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Review Article

Astrocytic Microdomain Dynamics in Circuit and Network Performance

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Abstract

Resting state networks reflect a highly structured, dynamical brain organization that is likely to undergird a diverse and unified suite of behavioral and mental functions. How this organization is generated and what mechanisms are employed to mediate brain functions remained unresolved questions. It is increasingly evident, however, that a key cellular element for constructing these networks is the astrocyte. While traditionally regarded as having a primarily homeostatic role, astrocytes are in fact uniquely qualified to regulate mechanisms of information exchange that are inherent in the structure and dynamics of resting state network operation. Among the chief ways enabling astrocytes to regulate information flow is the formation of compartmentalized, highly dynamic structures targeted to synaptic nodes, known as calcium microdomains, that modulate interneuronal transmission. Microdomains modulate communication between neurons by actively shaping domain specific calcium oscillatory signaling at several levels: morphologically, through activation of actin networks that alter synaptic coverage and local ion concentrations; spatially, by targeting calcium transducers like calcineurin; and molecularly, by regulating calcium influx via calcium transporters and channels, such as the sodium calcium exchanger. Gliotransmission appears to form the basis for the decoding of calcium encoded network influences, contributing to the synchronization of gamma oscillations, resonance based stabilization of noise perturbations, and global network functional activity, among other modulatory mechanisms. Due to the close association of RSNs with brain functioning, they have been identified as fundamental targets for personalized patient care, with the potential to improve the treatment of conditions of the brain and nervous system. Hence, knowledge of how calcium micro-domains regulate information flow in networks can be expected to assist in the development of therapy for cognitive dysfunctions.

Keywords: *Calcium Microdomains; Calcium Oscillations; Astrocytes; Resting State Networks; Calcineurin; Perisynaptic Processes; Actin Dynamics; Sodium Calcium Exchanger; Slow Wave; Attractors*

Introduction

Resting state networks (RSNs) are large-scale patterns of synchronized activity that shape the brain's dynamic organization and undergird diverse brain functions [1,2]. Computational brain network modelling based on neuroimaging data, for example, reveal that the brain's dynamic organization can be explained in terms of RSNs, while their topography closely corresponds to responses elicited by a wide variety of sensory, motor, and cognitive tasks.

How RSNs organize information processing is unknown. A dominant hypothesis relates network dynamics to the brain's ability, either locally or globally, to assume stable configurations that resist the tendency to destabilize when affected by perturbations [3]. Underlying activation patterns of resting networks have been linked to the presence of such stable switching attractors, which enable information maintenance and facilitate cognitive transitions. Accordingly, computational processes employing RSN dynamics are likely to accommodate such properties when engaged in information processing.

Although RSN stability is partly due to underlying neuroanatomical structures, the generation of RSNs is not primarily structural, but results from an interplay between dynamics and structure [4], which together elicit the functional connectivity that stabilizes the network. Physiologically, there is much support indicating that this interplay is due to interactions between large scale electrical patterns, which have frequently been identified with brain oscillations. Indeed, the distribution of RSNs throughout cortical regions has been posited to be grounded in patterned electrical coupling that underpins their highly ordered structure. The stability of resting state networks, for example, is typically conceived in the context of the synchronicity of oscillatory phenomena. RSNs are also posited to engage in the processing of information involving mechanisms of oscillatory modulation [5] where information flow can, for example, be disrupted or redirected to new destinations [6,7].

While these dynamics have often been attributed to inhibitory-excitatory neuron pairing [8], that is, involving neurons alone, an increasing number of findings suggest network dynamics is heavily influenced via non-neuronal elements identified as astrocytes [9,10]. Astrocytes, for example, can detect neuronal activity via their sensitivity to glutamate by metabotropic glutamate receptors and receptor activation can in turn mediate transient increases of astrocytic intracellular calcium concentration through inositol 1,4,5-trisphosphate production or calmodulin signaling pathways. Via gliotransmission, or the propagation of calcium changes to astrocyte networks, calcium signaling could affect synaptic information transfer, either focally at synapses or regionally between populations of neurons [11].

Supporting this, electron microscopy has shown that astrocytic fine processes ensheath synapses and imaging techniques have identified localized Ca transients occurring within the fine processes of the astrocyte structure, termed calcium microdomains. Gliotransmitter release, moreover, appears to parallel Ca oscillations, in contrast to long lasting Ca elevations, which are much less effective [12] for calcium -dependent glial output at synapses, thereby implicating calcium level, microdomain periodicity in modulating neuronal communication.

Accordingly, this paper will review current findings on micro-domain based, calcium modulatory mechanisms. Because these mechanisms are ultimately likely to affect RSNs, indeed to both sustain and modulate their performance, the review will place these findings in the context of extracellular mechanisms that could be expected to influence information related exchange within such networks.

Calcium Microdomains: Structural Targeting of Information Flow

The contribution of astrocytes to RSN function is multimodal. Among the factors likely to directly influence RSN function is the complex morphology of these cells. Astrocytes are characterized by an intricate arborization rivaling that of neurons and by anatomical specializations that control local communication with neurons. Because astrocytes are fundamental cellular elements of most synapses [13], their morphological structure can play a significant role in brain communication and information processing. By shielding the synapse from external sources of neurotransmitter, for example, astrocytes carry out the critical function of tuning neurotransmitter responsivity and sharpening the temporal window within which postsynaptic stimulation is effective. This tripartite structure, coupled with the fact that astrocytes form dense syncytia creating functional networks spanning the brain [14], enable large scale populations of neurons to be influenced by local events controlled at each synapse. Individual human astrocytes, for example, can cover ~2 million synapses, with a synaptic density of ~1100 million synapses mm. By means of this association astrocytes have been shown to induce slow and infra-slow oscillations [15].

Astrocyte morphology, moreover, is not static but undergoes a wide morphological range, which can dynamically modulate the physiological properties of local synapses. In response to strong behavioral stimuli, like that experienced during arousal and recovery from general anesthesia astrocytes show rapid and reversible structural remodeling occurring in perisynaptic astrocyte processes that changes the extent of the coverage of the neuropil [14]. Changes in intracellular volume have been notably shown to be coupled to the activity of the Na-Ca exchanger and are strongly correlated with the appearance of Ca oscillations within the perisynaptic zones [16,17], which appears to be a function of astrocytic depolarization that follows on channel mediated increases in intracellular Na. Synaptic plasticity also appears to be coupled to these dynamic morphological changes. Synaptic activation that generates an LTP is sufficient to induce rapid - within dozens of minutes - motility of PAPs accompanied with increased astrocytic coverage of spines [18]. These volume effects are thus of particular significance for their ability to focally enhance or restrict communication between neurons.

Within the perisynaptic zones, points of contact with dendritic spines display striking activity dependent and spontaneous changes in Ca levels. By virtue of their small dimensions, the Ca fluctuations occurring within them are termed "microdomains,". Significantly, genetically encoded Ca indicators, despite global astrocytic distribution, respond to Ca changes only locally, i.e. within microdomains, and fail to respond to Ca elevations occurring throughout the astrocyte. This suggests that synaptic events influencing or influenced by astrocytes reflect localized changes occurring within these compartments.

In line with this observation, a majority of perisynaptic astrocytic structures appear as nodes. A strict correlation has been demonstrated between nodes and the size of spines as well as between Ca transients and spine size [19], suggesting that the domains are morphologically and functionally correlated with a unique set of neuronal synaptic partners. The spatial spread of the Ca transients as a function of time course, for instance, is consistent with an area smaller than 1 μm^2, suggesting that most spontaneous astrocytic Ca events are compartmentalized to individual microdomains.

Serial electron microscopy (EM) with 3D reconstruction [20] has revealed the intracellular organization of cellular organelles that would be available for physiological events and would apparently provide for support of localized calcium modulation. From these reconstructions five distinct organelles are observed, including empty and full endosomes, phagosomes, mitochondria, and endoplasmic reticulum (ER) cisternae. Endosomes and phagosomes constitute the largest volume of organelle associated space, accounting for more than 60% of all the organelles detected. Mitochondria and ER cisternae make up most of the ~40% of the remaining organelle related volume. The known role of mitochondria and ER in calcium dynamics are consistent with the known roles of diverse astrocytic functions and so appear to constitute a "primary site" of astrocytic Ca signaling within tripartite synapses together with that of extracellular influx. Hence, these domains represent independently functioning, specialized compartments endowed with all the machinery needed for governing astrocyte-neuron dynamic interactions.

The Origin of Microdomain Ca^{2+} Events

These morphological data indicate that release of calcium into the microdomain cytosol can occur from several intracellular and extracellular sources, including mitochondrial and ER cell organelles as well as cellular membrane channels and transporters. However, confirmation of these sources has traditionally proved difficult due to the small size of the domains. The recent development of genetically encoded calcium indicators [21] has overcome the size restrictions imposed by these fine processes and the rupturing of the cell membrane that occurs with microinjection during dye loading.

Intracellular sources of calcium: ER and mitochondria

Stimulation of metabotropic excitatory and inhibitory, G-protein-coupled receptors (GPCRs) induces the production of IP_3, and activa-

tion of IP_3 receptors (IP_3Rs) on ER membranes results in the release of Ca^{2+} from the endoplasmic reticulum (ER) [22]. In line with the observation of ER in microdomain nodes, genetic deletion of the IP_3 R type 2 (IP_3R2), reduces the number or amplitude of spontaneous Ca^{2+} events by roughly half [23]. The elimination of some but not all Ca events suggests that other internal sources result in calcium elevation with stimulation. This suggestion has been confirmed in the case of brain sources bearing the genetic lesion for the IP_3R2 receptor. In these cases, cytosolic Ca^{2+} elevations were observed in node regions without corresponding decreases in ER Ca^{2+} levels.

Instead, Ca^{2+} transients were observed originating from mitochondria [24]. Implicated in these results are mitochondrial $IP_3R1/3$ and/or ryanodine receptors that act in concert with IP_3R2 ER receptors to release Ca to the node cytosol. In addition to these routes, intracellular sources of calcium events can arise from mitochondrial efflux in response to opening of the mitochondrial permeability transition pore (mPTP).

Extracellular sources

Although Ca^{2+} microdomain events rely in part on Ca^{2+} release from intracellular stores, accumulating evidence reveals a greater dependence of calcium based oscillatory signaling on the influx of extracellular, transmembrane Ca [25]. While GPCR pathways can evoke IP_3 signaling and internal release of Ca - the so called calcium initiated calcium release events (CICR) - the relative slowness of CICR changes with respect to fast onset calcium events suggests the presence of other calcium sources capable of providing for fast calcium level changes. These sources of rapid calcium change are now known to involve extracellular calcium influx that can occur through multiple and distinct ionotropic receptors and ion channels.

Among the chief ionotropic receptors are those sensitive to glutamate. These ionotropic receptors (iGluRs) are ligand-gated ion channels that become permeable to the cations Na, Ca, and K when activated by glutamate. They are generally classed into iGluRs according to their respective sensitivity to either kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropi-acid (AMPA), or N-methyl-D-aspartate (NMDA). Studies employing patch-clamping of astrocytes have shown the induction of both depolarization and Ca changes, an apparent indication of the extracellular origin of Ca in intracellular calcium changes [26]. While the functional significance of astrocyte AMPA receptors has remained uncertain, astrocyte NMDA receptors appear to have functionally significant roles in maintaining astrocyte Ca levels, which has implications for Ca microdomain activity pertinent to gliotransmission and/or the regulation of synaptic strength. Topical superfusion of either AMPA or NMDA receptor antagonists, for instance, when applied directly to the brain, reduces fast Ca events in astrocyte processes, but only the NMDA antagonist CNQX reduces fast Ca events in nodes [27].

Astrocytes also express alpha-7 nicotinic acetylcholine receptors in their membranes and acetylcholine activation of these receptors can induce intracellular Ca^{2+} transients [28] in culture or in hippocampal slices. These receptors have high Ca permeability, but are rapidly deactivated, suggesting their involvement in very short-lived astrocytic Ca events. Activation of astrocyte α7nAChRs has been shown to cause D-serine release, leading to nearby neuronal NMDA receptor modulation and the induction of patterned gliotransmission during the circadian cycle. Importantly, nicotinic receptor activation also induces morphological changes in perisynaptic processes, which affects spine and synapse coverage.

Another ligand gated, ionotropic receptor permitting Ca influx into astrocytes is the purinergic (P2X) class of receptors, which are sensitive to ATP. P2X activation has been shown to cause astrocyte Ca transients in cell soma in brain slices and isolated astrocytes, as measured with Ca dyes [29].

Finally, a Ca transporter, the Na/Ca exchanger (NCX), has been shown to have several notable functions in modulating domain located, calcium events. In astrocytes the Na/Ca exchanger (NCX) extrudes intracellular Ca in exchange for Na influx [30]. Increasing intracellular Na^+ levels, however, causes the NCX to reverse direction and to bring extracellular Ca^{2+} into the cell in exchange for Na^+ efflux, leading to Ca^{2+} increases in astrocytes. Hence, it functions as both a homeostatic and a calcium signaling regulator. NCX is primarily confined to fine

peri-synaptic astrocyte processes where it is frequently localized with the Na^+/K^+ ATPase and glutamate transporters that work together to take up glutamate and buffer ion gradients [31]. This creates an insular compartment for Ca^{2+} and Na^+ signaling that restricts calcium events to the astrocyte processes.

Combined intracellular and extracellular fine tuning of Ca changes

While Ca fluctuations in microdomains may have an exclusively intracellular or extracellular calcium source, cooperativity among multiple sources is known to exist in cases, which contributes to the amplitude and spatiotemporal dimensions shaping the overall features of transient calcium events [21]. Under typical conditions, for example, ER calcium release triggers activation of calcium influx via store operated calcium entry (SOCE). The depletion of ER calcium in such cases induces the migration of sensor stromal, interaction molecules (STIMs) to the plasma membrane and activates Ca - release-activated Ca^{2+} channels (CRAC channels) formed by Orai proteins, thereby enabling calcium influx from the extracellular space. Supporting this observation, Ca transients evoked by thrombin in PAPs from $Orai1^{-/-}$ mice are substantially attenuated, an indication that Ca^{2+} microdomains rely on the coordinated release of calcium from the ER and the extracellular space.

Although a complete picture of the Ca microdomain features contributing to calcium signaling is not fully resolved, it is significant that microdomain calcium events can be classed according to fast and slow events and implicates at least two functional mechanisms according to which astrocytes can govern communication between neurons. The dominant calcium influx due to NCX [32] indicates that this transporter is critical to the ability of astrocytes to initiate or respond quickly to neuronal events, and so likely affects interneuronal events on time scales comparable to those of neuronal transmission. Slower events can be expected to condition information flow over longer time scales, possibly related to plastic changes occurring in association with events like long term potentiation (LTP) or long term depression (LTD) [18].

Regulating Calcium Fluctuations in Micro domains

Cytosolic calcium concentration increases are among the earliest events that occur after stimulation of many different types of cells by endogenous signaling molecules such as hormones or neurotransmitters, frequently assuming a periodic patterning like that of oscillations or waves. Accumulating evidence indicates that the adoption of such patterning across a wide variety of cell types has functional relevance [33] and that deviations from an operational norm can result in cellular pathologies. In astrocytes, both neuronally driven and spontaneous Ca oscillations are implicated in modulating interneuronal communication [21,34].

Neuron driven and spontaneous oscillations

In the visual cortex and hippocampus neuronal afferents can trigger Ca oscillations within astrocytes, with the frequency of the oscillations changing as a function of afferent firing rates [35]. Either increased frequency or increased intensity of neuronal stimulation has been shown to induce an increase in the frequency of astrocytic oscillations. Repetitive episodes of neuronal stimulation or application of glutamate receptor agonists, additionally, can result in long-lasting increases in Ca oscillation frequency. These results suggest that the intracellular oscillation frequency of astrocytes can be dynamically controlled by neuronal activity since it changes with the pattern of stimulation and so could encode neuronal specific information.

Astrocytes also display intracellular oscillations that are not driven by neuronal activity [34]. Significantly, spontaneous calcium oscillations appear subject to non-linear dynamical properties, exhibiting bifurcations and synchronization, suggesting that calcium encoding within astrocytes may entail computational processing. These spontaneous oscillations can trigger NMDA receptor-mediated inward currents in neuron partners thereby influencing neuronal activity. Such observations reveal not only a bidirectional influence between astrocytes and neurons involving Ca oscillations but also the fact that astrocytes can act as primary sources for influencing neuronal activity.

Factors regulating calcium oscillations

Accumulating evidence indicates that astrocytic calcium oscillations are highly localized and under tight control within the microdomain nodes, suggesting an important functional role in influencing information transmission at synapses. Increasing evidence indicates that a primary candidate for coupling synaptic neuronal activity with microdomain calcium oscillations is the Na^+/ Ca^{2+} exchanger (NCX) [31], located on the astrocyte membrane of these domains, where its activity generates stable astrocytic Ca2+ oscillations [16,17].

The oscillations are not affected by changing the astrocytic resting membrane potential either to -90 or -50 mV from an initial -70 mV. However, changing the intracellular Na concentration slightly from 15 mM to 20 mM reverses the transporter, suggesting that it is very sensitive to changes in the intracellular astrocytic Na concentration, which ordinarily lies in the 15 - 20 mM range. Significantly, Ca fluctuations in thin astrocytic processes are preserved in IP_3 type 2 receptor (IP_3R2) knock-out animals, implying that local Ca oscillations are not primarily driven by ER store-operated mechanisms. Together these findings implicate the NCX as a primary mechanism driving spontaneous intracellular calcium oscillations within the microdomain regions.

The use of the NCX for driving intracellular calcium oscillations also appears to occur in magnocellular neurons, where many of the putative influx sources that may affect calcium oscillations have been explored pharmacologically [36], including voltage gated calcium channels, Na channels, signaling species, and neurotransmitters. In these studies, inhibitors of voltage gated calcium channels (VGCC) such as nicardipine, conotoxin GVIA, conotoxin MVIIC, agatoxin IVA (for L-, N-, P and P/Q-type channels, respectively) have been shown not to affect Ca oscillations, although a specific R type VGCC blocker, SNX-482, was observed to diminish their magnitude. Similarly, inhibition of the Na channel with TTX did not affect the presence of oscillations, whereas the elimination of extracellular Na or inhibition of NCX reverse mode operation with KB-R7943 blocked them. Intracellularly, the inhibitor of mitochondrial coupling, CCCP, irreversibly blocked spontaneous Ca fluctuations. Intracellular signaling attenuation by inhibitors of phospholipase C and adenyl cyclase had little effect, on the other hand. The neurotransmitter GABA, but not glutamate, also blocked oscillations. Pharmacological investigations thus indicate that spontaneous oscillations in a class of neurons are mediated by a concerted action of R-type Ca channels and the NCX exchanger. These findings suggest the presence of a general intracellular signaling mechanism linking these influx mechanisms with the information encoding and decoding of calcium oscillations, which is widely distributed among both glial and neuron cells of the brain and may undergird fast bidirectional communication between the two cell classes [37].

Regulation of the sodium calcium exchanger

Besides the role played by intracellular Na concentration changes on the orientation of NCX calcium mediated transport, several other factors also contribute to the magnitude of calcium influx imported by the exchanger. These include effects due to phosphorylation of the exchanger and dynamic morphological changes occurring in the microdomain region that affect exchanger dynamics.

Phosphorylation

ATP hydrolysis does not appear to be needed by the NCX exchanger to power net Ca^{2+} extrusion. However, cytosolic ATP does have a significant effect on exchanger kinetics [42], likely due to the phosphorylating of critical sites on the exchanger [38]. Removal of the nucleotide in the presence of Mg, for instance, is known to deactivate the exchanger. With thiophosphorylation, moreover, it is only partially deactivated after substrate removal [42] consistent with exchanger phosphorylation and the greater stability of the thio-phosphorylated residue relative to phosphorylation and a reduced access to phosphatase action. In dialyzed squid axons thiophosphorylation stimulates all exchange modalities, so long as Mg^{2+} is present. Together these data implicate the involvement of kinase action in exchanger transport. The ineffectiveness of inhibitors of PKA, PKC, TyrK, and CAMK, however, suggests the kinase is not a member of these kinase classes. Nonetheless, some involvement of PKA phosphorylation of exchanger may occur since cAMP signaling modulates a slow pattern of exchanger related calcium fluctuations.

Volume changes and actin mobilization effects on calcium fluctuations

Astrocytic perisynaptic processes are highly dynamic and these dynamic features have been linked to effects on interneuronal transmission. Oscillatory Ca fluctuations appear to be coupled to these morphological changes, which can involve greater or lesser synaptic coverage of microdomains over pre- or postsynaptic contacts [22]. Additionally, the occurrence of Ca fluctuations strongly depends on the astrocytic surface to volume ratio (SVR) and correlates with pre-and postsynaptic astrocytic coverage. High astrocytic SVR values are correlated with large amplitude astrocytic Ca fluctuations, whereas medium SVR values, in conjunction with high coverage of both presynaptic axon terminal and postsynaptic dendritic spine, are correlated with medium Ca fluctuations.

Significantly, power spectral density changes over a range of 100 to 500 Hz are directly correlated with increasing SVR. Correspondingly, rapid and dynamic changes occur in microdomain shape and size during neural activity. Together with the observations of the dependence of calcium oscillations on the surface volume ratio, these rapid changes in perisynaptic regions highlight a prominent role for a dynamic cytoskeleton during structural remodeling of astrocytes that is likely to influence neuronal communication.

In view of the fact that microdomains lack microtubules and intermediate filaments [20], their shape changes are likely to entail cytoskeletal reorganization involving actin. Mechanisms for regulating the microdomain actin cytoskeleton are thus likely to also influence the generation of calcium oscillations in perisynaptic zones. Analogous to typical lamellipodia with F-actin-rich subcellular compartments of migrating non-neuronal cells, the dynamic movements of astrocytic microdomains are significantly impaired upon inhibition of the GTPase Rac1 [40] an ubiquitous GTPase driving lamellipodia formation. Inhibition of Rac1 inactivates downstream targets of Arp2/3 actin branching leading to extensive changes in astrocyte morphology. For example, Arp2/3 inactivation *in situ* has been associated with the loss of fine extensions [41] and knockdown of Arp associated proteins like profilin reduce the astrocytic volume [42]. Invasion of the synaptic cleft is also modulated by actin linker proteins like ezrin and connexin30 that enhance synaptic potentiation. Synaptic proteomics, in line with this, show that new translation is required for changes in perisynaptic astrocyte protein composition that occur after fear conditioning [43].

Accordingly, the presence of rapid cytoskeletal remodeling in astrocytes in response to synaptic activity and LTP, suggests the presence of a transducing, excitable actin network. Since actin dynamics are known to be altered by chemophysical stimuli and topography these latter are likely to be predisposing factors that affect NCX induced, calcium fluctuations.

Encoding and Decoding in the Control of Information Flow

Calcium oscillations can, in principle, encode diverse and specific signals via differing modes of modulation. Oscillations could encode signals either by temporally modulating frequency, by altering magnitude, or by varying coupling regimes. Encoding by these means is seen in a wide group of cell types and cellular functions including oocyte fertilization, cell secretion, muscle contraction, neuronal migration, and neurite growth, development, and apoptosis [33,44,45]. In astrocytes encoding of information has been linked to rapidly occurring calcium oscillations linked to extracellular calcium influx.

Encoding information

Mechanosensory and spatial influences on rhythmical calcium changes

As noted, the spatial geometry of microdomain regions has significant influence on spontaneous Ca oscillations generated by the NCX exchanger, with increases in the surface area to volume ratio resulting in large amplitude calcium fluctuations, synaptic coverage changes, and frequency dependence. Additionally, while spatial influences on spontaneous calcium changes may primarily involve the sodium calcium exchanger, localized effects on calcium signaling due to astrocytic spatial geometry may be mediated by other influx mechanisms, albeit to lesser degrees and occurring more slowly. In one model the frequency of calcium signals is critically dependent on the spatial organization of the IP_3R ER channels, based on the dynamics of calcium induced calcium release (CICR) into small spatial volumes corresponding to microdomains [46]. Depending on the spatial distribution of the calcium channels, for example, the same channels can give

rise to different types of calcium signals. Simulations of channel kinetics, for example, show that spontaneous calcium signals are due to the interplay between the excitability of the system and its stochasticity and that this interplay is highly dependent on the spatial location of the channels. In still other cases mechanical stimulation-evoked Ca^{2+} responses in astrocytes of the rat brainstem were blocked by antagonists of connexin channels, connexin 43 (Cx43) blocking peptide Gap26 or Cx43 gene knock-down or antagonists of TRPV4 channels. These data suggest that some mechanosensory dependent Ca^{2+} events are also mediated by interaction between TRPV4 and Cx43 channels.

Important unsettled questions raised by these findings are the nature of the information that may be encoded by actin initiated and spatially informed, microdomain, calcium fluctuations and how this information may be decoded so as to modulate synaptic transmission.

Decoding calcium oscillations

Although it is uncertain what information may be encoded in calcium oscillations by the spatial dynamics of microdomains, there is increasing understanding of the mechanisms likely to be employed to decode this information. Such decoding can be expected to provide insight into the sources and nature of the encoding processes as well as the transmission of this information beyond the astrocyte. The involvement of the NCX in Ca-dependent, exocytotic glutamate release, for example, could be linked to the activation of plasmalemmal ionotropic glutamate receptors and glutamate transporters at the synapse to mediate fast and spatially localized gliotransmission [37], a mechanism that can be expected to be informed by calcium signaling.

Calmodulin and calcineurin

Accumulating evidence suggests that intermediate steps linked to gliotransmission employ the calcium binding protein calmodulin and the protein phosphatase, calcineurin.

Structure and activation of transducing molecules

Both calmodulin and calcineurin are known ubiquitous players in intracellular calcium signaling. Calmodulin is a small, highly conserved protein that becomes activated when bound to calcium. In the Ca^{2+}-free state, it is structurally collapsed but when bound to calcium becomes conformationally open, where it can interact with a wide range of target proteins. The predominantly hydrophobic nature of this binding enables the recognition of some 300 known target proteins having a broad variety of CaM-binding sequences. Key signaling targets include calmodulin kinase II and the phosphatase calcineurin.

Calcium binding of calmodulin precedes binding of the activated CaM unit to calcineurin, one of the most abundant proteins in the CNS [47]. Generation of the CaM:calcineurin complex in turn results in the active phosphatase. This process couples calcium signaling to dephosphorylation in a manner similar to the coupling of calcium signaling to phosphorylation via CaM-modulated kinases. One notable difference, however, is that there are multiple CaM-modulated kinases whereas CaN is the only phosphatase known to be directly activated by CaM. The affinity of CaM for calcineurin, nonetheless, is in the low picomolar range, which represents an extraordinarily tight binding and suggests that calcineurin is an especially important CaM substrate, as are its downstream targets.

Decoding of calcium signaling by calcineurin can be expected to be demonstrated by variability in the calcineurin activation response, a process of transduction, or may be observed as a cumulatively increasing level of dephosphorylated target protein, a process of signal integration [48]. In the first case, calcineurin targets may be rapidly rephosphorylated and the level of target dephosphorylation may be observed as an oscillatory dynamic paralleling the calcium oscillations, i.e. a direct transduction of the calcium signal. In pancreatic islet cells, for example, calcineurin activation more frequently transduces the oscillatory dynamics of the intracellular calcium fluctuations [48], suggesting that embedded codes are found in the oscillatory dynamic per se. In this cell system, transduction dynamics have been observed using a fluorescence resonance energy transfer (FRET) based reporter used for monitoring calcineurin activation. Using this

method, a robust increase in the cyan-to-yellow fluorescence emission ratio could be observed, which tracked the increase in cytosolic Ca both spatially and temporally. The Ca^{2+}-induced change was blocked by pretreatment with a CaM antagonist indicating that calcineurin activation was indeed tracking the changes in calcium occurring within the cell. In neurons, calcineurin activity is known to induce long term depression, although confirmation of similar activation dynamics that parallels calcium oscillations in either neurons or astrocytes remains to be determined.

Significantly, calculations of the kinetics of Ca^{2+} binding and dissociation from CaM indicate that the calcium bound form can only diffuse a short distance, about 0.1 μm, before calcium dissociation, suggesting that Ca^{2+}/CaM primarily acts as a highly localized signal [47]. Consistent with this, the subcellular spatial distribution of calcineurin activation is tightly coupled to CaM locations in cells [48]; that is, the differential calcineurin activity patterns are dictated by variations in the subcellular distribution of calmodulin (CaM), indicating that the localization of CaM actively shapes both the spatial and temporal aspects of calcineurin signaling.

In nervous tissue, calcineurin is apparently involved both in normal information processing and in pathological reactive gliosis [56]. Calcineurin deficient mice have been shown to exhibit abnormal spatial memory behaviors, adopting widely variant learning strategies, which suggests that information processing for memory acquisition requires CaN. CaN in astrocytes can also be strongly activated in response to the induction of LTP in tissue culture, revealing that astrocytic CaN may transduce neuronal activity.

Synapses and Networks: Possible Influences of Microdomain Calcium Signaling on Network Communication

Resting-state networks possess multiple intrinsic properties that identify them as brain networks and could be affected by calcium signaling. Besides neuroanatomical structure, documented findings also include local neuronal dynamics, signal transmission delays, physical features of the neuropil, and genuine noise. In principle it can be proposed that astrocyte Ca^{2+} signaling assists the establishment of self-coordinated spatio-temporal patterns affecting local fast responses or slow global responses, the latter the result of signal integration from multiple microdomain compartments [50]. These effects are likely to undergird the operational integrity of brain networks and enable computational processing and information exchange locally and regionally. Several such mechanisms now known to be initiated by astrocytic calcium events could modify circuit and network based operation.

Potentiation of synaptic connectivity: the LTP and LTD

Astrocytes are known to be associated with the mechanisms of long term potentiation (LTP) and long term depression (LTD), mechanisms that involve strengthening and weakening of synaptic connections and so also of regulating information flow [51]. For instance, the NMDAR-dependent LTP seen in hippocampal CA1 synapses requires transient d-serine gliotransmission from astrocytes. Altering the potentiation of synapses can be expected to shape the course of information flow, effects likely to be reflected in changes in functional and effective connectivity.

Network stabilization

The stability of RSN function, defined as a network's ability to sustain the brain's organizational order or to elicit robust functional outcomes despite perturbations, is a critical need for reliable brain operation. Neural networks undergo significant variability when exposed to sensory or noise based perturbations. Strikingly, despite such large-scale fluctuations in input and performance, the outputs of these networks are stable and are tuned to sustain brain functioning. Insight into how this stabilization occurs has been obtained in simulations of hippocampal grid cells. In this model, it has been shown that operational stability may be due to the presence of neuronal resonance that structures high pass, tunable filtering via targeted suppression of low-frequency perturbations [52]. Importantly, the slow kinetics of negative feedback loops are essential in stabilizing networks built with resonators, a process of temporal modulation that could be achieved by astrocytic regulation.

Tuning information transfer

Theoretical considerations as well as experimental evidence indicates that network oscillations in the gamma frequency range depends on rhythmic contributions from networks of synaptically connected GABAergic (inhibitory) interneurons, which impinge on excitatory neurons [6] thereby generating oscillations involved in information transfer. Imaging data from cultured hippocampal slices has revealed the participation of astrocytes in generating oscillations. When calcium levels in astrocytes are lowered by buffering there is a corresponding reduction in correlated neuron activity at network levels. By contrast, uncaging of intracellular astrocyte calcium can generate synchronized activity within the network.

These findings suggest that astrocytic gliotransmission contributes to information transfer by assisting neuronal synchronization. Consistent with this, calcium elevations in astrocytes and subsequent glutamate release have been shown to lead to the synchronous excitation of clusters of pyramidal neurons in the hippocampal network. In this system astrocytic regulation of signal transmission between neurons improved firing synchrony by tuning the coherency of network oscillations. In particular, astrocyte-mediated potentiation of inhibitory synaptic transmission together with astrocytic regulation of excitatory synaptic input greatly improved network oscillation coherence [53]. Since gamma oscillation synchrony is posited to ground information transfer, these findings directly implicate astrocytes in brain communication events.

Synchronizing network structure: Astrocyte calcium and slow wave oscillation

Among the most dramatic impacts of the astrocytes are those affecting whole networks and, potentially, brain states through their coordination of neuronal network activation.

Illustrative of such larger scale networks is the slow wave, a global activity state that incorporates cortical and, to a limited extent, subcortical activity occurring during NREM sleep. This slowly oscillating wave originates from both the thalamus and cortex with oscillations that take place roughly every second between an Up period of depolarization with spiking and a Down/Off period of hyperpolarization in which neurons are silent [54]. Studies of select, slow oscillation phases reveal that the negative peak is continuously shifted across the cortex [55].

Additionally, slow oscillations are found more frequently in anterior regions and propagate posteriorly. Streamline maps that condense the spatio-temporal dynamics of these slow oscillations reveal that the origin of the waves coincides with the position of the anterior electrodes, with the average delay map oriented predominantly in a fronto-occipital direction. Together these data show that the slow wave is a global, synchronized network phenomenon, involving neurons throughout the cortex and, to a lesser degree, neurons in subcortical areas, including the thalamus, striatum, and cerebellum.

Current work demonstrates that calcium fluxes in astrocytes are required for generating the Up state of the slow wave. Supporting this are the following salient findings [15]: electrical stimulation of astrocytes activates other astrocytes in the local circuits and triggers UP state synchronization of neighboring neurons; intracellular injections of a calcium chelator into individual astrocytes inhibit spontaneous and induced UP states; and finally, both astrocytic activity and neuronal UP states can be regulated by purinergic signaling in the circuit. Regional studies have further shown that activation of local ensembles via calcium can lead to slow wave dominated states.

Optogenetic activation of astrocytes, for instance, can convert irregular activity in local neuronal circuits to patterned, slowly oscillating activity. Together these results indicate that calcium fluxes in astrocytes are likely to be causally involved in regulating the synchronized activation of neuronal ensembles.

At global scales, such changes in synchronization function to drive the network toward a state of global functional connectivity. Blood oxygenation level-dependent (BOLD) responses that reveal a cortex-wide and spatially organized correlate of local neuronal activity, for example, are directly related to slow calcium waves [56].

Moreover, during slow wave activity, the slow wave events are correlated with the strength of functional connectivity between different cortical areas. These findings suggest that the transition from neuronal excitability to the synchronized slow wave state drives a cortex-wide increase in functional connectivity, which links the changes in functional connectivity directly to the generation of slow waves. In line with this, filtering of the BOLD signal at different frequency intervals prior to conducting a cross-correlation analysis has revealed a significant correlation decrease in the frequency interval associated with the UP Down phases, chiefly between 0.01 and 0.4 Hz. This latter finding shows that astrocytic induced, slow wave UP Down phases are likely to be main factors involved in the increase in cortical functional connectivity across the cortex.

Conclusion

Employing neuroimaging advances based on fMRI in a host of allied approaches, knowledge of resting state networks and their contribution to brain organization and operation is greatly expanding. On the other hand, knowledge of the underlying mechanisms for the construction of these networks and how they participate in information exchange and regulation remains at an early stage. Accumulating evidence indicates that these mechanisms are not exclusively neuronal and, indeed, may be equally distributed between neurons and non-neuronal partners, particularly the astrocyte. This review discusses the likely contribution to network and circuit performance of a highly specialized astrocyte region that is in intimate contact with synapses, the calcium microdomain.

Reviewed here are structural and molecular features of the microdomain that enable it to share in and modulate information exchange focally, not only between synaptic neuron partners, but also between neurons and their astrocyte partner. These features illustrate that the microdomain region is uniquely designed to actively influence brain communication, and that this design is holistically engaged, with structural and molecular features working in concert to encode communication specific information. The repercussions of targeted intervention in synaptic transmission can be expected to influence transmission in a variety of ways critical to the functioning of circuits and networks, including their stabilization against information degradation as well as their computational processing.

Due to the close association of RSNs with brain functioning, they have been identified as fundamental targets for personalized patient care, with the potential to improve the treatment of conditions of the brain and nervous system. Hence, knowledge of how astrocyte mi- crodomains regulate information flow in these networks can also be expected to assist in the development of therapies for cognitive dysfunctions.

Bibliography

1. Power JD., *et al*. "Functional network organization of the human brain". *Neuron* 72 (2011): 665-678.
2. Yeo BT., *et al*. "The organization of the human cerebral cortex estimated by intrinsic functional connectivity". *Journal of Neurophysiology* 106 (2011): 1125-1165.
3. Rabinovich M and Varona P. "Robust transient dynamics and brain functions". *Frontiers in Computational Neuroscience* 5.24 (2011): 1-10.
4. Sequin C., *et al*. "Navigation of brain networks". *Proceedings of the National Academy of Science, USA* 115.24 (2018): 6297-6302.
5. Vidaurre D., *et al*. "Brain network dynamics are hierarchically organized in time". *Proceedings of the National Academy of Science, USA* 114 (2017): 12827- 12832.

6. Lowet E., *et al*. "A quantitative theory of gamma synchronization in macaque V1". *eLIFE* 6 (2017): e26642.

7. Canavier C. "Phase-resetting as a tool of information transmission". *Current Opinion in Neurobiology* 31 (2015): 206-213.

8. X. "Neurophysiological and computational principles of cortical rhythms in cognition". *Physiological Review* 90 (2010): 1195-1268.

9. Santello M., *et al*. "Astrocyte function from information processing to cognition and cognitive impairment". *Nature Neuroscience* 22 (2019): 154-166.

10. Pannasch U and Rouach N. "Emerging role for astroglial networks in information processing: from synapse to behavior". *Trends in Neuroscience* 36 (2013): 405-417.

11. Pasti L., *et al*. "Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes *in situ*". *Journal of Neuroscience* 17 (1997): 7817-7830.

12. Todd KJ., *et al*. "Perisynaptic glia discriminate patterns of motor nerve activity and influence plasticity at the neuromuscular junction". *Journal of Neuroscience* 30 (2010): 11870-11882.

13. Araque A., *et al*. "Tripartite synapses: glia, the unacknowledged partner". *Trends in Neuroscience* 22 (1999): 208-215.

14. Zhou B., *et al*. "Astrocyte morphology: diversity, plasticity, and role in neurological diseases". *CNS Neuroscience and Therapeutics* 25.6 (2019): 665-673.

15. Poskanzer KE and Yuste R. "Astrocytes regulate cortical state switching *in vivo*". *Proceedings of the National Academy of Science, USA* 10113.19 (2016): E2675-2684.

16. Héja L., *et al*. "Spontaneous Ca^{2+} fluctuations arise in thin astrocytic processes with real 3D geometry". *Frontiers in Cell Neuroscience* 15 (2021): 617989.

17. Héja L and Kardos J. "NCX activity generates spontaneous Ca^{2+} oscillations in the astrocytic leaflet microdomain". *Cell Calcium* 86 (2020): 102137.

18. Wenzel J., *et al*. "The influence of long-term potentiation on the spatial relationship between astrocyte processes and potentiated synapses in the dentate gyrus neuropil of rat brain". *Brain Research* 560.1-2 (1991): 122-131.

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

20. Aboufares EAA., *et al*. "Characterization of subcellular organelles in cortical perisynaptic astrocytes". *Frontiers in Cell Neuroscience* 14 (2021): 573944.

21. Bindocci E., *et al*. "Three-dimensional calcium imaging advances in understanding of astrocyte biology". *Science* 356 (2017): 6339.

22. Mariotti L., *et al*. "The inhibitory neurotransmitter GABA evokes long-lasting Ca^{2+} oscillations in cortical astrocytes". *Glia* 64 (2016): 363-373.

23. Kanemaru K., *et al*. "*In Vivo* visualization of subtle, transient, and local activity of astrocytes using an ultrasensitive Ca^{2+} indicator". *Cell Reports* 8 (2014): 311-318.

24. Okubo Y., *et al*. "Inositol 1,4,5-trisphosphate receptor type 2-independent Ca^{2+} release from the endoplasmic reticulum in astrocytes". *Glia* 67 (2019): 113-124.

25. Rungta RL., *et al*. "Ca2+ transients in astrocyte fine processes occur via Ca^{2+} influx in the adult mouse hippocampus". *Glia* 64 (2016): 2093-2103.

26. Palygin O., *et al*. "Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes". *British Journal of Pharmacology* 163 (2011): 1755-1766.

27. Lind BL., *et al*. "Fast Ca^{2+} responses in astrocyte end-feet and neurovascular coupling in mice". *Glia* 66 (2017): 348-358.

28. Sharma G and Vijayaraghavan S. "Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores". *Proceedings of the National Academy of Science, USA* 98 (2001): 4148-4153.

29. Palygin O., *et al*. "Ionotropic NMDA and P2X1/5 receptors mediate synaptically induced Ca^{2+} signalling in cortical astrocytes". *Cell Calcium* 48 (2010): 225-231.

30. Verkhratsky A., *et al*. "Sodium fluxes and astroglial function". *Advances in Experimental Medicine and Biogeology* 961 (2013): 295-305.

31. Blaustein MP., *et al*. "Na/Ca exchanger and PMCA localization in neurons and astrocytes: Functional implications". *Annals of the New York Academy of Sciences* 976 (2002): 356-366.

32. Blaustein MP and Lederer WJ. "Sodium/calcium exchange: its physiological implications". *Physiological Reviews* 79.3 (1999): 763-855.

33. Su L., *et al*. "Cytosolic calcium oscillations in submandibular gland cells". *Acta Pharmacologica Sinica* 27.7 (2006): 843-847.

34. Parri HR., *et al*. "Spontaneous astrocytic Ca oscillations *in situ* drive NMDAR- mediated neuronal excitation". *Nature Neuroscience* 4.8 (2001): 803-812.

35. Volterra A., *et al*. "Astrocyte Ca^{2+} signalling: an unexpected complexity". *Nature Review Neuroscience* 15 (2014): 327-335.

36. Kortus S., *et al*. "Sodium-calcium exchanger and R-type Ca^{2+} channels mediate spontaneous [Ca^{2+}] i oscillations in magnocellular neurones of the rat supraoptic nucleus". *Cell Calcium* 59.6 (2016): 289-298.

37. Reyes RC., *et al*. "Plasmalemmal Na^{+}/Ca^{2+} exchanger modulates Ca^{2+}- dependent exocytotic release of glutamate from rat cortical astrocytes". *Association Neuroscience* 4.1 (2012): e00075.

38. Dipolo R. "Effect of ATP on the calcium efflux in dialyzed squid giant axons". *Journal of General Physiology* 64 (1974): 503-517.

39. Nishida H and Okabe S. "Direct astrocytic contacts regulate local maturation of dendritic spines". *Journal of Neuroscience* 27 (2007): 331-340.

40. Rottner K., *et al*. "Actin assembly mechanisms at a glance". *Journal of Cell Science* 130 (2017): 3427-3435.

41. Murk K., *et al*. "The antagonistic modulation of Arp2/3 activity by N-WASP, WAVE2 and PICK1 defines dynamic changes in astrocyte morphology". *Journal of Cell Science* 126 (2013): 3873-3883.

42. Molotkov D., *et al*. "Calcium- induced outgrowth of astrocytic peripheral processes requires actin binding by Profilin-1". *Cell Calcium* 53 (2013): 338-348.

43. Sapkota D., *et al*. "Activity-dependent translation dynamically alters the proteome of the perisynaptic astrocyte process". *Cell Reports* 41.3 (2022): 111474.

44. Malcuit C., *et al*. "Calcium oscillations and mammalian egg activation". *Journal of Cell Physiology* 206 (2006): 565-573.

45. Komuro H and Kumada T. "Ca^{2+} transients control CNS neuronal migration". *Cell Calcium* 37 (2005): 387-393.

46. Denizot A., *et al*. "Simulation of calcium signaling in fine astrocytic processes: Effect of spatial properties on spontaneous activity". *PLoS Computational Biology* 15.8 (2019): e1006795.

47. Creamer T. "Calcineurin". *Cell Communication and Signaling* 18 (2020): 137.

48. Mehta S., *et al*. "Calmodulin-controlled spatial decoding of oscillatory C signals by calcineurin". *eLife* (2014): e03765.

49. Lim D., *et al*. "Calcineurin signalling in astrocytes: from pathology to physiology and control of neuronal functions". *Neurochemical Research* (2022).

50. Gordleeva SY., *et al*. "Astrocyte as spatiotemporal integrating detector of neuronal activity". *Frontiers in Physiology* 10 (2019): 294.

51. Santello M., *et al*. "Astrocyte function from information processing to cognition and cognitive impairment". *Nature Neuroscience* 22 (2019): 154-166.

52. Mittal D and Narayanan R. "Resonating neurons stabilize heterogeneous grid-cell networks". *eLife* 10 (2021): e66804.

53. Makovkin S., *et al*. "Controlling synchronization of gamma oscillations by astrocytic modulation in a model hippocampal neural network". *Scientific Reports* 12.6970 (2022): 1-15.

54. Neske GT. "The slow oscillation in cortical and thalamic networks: mechanisms and functions". *Frontiers in Neural Circuits* 9 (2016): 88-113.

55. Tononi G and Cirelli C. "Sleep and synaptic down-selection". *European Journal of Neuroscience* 51 (2020): 413-421.

56. Bojarskaite L., *et al*. "Astrocytic Ca^{2+} signaling is reduced during sleep and is involved in the regulation of slow wave sleep". *Nature Communications* 11 (2020): 3240.

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