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Spatiotemporal pattern of calcium activity in astrocytic network

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ABSTRACT

Ca^{2+} influx through an astrocyte plasma membrane is mediated by ionotropic receptors and Ca^{2+} channels according the electrochemical gradient. These conductances allow astrocytes to sense the levels of neuronal activity and environmental changes. Na^+/Ca^{2+} exchanger (NCX) removes elevated Ca^{2+} from the cell but can reverse and bring Ca^{2+} in. Ca^{2+} entry through the plasma membrane produces local Ca^{2+} elevations that can be further amplified by Ca^{2+} induced activation of inositol-3-phosphate (IP_3) receptors and subsequent Ca^{2+} release from intracellular Ca^{2+} stores. These Ca^{2+} stores are located in astrocytic processes called branchlets, while perisynaptic astrocytic processes are formed by organelle-free leaflets. Such morphological structure suggests separate synaptic and extrasynaptic mechanisms of Ca^{2+} signaling in astrocytes. Astrocytic leaflets sense synaptic activity, astrocytic branchlets integrate signals arriving from the leaflets and from extrasynaptic inputs. The surface-to-volume ratio (SVR) of the branchlets sets the threshold for generation of spreading Ca^{2+} events. Therefore, morphological remodeling of the processes is an important regulator of astrocytic Ca^{2+} activity. Ca^{2+} events can propagate beyond single astrocytes and form complex spatiotemporal patterns of Ca^{2+} activity in the astrocytic network. Ca^{2+} events spread intercellularly through gap-junctions and via extracellular ATP diffusion. Spatially and temporarily organized Ca^{2+} events in astrocytic network influence variable numbers of synapses and neuronal compartments, gate excitation flow and synaptic plasticity in the neuronal network through the release of gliotransmitters. Thus, multiple patterns of Ca^{2+} activity in the astrocytic network (guiding templates) determine multiple states of the neuronal network. This phenomenon may be linked to learning, memory and information processing in the brain.

1. Introduction

Electrically non-excitable astrocytes employ ionic signaling as a substrate for their excitability [1]; spatially and temporally controlled fluctuations in the cytoplasmic Ca^{2+} concentration represent the major component of astrocytic signaling [2–5]. Astrocytic Ca^{2+} elevations (events) trigger release of gliotransmitters [6,7], regulate K^+ uptake [8], are involved in regulation of local blood flow [9] and morphological remodeling of these cells [10–12]. Despite numerous reports and reviews describing Ca^{2+} activity in astrocytes, the principles of Ca^{2+} events integration within single astrocytes and in the astrocytic network are still purely understood. Here I review the mechanisms that define and modulate the spatiotemporal properties of astrocytic Ca^{2+} activity, synaptic and extrasynaptic astrocytic signaling, and discuss a possible role of astrocytic Ca^{2+} pattern in guiding neuronal activity.

Astrocytic Ca^{2+} events are initiated because of Ca^{2+} entry to the cytosol through plasma membrane from the extracellular space and because of a Ca^{2+} release from intracellular Ca^{2+} stores – endoplasmic reticulum (ER) and mitochondria. Activation of these fluxes and their subcellular location determine the frequency and spatial distribution of Ca^{2+} events initiation points. After initiation, the Ca^{2+} event can spread within the cell and beyond. In the cell, Ca^{2+} event can spread both diffusively or regeneratively. In the astrocytic network, Ca^{2+} event can spread by chemical signal (ATP) or through the gap-junctions. Finally, Ca^{2+} event is terminated by Ca^{2+} extrusion through the plasma membrane and by uptake to Ca^{2+} stores. Some of the major mechanisms responsible for the generation and propagation of Ca^{2+} events are summarized below (Fig. 1).

2. Ionotropic Ca^{2+} permeable receptors and transient receptor potential (TRP) channels

Freshly isolated cortical astrocytes express functional N-methyl-D-aspartate (NMDA) and purinergic P2X(1/5) receptors [13,14]. Both

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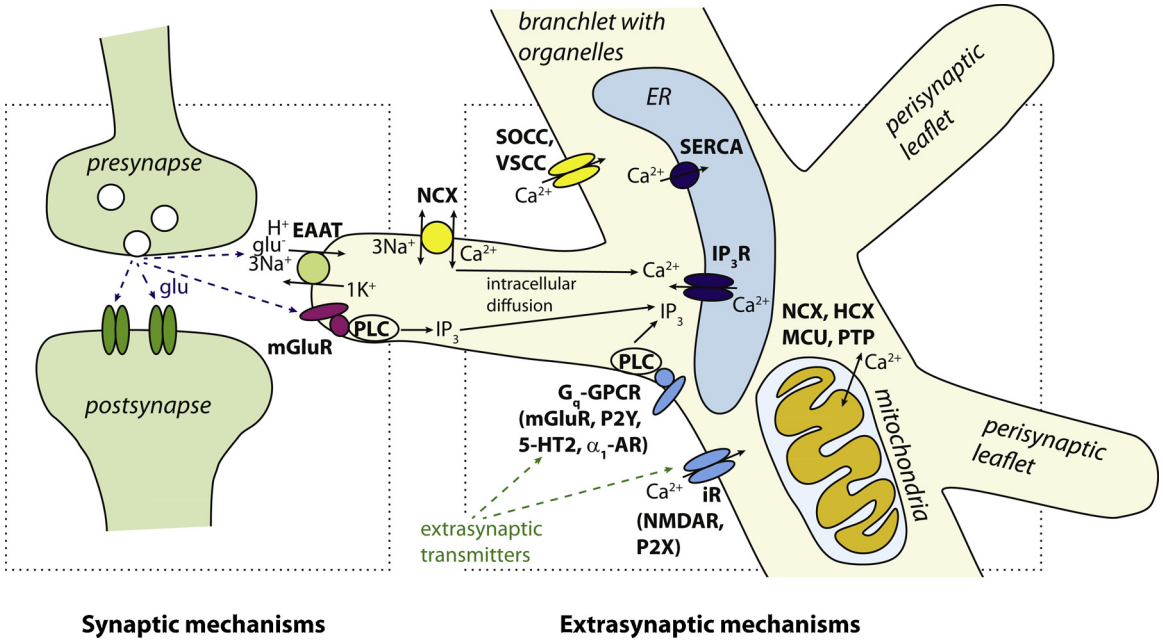

Fig. 1. Synaptic and extrasynaptic mechanisms of Ca^{2+} activity in astrocyte.
Schematics depictures astrocytic branchlet with leaflets and a glutamate synapse.

Synaptic mechanisms

Astrocytic leaflets extend towards glutamate synapses. These processes are devoid of organelles but are equipped with excitatory amino acid transporters (EAAT) that move one glutamate, one H^+ and three Na^+ in exchange for one K^+. Thus, during glutamate uptake local intracellular Na^+ concentration increases. Then Na^+ is removed from the cell by Na^+/Ca^{2+} exchanger (NCX), which in turn produces local Ca^{2+} elevation. Synaptically released glutamate can also target metabotropic glutamate receptors (mGluRs) on leaflets. mGluR activation triggers phospholipase C (PLC) -mediated production of inositol-3-phosphate (IP_3). Both IP_3 and Ca^{2+} diffuse towards parent astrocytic branchlet where they serve as co-agonists of IP_3 receptors, triggering Ca^{2+} release from endoplasmic reticulum (ER).

Integration of synaptic inputs

Local Ca^{2+} elevations triggered in multiple organelle-free leaflets are integrated and amplified in the parent branchlet through Ca^{2+} induced Ca^{2+} release via IP_3 receptors on ER. The amplified Ca^{2+} event can further propagate and integrate with Ca^{2+} events in neighboring branchlet compartments. Such integration and propagation of Ca^{2+} events form a complex spatiotemporal pattern of Ca^{2+} activity in single astrocytes and astrocytic network.

Extrasynaptic mechanisms

In addition to the integration of synaptic inputs at the leaflets, astrocytic branchlet can detect extrasynaptic diffuse signals mediated by various neuro- and gliotransmitters targeting both ionotropic (iR) and metabotropic astrocytic receptors. Astrocytic N-methyl-D-aspartate receptors (NMDAR) can sense glutamate spillover, P2X receptors can be activated by ATP released by neighboring astrocytes. G_q-protein coupled receptors (G_q-GPCR) such as mGluR, P2Y, 5-HT2, α1-adrenoreceptors (AR) trigger production of IP_3.

Intrinsic mechanisms

Store-operated Ca^{2+} channels (SOCCs), voltage-gated Ca^{2+} channels (VGCCs) are responsible for direct Ca^{2+} entry to the astrocytic branchlet. The branchlet also contains mitochondria, that can be involved in intracellular Ca^{2+} dynamics. When cytosolic Ca^{2+} is elevated to micromolar range in can be moved into mitochondria through mitochondrial Ca^{2+} uniporter (MCU). Ca^{2+} in nanomolar concentrations is taken from the cytosol by mitochondrial H^+/Ca^{2+} exchanger (HCX). Mitochondria can also release Ca^{2+} to the cytosol through the permeability transition pore (PTP). Finally, Ca^{2+} can be moved through the mitochondrial membrane by mitochondrial NCX.

Thus Ca^{2+} activity in astrocytic branchlets reflects synaptic activity, sampled by astrocytic leaflets, extrasynaptic signals sensed by all astrocytic compartments and intrinsic/metabolic processes.

receptors are permeable to cations, including Ca^{2+}. NMDA receptors are gated by glutamate, P2X receptors are gated by ATP. Both ligands can be released by neurons and astrocytes [6,15–18]. Unlike neuronal, astrocytic NMDA receptors are not blocked by Mg^{2+}, therefore do not require astrocytic depolarization for their activation.

In addition to ligand-activated ionotropic receptors, astrocytes express TRP channels that allow them to sense various changes in the environment [19]. TRPA1 channel-mediated Ca^{2+} influx contributes to the resting level of cytosolic Ca^{2+} and promotes D-serine release into the extracellular space, which in turn contributes to NMDA receptor-dependent LTP [20]. Blockade of TRPA1 reduces spontaneous Ca^{2+} activity in astrocytes and downregulates GABA uptake mediated by astrocyte-specific transporter, GAT3 [21]. GAT3 regulates the level of ambient GABA of both synaptic and non-synaptic origin and, therefore, the magnitude of tonic $GABA_A$ receptor-mediated conductance in neurons [22,23]. Mechanical stimulation of astrocytes triggers a Ca^{2+} influx through TRPC1 channels [24]. Astrocytes also express functional TRPC3 channels [25]. Another mechanosensitive Ca^{2+} channel expressed in astrocytes is TRPV4 [26]. Mechanical stimulation of astrocytes in vivo can occur due to vasomotion, making astrocytes sensitive to the local blood circulation level. These channels can respond to changes in osmotic pressure and mediate astrocytic response to cerebral hypoxia/ischemia and stroke [27,28]. Astrocytes also express temperature sensitive Ca^{2+} channel TRPV1 [29]. Significant energy consumption of the brain should produce metabolic heat, especially in the active regions [30]. Yet, spatial and temporal distributions of brain temperatures are purely understood [31]. TRPV1 expression makes astrocyte sensitive to the local temperature that can change both as a result of physiological activity and pathological processes (e.g. inflammation) [32].

TRP channels can operate as store-operated Ca^{2+} channels (SOCCs) [33]. Although Orai is another type of SOCCs, it is predominantly expressed in microglia, whereas astrocytes rely more on TRP channels. SOCCs are thought to require emptying of Ca^{2+} stores and to be instrumental for their refill. However, a recent report demonstrates that SOCCs can be activated without Ca^{2+} store depletion [34]. Thus, a

sustained SOCCs activity not only contributes to the maintenance of astrocytic Ca^{2+} store levels but can also be responsible for the generation of astrocytic Ca^{2+} events. Indeed, Ca^{2+} entry through SOCCs can trigger the Ca^{2+} release from ER.

3. Voltage-gated Ca^{2+} channels (VGCCs)

The role of VGCCs in astrocytic Ca^{2+} signaling remains controversial. These channels do not contribute to spontaneous Ca^{2+} events in astrocytes at the baseline conditions, but may be recruited upon membrane depolarization or under pathological conditions [35,36]

The expression of VGCCs is well documented in cultured astrocytes [37–41]. Functional VGCCs have been also reported in hippocampal slices [42]. VGCCs can serve as a sensor for astrocyte membrane depolarization [39,43]. in vivo, such depolarization can occur in response to activity-dependent K^+ accumulation. There are several sources of extracellular K^+ including K^+ efflux through postsynaptic ionotropic glutamate receptors, extrusion of K^+ along with Cl^- by K^+/Cl^- co-transporter 2 (KCC2) at inhibitory synapses, action potentials, glutamate uptake [44–47]. The activation of astrocytic VGCCs triggers release of gliotransmitters regulating synaptic strength [39,42].

However, there are reports casting doubt on the functional relevance of VGCCs in vivo [48,49]. Gene expression coding VGCCs is relatively low in astrocytes compared to neurons in the cortex [50]. No reduction in amplitude or frequency of spontaneous Ca^{2+} events has been observed in hippocampal astrocytes of $Ca_v1.3$ knockout mice [49]. L-type and T-type VGCCs blockers have no significant effect on the spontaneous Ca^{2+} events in astrocytes [49].

Carmignoto and coauthors argue that even astrocyte depolarization may not trigger Ca^{2+} entry through VGCCs [48]. They show that K^+ induced astrocytic Ca^{2+} events are attenuated by blockade of metabotropic glutamate receptors (mGluR) in hippocampal slices. Emptying intracellular Ca^{2+} stores or blocking the vesicular release with tetanus toxin also prevents these Ca^{2+} events. These findings suggest that K^+ elevation depolarizes the neurons, which release neurotransmitters producing Ca^{2+} elevations in astrocytes. An alternative explanation, however, is that Ca^{2+} entry through VGCCs is relatively small in astrocytes. It has to be amplified by Ca^{2+}-induced Ca^{2+} release from ER. This release is mediated by IP_3 receptors, that require both Ca^{2+} and IP_3 for their activation [51,52]. Blocking mGluRs and the vesicular release could affect IP_3 production, emptying Ca^{2+} stores would prevent Ca^{2+} release.

4. Na^+/Ca^{2+} exchanger (NCX)

NCX is considered the most important mechanism for Ca^{2+} removal from the astrocytes [53]. It uses Na^+ gradient bringing three Na^+ into the cell and taking one Ca^{2+} out of the cell. However, when intracellular Na^+ is elevated, NCX can operate in the reverse mode which leads to the Ca^{2+} influx. Na^+ enters the astrocyte through many pathways, including ionotropic receptors, Na^+-driven glutamate and GABA uptake [54,55]. In quiescent tissue, ambient glutamate concentration is maintained at a low level to prevent cytotoxic effects of this neurotransmitter [56]. Significant glutamate uptake occurs during synaptic transmission [57] Ambient GABA is present in quiescent tissue in higher concentrations and is responsible for persistent tonic $GABA_A$ receptor-mediated current [58]. Notably, astrocytic GABA transporters (GAT-3) are tuned to respond to extrasynaptic, rather than synaptic GABA [23]. Thus, astrocytic 'tonic GABA uptake' can potentially lead to constant Na^+ inflow contributing to the constitutive NCX reversal and Ca^{2+} entry [59]. Ca^{2+} entry through NCX can be further amplified by Ca^{2+}-induced Ca^{2+} release [60].

Notably, cytosolic Ca^{2+} and Na^+ also serve as allosteric modulators of NCX [61–63]. A modeling study predicts that Ca^{2+}-dependent activation of NCX and its relief from Na^+-dependent inactive state provides a positive feedback loop which can underlie regenerative Ca^{2+} influx [64]. Thus, NCX can mediate amplification and propagation of local Ca^{2+} events, even in the absence of Ca^{2+}-induced Ca^{2+} release from ER.

NCX has a complex relationship with K^+. Extracellular K^+ elevation depolarizes the astrocytes suppressing or even reversing electrogenic glutamate uptake [46,65]. Downregulation of glutamate uptake reduces Na^+ entry, hence, NCX-mediated Ca^{2+} influx. On the other hand, when intracellular Ca^{2+} is elevated, NCX removes it from the cell and loads the cell with Na^+. This activates Na^+/K^+ ATPase and promotes K^+ clearance [8].

5. IP_3 receptors

In astrocytes, Ca^{2+} release from ER is mediated by IP_3 receptors [66,67]. These receptors require both Ca^{2+} and IP_3 as co-agonists [51,68,69]. Thus, Ca^{2+} elevations, mediated by Ca^{2+} entry through the plasma membrane or by intracellular diffusion, can trigger the Ca^{2+} release from ER [43,52,70]. This process is known as Ca^{2+} -induced Ca^{2+} release and occurs when Ca^{2+} elevations reach the IP_3 receptor activation threshold. This threshold depends on the level of IP_3, which is produced by phospholipase C (PLC) [71]. PLC is a Ca^{2+}-dependent enzyme, and Ca^{2+} events can trigger IP_3 production [67,72]. However, PLC is classically activated by Gq-protein coupled mechanism. Astrocytes express several types of Gq-protein coupled receptors which can trigger IP_3 production. mGluR5 long thought to be the major signaling pathway triggering Ca^{2+} events in astrocytes following synaptic glutamate release [73,74]. However, it appeared that expression of mGluR5 significantly drops after postnatal week 3 and becomes negligible [75]. Interestingly, astrocytic mGluR5 re-emerge in animal models of temporal lobe epilepsy and in human patient epilepsy samples [76]. Gq-protein coupled purinergic P2Y receptors are also expressed in astrocytes [77,78]. Along with ionotropic P2X receptors, these receptors contribute to the propagation of Ca^{2+} events in the astrocytic network via ATP release. Serotoninergic 5HT2 A and adrenergic α1AR Gq-protein coupled receptors can also activate PLC in astrocytes [79–81].

When IP_3 level increases, it makes IP_3 receptors more sensitive to Ca^{2+} thus increasing the probability for amplification and propagation of Ca^{2+} events [52]. Indeed, application of mGluR agonist increases sizes and durations of Ca^{2+} events in astrocytic culture [82]. Similarly, low-frequency stimulation of glutamatergic Schaffer collaterals increased sizes and durations of astrocytic Ca^{2+} events in hippocampal slices [82,83]. This modulation of Ca^{2+} event properties was abolished by the mGluR antagonist.

IP_3 receptors are dynamically clustered along the ER by IP_3, which promotes intracellular Ca^{2+} wave propagation [84,85]. IP_3 buffering prevents the activity-dependent spread of astrocytic Ca^{2+} events in an inducible transgenic mouse expressing of an IP_3 absorbent, 'IP_3 sponge' [10]. Knock out of astrocyte-specific IP_3 receptors also limits the amplification and spread of Ca^{2+} events [49,61,86].

6. Ca^{2+} events and mitochondria

Another important player in astrocytic Ca^{2+} dynamics is mitochondria [87]. Mitochondria generally act as a Ca^{2+} buffer and respond to Ca^{2+} elevation either by increasing the energy supply or by triggering apoptosis [88]. Mitochondrial Ca^{2+} fluxes are mediated by mitochondrial NCX, H^+/Ca^{2+} exchanger (HCX), mitochondrial Ca^{2+} uniporter (MCU) and the permeability transition pore (PTP) [89]. These Ca^{2+} transport mechanisms are located in the inner mitochondrial membrane, since the outer mitochondrial membrane is permeable to soluble molecules smaller 5 kDa, including Ca^{2+}. MCU drives large Ca^{2+} entry to mitochondria, but it operates at micromolar cytosolic concentrations of this ion [90]. Because mitochondria are located in close proximity to the ER. MCU is well placed to contribute to the

removal of Ca^{2+} released to the cytosol through IP_3 receptors [91,92]. Unlike MSU, HCX operates at nanomolar Ca^{2+} concentrations and may influence such processes as Ca^{2+}-induced Ca^{2+} release [88]. A recent report has suggested that brief openings of PTP are responsible for astrocytic spontaneous Ca^{2+} events in the absence of IP_3 receptor-mediated Ca^{2+} release [87].

7. Synaptic and extrasynaptic mechanisms of Ca^{2+} activity in astrocytes

Astrocytic processes can be classified into three morphological types: (1) flat organelle-free astrocytic leaflets, (2) astrocytic branchlets hosting ER and mitochondria, and (3) perivascular endfeet contacting blood vessels [93–96]. In the context of Ca^{2+} signaling, astrocytic processes can be divided into active and passive. Active processes are branchlets and endfeet that contain Ca^{2+} stores [94,97]. These processes can generate propagating Ca^{2+} transients mediated by Ca^{2+} induced Ca^{2+} release. Despite previous beliefs, that synaptic activity directly triggers Ca^{2+} release from intracellular Ca^{2+} stores through mGluRs, active branchlets in the hippocampus have direct contact with only 22.8% of thin dendritic spines and 23.4% of mushroom dendritic spines [96]. Thus, neurotransmitter has to diffuse extrasynaptically to reach active processes for the majority of synapses, or the signal (Ca^{2+} or IP_3) has to propagate diffusively inside of the passive processes (Figs. 1 and 2A, B).

Passive processes are astrocytic leaflets that are flat cytoplasmic protrusions of branchlets which are devoid of organelles including Ca^{2+} stores [94]. Leaflets extend towards synapses and form perisynaptic astrocytic processes (PAPs) (Fig. 2A). The PAPs distribution is not homogenous, and their density is higher at the spine side than at the axonal varicosity side of the synapse in hippocampal CA1 [96,98]. The volume fraction of PAPs in the vicinity of dendritic shafts is relatively low, which promotes activation of extrasynaptic receptors and neurotransmitter spillover from the shaft synapses (e.g. GABAergic) [96]. Notwithstanding the lack of Ca^{2+} stores, Ca^{2+} transients can occur in passive leaflets due to Ca^{2+} entry through the plasma membrane. Astrocytic leaflets are thin, flat structures highly packed with glutamate transporters which ensure a rapid rise in intracellular Na^+ during glutamate uptake [99–101]. Then Na^+ is exchanged for Ca^{2+} with NCX which produces a Ca^{2+} elevation in the process.

Three-dimensional (3D) ultrastructural analysis demonstrated that the mean distance from postsynaptic density (PSD) to astrocytic Ca^{2+} stores is 1 μm in hippocampal neuropil [94]. This distance hardly can be overpassed by diffuse glutamate escaping synaptic cleft because of efficient glutamate uptake [102]. However, Ca^{2+} elevations may occur in the perisynaptic astrocytic leaflets. Then Ca^{2+} can diffuse inside of the leaflet towards the active branchlet. A mathematical model suggests that the flat structure of the leaflet favors intracellular diffusion, and the Ca^{2+} molecules can reach longer distances in the leaflet than in round astrocytic branchlet [3,103]. In addition, the ability of NCX to generate regenerative Ca^{2+} propagation may aid Ca^{2+} diffusion in the leaflet [64]. Activation of mGluRs in the leaflets by synaptically released glutamate is also possible. This would create a local source of IP_3 provided the presence of PLC in the vicinity of these receptors [71]. A mathematical model of IP_3 diffusion in perisynaptic astrocytic leaflet suggests that IP_3 forms a concentration gradient along the leaflet and reaches a steady-state in milliseconds, which is three orders of magnitude faster that IP_3 degradation [104]. Thus, both Ca^{2+} and IP_3 elevations in astrocytic leaflets can propagate into parent branchlet to trigger Ca^{2+} induced Ca^{2+} release [105]. The branchlet, therefore, serves as an integrator of synaptic activity sensed by the leaflets. In addition to synaptic signaling, both perisynaptic leaflets and active branchlets can receive multiple diffuse signals mediated by a range of neuro- and gliotransmitters (e.g. ATP, dopamine, noradrenaline, acetylcholine, GABA, etc.) [6]. They can activate both ionotropic and Gq-coupled receptors in the astrocytic membrane (Fig. 1). Both leaflets and branchlets can possess various Ca^{2+} channels that open spontaneously or respond to respond to changes in the local environment (K^+, P_{O2}, pH, vasomotion, osmotic pressure). However, the precise subcellular distribution of Ca^{2+} conductances in the plasma membrane requires further investigation.

8. Ca^{2+} activity and astrocyte morphology

Flat structure of astrocytic leaflets allows them to ensure a high density of astrocyte surface in the vicinity of synapses, which is necessary for the efficient glutamate uptake [94,98,106]. The perisynaptic leaflets can retract or extend towards synapses under different physiological and pathological conditions. Astrocytic processes retract from glutamate synapses in the hypothalamic supraoptic nucleus of lactating rats [107] and from cortical synapses during sleep [108]. This remodeling leads to reduced glutamate clearance. Following LTP induction perisynaptic leaflets also transiently withdraw from the spines to allow their growth [109–111]. Such leaflet retraction also temporarily increases glutamate spillover. How the retraction of astrocytic leaflets affects the Ca^{2+} response of astrocytes to synaptic activity remains poorly understood. On the other hand, the level of astrocytic Ca^{2+} activity correlates with the remodeling of PAPs. Suppression of astrocytic Ca^{2+} elevations in 'IP_3 sponge' expressing mouse reduces synaptic coverage by the leaflets and promotes glutamate spillover [10].

Astrocytic branchlets undergo significant remodeling both in physiological and pathological processes. Brain region-specific remodeling of astrocytic branchlets occurs with aging: the number of branchlets increases in astrocytes of hippocampal CA1 and dentate gyrus, and decreases in entorhinal cortex [112]. Reduction in the number of branchlets is observed 2 weeks after pilocarpine-induced *status epilepticus* in rat hippocampal astrocytes [12]. Similarly, astrocytic atrophy is reported in a mouse model of Alzheimer's disease (3xTG-AD) in various brain regions [113]. This atrophy is reversed by an enriched housing environment in mice and their physical activity [114].

The remodeling of astrocytic branchlets can affect astrocytic Ca^{2+} activity in two ways. First, changes in the number of branchlets and therefore the amount of Ca^{2+} stores will affect astrocyte ability to amplify local Ca^{2+} events. Indeed, the developmental increase in the complexity of astrocytic branchlets is associated with an increase in the sizes of astrocytic Ca^{2+} events, but not in their frequency [115]. Astrocytic atrophy after *status epilepticus* parallels a decrease in sizes of astrocytic Ca^{2+} events, but not in their frequency [12]. Second, the SVR of the branchlets determines the probability of Ca^{2+} events initiation (Fig. 2C) [43]. Thinner branchlets have higher SVR and therefore Ca^{2+} entering such branchlets will produce larger Ca^{2+} concentration change in the cytosol [49]. In the agreement, fluorescence lifetime imaging microscopy (FLIM) has demonstrated higher integral Ca^{2+} concentration in thin distal astrocytic branchlets than in thick proximal processes [116]. Therefore, changes in the thickness of astrocytic branchlets (or the proportion of thin and thick branchlets) can affect astrocyte ability to integrate synaptic and extrasynaptic inputs and generate Ca^{2+} events.

9. Intercellular Ca^{2+} event propagation

The astrocytic network can generate spontaneous Ca^{2+} events of different sizes and durations [83,117]. These events persist under the blockade of neuronal action potentials. However, their frequency is significantly reduced by the blockade of vesicular release by bafilomycin A1 in hippocampal slices [83]. Viral expression of tetanus toxin blocks the vesicular release in cultured astrocytes and prevents spread of Ca^{2+} events in astrocytic network triggered by mechanical stimulation [118]. These findings suggest that vesicular release of gliotransmitters is important for intercellular propagation of Ca^{2+} events in the astrocytic network. The vesicular ATP release has been identified as a mechanism for such signaling [18,119].

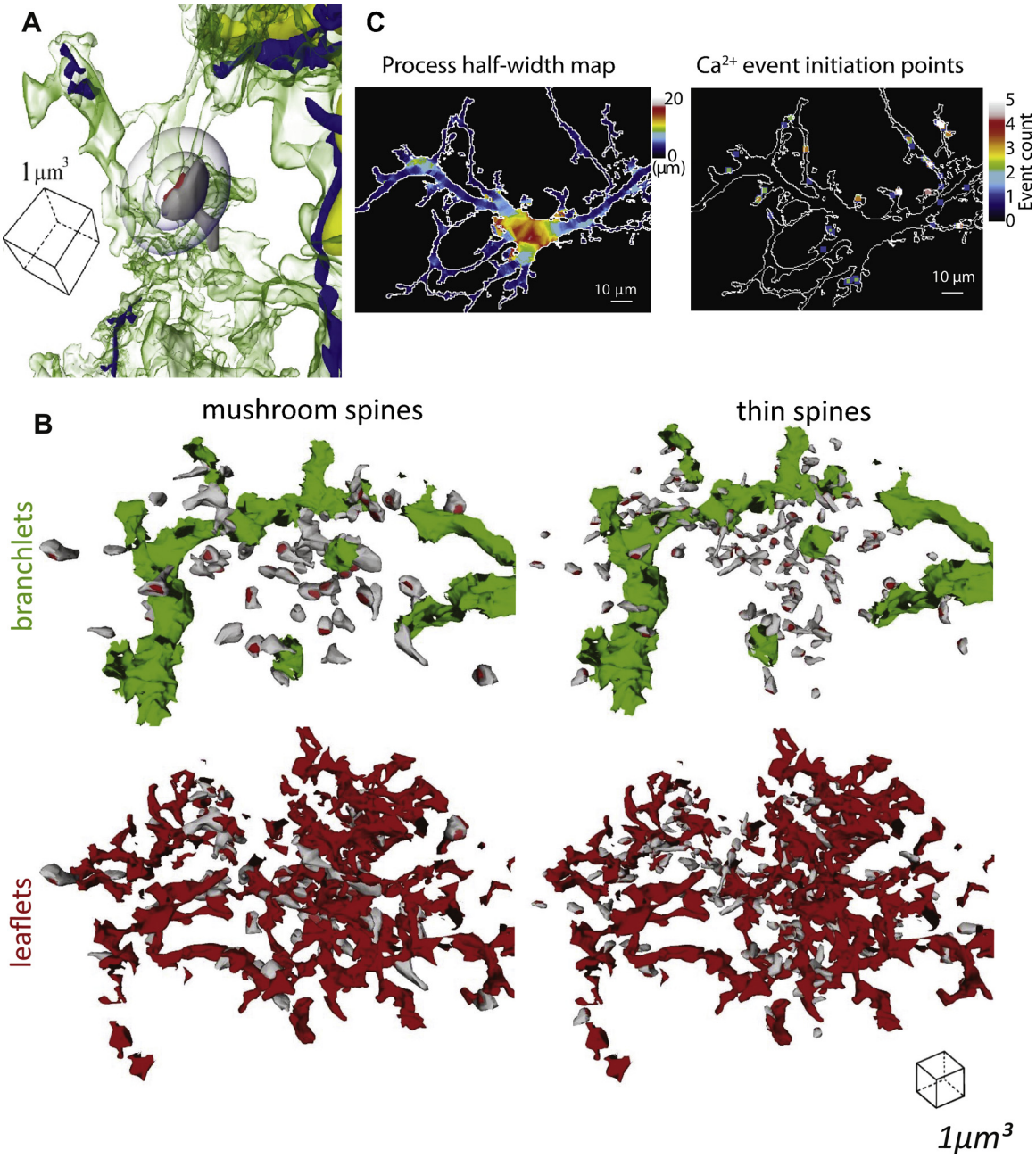

Fig. 2. Morphofunctional properties of astrocytic processes.

A. 3D reconstruction based on serial section electron microscopy of rat hippocampal neuropil. Two types of astrocytic processes can be identified: (1) organelle-free leaflets ('empty' green processes) approaching the synapse and (2) branchlets containing Ca^{2+} stores: ER (blue) and mitochondria (yellow). Gray – dendritic spine, red – postsynaptic density (PSD). Reproduced with permission from [94].

B. The spatial relationship between dendritic spines (gray), astrocytic branchlets (green) and astrocytic leaflets (red). 3D reconstruction based on serial section electron microscopy of rat hippocampal neuropil. Both mushroom and thin spines are colocalized with the leaflets and rarely contact the branchlets. Reproduced with permission from [96].

C. Initiation of astrocytic Ca^{2+} events depends on the surface-to-volume ratio (SVR) of the process. Ca^{2+} events more frequently start in thin astrocytic processes (with higher SVR), than in thick processes or soma. *Left*, a map of process half-width of rat cultured astrocyte. *Right*, distribution of Ca^{2+} events initiation points within this astrocyte. Adapted with permission from [43].

ATP mediates long-range Ca^{2+} event propagation within the astrocytic network, but its speed is limited by the gliotransmitter diffusion in tortuous extracellular space and, therefore, is relatively slow. Faster propagation of Ca^{2+} activity from one astrocyte to its immediate neighbors is mediated by gap-junctions [120]. Astrocytic gap-junctions contain connexins forming hemichannels or connexons. Each connexon consists of six identical connexins. The two connexons of neighboring cells form a gap-junction channel. In the mature brain, astrocytes express high levels of connexin 30 and connexin 43 and low level of connexin 26 [121]. Connexin 43 is regulated by phosphorylation following cerebral ischemia or neural activation [122–124]. Connexons are permeable to small molecules including fluorescent dyes and biocytin. Therefore, it is possible to count the number of stained cells following loading of the target astrocyte through the patch pipette. This method has demonstrated that each astrocyte is coupled via gap-junctions to 15.3 ± 2.8 its neighbors in rat hippocampal CA1 [12] and

18.1 ± 12.8 its neighbors in mouse hippocampal CA1 [125]. Gap-junction coupling is anisotropic and cell-specific [126]. It changes during development, in response to various stimuli and under pathologic conditions [127–130]. Although gap-junctions are permeable to both IP_3 and Ca^{2+}, they can be also closed by Ca^{2+} elevation in the cytosol [131]. However, Toyofuku and co-authors have directly demonstrated that Ca^{2+} wave can propagate through the gap-junctions [132]. They have developed a cellular system in which HEK293 cells expressing both connexin-43 and ryanodine receptor have been surrounded by HEK293 cells expressing only connexin 43. Application of caffeine produced a Ca^{2+} wave originating from caffeine-sensitive cells and propagating through caffeine-insensitive cells. This effect could be abolished by a gap-junction blocker.

10. The astrocytic Ca^{2+} pattern is modulated by neuronal activity and astrocyte environment

Astrocytes readily respond to single pulse synaptic stimulation with substantial transporter and K^+ currents recorded in soma [45,46,133,134]. This suggests that astrocytic processes must be exposed to significant concentrations of both glutamate and K^+. However, the detectable Ca^{2+} response is astrocyte often requires high-frequency synaptic stimulation (HFS) in slices [86,135,136] or observed when neurons fire a burst of action potentials in vivo [137,138]. Moreover, the astrocytes respond to neuronal bursts with a variable latency of several seconds [137,139]. Such long latency cannot be explained by intracellular Ca^{2+} diffusion, IP_3 production or Ca^{2+} release from Ca^{2+} stores which are much faster. A parsimonious explanation is that synaptically released glutamate does not directly trigger Ca^{2+} events in astrocytes, but modulates their properties: the frequency, the durations and the spreads [82,83]. An increase in the frequency will also raise the probability of Ca^{2+} event detection with variable latency following the stimulation. The appearance of such Ca^{2+} event may be misinterpreted as directly triggered astrocyte response. In addition, neuronal stimulation increases spread of Ca^{2+} events [82,83]. When Ca^{2+} event spreads, it can be detected in a neighboring region-of-interest (ROI) and be also interpreted as stimulation triggered Ca^{2+} transient.

Spontaneous Ca^{2+} events in astrocytes are modulated not only by neuronal activity but also by changes in the cell environment. For example, a decrease of the partial pressure of oxygen (P_{O2}) increases Ca^{2+} activity in astrocytes [118,140]. Brain stem astrocytes respond to a decrease in extracellular pH with elevations in intracellular Ca^{2+} and facilitated exocytosis of ATP-containing vesicles [141].

11. The timescale of astrocyte Ca^{2+} signaling

One of the major questions related to astrocyte Ca^{2+} signaling is its timescale. The duration of neuronal events such as action potentials (few ms) or excitatory/inhibitory postsynaptic potentials (EPSP/IPSP, tens of ms) is typically orders of magnitude shorter than that of Ca^{2+} events in astrocytes (from hundreds of ms to several s) (Fig. 3A–C). This raises a question if the astrocytic activity can be involved in real-time information processing in the brain: the average human reaction time to visual stimulus is 250 ms, to audio stimulus 170 ms and to touch stimulus 150 ms. In fact, this reaction is largely unconscious [142]. Conscious intention to move takes 1.42 s [143]. During such period shortest astrocytic Ca^{2+} events can participate in the information processing.

It is also important to compare not individual events, but encoding sequences. Several coding schemes are proposed for neuronal representation of information: rate coding, temporal coding, and population coding. In each case, a spike train is generated [144]. Although the duration of the sequences may vary, it substantially increases the coding timescale relatively for the duration of individual action potentials.

Astrocytes encode stimuli in spatial and temporal properties of Ca^{2+} events. These events form a spatial pattern within individual cells and within the astrocytic network (Fig. 3B, C) [12,43,115,145]. If such pattern serves a coding function in astrocytes as a temporal sequence of action potentials in neurons, one should consider the timescale of the pattern changes, not the durations of individual Ca^{2+} events in astrocytes. Because each Ca^{2+} event starts and ends at different times, the overall pattern of Ca^{2+} activity in the astrocytic network changes instantaneously. Thus, the sequences of neuronal firing and changes in the astrocytic Ca^{2+} activity pattern exist at the same timescale.

12. Guiding template

Astrocytes control synaptic circuits in several ways, that include the uptake of neurotransmitters, extracellular matrix remodeling, energy supply, K^+ clearance, the release of gliotransmitters [146]. Most of these mechanisms directly or indirectly related to astrocyte Ca^{2+} activity. For example, Ca^{2+} elevation triggers the vesicular release of gliotransmitters such as glutamate or D-serine [136,147]. Notably, the delay of vesicular release after Ca^{2+} elevation is orders of magnitude longer in astrocytes than in neurons [148]. Such loose excitation-secretion coupling prevents immediate discharge of all the vesicles at the onset of the astrocytic Ca^{2+} event and may ensure a consistent presence of gliotransmitter during astrocytic Ca^{2+} activity. In addition to the vesicular release, Ca^{2+} elevations enhance Ca^{2+}-dependent K^+ clearance [8], induce remodeling of perisynaptic astrocytic leaflets [149]. Na^+-driven uptake of neurotransmitters (GABA and glutamate) depends on the Na^+/Ca^{2+} exchange by NCX [54,55].

Interestingly, the Ca^{2+}-dependent release of gliotransmitters bidirectionally modulates neuronal signaling. This obvious contradiction may be resolved if the bidirectional effects are separated in space or time. It has been suggested, the level of neuronal activity could determine the release of different gliotransmitters from a single astrocyte [150]. However, it would not work if the same gliotransmitter causes bidirectional effects. For example, astrocytic glutamate can increase GABA release by targeting presynaptic kainate receptors and decrease GABA release by acting on presynaptic mGluRs [151,152]. In fact, these two actions of glutamate do not cancel each other: kainate receptor activation promotes action potential-dependent release of GABA, while activation of presynaptic mGluRs suppresses both spontaneous and action potential-dependent GABA release [153]. The overall effect of two receptor coactivation is bi-directional activity-dependent modulation of inhibitory signal propagation in the neuronal network [154].

Ultimately, astrocytic Ca^{2+} elevations may be linked to spatiotemporal patterns of glio- and neurotransmitters surrounding specific elements of neuronal and astrocytic networks. If each Ca^{2+} event within astrocytic domain produces a cloud of the gliotransmitter release of equal size, it is possible to estimate the number of synapses affected (Fig. 3D) [82]. The mean distance between glutamate synapses in hippocampal neuropil is 0.5 μm [102]. Thus, the single astrocytic Ca^{2+} event can target from tens to thousands of synapses. When the Ca^{2+} events propagate in the astrocytic network they can potentially target millions of spatially distributed synapses.

A decade ago I termed spatially and temporarily inhomogeneous pattern of ambient signaling molecules in the brain as a ‘guiding template’ [155]. This concept reflects the ability of diffuse molecules to guide the excitation flow in the neuronal networks by altering the properties of synapses and other neuronal compartments. Such guiding template is formed by superposition of release sources (spillover, astrocytes, non-vesicular release by neurons) and sinks (diffusion, uptake, enzymatic inactivation) of diffuse transmitters. Since many of these factors are directly triggered or influenced by astrocytic Ca^{2+} activity, it would be reasonable to consider the spatiotemporal properties of Ca^{2+} activity in the astrocytic network as a mechanism involved in the formation of a guiding template. Different levels of neuronal activity can summon different guiding templates, which in turn can alter the state of neuronal networks. Multiple states of the neuronal network will

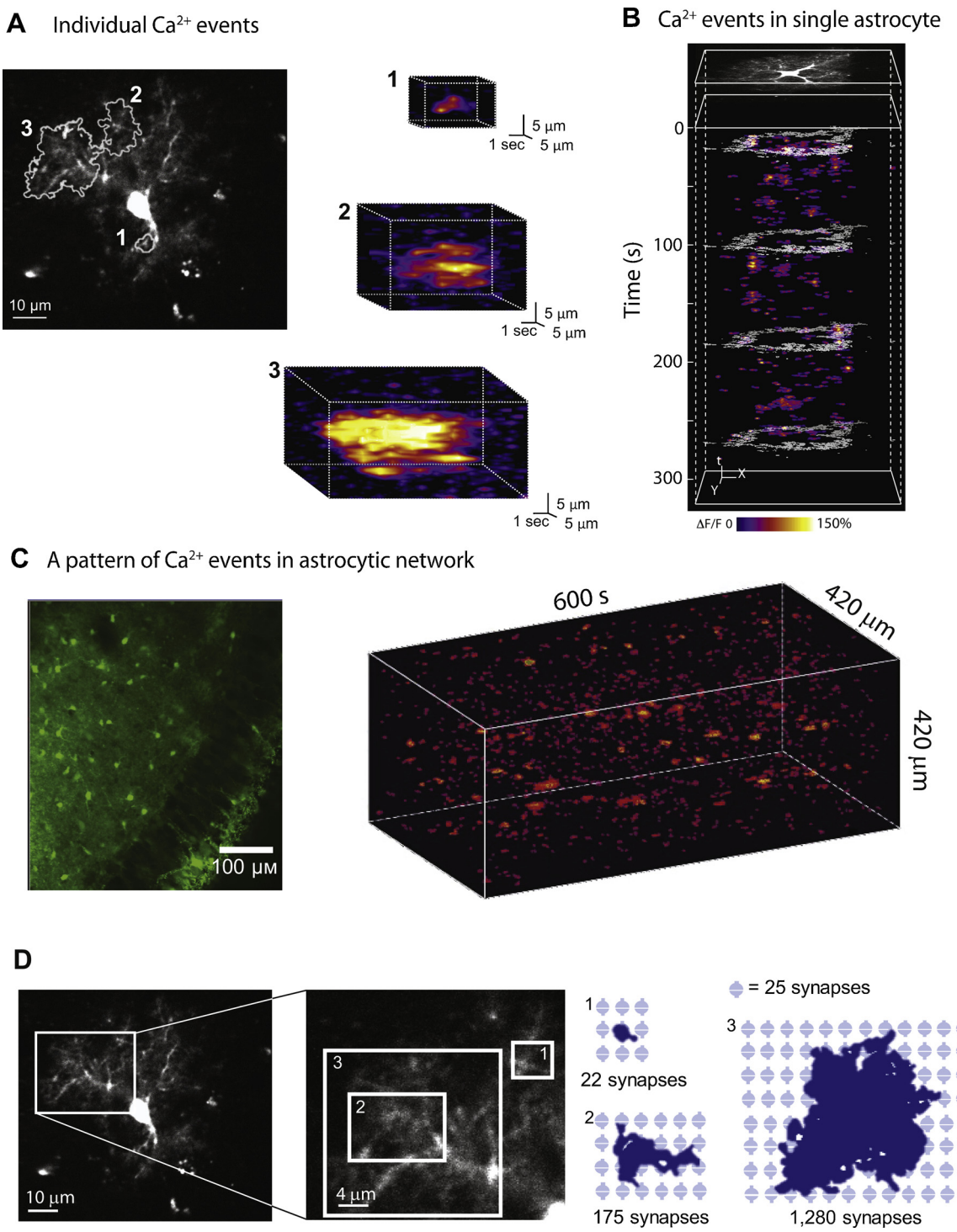

Fig. 3. Spatiotemporal pattern of Ca^{2+} events in astrocytes.

A. Spatiotemporal properties of individual Ca^{2+} events in single astrocyte expressing Ca^{2+} sensor GCaMP2 in mouse hippocampal slice. *Left*, fluorescent image of the astrocyte with outlined areas of three Ca^{2+} events. *Right*, 3D (x-y-time) reconstructions of three individual Ca^{2+} events outlined on the left. Note, variable Ca^{2+} event sizes and durations. Adapted with permission from [82].

B. Spatiotemporal pattern of Ca^{2+} events in single astrocyte expressing Ca^{2+} sensor GCaMP2 in mouse hippocampal slice. 3D (x-y-time) reconstruction of Ca^{2+} events within single astrocyte domain. Adapted with permission from [43].

C. A pattern of Ca^{2+} events in astrocytic network stained with Oregon Green 488 BAPTA-1 in rat hippocampal slice. *Left*, CA1 region of rat hippocampal slice stained with membrane-permeable Ca^{2+} dye, Oregon Green 488 BAPTA-1AM. This dye predominantly stains astrocytes. *Right*, 3D (x-y-time) reconstruction of astrocytic Ca^{2+} events (> 2 s) within hippocampal CA1. Adapted with permission from [12].

D. The number of synapses covered by astrocytic Ca^{2+} events induced in single astrocyte. *Left*, the same astrocyte as at the panel A. Square box indicated the zoomed in the region within the astrocytic domain. Square boxes within the zoomed in the region indicate the boundaries of Ca^{2+} events territories shown on the right. *Right*, three Ca^{2+} events detected in the zoomed in the area of the astrocytic domain. The territory of each event overlaid on the synapses. The synaptic density is assumed from a previously reported mean distance of 0.5 μm between neighboring synapses in hippocampal neuropil. Adapted with permission from [82].

significantly enhance the brain's ability for information processing and storage [144,156].

13. Concluding remarks

Many mechanisms governing Ca^{2+} dynamics in astrocytes have been well documented. It is established that Ca^{2+} enters the astrocytes through the plasma membrane and triggers Ca^{2+}-induced Ca^{2+} release. Astrocytic processes consist of active branchlets and passive perisynaptic leaflets. Ca^{2+} events in astrocytes appear in a variety of sizes and durations and form a pattern within individual cells and astrocytic network. This pattern rapidly changes and may induce multiple states of the local neuronal network.

However, many questions are still left for future research. Often an astrocytic Ca^{2+} pattern has been studied by two-dimensional (2D) confocal or two-photon imaging. This imaging provides only limited information about actual 3D Ca^{2+} events [116,157,158]. Indeed, it is unclear if Ca^{2+} 'microdomains' recorded with 2D imaging in brain slices or in vivo reflect the full size of Ca^{2+} events of just anatomical boundaries determined by the position of the focal plane [117,159]. In fact, more accurate information about spatiotemporal properties of Ca^{2+} pattern in the astrocytic network requires 4D (x-y-z-time) fast imaging combined with novel methods of analysis, which can possibly recruit multivariable regression analysis (also known as 'Big Data' analysis) and artificial intelligence (AI) systems [158].

The role of the astrocytic Ca^{2+} pattern in brain computation and memory need to be further investigated. The concept of guiding template can be possibly used in computational neuroscience. Most of current 'neuron only' models can be 'upgraded' with the astrocytic network. Experimental addressing of several questions related to population dynamics in the astrocytic network is also needed. (1) To guide signaling in the neuronal network, the astrocytic Ca^{2+} pattern should be reproducible. The same level or the sequence of neuronal activity and environmental factors should summon the same astrocytic Ca^{2+} pattern or a pattern with the same critical characteristics. (2) To store information, the astrocytic Ca^{2+} pattern should undergo some form of long-term plasticity. There should be a rule (analogous to Hebbian rule for synaptic plasticity) describing the conditions which should be met to alter the activity-dependent properties of the astrocytic Ca^{2+} pattern. In fact, long-term plasticity of the astrocytic Ca^{2+} pattern can be related to morphological remodeling of astrocytes such as retraction or extension of perisynaptic astrocytic leaflets, changes in the number and diameter of active branchlets, the gap-junction coupling of astrocytes.

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