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Review

Revisiting astrocytic calcium signaling in the brain

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ABSTRACT

Astrocytes, characterized by complex spongiform morphology, participate in various physiological processes, and abnormal changes in their calcium (Ca^{2+}) signaling are implicated in central nervous system disorders. However, medications targeting the control of Ca^{2+} have fallen short of the anticipated therapeutic outcomes in clinical applications. This underscores the fact that our comprehension of this intricate regulation of calcium ions remains considerably incomplete. In recent years, with the advancement of Ca^{2+} labeling, imaging, and analysis techniques, Ca^{2+} signals have been found to exhibit high specificity at different spatial locations within the intricate structure of astrocytes. This has ushered the study of Ca^{2+} signaling in astrocytes into a new phase, leading to several groundbreaking research achievements. Despite this, the comprehensive understanding of astrocytic Ca^{2+} signaling and their implications remains challenging area for future research.

Deciphering Ca^{2+} signaling in astrocytes is crucial for understanding the functional roles of the glial cells. A growing body of evidence suggests that astrocytic Ca^{2+} signaling contributes to a variety of astrocytic functions, including modulation of cerebral blood flow, synaptic transmission, synaptic plasticity, and gliotransmitter release [1]. In this context, our attention is dedicated to advancing our understanding of astrocytic Ca^{2+} events. Collectively, these advancements promise to yield fresh insights into the mechanisms through which astrocytes regulate the brain function and how changes in astrocytic Ca^{2+} signals influence physiological and pathological processes.

1. Astrocyte morphology

The types of glial cells in the central nervous system (CNS) include microglia, astrocytes, and oligodendrocyte precursors [2–4]. Astrocytes were first described by Rudolf Virchow in the 19th century. Decades later, researchers used the silver chromate staining technique to visualize the morphology of astrocytes, advancing the view that astrocytes act as a “glue” in the CNS [5]. These abundant and morphologically complex cells in mammalian brain are distributed in a lattice-like pattern [6,7], forming a dense network in which other glial cells, blood vessels, and neurons are embedded [6–10].

Astrocytes exhibit complex spongiform shapes, with volumes of approximately ~6.6×10^4 μm and diameters ranging from ~40 to 60 μm [11–14]. The fine structure of astrocytes, particularly protoplasmic astrocytes in the gray matter, consists of a soma, several major branches, numerous branchlets, leaflets, and end-feet (Fig. 1) [15]. Branches, the major processes emanating from the soma, have diameters in the micrometer range. Secondary to tertiary processes, branchlets have diameters in the sub-micrometer range. Leaflets, the terminal extensions of branchlets, show close contacts with synapses, while end-feet are distal extensions that contact blood vessels. Perisynaptic astrocyte processes (PAPs), a morphologically distinct leaflets adjacent to a synapse, affect information processing by the nerve circuits and the behaviours which rely on it [16,17]. However, emerging evidence has suggested that the morphology of astrocytes varies under different conditions. For instance, the phenotypes of reactive astrocytes show an increased number of processes and a denser morphology with altered soma volume compared to resting astrocytes [18].

As a heterogeneous population of cells, astrocytes vary in morphological appearance [19]. Generally, protoplasmic astrocytes are one of the major types found in grey matter, whereas fibrous astrocytes are found in white matter [20–22]. An increasing number of studies have shown that astrocytes exhibit morphological deficits that may contribute

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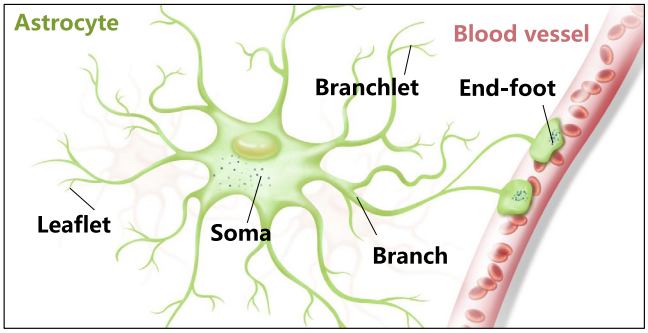

Fig. 1. Astrocytes present a complex morphological appearance. Schematic representation of the astrocyte structure.

to the progression of CNS disease [7,14]. For instance, at the onset of Alzheimer's disease (AD), progressive atrophy of astrocytes with decreased GFAP expression was observed in the hippocampus and cortex [23–25]. Furthermore, several studies have shown that the morphological changes of astrocytes in the substantia nigra pars compacta exhibited GFAP-astrogliosis in patients with Parkinson's disease (PD). These findings imply that astrogloiosis dysfunction is likely to be fundamental to dissecting the cellular mechanisms underlying CNS disease [26–28]. In summary, the study of astrocytes with varying morphologies has garnered increased attention due to their important contribution to the CNS.

2. Ca^{2+} signaling in astrocytes

Calcium (Ca^{2+}) is a crucial intracellular second messenger for the survival and fate of organisms [29,30]. The Ca^{2+} signaling system comprises numerous types of proteins, including channels, pumps, receptors, exchangers, and sensors, several of which are altered in expression or mutated in various diseases [31]. For example, excessive inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signaling contribute to the excitatory-inhibitory imbalance of neurons in bipolar disorder [32,33]. Notably, researchers have studied Ca^{2+} signaling in various cell types and model organisms to understand its physiological and pathological roles [34]. In the CNS, Ca^{2+} serves as a pivotal component in intracellular signaling, facilitating communication within both glial cells and neurons. Its role is integral to the maintenance of brain homeostasis [35].

It is well known that astrocytes are electrically non-excitable glial cells and Ca^{2+} fluctuations have been detected in astrocytes under physiological and pathological conditions [36–38]. Spatially, Ca^{2+} signaling potentially propagates to neighbouring cells *via* astrocytic gap junctions [39,40]. This Ca^{2+} wave propagation has been thoroughly proven *in vitro* systems. Instead of wave propagation, synchronized Ca^{2+} activities of many astrocytes have been detected in awake and naturally sleeping animals in response to different behavioral stimuli [41]. Within a single astrocyte, Ca^{2+} events in different cellular compartments also have different functional roles, and several studies have provided evidence for the diversity of Ca^{2+} signals [15,42].

Analyzing Ca^{2+} in the astrocytic soma is relatively straightforward due to its distinct identification in the soma region. Ca^{2+} signaling in astrocytes is frequent and short in the distal processes, but relatively rare and large in the soma [43]. Furthermore, Ca^{2+} signaling induced by the hypoosmotic test was detected in most compartments of astrocytes except the soma, indicating that the astrocytic soma does not respond to the hypoosmotic test. Interestingly, under selected experimental conditions, Ca^{2+} signals can still be detected in astrocytes at the soma. Thrane et al. reported that the astrocytic soma responded to osmotic swelling, in terms of Ca^{2+} spikes both *in vivo* and *in vitro* [44]. The mouse pups used in this study may explain why the somatic Ca^{2+} response occurred, demonstrating that Ca^{2+} signaling at the soma relied on developmentally regulated autocrine signaling pathways. Age is likely to be an important factor regulating somatic Ca^{2+} signaling in astrocytes. Moreover, Duffy et al. showed that osmotically induced Ca^{2+} responses at the soma occur in the late phase of brain swelling [44]. Furthermore, another study supported the idea that astrocytes exhibited an increase in Ca^{2+}, mediated by voltage-dependent influx and released from internal stores in response to ischemia [45].

Notably, most Ca^{2+} activity in astrocytes is spatially restricted to microdomains, occurring in fine processes that form a complex meshwork [46]. These localized Ca^{2+} signals were observed to occur more frequently than somatic Ca^{2+} elevations and were firstly described as microdomains in the processes of Bergmann glia [47]. Researchers demonstrated that these astrocyte Ca^{2+} signals were frequently far removed from the soma, such as in branches, branchlets, and end-feet [9,48–50]. Ca^{2+} released from the endoplasmic reticulum (ER) store in an inositol triphosphate receptor 2 (IP_3R2)-dependent manner is one of the important sources of Ca^{2+} microdomain signals [50]. Microdomain activity, which occurs in the absence of IP3-dependent release from the endoplasmic reticulum, is mediated by Ca^{2+} efflux from the mitochondria during brief openings of the mitochondrial permeability transition pore [9]. The Ca^{2+} influx-dependent events are also detected in the fine processes of adult hippocampal astrocytes [51]. Many studies have focused on understanding the Ca^{2+} events at microdomains [1,52,53]. For instance, a fraction of astrocytic Ca^{2+} signaling is caused by spontaneous and action potential–dependent synaptic activity [54]. Microdomain Ca^{2+} signal has been detected in end-feet, mediated by Ca^{2+} entry *via* TRPV4 channels and Ca^{2+} release from IP_3R2-dependent stores [55,56]. Finally, another type of Ca^{2+} signaling occurs during startle responses and locomotion, and is driven by the volumetric release of neuromodulators [57,58].

3. Genesis of astrocytic Ca^{2+} events

Astrocytic Ca^{2+} signaling is regulated by multiple mechanisms, although these remain to be fully elucidated [59]. To date, numerous studies have shown that astrocytic Ca^{2+} signaling depended not only on intracellular Ca^{2+}resources, but also on extracellular transmembrane Ca^{2+} influxes, as shown in Fig. 2. The sources of Ca^{2+} also show spatiotemporal characteristics. For instance, transients in the soma of astrocytes rely on the coordination between the release of intracellular Ca^{2+} stores and extracellular influxes. However, some of the events in fine processes can be solely generated by Ca^{2+} influxes [51].

3.1. Intracellular Ca^{2+} source

The regulated release of Ca^{2+} from the internal stores of the ER or mitochondria is one of the major mechanisms contributing to astrocytic Ca^{2+} events. Among them, astrocytic Gq-G protein-coupled metabotropic receptor (GPCR)-linked IP_3R-dependent Ca^{2+} signaling is the most extensively studied molecular mechanism of Ca^{2+} signals, which has been investigated in an IP_3R2 knockout mouse [19,50,60–64]. Nagai et al. reported that mice lacking intracellular IP_3R2s showed downregulation of spontaneous and GPCR-mediated Ca^{2+} signaling [64]. Notably, astrocytic Ca^{2+} events in the soma are reduced by silencing IP_3R2 in the ER. However, both the proportion of fast-onset microdomain Ca^{2+} events evoked by nearby synaptic activity and the Ca^{2+} responses in fine processes to sensory stimulation are unaffected [50,60,65]. Nevertheless, IP_3R2 is unlikely to be the only type of receptor mediating Ca^{2+} release from the ER in astrocytes. Okubo et al. recently re-evaluated the assumption that Ca^{2+} release from the ER was abolished in IP_3R_2-KO astrocytes using a highly sensitive imaging technique and demonstrated that IP_3R_2-independent Ca^{2+} release induced small cytosolic Ca^{2+} elevations but robust Ca^{2+} transients in the mitochondria [66]. Accordingly, in IP_3R2-KO mice, ryanodine and $IP_3R1/3$ receptors may facilitate IP_3R2-independent Ca^{2+} release [67,68].

Mitochondria are another key player in the astrocytic Ca^{2+} microdomain, acting as a single electrically coupled continuum or as mul-

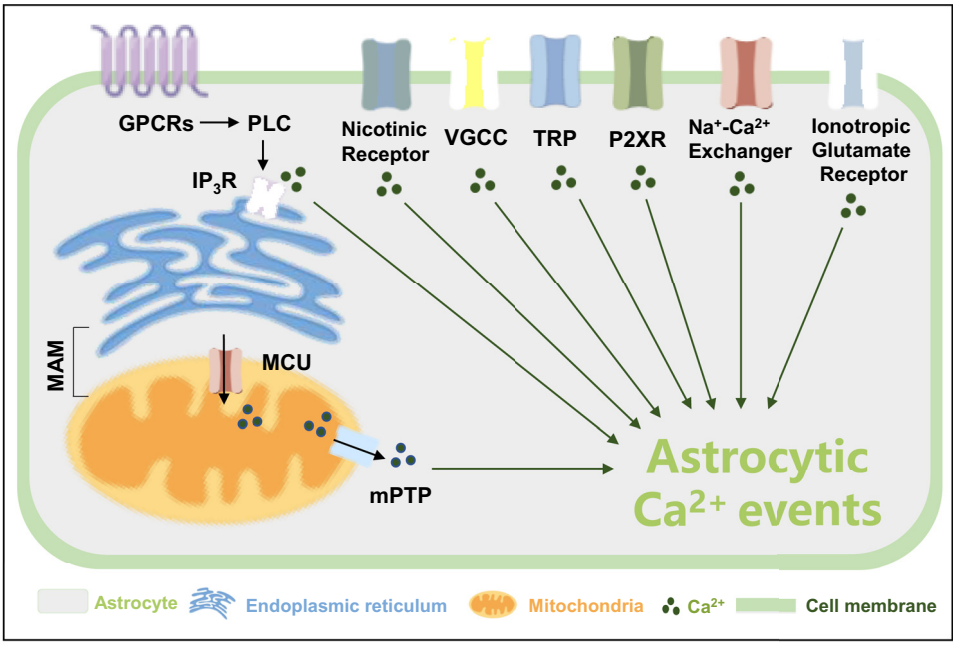

Fig. 2. Different Ca^{2+} channels exist in the cell and organelle membranes. Ca^{2+} channels are expressed on both the plasma membrane and the membrane of organelles, as discussed in this review. GPCRs: G-protein coupled metabotropic receptors, PLC: phospholipase C, IP_3R: inositol triphosphate receptor, MAM: mitochondria-associated ER membranes, MCU: mitochondrial Ca^{2+} uniporter, mPTP: mitochondrial permeability transition pore, VGCC: voltage-gated Ca^{2+} channel, TRP: transient receptor potential channels, P2XR: P2X receptors.

tiple separate organelles to modulate Ca^{2+} signals [69]. Recently, the Bergles laboratory demonstrated that mitochondria were critical for the Ca^{2+} activities in astrocytic microdomains and were used to store Ca^{2+}, which can influence signals by buffering receptor-induced Ca^{2+} transients. In this study, scientists have shown that spatially restricted Ca^{2+} events in astrocytic processes, which occurred independently of ER Ca^{2+} release, were caused by Ca^{2+} efflux from mitochondria in response to the transient opening of the mitochondrial permeability transition pore (mPTP) [9]. Moreover, studies indicated that opening of the mPTP maintained the levels of mitochondrial Ca^{2+}. Reversible mPTP opening-dependent ROS release is an adaptive housekeeping function of mitochondria to avoid potentially toxic levels of ROS (and Ca^{2+}) [70]. Agarwal et al. showed that inhibiting the function of astrocytic mPTP or disrupting the mitochondrial membrane potential markedly reduced Ca^{2+} events in the microdomain, whereas increasing mPTP opening increased Ca^{2+} transients [9]. Collectively, these studies showed that opening of mPTP was the cause of spontaneous Ca^{2+} signals in astrocytes lacking IP_3R-mediated Ca^{2+} release. Mitochondrial Ca^{2+} homeostasis is also achieved by the mitochondrial Ca^{2+} uniporter (MCU), mitochondrial H^+-Ca^{2+} exchangers, and Na^+-Ca^{2+} exchangers [71].

Interestingly, functional coupling also exists between mitochondria and ER Ca^{2+} stores in astrocytes. The ER and mitochondria are frequently in close proximity and have been shown to form mitochondria-associated ER membranes (MAMs) that promote the exchange of metabolites and ions [72]. This specialized junction may synergistically promote Ca^{2+} release. In PD, the interface of the ER–mitochondria was disrupted, leading to abnormal ER-to-mitochondria Ca^{2+} transfers in patients with PD and Parkin knockout mice with Parkin mutations [73]. Parkinson's disease protein 7 (Park7/DJ-1) which is another PD-related protein closely related to MAMs has also been implicated in Ca^{2+} events and upregulating DJ-1 prevents these alterations by re-establishing the ER-mitochondria tethering [74,75]. Liu et al. identified DJ-1 as a key component of the IP_3R–Grp75–VDAC1 complex, the loss of which resulted in decrease in mitochondrial Ca^{2+} upon IP_3R stimulation [75]. In AD, exposure of neurons to Aβ triggered the upregulation of MAM-associated proteins and increased ER–mitochondrial contact sites [76]. Moreover, MAM-localized Ca^{2+} signals have been implicated in the pathogenesis of other diseases and warrants further investigation [72].

3.2. *Extracellular Ca^{2+} influx*

Numerous studies have suggested that Ca^{2+} activity in astrocytes also depended on extracellular Ca^{2+} sources. In particular, astrocytes exhibited Ca^{2+} events close to the membrane [49,77]. Genetic and pharmacological evaluations have shown that extracellular Ca^{2+} influx in astrocytes occurred through transient receptor potential cation channel A1 (TRPA1) and others (e.g., TRPC3, TRPC4, TRPC5, TRPV1, TRPV4 and TRPC1) [55,77–84]. Importantly, the studies conducted by Freeman's group on TPR channels contributing to microdomain Ca^{2+} signals deserve attention. They demonstrated that astrocytic Ca^{2+} events mediated by different TRP channels were indispensable for multiple sensory-driven behaviors as well as the regulation of CNS gas exchange [85,86]. However, despite the dedication of numerous scientists to the investigation of these receptors, the molecular basis of this process has not been systematically elucidated. Ca^{2+} signaling in astrocytes in different brain regions may be mediated by different sources.

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that can be divided into three types (NMDA receptor, AMPA receptor, and Kainate receptor). iGluRs are also essential mediators of astrocytic Ca^{2+} influx [87–91]. Porter et al. demonstrated that hippocampal astrocytes responded to the neuronal release of the neurotransmitter glutamate by increasing in Ca^{2+} through iGluRs [92]. Similarly, NMDA triggered local increases in astrocytic Ca^{2+} in the mouse neocortex *via* functional NMDA receptors [93].

The Na^+-Ca^{2+} exchanger, also known as "NCX," exchanges the Ca^{2+} and Na^+ in the astrocytes. Multitudinous researches have attempted to model the function of NCX in astrocytic Ca^{2+} microdomain events, which revealed a novel form of astrocytic Ca^{2+} signaling [94–96].

P2X receptors, which are thought to contain seven subunits, are ion channels that open in response to the binding of extracellular ATP in astrocytes from the hippocampus, cortex, spinal cord, cerebellum, brain stem, and retina [19,97–99]. $P2X_{1/5}$ and $P2X_7$ are the major subunits

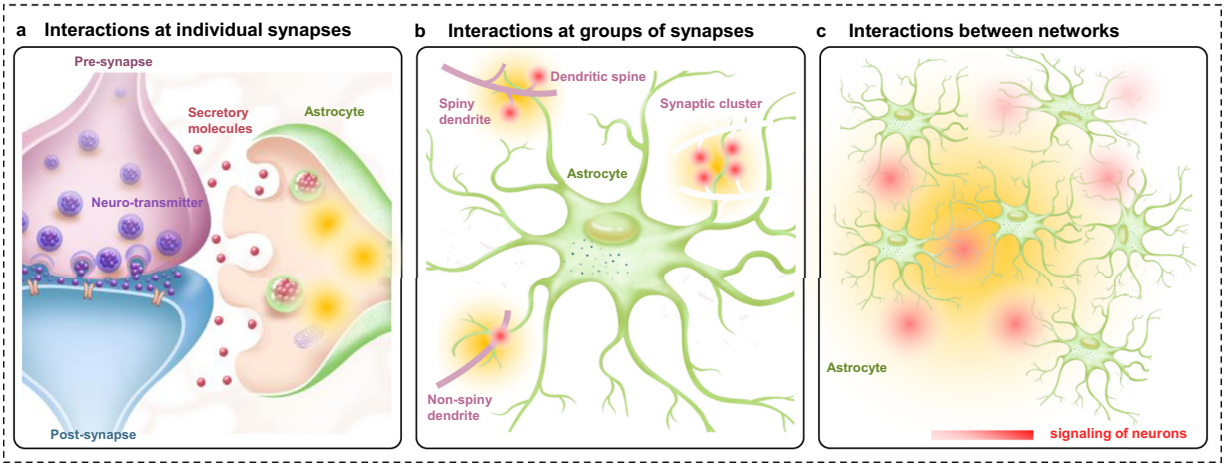

Fig. 3. Three modes of interaction between neurons and astrocytes. (a) Ca^{2+} events in perisynaptic astrocytic leaflets induce the release of signaling molecules that affect neuronal excitability, synaptic plasticity, and transmission. (b) Astrocytic Ca^{2+} signaling involves the territories of multiple synapses, which affect their plasticity/ activity. (c) Ca^{2+} events propagate through the astrocytic network and control information processing throughout the network of neurons.

associated with Ca^{2+} signals. Rat cortical astrocytes have been reported to express ligand-gated P2X (i.e., $P2\times_1$-$P2\times_5$ and $P2\times_7$) for all clones except the $P2\times_6$ receptor [88,100]. In addition to the cortex, $P2\times_7$ receptors were found to be expressed in the hippocampus and retina [101,102].

Voltage-Gated Ca^{2+} Channels (VGCC), documented at the molecular level, are classified as $Ca_v1.1$–1.4 or L-type Ca^{2+} channels, $Ca_v2.1$–2.3 or P/Q/N/R-type channels, and $Ca_v3.1$–3.3 or T-type Ca^{2+} channels [19,103]. Previous studies have found $Ca_v1.2$ and $Ca_v1.3$ channels in rodent cortical astrocytes, while VGCCs subtypes of α1B (N-type), α1C (L-type), α1D (L-type), α1E (R-type), and α1G (T-type) have been found in neonatal cultured cortical astrocytes [104–107]. Additionally, the primary pituicytes express immunoreactivity for the $Ca_v1.2$ L-type, $Ca_v2.1$ P/Q-type, Cav2.2 N-type, $Ca_v2.3$ R-type, and Cav3.1 T-type subunits [108].

The homomeric α-7 nicotinic acetylcholine receptors (α7nAChRs) are expressed in astrocytes, and activation of α7nAChRs in hippocampal slices or in culture has been shown to induce intracellular Ca^{2+} transients [109,110]. Other Ca^{2+} channels, such as Ca^{2+} release-activated Ca^{2+} channels and plasmalemmal Ca^{2+}-ATPase, also contribute to the astrocyte microdomain Ca^{2+} signals and other astrocytic functions [111]. While astrocytic Ca^{2+} events can originate from either pure intracellular or extracellular Ca^{2+} resources, they may also arise from the collaborative action of different sources [112,113].

4. Function of astrocytic Ca^{2+} signals

Ca^{2+} signaling assumes a fundamental role in diverse astrocytic functions, including the modulation of cerebral blood flow, synaptic transmission, synaptic plasticity, release of gliotransmitters, and neuronal spiking [1]. Additionally, the ability to generate Ca^{2+} events promotes ATP production by enhancing glycogenolysis [114]. Significantly, the complex morphology and wide distribution of astrocytes makes them well placed to exert local control over synapses and provide global support to neural circuits [9,115]. Astrocytes express numerous neurotransmitter receptors, particularly metabotropic receptors, which sense the microenvironment and induce intracellular Ca^{2+} signaling. Therefore, there exists a significant relationship between astrocytes and neurons that is mediated by Ca^{2+} signals. From the perspective of astrocytic Ca^{2+}, the interaction between astrocytes and neurons can be divided into three models, interactions at individual synapses, interactions at groups of synapses, and interactions between networks (Fig. 3). Delving deeper into the intricacies of these interactions can contribute to a better understanding of synapse-astrocyte interactions.

4.1. Synaptic transmission

It is known that some astrocytic Ca^{2+} signals are intrinsic, while others are driven by ATP or neurotransmitters such as norepinephrine (NA), acetylcholine, and dopamine. Indeed, NA regulates Ca^{2+} events in astrocytes [53,57,58,116]. Paukert et al. showed that the NA released after light stimulation greatly enhanced astrocytic Ca^{2+} signals and shifted the gain of astrocytic networks according to the behavioral state, enabling astrocytes to respond to local changes in activity of neuron [57]. Similarly, Chen et al. characterized a previously undescribed glial type in zebrafish that resembles mammalian astrocytes and demonstrated that zebrafish astrocytes exhibit microdomain Ca^{2+} transients and respond to NA [117]. Another study further confirmed that Ca^{2+} levels in radial astrocytes of zebrafish increased with the number of failed swimming attempts [118]. Recently, Poskanzer's group reported that NA-driven astrocytic Ca^{2+} events acted as a distinct neuromodulatory signal to regulate state of cortex and linking arousal-associated desynchrony to cortical circuit resynchronization [119]. Moreover, Ca^{2+} transients were induced by acetylcholine in hippocampal and cortical astrocytes and modulate synaptic transmission [120,121]. Dopamine, which is produced in dopaminergic neurons, induced Ca^{2+} responses in astrocytes in the nucleus accumbens or hippocampus [122,123]. In the nucleus accumbens, dopamine increased Ca^{2+} in astrocytes, stimulated adenosine/ATP release and inhibited excitatory synaptic transmission by activating presynaptic A1 receptors [122]. In the hippocampus, dopamine triggered changes in the concentration of intracellular astrocytic calcium with enhanced associated effects on local synaptic function [123].

4.2. Synaptic plasticity

Long-term potentiation (LTP) is the most researched form of synaptic plasticity and is thought to be achieved by Ca^{2+} signals in astrocytes and neurons [124–128]. Interestingly, different Ca^{2+} signaling pathways in astrocytes are involved in different forms of LTP [52]. The classical form of LTP relies on NMDA receptors and Goshen's group reported that astrocytic activation induced *de novo* NMDA-dependent LTP in CA1 to further confirm this concept [129,130]. D-serine is an endogenous ligand for NMDA receptors and it has been reported that astrocytes regulated activation of LTP through Ca^{2+}-dependent release of D-serine [131–133]. Additionally, Shigetomi et al. indicated that astrocytes contributed to NMDA receptor-dependent LTP, the activation of which caused Ca^{2+} influx, possibly through plasma membrane TRPA1 channels [78]. Navarrete et al. showed that cholinergic activity *in vivo* evoked by electrical or sensory stimulation increased astrocytic Ca^{2+} events and induced

LTP in the hippocampus. Similarly, in hippocampal slices, stimulation of cholinergic pathways evoked astrocytic Ca^{2+} signals and induced LTP at single CA3-CA1 synapses [134]. Recently, Liu et al. demonstrated that through the integration of synaptic inputs, astrocyte inositol IP_3R2-dependent Ca^{2+} signaling was crucial for late-phase LTP, which was mediated by astrocyte- and brain-derived neurotrophic factor [135].

4.3. Other functions of Ca^{2+} signals in astrocytes

Previous research has shown that changes in Ca^{2+} signals of astrocytes altered hemodynamics, increase glucose mobilization and influence cell activity by releasing neuroactive substances (e.g., glutamate, D-serine, and ATP) [136]. Moreover, Ca^{2+} signals in astrocytes stimulate the Na^+- and K^+-dependent adenosine triphosphatase, leading to a decrease in the extracellular concentration of K^+ [36]. Furthermore, researchers have discovered widespread astrocytic Ca^{2+} events in the cortex, showing that 11% of astrocytes exhibited Ca^{2+} signaling closely correlated with the behavior of running [137]. Interestingly, some substances can also affect the function of Ca^{2+} events to mediate responses in astrocytes. Nagai et al. reported that a 122-residue inhibitory peptide of β-adrenergic receptor kinase 1 attenuated astrocyte G_q-GPCR-mediated Ca^{2+} signals and contributed to behavioral adaptation and spatial memory [64].

Notably, astrocytic Ca^{2+} signaling has been proposed to be involved in disease progression [59,138]. In AD mice, Kuchibhotla et al. reported that astrocytic Ca^{2+} events were more frequent after CNS damage and were enhanced in the amyloid deposition regions [139]. P2Y1 receptors, highly expressed by reactive astrocytes surrounding plaques, are reported to mediate astrocyte hyperactivity, and blockade of P2Y1 receptors significantly reduced astrocytic Ca^{2+} activity and normalized astrocytic dysfunction. In addition to P2Y1 receptor-mediated Ca^{2+} signals, Bosson et al. demonstrated that astrocytes contributed to early Aβo toxicity by exhibiting a global and local Ca^{2+} hyperactivity involving TRPA1 channels [140,141]. Additionally, Lee et al. demonstrated that picomolar amounts of Aβ peptides was detected *via* an astrocytic α7-nAChR-dependent mechanism, which responded with increased Ca^{2+} transients [142]. However, astrocytic Ca^{2+} signals were reduced during the early stages of Aβ deposition, while neurons were hyperactive and specific tactile memory loss occurred. Restoration of deficient Ca^{2+} signals attenuated neuronal hyperactivity and alleviated the clinical phenotypes [143,144]. In an HD mouse model, the amplitude, duration, and frequency of astrocyte Ca^{2+} events significantly reduced, but astrocytes responded vigorously to cortical stimulation with evoked action potential-dependent Ca^{2+} signaling [145].

In short, astrocytic Ca^{2+} events are vital for optimal physiological and pathological function. Nevertheless, the underlying mechanisms and the physiological and pathophysiological significance of Ca^{2+} in astrocytes remain poorly understood. Therefore, the study of Ca^{2+} events in astrocytes remains elusive and is envisioned as the focus of future biological research after the development of more advanced detection methods.

5. Analysis tools of astrocytic Ca^{2+} signaling

Compared to the easy identification of somas, astrocytic Ca^{2+} microdomain signaling is difficult to define. The processes of astrocytes extend thin lamellar sheets that contain minimal cytoplasm, posing a challenge to the detection of Ca^{2+} changes with cytosolic indicators. Due to technical difficulties in accessing the small spatial area of calcium microdomains, the role of astrocytic Ca^{2+} microdomain activity remains poorly understood. As a result, many experiments and analysis methods are constantly being updated, to better dissect the characteristics of Ca^{2+} signaling in astrocytes.

5.1. Indicators for detecting Ca^{2+} signals

Observing changes and timelines in intracellular Ca^{2+} signaling is essential for exploring the physiological and pathological states in the CNS. Ca^{2+} activity is varied in a single astrocyte and in astrocytic networks at different locations and times. Various indicators are used to determine the number, size, and position of detected Ca^{2+} signals, which are powerful tools for visualizing the activity of Ca^{2+} signals. Visualization and quantification of intracellular Ca^{2+} signals can be achieved using chemical Ca^{2+} indicators (e.g., Fura-2, Fluo-4, and Mag-Fura-Red) or genetically encoded Ca^{2+} indicators (GECI) (e.g., GCaMPs, Pericams, and TN-XXL).

5.1.1. Chemical Ca^{2+} indicators

Beginning with the studies of Cornell-Bell et al., chemical Ca^{2+} indicators have been introduced into the study of astrocytic Ca^{2+} signal functions. These indicators have been widely used and the resulting data have significantly advanced the understanding of astrocyte function [42,146]. Importantly, the concentrations of the indicators should not exceed the buffering capacity of the cells [147,148]. Additionally, the selection of the most appropriate chemical Ca^{2+} indicator requires multiple considerations, including Ca^{2+} affinities, spectral properties, and the forms of different indicators [149].

Compared to GECIs, chemical Ca^{2+} indicators offer a notable advantage in that they are readily accessible commercially and can be easily employed without the need for cellular transfection. Moreover, cell-loading protocols have been well-established [150]. Thus, traditional chemical synthesis indicators have been widely used to investigate intracellular Ca^{2+} signaling. Selecting an appropriate chemical Ca^{2+} indicator requires various considerations, including Ca^{2+} affinities and spectral properties. However, these indicators have many limitations and problems, such as leakage, uneven dye loading photobleaching, and cytotoxicity. In particular, the cellular localization of the indicators cannot be specifically targeted to a particular organelle [149]. Therefore, they may be inappropriate indicators for the precise study of Ca^{2+} signaling [149].

5.1.2. Genetically-encoded Ca^{2+} indicators

Imaging of Ca^{2+} with protein-based indicators has been extensively used to follow neural activity in intact nervous systems. In recent years, GECIs have become one of the most comprehensive calcium indicators and have made outstanding contributions to the study of physiological functions and developmental mechanisms of different tissues and organisms [151]. GECIs have been particularly valuable in studying Ca^{2+} signals in branches and branchlets of astrocytes [59,152]. According to the luminescence principle, GECIs can be divided into two categories: single-fluorescent protein-based GECIs and fluorescence resonance energy transfer (FRET)-based GECIs [153]. The most commonly used single-fluorescent protein-based GECIs include GCaMPs, Camgaroos, and Pericams, while FRET-based GECIs include TN-XXL, D3cpV, and Cameleons [151,154].

Notably, GCaMP indicators are the most broadly used GECIs for monitoring Ca^{2+} in astrocytes. GCaMP indicators consist of a circularly permuted enhanced GFP moiety linked to the calcium-binding protein calmodulin (CaM) and the CaM-binding peptide M13pep [155]. The first GCaMP indicator was terribly dim and poorly folded at 37 °C, limiting its effectiveness for imaging. Improvement by grafting of GFP-stabilizing mutations and random mutagenesis led to the generation of GCaMP2, which showed significant improvements in the characteristics of fluorescence and folding [156–158]. Since then, groups at the Janelia Research Campus have continued to develop and optimize several rapid and sensitive GCaMP-type indicators by using structure-guided mutagenesis and large-scale screening. The main approaches to the introduction of single wavelength GECIs for the detection of Ca^{2+} signals are summarized in Table 1.

Table 1
Summary of the single wavelength GECIs for detecting astrocytic Ca^{2+} signaling.

Name		Wavelength (nm)		Ref.
		Excitation	Emission	PMID
GCaMP	GCaMP3	485	510	23868258
	GCaMP5G	485	510	23868258
	GCaMP5K	485	510	23868258
	GCaMP6f	485	510	23868258
	GCaMP6s	485	510	23868258
	GCaMP6m	485	510	23868258
	jGCaMP7s	485	510	31209382
	jGCaMP7f	485	510	31209382
	jGCaMP7b	485	510	31209382
	jGCaMP7c	485	510	31209382
	jGCaMP8s	485	510	36922596
	jGCaMP8m	485	510	36922596
	jGCaMP8f	485	510	36922596
Lck-GCaMP6f		488	510	27939582
jRCaMP1	jRCaMP1a	575	595	27011354
	jRCaMP1b	570	595	27011354
jRGECO1a		565	590	27011354
XCaMP-B	XCaMP-B	405	446	31080068
	XCaMP-G	488	514	31080068
	XCaMP-Gf	488	514	31080068
	XCaMP-Gf_0	488	514	31080068
	XCaMP-Y	488	527	31080068
	XCaMP-R	561	593	31080068

Crucially, given the swift advancement of new techniques, there is a growing need for more precise localization of astrocytic Ca^{2+} signals. Scientists have developed membrane and organelle-targeted indicators that have been used in various studies [159–161]. In 2013, Khakh and his colleagues compared two types of GECIs, cyto-GCaMP3 and Lck-GCaMP3, and provided detailed descriptions to facilitate the systematic research on astrocytic Ca^{2+} signaling [48]. A few years later, O'Donnell et al. observed mitochondrially centered and extramitochondrial Ca^{2+} signals using Lck-GCaMP6s [162]. Mitochondria, as key organelles regulating Ca^{2+} signals, have been considered in the construction of DNA plasmids encoding GECIs. Chen *et al.* constructed a mito-GCaMP2 by genetic manipulation and demonstrated that the response of mitochondrial Ca^{2+} signals was diverse in different cell lines [163]. Similarly, Zhang et al. observed mitochondrial Ca^{2+} imaging of cultured astrocytes by transfecting with mito-GCaMP5G/6s in astrocytes [164]. The ER acts as a store that maintains Ca^{2+} homeostasis [165]. Aryal et al. developed ER-GCaMP6f and indicated that ER-GCaMP6f was expressed *in vivo* and used to measure Ca^{2+} activity in brain slices [160]. With the development of technology and the need for ongoing research, numerous membrane- and organelle-targeted indicators are being developed and applied.

5.2. Software

In order to investigate astrocytic Ca^{2+} signals in depth, various software tools that employ an event-based perspective to accurately quantify Ca^{2+} in fluorescence imaging datasets have been developed. Here, we discussed the most commonly used image analysis toolboxes tailored for astrocytic Ca^{2+} image data.

5.2.1. GECIquant

In 2015, Khakh et al. developed GECIquant, which allows for rapid semi-automated detection of regions of interest (ROIs) for Ca^{2+} signaling [50]. As the first software used to specifically analyze Ca^{2+} in GCaMP expressing astrocytes, GECIquant was able to automatically detect the microdomain and expand wave ROIs based on the provided area criteria. The GECIquant software focuses on the single cell level, with the ability to provide raw fluorescence data from different regions. Moreover, traces can be processed by the software to obtain additional features [166].

5.2.2. Ca^{2+} signal classification and decoding (CaSCaDe)

Subsequently, in 2017, CaSCaDe was developed, which was similar to GECIquant and used a machine learning-based algorithm to identify Ca^{2+} signaling. Significantly, the CaSCaDe software can represent the regions that exhibit dynamic fluorescence changes and provide information on frequency, number, time course, and amplitude [9].

5.2.3. Astrocyte quantitative analysis (AQuA)

Both GECIquant and CaSCaDe rely on ROIs and measure along time. Nevertheless, their shortcoming lies in the defined boundaries of microdomains, which would lead to inaccurate signals. Accordingly, Wang et al. presented a new analytical framework that releases researchers from the limitations of ROI-based tools. The AQuA software accurately quantifies complex Ca^{2+} signaling and neurotransmitter activity in fluorescence imaging datasets [167]. In this study, researchers demonstrated that the AQuA software outperformed other analysis methods on simulated datasets and described event detection using multiple GECIs. Further, it applies not only to fluorescent indicators, but also to others tested here, especially those with complex dynamics.

5.2.4. Cellular and hemodynamic image processing suite (CHIPS)

CHIPS is an open source toolbox developed to analyze the cells and blood vessels, primarily from two-photon microscopy [168]. Significantly, it can integrate a range of algorithms and streamline image analysis pipelines. In detail, CHIPS is best suited to examine a dataset using multiple approaches simultaneously. For instance, it can simultaneously analyze cell volume or vascular diameter [169].

5.2.5. Begonia

Begonia is a MATLAB-based two-photon imaging analysis toolbox developed for astrocytic Ca^{2+} signals [170]. The analysis suite includes an automatic, event-based algorithm with few input parameters that can capture a high level of spatio-temporal complexity of astrocytic Ca^{2+} signals. Furthermore, begonia enables the experimentalist to accurately quantify astrocytic Ca^{2+} signals and examine Ca^{2+} transients in conjunction with other time series data [169].

5.2.6. Deconvolution of Ca^{2+} fluorescent patterns (deCLUTTER)

Recently, Grochowska et al. developed a new pipeline for calcium imaging data analysis called deCLUTTER^{2+}, which can be used to discover spontaneous or cue-dependent patterns of Ca^{2+} transients [171]. In their study, to better integrate the data from the different cell lines, deCLUTTER was used to analyze the variability of Ca^{2+} at different time points, with older astrocytes contributing to the clusters of more responsive cells.

6. Conclusion and perspectives

This review focuses on the recent advances in astrocytic Ca^{2+} signaling, contributing to more comprehensive understanding of Ca^{2+} events in astrocytes. In particular, we describe the main characteristics of astrocytic Ca^{2+} signaling, exploring its genesis and functions in the CNS. Finally, we provide an overview of the analytical tools used to study astrocytic Ca^{2+} signaling.

Despite these advances, research in this field is still in its infancy, leaving several fundamental questions unanswered. Firstly, the heterogeneity of astrocytes may be reflected in various types of Ca^{2+} signaling [172,173]. Supporting this perspective, given the demonstrated astrocytic heterogeneity across different brain regions, Khakh et al. identified distinct astrocytic Ca^{2+} signals in the CA3 region compared to other regions of the hippocampus [54,174–178]. Secondly, *in vivo* changes in astrocytic Ca^{2+} signals are diverse and complex over both short and

long time periods. Therefore, a pressing need exists for enhanced experiments that can comprehensively investigate astrocyte responses across different temporal scales, building on the refined comprehension of Ca^{2+} signals *in vivo*. When scientists can consistently and accurately observe and measure specific astrocyte Ca^{2+} microdomain signaling with spatial and temporal resolution, the field will be well-positioned to delve into the functions of astrocytes in the CNS. Thirdly, the investigation of the properties and biophysics of astrocyte Ca^{2+} signals in processes deserves further attention. Notably, anesthesia, a critical confounding factor, has been confirmed to affect Ca^{2+} responses within astrocytes. Therefore, maintaining mice in an awake state is imperative when researchers investigate Ca^{2+} signals [172,179,180]. Selection of sensitive critical indicators, in particular GECIs, allows *in vivo* monitoring of astrocytic Ca^{2+} activity in precise regions. Fourth, the present studies may not systematically reflect the functions of astrocytic Ca^{2+} events throughout the entire brain. More attention should be directed towards understanding the synergistic effect between astrocytic Ca^{2+} events and other components of the neurovascular unit. Takano et al. reported that astrocytic Ca^{2+} signals mediated vasodilation in response to increased neural activity [181]. In essence, the study highlights the necessity for more systematic research on astrocytic Ca^{2+} signals in the future. Consequently, it is crucial to adopt more comprehensive strategies and develop new technologies to enhance our understanding of astrocytic Ca^{2+} signaling *in vivo*.

Numerous challenges in this field warrant in-depth exploration. Key questions that require addressing include: (1) What is the precise molecular mechanism by which astrocytes control Ca^{2+}? (2) Can novel methods be developed to regulate astrocyte Ca^{2+} signaling in specific brain regions? (3) Do altered astrocyte Ca^{2+} signals contribute to specific CNS diseases *via* different pathways?

In consideration of the aforementioned points, despite clear progress, further in-depth studies are needed to explore the molecular mechanisms of Ca^{2+} signals in astrocytes. Hence, the next stage of research into astrocytic Ca^{2+} signals should persistently emphasize investigations into astrocyte-related physiology, pathology, and animal behaviors. This strategic focus will undoubtedly contribute to a more nuanced understanding of the pivotal roles of astrocytic Ca^{2+} signaling in both normal and pathological conditions, paving the way for significant advancements in the foreseeable future.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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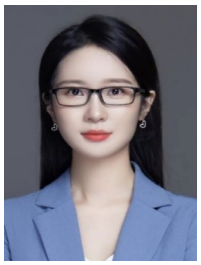
References

[1] N. Bazargani, D. Attwell, Astrocyte calcium signaling: The third wave, Nat. Neurosci. 19 (2) (2016) 182–189.
[2] N.J. Allen, D.A. Lyons, Glia as architects of central nervous system formation and function, Science 362 (6411) (2018) 181–185.
[3] L. Schirmer, D.P. Schafer, T. Bartels, et al., Diversity and function of glial cell types in multiple sclerosis, Trends. Immunol. 42 (3) (2021) 228–247.
[4] Q. Guo, A. Scheller, W. Huang, Progenies of NG2 glia: What do we learn from transgenic mouse models ? Neural Regen. Res. 16 (1) (2021) 43–48.
[5] H.G. Lee, M.A. Wheeler, F.J. Quintana, Function and therapeutic value of astrocytes in neurological diseases, Nat. Rev. Drug Discov. 21 (5) (2022) 339–358.
[6] M.R. Freeman, Specification and morphogenesis of astrocytes, Science 330 (6005) (2010) 774–778.
[7] F. Endo, A. Kasai, J.S. Soto, et al., Molecular basis of astrocyte diversity and morphology across the CNS in health and disease, Science 378 (6619) (2022) eadc9020.
[8] T. Kosaka, K. Hama, Three-dimensional structure of astrocytes in the rat dentate gyrus, J. Comp. Neurol. 249 (2) (1986) 242–260.
[9] A. Agarwal, P.H. Wu, E.G. Hughes, et al., Transient opening of the mitochondrial permeability transition pore induces microdomain calcium transients in astrocyte processes, Neuron 93 (3) (2017) 587–605.e7.
[10] X. Yu, J. Nagai, B.S. Khakh, Improved tools to study astrocytes, Nat. Rev. Neurosci. 21 (3) (2020) 121–138.
[11] K. Ogata, T. Kosaka, Structural and quantitative analysis of astrocytes in the mouse hippocampus, Neuroscience 113 (1) (2002) 221–233.
[12] H. Chai, B. Diaz-Castro, E. Shigetomi, et al., Neural circuit-specialized astrocytes: Transcriptomic, proteomic, morphological, and functional evidence, Neuron 95 (3) (2017) 531–549.e9.
[13] E.A. Bushong, M.E. Martone, Y.Z. Jones, et al., Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains, J. Neurosci. 22 (1) (2002) 183–192.
[14] B. Zhou, Y.X. Zuo, R.T. Jiang, Astrocyte morphology: Diversity, plasticity, and role in neurological diseases, CNS. Neurosci. Ther. 25 (6) (2019) 665–673.
[15] B.S. Khakh, M.V. Sofroniew, Diversity of astrocyte functions and phenotypes in neural circuits, Nat. Neurosci. 18 (7) (2015) 942–952.
[16] T. Takano, J.T. Wallace, K.T. Baldwin, et al., Chemico-genetic discovery of astrocytic control of inhibition *in vivo*, Nature 588 (7837) (2020) 296–302.
[17] M. Saint-Martin, Y. Goda, Astrocyte-synapse interactions and cell adhesion molecules, FEBS. J. 290 (14) (2023) 3512–3526.
[18] S.P. Aryal, K.R. Neupane, A.A. Masud, et al., Characterization of astrocyte morphology and function using a fast and reliable tissue clearing technique, Curr. Protoc. 1 (10) (2021) e279.
[19] A. Verkhratsky, M. Nedergaard, Physiology of astroglia, Physiol. Rev. 98 (1) (2018) 239–389.
[20] V. Gallo, B. Deneen, Glial development: The crossroads of regeneration and repair in the CNS, Neuron 83 (2) (2014) 283–308.
[21] R.B. Rodnight, C. Gottfried, Morphological plasticity of rodent astroglia, J. Neurochem. 124 (3) (2013) 263–275.
[22] I. Lundgaard, M.J. Osorio, B.T. Kress, et al., White matter astrocytes in health and disease, Neuroscience 276 (2014) 161–173.
[23] J.E. Simpson, P.G. Ince, G. Lace, et al., Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain, Neurobiol. Aging 31 (4) (2010) 578–590.
[24] J. Beauquis, P. Pavia, C. Pomilio, et al., Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease, Exp. Neurol. 239 (2013) 28–37.
[25] C.Y. Yeh, B. Vadhwana, A. Verkhratsky, et al., Early astrocytic atrophy in the entorhinal cortex of a triple transgenic animal model of Alzheimer's disease, ASN Neuro 3 (5) (2011) 271–279.
[26] H.D.E. Booth, W.D. Hirst, R. Wade-Martins, The role of astrocyte dysfunction in Parkinson's disease pathogenesis, Trends. Neurosci. 40 (6) (2017) 358–370.
[27] J. Tong, L.C. Ang, B. Williams, et al., Low levels of astroglial markers in Parkinson's disease: Relationship to alpha-synuclein accumulation, Neurobiol. Dis. 82 (2015) 243–253.
[28] I. Lastres-Becker, A. Ulusoy, N.G. Innamorato, et al., alpha-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage Parkinson's disease, Hum. Mol. Genet. 21 (14) (2012) 3173–3192.
[29] D.E. Clapham, Calcium signaling, Cell 131 (6) (2007) 1047–1058.
[30] S. Patergnani, A. Danese, E. Bouhamida, et al., Various aspects of calcium signaling in the regulation of apoptosis, autophagy, cell proliferation, and cancer, Int. J. Mol. Sci. 21 (21) (2020) 8323.
[31] E. Izquierdo-Torres, A. Hernandez-Oliveras, G. Fuentes-Garcia, et al., Calcium signaling and epigenetics: A key point to understand carcinogenesis, Cell Calcium 91 (2020) 102285.
[32] C. Schlecker, W. Boehmerle, A. Jeromin, et al., Neuronal calcium sensor-1 enhancement of InsP3 receptor activity is inhibited by therapeutic levels of lithium, J. Clin. Invest. 116 (6) (2006) 1668–1674.
[33] B.J. Eickholt, G.J. Towers, W.J. Ryves, et al., Effects of valproic acid derivatives on inositol trisphosphate depletion, teratogenicity, glycogen synthase kinase-3beta inhibition, and viral replication: A screening approach for new bipolar disorder drugs derived from the valproic acid core structure, Mol. Pharmacol. 67 (5) (2005) 1426–1433.
[34] M.S. Islam, Calcium signaling: From basic to bedside, Adv. Exp. Med. Biol. 1131 (2020) 1–6.
[35] V.S. Alves, H.S. Alves-Silva, D.J.B. Orts, et al., Calcium signaling in neurons and glial cells: Role of cav1 channels, Neuroscience 421 (2019) 95–111.
[36] F. Wang, N.A. Smith, Q. Xu, et al., Astrocytes modulate neural network activity by Ca(2)+-dependent uptake of extracellular K^+, Sci. Signal. 5 (218) (2012) ra26.
[37] A.I. Borrachero-Conejo, E. Saracino, M. Natali, et al., Electrical stimulation by an organic transistor architecture induces calcium signaling in nonexcitable brain cells, Adv. Healthc. Mater. 8 (3) (2019) e1801139.
[38] J. Kang, N. Kang, D. Lovatt, et al., Connexin 43 hemichannels are permeable to ATP, J. Neurosci. 28 (18) (2008) 4702–4711.
[39] A. Verkhratsky, H. Kettenmann, Calcium signalling in glial cells, Trends. Neurosci. 19 (8) (1996) 346–352.

[40] D.N. Bowser, B.S. Khakh, Two forms of single-vesicle astrocyte exocytosis imaged with total internal reflection fluorescence microscopy, Proc. Natl. Acad. Sci. U. S. A. 104 (10) (2007) 4212–4217.
[41] L. Bojarskaite, D.M. Bjornstad, K.H. Pettersen, et al., Astrocytic $Ca^{(2+)}$ signaling is reduced during sleep and is involved in the regulation of slow wave sleep, Nat. Commun. 11 (1) (2020) 3240.
[42] B.S. Khakh, K.D. McCarthy, Astrocyte calcium signaling: From observations to functions and the challenges therein, Cold. Spring. Harb. Perspect. Biol. 7 (4) (2015) a020404.
[43] D. Lim, A. Semyanov, A. Genazzani, et al., Calcium signaling in neuroglia, Int. Rev. Cell Mol. Biol. 362 (2021) 1–53.
[44] A.S. Thrane, P.M. Rappold, T. Fujita, et al., Critical role of aquaporin-4 (AQP4) in astrocytic Ca^{2+} signaling events elicited by cerebral edema, Proc. Natl. Acad. Sci. U. S. A. 108 (2) (2011) 846–851.
[45] S. Duffy, B.A. MacVicar, *In vitro* ischemia promotes calcium influx and intracellular calcium release in hippocampal astrocytes, J. Neurosci. 16 (1) (1996) 71–81.
[46] A. Denizot, M. Arizono, U.V. Nagerl, et al., Control of $Ca^{(2+)}$ signals by astrocyte nanoscale morphology at tripartite synapses, Glia 70 (12) (2022) 2378–2391.
[47] J. Grosche, V. Matyash, T. Moller, et al., Microdomains for neuron-glia interaction: Parallel fiber signaling to Bergmann glial cells, Nat. Neurosci. 2 (2) (1999) 139–143.
[48] E. Shigetomi, E.A. Bushong, M.D. Haustein, et al., Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses, J. Gen. Physiol. 141 (5) (2013) 633–647.
[49] E. Shigetomi, S. Kracun, M.V. Sofroniew, et al., A genetically targeted optical sensor to monitor calcium signals in astrocyte processes, Nat. Neurosci. 13 (6) (2010) 759–766.
[50] R. Srinivasan, B.S. Huang, S. Venugopal, et al., $Ca^{(2+)}$ signaling in astrocytes from Ip3r2(-/-) mice in brain slices and during startle responses *in vivo*, Nat. Neurosci. 18 (5) (2015) 708–717.
[51] R.L. Rungta, L.P. Bernier, L. Dissing-Olesen, et al., $Ca^{(2+)}$ transients in astrocyte fine processes occur *via* $Ca^{(2+)}$ influx in the adult mouse hippocampus, Glia 64 (12) (2016) 2093–2103.
[52] A. Volterra, N. Liaudet, I. Savtchouk, Astrocyte Ca(2)(+) signalling: An unexpected complexity, Nat. Rev. Neurosci. 15 (5) (2014) 327–335.
[53] A. Semyanov, C. Henneberger, A. Agarwal, Making sense of astrocytic calcium signals - from acquisition to interpretation, Nat. Rev. Neurosci. 21 (10) (2020) 551–564.
[54] M.A. Di Castro, J. Chuquet, N. Liaudet, et al., Local Ca^{2+} detection and modulation of synaptic release by astrocytes, Nat. Neurosci. 14 (10) (2011) 1276–1284.
[55] K.M. Dunn, D.C. Hill-Eubanks, W.B. Liedtke, et al., TRPV4 channels stimulate Ca^{2+}-induced Ca^{2+} release in astrocytic endfeet and amplify neurovascular coupling responses, Proc. Natl. Acad. Sci. U. S. A. 110 (15) (2013) 6157–6162.
[56] S.V. Straub, A.D. Bonev, M.K. Wilkerson, et al., Dynamic inositol trisphosphate-mediated calcium signals within astrocytic endfeet underlie vasodilation of cerebral arterioles, J. Gen. Physiol. 128 (6) (2006) 659–669.
[57] M. Paukert, A. Agarwal, J. Cha, et al., Norepinephrine controls astroglial responsiveness to local circuit activity, Neuron 82 (6) (2014) 1263–1270.
[58] F. Ding, J. O'Donnell, A.S. Thrane, et al., alpha1-Adrenergic receptors mediate coordinated Ca^{2+} signaling of cortical astrocytes in awake, behaving mice, Cell Calcium 54 (6) (2013) 387–394.
[59] E. Shigetomi, S. Patel, B.S. Khakh, Probing the complexities of astrocyte calcium signaling, Trends. Cell Biol. 26 (4) (2016) 300–312.
[60] J.L. Stobart, K.D. Ferrari, M.J.P. Barrett, et al., Cortical circuit activity evokes rapid astrocyte calcium signals on a similar timescale to neurons, Neuron 98 (4) (2018) 726–735.e4.
[61] K. Nizar, H. Uhlirova, P. Tian, et al., *In vivo* stimulus-induced vasodilation occurs without IP3 receptor activation and may precede astrocytic calcium increase, J. Neurosci. 33 (19) (2013) 8411–8422.
[62] Q. Wang, Y. Kong, D.Y. Wu, et al., Impaired calcium signaling in astrocytes modulates autism spectrum disorder-like behaviors in mice, Nat. Commun. 12 (1) (2021) 3321.
[63] C. Agulhon, T.A. Fiacco, K.D. McCarthy, Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling, Science 327 (5970) (2010) 1250–1254.
[64] J. Nagai, A. Bellafard, Z. Qu, et al., Specific and behaviorally consequential astrocyte G(q) GPCR signaling attenuation *in vivo* with ibetaARK, Neuron 109 (14) (2021) 2256–2274.e9.
[65] J.L. Stobart, K.D. Ferrari, M.J.P. Barrett, et al., Long-term *in vivo* calcium imaging of astrocytes reveals distinct cellular compartment responses to sensory stimulation, Cereb. Cortex 28 (1) (2018) 184–198.
[66] Y. Okubo, K. Kanemaru, J. Suzuki, et al., Inositol 1,4,5-trisphosphate receptor type 2-independent $Ca^{(2+)}$ release from the endoplasmic reticulum in astrocytes, Glia 67 (1) (2019) 113–124.
[67] V. Parpura, V. Grubisic, A. Verkhratsky, $Ca^{(2+)}$ sources for the exocytotic release of glutamate from astrocytes, Biochim. Biophys. Acta 1813 (5) (2011) 984–991.
[68] M.W. Sherwood, M. Arizono, C. Hisatsune, et al., Astrocytic IP(3) Rs: Contribution to $Ca^{(2+)}$ signalling and hippocampal LTP, Glia 65 (3) (2017) 502–513.
[69] M.L. Olson, S. Chalmers, J.G. McCarron, Mitochondrial organization and Ca^{2+} uptake, Biochem. Soc. Trans. 40 (1) (2012) 158–167.
[70] D.B. Zorov, M. Juhaszova, S.J. Sollott, Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release, Physiol. Rev. 94 (3) (2014) 909–950.
[71] P. Bernardi, V. Petronilli, The permeability transition pore as a mitochondrial calcium release channel: A critical appraisal, J. Bioenergy Biomembr. 28 (2) (1996) 131–138.
[72] J. Loncke, A. Kaasik, I. Bezprozvanny, et al., Balancing ER-mitochondrial $Ca^{(2+)}$ fluxes in health and disease, Trends. Cell Biol. 31 (7) (2021) 598–612.
[73] C.A. Gautier, Z. Erpapazoglou, F. Mouton-Liger, et al., The endoplasmic reticulum-mitochondria interface is perturbed in PARK2 knockout mice and patients with PARK2 mutations, Hum. Mol. Genet. 25 (14) (2016) 2972–2984.
[74] D. Ottolini, T. Cali, A. Negro, et al., The Parkinson disease-related protein DJ-1 counteracts mitochondrial impairment induced by the tumour suppressor protein p53 by enhancing endoplasmic reticulum-mitochondria tethering, Hum. Mol. Genet. 22 (11) (2013) 2152–2168.
[75] Y. Liu, X. Ma, H. Fujioka, et al., DJ-1 regulates the integrity and function of ER-mitochondria association through interaction with IP3R3-Grp75-VDAC1, Proc. Natl. Acad. Sci. U. S. A. 116 (50) (2019) 25322–25328.
[76] L. Hedskog, C.M. Pinho, R. Filadi, et al., Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models, Proc. Natl. Acad. Sci. U. S. A. 110 (19) (2013) 7916–7921.
[77] E. Shigetomi, X. Tong, K.Y. Kwan, et al., TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3, Nat. Neurosci. 15 (1) (2011) 70–80.
[78] E. Shigetomi, O. Jackson-Weaver, R.T. Huckstepp, et al., TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation *via* constitutive D-serine release, J. Neurosci. 33 (24) (2013) 10143–10153.
[79] E.B. Malarkey, Y. Ni, V. Parpura, Ca^{2+} entry through TRPC1 channels contributes to intracellular Ca^{2+} dynamics and consequent glutamate release from rat astrocytes, Glia 56 (8) (2008) 821–835.
[80] V. Benfenati, M. Amiry-Moghaddam, M. Caprini, et al., Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes, Neuroscience 148 (4) (2007) 876–892.
[81] P. Pizzo, A. Burgo, T. Pozzan, et al., Role of capacitative calcium entry on glutamate-induced calcium influx in type-I rat cortical astrocytes, J. Neurochem. 79 (1) (2001) 98–109.
[82] M. Grimaldi, M. Maratos, A. Verma, Transient receptor potential channel activation causes a novel form of $[Ca^{2+}]$I oscillations and is not involved in capacitative Ca^{2+} entry in glial cells, J. Neurosci. 23 (11) (2003) 4737–4745.
[83] S.J. Oh, J.M. Lee, H.B. Kim, et al., Ultrasonic neuromodulation *via* astrocytic TRPA1, Curr. Biol. 29 (20) (2019) 3386–3401.e8.
[84] M. Uchiyama, A. Nakao, Y. Kurita, et al., O(2)-dependent protein internalization underlies astrocytic sensing of acute hypoxia by restricting multimodal TRPA1 channel responses, Curr. Biol. 30 (17) (2020) 3378–3396.e7.
[85] Z. Ma, T. Stork, D.E. Bergles, et al., Neuromodulators signal through astrocytes to alter neural circuit activity and behaviour, Nature 539 (7629) (2016) 428–432.
[86] Z. Ma, M.R. Freeman, TrpML-mediated astrocyte microdomain Ca^{2+} transients regulate astrocyte-tracheal interactions, Elife 9 (2020).
[87] M.K. Shelton, K.D. McCarthy, Mature hippocampal astrocytes exhibit functional metabotropic and ionotropic glutamate receptors *in situ*, Glia 26 (1) (1999) 1–11.
[88] O. Palygin, U. Lalo, A. Verkhratsky, et al., Ionotropic NMDA and P2×1/5 receptors mediate synaptically induced Ca^{2+} signalling in cortical astrocytes, Cell Calcium 48 (4) (2010) 225–231.
[89] U. Lalo, O. Palygin, S. Rasooli-Nejad, et al., Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex, PLOS Biol. 12 (1) (2014) e1001747.
[90] D. Dzamba, P. Honsa, M. Valny, et al., Quantitative analysis of glutamate receptors in glial cells from the cortex of GFAP/EGFP mice following ischemic injury: Focus on NMDA receptors, Cell. Mol. Neurobiol. 35 (8) (2015) 1187–1202.
[91] E.M.F. Mehina, C. Murphy-Royal, G.R. Gordon, Steady-state free $Ca^{(2+)}$ in astrocytes is decreased by experience and impacts arteriole tone, J. Neurosci. 37 (34) (2017) 8150–8165.
[92] J.T. Porter, K.D. McCarthy, Hippocampal astrocytes *in situ* respond to glutamate released from synaptic terminals, J. Neurosci. 16 (16) (1996) 5073–5081.
[93] C.G. Schipke, C. Ohlemeyer, M. Matyash, et al., Astrocytes of the mouse neocortex express functional N-methyl-D-aspartate receptors, FASEB J. 15 (7) (2001) 1270–1272.
[94] A.R. Brazhe, A.Y. Verisokin, D.V. Verveyko, et al., Sodium-calcium exchanger can account for regenerative $Ca^{(2+)}$ entry in thin astrocyte processes, Front. Cell Neurosci. 12 (2018) 250.
[95] L. Heja, J. Kardos, NCX activity generates spontaneous $Ca^{(2+)}$ oscillations in the astrocytic leaflet microdomain, Cell Calcium 86 (2020) 102137.
[96] J.J. Wade, K. Breslin, K. Wong-Lin, et al., Calcium microdomain formation at the perisynaptic cradle due to NCX reversal: A computational study, Front. Cell Neurosci. 13 (2019) 185.
[97] R.A. North, Molecular physiology of P2X receptors, Physiol. Rev. 82 (4) (2002) 1013–1067.
[98] S.J. Dixon, R. Yu, N. Panupinthu, et al., Activation of P2 nucleotide receptors stimulates acid efflux from astrocytes, Glia 47 (4) (2004) 367–376.
[99] H. Franke, J. Grosche, H. Schadlich, et al., P2X receptor expression on astrocytes in the nucleus accumbens of rats, Neuroscience 108 (3) (2001) 421–429.
[100] M. Fumagalli, R. Brambilla, N. D'Ambrosi, et al., Nucleotide-mediated calcium signaling in rat cortical astrocytes: Role of P2X and P2Y receptors, Glia 43 (3) (2003) 218 -03.
[101] L.Q. Chen, X.J. Lv, Q.H. Guo, et al., Asymmetric activation of microglia in the hippocampus drives anxiodepressive consequences of trigeminal neuralgia in rodents, Br. J. Pharmacol. 180 (8) (2023) 1090–1113.
[102] T. Pannicke, W. Fischer, B. Biedermann, et al., P2×7 receptors in Muller glial cells from the human retina, J. Neurosci. 20 (16) (2000) 5965–5972.
[103] W.A. Catterall, Voltage-gated calcium channels, Cold. Spring. Harb. Perspect. Biol. 3 (8) (2011) a003947.

[104] J.D. Cahoy, B. Emery, A. Kaushal, et al., A transcriptome database for astrocytes, neurons, and oligodendrocytes: A new resource for understanding brain development and function, J. Neurosci. 28 (1) (2008) 264–278.
[105] Y. Zhang, K. Chen, S.A. Sloan, et al., An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex, J. Neurosci. 34 (36) (2014) 11929–11947.
[106] I. Latour, J. Hamid, A.M. Beedle, et al., Expression of voltage-gated Ca^{2+} channel subtypes in cultured astrocytes, Glia 41 (4) (2003) 347–353.
[107] M. D'Ascenzo, M. Vairano, C. Andreassi, et al., Electrophysiological and molecular evidence of l-(Cav1), N- (Cav2.2), and R- (Cav2.3) type Ca^{2+} channels in rat cortical astrocytes, Glia 45 (4) (2004) 354–363.
[108] D. Wang, B. Yan, W.R. Rajapaksha, et al., The expression of voltage-gated ca^{2+} channels in pituicytes and the up-regulation of L-type ca^{2+} channels during water deprivation, J. Neuroendocrinol. 21 (10) (2009) 858–866.
[109] J.X. Shen, J.L. Yakel, Functional alpha7 nicotinic ACh receptors on astrocytes in rat hippocampal CA1 slices, J. Mol. Neurosci. 48 (1) (2012) 14–21.
[110] J. Corradi, C. Bouzat, Understanding the bases of function and modulation of alpha7 nicotinic receptors: Implications for drug discovery, Mol. Pharmacol. 90 (3) (2016) 288–299.
[111] B. Moreau, S. Straube, R.J. Fisher, et al., Ca^{2+}-calmodulin-dependent facilitation and Ca^{2+} inactivation of Ca^{2+} release-activated Ca^{2+} channels, J. Biol. Chem. 280 (10) (2005) 8776–8783.
[112] A. Lia, V.J. Henriques, M. Zonta, et al., Calcium signals in astrocyte microdomains, a decade of great advances, Front. Cell Neurosci. 15 (2021) 673433.
[113] A.B. Toth, K. Hori, M.M. Novakovic, et al., CRAC channels regulate astrocyte $Ca^{(2+)}$ signaling and gliotransmitter release to modulate hippocampal GABAergic transmission, Sci. Signal. (582) (2019) 12.
[114] D. Ververken, P. Van Veldhoven, C. Proost, et al., On the role of calcium ions in the regulation of glycogenolysis in mouse brain cortical slices, J. Neurochem. 38 (5) (1982) 1286–1295.
[115] A. Araque, G. Carmignoto, P.G. Haydon, et al., Gliotransmitters travel in time and space, Neuron 81 (4) (2014) 728–739.
[116] M. Slezak, S. Kandler, P.P. Van Veldhoven, et al., Distinct mechanisms for visual and motor-related astrocyte responses in mouse visual cortex, Curr. Biol. 29 (18) (2019) 3120–3127.e5.
[117] J. Chen, K.E. Poskanzer, M.R. Freeman, et al., Live-imaging of astrocyte morphogenesis and function in zebrafish neural circuits, Nat. Neurosci. 23 (10) (2020) 1297–1306.
[118] Y. Mu, D.V. Bennett, M. Rubinov, et al., Glia accumulate evidence that actions are futile and suppress unsuccessful behavior, Cell 178 (1) (2019) 27–43 e19.
[119] M.E. Reitman, V. Tse, X. Mi, et al., Norepinephrine links astrocytic activity to regulation of cortical state, Nat. Neurosci. 26 (4) (2023) 579–593.
[120] T. Papouin, J.M. Dunphy, M. Tolman, et al., Septal cholinergic neuromodulation tunes the astrocyte-dependent gating of hippocampal NMDA receptors to wakefulness, Neuron 94 (4) (2017) 840–854.e7.
[121] N. Takata, T. Mishima, C. Hisatsune, et al., Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity *in vivo*, J. Neurosci. 31 (49) (2011) 18155–18165.
[122] M. Corkrum, A. Covelo, J. Lines, et al., Dopamine-evoked synaptic regulation in the nucleus accumbens requires astrocyte activity, Neuron 105 (6) (2020) 1036–1047.e5.
[123] A. Jennings, O. Tyurikova, L. Bard, et al., Dopamine elevates and lowers astroglial $Ca^{(2+)}$ through distinct pathways depending on local synaptic circuitry, Glia 65 (3) (2017) 447–459.
[124] W.A.J. Vints, O. Levin, H. Fujiyama, et al., Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity, Front. Neuroendocrinol. 66 (2022) 100993.
[125] J.A. Kauer, R.C. Malenka, Synaptic plasticity and addiction, Nat. Rev. Neurosci. 8 (11) (2007) 844–858.
[126] G. Chindemi, M. Abdellah, O. Amsalem, et al., A calcium-based plasticity model for predicting long-term potentiation and depression in the neocortex, Nat. Commun. 13 (1) (2022) 3038.
[127] G. Saw, K. Krishna, N. Gupta, et al., Epigenetic regulation of microglial phosphatidylinositol 3-kinase pathway involved in long-term potentiation and synaptic plasticity in rats, Glia 68 (3) (2020) 656–669.
[128] H. Jorntell, Cerebellar synaptic plasticity and the credit assignment problem, Cerebellum. 15 (2) (2016) 104–111.
[129] C. Henneberger, T. Papouin, S.H. Oliet, et al., Long-term potentiation depends on release of D-serine from astrocytes, Nature 463 (7278) (2010) 232–236.
[130] A. Adamsky, A. Kol, T. Kreisel, et al., Astrocytic activation generates de novo neuronal potentiation and memory enhancement, Cell 174 (1) (2018) 59–71 e14.
[131] M.J. Schell, M.E. Molliver, S.H. Snyder, D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release, Proc. Natl. Acad. Sci. U. S. A., 92 (9) (1995) 3948–3952.
[132] J.P. Mothet, A.T. Parent, H. Wolosker, et al., D-serine is an endogenous ligand for the glycine site of the N-methyl-d-aspartate receptor, Proc. Natl. Acad. Sci. U. S. A. 97 (9) (2000) 4926–4931.
[133] A. Panatier, D.T. Theodosis, J.P. Mothet, et al., Glia-derived D-serine controls NMDA receptor activity and synaptic memory, Cell 125 (4) (2006) 775–784.
[134] M. Navarrete, G. Perea, D. Fernandez de Sevilla, et al., Astrocytes mediate *in vivo* cholinergic-induced synaptic plasticity, PLOS Biol. 10 (2) (2012) e1001259.
[135] J.H. Liu, M. Zhang, Q. Wang, et al., Distinct roles of astroglia and neurons in synaptic plasticity and memory, Mol. Psychiatry 27 (2) (2022) 873–885.
[136] P.G. Haydon, GLIA: Listening and talking to the synapse, Nat. Rev. Neurosci. 2 (3) (2001) 185–193.
[137] D.A. Dombeck, A.N. Khabbaz, F. Collman, et al., Imaging large-scale neural activity with cellular resolution in awake, mobile mice, Neuron 56 (1) (2007) 43–57.
[138] M. Nedergaard, J.J. Rodriguez, A. Verkhratsky, Glial calcium and diseases of the nervous system, Cell Calcium 47 (2) (2010) 140–149.
[139] K.V. Kuchibhotla, C.R. Lattarulo, B.T. Hyman, et al., Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice, Science 323 (5918) (2009) 1211–1215.
[140] A. Bosson, A. Paumier, S. Boisseau, et al., TRPA1 channels promote astrocytic $Ca^{(2+)}$ hyperactivity and synaptic dysfunction mediated by oligomeric forms of amyloid–beta peptide, Mol. Neurodegener. 12 (1) (2017) 53.
[141] A. Paumier, S. Boisseau, M. Jacquier-Sarlin, et al., Astrocyte-neuron interplay is critical for Alzheimer's disease pathogenesis and is rescued by TRPA1 channel blockade, Brain 145 (1) (2022) 388–405.
[142] L. Lee, P. Kosuri, O. Arancio, Picomolar amyloid-beta peptides enhance spontaneous astrocyte calcium transients, J. Alzheimers Dis. 38 (1) (2014) 49–62.
[143] D. Shah, W. Gsell, J. Wahis, et al., Astrocyte calcium dysfunction causes early network hyperactivity in Alzheimer's disease, Cell Rep. 40 (8) (2022) 111280.
[144] A. Lia, G. Sansevero, A. Chiavegato, et al., Rescue of astrocyte activity by the calcium sensor STIM1 restores long-term synaptic plasticity in female mice modelling Alzheimer's disease, Nat. Commun. 14 (1) (2023) 1590.
[145] R. Jiang, B. Diaz-Castro, L.L. Looger, et al., Dysfunctional calcium and glutamate signaling in striatal astrocytes from Huntington's disease model mice, J. Neurosci. 36 (12) (2016) 3453–3470.
[146] A.H. Cornell-Bell, S.M. Finkbeiner, M.S. Cooper, et al., Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling, Science 247 (4941) (1990) 470–473.
[147] R.Y. Tsien, Fluorescence measurement and photochemical manipulation of cytosolic free calcium, Trends. Neurosci. 11 (10) (1988) 419–424.
[148] E. Neher, Quantitative aspects of calcium fluorimetry, Cold. Spring. Harb. Protoc. 2013 (10) (2013) 918–924.
[149] R.M. Paredes, J.C. Etzler, L.T. Watts, et al., Chemical calcium indicators, Methods 46 (3) (2008) 143–151.
[150] A. Takahashi, P. Camacho, J.D. Lechleiter, et al., Measurement of intracellular calcium, Physiol. Rev. 79 (4) (1999) 1089–1125.
[151] M.I. Kotlikoff, Genetically encoded Ca^{2+} indicators: Using genetics and molecular design to understand complex physiology, J. Physiol. 578 (Pt 1) (2007) 55–67.
[152] A.M. Reeves, E. Shigetomi, B.S. Khakh, Bulk loading of calcium indicator dyes to study astrocyte physiology: Key limitations and improvements using morphological maps, J. Neurosci. 31 (25) (2011) 9353–9358.
[153] S.A. Hires, L. Tian, L.L. Looger, Reporting neural activity with genetically encoded calcium indicators, Brain Cell Biol. 36 (1–4) (2008) 69–86.
[154] M. Krebs, K. Held, A. Binder, et al., FRET-based genetically encoded sensors allow high-resolution live cell imaging of Ca(2)(+) dynamics, Plant J. 69 (1) (2012) 181–192.
[155] J. Akerboom, J.D. Rivera, M.M. Guilbe, et al., Crystal structures of the GCaMP calcium sensor reveal the mechanism of fluorescence signal change and aid rational design, J. Biol. Chem. 284 (10) (2009) 6455–6464.
[156] Y.N. Tallini, M. Ohkura, B.R. Choi, et al., Imaging cellular signals in the heart *in vivo*: Cardiac expression of the high-signal Ca^{2+} indicator GCaMP2, Proc. Natl. Acad. Sci. U. S. A. 103 (12) (2006) 4753–4758.
[157] J. Diez-Garcia, S. Matsushita, H. Mutoh, et al., Activation of cerebellar parallel fibers monitored in transgenic mice expressing a fluorescent Ca^{2+} indicator protein, Eur. J. Neurosci. 22 (3) (2005) 627–635.
[158] M. Ohkura, M. Matsuzaki, H. Kasai, et al., Genetically encoded bright Ca^{2+} probe applicable for dynamic Ca^{2+} imaging of dendritic spines, Anal. Chem. 77 (18) (2005) 5861–5869.
[159] R. Srinivasan, T.Y. Lu, H. Chai, et al., New transgenic mouse lines for selectively targeting astrocytes and studying calcium signals in astrocyte processes *in situ* and *in vivo*, Neuron 92 (6) (2016) 1181–1195.
[160] S.P. Aryal, M. Xia, E. Adindu, et al., ER-GCaMP6f: An endoplasmic reticulum–targeted genetic probe to measure calcium activity in astrocytic processes, Anal. Chem. 94 (4) (2022) 2099–2108.
[161] H. Li, X. Wang, N. Zhang, et al., Imaging of mitochondrial Ca^{2+} dynamics in astrocytes using cell-specific mitochondria-targeted GCaMP5G/6s: Mitochondrial Ca^{2+} uptake and cytosolic Ca^{2+} availability *via* the endoplasmic reticulum store, Cell Calcium 56 (6) (2014) 457–466.
[162] J.C. O'Donnell, J.G. Jackson, M.B. Robinson, Transient oxygen/glucose deprivation causes a delayed loss of mitochondria and increases spontaneous calcium signaling in astrocytic processes, J. Neurosci. 36 (27) (2016) 7109–7127.
[163] M. Chen, Y. Wang, T. Hou, et al., Differential mitochondrial calcium responses in different cell types detected with a mitochondrial calcium fluorescent indicator, mito-GCaMP2, Acta Biochim. Biophys. Sin. (Shanghai) 43 (10) (2011) 822–830.
[164] N. Zhang, Z. Zhang, I. Ozden, et al., Imaging mitochondrial Ca^{2+} uptake in astrocytes and neurons using genetically encoded Ca^{2+} indicators (GECIs), J. Vis. Exp. 22 (179) (2022).
[165] S.J. Park, C. Li, Y.M. Chen, Endoplasmic reticulum calcium homeostasis in kidney disease: Pathogenesis and therapeutic targets, Am. J. Pathol. 191 (2) (2021) 256–265.
[166] L. Mariotti, G. Losi, A. Lia, et al., Interneuron-specific signaling evokes distinctive somatostatin-mediated responses in adult cortical astrocytes, Nat. Commun. 9 (1) (2018) 82.

[167] Y. Wang, N.V. DelRosso, T.V. Vaidyanathan, et al., Accurate quantification of astrocyte and neurotransmitter fluorescence dynamics for single-cell and population-level physiology, Nat. Neurosci. 22 (11) (2019) 1936–1944.
[168] M.J.P. Barrett, K.D. Ferrari, J.L. Stobart, et al., CHIPS: An extensible toolbox for cellular and hemodynamic two-photon image analysis, Neuroinformatics 16 (1) (2018) 145–147.
[169] K.A. Gorzo, G.R. Gordon, Photonics tools begin to clarify astrocyte calcium transients, Neurophotonics 9 (2) (2022) 021907.
[170] D.M. Bjornstad, K.S. Abjorsbraten, E. Hennestad, et al., Begonia-a two-photon imaging analysis pipeline for astrocytic Ca(2+) signals, Front. Cell Neurosci. 15 (2021) 681066.
[171] M.M. Grochowska, F. Ferraro, A. Carreras Mascaro, et al., deCLUTTER2+ pipeline to analyze calcium traces in a novel stem cell model for ventral midbrain patterned astrocytes, Dis. Model. Mech. 16 (6) (2023) dmm049980.
[172] A. Nimmerjahn, E.A. Mukamel, M.J. Schnitzer, Motor behavior activates Bergmann glial networks, Neuron 62 (3) (2009) 400–412.
[173] X. Tong, E. Shigetomi, L.L. Looger, et al., Genetically encoded calcium indicators and astrocyte calcium microdomains, Neuroscientist. 19 (3) (2013) 274–291.
[174] M.D. Haustein, S. Kracun, X.H. Lu, et al., Conditions and constraints for astrocyte calcium signaling in the hippocampal mossy fiber pathway, Neuron 82 (2) (2014) 413–429.
[175] A. Panatier, J. Vallee, M. Haber, et al., Astrocytes are endogenous regulators of basal transmission at central synapses, Cell 146 (5) (2011) 785–798.
[176] M. Bugiani, B.C. Plug, J.H.K. Man, et al., Heterogeneity of white matter astrocytes in the human brain, Acta Neuropathol. 143 (2) (2022) 159–177.
[177] S. Kohler, U. Winkler, J. Hirrlinger, Heterogeneity of astrocytes in grey and white matter, Neurochem. Res. 46 (1) (2021) 3–14.
[178] B.S. Khakh, B. Deneen, The emerging nature of astrocyte diversity, Annu. Rev. Neurosci. 42 (2019) 187–207.
[179] J. Schummers, H. Yu, M. Sur, Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex, Science 320 (5883) (2008) 1638–1643.
[180] A.S. Thrane, V. Rangroo Thrane, D. Zeppenfeld, General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex, Proc. Natl. Acad. Sci. U. S. A. 109 (46) (2012) 18974–18979.
[181] T. Takano, G.F. Tian, W. Peng, et al., Astrocyte-mediated control of cerebral blood flow, Nat. Neurosci. 9 (2) (2006) 260–267.



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