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HOMEOSTATIC FUNCTION OF ASTROCYTES: Ca^{2+} AND Na^+ SIGNALLING

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Abstract

The name astroglia unifies many non-excitable neural cells that act as primary homeostatic cells in the nervous system. Neuronal activity triggers multiple homeostatic responses of astroglia that include increase in metabolic activity and synthesis of neuronal preferred energy substrate lactate, clearance of neurotransmitters and buffering of extracellular K^+ ions to name but a few. Many (if not all) of astroglial homeostatic responses are controlled by dynamic changes in the cytoplasmic concentration of two cations, Ca^{2+} and Na^+. Intracellular concentration of these ions is tightly controlled by several transporters and can be rapidly affected by the activation of respective fluxes through ionic channels or ion exchangers. Here, we provide a comprehensive review of astroglial Ca^{2+} and Na^+ signalling.

Keywords

• Astrocyte • Homeostasis • Excitability • Ca^{2+} signalling • Na^+ signalling

Abbreviations:

AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
$[Ca^{2+}]_i$ - cytoplasmic free Ca^{2+} concentration
$[Ca^{2+}]_L$ - intra-ER (or intraluminal) free Ca^{2+} concentration
CNS - central nervous system
CRAC - Ca^{2+}-release activated Ca^{2+}
ER - endoplasmic reticulum
GABA - γ-aminobutyric acid
$InsP_3R$ - inositol 1,4,5 trisphosphate ($InsP_3$)-gated Ca^{2+} channel/receptor
$[Na^+]_i$ - cytoplasmic Na^+ concentration
NCX - Na^+/Ca^{2+} exchanger
NKA - Na^+/K^+ ATPase
NMDA - N-methyl D-aspartate
PLC - phospholipase C
PMCA - plasmalemmal Ca^{2+} ATPase
RyR - ryanodine receptor
SERCA - sarco(endoplasmic) reticulum Ca^{2+} ATPase
SLC - solute carrier
TRP - transient receptor potential

1. Astroglia - the homeostatic cells of the brain

The nervous system in mammals represents complex network formed by several distinct cell types of neural crest and non-neural crest origin. In the central nervous system (CNS) the neural cells are neurones, astrocytes, NG2 glia and myelinating oligodendrocytes, whereas the non-neural cells are the microglia. In the peripheral nervous system the neural elements include sensory, sympathetic and parasympathetic as well as enteric neurones and highly diversified peripheral glia represented by satellite glial cells, enteric glia, and myelinating, non-myelinating and perisynaptic Schwann cells. All these diverse cells are unified through several levels of intercellular signalling accomplished by inter- and intracellular diffusion of various molecules that either bind and stimulate the plasmalemmal receptors or penetrate cellular membranes through specific ion channels, thus initiating electrical excitation (by virtue of charges carried by ions) or triggering local cytoplasmic responses by interacting with variety of intracellular targets.

Among many neural cells forming the CNS astroglial cells have a specific role of a main homeostatic element. Astrocytes (which means star-like cells, the name invented by Michael von Lenhossek [1,2]) represent a highly heterogeneous cell population, which include protoplasmic astrocytes of grey matter of the brain and the spinal cord, fibrous astrocytes localised in the white matter, classical radial glia that acts as a pluripotent neural precursor cell during development, radial Müller retinal glial cells, pseudo-radial cerebellar Bergmann glial cells, velate astrocytes of cerebellum, tanycytes that connect ventricular walls with parts of hypothalamus and spinal cord, pituicytes in the neuro-hypophysis, and perivascular and marginal astrocytes. The brain of higher primates also contains interlaminar, polarised and varicose projection astrocytes, with all these types being particularly developed in the human brain (see [3-14] for details and

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relevant references). In addition, astroglia cover several types of specialised cells such as ependymocytes, choroid plexus cells and retinal pigment epithelial cells.

Astrocytes appeared early in evolution, first as supporting cells and then gained their functional importance in the course of specialisation of the nervous system. Functions of astrocytes are many: i) they define the brain microanatomy and provide structural support for other cellular elements of the CNS; ii) they synthesise and store glycogen and produce lactate, the latter being preferred metabolic substrate for neurones; iii) they control homeostasis and turnover of several key neurotransmitters and neuromodulators (for example, glutamate and adenosine); and iv) they regulate synaptic connectivity through supporting synaptogenesis, controlling ion/transmitter concentrations in the synaptic cleft and regulating synaptic plasticity via secreting various neuroactive factors [14-16].

2. Specific nature of astroglial excitability

Excitability of neurones and neuroglia are fundamentally different. Signalling in neuronal networks is mainly accomplished through propagating waves of transient opening of the plasmalemmal ion channels that provide fluxes of ions underlying electrical excitability. These propagating waves, manifested by action potentials, can rapidly (up to 100 m/s) convey excitation through neuronal axons. Electrical signals, when reaching neuronal terminals, activate local Ca^{2+} entry that initiates exocytotic release of neurotransmitters. The latter diffuse through the synaptic cleft and transfer excitation to the postsynaptic neuronal structures through the activation of specific receptors [17-22].

Glial cells, in contrast to neurones, cannot generate plasmalemmal action potentials, because of low densities of voltage-operated ion channels and high expression of K^+ channels that prevent substantial depolarisations of glial membranes. Instead, glial cells use intracellular signalling routes where local gradients of ions interact with intracellular targets and trigger physiological reactions. In addition, glial cells, and astrocytes in particular, are physically connected into syncytia by means of intercellular contacts represented by gap junctions. The gap junctions are made by closely apposing plasmalemma of two neighbouring cells with a narrow (~ 2 - 2.5 nm) intercellular cleft. Here, specialised intercellular channels, which span both membranes and establish direct intercellular contacts, are concentrated. These junctional channels are composed of two aligned "hemichannels" or connexons each made up from 6 subunits or connexins [23,24]. Out of about 20 known connexins [25,26] astroglial cells express mainly connexins Cx43, and to a lesser extent Cx30 and Cx26; as minor constituents of their gap junctions, astrocytes can also express Cxs 45, 40 and 46 [27]. These gap junctional channels have a large pore (with a diameter of ~ 1.5 nm), which allows intercellular passage of ions and various active substances such as second messengers [e.g., inositol 1,4,5 trisphosphate ($InsP_3$)], nucleotides (ATP, ADP) or metabolic substrates (glucose). As a result, the gap junctions provide a specific route for intercellular and long-range signalling, which is manifested in propagating waves of ionic (Ca^{2+} or Na^+) signals, or metabolic waves. The communication through gap junctions is of course much slower when compared with the spread of action potentials (on average, for example, Ca^{2+} waves propagate through astroglial syncytia with a speed of ~ 20 - 40 μm/s); however, it is more diversified and may provide a substrate for integrating intercellular signalling. It should be noted, however, that, although the above described gap junctional communication contributes to intercellular Ca^{2+} waves, it is ATP, which gets released from astrocytes, that serves as an extracellular signal to majorly support the intercellular Ca^{2+} waves [28-31].

Fast signalling in astroglial cells, which can be initiated by neuronal activity or many different neurotransmitters and neuromodulators, is mediated by intracellular ion gradients. In this essay, we shall concentrate on two ions, Ca^{2+} and Na^+, which mediate signalling in astroglia and discuss in detail glial signalling mediated by them. It should be noted that the regulation of the Ca^{2+} dynamics (and possibly also of Na^+) could differ in various subcellular locations of astrocytes, which could result in local signaling, rather than the above long-range intercellular waves. For instance, astrocyte perisynaptic processes are the sites where synapses can evoke local Ca^{2+} elevations that could result in a local feedback signalling via the gliotransmitter release [32-34].

3. Ca^{2+} signalling in astroglia

3.1. Molecular machinery of Ca^{2+} signalling

Evolutionary, Ca^{2+} has emerged as one of the most universal intracellular second messengers, due to its unique qualities (flexible coordination chemistry, high affinity for carboxylate oxygen, which is the most frequent motif in amino acids, and rapid binding kinetics) and by its availability in the primordial ocean [35,36]. At high concentrations, Ca^{2+} ions cause numerous anti-life effects such as protein and nucleic acid aggregations or precipitation of phosphates; in addition ATP-based energetics require low levels of Ca^{2+} in the cytosol. These factors stipulated the general principle of Ca^{2+} signalling, which is based around steep concentration gradients for Ca^{2+} between the cytosol and the extracellular environment as well as various intracellular compartments. These concentration gradients create electro-driving force for Ca^{2+} aimed at the cytosol where resting Ca^{2+} concentration is kept at level between 50 and 100 nM. Ca^{2+} movements across cellular membranes occur either via diffusion through Ca^{2+} permeable channels or by transport with ATP-consuming pumps or ion-dependent exchangers; the former underlie downhill Ca^{2+} translocation (i.e. in the direction of electro-chemical gradient) whereas the latter provides for up-hill (i.e. against electro-chemical gradient) Ca^{2+} flux. Ca^{2+}-permeable ion channels are represented by several families, which include highly Ca^{2+} selective voltage-gated Ca^{2+} channels, intracellular Ca^{2+} channels ($InsP_3$ receptors or $InsP_3$Rs and ryanodine receptors or RyRs), and plasmalemmal Ca^{2+}-release activated Ca^{2+} channels (CRAC channels, that on a molecular level represent activity of Orai proteins) and cationic channels with various degrees of Ca^{2+} permeability (Figure 1). The latter cationic channels are represented by ligand-gated

channels (or ionotropic neurotransmitter receptors such as, for example, glutamate, ATP or nicotinic acetylcholine receptors), by extended family of transient receptor potential (TRP) channels and some other types of cationic channels. Ca^{2+} transport against concentration gradients is mainly accomplished by plasmalemmal Ca^{2+} ATPases (PMCA or plasmalemmal Ca^{2+} pumps), by SarcoEndoplasmic reticulum ATPases (SERCA or endoplasmic reticulum Ca^{2+} pumps) and by ion exchangers of which the Na^+/Ca^{2+} exchanger (NCX) is by far the most important. Inside the cell, Ca^{2+} is buffered by Ca^{2+} binding proteins (CBPs), affinity of which to Ca^{2+} differs in different cellular compartments. For example, Ca^{2+} affinity of cytosolic CBPs lies in a low nM range, whereas endoplasmic reticulum (ER) CBPs have K_D for Ca^{2+} at ~ 0.5 mM. These different affinities determine the range of diffusion of Ca^{2+} ions. In the cytosol, CBPs limit diffusion and favour development of local high-Ca^{2+} concentration microdomains, whereas, in the ER, CBPs allow almost free and long-distance Ca^{2+} diffusion that being instrumental for making ER Ca^{2+} tunnels [37,38]. Cellular Ca^{2+} homeostasis is also regulated by mitochondria which are able to accumulate Ca^{2+} (via electrochemically driven diffusion through Ca^{2+} selective channel generally referred to as Ca^{2+} uniporter) and to release Ca^{2+} through mitochondrial Na^+/Ca^{2+} exchanger as well as transient openings of mitochondrial permeability transition pore [39,40].

Effectors of Ca^{2+} signals are Ca^{2+} regulated enzymes (also known as "Ca^{2+} sensors"), binding of Ca^{2+} to which affects functional activity. These Ca^{2+} sensors are many; they have different affinities to Ca^{2+} and are heterogeneously distributed between cellular compartments. These specificities of Ca^{2+} sensors sensitivity to Ca^{2+} and their cellular distribution underlie amplitude and spatial coding of Ca^{2+} signals.

The shape and spatio-temporal organisation of Ca^{2+} signals are defined by the interplay between Ca^{2+} diffusional fluxes and Ca^{2+} transport (Figure 1). Combinations of these are multiple and labile; as was conceptualised by Michael Berridge, cells can create and rapidly modify "Ca^{2+} signalling toolkits" that adapt Ca^{2+} signalling to the environmental

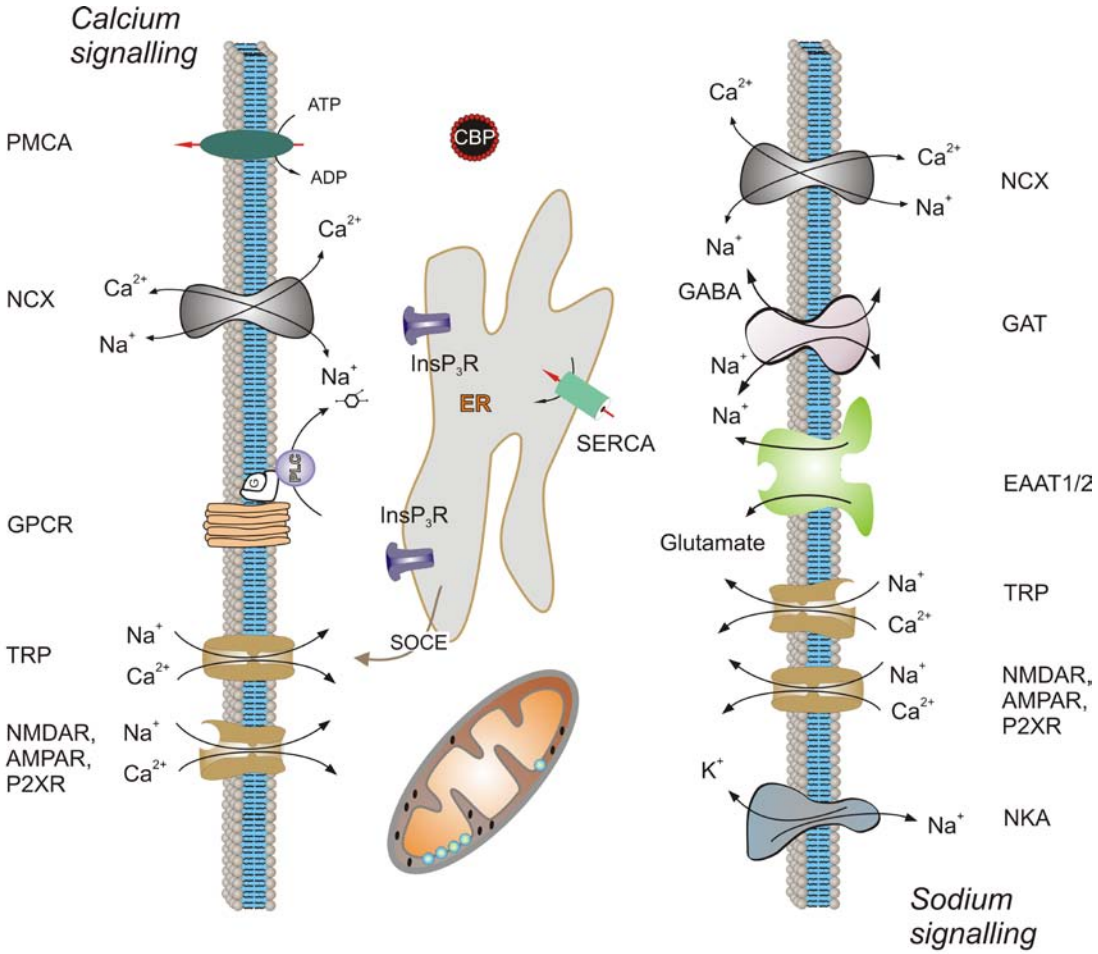

Figure 1. Molecular cascades of Ca^{2+} and Na^+ signalling in astroglia (see text for details). Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CBP, Ca^{2+} binding protein; EAAT, excitatory amino acid transporter; ER, endoplasmic reticulum; G, G-protein; GABA, γ-aminobutyric acid; GAT, GABA transporter; GPCR, G-protein coupled receptor; InsP3R, inositol 1,4,5 trisphosphate-gated Ca^{2+} channel/receptor; NCX, Na^+/Ca^{2+} exchanger; NKA, Na^+/K^+ ATPase; NMDAR, N-methyl D-aspartate receptor; PLC, phospholipase C; PMCA, plasmalemmal Ca^{2+}-ATPase; P2XR, purinergic 2X receptor, SERCA, sarco(endoplasmic) reticulum Ca^{2+} ATPase; SOCE, store-operate Ca^{2+} entry; TRP, transient receptor potential.

requirements [41,42]. Another important feature of Ca^{2+} homeostatic/signalling system is its autoregulation by Ca^{2+} ions themselves, as transient changes in Ca^{2+} concentration establish multiple feedback mechanisms that modify the handling of itself. As a rule, most of Ca^{2+} permeable channels are subject to Ca^{2+}-dependent inactivation, which develops either through direct binding of Ca^{2+} ions to the channel or Ca^{2+}-dependent channel phosphorylation. Similarly, Ca^{2+} pumping by SERCA is regulated by Ca^{2+} concentration within the ER lumen; this intraluminal Ca^{2+} concentration also controls the availability of intracellular Ca^{2+} channels for activation. Conceptually, lowering Ca^{2+} concentration in the ER facilitates Ca^{2+} uptake and reduces channels activation, whereas increase in intra-ER Ca^{2+} concentration facilitates channels opening and reduces SERCA activity (see [43,44] for detailed discussion). Finally Ca^{2+} fluxes are modulated by mitochondria which, by providing ATP and dynamic Ca^{2+} buffering, regulate plasmalemmal Ca^{2+} entry and ER Ca^{2+} uptake [45,46].

3.2. Endoplasmic reticulum as a main source of astroglial Ca^{2+} signalling

Astroglial cells respond with intracellular Ca^{2+} elevation to a broad variety of external stimuli from direct mechanical stimulation to a multitude of neurotransmitters, neuromodulators, hormones and other biologically active substances. The ability of astroglia to react with $[Ca^{2+}]_i$ elevation to almost every neuroligands it encounters was firmly established in experiments in cell cultures [47-52]. These early experiments were fundamental for glial research because, they demonstrated that astrocytes are potentially capable of expressing virtually every receptor modality and that most of these receptors are coupled to ER through the phospholipase C (PLC)/$InsP_3$ signalling cascade. These experiments also highlighted remarkable plasticity of astroglial cells in vitro, as indeed, these cells were able to rapidly modify receptor expression pattern. First studies of astrocytes *in situ*, in brain slices, confirmed the primary importance of $InsP_3$-ER link in generation of astroglial Ca^{2+} signals [53-56]. At the same time, these experiments also found that receptor expression in astroglia in neural tissue is restricted to match that of neurons, i.e. immediate neurotransmitter environment [13,16,52,57].

The ER is one of the largest intracellular organelles, which is involved in a variety of fundamental cellular processes such as protein synthesis, protein folding and trafficking haulage of secretory products etc. [58-61]. The ER is also a key organelle of Ca^{2+}signalling, being arguably the largest dynamic Ca^{2+} store able to accumulate, store and release Ca^{2+} ions in response to (patho)physiological stimulation. Ca^{2+} accumulation into the ER lumen is accomplished by SERCA pumping; the Ca^{2+} concentration in the ER at rest (also known as intraluminal Ca^{2+} concentration, $[Ca^{2+}]_L$) is maintained at 0.2 - 1.0 mM range [62-66]. Release of Ca^{2+} from ER in astroglia is primarily mediated by $InsP_3$ receptors; and their inhibition by pharmacological agents (e.g., heparin) or by genetic deletion often prevents development of Ca^{2+} signals in astrocytes [55,67]. Functional role of second type of ER Ca^{2+} release channel, the RyR, a Ca^{2+}-gated Ca^{2+} channel, in astroglial Ca^{2+} dynamics remains controversial, although astrocytes express RyR both *in vitro* and *in situ* [68-70] and RyRs contribute to Ca^{2+} signals necessary for glutamate release via regulated exocytotic pathway [71]. The $InsP_3Rs$ are simultaneously controlled by $InsP_3$ and Ca^{2+} ions and therefore local increase in $[Ca^{2+}]_i$ facilitates receptor opening and promotes Ca^{2+}-induced Ca^{2+} release through $InsP_3R$. This feature underlies the occurrence of propagating Ca^{2+} waves which, in essence, represent a wave of ER membrane excitation manifested by propagating recruitment of $InsP_3$ receptors and coordinated with the extracellular spread of ATP. These Ca^{2+} waves are important for astrocyte physiology; astroglial stimulation usually occurs at the level of distal processes, and Ca^{2+} waves convey this excitation to the soma. Furthermore, astroglial Ca^{2+} wave travels through astroglial syncytia, being therefore a substrate for astroglial long-range signalling [72,73]. In addition to $InsP_3$, astrocytes can also utilize other ER Ca^{2+}-mobilizing second messengers in response to external stimuli, most notably cyclic adenosine diphosphoribose (cyclic ADP-ribose) [74,75] and nicotinic acid adenine dinucleotide phosphate, (NAADP) [76-78].

Ca^{2+} signals, produced by activation of ER Ca^{2+} release, control many functions of astroglia. In particular, ER-originated Ca^{2+} signals are critical for inducing exocytotic release of neurotransmitters (such as, for example, ATP, glutamate or D-serine) from astrocytes (see [79,80] for review and references). Inhibition of Ca^{2+} accumulation into the ER by specific blockade of SERCA pumps with thapsigargin, that leads to exhaustion of the ER Ca^{2+} content due to an unopposed leak through the endomembrane, effectively eliminated Ca^{2+}-dependent release of glutamate from cultured astrocytes [71]. The same effect was achieved after inhibition of $InsP_3$ receptors by membrane-permeable antagonist diphenylboric acid 2-aminoethyl ester (2-APB), which can also affect the store-operated Ca^{2+} entry discussed next. The role for ER Ca^{2+} signalling cascade in controlling astroglial gliotransmission was subsequently corroborated in experiments in acute slices (e.g., [81-83]].

3.3. Plasmalemmal Ca^{2+} influx in astrocytes: role of TRP channels and ionotropic receptors

Despite the fact that ER Ca^{2+} store acts as a main source for astroglial Ca^{2+} signalling, astrocytes also possess several mechanisms for Ca^{2+} entry that produce physiologically relevant Ca^{2+} signals (Figure 1). There is little evidence that astrocytes *in situ* can express functional voltage-gated Ca^{2+} channels, although these channels have been detected in several *in vitro* experiments (see [80] for detailed review). Two major pathways controlling plasmalemmal Ca^{2+} entry in astroglial cells are represented by store-operated and ligand-operated ion channels.

The store-operated Ca^{2+} entry is generally present in a majority of electrically non-excitable cells. This Ca^{2+} influx pathway (initially described as a "capacitative" Ca^{2+} entry) [84,85] is controlled by the Ca^{2+} content in the ER lumen, where decrease in $[Ca^{2+}]_L$ results in the opening of plasmalemmal Ca^{2+}-permeable channels [86]. Activation of the store-operated Ca^{2+} entry fulfils two functions: first, it provides Ca^{2+} for replenishment of

the ER store (the capacitative function), and second, it is important for producing the sustained ("plateau") phase of the Ca^{2+} signal that often outlasts the period of cell stimulation. There are several molecular determinants of the store-operated Ca^{2+} entry. Many types of cells express specific (I_{CRAC}) store-operated channels characterised by extremely high Ca^{2+} selectivity and very low single channel conductance. Activation of these channels reflects interaction of stromal interaction molecule (STIM) proteins (that detect ER Ca^{2+} concentration) with Orai (named after Greek gate-keeping goddesses [87]) proteins that form the plasmalemmal channel [88]. Alternatively store-operated Ca^{2+} influx may involve activation of TRPC channels [89].

The store-operated Ca^{2+} entry is functionally expressed in astroglia [90,91]. Initial experimental evidence indicates the role for TRPC channels. They are expressed in astrocytes at both mRNA and protein levels, and TRPC activity is involved in shaping astroglial Ca^{2+} signals [92-94]. Further analysis revealed that in astrocytes the TRPC channels are assembles of brain native heteromultimers [92,95] containing obligatory TRPC1 (channel forming subunit) and TRPC4 and/or TRPC5 (auxiliary subunits). Inhibition of TRPC1 channel expression by antisense mRNA or its occlusion with blocking antibody directed at an epitope in the pore forming region of the TRPC1 protein significantly decreased store-operated Ca^{2+} influx in cultured astrocytes [92,95] and reduced plateau phase of ATP-activated $[Ca^{2+}]_i$ transients [95]. Likewise, immunological inhibition of TRPC1 protein substantially decreased mechanically-induced Ca^{2+} signalling in astrocytes and suppressed Ca^{2+}-dependent glutamate release [95].

It has been recently demonstrated that astrocytes possess functional STIM1 and Orai1 molecules which play a role in thrombin-induced cytosolic Ca^{2+} dynamics [96]. This was demonstrated in cultured astrocytes, investigated using immunocytochemisty and Ca^{2+} imaging. Overexpression and silencing (using short interfering RNA) of STIM1/Orai1 led to enhanced or muted Ca^{2+} dynamics in astrocytes, respectively.

The second pathway for plasmalemmal Ca^{2+} entry in astrocytes is associated with ionotropic receptors (ligand-gated Ca^{2+}-permeable channels). Several types of ionotropic receptors are present in astrocytes *in vitro*, *in situ* and *in vivo* (see [97-99]. The most important astroglial ionotropic receptors are α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl D-aspartate (NMDA) glutamate receptors and P2X purinoceptors. Often, astroglial AMPA receptors do not express GluR-B (GluR2) subunit, which makes these receptors moderately Ca^{2+} permeable [100,101]. The NMDA receptors identified in cortical astrocytes [102-105] differ in their biophysics and pharmacology from the neuronal ones. In particular, astroglial NMDA receptors are weakly (if at all) sensitive to Mg^{2+} block at physiological resting potential, and their Ca^{2+} permeability is ~ 2 times lower as compared to neurones ($P_{Ca}/P_{monovalent}$ ~ 3 *vs.* ~10 in neurones [106]. Nonetheless, synaptic activation of astroglial NMDA receptors in cortical slices results in substantial Ca^{2+} signals [106].

Astrocytes express $P2X_{1/5}$ and $P2X_7$ purinoceptors, which may create Ca^{2+} fluxes [102,105,107]. The $P2X_{1/5}$ have moderate Ca^{2+} permeability ($P_{Ca}/P_{monovalent}$ ~ 2), which is sufficient to produce physiologically relevant Ca^{2+} signals upon appropriate stimulation [106]. The $P2X_7$ receptors activation may result in massive Ca^{2+} influx, although this signalling is most likely present only in pathology [108].

Astrocytes also express TRPA1 channels, which generate frequent occurrence of local, punctate Ca^{2+} influx. This activity of TRPA1 contributes to the resting Ca^{2+} levels in astrocytes [34]. This ion channel is best known as a chemosensor for various environmental noxious stimuli causing pain [109-111], albeit it can also be activated by cold [112] or heat [113].

4. Na^+ signalling in astroglia

4.1. Dynamic changes in cytoplasmic Na^+ concentration in astrocytes

At rest, astrocytes have relatively high cytosolic Na^+ concentration ($[Na^+]_i$); in various astroglial preparations (i.e. in culture and in acute slices) it was determined at ~ 15 - 20 mM (cultured hippocampal astrocytes, 15 - 16 mM [114]; cultured astrocytes from visual cortex, 17 mM [115]; astrocytes in cortical slices, 17 - 20 mM [116]; see also [117] for comprehensive review). These levels of resting $[Na^+]_i$ in astrocytes are almost twice higher when compared to neurones (~ 4 - 10 mM; see e.g., [114,118-120]); high cytosolic Na^+ in astrocytes also has functional consequences because it sets reversal potential for many Na^+-dependent transporters/exchangers, which shall be discussed below.

Stimulation of astrocytes (either mechanical or chemical) induces transient and complex changes in $[Na^+]_i$. For example, application of glutamate to astrocytes *in vitro* evoked local $[Na^+]_i$ transients and propagating Na^+ waves spreading though astroglial syncytium [121-123]. Similarly, both single-cell $[Na^+]_i$ transients and astroglial Na^+ waves were observed in astroglial preparations *in situ*. In cerebellar Bergmann glia, glutamate induced $[Na^+]_i$ increase by 10 - 25 mM above the resting level [124,125]; in hippocampus, glutamate induced $[Na^+]_i$ rise and astroglial Na^+ waves [126]. Astroglial $[Na^+]_i$ in hippocampus was also reported to rise by ~ 7 mM following stimulation with γ-aminobutyric acid (GABA) [116]. Finally, astroglial $[Na^+]_i$ increases are induced by stimulation of synaptic inputs which has been detected in both cerebellum and hippocampus [125,127,128].

4.2. Molecular mechanisms controlling $[Na^+]_i$ in astroglia

The cytosolic Na^+ concentration in astrocytes is regulated by Na^+ diffusion through plasmalemmal channels, by Na^+ transport through ATP-dependent pumps and by Na^+ translocation by multiple ion exchangers (Figure 1). Main route for plasmalemmal diffusion of Na^+ across the plasmalemma is associated with ionotropic glutamate and purinoceptors, which produce substantial Na^+ fluxes upon activation. In Bergmann glia, for example, stimulation of AMPA receptors with kainate increases $[Na^+]_i$ by ~ 20 -25 mM [124]. Na^+ can also enter astrocytes through TRP channels, non-specific mechanosensitive cationic channels and possibly through Epithelial Na^+ Channel (ENaC)/Degenerin family 21 channels or proton-activated Acid

Sensing Ion Channels (ASICs); for review see [117]. Astrocytes in subfornical organ express specific type of Na^+ channels sensitive to fluctuations in extracellular Na^+ concentration. These channels (classified as Na_x channels) are involved in astroglial chemosensing and regulation of body Na^+ homeostasis [129]. All in all, physiological stimulation of astrocytes trigger substantial Na^+ influx, which is mainly mediated by ionotropic receptors and possibly by TRPC channels activated following depletion of ER Ca^{2+} stores.

The Na^+- K^+ pump or Na^+/K^+ ATPase (NKA) is the main energy-dependent astroglial Na^+ transporter. Astrocytes throughout the CNS express the NKA a1/a2 subunits. The Na^+/K^+ ATPase is activated following an increase in $[Na^+]_i$ and hence every transient $[Na^+]_i$ elevation promotes Na^+ efflux in exchange for K^+ influx, which may represent a link between cytosolic Na^+ fluctuations and K^+ buffering. Astrocytes are also in possession of multiple ion exchangers or solute carriers (SLC; of which more than 50 families embracing ~ 380 members are known [130,131]) that utilise the energy stored in pre-existing ion concentration gradients.

Arguably, the most physiologically important ion exchanger is the Na^+/Ca^{2+} exchanger or NCX, which belongs to the SLC8 family [132]. All 3 main isoforms, NCX1, NCX2 and NCX3 are expressed in astroglia. Importantly, the NCX proteins are often concentrated in astroglial perisynaptic processes where they co-localise with NKA and plasma membrane glutamate transporters [133]. The NCX can mediate the transport of both Na^+ and Ca^{2+} in both directions; generally NCX may operate either in the forward mode (Ca^{2+} extrusion associated with Na^+ influx) or in the reverse mode (Ca^{2+} entry associated with Na^+ extrusion). This is determined by (i) stoichiometry of the exchanger, which is $3Na^+$:$1Ca^{2+}$, (ii) transmembrane concentration gradients for Na^+ and Ca^{2+}, and (iii) the level of membrane potential. High resting $[Na^+]_i$ in astrocytes sets the reversal potential of the NCX ~ -80 mV (see [117] for calculations and further details), which is very close to the resting membrane potential. Consequently, the NCX in astrocytes dynamically fluctuates between forward/reverse modes and mediates both Ca^{2+} entry and $[Ca^{2+}]_i$ clearance as well as Na^+ influx/efflux [115,124,134,135]. The reverse mode of the NCX is triggered by mild depolarisation and by Na^+ influx through either ionotropic receptors or neurotransmitter transporters discussed below.

The Na^+-dependent neurotransmitter transporters in astrocytes are mainly represented by plasma membrane transporters for glutamate and GABA. The glutamate transporters, generally classified as the excitatory amino acid transporters 1 to 5 (EAAT1 to EAAT5 belonging to SLC1 family), are fundamental for glutamate homeostasis. Astrocytes, which specifically express EAAT1 and EAAT2 (homologues of which in rodents are known as glutamate transporter 1, or GLT1, and glutamate-aspartate transporter or GLAST) act as the main sink for glutamate in the CNS accumulating ~80% of glutamate released in the course of synaptic transmission [136]. Glutamate accumulated into astrocytes is rapidly converted (by another astroglia-specific enzyme glutamine synthetase [137,138]) into glutamine; the latter is either transported to neurones, where it acts as the major precursor for glutamate and GABA and thus is indispensable for sustained synaptic activity (the glutamate-glutamine or GABA-glutamine shuttles), or is utilised for astroglial energetics [137]. The stoichiometry of EAAT1/2 is 1 Glu^-:3 Na^+:1K^+:1H^+, of which Na^+, proton and glutamate enter the cell in exchange to K^+ efflux. As a result of this stoichiometry and transmembrane gradients of relevant ions, the reversal potential for glutamate transporters is more positive than 50 mV [117]. This makes the reversal of glutamate transport impossible in physiological conditions; only during strong pathological insults accompanied by massive $[Na^+]_i$ overload and very high extracellular K^+ accumulation can the glutamate transport change direction and provide additional glutamate, which may exacerbate excitotoxicity [139]. In physiological conditions, activation of glutamate transport in astrocytes triggers inward Na^+ current which can elevate $[Na^+]_i$ by 10 - 20 mM [117,125]. Astrocytes also express GABA transporters of GAT1 and GAT3 types (SLC6 family), which are localised predominantly in astroglial processes surrounding inhibitory synapses. GABA transport via GAT3 can be affected by TRPA1 activity (decreased TRPA1 function leads to reduction in GABA uptake), which subsequently affects nearby GABA-ergic synaptic transmission [34]. GABA transporters provide for a transmembrane symport of 1 GABA molecule (uncharged in physiological conditions), 2 Na^+ ions and 1 Cl^- anion. Activation of GABA transporters also result in Na^+ influx that can elevate $[Na^+]_i$ by ~ 7 mM [116]. Importantly, the reversal potential for GABA transporters lies very close to astrocytic resting membrane potential and therefore even small elevation in $[Na^+]_i$ can switch the transporter into reverse mode and hence facilitate GABA release from astrocytes; this release which can inhibit neuronal excitability was indeed detected in cortical slices [116].

Cellular Na^+ homeostasis is also regulated by mitochondria which are able to accumulate Na^+ through mitochondrial Na^+/Ca^{2+} exchanger [40]. The NCLX, the solute carrier SLC24A6, is essential molecular component of this exchanger [140].

4.3. Functional role of astroglial Na^+ signalling

Dynamic fluctuations in cytoplasmic Na^+ concentration can affect surprisingly wide array of molecular targets and cascades that are critical for the homeostatic function of astroglia. First of all, $[Na^+]_i$ modulates homeostasis of several neurotransmitters, that include principal excitatory transmitter glutamate and inhibitory transmitters GABA and glycine. Glutamate uptake is critical for termination of excitatory transmission and as the first step in glutamate-glutamine/GABA-glutamine shuttle. Increase in $[Na^+]_i$ decreases the efficacy of glutamate transport; as it were glutamatergic transmission activates Na^+ influx into astrocytes via ionotropic receptors and EAATs. Thus increased $[Na^+]_i$, which coincides with the peak of glutamatergic synaptic transmission event, temporarily decreases glutamate uptake, thus transiently increasing the effective glutamate concentration in the synaptic cleft. Levels of $[Na^+]_i$ also influence glutamine synthetase as well as export of glutamine from astrocytes to neurones. The latter is mediated by Na^+-coupled neutral amino acid transporter SNAT3/SLC38A3 and is directly controlled by $[Na^+]_i$ [141].

Astroglial $[Na^+]_i$ also regulates GABA-ergic transmission through (i) controlling astroglial GABA uptake via GAT1/3 pathway and (ii) by maintaining GABA synthesis in neuronal terminals by supplying glutamine. Astroglial GABA transport system is easy to reverse, because (as mentioned before) its reversal potential is set close to the resting potential of astrocyte. Thus, mild depolarisation and even small increases in $[Na^+]_i$ may reverse the GAT-dependent transport making astrocytes a source of GABA. Additionally, GABA-ergic transmission turned out very sensitive to astroglial glutamine supply, and inhibition of glutamine synthetase substantially suppresses GABA-ergic inhibitory transmission [142]. Similarly astroglial $[Na^+]_i$ regulates the efficacy of glycine clearance from the relevant synapses.

Dynamic changes in astroglial $[Na^+]_i$ modulate Ca^{2+} signalling by defining the mode of operation of NCX. Increase in $[Na^+]_i$ were shown to induce additional Ca^{2+} influx that contributed to neurotransmitter-evoked $[Ca^{2+}]_i$ transients [124]. Such Ca^{2+} entry through NCX was even demonstrated to induce exocytotic release of neurotransmitters from astroglia [115,134,143].

Astroglial Na^+ signals are coupled to several important homeostatic pathways. In particular $[Na^+]_i$ levels directly control the activity of NKA and $Na^+/K^+/Cl^-$ co-transporter NKCC1, thus regulating K^+ buffering. The $[Na^+]_i$ controls the activity of Na^+-proton exchanger and Na^+-bicarbonate transporter, both being critical for pH homeostasis (see [117] for further discussion).

Finally, $[Na^+]_i$ controls one of the most fundamental astroglial functions - that is the metabolic support of neurones. The latter occurs in the form of astrocyte-neurone lactate shuttle, when astrocytes supply active neurones with their preferred energy substrate lactate [144-146]. Neuronal activity-induced elevation of astroglial $[Na^+]_i$ triggers lactate synthesis mediated through NKA; and therefore astroglial Na^+ signalling is fundamental for neuronal metabolic support.

5. Concluding remarks

Rapid astroglial signalling, that is fundamental for neuronal-glial communications, is mediated through fluctuations of cytoplasmic concentrations of two principal cations Ca^{2+} and Na^+. Neuronal activity can trigger complex spatio-temporal changes of $[Ca^{2+}]_i$ and $[Na^+]_i$ in astrocytes, which in turn regulate multiple effector pathways that control homeostatic function of these glial cells.

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VP and AV conceptualized and wrote the manuscript; both authors approve the submitted version.

Competing financial interests

The authors declare that they have no competing financial interests.

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