.* (2012) TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. *Cell* 151, 25-40
43 Arizono, M., *et al.* (2021) Super-resolution shadow imaging reveals local remodeling of astrocytic microstructures and brain extracellular space after osmotic challenge. *Glia*
44 Wu, Y.W., *et al.* (2019) Morphological profile determines the frequency of spontaneous calcium events in astrocytic processes. *Glia* 67, 246-262
45 Shih, P.Y., *et al.* (2013) Retrograde synaptic signaling mediated by K^+ efflux through postsynaptic NMDA receptors. *Cell Rep* 5, 941-951
46 Semyanov, A., *et al.* (2020) Making sense of astrocytic calcium signals - from acquisition to interpretation. *Nat Rev Neurosci* 21, 551-564
47 Verkhratsky, A., *et al.* (2020) Ionic signalling in astroglia beyond calcium. *J Physiol* 598, 1655-1670
48 Semyanov, A. (2019) Spatiotemporal pattern of calcium activity in astrocytic network. *Cell Calcium* 78, 15-25
49 Kirischuk, S., *et al.* (2007) Membrane currents and cytoplasmic sodium transients generated by glutamate transport in Bergmann glial cells. *Pflugers Arch* 454, 245-252
50 Rose, C.R., *et al.* (2020) On the special role of NCX in astrocytes: Translating Na^+-transients into intracellular Ca^{2+} signals. *Cell Calcium* 86, 102154
51 Verkhratsky, A., *et al.* (2012) Calcium signalling in astroglia. *Mol Cell Endocrinol* 353, 45-56
52 Losonczy, A., *et al.* (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. *Nature* 452, 436-441
53 Shinohara, T., *et al.* (2011) Mechanistic basis of bell-shaped dependence of inositol 1,4,5-trisphosphate receptor gating on cytosolic calcium. *Proc Natl Acad Sci U S A* 108, 15486-15491
54 Agarwal, A., *et al.* (2017) Transient Opening of the Mitochondrial Permeability Transition Pore Induces Microdomain Calcium Transients in Astrocyte Processes. *Neuron* 93, 587-605 e587
55 Okubo, Y. and Iino, M. (2020) Visualization of astrocytic intracellular Ca^{2+} mobilization. *J Physiol* 598, 1671-1681
56 Molotkov, D., *et al.* (2013) Calcium-induced outgrowth of astrocytic peripheral processes requires actin binding by Profilin-1. *Cell Calcium* 53, 338-348
57 Tanaka, M., *et al.* (2013) Astrocytic Ca^{2+} signals are required for the functional integrity of tripartite synapses. *Mol Brain* 6, 6
58 Cserep, C., *et al.* (2020) Shaping Neuronal Fate: Functional Heterogeneity of Direct Microglia-Neuron Interactions. *Neuron*
59 Domingues, H.S., *et al.* (2016) Oligodendrocyte, Astrocyte, and Microglia Crosstalk in Myelin Development, Damage, and Repair. *Front Cell Dev Biol* 4, 71
60 Wigley, R., *et al.* (2007) Morphological and physiological interactions of NG2-glia with astrocytes and neurons. *J Anat* 210, 661-670
61 Verkhratsky, A. and Nedergaard, M. (2014) Astroglial cradle in the life of the synapse. *Philos Trans R Soc Lond B Biol Sci* 369, 20130595
62 Cali, C., *et al.* (2019) The Strategic Location of Glycogen and Lactate: From Body Energy Reserve to Brain Plasticity. *Front Cell Neurosci* 13, 82

63 Mohammed, H., *et al.* (2018) Abstractocyte: A Visual Tool for Exploring Nanoscale Astroglial Cells. *IEEE Trans Vis Comput Graph* 24, 853-861
64 Theodosis, D.T., *et al.* (2008) Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol Rev* 88, 983-1008
65 Popov, A., *et al.* (2021) Astrocyte dystrophy in ageing brain parallels impaired synaptic plasticity. *Aging Cell* 20, e13334
66 Popov, A., *et al.* (2020) Caloric restriction triggers morphofunctional remodeling of astrocytes and enhances synaptic plasticity in the mouse hippocampus. *Cell Death Dis* 11, 208
67 Woo, J., *et al.* (2020) The molecular mechanism of synaptic activity-induced astrocytic volume transient. *J Physiol* 598, 4555-4572
68 Haj-Yasein, N.N., *et al.* (2012) Aquaporin-4 regulates extracellular space volume dynamics during high-frequency synaptic stimulation: a gene deletion study in mouse hippocampus. *Glia* 60, 867-874
69 Schiweck, J., *et al.* (2018) Important Shapeshifter: Mechanisms Allowing Astrocytes to Respond to the Changing Nervous System During Development, Injury and Disease. *Front Cell Neurosci* 12, 261
70 Matejuk, A. and Ransohoff, R.M. (2020) Crosstalk Between Astrocytes and Microglia: An Overview. *Front Immunol* 11, 1416
71 Wiese, S., *et al.* (2012) Astrocytes as a source for extracellular matrix molecules and cytokines. *Front Pharmacol* 3, 120
72 Vargova, L. and Sykova, E. (2014) Astrocytes and extracellular matrix in extrasynaptic volume transmission. *Philos Trans R Soc Lond B Biol Sci* 369, 20130608
73 Song, I. and Dityatev, A. (2018) Crosstalk between glia, extracellular matrix and neurons. *Brain Res Bull* 136, 101-108
74 Johnson, K.M., *et al.* (2015) Extracellular matrix composition determines astrocyte responses to mechanical and inflammatory stimuli. *Neurosci Lett* 600, 104-109
75 Savtchenko, L.P., *et al.* (2017) Electrodiffusion phenomena in neuroscience: a neglected companion. *Nat Rev Neurosci* 18, 598-612
76 Rusakov, D.A. (2012) Depletion of extracellular Ca^{2+} prompts astroglia to moderate synaptic network activity. *Sci Signal* 5, pe4
77 Nicholson, C. and Hrabetova, S. (2017) Brain Extracellular Space: The Final Frontier of Neuroscience. *Biophys J* 113, 2133-2142
78 Thorne, R.G. and Nicholson, C. (2006) *In vivo* diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proc Natl Acad Sci U S A* 103, 5567-5572
79 Sykova, E. and Nicholson, C. (2008) Diffusion in brain extracellular space. *Physiol Rev* 88, 1277-1340
80 Dembitskaya, Y., *et al.* (2021) Attenuation of the extracellular matrix increases the number of synapses but suppresses synaptic plasticity through upregulation of SK channels. *Cell Calcium* 96, 102406
81 Dansie, L.E. and Ethell, I.M. (2011) Casting a net on dendritic spines: the extracellular matrix and its receptors. *Dev Neurobiol* 71, 956-981
82 Hrabetova, S., *et al.* (2018) Unveiling the Extracellular Space of the Brain: From Super-resolved Microstructure to *In Vivo* Function. *J Neurosci* 38, 9355-9363

83 Xie, L.*, et al.* (2013) Sleep drives metabolite clearance from the adult brain. *Science* 342, 373-377
84 Verkhratsky, A.*, et al.* (2020) Astroglial asthenia and loss of function, rather than reactivity, contribute to the ageing of the brain. *Pflugers Arch*
85 Dinh Duong, T.A.*, et al.* (2019) FGF Signaling Directs the Cell Fate Switch from Neurons to Astrocytes in the Developing Mouse Cerebral Cortex. *J Neurosci* 39, 6081-6094
86 Loewenstein, Y.*, et al.* (2011) Multiplicative dynamics underlie the emergence of the log-normal distribution of spine sizes in the neocortex in vivo. *J Neurosci* 31, 9481-9488
87 Buzsaki, G. and Mizuseki, K. (2014) The log-dynamic brain: how skewed distributions affect network operations. *Nat Rev Neurosci* 15, 264-278
88 Yasumatsu, N.*, et al.* (2008) Principles of long-term dynamics of dendritic spines. *J Neurosci* 28, 13592-13608
89 Grunditz, A.*, et al.* (2008) Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. *J Neurosci* 28, 13457-13466
90 Yuste, R. (2013) Electrical compartmentalization in dendritic spines. *Annu Rev Neurosci* 36, 429-449
91 Nagerl, U.V.*, et al.* (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 44, 759-767
92 Rusakov, D.A.*, et al.* (2011) Astrocytes as regulators of synaptic function: a quest for the Ca^{2+} master key. *Neuroscientist* 17, 513-523
93 Larkum, M.E. and Nevian, T. (2008) Synaptic clustering by dendritic signalling mechanisms. *Curr Opin Neurobiol* 18, 321-331
94 Spruston, N.*, et al.* (2013) Information Processing in Dendrites and Spines. In *Fundamental Neuroscience (Fourth Edition)*, pp. 231-260, Academic Press

Figure legends

Figure 1. Astrocyte-neuron communication in the active milieu

The active milieu concept integrates multiple theories that address different aspects of local function organization of the brain: multipartite synapse, neuro(glio)vascular unit, extrasynaptic signaling and volume transmission. The active milieu is formed through dynamic interactions between neuronal elements (somata, axons, dendrites, and spines), non-neuronal cell elements (astrocytic and microglial processes), vasculature (capillary), extracellular space (ECS) and extracellular matrix (ECM). In an active milieu, synapses can contact, signal and be homeostatically controlled by astrocyte branches (**a**), by single or by several leaflets (**b**). A single astrocytic branch or leaflet may be contacted by several synapses (**c**). Dynamic changes in the morphology of astrocytic processes affect diffusional barriers, neurotransmitter clearance, and K^+ dynamics, the supply of glutamine or energy substrates, thus regulating neuronal plasticity. Astrocytic processes form loop-like structures through reciprocal gap junctions (GJ).

Figure 2. Morphological classification of astrocytic processes

(**A**) Three-dimensional reconstruction of an astrocyte loaded with fluorescent dye Alexa Fluo 594 through a patch-pipette (*centre*) [84]. The cell consists of soma with optically resolved branches of primary, secondary, and higher orders. These branches are surrounded by fine terminal leaflets that cannot be resolved with diffraction-limited optical imaging and appear as a spongiform cloud. Astrocytic leaflets occupy most of the astrocyte territory also known as an anatomic domain). Two insets (*left* and *right*) are zooming onto branches and leaflets reconstructed from serial section transmission electron microscopy (TEM) image stacks [33]. Green – astrocyte membrane, blue – endoplasmic reticulum (ER), yellow – mitochondria, grey – dendritic spine, red – postsynaptic density. Astrocytic branches contain organelles, whereas leaflets are organelle-free. Leaflets are interspaced with dendritic spines, filling the space between them like paper cushions around pears packed for shipping. Thus, most of the synapses hosted by dendritic spines interact with organelle-free leaflets, while individual leaflets may interact with several synapses. Some dendritic spines, however, reside in the vicinity of astrocytic branches and soma, and those synapses may have a different effect on astrocytic Ca^{2+} activity [16, 17].

(**B**) Analysis of local astrocytic surface-to-volume ratio (SVR) with the method of spheres (for more detail, see [33]) in serial section TEM reconstruction. Astrocytic branches and leaflets have significantly different SVR. High SVR correlates with a low volume of cytoplasm in astrocytic leaflets, which significantly affects the dynamics of ionic gradients. Ions (e.g. Na^+, Ca^{2+}) entering the leaflet through plasma membrane achieve high concentration faster than those entering branches with lower SVR. Hence, the amplitude and time course of intracellular Ca^{2+} and Na^+ transients representing a major form of astrocytic excitability is determined by local SVR [44, 48].

(**A** and **B**) The absence of organelles and high SVR represent two morphological criteria that distinguish between astrocytic leaflets and branches.

(**B**) reproduced, with permission from [33].

Figure 3. Homology between astrocytic processes and neuronal dendrites

(**A**) Dendritic shaft is homologous to astrocytic branches; dendritic spines are homologous to astrocytic leaflets. The spines possess neurotransmitter receptors, whereas the leaflets bear glutamate transporters and Na^+/Ca^{2+} exchanger, as well as Na^+/K^+ ATPase and K^+ channels; the leaflets also house neurotransmitter receptors and other SLC transporters, which are not shown. Astrocytic leaflets fill the space between spines with lesser density around the axon (presynapse) and are even less dense in the vicinity of the dendritic shaft [11, 16]. Synaptically released glutamate targets glutamate receptors on both dendritic spines and leaflets. Astrocytic glutamate transporters in leaflets generate Na^+/glutamate co-transport that produces Na^+ transient and subsequent $[Ca^{2+}]_i$ elevation mediated by reversal of Na^+/Ca^{2+} exchanger (NCX) [48, 50]. In the dendritic spine, excitatory postsynaptic potential (EPSP) propagates to the dendritic shaft, where it integrates with other EPSPs, eventually triggering a dendritic spike [94]. Similarly, Ca^{2+} transients in the astrocytic leaflet propagate to the astrocytic branch triggering Ca^{2+}-induced Ca^{2+} release from ER through $InsP_3R$ [46]. While Ca^{2+} events in leaflets are focal, ER-mediated Ca^{2+} events have regenerative features and propagate through branches like dendritic spikes propagate through dendritic shafts. Mitochondria located in branches can also contribute to Ca^{2+} events both as a source and the sink (mitochondrial firewall) of Ca^{2+} [54].

(**B, C**) Astrocytic leaflets and dendritic spines and have a homologous structure. Dendritic spine sizes are described by single log-normal distribution [86]. A similar parameter for astrocytic leaflets linking morphology and function is the SVR. The distribution of astrocytic leaflet SVR is the same as the distribution of dendritic spine radius [16].

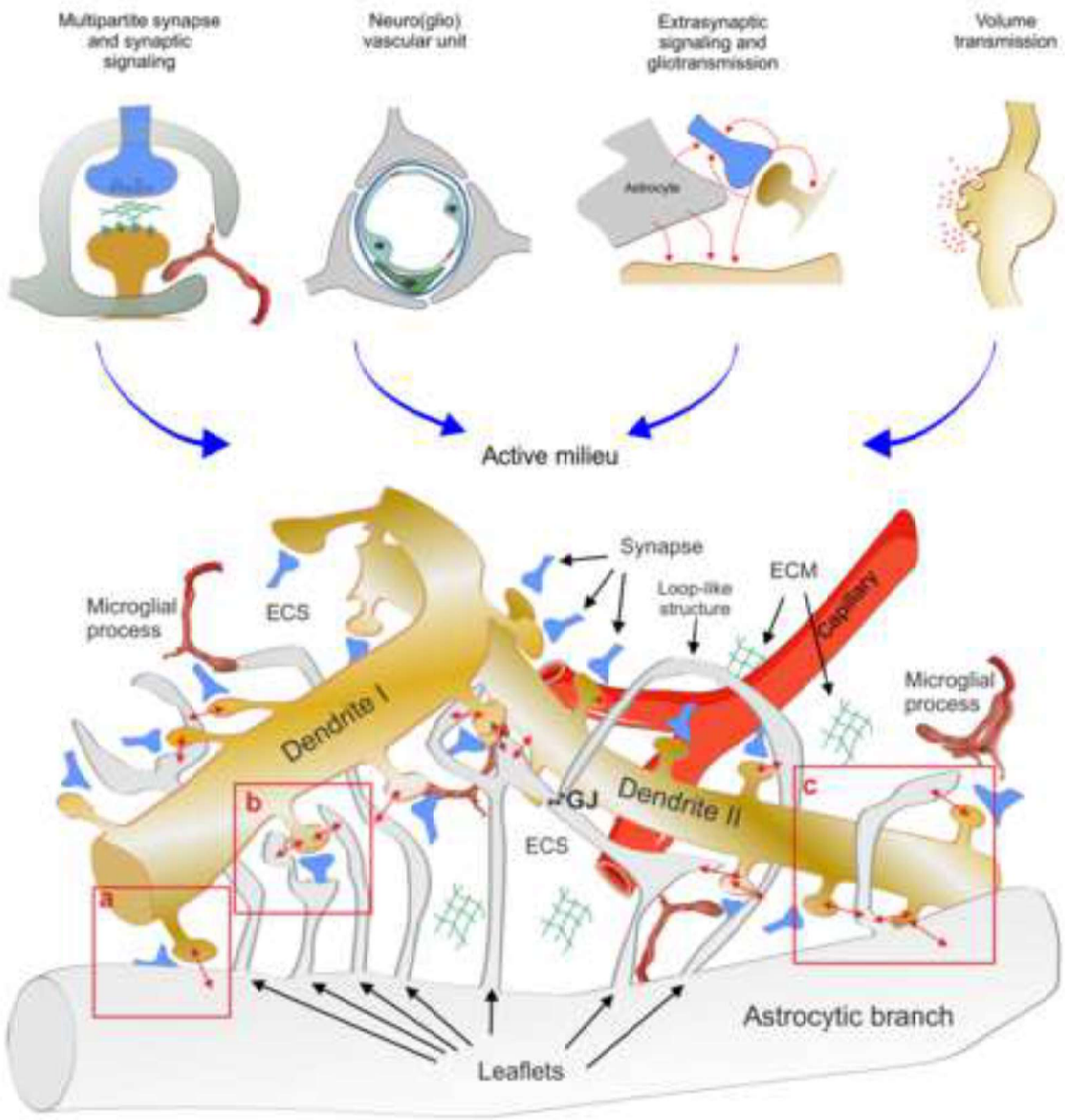
(**B, C**) modified from [16].

Outstanding questions Box

- How different elements of active milieu interact with each other?
- Do interactions within the active milieu undergo long-lasting changes in their efficiency beyond synaptic plasticity?
- Can plasticity of active milieu represent a mechanism for information storage in the brain and contribute to learning and memory being an intrinsic part of an engram? If that is the case, the neuronal only basis of the engram needs revision.
- How spatiotemporal pattern of synaptic activity affects the astrocytic network? Can different levels of neuronal activity translate into distinct spatiotemporal patterns of astrocytic activity?
- What is the functional relevance of astrocytic activity patterns? Can these patterns form 'guiding templates' for excitation propagation in the neuronal networks (e.g., by modifying the synaptic efficacy, plasticity, release, and uptake of neurotransmitters, etc.)?
- How do astrocytic branches grow? Can leaflets convert into branches and back? What triggers the appearance of new leaflets?

Figure 1

Click here to access/download;Figure;Figure_1.tif



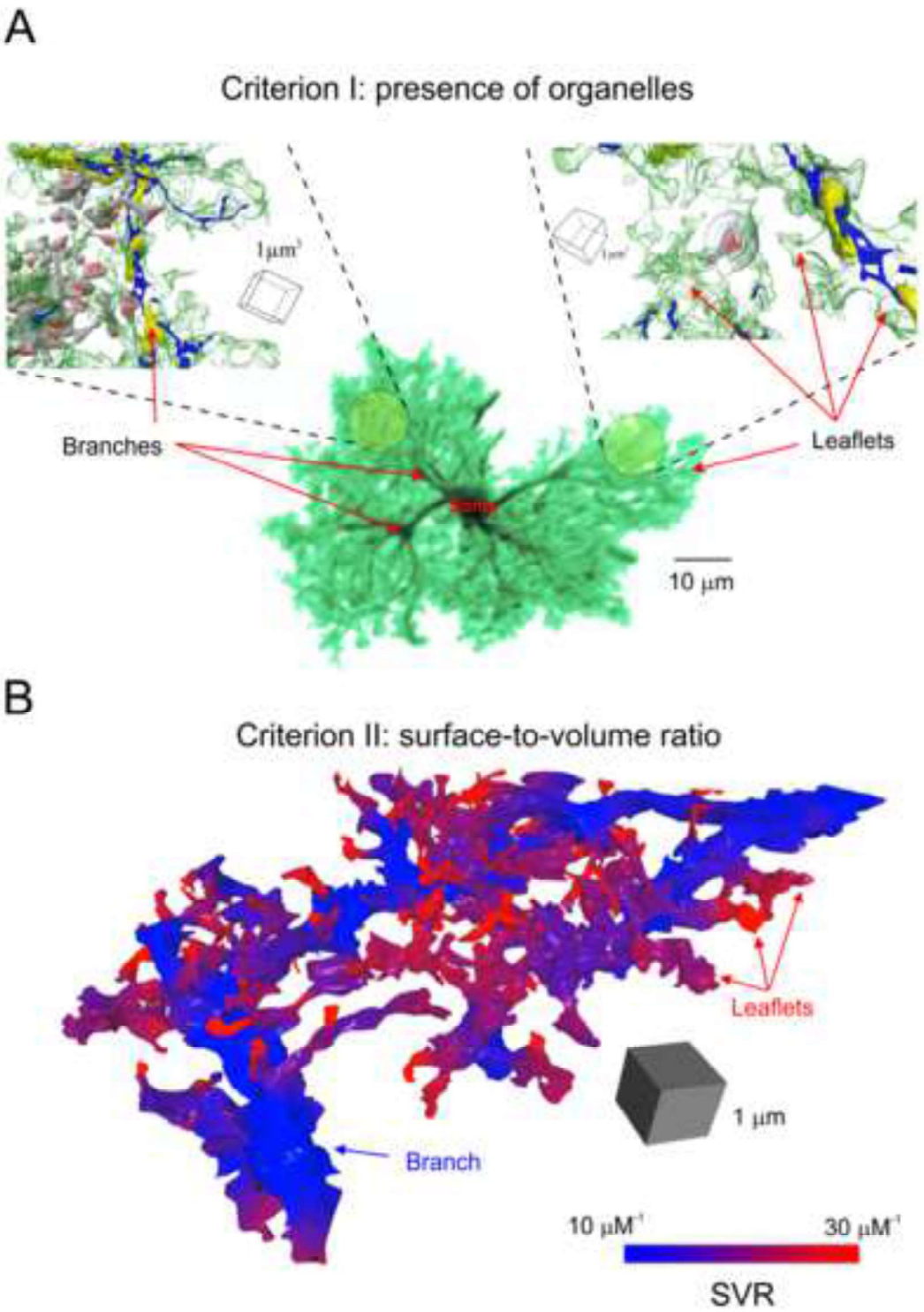


Figure 3