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Differential calcium signalling in neuronal-glial networks

Alexei Verkhratsky[1,2], Miroslava Anderova[1], Alexandr Chvatal[1]

[1]Institute of Experimental Medicine, ASCR, Videnska 1083, 142 20 Prague 4, Czech Republic, [2]Faculty of Life Sciences, The University of Manchester, Manchester M13 9PT, UK

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1. ABSTRACT

Calcium ions are the probably the most ancient, the most universal and omnipresent intracellular signalling molecules, which are involved in regulation of a host of cellular functional reactions. In the nervous system Ca^{2+} signalling is intimately involved in information transfer and integration within neural circuits. Local Ca^{2+} signals or Ca^{2+} microdomains control neurotransmitter release; more global Ca^{2+} signals regulate synaptic strength and accomplish postsynaptic processing. In the glial syncytium Ca^{2+} ions provide for glial "Ca^{2+} excitability", convey long-range signalling by means of propagating Ca^{2+} waves and control the release of gliotransmitters. Differential Ca^{2+} signals in various elements of neural circuits represent therefore molecular mechanisms of integration in the nervous system.

2. INTRODUCTION: GLIAL-NEURONAL NETWORKS AS A SUBSTRATE FOR BRAIN FUNCTION

Human brain, where our thoughts, emotions and hopes dwell, is formed by an exceedingly complex cellular circuitry, which comprises more than 100 billion neurones and probably about 1 trillion glial cells (37, 45, 73, 124, 125, 145). Glial-neuronal circuits form dynamic ensembles, which act as a substrate for brain function. Integration and communications between glial and neuronal networks is generally achieved through extracellular space via the release of chemical neurotransmitters from synaptic terminals or gliotransmitters from astroglial processes (71, 72, 143, 154, 156); signal transduction within the circuits is, however, accomplished by two fundamentally distinct mechanisms. The neuronal networking relies upon rapidly

propagating electrical signals, the action potentials, which are generated by voltage-gated channels residing in the plasmalemma (60-62). When reaching the synaptic terminals, electrical signals transform into the release of neurotransmitters, which, by activating receptors expressed in postsynaptic neurones or perisynaptic astroglia, accomplish information transfer within neuronal-glial network. Glial cells in contrast, are unable to generate action potentials, chiefly due to a very low density of voltage-gated channels in their membrane (152). Nevertheless, glial circuits are integrated via intracellular route, through the excitable media formed by the membrane of the endoplasmic reticulum (28, 146, 149) in combination with intercellular volume transmission through gap junctions (33, 44). Combination of plasmalemmal and intracellular excitability, release of neuro- and gliotransmitters and intercellular volume transmission are central for integration within neuronal-glial circuits. On the molecular level the central stage is occupied by Ca^{2+} ions, which control the release of chemical transmitters, regulate synaptic plasticity and provide glia with calcium excitability.

3. MOLECULAR PHYSIOLOGY OF CALCIUM SIGNALLING AND CALCIUM EXCITABILITY

Calcium signalling system is unique in its omnipresence and pluripotency; it has developed initially at the very dawn of life as an ultimate survival mechanism, which protected intracellular environment against Ca^{2+} ions of the primordial ocean (25). Indeed, at the moment polyphosphates (in the form of ATP) were chosen as energy accumulators, protection against Ca^{2+} became vital because Ca^{2+} phosphates are insoluble (160). Therefore, survival of proto-cells was critically dependent on their ability to rigidly control movements of Ca^{2+} ions through their membrane and to keep Ca^{2+} low within the cytoplasmic compartment. Thus the foundation for Ca^{2+} homeostatic/signalling system was laid. Ca^{2+} however, comes not only in the disguise of universal killer; as an ion it has highly flexible coordination number (6 to 10) and can therefore interact with a huge variety of biological molecules (65). It was not surprising, hence, that evolution swiftly utilised calcium as a universal signalling molecule.

Conceptually, calcium homeostasis/signalling system is operated by several molecular cascades, which provide for Ca^{2+} transport across membranes that create and delineate cellular compartments (see (9-11, 81, 118, 119, 144, 153) and Figure 1 for review). These compartments, represented by the cytoplasm, the endoplasmic reticulum (ER) with nuclear envelope, the Golgi complex and mitochondria are endowed with distinct complements of Ca^{2+} homeostatic molecules that are responsible for creating steep Ca^{2+} gradients. For example, free Ca^{2+} concentration in the cytosol is ~ 10 - 20 thousand times lower compared with both extracellular space and the ER lumen; resulting transmembrane gradients determine the direction of Ca^{2+} diffusion.

Molecules of Ca^{2+} homeostasis/signalling are relatively few; they are represented by evolutionary conserved families of Ca^{2+} channels, Ca^{2+} transporters (comprising Ca^{2+} pumps and Ca^{2+} exchangers) and Ca^{2+} buffers; these molecular cascades, working in concert, provide for spatially and temporally organised fluctuations of intracellular Ca^{2+} concentration, generally known as Ca^{2+} signals (11, 81, 117, 118).

Calcium channels are responsible for diffusion-based Ca^{2+} movements down their electro-chemical gradients and can be generally subdivided into plasmalemmal Ca^{2+} channels (e.g. voltage- or ligand-gated channels, non-selective channels, store-operated channels, etc. (19, 26, 102, 108, 116)) and intracellular Ca^{2+} channels residing in the endomembrane (such as $InsP_3$ receptors, Ca^{2+}-gated channels, generally referred to as ryanodine receptors, and possibly some other channels, for example NAADP receptors, pannexins or indeed some types of TRP channels) or mitochondrial Ca^{2+} uniporter (12, 42, 55, 68, 70, 74, 142)). Calcium transporters, represented by Ca^{2+} ATP-ases (plasmalemmal - PMCA and endomembrane - SERCA) and Ca^{2+} exchangers (operative in both plasmalemma and in mitochondrial membrane) transport Ca^{2+} ions against concentration gradients using energy from either ATP hydrolysis or from pre-existing ion gradients (50, 161). Finally, Ca^{2+} buffers regulate Ca^{2+} diffusion within various cellular compartments. At the receiving end of Ca^{2+}-signalling chain a host of Ca^{2+}-sensitive enzymes (or "Ca^{2+} sensors") act as effectors, responsible for various physiological responses. Most importantly all components of Ca^{2+} homeostatic/signalling are regulated by Ca^{2+} ions themselves (via e.g. Ca^{2+}-dependent inactivation of Ca^{2+} channels; control of SERCA pumping activity by intraluminal free Ca^{2+} etc. - see e.g. (17)), which determines high versatility of this molecular machinery.

4. CALCIUM SIGNALLING IN NEURONES

Neuronal calcium signalling, in contrast to the majority of other cell types, very much relies on the Ca^{2+} entry through plasmalemmal channels of both voltage-gated and ligand gated varieties (10, 81), which, most likely, is dictated by the rapid nature of signalling transfer within neuronal networks. Indeed the binary code imposed by the excitable properties of neuronal membrane necessitates high velocity and temporal confinement of signalling events. The basis for signal transfer between neurones is formed by Ca^{2+} entry through voltage-gated channels (VGCCs) located in the presynaptic terminals, which in turn provide for the formation of local microdomains of high Ca^{2+} concentration that govern exocytosis of neurotransmitters (130). The postsynaptic membrane, being the place for the primary integration of incoming information, requites more complex Ca^{2+} regulation, which is achieved by virtue of multitude of ionotropic Ca^{2+} receptors with different Ca^{2+} permeabilities and biophysical properties and a host of metabotropic receptors regulating Ca^{2+} release from the intracellular source. All in all neurones must re-conciliate the need for both highly localised and propagating signalling, which are required for effective synaptic transmission and postsynaptic integration.

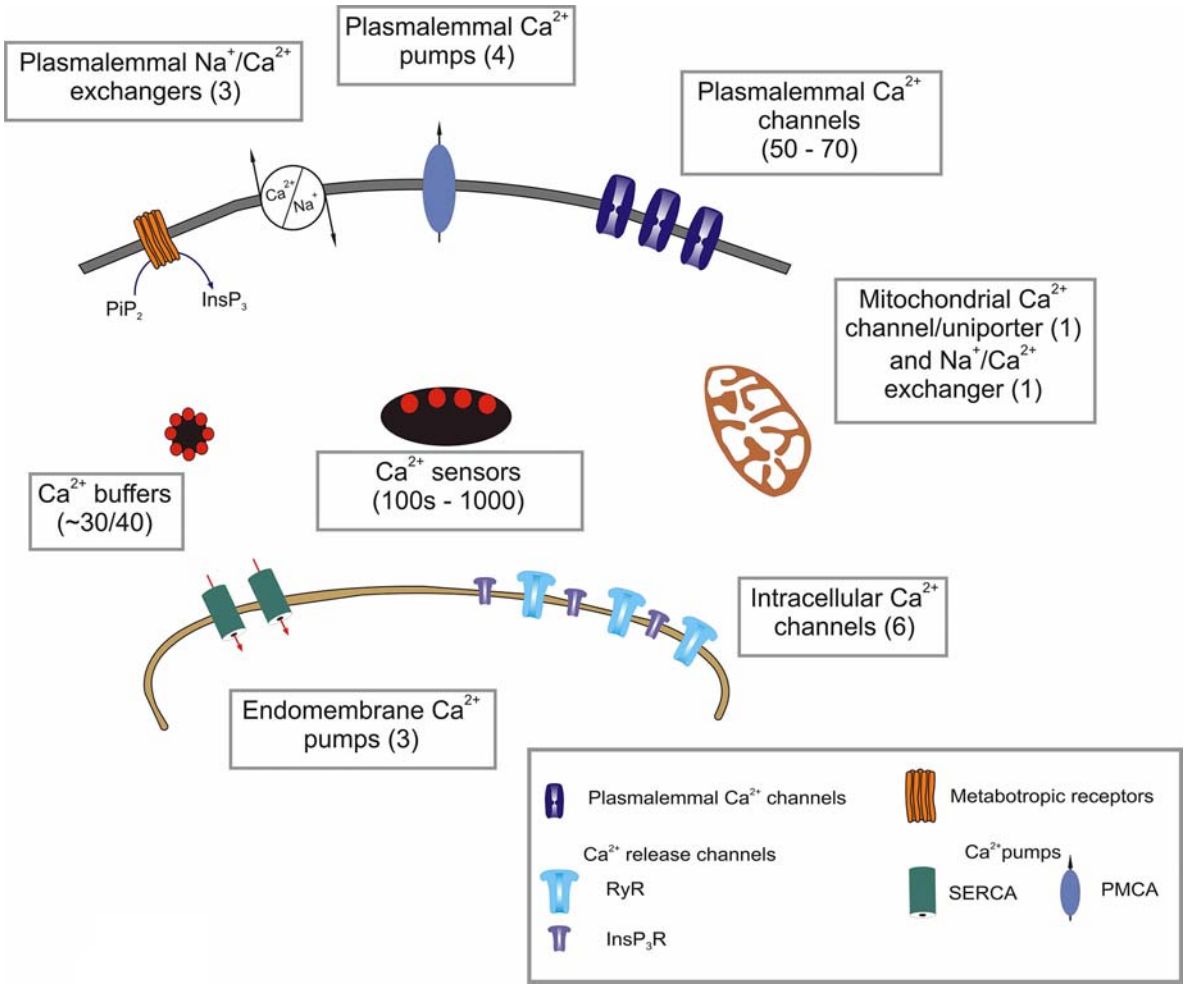

Figure 1. Principles of Ca^{2+} signalling. Ca^{2+} signals are generated by Ca^{2+} movements across cellular membranes, which delineate intracellular compartments, the cytosol, the ER and mitochondria; these movements are driven either by electro-chemical gradients (diffusion via membrane Ca^{2+} channels and transport through Na^+/Ca^{2+} exchanger) or by ATP energy (Ca^{2+} ATP-ases or pumps). Transmembrane Ca^{2+} fluxes are controlled by several highly conserved families of Ca^{2+} channels and transporters, represented by relatively few molecules. These are (i) plasmalemmal Ca^{2+}-permeable channels (voltage-gated, ligand-gated channels, store-operated channels and several classes of non-selective ion channels; all in all these are about 50 to 70 distinct molecular structures), (ii) intracellular Ca^{2+} channels (3 types of ryanodine receptors, 3 types of $InsP_3$ receptors and mitochondrial Ca^{2+} uniporter); (iii) Ca^{2+} ATP-ases/pumps (4 types of plasmalemmal pumps, PMCA and 3 types of intracellular pumps, SERCA) and (iv) 3 types of plasmalemmal Na+/Ca^{2+} exchangers, NCX, and mitochondrial NCX. In the cytosol and in the lumen of the ER Ca^{2+} concentration is also controlled by "Ca^{2+} buffers", represented by several 10s of Ca^{2+} binding proteins with different Ca^{2+} affinity/capacity. Fluctuations of free Ca^{2+} concentration within cellular compartments are detected by multitude of "Ca^{2+} sensors", which essentially are Ca^{2+} -regulated enzymes; Ca^{2+}-dependent activation/inhibition of these enzymes results in functional cellular responses.

The locality of Ca^{2+} signalling is accomplished by spatial segregation of voltage-gated Ca^{2+} channels, which are often clustered in strategically important sites - e.g. in the vicinity of synaptic vesicles (157) and by relatively high concentration of cytosolic Ca^{2+} buffers that very much limits Ca^{2+} diffusion (150). Generation of propagating Ca^{2+} signals is mainly confined to the endoplasmic reticulum, whose membrane forms an intracellular excitable media (10).

Neuronal ER is one of the largest organelles, formed by the continuous endomembrane; it extends from the nuclear envelope to peripheral dendrites and presynaptic terminals (144). The ER acts as a universal signalling organelle, accommodating variety of incoming signals, matching cellular activity with protein synthesis and posttranslational processing and generating output signalling cascades (9). Further, the ER serves as a dynamic Ca^{2+} store (9, 16, 117, 144), containing very high intraluminal free Ca^{2+} concentration, which may reach levels of 0.5 - 0.8 mM (3, 135, 137, 140). The ER acts as both generator and amplifier of Ca^{2+} signals (through Ca^{2+} release produced by $InsP_3$ and ryanodine receptors) and as a powerful Ca^{2+} buffer (through Ca^{2+} uptake via SERCA

pumps). In addition, the ER forms a substrate for Ca^{2+} signal propagation by (i) creating Ca^{2+} waves via Ca^{2+} assisted recruitment of Ca^{2+} release channels (both RyRs and $InsP_3Rs$ – see (10, 11)) and (ii) by Ca^{2+} diffusion through Ca^{2+} tunnels formed by continuous ER lumen (120, 121, 144).

Finally, termination of neuronal Ca^{2+} signals involves a coordinated activity of plasmalemmal Ca^{2+} pumps, sodium-calcium exchanger, SERCA pumps of endomembrane and mitochondria (50); failure of any of the components of Ca^{2+} extrusion system can initiate Ca^{2+} excitotoxicity (7).

5. CALCIUM SIGNALLING IN GLIAL CELLS

Glial cells are endowed with a full complement of Ca^{2+} homeostatic/signalling molecules, which have been reviewed in detail in numerous publications (e.g. (22, 32, 39, 40, 87, 93, 146, 149) to name but a few). Here therefore we shall only briefly outline the main properties of Ca^{2+} signalling pathways expressed in glia. It has to be stressed however, that the heterogeneity of glial cells is truly remarkable and glia residing in different brain regions often display very specific and distinct physiological properties.

5.1. Ca^{2+}-permeable plasmalemmal channels

Both types of macroglial cells, oligodendrocytes and astrocytes express various types of plasmalemmal Ca^{2+}-permeable channels. Voltage-gated Ca^{2+} channels have been identified in several glial preparations, both in culture and *in situ* (2, 31, 80, 85, 155). Glial cells demonstrated low- and high-voltage activated Ca^{2+} currents; more detailed pharmacological (31) and RT-PCR analysis (85) revealed expression of L-, N-, R- and T- types of VGCCs in cultured astrocytes. The role of VGCC-mediated Ca^{2+} influx in mature glial cells remains unclear; in thalamic astrocytes *in situ*, for example, inhibition of VGCCs by cobalt and nifedipine reduced spontaneous Ca^{2+} oscillations (111, 112). In contrast in other brain regions the role of voltage-activated Ca^{2+} influx pathway is negligible (24, 39). In immature glial cells VGCCs may have a specific role; in oligodendroglial precursors, for example, T-type Ca^{2+} channels are concentrated in the tips of processes which might be important for recognition of active axons in the neighborhood (80). Marked changes in the expression of glial VGCCs occur in response to different types of injury, such as ischemia, traumatic brain injury, hypomyelination or epilepsy; as a consequence VGCCs may play an important role in CNS pathophysiology (27, 159, 162).

Many types of ligand-gated channels expressed in glia are also Ca^{2+} permeable (152). Among these, particularly important are ionotropic glutamate receptors. AMPA receptors expressed in many types of glial cells are devoid of GluR-B subunit and thus have appreciable Ca^{2+} permeability ($P_{Ca}/ P_{Na} \sim 1$ - (20, 147)). In addition both astrocytes (in spinal cord and cortex) and oligodendrocytes express NMDA receptors, which (i) have high Ca^{2+} permeability and (ii) demonstrate a very weak Mg^{2+} block, which permits their activation at negative membrane potentials, so characteristic for glial cells (69, 84, 94, 126, 148, 166).

The second class of highly Ca^{2+} permeable ionotropic receptors is represented by P2X purinoreceptors (103), which are expressed in certain types of glial cells. Functional presence of P2X receptors in astrocytes is somewhat controversial. The ATP-mediated ion currents were detected in cultured astrocytes (158), and mRNA specific for $P2X_{1-4}$ and $P2X_6$ receptors were found in astrocytes from hippocampus and nucleus accumbens (41, 83). At the same time exhaustive investigations failed to detect P2X-mediated responses in hippocampal astrocytes (63), although ATP-induced currents, carried through presumed $P2X_{1/5}$ heteromeric receptors were identified in cortical astrocytes (Lalo, Pankratov, Kirchhoff, North & Verkhratsky, own observations). The P2X receptors, however, are abundant in microglia (38, 52, 56, 97), and $P2X_7$ receptors can trigger large Ca^{2+} influx into oligodendrocytes, which can be, under certain circumstances, excitotoxic (91).

Further plasmalemmal pathways participating in Ca^{2+} entry in glia are represented by store-operated channels. Molecular identity of these channels in glia is still unknown, yet they are functionally expressed in many types of astrocytes and oligodendrocytes both in culture and *in situ* (e.g. (46, 66, 134, 141)). In cultured astrocytes the store-operated channels were reported to cluster in plasma membrane-ER junctions, providing thus Ca^{2+} influx into the restricted space (46).

5.2. Endoplasmic reticulum takes the leading role in glial Ca^{2+} signalling

The main route for glial Ca^{2+} signalling is associated with the ER, and in particular with metabotropic receptor-driven $InsP_3$-induced Ca^{2+} release (32, 39, 77-79, 122, 123, 149). The role for ryanodine receptors and Ca^{2+}-induced Ca^{2+} release (CICR) is much less clear. Astrocytes express ryanodine receptors, as indicated by staining with fluorescent ryanodine (151) by RT-PCR analysis (92) and by immunocytochemistry (89, 133). Nonetheless, functional role for glial RyRs remains uncertain. Several groups have demonstrated caffeine-induced Ca^{2+} signals in cultured astrocytes (47, 48); however others were not able to confirm these observations (8, 32, 35, 151).

Expression of functional CICR, however, can be different in different brain areas; for example CICR was virtually absent in hippocampus (8), but present in ventrobasal thalamus (112). In oligodendrocytes from spinal cord functional CICR was identified; interestingly it involved direct coupling between plasmalemmal VGCCs and ryanodine receptors (106), being thus similar to depolarization-induced Ca^{2+} release operational in skeletal muscle. In addition the ER, being internally continuous Ca^{2+} store (120, 136), may play an important role in long-range Ca^{2+} transport through Ca^{2+} tunnels (96, 121).

5.3. Ca^{2+} extrusion

Termination of Ca^{2+} signals in glia is achieved by combined activity of plasmalemmal and endomembrane

pumps and mitochondrial buffering. Interestingly, astrocytes also express sodium-calcium exchanger, NCX (76, 139). Astroglial expression of NCX is somehow surprising, as the NCX usually operates under conditions of high and rapid Ca^{2+} loads, as for example in cardiac cells (36). It can well be, however, that astroglial NCX plays a very specific role, by removing excess of Na^+ ions, which accumulate in astroglia following activation of Na^+/glutamate transporters (75). Increase in $[Na^+]_i$ hampers glutamate uptake by reducing transmembrane Na^+ gradient; rapid removal of Na^+ ions from the cytoplasm can therefore be critically important for glutamate clearance. The NCX working in the reverse mode can accomplish this function (75). The possibility of functional coupling between Na^+/glutamate transporter and NCX is indirectly supported by demonstration of co-localisation of glutamate transporters and NCX in astroglial processes (95). Even more intriguing is the recent observation that Ca^{2+} influx supported by the reversed NCX may drive the exocytotic release of glutamate in cultured cortical astrocytes (107).

6. PROPAGATING GLIAL Ca^{2+} WAVES

Although glial cells, and particularly astrocytes, express plasmalemmal voltage- and ligand-gated channels, the excitability of glia is intracellular, because it is associated with the endomembrane forming the endoplasmic reticulum. The endomembrane, by virtue of Ca^{2+} release channels and SERCA pumps, forms the excitable media, tightly controlled by Ca^{2+} concentration gradients and local free Ca^{2+} microdomains. Indeed, the SERCAs provide for Ca^{2+} concentration gradient and build up high intraluminal Ca^{2+} concentration; free Ca^{2+} in the ER in turn controls both SERCA pumping velocity and availability of Ca^{2+} release channels for activation (17, 18, 23). From the cytosolic side, the $InsP_3$ receptor is positively regulated by free cytosolic Ca^{2+}, so that local increases in $[Ca^{2+}]_i$ can generate openings of $InsP_3Rs$ even at sub-threshold concentrations of $InsP_3$ (12). This Ca^{2+}-dependece forms the basis for the propagating activation of Ca^{2+} release channels along the endomembrane.

In glia, activation of metabotropic receptors often triggers initial $[Ca^{2+}]_i$ rises in the distal processes; where they can either localise (49), or initiate propagating intracellular Ca^{2+} wave, resulting from Ca^{2+}-assisted recruiting of $InsP_3Rs$ along the ER membrane (78, 163). In addition astroglial cells are capable of generating spontaneous Ca^{2+} oscillations (101), although in many cases these oscillations are driven by neuronal activity (1, 39). Propagating Ca^{2+} signals either initiated by activation of metabotropic receptors or occurring spontaneously can cross cell-to-cell boundaries and thus serve as a means for long-range astroglial communications. Intercellular Ca^{2+} waves were initially discovered in cultured astroglia (28); experimental evidence supporting the occurrence of propagating Ca^{2+} signals in astrocytes *in situ*, in brain slices begun to accumulate recently (51, 111, 129).

Mechanisms of glial Ca2+ waves propagation are complex and may involve (i) direct intercellular diffusion of InsP3 via gap junctions; (ii) regenerative release of a diffusible extracellular messenger (e.g. ATP) triggering metabotropic receptor-mediated Ca2+ release in neighbouring cells; (iii) diffusion of an extracellular messenger after release from a single cell (which may be important in microglia as well as astrocytes); and (iv) any combination of the above (6, 127, 138).

Importantly, mechanisms of intercellular Ca2+ wave propagation can be different in astroglial networks from different areas of the brain. For example, genetic deletion of Cx43, which forms gap junctions between brain astrocytes, results in the complete disappearance of astroglial Ca2+ waves in the neocortex, but not in the corpus callosum or hippocampus, where Ca2+ wave propagation relies primarily on ATP release (51).

7. Ca^{2+} SIGNALS CONTROL COMMUNICATIONS IN NEURONAL-GLIAL NETWORKS VIA RELEASE OF GLIOTRANSMITTERS

It is now well established that neuronal activity triggers Ca2+ signalling in glia (1, 29, 39, 49, 101, 123). The leading mechanism of this neuron to glia signalling is associated with the release of neurotransmitters and activation of glial receptors. Neurotransmitters can either diffuse from the synaptic cleft, and interact with glial membranes enwrapping synapses, or can mediate transmission in specialised neuronal-glial synapses (43, 64, 86, 115) or can be secreted from the ectopic release sites in neuronal terminals (90). In most of the cases the glial Ca2+ signalling results from activation of metabotropic receptors and subsequent InsP3-induced Ca2+ release from the ER (see above); although some alternative mechanisms (e.g. through extracellular K+ accumulation and subsequent activation of glial VGCCs - (57)) may also be operative.

Astroglial Ca2+ signals in turn, directly control information transfer from glia to neurones, as glial [Ca2+]i elevation trigger vesicular release of gliotransmitters, which act upon both neighbouring astrocytes and closely associated neurones. It is yet unclear whether glial cells can form "synapses" either with other glia or with neurones; although this cannot be excluded at present. The release of gliotransmitters is different from exocytosis in neuronal terminals in respect to the source of trigger Ca^{2+}: in the latter case Ca^{2+} enters the cytosol via plasmalemmal channels, whereas in the former Ca^{2+} comes from the intracellular stores; this difference determines slower exocytosis in glia (143, 156). On molecular level, astrocytes do possess all components of Ca^{2+}-regulated vesicular release. Astroglial cells contain vesicles, which can be concentrated in their processes; these vesicles are endowed with vesicle glutamate transporters of VGLUT1, 2 and 3 types, and bear vesicle-associated protein 3 (VAMP3 or cellubrevin), which allows vesicle to perform exocytotic fusion (14, 98, 110, 156). Physiologically, exocytotic release of glutamate from astrocytes was identified in several ways, including biochemical (109) and functional (4, 5, 13); it was also directly monitored by total internal reflection fluorescence

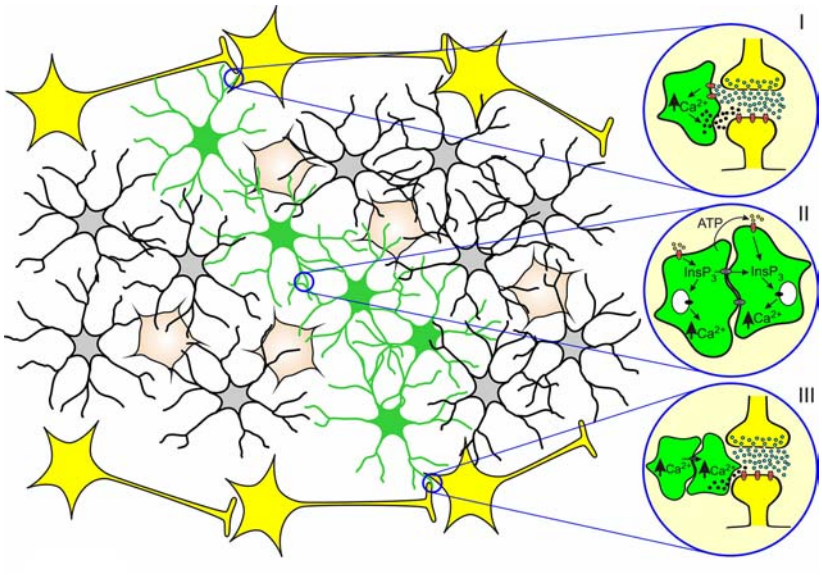

Figure 2. Local and distal gliotransmission. The figure shows neuro-glial communication over short and long distances. (I): local or short distance neuro-glial communication, synaptic activity is modulated by glial cells enwrapping the synapse. (II): long-distance gliotransmission represented by the calcium waves within the glial syncytium. (III): long distance neuro-glial communication, transmission in distant synapses is modulated.

imaging and by membrane capacitance recordings (14, 82, 156).

Transient Ca^{2+} increases in astrocytes trigger release of several gliotransmitters, which include not only glutamate but also ATP, D-serine and taurine (113, 128, 164, 165); release of gliotransmitters actively modulate neuronal excitability and synaptic transmission. Astroglial Ca^{2+} signals and related gliotransmitter release were shown to trigger variety of neuronal responses (111, 114) and affect synaptic transmission in neuronal glial co-cultures (5) and in brain slices (13, 67). It was shown that variations in glial intracellular Ca^{2+} may affect neuronal signal transduction in two ways: local and distal, (for reviews see (15, 53) and Figure 2). For example, intracellular Ca^{2+} signals observed in Bergmann glial cells in response to neuronal stimulation were restricted to cell microdomains adjacent to active synapses (49). It was suggested, that these rapid and spatially restricted Ca^{2+} transients underlie quick responses to the neuronal activity and provide for modulation at a local, probably synaptic, level. On the other hand, transient Ca^{2+} increases represented by spreading "calcium waves" over the astrocytic functional syncytium, i.e. gliotransmission, may represent the second, distal, type of neural modulation (Figure 2). Gliotransmitters released from astrocytes far away from the active synapses may modulate neuronal activity in distant areas of the nervous tissue. Recent studies demonstrated prolonged Ca^{2+} signals mediated by activation of mGluR5 in astrocytes, which resulted in Ca^{2+}-dependent release of gliotransmitters, which for minutes outlasted the initial stimulus (30). The function of D-serine, which is synthesised in glial cells and released upon activation of glutamate receptors, was emphasized in regulating synaptic excitatory transmission and plasticity in different brain areas (88, 105).

In addition to the possible physiological role of gliotransmission, the dysregulation of the latter could be involved in variety of pathological states, such as for example schizophrenia and epilepsy (53). Indeed, enhanced astroglial Ca^{2+} signals contributed to neuronal excitotoxicity after status epilepticus corroborating thus the neurotoxic role of astrocytic gliotransmission (34).

8. CALCIUM SIGNALLING INTEGRATE NEURONAL-GLIAL-VASCULAR UNITS

8.1. Astroglia define brain microarchitecture

Astroglia determine the functional micro-architecture throughout the grey matter, by creating relatively independent domains confined to the territories of individual astrocytes. Indeed recent experiments employing *in situ* high-resolution imaging of astrocytes infused with fluorescent dues or genetically labelled by selectively targeted fluorescent proteins (59, 156) revealed this specific spatial organisation of astrocytes in the grey matter. It was demonstrated that every protoplasmic astrocyte occupies a clearly defined territorial domain, which is free from the processes of other astrocytes (21, 104). The contacts between astrocytes, where the astroglial syncytium is formed, occur only at the level of very fine and most distant processes. This particular morphological arrangement creates grey matter compartments in which a single astrocyte forms contacts with all neuronal membranes and synapses residing within its confines (100). The fine processes formed by astrocytes enwrap neuronal terminals and form tripartite synapses, which are grouped into functional islands (54). Incidentally, astroglial processes also appear as highly dynamic structures as they produce filopodia and lamellopodia, which are able either

glide along neuronal surfaces or extend and retract between astroglial and neuronal membranes (58).

8.2. Astrocytes form neuronal-glial-vascular units

The specific territorial organisation of astroglia in the grey matter provides for a further functional compartmentalisation as every astrocyte extends processes towards neighbouring capillaries where these processes form endfeet. These astroglial endfeet completely cover the capillary wall from the side of brain parenchyma creating thus a glial-vascular interface; moreover astroglia release yet undefined factors which ascertain formation of tight junctions between vascular endothelial cells thus sealing the blood-brain barrier. Membranes of astroglial endfeet express numerous receptors, transporters and channels, which are instrumental for glial-capillary communications (132). In this way every astrocyte integrates neuronal membranes residing within its territory with nearby capillary, forming an independent glial-neuronal-vascular unit. This unit provides for morphological and functional link between brain parenchyma and microcirculation and accomplishes dynamic regulation of blood supply associated with neural activity (132).

Calcium ions represent the functional substrate for signalling within glial-neuronal vascular unit, being for example responsible for initiation of functional hyperaemia; the latter representing rapid increase in local circulation, which follows an increase in neural activity (131). Mechanisms of functional signalling linking increases in synaptic activity with circulation involve activation of receptors residing in perisynaptic astrocytes with subsequent generation of Ca^{2+} wave, which triggers release of either vasodilating agents (for example prostaglandin derivatives produced from arachidonic acid - (167)), or vasoconstrictors (e.g. 20-hydroxyeicosatetraenois acid also derived from arachidonic acid - (99)) . Therefore astrocytes, through local endfeet-vascular interactions, regulate focal changes in blood supply to support the functional activity of a single neuron-glia-vascular unit they delineate and control.

9. CONCLUSIONS

Calcium signalling machinery represents one of the most ancient, versatile and omnipresent systems providing multi-level regulation of cellular functions. In the brain, Ca^{2+} signals generated in neurones and glia act as a universal molecular mechanism, which regulates inter- and intracellular communications and determines integration within neuronal-glial networks. This is achieved by either highly compartmentalised Ca^{2+} microdomains, which control release of transmitters or by long-range propagating Ca^{2+} waves, which determine post-synaptic integration and signal transfer within glial syncytium.

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Abbreviations: AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, ATP: adenosine triphosphate, CICR: calcium-induced calcium release, Cx: connexin, ER: endoplasmic reticulum, $InsP_3R$: inositol trisphosphate receptor, NAADP: Nicotinic acid adenine dinucleotide phosphate, NCX: sodium-calcium exchanger, NMDA: N-methyl-D-aspartate, PMCA: plasmalemmal calcium ATP-ase, RyR: ryanodine receptor, SERCA: sarco(endo)plasmic reticulum calcium ATP-ase, TRP: transient receptor potential, VAMP: vesicle-associated protein, VGCC: voltage-gated calcium channel, VGLUT: vesicle glutamate transporters

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Send correspondence to: Alexei Verkhratsky, Faculty of Life Sciences, The University of Manchester, 1.124 Stopford Building, Oxford Road, Manchester M13 9PT, UK, Tel: 44161-2755414, E-mail: alex.verkhratsky@manchester.ac.uk

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