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Review

Probing the Complexities of Astrocyte Calcium Signaling

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Astrocytes are abundant glial cells that tile the entire central nervous system and mediate well-established functions for neurons, blood vessels, and other glia. These ubiquitous cells display intracellular Ca^{2+} signals, which have been intensely studied for 25 years. Recently, the use of improved methods has unearthed the panoply of astrocyte Ca^{2+} signals and a variable landscape of basal Ca^{2+} levels. *In vivo* studies have started to reveal the settings under which astrocytes display behaviorally relevant Ca^{2+} signaling. Studies in mice have emphasized how astrocyte Ca^{2+} signaling is altered in distinct neurodegenerative diseases. Progress in the past few years, fueled by methodological advances, has thus reignited interest in astrocyte Ca^{2+} signaling for brain function and dysfunction.

Trends

Astrocytes display intracellular Ca^{2+} signals.

Improved methods have unearthed new types of astrocyte Ca^{2+} signals.

Astrocyte basal Ca^{2+} levels are variable.

In vivo studies suggest that astrocytes display behaviorally relevant Ca^{2+} signaling.

Astrocyte Ca^{2+} signaling is altered in neurodegenerative diseases.

Introduction

Astrocytes tile the central nervous system and may represent ~20–40% of the total number of brain cells [1]. Their highly branched morphology, including proximity to neurons and blood vessels, has been the subject of intrigue, speculation, and study ever since astrocytes were discovered ~150 years ago. It is now well established that astrocytes serve diverse and important roles for the brain to function as an organ. These include roles for astrocytes in ion homeostasis, neurotransmitter clearance, synapse formation/removal, and synaptic modulation and contributions to neurovascular coupling, all of which are reviewed elsewhere [2–4]. From these perspectives, attention has focused on astrocyte intracellular Ca^{2+} signals as a basis to measure, interrogate, and ultimately understand their roles within neural circuits [2,5]. The strong focus on Ca^{2+} is based on the knowledge that Ca^{2+} is a widely used and important second messenger and on the realization that astrocytes, unlike neurons, are not electrically excitable [6].

The possibility that astrocytes may contribute to neural circuit function was first proposed almost 25 years ago [7,8], soon after astrocyte Ca^{2+} signals were discovered [5]. Since then, astrocyte Ca^{2+} signals have been studied in detail. In this review, we focus on recent insights on the properties of astrocyte Ca^{2+} signals, on emerging studies of astrocyte basal Ca^{2+} levels, and on their relation to two exemplar neurodegenerative disease models and behavior. Recent breakthroughs in the astrocyte field over the past few years have been fueled by technical advances that allow Ca^{2+} signals to be studied reliably in physiologically relevant compartments both *ex vivo* in brain slices and *in vivo* in adult mice.

Genetically Encoded Calcium Indicators (GECIs)

Organic Ca^{2+} indicator dyes, delivered to astrocytes by bulk loading or by intracellular dialysis using patch pipettes, have proven useful to study astrocytes [5]. Fundamental insights have emerged from their use [9–13]. These include the discovery of astrocyte Ca^{2+} signals [14–16] and two decades of work on their relation to neuronal activity [10,17], including the first studies of astrocyte Ca^{2+} dynamics *in vivo* [18]. However, astrocytes are problematic to load with organic

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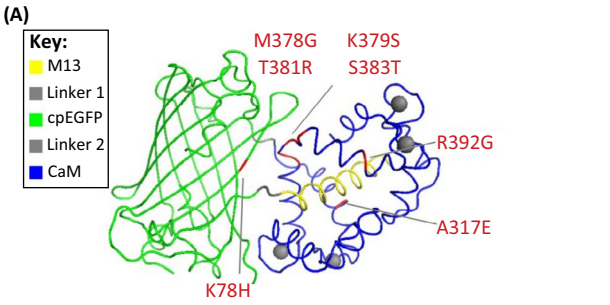
Ca^{2+} indicator dyes in adult brain slices. Moreover, *in vivo* repeated chronic imaging experiments are problematic because the dyes are lost from the cells over time and organic dyes clearly only reveal the astrocyte's somata [19]. Hence, additional approaches were needed. We focus here on recent studies employing GECIs, which comprise a single polypeptide chain of a fluorescent protein (or proteins) and a Ca^{2+}-binding motif [20–22].

Two types of GECI have been used in astrocyte studies: single-wavelength GECIs such as the GCaMP series (Figure 1) and FRET-based ratiometric GECIs such as the Yellow Cameleons [20]. GCaMPs are derived from circularly permuted enhanced GFP, the M13 peptide from myosin light chain kinase, and calmodulin (Figure 1) [23]. In the absence of Ca^{2+}, GCaMPs display weak fluorescence, but on Ca^{2+} binding (to calmodulin) increased green fluorescence is observed. GCaMPs have been optimized in terms of brightness, signal to noise, dynamic range, Ca^{2+} affinity, and photostability [24–27]. The latest GCaMP6 series developed by the Janelia GENIE Project performs as well as some organic Ca^{2+} indicator dyes [24]. The properties of GCaMPs are further leveraged by targeting them to subcellular compartments such as the plasma membrane [28–31], mitochondria [32–34], and endoplasmic reticulum [33,35]. Ratiometric FRET-based GECIs are also widely used and have been improved to tune Ca^{2+} affinity to desirable levels [36]. The design and evolution of GECIs has been reviewed [20,22].



	cpEGFP	CaM					
GCaMP5G	K78	A317	M378	K379	T381	S383	R392
GCaMP6s	K78H				T381R	S383T	R392G
GCaMP6m			M378G	K379S	T381R	S383T	R392G
GCaMP6f		A317E			T381R	S383T	R392G

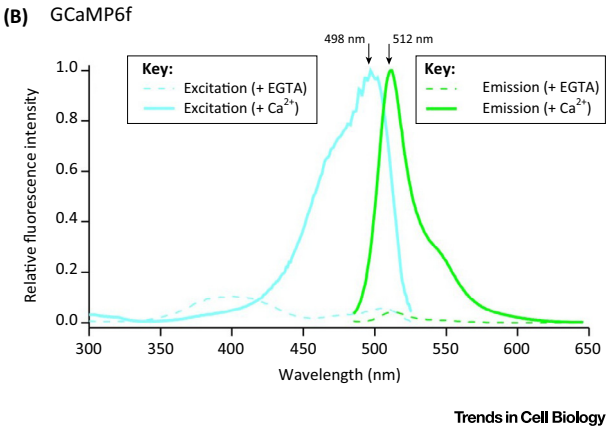


Figure 1. GCaMPs. (A) Structure of GCaMP showing the modular nature of the molecule and the locations of mutations in different GCaMP6 variants relative to GCaMP5G. The panel is reproduced from the paper reporting the development of GCaMP6f [24]. (B) Excitation and emission spectra of GCaMP6f in solution in the absence and presence of Ca^{2+}. The spectra were provided by the Janelia GENIE Project; further biophysical properties have been published [24].