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Reinforcing interdisciplinary collaborations to unravel the astrocyte “Calcium Code”

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Abstract

In this review article, we present the major insights from and challenges faced in the acquisition, analysis and modeling of astrocyte calcium activity, aiming at bridging the gap between those fields to crack the complex astrocyte “Calcium Code”. We then propose strategies to reinforce interdisciplinary collaborative projects to unravel astrocyte function in health and disease.

Keywords: astrocyte, glia, calcium, interdisciplinary

Introduction

Astrocytes, the most abundant non-neuronal cells of the nervous system, are essential to brain function, from synaptogenesis and neurotransmission to higher brain functions such as memory and learning [1]. Those functions of astrocytes are altered in various brain diseases such as epilepsy, brain tumours, neurodegenerative diseases, Down syndrome, major depressive

28 disorder and schizophrenia [1]. Astrocytes notably respond to stimuli with
29 transient elevations in cytosolic calcium concentration, referred to as calcium
30 signals. Those calcium signals are essential to brain function and are altered
31 in various brain diseases [1, 2]. Importantly, astrocyte calcium signals can
32 trigger the release of molecules referred to as gliotransmitters that modulate
33 neuronal communication at synapses (for recent reviews on gliotransmission
34 and the associated controversies, see [3, 4]). Better understanding astrocyte
35 physiology and the communication between astrocytes and other cells of the
36 central nervous system thus relies on our ability to make sense of those cal-
37 cium signals. Astrocyte calcium signals are characterized by diverse spatial
38 (from microdomains to signals spreading within astrocyte networks) and tem-
39 poral characteristics (from hundreds of milliseconds to tens of seconds) [2].
40 The majority of those signals occur in fine astrocyte compartments (50-200
41 nm), referred to as processes, that account for as much as 80 % of the volume
42 of an astrocyte, yet cannot be resolved by diffraction-limited light microscopy
43 [2, 5] (see Fig. 1). This strongly hinders our ability to characterize astrocyte
44 calcium activity, from the molecular pathways involved to the quantification
45 of the spatio-temporal properties of the signals. Consequently, the functions
46 of the various signals observed, referred to as the astrocyte “Calcium Code”,
47 remain unclear. Better characterizing astrocyte activity concomitantly with
48 the activity of other brain cells will be essential to unravel the roles of astro-
49 cyte calcium signals in brain function [6]. Please refer to the review [2] for
50 more details on the current challenges associated with the study of calcium
51 signals in astrocytes.

52

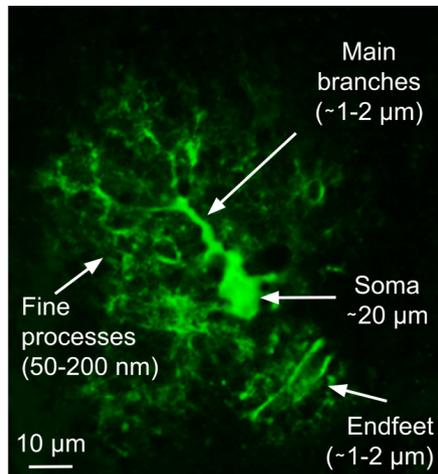


Fig. 1 Confocal image of an astrocyte expressing GCaMP6f (maximum intensity projection over time) that shows its different structural compartments and their size.

53 In this review article, we highlight the importance of reinforcing interdis-
54 plinary collaborations to crack the astrocyte “Calcium Code”, with a focus on
55 the characterization of the properties of astrocyte calcium signals. We present
56 the major insights from and challenges faced in data acquisition, analysis and
57 modeling of astrocyte calcium activity and propose strategies to facilitate and
58 strengthen collaborations between these fields, which are essential to unravel
59 the functions of astrocyte calcium signals in health and disease.

60 Acquisition of astrocyte calcium signals

61 Data acquisition is the first step to characterize astrocyte calcium activity.
62 In this section, we present a brief overview of the tools that are available
63 for imaging astrocyte calcium signals, both in slices and *in vivo*. We further
64 highlight the insights, challenges and perspectives associated with measuring
65 calcium signals in astrocytes.

66 Imaging tools for astrocyte calcium acquisition

67 The development of calcium indicators, which change their fluorescence prop-
68 erties when binding to calcium ions, allowed neuroscientists to study astrocyte
69 calcium activity. Numerous indicators exist, characterized by diverse kinetics
70 and diffusion properties, so that they should be chosen carefully. In the early
71 days, chemical calcium dyes, such as Fluo-4 or Oregon Green BAPTA, were
72 commonly used [7–12]. One of the main caveats of these chemical sensors is
73 the low signal to background noise ratio of the resulting signals, which only
74 allows visualizing calcium signals in the soma and the main thick branches
75 of astrocytes (see Fig. 1), unless loaded through a patch-clamp recording
76 pipette and visualized with high-resolution microscopy [13–15]. More recently,
77 the development of genetically-encoded calcium indicators (GECIs) [16] has
78 considerably improved our understanding of astrocyte calcium dynamics.
79 Various GECIs have been developed in the last years that can be imaged by
80 different tools, for precise or wide imaging at cellular or subcellular levels
81 [17–21]. GECIs have several advantages compared to classical calcium dyes.
82 First, they can be easily targeted to be expressed specifically in astrocytes.
83 Moreover, they provide a higher signal to background noise ratio compared to
84 classical calcium dyes and diffuse better into the fine processes. Additionally,
85 GECIs can be expressed in live organisms, thus allowing *in vivo* calcium
86 imaging in anesthetized [21–24], awake head-fixed [17, 25–27] or freely-moving
87 mice during consecutive behavioral sessions [25, 28, 29]. While many GECIs
88 have been designed in the last years for neurons, only a few are available to
89 target astrocytes specifically (reviewed in [30]). These GECIs have different
90 spectral, temporal and spatial properties that make them suitable for specific
91 experimental applications [31]. Importantly, they yield calcium signals with
92 different spatio-temporal properties that may not be comparable and may be
93 difficult to analyze with certain software (see section [Analysis of astrocyte](#)

94 calcium signals).

95
96 Astrocytes display most of their activity in their fine processes. The major-
97 ity of those signals are spatially-restricted, forming so-called microdomains,
98 and display strikingly diverse spatio-temporal properties [32]. Understanding
99 the physiological relevance of those calcium signals requires powerful imaging
100 techniques that can be used in combination with complementary methods to
101 manipulate astrocyte and neuronal activity, such as electrophysiology, optoge-
102 netics, pharmacology and behavioral tests. Notably, because of the small size
103 of astrocyte processes, *high-resolution microscopy* is needed to obtain a thor-
104 ough view of the astrocyte calcium activity. Both confocal and two-photon
105 microscopy are good options for imaging astrocyte calcium activity because
106 these setups are generally compatible with other techniques, allowing for the
107 study of calcium signals at the tripartite synapse level in slices and *in vivo* in
108 anesthetized [21–23, 27] or awake head-fixed mice [17, 24–26]. Light sheet fluo-
109 rescence microscopy (LSFM) and Lattice LSFM are novel imaging techniques
110 that allow fast 3D scanning with low phototoxicity and a resolution compara-
111 ble to confocal microscopy [33, 34]. Therefore, those techniques are excellent
112 imaging options for experiments in brain slices. Please refer to Table 1 for
113 an overview of optical resolution, phototoxicity/photobleaching, and compat-
114 ibility of the different imaging techniques. *High-resolution microscopy* allows
115 recording calcium signals at a high acquisition speed (in the order of ms) but
116 its spatial resolution is limited by diffraction (x-y: 0.2-0.3 μm and z: 0.5 μm
117 at best) and, thus, does not allow visualizing fine processes in detail. Recent
118 studies have used *super-resolution microscopy* such as stimulated emission
119 depletion (STED) and stochastic optical reconstruction microscopy (STORM)
120 to study astrocyte morphology at the tripartite synapse level in live tissue [35–
121 37]. STED microscopy has revealed that the complex spongiform morphology
122 of astrocyte processes contains functionally-isolated nanostructures that are
123 characterized by spatially-restricted calcium signals [37]. Currently, because of
124 their low acquisition speed and high laser intensity, which induces high photo-
125 bleaching of calcium sensors, *super-resolution microscopy* techniques cannot be
126 used to perform calcium imaging. Thus, in the aforementioned study, calcium
127 signals were acquired using *high-resolution microscopy* and were then mapped
128 onto super-resolution structural images. Super-resolution imaging requires
129 powerful computational tools, both for acquisition and analysis, which are
130 not broadly available in the experimental community (in terms of knowledge,
131 software and hardware) and emphasizes the need to establish collaborations
132 between experimental and computer scientists.

133 The need for interdisciplinary approaches

134 It is important to keep in mind that experimental approaches have inherent
135 limitations. First, calcium indicators are calcium buffers. Therefore, calcium
136 indicators compete with calcium binding sites in the cell, altering calcium sig-
137 nals and the normal functioning of the cell. Second, the spatial and temporal

138 characteristics of the measured signals are constrained by the imaging tech-
139 nique as well as the kinetics of the calcium indicator used. It is thus possible
140 that some faster or smaller calcium signals than those currently reported exist
141 in astrocytes that cannot be detected by the calcium imaging tools that are cur-
142 rently available. Importantly, this effect can be amplified during 3D scanning
143 for calcium signals that are faster than the z-scanning time. Lastly, experi-
144 mental manipulations, such as using a knock-out mouse line or bath applying
145 drugs, can have unexpected off-site effects that can impact the results, making
146 it difficult to extract causal relationships between the experimental manipula-
147 tion and the obtained results. Collaborative work with computational scientists
148 is essential to build mechanistic models to go beyond those limitations. For
149 example, models have been essential to characterize the effect of the concen-
150 tration, kinetics and diffusion coefficient of calcium buffers, such as calcium
151 indicators, on calcium dynamics [38, 39]. Models can thus be used to predict
152 the free calcium signals that would occur in the absence of indicators. Further,
153 models can measure *in silico* calcium signals at very high spatial and tempo-
154 ral resolution (depending on the method used, see section [Modeling astrocyte](#)
155 [calcium signals](#)), thus predicting the range of calcium signals that could not
156 be resolved experimentally.

157 Analysis of astrocyte calcium signals

158 In the quest of characterizing astrocyte calcium signals, the key role of analy-
159 sis is to provide tools to experimentalists and modelers to process their data,
160 of increasing size and complexity. Statistical as well as advanced computa-
161 tional image analysis tools are thus needed. In this section, we focus on the
162 analysis of calcium images, which is meant to quantify what is observed, *i.e.*,
163 to extract meaningful information or measurements from images. In particu-
164 lar, we emphasize the importance of developing computational image analysis
165 tools dedicated to the quantification of astrocyte calcium signals, and the
166 challenges to get there.

168 Image analysis to characterize astrocyte calcium signals

169 Decoding the astrocyte “Calcium Code” involves the characterization of the
170 spatio-temporal dynamics of astrocyte calcium signals. Computational image
171 analysis tools aim at accurately detecting all astrocyte calcium signals in a
172 sequence of microscopy images and, for each signal, extracting its dynamical
173 and spatial features, such as its amplitude, its duration, its trajectory, its propa-
174 gation speed, the location from which it originates and its volume. Various
175 information, such as the number of calcium signals in a specified region or cell,
176 their frequency at a position, and the different types of signals induced by a
177 stimulus, can be deduced from those measurements.

178 From an image analysis point of view, reaching this ideal of output infor-
179 mation requires preprocessing steps (*e.g.*, denoising, deconvolution, motion

Table 1 Overview of the main calcium imaging techniques used to study astrocyte calcium signals. * The ability to perform 3D fast scanning depends on the scanning method that the microscope uses, which varies depending on its hardware settings. ** Photobleaching and phototoxicity can be high at the focal plane with two-photon microscopy because it uses high intensity lasers, but it is low if the whole sample is considered (see [40]). *** Note that Light sheet fluorescence microscopy (LSFM) and Lattice LSFM cannot be used *in vivo* in postnatal murine models but can be used *in vivo* in embryos.

Imaging method	Optical resolution	Photobleaching & phototoxicity	Preparation	Compatible with fast 3D scanning *	Compatible with other techniques
Wide-field	Soma & main branches	High	<i>In vitro</i> & <i>in vivo</i> (anaesthetised & head-fixed)	No	Electrophysiology, pharmacology, wide-field photostimulation
Confocal	Soma, main branches & fine processes	High	<i>In vitro</i> & <i>in vivo</i> (anaesthetised & head-fixed)	No	Electrophysiology, pharmacology, localized photostimulation
Two-photon	Soma, main branches & fine processes	** Low	<i>In vitro</i> & <i>in vivo</i> (anaesthetised & head-fixed)	Yes, depending on the microscope	Electrophysiology, pharmacology, localized photostimulation
LSFM	Soma, main branches & fine processes	Low	*** <i>In vitro</i>	Yes, faster than two-photon	Electrophysiology, pharmacology
Lattice LSFM	Soma, main branches & fine processes	Very low	*** <i>In vitro</i>	Yes, faster than LSFM	Electrophysiology, pharmacology
Fiber photometry	Population	High	<i>In vivo</i> (freely behaving)	No	Electrophysiology, wide-field photostimulation
Miniscopes	Soma	High	<i>In vivo</i> (freely behaving)	No	Wide-field photostimulation

181 of the calcium signals in a sequence of microscopy images, which is very chal-
182 lenging due to the complex nature of these signals. First, calcium signals are
183 characterized by various durations (from milliseconds to tens of seconds), fre-
184 quencies and signal-to-noise ratios. Second, their spatial spreads vary from
185 microdomains to signals that propagate within the astrocyte in regions of
186 various sizes and shapes. Third, they can overlap in space and time [41]. As
187 most signals occur in fine astrocyte processes that cannot be fully resolved by
188 diffraction-limited light microscopy techniques, image analysis methods cannot
189 rely on morphological criteria to detect calcium signals, which also complex-
190 ifies their quantification. In addition, the developed image analysis methods
191 should ideally operate across data with different spatial scales, taken *in vivo*
192 or *in vitro*, and acquired with different imaging techniques.

193 **Lack of computational image analysis tools adapted to** 194 **the complexity and diversity of the data**

195 Recently, several image analysis algorithms have been developed to quantify
196 astrocyte calcium signals in 2D+time microscopy images. Among them, we
197 can cite GECIquant [42], CaSCaDe [17], FASP [43], AQuA [44] and, more
198 recently, Begonia [45] and Astral [46]. Most of these methods are ROI-based
199 approaches (ROI: region of interest), meaning that calcium signals are ana-
200 lyzed through fixed spatial boundaries in the image. As the spatial spread of
201 calcium signals can vary over time and become larger than or get out of the
202 ROI, those approaches can lead to inaccurate or partial detection of the sig-
203 nals. To solve this issue, event-based algorithms have been developed, such as
204 AQuA [44]. For more details about these algorithms (*e.g.*, analysis approach
205 and outputs), please refer to the dedicated section in the review article from
206 [47].

207
208 The aforementioned analysis tools have considerably improved the detec-
209 tion and characterization of astrocyte calcium signals. Yet, their use can be
210 restricted, either because they are not adapted to the diversity of acquisition
211 modes and calcium indicators (see section [Acquisition of astrocyte calcium](#)
212 [signals](#)) or because they are not open-source or not user-friendly [48]. This can
213 constrain some neuroscientists to implement “in-house” analysis pipelines,
214 which is time-consuming and less reproducible, or to use tools that were ini-
215 tially developed for neuronal calcium imaging analysis, such as CaImAn [49],
216 Suite2P [50] and LC_Pro [51]. This latter approach is not optimal as astrocytes
217 differ from neurons in many ways. For example, they have a different morphol-
218 ogy. Further, notably because astrocytes are not polarized cells, their calcium
219 signals display different spatio-temporal properties than the ones of neurons.

220
221 The continuous scientific and technical advances in calcium imaging will
222 always call for new and adapted image analysis algorithms. Until now, most of
223 the quantification of calcium signals has been performed on 2D+time fluores-
224 cence microscopy data. The recent emergence of 3D+time imaging techniques

225 gives access to new and major structural and dynamical information, such as
226 the number of calcium signals occurring in an entire astrocyte volume, their
227 synchronization, their trajectory and the location from which they originate
228 [5]. To the best of our knowledge, there is currently no image analysis tool to
229 detect, segment and quantify astrocyte calcium signals in 3D+time microscopy
230 images.

231 **Challenges hindering the development of 3D+time image** 232 **analysis tools**

233 The main reason why the quantification of astrocyte calcium signals has been
234 so far restricted to 2D+time images is because of the trade-off between tempo-
235 ral and spatial resolution in microscopy techniques. The access to a refined
236 3D imaging of the dynamical behavior of calcium signals in astrocytes is quite
237 recent, owing to the emergence of microscopes enabling a high 3D spatio-
238 temporal resolution with low phototoxicity (*e.g.*, lattice light sheet fluorescence
239 microscopy (LSFM) [33, 34] and of genetically encoded calcium indicators
240 (GECIs) [16]. Despite these scientific and technical advances, the development
241 of 3D+time image analysis tools tailored for the astrocyte calcium activity
242 is not straightforward and calls for new quantitative analysis algorithms
243 with new constraints and challenges. First, a key challenge in the develop-
244 ment of 3D+time image analysis tools is the memory and computational costs
245 required to process large 3D+time data. To give the reader an idea, the equiv-
246 alent of one hour of acquisition of Lattice LSFM data represents about 1 To
247 of data. Importantly, the developed analysis tools should be accessible and
248 thus ideally be able to run on standard desktop computers. To tackle this
249 challenge, ingenious solutions for image processing are needed such as using
250 data-dimensionality reduction techniques. Second, and more critical, reliable
251 and large amounts of labeled data are not available. Such datasets are crucial
252 to evaluate image analysis tools and to train data-driven tools, which are
253 increasingly common with the emergence of deep learning in biological image
254 analysis [52]. Manually annotating 3D time-lapse images is a tedious task –
255 mainly because of the complex visualization in 4D space – that cannot be per-
256 formed reliably. There is a significant intra- and inter-experimenter variability.
257 There is currently a major lack of annotations of astrocyte calcium activity
258 images. Note that this is also true for other datasets of 3D images in live tis-
259 sue [53]. For all of those reasons, a common and promising approach is to use
260 realistic synthetic datasets with known ground-truths (*i.e.*, all morphological
261 and dynamical properties are known and controlled) to train and quantita-
262 tively assess the performance of analysis software. This highlights the need for
263 developing models and simulators that are able to mimic real image sequences.

Need for public realistic synthetic datasets: join the forces!

To solve the difficulty to reliably label calcium signals in microscopy images, a promising approach consists in generating 3D+time synthetic datasets that realistically depict astrocyte calcium signals observed in real microscopy images. To be as realistic as possible, the simulation should be driven by a biophysical model that describes the calcium signals at the nanoscale, which requires close collaboration between image analysts, modelers (see section [Modeling astrocyte calcium signals](#)) and experimentalists (see section [Acquisition of astrocyte calcium signals](#)). For instance, a recent interdisciplinary collaboration [54] has resulted in the creation of a simulator to generate realistic sequences of 3D lattice LSFM images depicting calcium activity in the sponge-like network of astrocyte processes by integrating a simplified version of the kinetic model developed by Denizot *et al.*, 2019 [55]. In addition to hopefully opening the door to the deployment of 3D+time image analysis tools to quantify astrocyte calcium activity, these simulators could also help modelers tuning their models and the parameters in a faster way than using computational simulations, which are often time and computationally expensive. A major challenge to develop such simulators is the complexity of evaluating the similarity between the generated synthetic images and real images. Implementing rigorous methods to evaluate synthetic astrocyte calcium images will thus be essential to ensure the success of this approach. Note that these synthetic data are essential to guide analysts in the development of their algorithms, but final qualitative validation on real images is still required.

Modeling astrocyte calcium signals

Models correspond to simplifications that describe relevant parameters of a system of interest (its elements, their states and their interactions), allowing for better quantification, visualization, and understanding of the system. The famous quotation from George Box, “All models are wrong but some are useful” [56], highlights that models are incomplete representations of the system as a whole, yet provide crucial insights into the system’s behavior and dynamics. Such insights would not be grasped by a model as complex as the system of interest itself.

Depending on the question and hypothesis that emerge from experimental data, modelers choose different approaches and toolkits (see Table 2). For example, models studying calcium activity in microdomains will need a higher spatial resolution than models of somatic signals. Further, the modeling approaches that are well-suited to study calcium microdomains, such as particle-based methods (see [55] for a review), are more accurate but extremely demanding in terms of computational power and simulation time. Simulating hundreds of seconds of calcium activity in a fine astrocyte process (*e.g.*, 1 μm long, 200 nm in radius) can take days to compute, so that using those tools

to simulate signals in a whole cell or in a network of cells is currently unfeasible. Please note that the computation time to simulate *e.g.*, 1 millisecond of calcium activity varies not only depending on the modeling technique used, but also on the computational resources available in each laboratory, on the volume and number of reactions modeled as well as the simulation time. To learn more about the different approaches that can be used to model reactions, their insights and limitations, please refer to dedicated reviews [55, 57]. The goal of this section is not to present an exhaustive list of astrocyte models (see [55, 58, 59]), to review existing models of calcium signaling (reviewed in [60–65]), or to present a detailed list of modeling tools to model calcium signals [66, 67]. Rather, we emphasize the key insights that can be gained from models of astrocyte calcium activity as well as the challenges that computational neuroscientists are currently facing.

Insights from modeling into biological processes

Mathematical and computational models are powerful tools that provide new insights in the mechanisms that regulate calcium activity in astrocytes and generate testable predictions. First, models can be used to conduct *in silico* experiments that are time-consuming or unfeasible experimentally. Models have for example been used to finely tune the spatial distribution of calcium channels (molecules that, when open, result in a calcium influx into the cytosol, forming a calcium signal) within the cell and explore its impact on astrocyte activity [68, 69]. Moreover, models can be used to generate realistic datasets that can be used to train tools developed to characterize the system’s behavior (see section [Analysis of astrocyte calcium signals](#)) [54]. Lastly, computational models are useful to go beyond correlational observations and to propose mechanistic principles that explain experimentally-observed data. For example, models have shown the effect of cellular morphology on the compartmentalization of calcium signals in dendritic spines [70–73] as well as in astrocyte processes [74]. For a recent review on the insights gained from computational approaches on astrocyte function as well as strategies to start incorporating astrocyte calcium signals in systems neuroscience to better understand how astrocytes contribute to brain computation, see [75]. Overall, modeling approaches can provide key insights to astrocyte physiology.

Main challenges associated with the development of models of astrocyte calcium activity

Computational neuroscientists are facing major challenges to build models of astrocyte calcium activity. First, a lot of data are currently missing or not shared publicly, so that most parameter values used in the astrocyte models that are currently available are derived from data obtained in other cell types. Those data include the concentration and sub-cellular distribution of endogenous buffers, the diffusion coefficient of diffusing molecules involved in calcium dynamics in astrocytes, the distribution of the major calcium channels and

Table 2 Brief summary of the main modeling approaches that are commonly used to model astrocyte calcium activity, their insights, limitations and examples.

Biological processes are inherently noisy. When the system that is modeled contains a large number of molecules, this noise can be averaged. Such models are called deterministic and describe the variation of molecular concentrations over time. They are often used to describe calcium signals at the whole cell and at the network levels. When the system of interest contains a small number of molecules or ions, typically small subcellular compartments like astrocyte processes, this approximation is no longer valid and the stochastic nature of molecular reactions has to be taken into account in the model. Further, models can be spatial, *i.e.* take into account the position and potential diffusion of molecules in the cell, or well-mixed, *i.e.* at each time step, any molecule can virtually move anywhere in the cell. The location of the molecules and cell morphology is thus not taken into account in well-mixed models. * Calcium concentration in spatial stochastic simulations can be deduced from the number of molecules tracked and the system’s volume. ** Some spatial stochastic techniques track individual molecules (particle-based) while others track the number of molecules in small sub-compartments (voxel-based). See *e.g.* [76] for a review.

Note that the characteristics presented in this table are indicative as the usage and computational cost of a given model vary greatly depending on the precise method implemented and the number of molecules/reactions modeled (see [55, 57] for

Name of the modeling approach	Spatial	Describe variation of concentration	Tracks individual molecules	Computational cost	Common use	Example
Well-mixed, deterministic	No	Yes	No	Very low	Astrocyte network/w-hole cell	[77–79]
Well-mixed, stochastic	No	Yes	No	Low	Astrocyte network/w-hole cell	[80–82]
Spatial, deterministic	Yes	Yes	No	Low-intermediate	Whole cell/Signal propagation in major branches	[83–89]
Spatial, stochastic	Yes	Yes *	Yes **	High	Spongiform domain	[68, 90]

348 pumps in the cell, the dynamic remodeling of the morphology of the cell and
 349 of its internal calcium stores in live tissue. Second, the computational cost
 350 of simulations increases drastically as the accuracy of the model increases, so
 351 that a trade-off often has to be made by the modeler, resulting in models with
 352 few reactions or low spatial resolution. Further, some of the currently avail-
 353 able models suffer from a lack of availability, replicability, and reproducibility
 354 [58]. Lastly, models often focus on specific spatio-temporal scales of astrocyte

355 activity. Bridging those models together is critical to better understand the
356 involvement of local calcium signals in higher-level brain functions such as cog-
357 nition and learning. Building such multi-scale models is challenging but should
358 provide unprecedented insights in the involvement of astrocyte calcium signals
359 in the activity of neural circuits and overall in brain (dys-)function.

360 **Is there such a thing as a generic astrocyte model?**

361 Although astrocytes share common morphological and biochemical char-
362 acteristics, they are remarkably heterogeneous. The diversity of astrocyte
363 morphology has been described as early as 100 years ago by Cajal and
364 morphology-based classifications of astrocytes have been proposed [91]. Astro-
365 cyte electrophysiological properties [92–94], gene [95–97] and protein expres-
366 sion levels [98] also vary drastically depending on the brain region under study.
367 Those observations suggest that astrocytes are a heterogeneous cell popula-
368 tion, questioning the specificities and roles of individual sub-types. For more
369 details, see dedicated reviews on astrocyte heterogeneity [1, 99–101]. Whether
370 the diverse functions of astrocytes in the brain rely on molecularly and mor-
371 phologically distinct sub-populations of astrocytes is still poorly understood,
372 yet crucial to uncover the functions of astrocytes in the healthy and diseased
373 brain. A recent study identified sub-populations of astrocytes that selectively
374 contributed to specific functions such as synaptogenesis and tumor invasion of
375 glioma [102]. Incorporating this diversity in astrocyte models by building mod-
376 els of specific sub-populations of astrocytes rather than the currently available
377 generic astrocyte models will be essential to provide insights into the functional
378 implications of the molecular and morphological heterogeneity of astrocytes
379 that have been reported recently.

380 **Need for interdisciplinary collaborations to improve** 381 **models of astrocyte calcium activity**

382 Several strategies and perspectives could be developed to go beyond the afore-
383 mentioned challenges to model astrocyte calcium activity. First, computational
384 neuroscientists would highly benefit from the existence of open-source datasets,
385 which could be used to build and test models. Such datasets are crucial for
386 data-driven modeling practices, which rely on strong iterative collaborative
387 work between experimentalists and computational neuroscientists. Moreover,
388 several good practices and step-by-step modeling guides have been published
389 to ensure the reproducibility of models [103–105]. Lastly, initiatives such as the
390 Neuromatch Academy courses and conferences [106, 107] provide an unprece-
391 dented opportunity to build an accessible, democratic, inclusive, international
392 and interdisciplinary community aiming at using computational approaches to
393 improve our understanding of brain function.

Perspectives

Astrocytes are cells that display a highly complex activity that is essential to brain function. Characterizing the diverse signals displayed by active astrocytes and understanding their physiological roles, the “Calcium Code”, are the biggest challenges of the field and are crucial to understand the involvement of astrocytes in brain function. In this short review article, we highlighted the different insights that can be gained from each field that studies calcium signals in astrocytes and the major challenges that they are facing. Key challenges that prevent us from making sense of astrocyte calcium activity have arisen from our discussions during our interdisciplinary workshop, hosted by the 1st Virtual Conference of the European Society for Neurochemistry “Future perspectives for European neurochemistry – a young scientist’s conference”, in May 2021, entitled “Let’s join forces - Bridging the gap between experimental, computational and data sciences to disentangle astrocyte calcium activity”. Those challenges include:

- The development of analysis tools allowing accurate detection and characterization of individual calcium signals in astrocytes are lacking, notably in 3D+time.
- There is no consensus in key definitions and terminology, which further hinders efficient communication across fields (*e.g.*, calcium microdomain/-nanodomain, processes/leaflets, Calcium Code, gliapil/spongiform domain).
- A lot of data is missing to fully grasp the mechanisms regulating astrocyte calcium signals and their physiological roles. For example, local and regional variability of the expression levels of proteins involved in calcium signaling, both in health and disease, remain to be characterized. The morphology of perisynaptic astrocyte processes and their organelles, together with their dynamical remodeling, also remain to be uncovered in live tissue.
- Raw data are rarely shared in public repositories. Notably, labeled datasets are needed to evaluate image analysis tools and to train data-driven tools. Providing public access to such datasets has contributed to fast improvements in other fields, such as the development of tools detecting the onset of epileptic seizures [108].
- Interdisciplinary events and projects are rare, which constitutes a major barrier to our efforts to unravel the astrocyte “Calcium Code”. Indeed, scientists from different fields lack opportunities to discuss, share ideas and knowledge. The interactions between fields working on astrocyte calcium signals and opportunities for improvements are highlighted in Fig. 2. We believe that such joint efforts are essential to fully grasp the complex properties and functions of astrocytes.

Reinforcing interdisciplinary projects, bringing together experts from different fields, will be crucial to ensure our success in cracking the astrocyte “Calcium Code”. Such collaborative projects are still rare in the field, which might result from the high fragmentation of research projects and fields working on astrocyte physiology, often presenting their work in different, highly

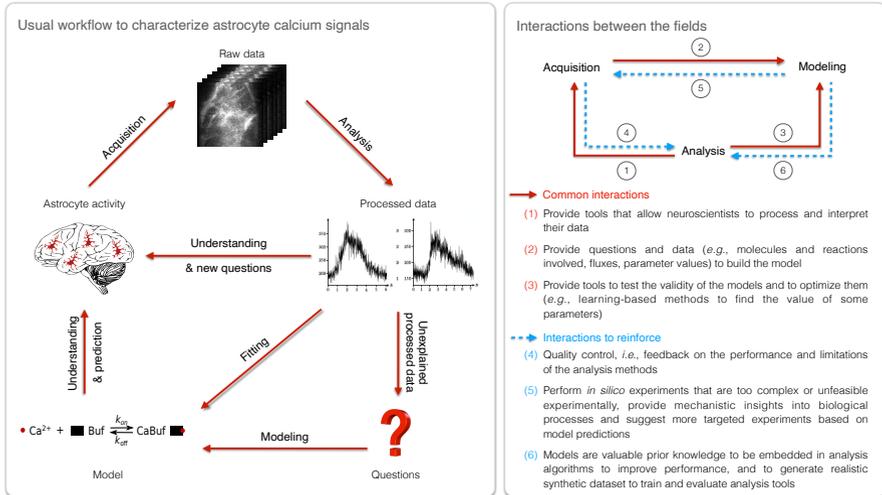


Fig. 2 Reinforcing interdisciplinary collaborations to unravel the astrocyte “Calcium Code”. Left: workflow for the characterization of calcium signals involving the fields of acquisition, analysis and modeling. The raw data acquired by experimentalists include *e.g.*, calcium images, structural images or omics data. Raw data processing by analysts results in dynamical (*e.g.*, duration, trajectory, frequency) and structural characterization (*e.g.*, protein localization, cell morphology) of astrocytes as well as the quantification of protein expression levels, for example. Right: schematic representation of the interactions between the fields. Interactions to reinforce are highlighted in dashed blue lines (4, 5, 6).

438 specialized conferences and journals. We propose initiatives that will facilitate
439 the emergence of new interdisciplinary projects:

- 440 • Agreement on shared definitions and terminology across fields.
- 441 • Sharing datasets, together with all the relevant information on the data
442 acquisition, processing and modeling (if relevant) methods used. This might
443 require the creation of an online platform to store and discuss data on
444 astrocytes.
- 445 • Sharing user-friendly data analysis tools, including providing the code in
446 open-access and the dataset(s) used to facilitate their dissemination to the
447 whole community.
- 448 • Organization of recurrent meetings and events that bring together experts
449 from various fields of expertise.

450 Because of the complexity of astrocyte morphology and signaling, interdis-
451 ciplinary projects will be essential to not only crack the astrocyte “Calcium
452 Code”, but also to successfully improve our understanding of astrocyte
453 (patho-)physiology and to propose models of astrocyte function.

454 **Data availability statement**

455 Data sharing is not applicable to this article as no datasets were generated or
456 analysed during this study.

457 **Ethical statement**

458 **Ethics approval and consent to participate**

459 Not Applicable.

460 **Consent for publication**

461 Not Applicable.

462 **Availability of data and materials**

463 Not Applicable.

464 **Competing interests**

465 The authors have no relevant financial or non-financial interests to disclose.

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472 **Author contributions**

473 Ana Covelo wrote the first draft of the data acquisition section, Anaïs Badoual
474 wrote the first draft of the data analysis section and Audrey Denizot wrote the
475 first draft of the introduction, modeling and perspectives sections. All authors
476 commented on previous versions of the manuscript, read and approved the
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