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1 Control of Ca^{2+} signals by astrocyte 2 nanoscale morphology at tripartite 3 synapses

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12 **Abstract** Astrocytic Ca^{2+} signals regulate synaptic activity. Most of those signals are spatially
13 restricted to microdomains and occur in fine processes that cannot be resolved by
14 diffraction-limited light microscopy, restricting our understanding of their physiology. Those fine
15 processes are characterized by an elaborate morphology, forming the so-called spongiform
16 domain, which could, similarly to dendritic spines, shapes local Ca^{2+} dynamics. Because of the
17 technical limitations to access the small spatial scale involved, the effect of astrocyte morphology
18 on Ca^{2+} microdomain activity remains poorly understood. Here, we use computational tools and
19 realistic 3D geometries of fine processes based on recent super-resolution microscopy data to
20 investigate the mechanistic link between astrocytic nanoscale morphology and local Ca^{2+} activity.
21 Simulations demonstrate that the nano-morphology of astrocytic processes powerfully shapes
22 the spatio-temporal properties of Ca^{2+} signals and promotes local Ca^{2+} activity. The model
23 predicts that this effect is attenuated upon astrocytic swelling, hallmark of brain diseases, which
24 we confirm experimentally in hypo-osmotic conditions. Upon repeated neurotransmitter release
25 events, the model predicts that swelling hinders astrocytic signal propagation. Overall, this study
26 highlights the influence of the complex morphology of astrocytes at the nanoscale and its
27 remodeling in pathological conditions on neuron-astrocyte communication at tripartite synapses.

29 Introduction

30 Astrocytes are glial cells of the central nervous system that are essential for brain develop-
31 ment and function, playing key roles in e.g ion homeostasis, neurotransmitters uptake and lactate
32 metabolism (see (*Verkhratsky and Nedergaard, 2018*) for more details). Furthermore, astrocytes
33 can modulate neuronal communication at synapses. In response to the release of neurotransmit-
34 ters from neurons, Ca^{2+} signals are observed in astrocytes, which can trigger the release of neu-
35 roactive molecules by the astrocyte, gliotransmitters. The first type of astrocytic Ca^{2+} signals that
36 has been observed was Ca^{2+} waves that propagate through gap junctions in astrocyte networks (*Gi-
37 aume and Venance, 1998*). Ca^{2+} waves are also observed within the branches of single astrocytes,
38 sporadically propagating to the soma (*Haustein et al., 2014; Bindocci et al., 2017*). The recent de-
39 velopment of Ca^{2+} imaging techniques that provide better spatial and temporal resolution has re-
40 vealed the existence of spatially-restricted Ca^{2+} signals in astrocytes, referred to as microdomains

41 or hotspots (*Di Castro et al., 2011; Panatier et al., 2011; Stobart et al., 2018a; Srinivasan et al.,*
42 *2015; Shigetomi et al., 2013; Sherwood et al., 2017; Otsu et al., 2015; Lind et al., 2013; Bindocci*
43 *et al., 2017; Agarwal et al., 2017; Arizono et al., 2020*). Those local Ca^{2+} signals account for as
44 much as 80 % of astrocytic Ca^{2+} activity and occur in fine astrocytic processes, also referred to as
45 branchlets, which occupy 75 % of the astrocytic volume (*Bindocci et al., 2017*), referred to as the
46 spongiform domain or gliapil. Given that one astrocyte can contact tens of thousands of synapses
47 simultaneously in the mouse hippocampus (*Bushong et al., 2002*) and up to two million in the
48 human brain (*Oberheim et al., 2006*) through these fine processes, local, fast Ca^{2+} signals might
49 enable the astrocyte to powerfully yet precisely control the flow of information through synaptic
50 circuits.

51

52 Elaborate cellular morphologies lend themselves to the fine compartmentalization of signals.
53 For example, the anatomical design of dendritic spines restricts the spread of Ca^{2+} to the acti-
54 vated synapse, ensuring that there is little cross-talk between different synapses and that the local
55 Ca^{2+} concentration can go very high (*Santamaria et al., 2011; Tønnesen et al., 2014; Yuste et al.,*
56 *2000; Noguchi et al., 2005; Yasuda, 2017; Holcman and Schuss, 2011*). It is thus likely that the
57 complex organization of the reticular structure of astrocytes plays a key role in shaping local Ca^{2+}
58 microdomains (*Rusakov, 2015*). For example, the complex shape of Bergmann glia has been pro-
59 posed to potentially restrict Ca^{2+} signals to the vicinity of synapses (*Grosche et al., 1999*). Similarly,
60 perisynaptic enlargements called “astrocytic compartments” along major branches of astrocytes
61 also exhibit Ca^{2+} signals (*Panatier et al., 2011*). Fine processes of the spongiform domain, however,
62 cannot be resolved by diffraction-limited light microscopy (*Rusakov, 2015*), so that the contribution
63 of their nanoscale morphology to shaping local Ca^{2+} signals is poorly understood. Our recent 3D
64 super-resolution Stimulated Emission Depletion (STED) microscopy study (*Arizono et al., 2020*) re-
65 vealed that particular shapes of the complex meshwork of astrocytic processes, reminiscent of
66 dendritic spines, are associated with compartmentalized Ca^{2+} signals. Furthermore, aberrant Ca^{2+}
67 signals (*Shigetomi et al., 2019*) and changes in astrocytic morphology, such as “astrocytic swelling”,
68 occur in pathological conditions such as brain injury, including ischemic, traumatic, hemorrhagic
69 and metabolic injuries, stroke and epilepsy (*Lafrenaye and Simard, 2019*). Interestingly, our recent
70 STED study reported that swelling can also occur at the level of fine astrocytic processes (*Arizono*
71 *et al., 2021a*). The effect of such a remodeling of astrocytic nano-morphology on the local Ca^{2+}
72 signals involved in regulating synapses yet remains unclear. Together, there is a great interest
73 in understanding the mechanistic link between the nano-morphology of astrocytic processes and
74 Ca^{2+} profiles in health and disease. Changing cell morphology experimentally is not straightforward
75 and can affect cell physiology, notably when the osmolarity of the extracellular space is altered. By
76 contrast, computational approaches can finely tune geometrical properties, resulting in more con-
77 trolled investigations than what could be done experimentally. Computational models are thus
78 tools of choice to investigate the isolated impact of morphological parameters on Ca^{2+} dynamics.

79 Here, we use computational tools to investigate the effect of astrocyte nanoscale morphology
80 on local Ca^{2+} activity, in healthy and swelling conditions. To account for the stochasticity of molec-
81 ular interactions in small volumes such as those of astrocyte processes, we use the stochastic
82 voxel-based model of Ca^{2+} signals from Denizot et al. (*Denizot et al., 2019a*). Idealized geome-
83 tries of branchlets were designed based on super-resolution microscopy images recently reported
84 in live tissue (*Arizono et al., 2020*). Branchlets consist in alternations of structures referred to as
85 nodes and shafts. At this spatial scale, swelling resulting from hypo-osmotic conditions results in
86 an increased diameter of shafts, without altering node morphology (see Fig 3 from (*Arizono et al.,*
87 *2021b*)). To explore how such changes in morphology affect Ca^{2+} signalling, we performed simula-
88 tions in geometrical models of branchlets of varying shaft widths. Our simulation results suggest
89 that astrocyte swelling results in an increased diffusion flux, which decreases Ca^{2+} peak probabil-
90 ity, duration and amplitude in the stimulated and neighboring nodes. To test those predictions,
91 we performed Ca^{2+} recordings in organotypic hippocampal cultures in hypo-osmotic conditions,

92 which result in increased shaft width without altering node width. Ca^{2+} activity in hypo-osmotic
93 conditions was decreased compared to Ca^{2+} activity in healthy tissue, in line with our model pre-
94 dictions. In case of repeated node stimulation, thin shafts allowed signal propagation even if some
95 stimuli were omitted. Thin shafts thus allow for more robust signal propagation within the gliapil,
96 which is hindered in swelling conditions.

97

98 Overall, this study sheds light on the influence of the nanoscale morphology of the complex
99 spongiform domain of astrocytes on Ca^{2+} microdomain activity. Importantly, our results provide
100 evidence that node compartmentalization, resulting from a reduced diffusion efflux from nodes
101 connected to thin shafts, can increase Ca^{2+} activity locally. Our study indicates that pathological
102 changes in nanoscale astrocyte morphology may substantially affect their Ca^{2+} activity.

103 Results

104 Geometrical representation of typical astrocyte processes

105 In order to investigate the effect of astrocyte nanoscale morphology on the spatio-temporal
106 properties of Ca^{2+} signals, we have designed geometries of typical astrocyte processes, derived
107 from the recent characterization of their ultrastructure at a high spatial resolution (50 nm in x-
108 y) in organotypic hippocampal culture as well as in acute slices and *in vivo* (Arizono *et al.*, 2020).
109 Geometries consist of alternations of bulbous structures, nodes, connected to each other with
110 cylindrical structures, referred to as shafts. To reproduce the morphological changes of processes
111 associated with cell swelling reported in hypo-osmotic conditions (Fig 3 in (Arizono *et al.*, 2021b)),
112 geometries with different shaft width d_{shaft} and constant node width were designed (Fig 1A). In
113 accordance with node-shaft structures observed experimentally (Arizono *et al.*, 2020), node width
114 was set to 380 nm. To model astrocytic Ca^{2+} activity with a high spatial resolution while taking into
115 account the randomness of reactions in small volumes, we used the stochastic voxel-based model
116 from Denizot *et al.* (Denizot *et al.*, 2019a) (see Methods). The reactions included in the model
117 are presented in Fig 1B and in the Methods section. As the majority of Ca^{2+} signals in astrocytes
118 result from the opening of Inositol 3-Phosphate receptors (IP_3Rs), located at the membrane of the
119 endoplasmic reticulum (ER) (Srinivasan *et al.*, 2015), signals in the model result from the opening
120 of IP_3Rs (see Denizot *et al.* (Denizot *et al.*, 2019a) for discussion on the model's assumptions and
121 limitations).

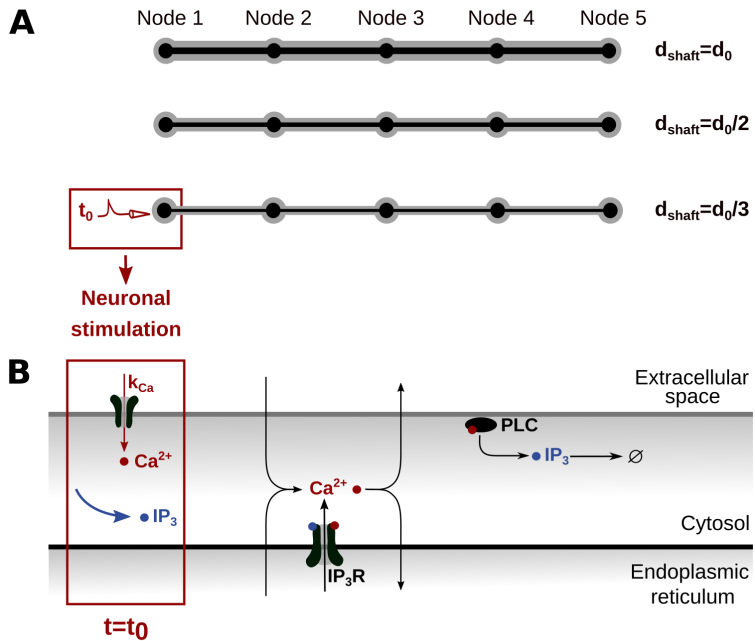


Figure 1. Geometries and kinetic scheme used for simulating Ca^{2+} dynamics in node/shaft structures of the gliapil. (A) Geometries reproducing node/shaft geometries of the gliapil (Arizono *et al.*, 2020; Panatier *et al.*, 2014) were designed. Nodes are approximated as spheres of diameter 380 nm and shafts as 1 μm -long cylinders. The geometries designed in this study, referred to as “5 nodes”, contain 5 identical nodes and 4 identical shafts. Geometries were characterized by different shaft widths: $d_{\text{shaft}} = d_0 = 380\text{nm}$, $d_{\text{shaft}} = \frac{d_0}{2}$ and $d_{\text{shaft}} = \frac{d_0}{3}$. The associated cytosolic volume, plasma and ER membrane areas are presented in Table 1. (B) Biochemical processes included in the model. Ca^{2+} can enter/exit the cytosol from/to the extracellular space or the endoplasmic reticulum (ER), resulting from the activity of Ca^{2+} channels/pumps. Ca^{2+} and IP_3 diffuse in the cytosol following Brownian motion. The kinetics of IP_3R channels corresponds to the 8-state Markov model from (Denizot *et al.*, 2019b), adapted from (De Young and Keizer, 1992; Bezprozvanny *et al.*, 1991). When both IP_3 and Ca^{2+} are bound to IP_3R activating binding sites, the IP_3R is in open state and Ca^{2+} enters the cytosol. Ca^{2+} can activate Phospholipase C δ (PLC δ), which results in the production of IP_3 . For more details, please refer to (Denizot *et al.*, 2019b). Neuronal stimulation is simulated as an infusion of IP_3 in the cytosol and the opening of Ca^{2+} channels at the plasma membrane with an influx rate k_{Ca} (see Methods).

Figure 1–Figure supplement 1. Sensitivity study of the effect of voxel size on Ca^{2+} signals.

Figure 1–source data 1. Characteristics of meshes used to investigate the effect of voxel size on Ca^{2+} signals (Table Supplement 1).

122 Thin shafts favor node compartmentalization

123 In order to test whether the geometries designed in this study are a good approximation of
 124 the ultrastructure of the gliapil, we have compared molecular diffusion flux in those geometries
 125 with those reported experimentally. To do so, we simulated photobleaching experiments and com-
 126 pared our results to experimental results from Arizono *et al.* (Arizono *et al.*, 2020). The principle
 127 of bleaching simulations is presented in the Methods section and in Supplemental Movie 1. Here,
 128 we refer to an increased node compartmentalization when the time to recovery after bleaching, τ ,
 129 increases (see Fig 2B).

130
 131 Bleaching traces in simulations are both qualitatively (Fig 2B) and quantitatively (Fig 2C) similar
 132 to experimental bleaching traces, for shaft width $d_{\text{shaft}} = d_0$ and $d_{\text{shaft}} = \frac{d_0}{2}$. Indeed, no significant
 133 difference of I_0 (Fig 2C1), I_{inf} (Fig 2C2) and τ (Fig 2C3) was observed between simulations and ex-
 134 perimental traces. Simulations were also performed with $d_{\text{shaft}} = \frac{d_0}{3}$. Our simulations successfully
 135 reproduce experimental bleaching experiments and suggest that τ , and thus node compartmental-

Table 1. Characteristics of the geometries of astrocyte branchlets used in this study. V_{cyt} is the cytosolic volume, S_{PM} is the area of the plasma membrane and S_{ER} is the area of the ER membrane. Volumes are expressed in nm^3 and areas in nm^2 . Meshes are available at <http://modeldb.yale.edu/266928>, access code: Ito42@tpk3D?

Geom	$V_{\text{cyt}} (\text{nm}^3)$	$S_{\text{PM}} (\text{nm}^2)$	$S_{\text{ER}} (\text{nm}^2)$
"5nodes" $d_{\text{shaft}} = d_0$	6.20×10^8	7.73×10^6	2.19×10^6
"5nodes" $d_{\text{shaft}} = d_0/2$	2.63×10^8	4.95×10^6	1.28×10^6
"5nodes" $d_{\text{shaft}} = d_0/3$	1.95×10^8	4.10×10^6	9.99×10^5
"No ER" $d_{\text{shaft}} = d_0$	6.73×10^8	7.73×10^6	0.00
"No ER" $d_{\text{shaft}} = d_0/2$	2.83×10^8	4.95×10^6	0.00
"No ER" $d_{\text{shaft}} = d_0/3$	2.10×10^8	4.09×10^6	0.00
"Node ER" $d_{\text{shaft}} = d_0$	6.67×10^8	7.75×10^6	4.17×10^5
"Node ER" $d_{\text{shaft}} = d_0/2$	2.74×10^8	4.96×10^6	4.37×10^5
"Node ER" $d_{\text{shaft}} = d_0/3$	2.00×10^8	4.11×10^6	4.41×10^5
"Cyl ER" $d_{\text{shaft}} = d_0$	6.27×10^8	7.74×10^6	2.03×10^6
"Cyl ER" $d_{\text{shaft}} = d_0/2$	2.77×10^8	4.95×10^6	8.78×10^5
"Cyl ER" $d_{\text{shaft}} = d_0/3$	2.07×10^8	4.09×10^6	5.86×10^5

136 ization, increases when shaft width decreases (Fig 2C3). This result is not surprising as a decreased
137 shaft width results in a smaller size of the exit point for diffusing molecules from the node. This
138 is similar to e.g dendritic spines, which compartmentalization is increased for thinner spine necks
139 (*Santamaria et al., 2011; Tønnesen et al., 2014*). The geometries that we have designed can thus
140 be considered as a reasonable approximation of the ultrastructure of the gliapil observed experi-
141 mentally in live tissue.

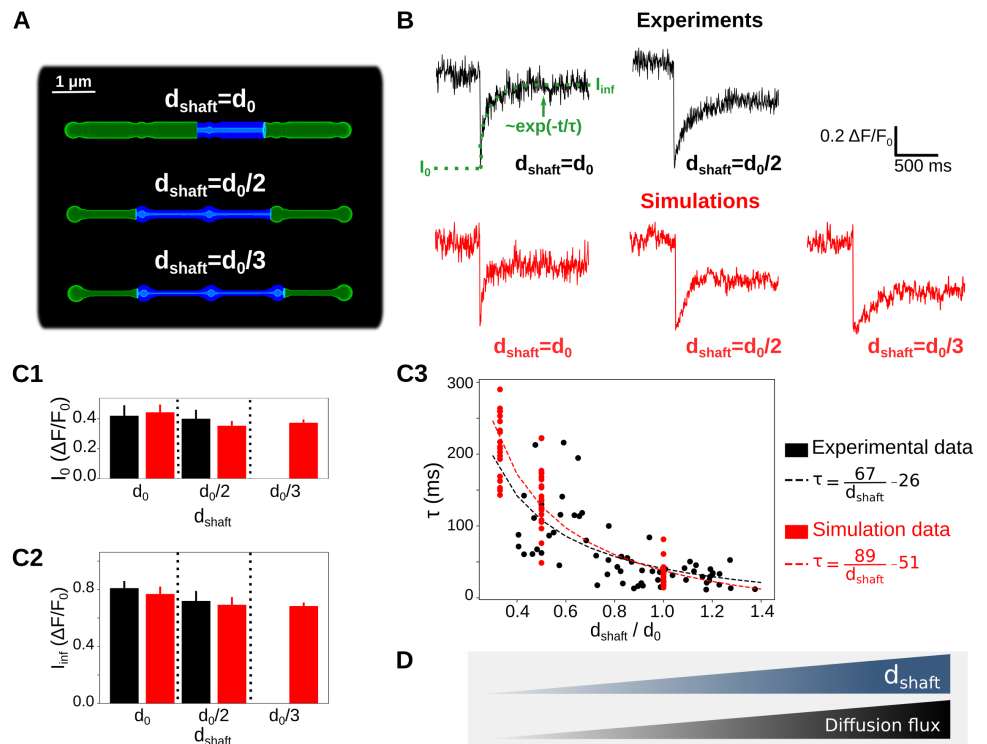


Figure 2. Simulations confirm that thin shafts favor node compartmentalization. (A) Geometries of different shaft widths d_{shaft} , $d_{\text{shaft}} = d_0$, $\frac{d_0}{2}$ and $\frac{d_0}{3}$, used in the bleaching simulations. Blue color represents the bleached volume, which varied depending on the value of d_{shaft} in order to fit experimental values of I_0 and I_{inf} . (B) Representative experimental (top) and simulation (bottom) traces for different shaft width values. I_0 , I_{inf} and τ were calculated using Eq 1. Note that simulations were also performed for $d_{\text{shaft}} = \frac{d_0}{3}$. (C) Quantification of I_0 (C1), I_{inf} (C2) and τ (C3) values in simulations (red) compared to experiments (black). Note that no experimental data was available for $d_{\text{shaft}} = \frac{d_0}{3}$. In C1 and C2, $n=5 \times 2$ and 20×3 for experiments and simulations, respectively. Data are presented as mean \pm STD. In C3, $n=66$ and $n=20 \times 3$ for experiments and simulations, respectively. τ is negatively correlated to d_{shaft} in experiments ($n=66$ from 7 slices; Spearman $r=-0.72$, $p<0.001$ ***) and simulations ($n=60$; Spearman $r=-0.89$, $p<0.001$ ***). Black and red lines represent curve fit of τ as a function of d_{shaft} of the form $\tau = a * \frac{1}{d_{\text{shaft}}} + b$ for experiments and simulations, respectively. (D) Schematic summarizing the conclusion of this figure: diffusion flux increases with d_{shaft} . In that sense, thin shafts favor node compartmentalization. Data in panels C1 and C2 are represented as mean \pm STD, $n=20$ for each geometry.

Figure 2-video 1. Movie of a simulated bleaching experiment, with $d_{\text{shaft}} = \frac{d_0}{2}$. Fluorescing ZsGreen molecules are in red and bleached, non-fluorescing, ZsGreen are in yellow.

142 Thin shafts enhance Ca^{2+} activity in nodes

143 80% of astrocyte Ca^{2+} activity occurs in the gliopil (*Bindocci et al., 2017*), which suggests that
 144 most neuron-astrocyte communication occurs at fine astrocytic processes. As we observed that
 145 a decreased shaft width is associated with a decreased diffusion flux, i.e an increased compart-
 146 mentalization of nodes, we have tested whether this effect influences Ca^{2+} activity upon neuronal
 147 stimulation. To do so, we have first analyzed Ca^{2+} signals resulting from a single neuronal stimula-
 148 tion, which was simulated as an infusion of IP_3 and Ca^{2+} in the stimulated node, node 1 (see Meth-
 149 ods). Those parameters encompass the IP_3 production by phospholipase C following the activation
 150 of G_q -G-protein-coupled receptors (GPCRs) resulting from the binding of neuronal glutamate, ATP
 151 and noradrenaline to G_q proteins and Ca^{2+} entry at the plasma membrane through Ca^{2+} channels,
 152 ionotropic receptors or the sodium/calcium exchanger (NCX) functioning in reverse mode (*Ahmad-*
 153 *pour et al., 2021; Semyanov et al., 2020*). Signals were recorded both in the stimulated node, node
 154 1, and in the neighboring node, node 2 (Supplemental Movie 1). Representative Ca^{2+} traces in
 155 node 2 for $d_{\text{shaft}} = d_0$, $\frac{d_0}{2}$ and $\frac{d_0}{3}$ are displayed in Fig 3A. Our first result is that Ca^{2+} peak probability

156 increases when d_{shaft} decreases (Fig 3B1). The time to 1st peak increases with d_{shaft} (Fig 3B2). By con-
157 trast, peak amplitude (Fig 3B3) and duration (Fig 3B4) increase when d_{shaft} decreases. In order to
158 better understand the mechanisms responsible for the increased Ca^{2+} peak probability, amplitude
159 and duration when d_{shaft} decreases, we have measured the frequency of IP_3R opening in nodes
160 1 and 2. The frequency of IP_3R opening increases when d_{shaft} decreases (Fig 3B5). Note that the
161 duration of IP_3R opening and the number of IP_3Rs open per Ca^{2+} peak did not vary with d_{shaft} . The
162 increased IP_3R opening frequency associated with small values of d_{shaft} probably results from the
163 increased residency time of molecules in nodes connected to thin shafts (Fig 3C). Indeed, a thin
164 shaft can "trap" Ca^{2+} and IP_3 longer in the node, thus locally increasing the probability of IP_3Rs
165 to open, resulting in larger Ca^{2+} peaks. For more details, the reader can refer to the theoretical
166 work investigating the narrow escape problem for diffusion in microdomains (*Schuss et al., 2007*).
167 Nodes connected to thinner shafts, despite being characterized by a lower diffusion flux (Fig 2),
168 could thus consist of signal amplification units, favoring the generation of larger signals, therefore
169 increasing Ca^{2+} peak probability, amplitude and duration both in the stimulated node and in neigh-
170 boring nodes.

171

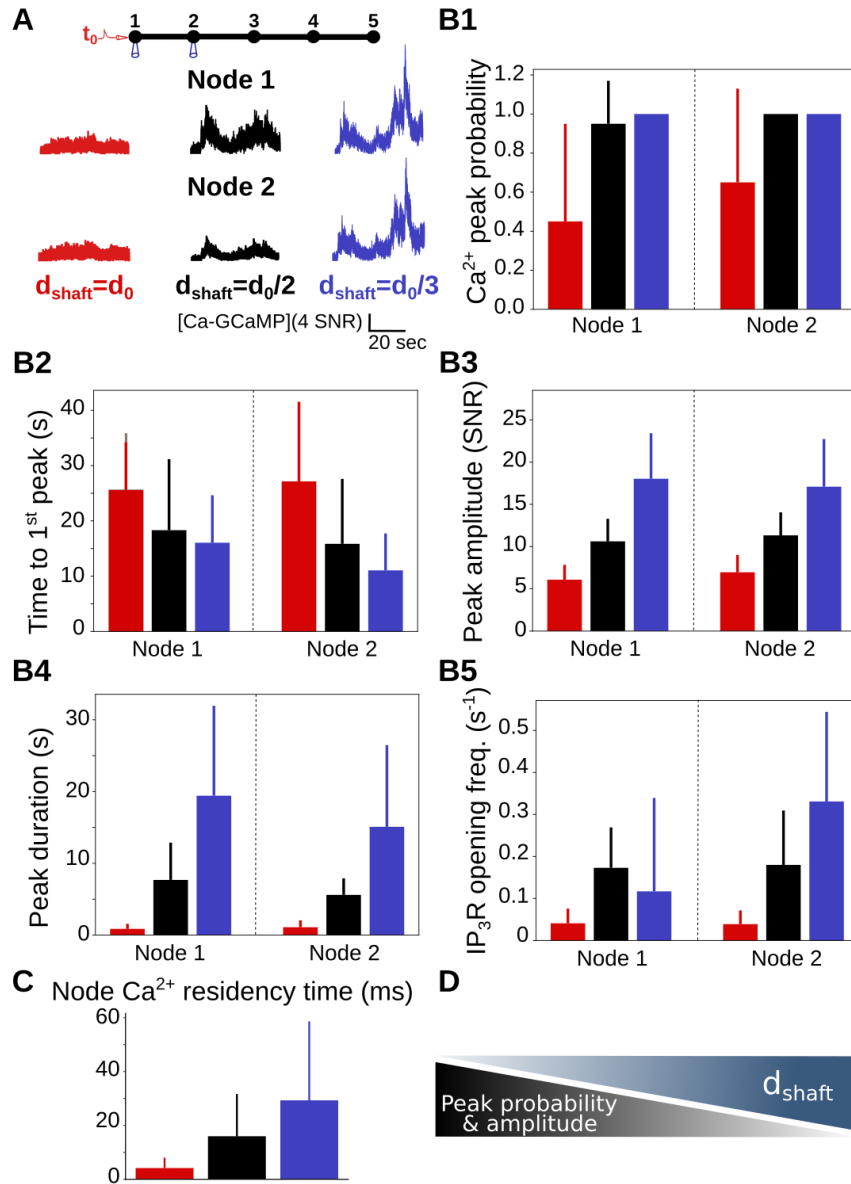


Figure 3. Ca²⁺ peak probability, amplitude and duration increase when shaft width decreases. (A) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1s$, while Ca²⁺ activity was monitored in node 2. Representative Ca²⁺ traces for shaft width $d_{\text{shaft}} = d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue), expressed as SNR (see Methods). (B) Quantification of the effect of d_{shaft} on Ca²⁺ signal characteristics. Data are represented as mean \pm STD, $n=20$. Ca²⁺ peak probability increases (***, B1), Time to 1st peak decreases (***, B2), peak amplitude (***, B3) and duration (***, B4) increase when d_{shaft} decreases. (C) Ca²⁺ residency time in node 1 increases when d_{shaft} decreases (***, $n=300$). (D) Schematic summarizing the main result from this figure: Ca²⁺ peak probability and amplitude increase when shaft width decreases. The effect of d_{shaft} on each Ca²⁺ signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

Figure 3-video 1. Movie of a simulation of the neuronal stimulation protocol, with $d_{\text{shaft}} = \frac{d_0}{2}$, $k_{Ca} = 0 s^{-1}$.

Figure 3-Figure supplement 1. Sensitivity study of the effect of d_{shaft} on local Ca²⁺ signals.

Figure 3-Figure supplement 2. Effect of Ca²⁺ influx rate at the plasma membrane on local Ca²⁺ signals.

Figure 3-Figure supplement 3. Sensitivity study of the effect of boundary conditions on local Ca²⁺ activity upon single node stimulation.

Figure 3-Figure supplement 4. Node/shaft width ratio and node volume control local Ca²⁺ activity.

Figure 3-Figure supplement 5. Thin shafts enhance local spontaneous Ca²⁺ activity.

Figure 3-Figure supplement 6. Ca²⁺ peak probability, amplitude and duration decrease with ER surface area.

Figure 3-Figure supplement 7. Local Ca²⁺ activity increases when shaft diameter decreases, for all ER geometries tested.

Figure 3-Figure supplement 8. Sensitivity study of the effect of ER morphology on Ca²⁺ dynamics.

Figure 3-Figure supplement 9. Ca²⁺ indicators alter Ca²⁺ peak characteristics.

172 To identify the cause of the increased Ca^{2+} activity when shaft width decreases (Fig 3), we per-
173 formed simulations in which we altered the stimulated node (Supplemental Fig 1 D-E), Ca^{2+} influx at
174 the plasma membrane (Supplemental Fig 2) and boundary conditions (Supplemental Fig 3). Those
175 parameters did not affect the observed effects of d_{shaft} on Ca^{2+} dynamics. Furthermore, we re-
176 peated simulations of Fig 3 with constant cytosolic volume (Supplemental Fig 4) and constant num-
177 ber of IP_3 Rs (Supplemental Fig 1 A-C), irrespective of the value of d_{shaft} . Our results confirm that the
178 relevant parameter responsible for the observed effects of d_{shaft} on Ca^{2+} signal characteristics is
179 the node/shaft width ratio or the cytosolic volume rather than d_{shaft} itself. Spontaneous Ca^{2+} sig-
180 nals were affected by shaft width in the same way as neuronal-induced Ca^{2+} signals (Supplemental
181 Fig 5) and, in particular, reproduced the increase of the amplitude ratio of spontaneous Ca^{2+} sig-
182 nals between node 2 and node 1 with shaft width observed in hippocampal organotypic cultures
183 (*Arizono et al., 2020*). Note that ER morphology, in particular ER surface area (Supplemental Fig 6,
184 7 and 8), and Ca^{2+} buffering by Ca^{2+} indicators (Supplemental Fig 9), consistent with previous re-
185 ports (*Denizot et al., 2019a; Bartol et al., 2015; Majewska et al., 2000*), can also alter local Ca^{2+}
186 dynamics.

187 Overall, our results suggest that a decreased shaft width, resulting in a decreased diffusion ef-
188 flux from nodes, increases Ca^{2+} peak probability, amplitude and duration. Conversely, the swelling
189 of fine processes, resulting in an increase of shaft width, attenuates local Ca^{2+} peak probability,
190 amplitude and duration.

191 Ca^{2+} imaging confirms that swelling attenuates local spontaneous Ca^{2+} activity

192 Our recent super-resolution study revealed that the nano-architecture of fine processes is re-
193 modeled in hypo-osmotic conditions (*Arizono et al., 2021b*). At this spatial scale, hypo-osmotic
194 conditions resulted in heterogeneous swelling of processes, where shaft width increased while
195 node width remained unaltered (see Fig 3 in (*Arizono et al., 2021b*)). Simulation results presented
196 in Fig 3 suggest that processes with increased shaft width d_{shaft} are associated with decreased Ca^{2+}
197 peak probability, duration and amplitude. To test whether this effect can be observed in live tis-
198 sue, we thus performed experimental measurements of calcium dynamics in fine astrocytic pro-
199 cesses under basal and hypo-osmotic conditions. To resolve Ca^{2+} signals in fine branchlets, we
200 performed experiments using confocal microscopy in organotypic cultures, which provides the
201 best optical access and sample stability in live tissue to date (resolution ≈ 200 nm in x-y versus \approx
202 500 nm for two-photon microscopy). In accordance with our model's predictions, Ca^{2+} peak ampli-
203 tude and duration were lower in hypo-osmotic compared to basal conditions (Fig 4A-D). Note that
204 peak amplitude and duration did not significantly vary when measured repeatedly in basal condi-
205 tions (Fig 4E-F). Overall, our experimental results confirm that the complex nano-architecture of
206 fine astrocytic processes and its alteration, such as cell swelling, shapes local Ca^{2+} activity.

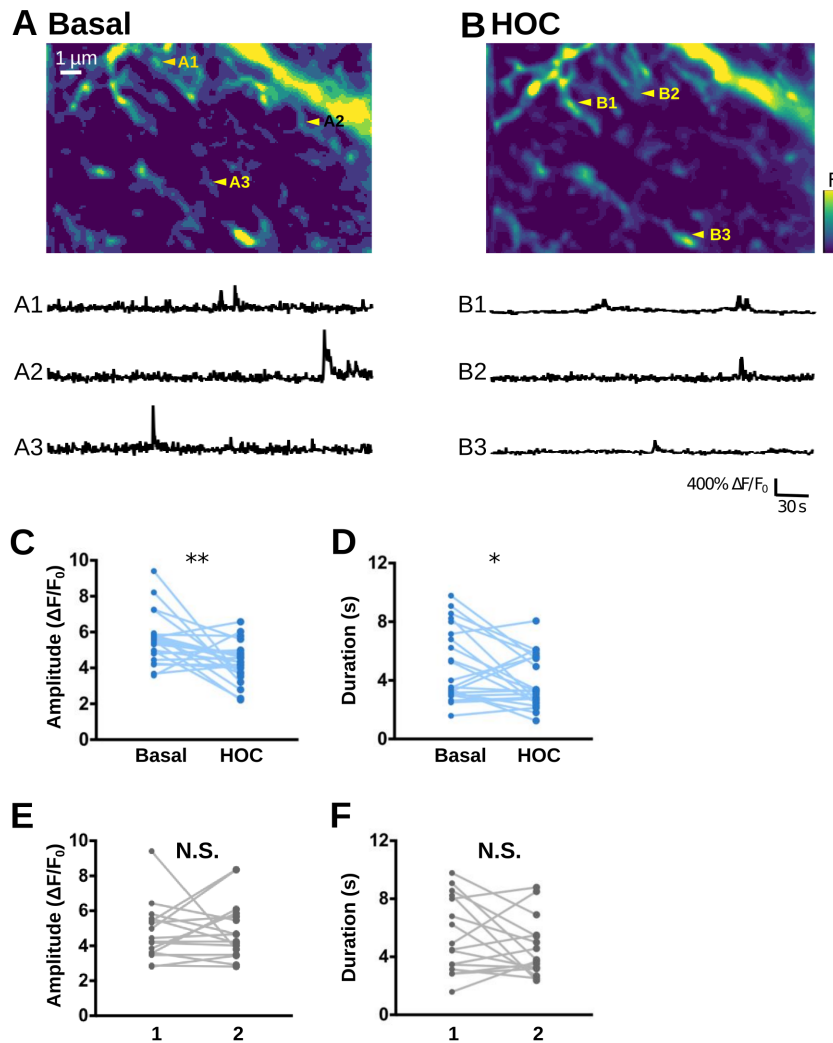


Figure 4. Ca²⁺ imaging confirms that swelling attenuates local spontaneous Ca²⁺ activity. (A, B) (Top) Confocal images of the astrocytic spongiform domain expressing GCaMP6s at baseline (basal, A) and in hypo-osmotic condition (HOC, B), measured in organotypic hippocampal cultures. (Bottom) Representative traces of spontaneous Ca²⁺ events from ROIs indicated in A (A1-A3) and B (B1-B3). (C, D) Ca²⁺ peak amplitude (C) and duration (D) of spontaneous Ca²⁺ events are significantly smaller in hypo-osmotic conditions (HOC) compared to basal conditions (Basal). (E, F) Amplitude (E) and duration (F) of spontaneous Ca²⁺ events do not significantly vary when measured twice in a row (1, 2) in the absence of HOC. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

Thin shafts favor more robust signal propagation

207 A single astrocyte branchlet can contact and modulate the activity of multiple synapses, re-
 208 ferred to as synaptic clusters, which can belong to the same or to different dendrites or even to
 209 different neurons (Arizono et al., 2020; Reichenbach et al., 2010; Witcher et al., 2007; Cali et al.,
 210 2019; Semyanov et al., 2020). The frequency of stimulation of the branchlet can vary depending
 211 on the activity of the adjacent synapses. For example, a connection of adjacent astrocytic nodes
 212 to co-active synapses would result in a high node stimulation frequency. We have thus tested the
 213 influence of the frequency of neuronal stimulation of neighboring nodes on the propagation of
 214 Ca²⁺ signals in branchlets. To do so, we have performed simulations in which neighboring nodes
 215 were repeatedly stimulated after a time period τ_{IP_3} , that varied from 50 ms to 5s, while Ca²⁺ signals
 216 were recorded in a remote node, node 5 (Fig 5A). Neuronal stimulation is simulated as an infusion
 217 of 50 IP₃ molecules in the stimulated node. Representative Ca²⁺ traces in node 5 in branchlets with
 218

219 various shaft widths d_{shaft} are presented in Fig 5A for $\tau_{\text{IP3}}=250$ and 3000 ms. Our first notable result
220 is that the time to 1st peak in node 5 decreases with d_{shaft} , whatever the value of τ_{IP3} (Fig 5B1). More
221 specifically, time to 1st peak is higher for $d_{\text{shaft}}=d_0$ compared to both $d_{\text{shaft}}=\frac{d_0}{2}$ and $\frac{d_0}{3}$, while differ-
222 ences between $d_{\text{shaft}}=\frac{d_0}{2}$ and $\frac{d_0}{3}$ are not as striking. Moreover, the difference between $d_{\text{shaft}}=d_0$, $\frac{d_0}{2}$
223 and $\frac{d_0}{3}$ increases with τ_{IP3} . This suggests that geometries with $d_{\text{shaft}}=d_0$ better discriminate slow from
224 fast frequency of node stimulation compared to geometries with thinner shafts. Geometries with
225 $d_{\text{shaft}}=d_0$ are further characterized by a lower Ca^{2+} peak probability in node 5 compared to geome-
226 tries with $d_{\text{shaft}}=\frac{d_0}{2}$ and $\frac{d_0}{3}$ (Fig 5B2). More precisely, Ca^{2+} peak probability decreases as τ_{IP3} increases
227 for $d_{\text{shaft}}=d_0$, which was observed independently of our boundary conditions (Supplemental Fig 1,
228 see Methods). This suggests that geometries with larger shafts could be associated with decreased
229 signal propagation to remote nodes in case of repeated node stimulation at low frequency ($\tau_{\text{IP3}} >$
230 2s).

231

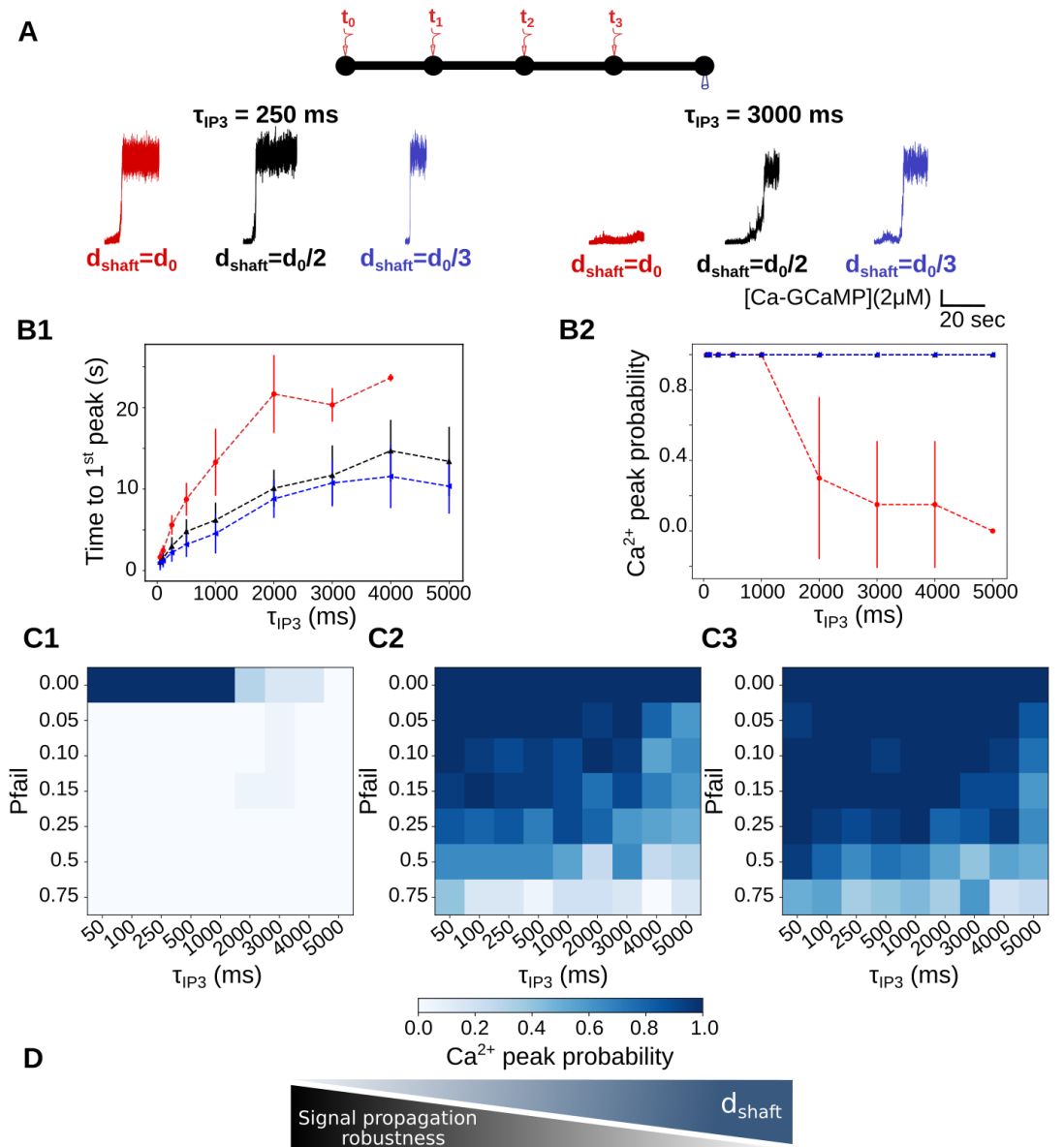


Figure 5. Thin shafts are associated with a more robust signal propagation upon repeated neuronal stimuli. (A) (Top) Neuronal stimulation protocol: node 1 is stimulated at $t=t_0=5s$, node 2 at $t_0 + \tau_{IP3}$, node 3 at $t_0 + 2\tau_{IP3}$ and node 4 at $t_0 + 3\tau_{IP3}$, $k_{Ca}=0 \text{ s}^{-1}$. Ca^{2+} activity is recorded in node 5. (Bottom) Representative Ca^{2+} traces in node 5 for shaft width $d_{shaft} = d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue), with $\tau_{IP3}=250\text{ms}$ (left) and 3000ms (right), expressed as SNR (see Methods). (B1) Time to 1st peak increases with τ_{IP3} for $d_{shaft} = d_0$ (***), $\frac{d_0}{2}$ (***) and $\frac{d_0}{3}$ (***). T-tests revealed that for any value of τ_{IP3} , time to 1st peak is higher for $d_{shaft} = d_0$ compared to $d_{shaft} = \frac{d_0}{2}$ and $\frac{d_0}{3}$. Time to 1st peak is significantly higher when $d_{shaft} = \frac{d_0}{2}$ compared to $d_{shaft} = \frac{d_0}{3}$, for most values of τ_{IP3} ($p=0.032^*$, 0.0025^{**} , 0.034^* , 0.016^* and 0.019^* for $\tau_{IP3}=250, 500, 1000, 4000$ and 5000 , respectively). (B2) Ca^{2+} peak probability in node 5 is lower for $d_{shaft} = d_0$ compared to $d_{shaft} = \frac{d_0}{2}$ and $\frac{d_0}{3}$. Ca^{2+} peak probability decreases as τ_{IP3} increases for $d_{shaft} = d_0$ (***). (C) Ca^{2+} peak probability in node 5 (colorbar) as a function of τ_{IP3} and of the probability of failure of node stimulation p_{fail} , for $d_{shaft} = d_0$ (C1), $d_{shaft} = \frac{d_0}{2}$ (C2) and $d_{shaft} = \frac{d_0}{3}$ (C3), with $p_{fail} \in [0, 1]$. (D) Schematic summarizing the main conclusion of this figure: decreased shaft width allows signal propagation despite omitted node stimulation, thus favoring more robust signal propagation. Data are represented as mean \pm STD, $n=20$ for each value of d_{shaft} and of τ_{IP3} . Lines in panel B are guides for the eyes. The effect of d_{shaft} on each Ca^{2+} signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

Figure 5-Figure supplement 1. Sensitivity study of the effect of boundary conditions on local Ca^{2+} activity upon repeated neuronal stimuli.

232 For $\tau_{IP3}=4s$ and $d_{shaft}=\frac{d_0}{3}$, signals were detected in node 5 11.55 ± 3.89 s after the stimulation of
233 node 1, which means that they occurred before the stimulation of node 4 ($t=t_0 + 12s$ for $\tau_{IP3}=4s$).
234 This phenomenon was not observed for $d_{shaft}=d_0$, for which time to 1st peak when $\tau_{IP3}=4s$ was 23.67
235 ± 0.47 s. This suggests that for $d_{shaft}=\frac{d_0}{3}$, contrary to $d_{shaft}=d_0$, one node stimulation could be omitted
236 without having any consequence on Ca^{2+} peak probability in node 5. In order to test this hypothesis,
237 we have performed simulations in which the stimulation of nodes 2, 3 and 4 occurred with a given
238 probability of failure p_{fail} . Simulations were performed for $p_{fail}=0, 0.05, 0.1, 0.15, 0.25$ and 0.75 . Ca^{2+}
239 peak probability in node 5, depending on p_{fail} and on τ_{IP3} is presented in Fig 5C, for $d_{shaft}=d_0$ (Fig 5C1),
240 $d_{shaft}=\frac{d_0}{2}$ (Fig 5C2) and $d_{shaft}=\frac{d_0}{3}$ (Fig 5C3). As expected, Ca^{2+} peak probability, despite high values of
241 p_{fail} , increases when d_{shaft} decreases. Thus, thin shafts can favor signal propagation by allowing the
242 omission of a node stimulation. In that sense, geometries displaying thin shafts are characterized
243 by a more robust signal propagation (Fig 5D).

244
245 Together, our results suggest that, in the context of repeated node stimulation, thin shafts are
246 associated with an increase of Ca^{2+} peak probability in more remote nodes, with an earlier sig-
247 nal onset, suggesting increased signal propagation. Astrocytic processes with thicker shafts (here
248 $d_{shaft}=d_0$), such as observed in hypo-osmotic conditions (**Arizono et al., 2021b**), are associated with
249 lower signal propagation in case of low stimulation frequency (time period $> 2s$), potentially fa-
250 voring the formation of local Ca^{2+} hotspots. Our results suggest that geometries with thick shafts
251 could impair signal propagation when a branchlet is stimulated at a low frequency. In that sense,
252 astrocyte branchlets with thicker shafts would be better detectors of the surrounding level of neu-
253 ronal activity. By contrast, branchlets with thin shafts would be less discriminating and provide
254 more robust signal propagation.

255 Discussion

256 Fine astrocytic processes are responsible for most astrocytic Ca^{2+} signals (**Bindocci et al., 2017**)
257 and are preferential sites of neuron-astrocyte communication (**Arizono et al., 2020**). A better un-
258 derstanding of the mechanistic link between their morphology and the spatio-temporal properties
259 of local Ca^{2+} signals is crucial, yet hard to test experimentally. Here, we perform reaction-diffusion
260 simulations in idealized morphologies of astrocytic processes derived from 3D super-resolution mi-
261 croscopy to investigate the effect of astrocyte nanoscale morphology on Ca^{2+} activity in the gliapil.
262 Our simulation results suggest that the morphology of processes, alternating between large and
263 thinner cellular compartments (**Panatier et al., 2014; Arizono et al., 2020; Grosche et al., 1999**), in-
264 creases Ca^{2+} peak probability, duration, amplitude and propagation. Conversely, astrocyte swelling
265 attenuates local Ca^{2+} activity and signal propagation (Fig 6). Our simulation results, in accordance
266 with experimental data, suggest that thin shafts are associated with a decreased diffusion flux, i.e
267 an increased compartmentalization of nodes. Thus, nodes, similarly to dendritic spines (**Santa-**
268 **maria et al., 2011**), act as diffusion traps when shaft width is low. Note that, more than the value
269 of shaft width itself, our results emphasize the effect of the ratio between node and shaft diameter
270 on Ca^{2+} activity. The geometries designed and used in this study emerge as interesting tools to in-
271 vestigate the influence of the ultrastructure of fine astrocyte branchlets on the local Ca^{2+} dynamics
272 in health and disease. By recording the mechanisms resulting in Ca^{2+} signals upon neuronal stim-
273 ulation in small cellular compartments of the gliapil, which cannot be performed experimentally,
274 our simulation results shed light on the mechanisms by which astrocyte swelling influences the
275 frequency, amplitude and propagation of Ca^{2+} signals at the nanoscale.

276

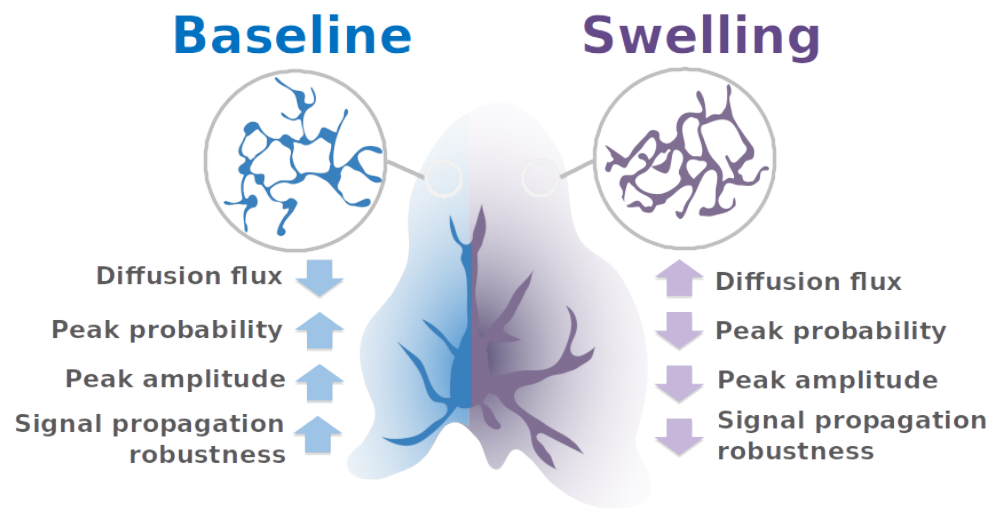


Figure 6. Proposed mechanisms that regulate astrocytic Ca^{2+} activity at tripartite synapses. Our simulation results demonstrate that the nano-morphology of astrocytic processes, consisting in the alternation of nodes and shafts, powerfully shapes the spatio-temporal properties of Ca^{2+} signals and promotes local Ca^{2+} activity. Astrocyte swelling, observed in pathological conditions such as brain injury, stroke and epilepsy, results in an increased shaft width without altering node size. Our results suggest that such pathological alterations of the nanoscale morphology of astrocytes result in a decreased local Ca^{2+} activity, which we confirm experimentally in hypo-osmotic conditions. Upon repeated neuronal stimuli, we predict that swelling hinders astrocytic signal propagation. Overall, this study highlights the impact of astrocyte nano-morphology on astrocyte activity at tripartite synapses, in health and disease.

277 Experimental Ca^{2+} recordings of astrocyte activity have established that astrocyte processes display both highly localized microdomain signals and propagating Ca^{2+} waves (*Srinivasan et al., 2015;*
 278 *Bindocci et al., 2017*). Our simulations suggest that the morphology of the cell and of its organelles
 279 can strongly influence the formation of these patterns of astrocytic Ca^{2+} signaling. Notably, thinner
 280 shafts allow less discriminating and more robust signal propagation upon repeated stimuli compared
 281 to larger shafts. Thinner shafts indeed allow signal propagation even in the absence of some
 282 stimuli or when the time interval between repeated stimuli is large. On the contrary, geometries
 283 with thick shafts seem to be more discriminating, potentially favoring the propagation of signals
 284 resulting from repeated stimuli from co-active synapses. Cellular morphology thus emerges as
 285 a key parameter that regulates the active propagation of Ca^{2+} signals. The ultrastructure of the
 286 spongiform domain of astrocytes is very complex, characterized by abundant branching points,
 287 conferring a reticular morphology (*Arizono et al., 2020*). Those branching points are reportedly
 288 sometimes arranged into ring-like structures, although their occurrence and shape are still debated
 289 and could differ depending on the brain region under study (*Arizono et al., 2020; Panatier*
 290 *et al., 2014; Kiyoshi et al., 2020; Salmon et al., 2021; Arizono and Nägerl, 2021*). The effect of this
 291 reticular ultrastructure on the propagation of Ca^{2+} signals remains to be uncovered. Further characterization
 292 of the shape of fine astrocytic processes of the spongiform domain, their variability
 293 as well as their connectivity to the neighboring synapses are thus required. Pairing those observations
 294 with biophysically-detailed models such as the one presented in this study stands to deepen
 295 our understanding of the roles of astrocytic and neuronal morphology at tripartite synapses on
 296 neuron-astrocyte communication.
 297

298
 299 In neurons, both experimental (*Yuste et al., 2000; Noguchi et al., 2005; Tønnesen et al., 2014*)
 300 and modelling (*Schmidt and Eilers, 2009; Biess et al., 2007; Simon et al., 2014; Bell et al., 2019; Hol-*
 301 *cman and Schuss, 2005, 2011; Santamaria et al., 2011; Cugno et al., 2019*) studies have suggested
 302 that thin spine necks favor the compartmentalization of Ca^{2+} signals within the spine head. This
 303 compartmentalization of synapses allows neurons to discriminate various inputs and to process

304 information locally (*Wybo et al., 2019; Poirazi and Papoutsis, 2020*), increasing the computational
305 power of the neuronal circuits. According to our simulation results, nodes connected to thin shafts
306 could favor the emergence of large signals at the site of neuron-astrocyte communication. Interest-
307 ingly, we further propose that those amplified signals in nodes, instead of resulting in Ca^{2+} hotspots,
308 favor active signal propagation. In response to spike timing-dependent plasticity (STDP) protocols
309 (*Araya et al., 2014*) and after long-term potentiation (*Tønnesen et al., 2014*), the neck of dendritic
310 spines become wider and shorter. Recent *in vitro* and *in vivo* studies have reported that the geom-
311 etry of the ER in neurons is also highly dynamic (*Kucharz et al., 2013*), experiencing fast fission and
312 fusion events. Further, the ER is variably distributed in spines, being preferentially present in large
313 spines associated with strong synapses (*Holbro et al., 2009*). The geometries of dendrites and of
314 their organelles, which influence local signals, are thus highly dynamic. Similarly, fine astrocytic
315 processes encounter morphological rearrangements that are activity-dependent, which notably
316 influence synaptic maturation, efficacy and spine stability (*Theodosis et al., 2008; Zhou et al., 2019;*
317 *Henneberger et al., 2020*). Our study sheds light on the influence of rearrangements of the reticular
318 morphology of fine processes on signal computation by astrocytes. Further investigation manipu-
319 lating astrocyte morphology *in situ* as well as *in vivo* is required to better characterize the variability
320 of astrocyte ultrastructure and the associated integration of Ca^{2+} signals.

321
322 The morphology of the complex spongiform domain of astrocytes is highly dynamic, subject to
323 activity-dependent as well as pathological remodeling. Our study, providing mechanisms by which
324 an altered astrocyte morphology influences neuron-astrocyte communication at the nanoscale,
325 gives new insights into the involvement of astrocytes in brain function in health and disease.

326 **Methods and Materials**

327 **Stochastic spatially-explicit voxel-based simulations**

328 In order to model astrocyte Ca^{2+} signals in astrocyte branchlets, we have used the voxel-based
329 “GCaMP” implementation of the Inositol 3-Phosphate (IP_3) receptor-dependent Ca^{2+} signaling model
330 from Denizot et al (*Denizot et al., 2019a*), using the same reaction scheme and parameter values
331 (Fig 1B). Briefly, we model Ca^{2+} fluxes in and out of the cytosol, mediated by Ca^{2+} channels and
332 pumps on the endoplasmic reticulum (ER) and on the plasma membrane. Ca^{2+} signals occur when
333 some IP_3 R channels are in the open state. IP_3 can be synthesized by the Ca^{2+} -dependent activ-
334 ity of phospholipase C δ ($\text{PLC}\delta$) and the removal of IP_3 molecules from the cytosol is expressed
335 as a single decay rate. IP_3 R kinetics is described by a Markov model, derived from De Young &
336 Keizer’s model (*De Young and Keizer, 1992*). Each IP_3 R molecule contains one IP_3 binding site and
337 two Ca^{2+} binding sites. An IP_3 R is in the open state when in state {110} (first Ca site and IP_3 bound,
338 second Ca site free). Depending on the simulation, other diffusing molecules were added to the
339 model, such as the fluorescent molecule ZsGreen and fluorescent Ca^{2+} indicators, here 10 μM of
340 GCaMP6s. GCaMPs are genetically-encoded Ca^{2+} indicators (GECIs) that are derived from the flu-
341 orescent protein GFP and the Ca^{2+} buffer calmodulin (see (*Shigetomi et al., 2016*) for a review on
342 GECIs). For further details on the kinetic scheme and model assumptions, please refer to Denizot
343 et al. 2019 (*Denizot et al., 2019a*).

344
345 The model was implemented using STEPS (<http://steps.sourceforge.net/>), a python package per-
346 forming exact stochastic simulation of reaction-diffusion systems (*Hepburn et al., 2012*). More
347 precisely, STEPS uses a spatialized implementation of Gillespie’s SSA algorithm (*Gillespie, 1977;*
348 *Isaacson and Isaacson, 2009; Smith and Grima, 2018*). Simulations in STEPS can be performed in
349 complex geometries in 3 spatial dimensions. Space is divided into well-mixed tetrahedral compart-
350 ments, referred to as voxels. Reactions between 2 molecules can only occur if they are located
351 within the same voxel. Diffusion events are modeled as a decrease of the number of molecules in
352 the original voxel and an increase in the number of molecules in its neighboring voxel. Boundary

353 conditions, except when specified otherwise, were reflective. In other words, mobile molecules
354 could not diffuse away from the geometry, as if they were “bouncing” onto the plasma membrane.
355 STEPS enables to compute, in complex 3D geometries, reactions and diffusion in the cytosol as well
356 as reactions between cytosolic molecules and molecules located at the plasma or ER membrane.

357

358 Geometries

359 Typical astrocyte branchlet geometries were designed from their recent experimental charac-
360 terization in live tissue at high spatial resolution (50 nm in x-y) (*Arizono et al., 2020*). Those geome-
361 tries consist in alternations of bulbous structures, nodes, connected to each other with cylindrical
362 structures, shafts. Geometries with different shaft widths d_{shaft} were designed using Trelis software
363 (<https://www.csimsoft.com/trelis>, Fig 1A). The geometry of a node was approximated as being a
364 sphere of diameter 380 nm. Shaft geometry consisted in a 1 μm long cylinder. Shaft diameter was
365 defined relative to node diameter. For example, shaft diameter was the same as node diameter, i.e
366 $d_{\text{shaft}} = d_0 = 380$ nm. Similarly, shaft diameter was 190 nm and 127 nm for $d_{\text{shaft}} = \frac{d_0}{2}$ and $\frac{d_0}{3}$, respectively.
367 Cones were positioned between spheres and cylinders in order to create a smoother transition be-
368 tween nodes and shafts, better approximating the geometry observed experimentally. Cytosolic
369 volume was thus $V_1 = 0.620 \mu\text{m}^3$, $V_2 = 0.263 \mu\text{m}^3$ and $V_3 = 0.195 \mu\text{m}^3$, for $d_{\text{shaft}} = d_0$, $\frac{d_0}{2}$ and $\frac{d_0}{3}$, respectively.
370 A subset of simulations were performed in a geometry with $V_1 = 0.258 \mu\text{m}^3$. This geometry is charac-
371 terized, similarly to geometries with $d_{\text{shaft}} = d_0$, by a node/shaft width ratio of 1. It contains cylinders
372 of length 750 nm, diameter 285 nm and spheres of diameter 285 nm. As a first approximation,
373 ER geometry was considered to be similar to the geometry of the astrocyte branchlet: node/shaft
374 successions. ER nodes were aligned with cytosolic nodes. As no quantification of the ER volume
375 compared to cellular volume was found for astrocytes in the literature, ER volume was 10% of the
376 total branchlet volume, based on available data in neurons (*Spacek and Harris, 1997*). The cytosolic
377 volume, plasma and ER membrane areas of the different “5Nodes” geometries are presented in
378 Table 1.

379

380 A sensitivity study was performed to investigate the effect of voxel size on the kinetics of the
381 molecular interactions modeled. Information on the voxel sizes of the different meshes used is
382 presented in Supplemental Table 1. Results are presented in Supplemental Fig 1. Meshes that
383 contained voxels that were $< 50 \text{ nm}^3$ were characterized by aberrant kinetics, resulting in aberrant
384 average numbers of molecules in a given state. Those results thus suggest that to prevent errors
385 due to voxel size, meshes should not display voxel sizes that are $< 50 \text{ nm}^3$. We have thus made
386 sure, while meshing the geometries in which simulations were ran, that no voxels were $< 50 \text{ nm}^3$.
387 Minimum voxel size was 443 nm^3 , 1100 nm^3 and 447 nm^3 , for $d_{\text{shaft}} = d_0$, $\frac{d_0}{2}$ and $\frac{d_0}{3}$ geometries, re-
388 spectively.

389

390 In a subset of simulations, ER geometry varied. The shape of the cell was the same as in “5nodes”
391 geometries (Fig 1). ER geometry consisting of node/shaft alternations, described above, is referred
392 to as “Node/shaft ER”. “No ER” geometry contains no ER. “Node ER” is characterized by a discon-
393 tinuous ER geometry, consisting in spheres of diameter 54 nm, located in cellular nodes. “Cyl ER”
394 corresponds to a cylindrical ER, of length $l_{\text{ER}} = 6274$ nm and a diameter of 108, 54 and 36 nm, for
395 $d_{\text{shaft}} = d_0$, $\frac{d_0}{2}$ and $\frac{d_0}{3}$, respectively. The associated cytosolic volume, ER and plasma membrane area
396 are presented in Table 1.

397 Protocol for simulating bleaching experiments

398 In order to test whether the idealized geometries presented in Fig 1 are a good approximation
399 of the spongiform ultrastructure of astrocyte branchlets, we have simulated their fluorescence
400 recovery after photobleaching (FRAP) experiments. Briefly, laser pulses are simulated on a node
401 (region of interest) while the fluorescence level is being recorded. At bleaching time, the fluores-

402 cence level in the region of interest decreases to I_0 . Then, because of the diffusion of fluorescent
 403 molecules into the region of interest, fluorescence increases until it reaches a new steady state,
 404 I_{inf} . We characterize node compartmentalization by measuring the time τ taken by fluorescing
 405 molecules to diffuse into the node to reach I_{inf} . In other words, a high node compartmentalization
 406 will be associated with a high value of τ . Thus, 3 main parameters characterize bleaching traces:
 407 I_0 , τ and I_{inf} .

408
 409 To mimic bleaching experiments in fine branchlets performed by Arizono et al (Arizono et al.,
 410 2020), ZsGreen molecules were added to simulation space. After 2 seconds of simulation, providing
 411 the basal level of fluorescence, 60% of ZsGreen molecules were bleached. In order to fit I_0 and I_{inf}
 412 that were measured experimentally, and as bleaching time lasted 10 ms in experiments and 1
 413 ms in simulations, the bleached volume in simulations was adjusted depending on the geometry
 414 (see Fig 2A). Bleaching was simulated as a transition from ZsGreen molecules to ZsGreen-bleached
 415 molecules, the latter being considered as non-fluorescing molecules. Screenshots of simulations,
 416 illustrating the diffusion of ZsGreen and ZsGreen-bleached molecules, are presented in Fig S2B.
 417 The number of ZsGreen molecules in the central node was recorded over simulation time and a fit
 418 was performed following equation 1 to determine the values of I_0 , I_{inf} and τ .

$$I(t) = I_0 - (I_0 - I_{inf})e^{-t/\tau} \quad (1)$$

419 , where $I(t)$ is the level of fluorescence measured at time t . The coefficient of diffusion, $D_{ZsGreen}$, and
 420 the concentration, $[ZsGreen]$, of ZsGreen were adjusted to fit experimental data. Indeed, the am-
 421 plitude of $[ZsGreen]$ fluctuations at steady state is inversely proportional to the number of ZsGreen
 422 molecules in the geometry. In other words, fluorescence signals are more noisy when $[ZsGreen]$
 423 is low. Moreover, the autocorrelation of those fluctuations depends on the coefficient of diffusion
 424 of ZsGreen, $D_{ZsGreen}$. If $D_{ZsGreen}$ increases, the autocorrelation of Lag, where Lag is the autocorrela-
 425 tion delay, will decrease faster as Lag increases. Comparing the fluctuations of $[ZsGreen]$ and its
 426 autocorrelation in experiments and in simulations thus enabled to find the values of $D_{ZsGreen}$ and
 427 of $[ZsGreen]$ that allowed for the best fit to experimental data. In the simulations presented here,
 428 $D_{ZsGreen} = 90 \mu m^2 \cdot s^{-1}$ and $[ZsGreen] = 25 \mu M$.

429 **Protocols for simulating neuronal stimulation**

430 In order to investigate the propagation of Ca^{2+} signals from nodes that contact neuronal spines,
 431 we have developed 2 different protocols for our simulations, performed in the geometries pre-
 432 sented in Fig 1. As nodes were the site of Ca^{2+} signal initiation (Arizono et al., 2020) and as most
 433 spines contacted nodes rather than shafts, we have simulated neuronal stimulation in nodes. To
 434 simulate neuronal stimulation, IP_3 and Ca^{2+} were infused in the cytosol at stimulation time. IP_3
 435 infusion reflects the production of IP_3 by phospholipase C that results from the activation of G_q -G-
 436 protein-coupled receptors (GPCRs). Ca^{2+} infusion mimics the influx of Ca^{2+} in the cytosol through
 437 Ca^{2+} channels at the plasma membrane. The rate of this neuronal activity-induced Ca^{2+} influx, k_{Ca} ,
 438 varied within a physiological range of values, from 0 to $1000 s^{-1}$ (Wu et al., 2018; Brazhe et al., 2018).
 439 Signals were recorded both in the stimulated node, Node 1, and in the neighboring node, Node 2.

- 440 • In the first protocol, 100 IP_3 molecules were infused in Node 1, at $t=t_0=1s$, while Ca^{2+} activity
 441 was monitored in Node 1 and in the neighboring node, Node 2 (see e.g Fig 3A). Neuronal
 442 activity-induced Ca^{2+} influx was mediated by generic Ca^{2+} channels at the plasma membrane.
 443 25 of those Ca^{2+} channels were placed on the plasma membrane of Node 1, corresponding
 444 to a similar density to the IP_3R density on the ER membrane, and were set to an inactive state.
 445 At stimulation time $t=t_0$, Ca^{2+} channels were set to an active state, resulting in an influx of Ca^{2+}
 446 within the cytosol at rate k_{Ca} . At $t=t_0 + 1$, Ca^{2+} channels were set back to their initial inactive
 447 state. Simulations were performed in geometries with varying shaft width d_{shaft} .

448 • In the second protocol, we have investigated signal propagation in the node/shaft geometry
449 depending on shaft width d_{shaft} when several nodes were successively stimulated. In “5nodes”
450 geometries, 50 IP₃ molecules were infused at $t_0=5s$, $t_0 + \tau_{\text{IP}_3}$, $t_0 + 2\tau_{\text{IP}_3}$, $t_0 + 3\tau_{\text{IP}_3}$ in Nodes 1, 2,
451 3 and 4, respectively. During the whole simulation time, Ca²⁺ activity was recorded in Node
452 5 (see Fig 5). In a subset of simulations, stimulation of Nodes 2, 3 and 4 occurred with a
453 probability $1 - p_{\text{fail}}$, with $p_{\text{fail}} \in [0, 1]$.

454 **Peak detection and analysis**

455 The same strategy as developed by Denizot et al. (*Denizot et al., 2019a*) was used for detect-
456 ing and analysing Ca²⁺ signals. Briefly, basal concentration of Ca²⁺, $[Ca]_b$, was defined based on a
457 histogram of the number of Ca²⁺ ions in the absence of neuronal stimulation. Peak initiation cor-
458 responded to the time when $[Ca^{2+}]$ was higher than the following threshold: $[Ca]_b + n\sigma_{Ca}$, where σ_{Ca}
459 is the standard deviation of $[Ca^{2+}]$ histogram in the absence of neuronal stimulation. The value of
460 n was set by hand depending on signal/noise ratio of the simulation of interest. Peak termination
461 corresponded to the time when $[Ca^{2+}]$ decreased below the peak threshold.

462 Several parameters were analyzed to characterize Ca²⁺ signals. Peak amplitude, A , corresponds to
463 the maximum $[Ca^{2+}]$ measured during the peak duration. It is expressed as signal to noise ratio
464 $SNR = \frac{A - [Ca]_b}{[Ca]_b}$. Peak duration corresponds to the time between peak initiation and peak termi-
465 nation. Time to 1st peak corresponds to the delay between the beginning of the simulation and
466 the first peak detection, measured in the cellular compartment of interest. Peak probability corre-
467 sponds to the fraction of simulations in which at least one peak was detected during simulation
468 time in the region of interest. Ca²⁺ residency time was measured by performing $n=300$ simulations
469 for each value of d_{shaft} , in which only 1 Ca²⁺ ion was added to node 1, without other molecular
470 species. Ca²⁺ residency time corresponds to the time taken for the ion to diffuse away from node
471 1.

472 **Organotypic hippocampal slice cultures**

473 All experiments were performed as described in (*Arizono et al., 2020*) and were in accordance
474 with the European Union and CNRS UMR5297 institutional guidelines for the care and use of lab-
475 oratory animals (Council directive 2010/63/EU). Organotypic hippocampal slices (Gähwiler type)
476 were dissected from 5–7-d-old wild-type mice and cultured 5–8 week in a roller drum at 35°C, as
477 previously described (*Gähwiler, 1981*).

478 **Viral infection**

479 AAV9-GFAP-GCaMP6s (*Stobart et al., 2018b*) was injected by brief pressure pulses (40ms; 15
480 psi) into the stratum radiatum of 2-3-week old slices from Thy1-YFP-H (JAX:003782) mice 4-6 weeks
481 prior to the experiment.

482 **Image acquisition**

483 For Ca²⁺ imaging, we used a custom-built setup based on an inverted microscope body (Leica
484 DMI6000), as previously described in (*Tønnesen et al., 2011*). We used a 1.3 NA glycerol immersion
485 objective equipped with a correction collar to reduce spherical aberrations and thereby allow imag-
486 ing deeper inside brain tissue (*Urban et al., 2011*). The excitation light was provided by a pulsed
487 diode laser ($\lambda = 485$ nm, PicoQuant, Berlin, Germany). The fluorescence signal was confocally de-
488 tected by an avalanche photodiode (APD; SPCM-AQRH-14-FC; PerkinElmer). The spatial resolution
489 of the setup was around 175 nm (in x-y) and 450 nm (z). Confocal time-lapse imaging (12.5 x 25
490 μm , pixel size 100 nm) was performed at 2Hz for 2.5 min in artificial cerebrospinal fluid containing
491 125 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl₂, 2 mM CaCl₂, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 20 mM
492 D-glucose, 1 mM Trolox; 300 mOsm; pH 7.4. Perfusion rate was 2 mL/min and the temperature 32

493 °C. Hypo-osmotic stress (300 mOsm to 200 mOsm) was applied by perfusing ACSF with reduced
494 NaCl concentration (119 to 69 mM NaCl).

495 **Quantification and statistical analysis**

496 For each parameter set, 20 simulations, with different seeds, were generated. Each parameter
497 describing Ca²⁺ dynamics was expressed as mean ± standard deviation. The effect of d_{shaft} on each
498 Ca²⁺ signal characteristic was tested using one-way ANOVA. Comparison between two different
499 conditions was performed using unpaired Student T-test if values followed a Gaussian distribution,
500 Mann-Whitney test otherwise. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for
501 $p \leq 0.001$.

502 **Simulation code**

503 The simulation code, implemented with STEPS 3.5.0, and the meshes are available on ModelDB
504 (*McDougal et al., 2017*) at <http://modeldb.yale.edu/266928>, access code: Ito42@tpk3D?. The origi-
505 nal model from Denizot et al. (*Denizot et al., 2019a*) is available at <http://modeldb.yale.edu/247694>.

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Table 2. Characteristics of meshes used to investigate the effect of voxel size on Ca^{2+} signals. Cylinder geometries were generated with various total number of tetrahedra (geometry Cyl_i with $i \in [1, 8]$, see also Fig 1A), resulting in different voxel sizes. V_{mean} corresponds to the average voxel volume, V_{std} to its standard deviation, V_{max} to the maximum voxel volume and V_{min} to the minimum voxel volume in the mesh. Volumes are expressed in nm^3 . Diff (nm^3) corresponds to $V_{\text{max}} - V_{\text{min}}$ while Diff (%) is the ratio $\frac{V_{\text{max}} - V_{\text{min}}}{V_{\text{mean}}} * 100$

Geom	# tet	V_{mean}	V_{std}	V_{max}	V_{min}	Diff (nm^3)	Diff (%)
Cyl_1	372148	84	25	296	15	281	335
Cyl_2	274233	115	34	389	20	369	321
Cyl_3	125927	249	71	761	33	728	292
Cyl_4	40077	782	229	2200	67	2133	273
Cyl_5	11523	2700	790	7521	177	7344	272
Cyl_6	3103	9900	3200	24000	94	23906	241
Cyl_7	1417	21000	11000	62000	241	61759	294
Cyl_8	945	32000	28000	145000	241	144759	452

729 Supplemental Movie 1

730 Movie presenting a simulation of the bleaching protocol, with $d_{\text{shaft}} = \frac{d_0}{2}$. Fluorescing ZsGreen
731 molecules are in red and bleached, non-fluorescing, ZsGreen are in yellow. Molecule position
732 was updated every 1 ms. The plot presents the variation of the number of fluorescing ZsGreen
733 molecules with time in the central node. Note that, to facilitate visualization, here $[\text{ZsGreen}] = 5$
734 μM . Before the bleaching event, all ZsGreen proteins (red) fluoresce. At bleaching time, fluorescing
735 molecules in the bleached region encounter conformational modifications that prevent them from
736 fluorescing (yellow molecules). Consequently, the fluorescence level drops to I_0 . Because of the
737 diffusion of molecules in the cell, the fluorescence level in the central node then increases until it
738 reaches a new baseline I_{inf} , after a recovery time τ . Fluorescing ZsGreen molecules are in red and
739 bleached, non-fluorescing, ZsGreen are in yellow.

740 Supplemental Movie 2

741 Movie presenting a simulation of the neuronal stimulation protocol, with $d_{\text{shaft}} = \frac{d_0}{2}$ and Ca^{2+} influx
742 rate at the plasma membrane $k_{\text{Ca}} = 0 \text{ s}^{-1}$. 100 IP_3 molecules are injected in Node 1 at $t_0 = 0.1 \text{ s}$, while
743 the number of Ca-GCaMP, IP_3 and IP_3R channels in the open state are monitored in nodes 1 and
744 2. Molecule position was updated every 0.1 ms. Ca-GCaMP molecules are in yellow, IP_3 in red and
745 IP_3R in blue. Note that the size of molecules in the movie was increased to facilitate visualization.
746 Speed was increased 5-fold for visualization purposes.

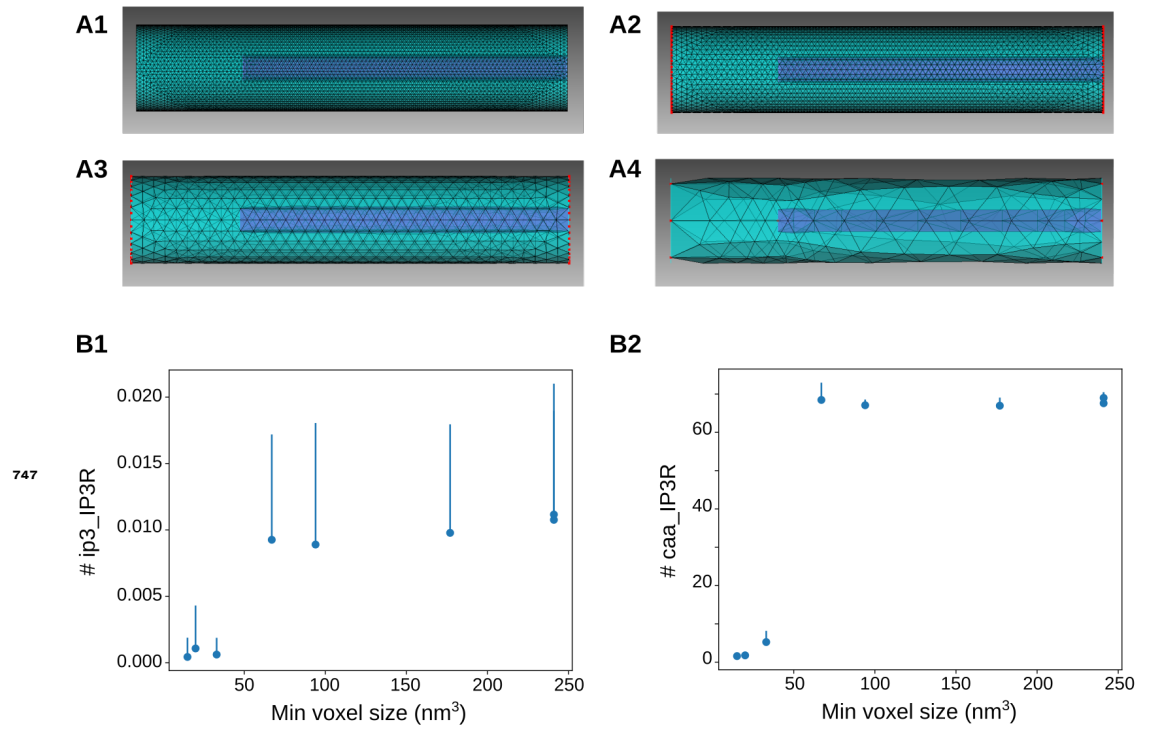
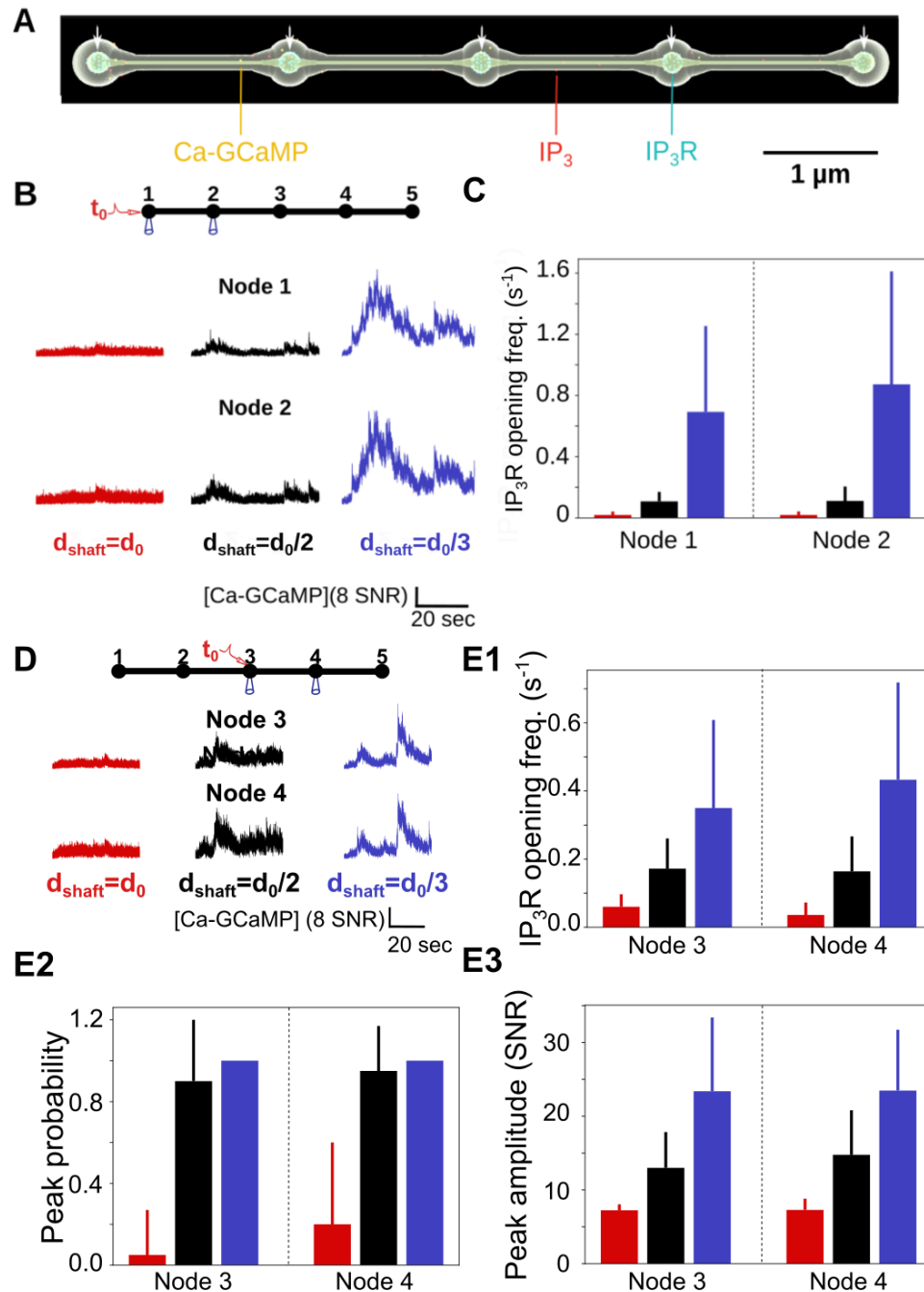


Figure 1-Figure supplement 1. Sensitivity study of the effect of voxel size on Ca^{2+} signals. (A) Simulations were performed in a cylinder geometry with various numbers of tetrahedra. 4 examples are presented here, "*Cyl*₁" (A1), "*Cyl*₃" (A2), "*Cyl*₅" (A3) and "*Cyl*₈" (A4). Please refer to Table 2 for details on the characteristics of each mesh. (B) Study of the average number of IP₃R in the ip3_IP3R state (IP₃ bound to its binding site on IP₃R, B1) and in the caa_IP3R state (Ca²⁺ bound to the activating site of IP₃R, B2) depending on the minimum voxel size of the mesh.



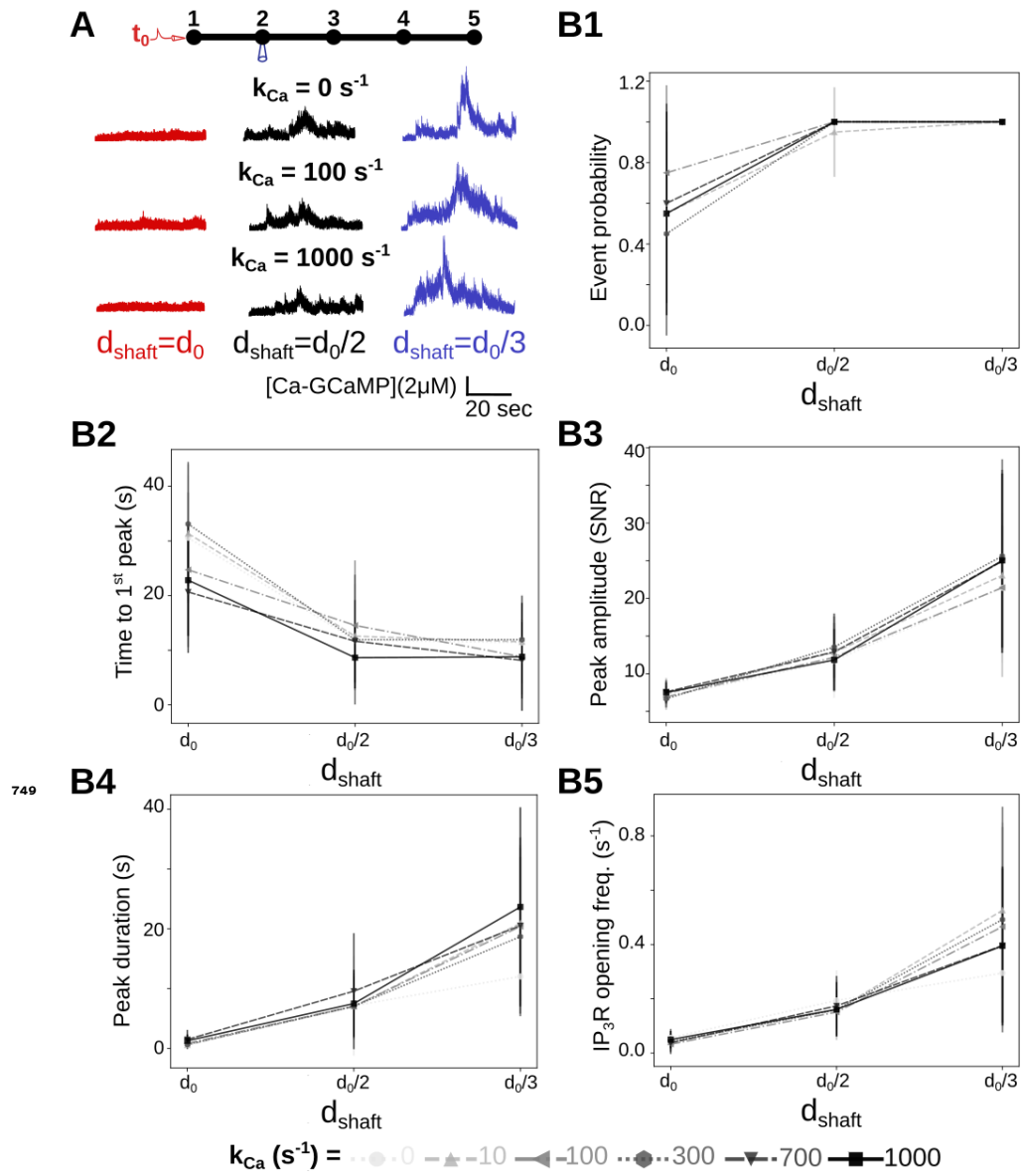
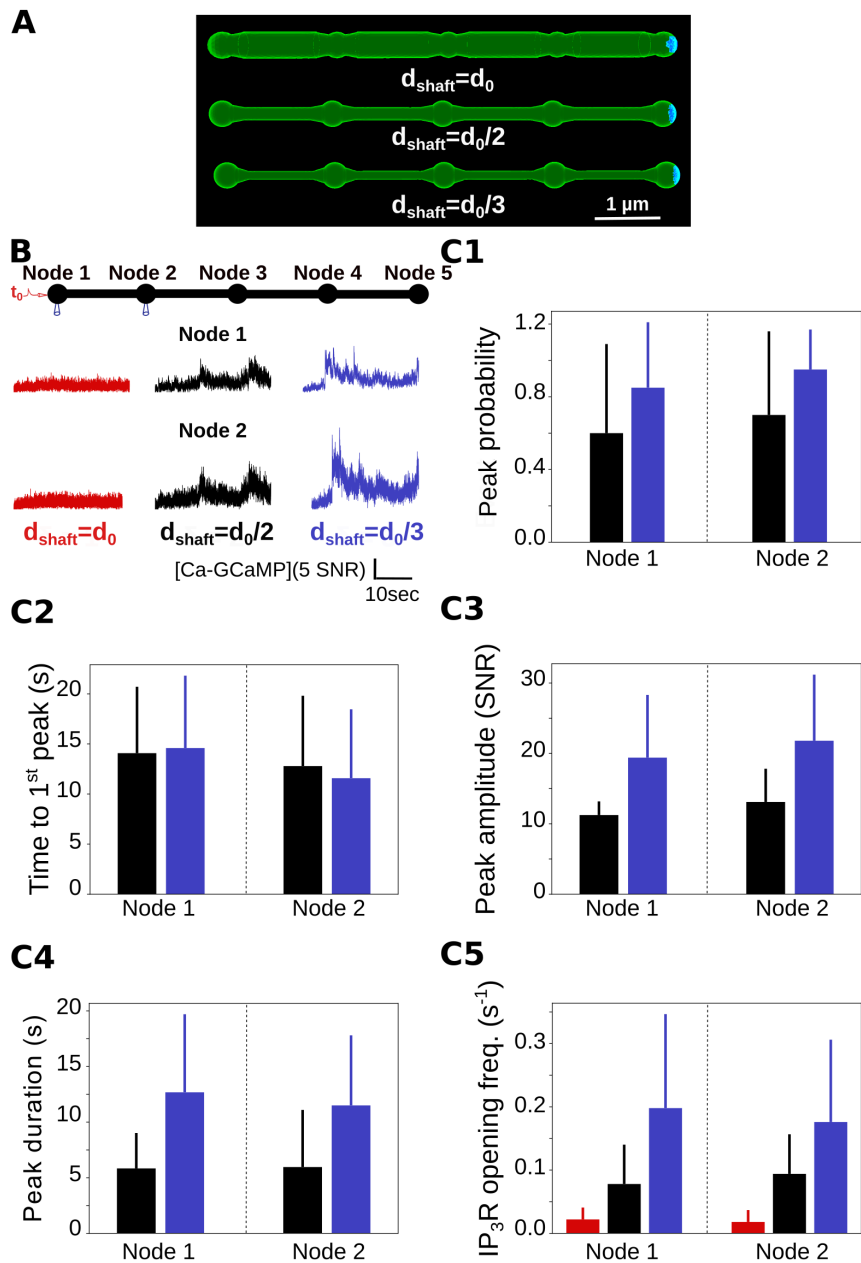


Figure 3-Figure supplement 2. Effect of Ca^{2+} influx rate at the plasma membrane on local Ca^{2+} signals. A) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1\text{s}$, while Ca^{2+} activity was monitored in node 2. Representative Ca^{2+} traces for shaft width $d_{shaft} = d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue), with a Ca^{2+} influx rate at the plasma membrane $k_{Ca}=0$ (top), 100 (middle) and 1000 s^{-1} (bottom), expressed as SNR (see Methods). (B) Quantification of the effect of d_{shaft} on Ca^{2+} signal characteristics for $k_{Ca}=0, 10, 100, 300, 700$ and 1000 s^{-1} . Data are represented as mean \pm STD, $n=20$ for each set of parameters tested. Ca^{2+} peak probability increases (***, B1), Time to 1st peak decreases (***, B2), peak amplitude (***, B3) and duration (***, B4) increase when d_{shaft} decreases, for $k_{Ca}=0, 10, 100, 300, 700$ and 1000 s^{-1} . Ca^{2+} peak characteristics did not statistically vary with k_{Ca} . The effect of k_{Ca} on each Ca^{2+} signal characteristic was tested using one-way ANOVA.



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Figure 3-Figure supplement 3. Sensitivity study of the effect of boundary conditions on local Ca²⁺ activity upon single node stimulation. (A) Geometries used in the simulations were the “5nodes” geometries (Fig 1A). Boundary conditions were absorbing at the extremity of node 5 (blue), while the remaining boundaries were reflective (green). This mimics the connection of the modeled astrocyte branchlet to the rest of the cell. (B) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1\text{s}$ ($k_{Ca}=0\text{ s}^{-1}$), while Ca²⁺ activity was monitored in nodes 1 and 2. (Bottom) Representative [Ca-GCaMP] traces in nodes 1 and 2, expressed as SNR (see Methods), for $d_{\text{shaft}}=d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue). (C1) Ca²⁺ peak probability increases when d_{shaft} decreases in nodes 1 (***) and 2 (**). (C2) Time to 1st peak does not change with d_{shaft} , both in node 1 (p-value=0.85) and 2 (p-value=0.90). (C3) Peak amplitude increases when d_{shaft} decreases in nodes 1 (p-value=0.0056 **) and 2 (p-value=0.0021 **). (C4) Peak duration increases when d_{shaft} decreases in nodes 1 (p-value=0.0051 **) and 2 (p-value=0.014 *). (C5) The frequency of IP₃R opening increases when d_{shaft} decreases in both nodes 1 and 2 (**). Note that there were no Ca²⁺ peak in simulations with $d_{\text{shaft}}=d_0$. Ca²⁺ peak characteristics were not statistically different between node 1 and 2. Data are represented as mean \pm STD, n=20 for each geometry. The effect of d_{shaft} on each Ca²⁺ signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

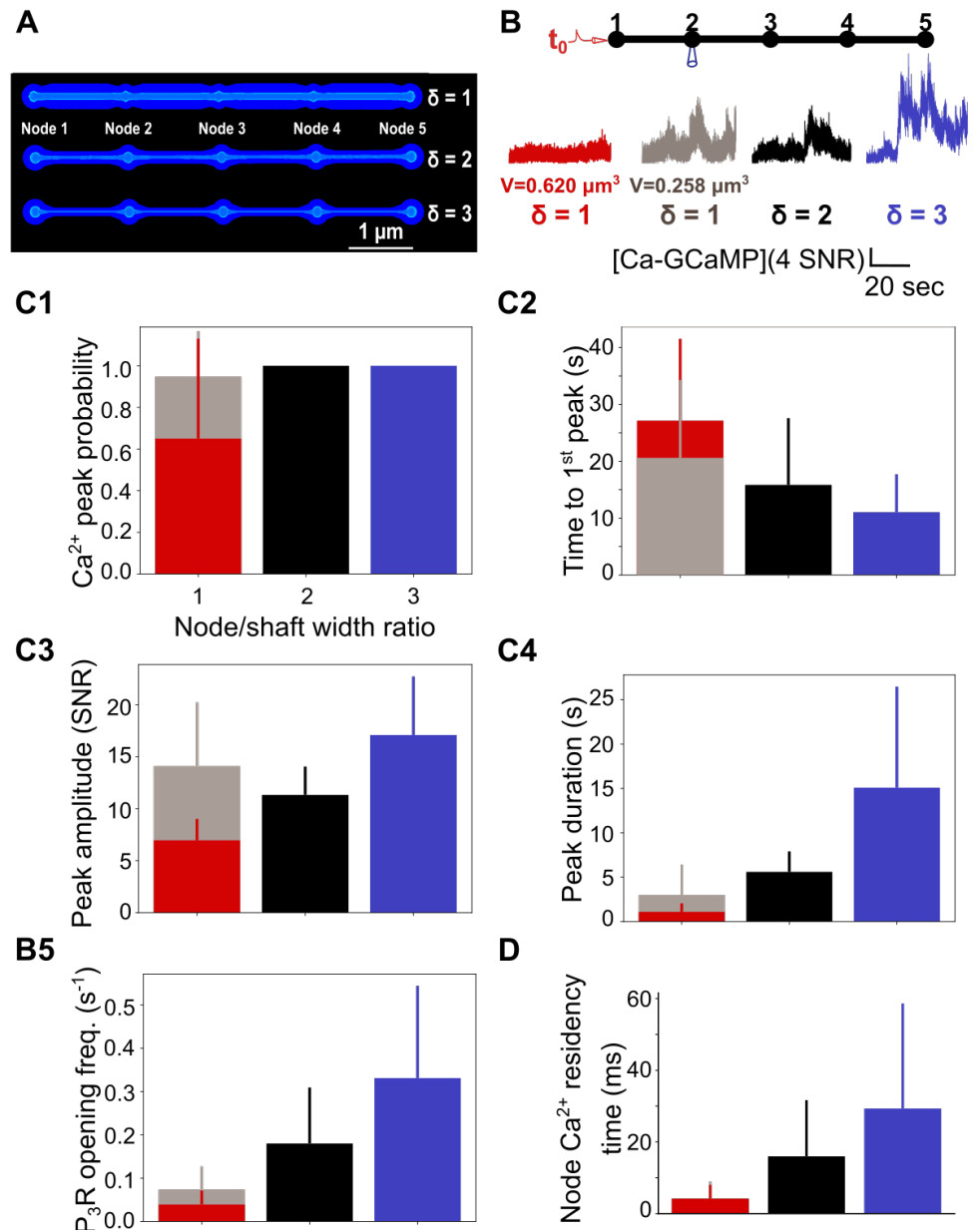
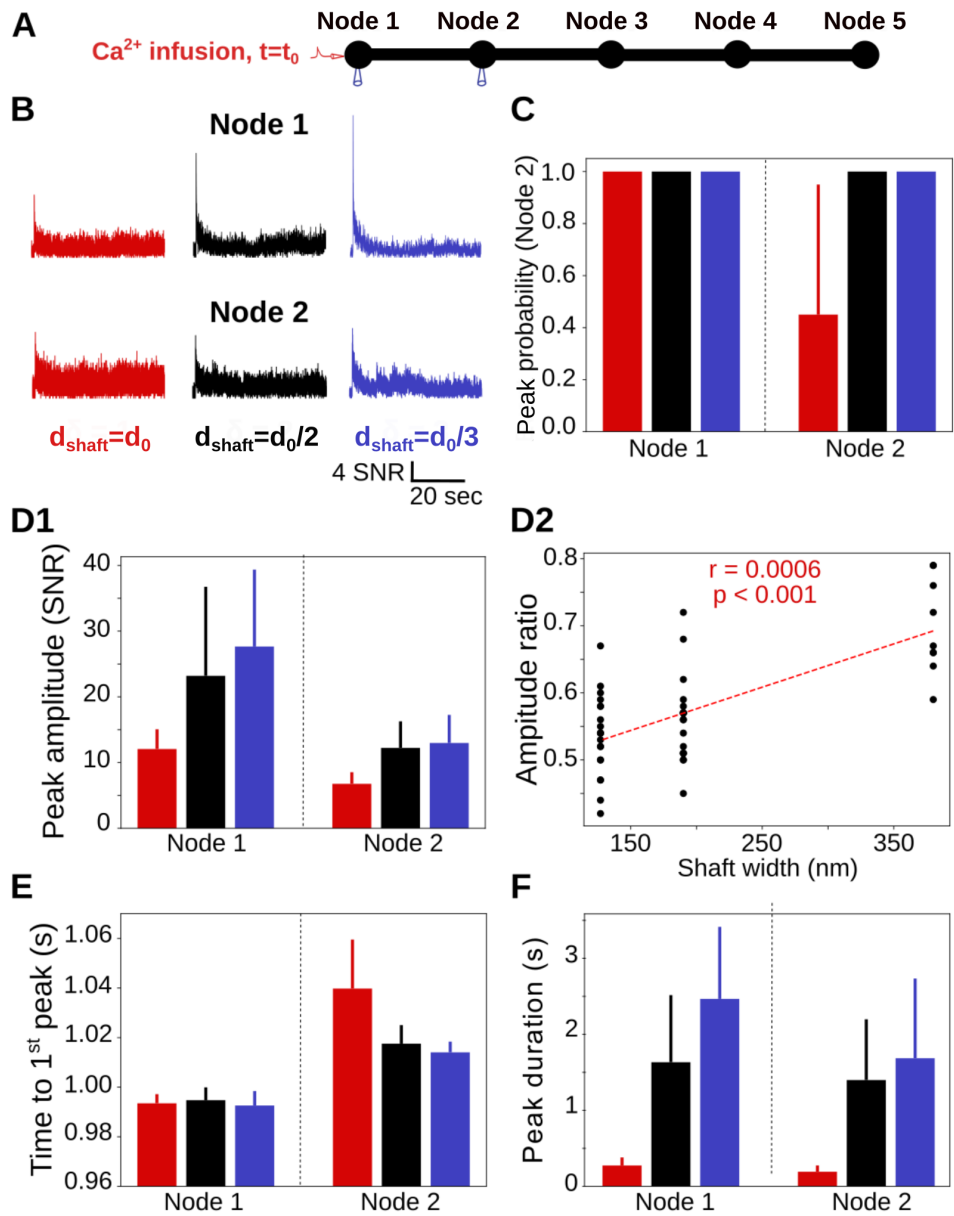
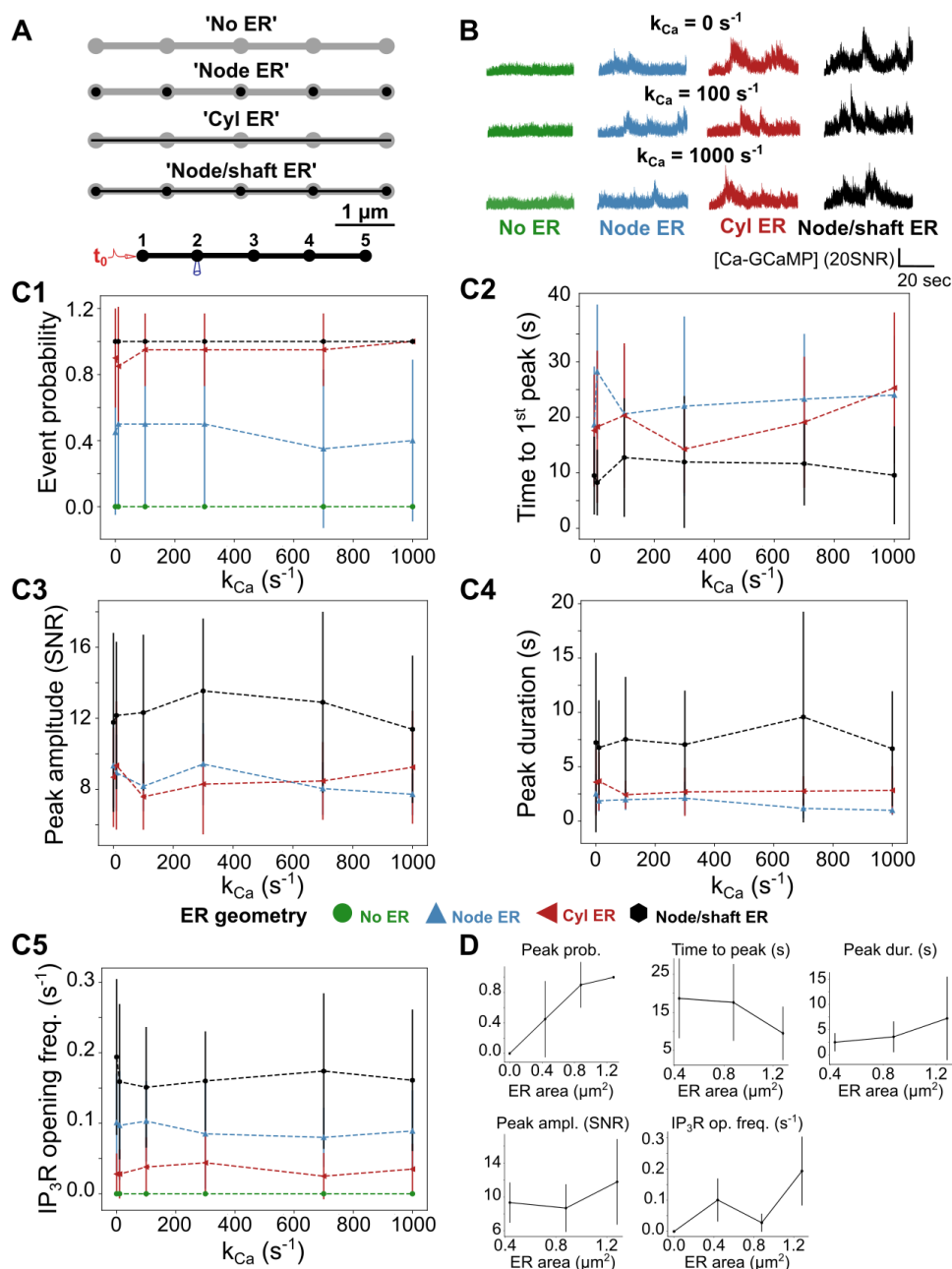


Figure 3-Figure supplement 4. Node/shaft width ratio and node volume control local Ca^{2+} activity. (A) Screenshots from STEPS visualization toolkit revealing meshes used in this study, that contain 5 identical nodes and 4 identical shafts. (B) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1\text{s}$ ($k_{Ca}=0\text{ s}^{-1}$), while Ca^{2+} activity was recorded in node 2. (Bottom) Representative Ca^{2+} traces in node 2 in geometries with node/shaft width ratio $\delta = \frac{d_0}{d_{shaft}}$ = 1 (red and gray), 2 (black) and 3 (blue), expressed as SNR (see Methods). Red and gray traces correspond to Ca^{2+} signals in geometries with $\delta=1$ and a volume $V_1=0.620\ \mu\text{m}^3$ and $V_1=0.258\ \mu\text{m}^3$, respectively. Black and blue traces were recorded in geometries with $V_2=0.263\ \mu\text{m}^3$ and $V_3=0.195\ \mu\text{m}^3$, respectively. (C) Quantification of Ca^{2+} signal characteristics depending on node/shaft width ratio δ and on process volume. Data are represented as mean \pm STD, $n=20$ for each geometry. (C1) Ca^{2+} peak probability does not vary with δ when $V_1=0.258\ \mu\text{m}^3$ (p-value=0.22). (C2) Time to 1st peak decreases with δ (p-value=0.0018 *). (C3) Peak amplitude does not vary with δ when $V_1=0.258\ \mu\text{m}^3$ (p-value=0.16). (C4) Peak duration increases with δ , for both $V_1=0.620\ \mu\text{m}^3$ and $V_1=0.258\ \mu\text{m}^3$ (***). (C5) The frequency of IP_3R opening increases with δ , for both $V_1=0.620\ \mu\text{m}^3$ and $V_1=0.258\ \mu\text{m}^3$ (***). (D) Ca^{2+} residency time in nodes increases with δ , for both $V_1=0.620\ \mu\text{m}^3$ and $V_1=0.258\ \mu\text{m}^3$ (***, $n=300$). The effect of node/shaft width ratio on each Ca^{2+} signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.



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Figure 3-Figure supplement 5. Thin shafts enhance local spontaneous Ca²⁺ activity. (A) Neuronal stimulation protocol simulated for each geometry. 200 Ca²⁺ ions were infused in Node 1 at time t=t₀, while Ca²⁺ activity was recorded in nodes 1 and 2. (B) Representative Ca²⁺ traces in node 1 (top) and node 2 (bottom) for $d_{\text{shaft}}=d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue), expressed as SNR (see Methods). (C) Ca²⁺ peak probability increases when d_{shaft} decreases in node 2 (***). Note that peak probability is 1 in node 1 as the injection of 200 Ca²⁺ ions in node 1 results in a local Ca-GCaMP peak for all seed values tested. (D1) Peak amplitude increases when d_{shaft} decreases in node 1 (***) and 2 (***). (D2) The ratio between peak amplitude in node 2 and in Node 1 increases with shaft width (Spearman $r=0.0006$, p-value < 0.001 ***). (E) Time to 1st peak in node 2 increases with d_{shaft} (***). Note that time to 1st peak does not vary with d_{shaft} in node 1 as it occurs quickly after Ca²⁺ injection, through the binding of the injected Ca²⁺ ions to GCaMP molecules. (F) Peak duration increases when d_{shaft} decreases in node 1 (***) and 2 (***). Note that Ca²⁺ peak characteristics were not statistically different between nodes 1 and 2. Data are represented as mean ± STD, n=20 for each geometry. The effect of d_{shaft} on each Ca²⁺ signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.



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Figure 3-Figure supplement 6. Ca^{2+} peak probability, amplitude and duration decrease with ER surface area. (A) As the shape and distribution of the ER in fine processes have not been characterized in live tissue so far but are likely highly variable (Aboufaires El Alaoui et al., 2021), simulations were performed in meshes with various ER shapes: "No ER", "Node ER" and "Node/shaft ER", in which there was no ER, discontinuous ER in nodes or an ER consisting in node/shaft alternations, respectively. (B) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1\text{s}$, while Ca^{2+} activity was monitored in nodes 1 and 2. (Bottom) Representative Ca^{2+} traces in node 2, in "No ER" (green), "Node ER" (blue) and "Node/shaft ER" (black) geometries $d_{\text{shaft}} = \frac{d_0}{2}$, for $k_{Ca} = 0, 10, 100, 300, 700$ and 1000 s^{-1} , expressed as SNR (see Methods). (C) Quantification of peak characteristics depending on ER geometry and k_{Ca} . Ca^{2+} peak probability (***, C1), time to 1st peak (***, C2), peak amplitude (***, C3), duration (***, C4) and the frequency of IP₃R opening (***, C5) vary with the ER morphology. Note that Ca^{2+} peak characteristics did not vary with k_{Ca} . (D) Time to 1st decreases as ER surface area increases (***), while Ca^{2+} peak probability (***), amplitude (***), and duration (***), as well as the frequency of IP₃R opening (***), increase as ER surface area increases, $k_{Ca} = 0 \text{ s}^{-1}$. Data are represented as mean \pm STD, $n=20$ for each geometry. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

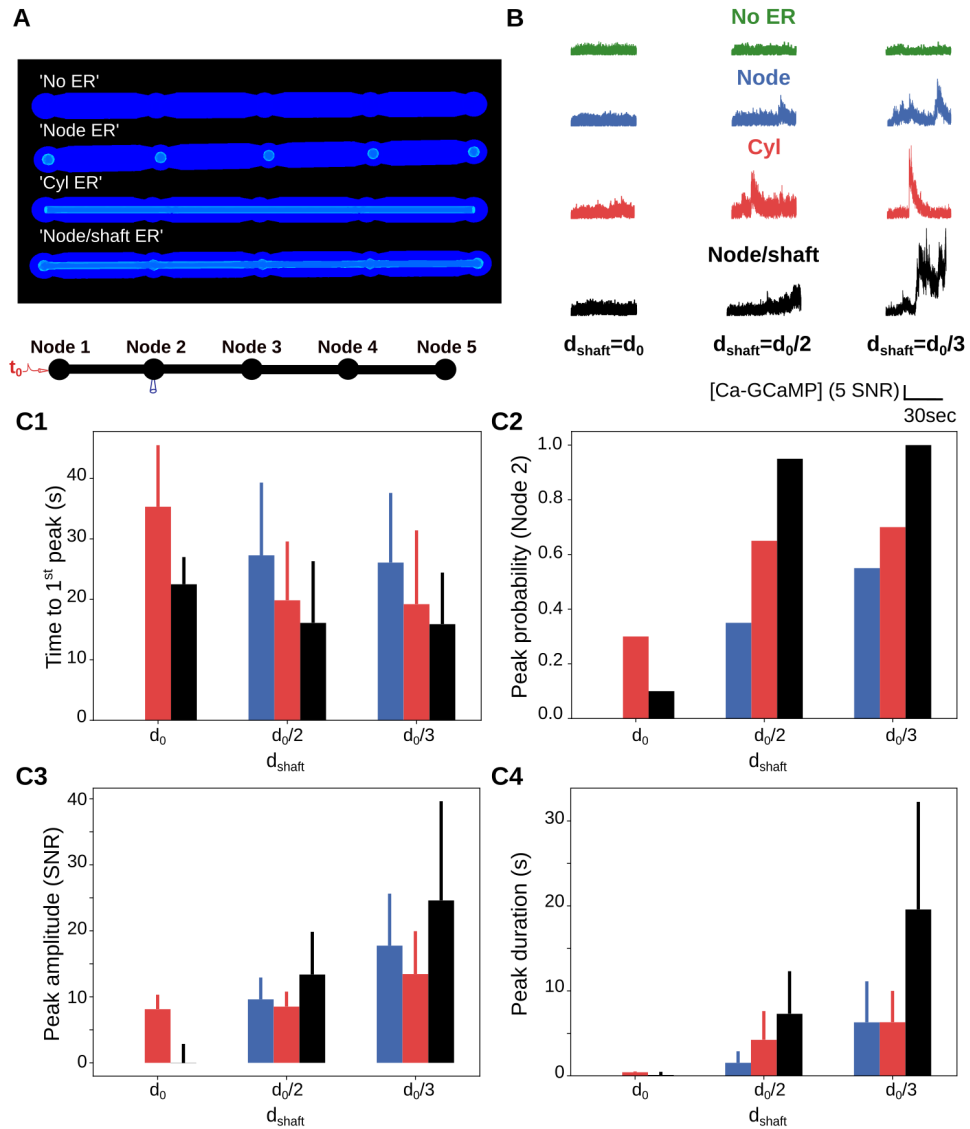


Figure 3-Figure supplement 7. Local Ca^{2+} activity increases when shaft diameter decreases, for all ER geometries tested. (A) Screenshots displaying the geometries used to investigate the effect of ER geometry on Ca^{2+} dynamics: "No ER", "Node ER", "Node/shaft ER" and "Cyl ER". (B) (Top) Neuronal stimulation protocol simulated for each geometry: node 1 was stimulated at $t=t_0=1s$ ($k_{Ca}=0 s^{-1}$), while Ca^{2+} activity was monitored in node 2. (Bottom) Representative Ca^{2+} traces in node 2 for $d_{shaft}=d_0$ (left), $\frac{d_0}{2}$ (center) and $\frac{d_0}{3}$ (right), in "No ER" (green), "Node ER" (blue), "Cyl ER" (red) and "Node/shaft ER" (black) geometries, expressed as SNR (see Methods). (C) Quantification of the effect of d_{shaft} on Ca^{2+} dynamics. Note that no bar is visible for simulations in "Node ER" $d_{shaft}=d_0$ geometries as no peak was detected. Results in "Node/shaft ER" geometries are presented in Fig3 but are added here (black bars) for comparison. (C1) Time to 1st peak in node 2 does not vary with d_{shaft} in "Node ER" simulations (p-value=0.85) but increases with d_{shaft} in "Cyl ER" simulations (p-value= 0.021 *). (C2) Ca^{2+} peak probability in node 2 increases when d_{shaft} decreases in "Node ER" simulations (***) and in "Cyl ER" simulations (p-value=0.01 *). (C3) Peak amplitude increases when d_{shaft} decreases in "Node ER" geometries (p-value=0.027 *) and in "Cyl ER" (p-value=0.01 *). (C4) Peak duration increases when d_{shaft} decreases in "Node ER" simulations (p-value=0.028 *) and in "Cyl ER" simulations (p-value=0.001 **). Data are represented as mean \pm STD, n=20, for each geometry. The effect of d_{shaft} on each Ca^{2+} signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

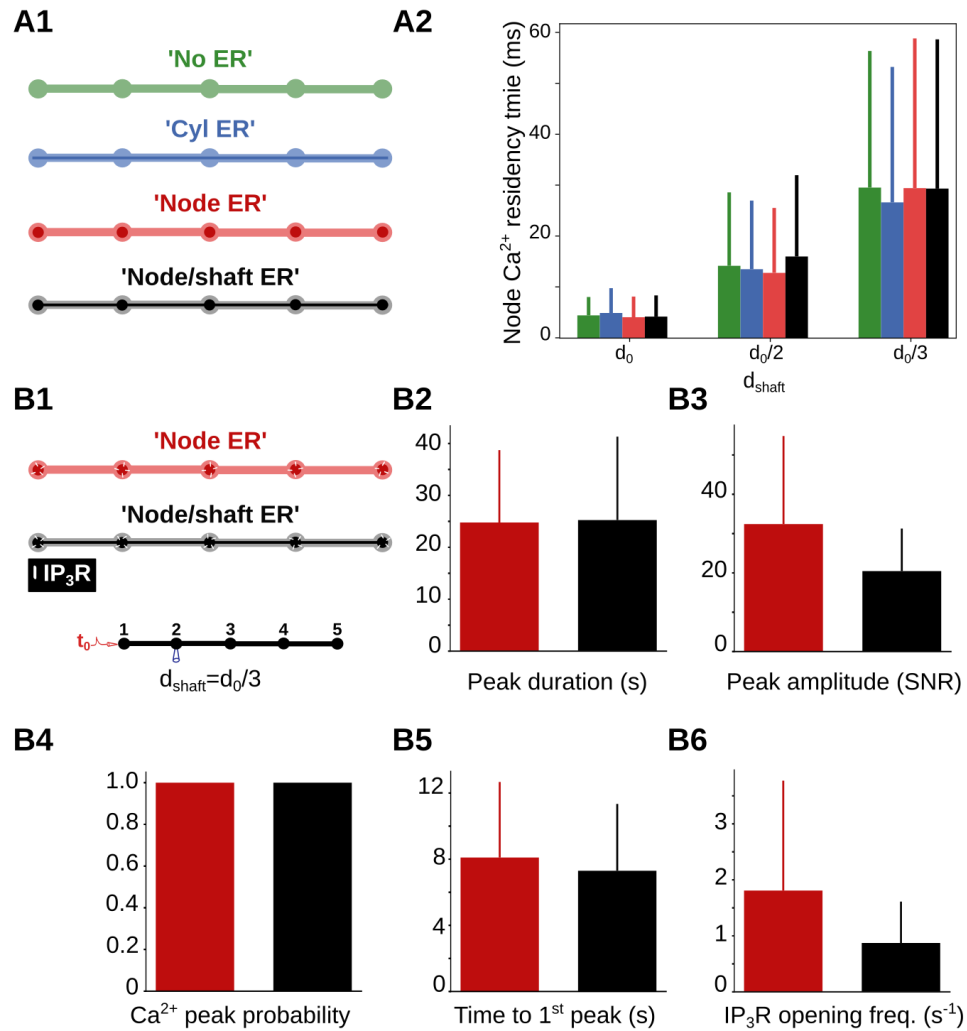


Figure 3-Figure supplement 8. Sensitivity study of the effect of ER morphology on Ca^{2+} dynamics. (A) Node Ca^{2+} residency time is not influenced by ER morphology. (A1) One Ca^{2+} ion was added in node 1 in geometries with different ER shapes: "No ER" (green), "Node ER" (blue), "Cyl ER" (red) and "Node/shaft ER" (black). (A2) For all ER shapes tested, Ca^{2+} residency time in nodes increases when d_{shaft} decreases. Node Ca^{2+} residency time does not change depending on ER morphology, suggesting that cellular morphology has a greater influence on compartmentalization than ER morphology in the meshes used in this study. Data are represented as mean \leq STD, $n=300$ for each geometry. (B) Sensitivity study of the effect of ER morphology on Ca^{2+} dynamics with constant number of IP_3R s. (B1) Simulations were performed in 'Node ER' (red) and "Node/shaft ER" (black) geometries with IP_3R channels (white) located in nodes (Top), resulting in a constant number of IP_3R channels for both ER shapes. Node 1 was stimulated at $t=t_0=1\text{ s}$ ($k_{\text{Ca}}=0\text{ s}^{-1}$), while Ca^{2+} activity was monitored in node 2 (bottom). (B2-6) Quantification of Ca^{2+} signals depending on ER morphology with constant number of IP_3R in node 2, $d_{\text{shaft}} = \frac{d_0}{3}$. Data are represented as mean \leq STD, $n=20$ for each geometry. Ca^{2+} peak duration (B2), amplitude (B3), probability (B4) and delay (B5) do not vary with ER morphology when IP_3R channels are located in nodes. (B6) IP_3R opening frequency does not significantly differ between "Node ER" and "Node/shaft ER" geometries ($p\text{-value}=0.06$). Those results suggest that the decreased Ca^{2+} activity in branchlets that contain discontinuous ER reported in Fig 4 mainly results from the decreased number of IP_3R channels associated with the decreased ER surface area of "Node ER" compared to "Node/shaft ER" morphology.

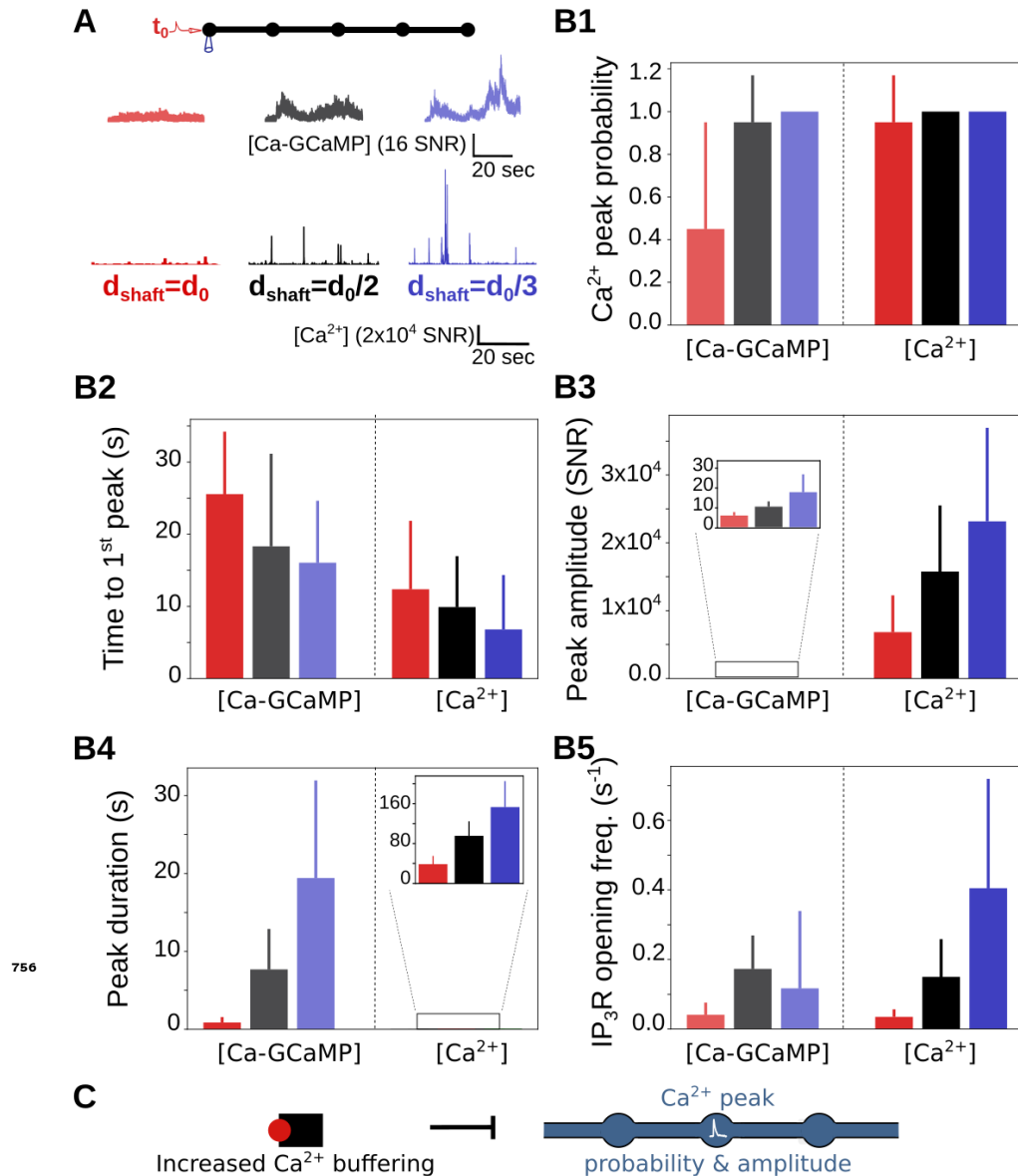


Figure 3-Figure supplement 9. Ca²⁺ indicators alter Ca²⁺ peak characteristics. (A) In order to investigate free Ca²⁺ peak probability, no GCaMP molecules were added to the model. (Top) At $t=t_0=1s$, node 1 was stimulated ($k_{Ca}=0 s^{-1}$), while Ca²⁺ concentration was monitored in node 1. The geometry used for $d_{\text{shaft}}=d_0$ corresponds to the geometry presented in Fig 1A. (Middle) Representative Ca-GCaMP traces in node 1, in simulations containing GCaMP molecules in geometries with shaft width $d_{\text{shaft}}=d_0$ (light red), $\frac{d_0}{2}$ (grey) and $\frac{d_0}{3}$ (light blue). (Bottom) Representative free Ca²⁺ traces in node 1, in the absence of GCaMP molecules, in geometries with shaft width $d_{\text{shaft}}=d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue). The amplitude of Ca-GCaMP and free Ca²⁺ signals is expressed as SNR (see Methods). (B) Characteristics of free Ca²⁺ signals depending on d_{shaft} . Ca-GCaMP signals measured in simulations from Fig 3 are presented for comparison. (B1) Free Ca²⁺ peak probability does not vary with d_{shaft} (p-value=0.22). Ca-GCaMP peak probability is lower than free Ca²⁺ peak probability for $d_{\text{shaft}}=d_0$ (***). (B2) Time to 1st free Ca²⁺ peak increases with d_{shaft} (***). Time to 1st Ca-GCaMP peak is higher than time to 1st free Ca²⁺ peak, for any value of d_{shaft} (***). (B3) Free Ca²⁺ peak amplitude increases when d_{shaft} decreases (***). Ca-GCaMP peak amplitude is lower than free Ca²⁺ peak amplitude, for any value of d_{shaft} (***). (B4) Free Ca²⁺ peak duration increases when d_{shaft} decreases (***). Ca-GCaMP peak duration is higher than free Ca²⁺ peak duration, for any value of d_{shaft} (***). (B5) The frequency of IP₃R opening increases when d_{shaft} decreases (***). (C) Schematic summarizing the main result from this figure: increased Ca²⁺ buffering is associated with decreased Ca²⁺ peak probability and amplitude. Data are represented as mean \pm STD, n=20 for each geometry. The effect of d_{shaft} on each free Ca²⁺ signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.

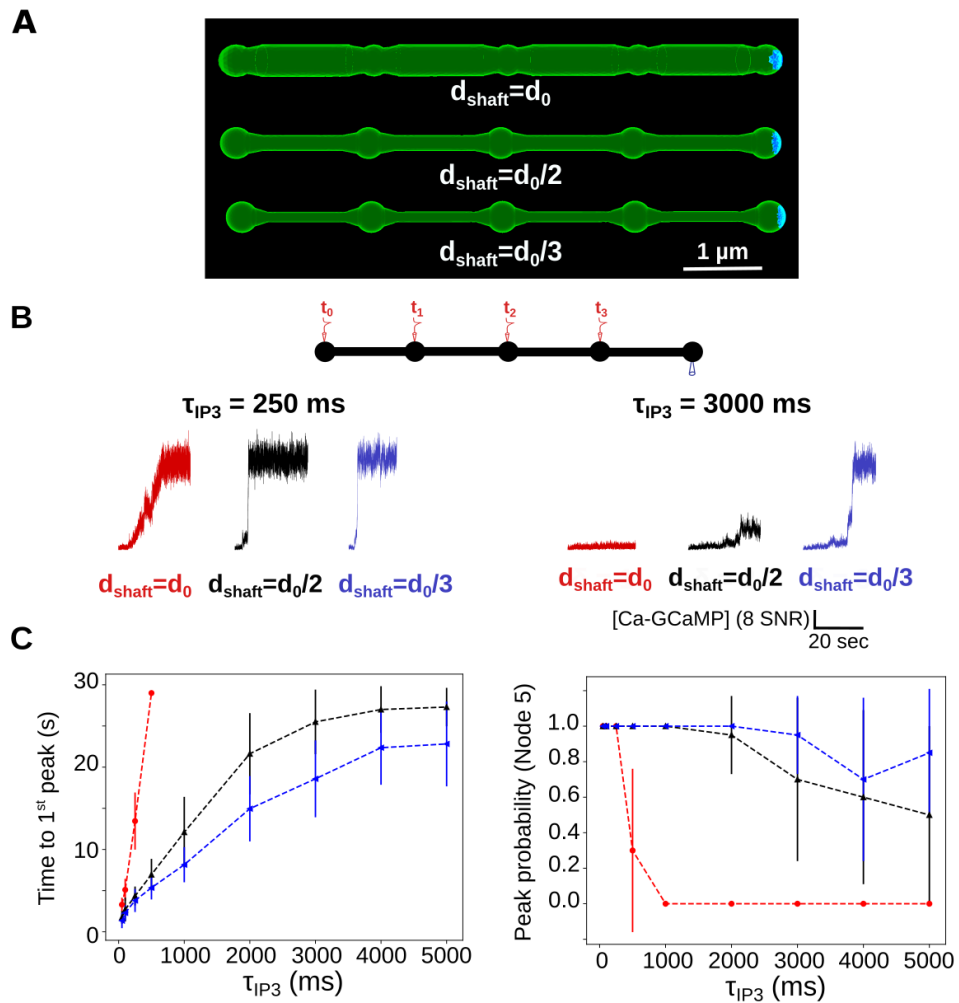


Figure 5-Figure supplement 1. Sensitivity study of the effect of boundary conditions on local Ca^{2+} activity upon repeated neuronal stimuli. (A) Simulations were performed in the “5nodes” geometries (Fig1). Boundary conditions were absorbing at the extremity of node 5 (blue) while the remaining boundaries were characterized by reflective boundary conditions (green). Those boundary conditions mimic the connection of the modeled astrocyte branchlet to the rest of the cell. (B) (Top) node 1 was stimulated at $t=t_0=5s$, node 2 at $t_0 + \tau_{IP3}$, node 3 at $t_0 + 2\tau_{IP3}$ and node 4 at $t_0 + 3\tau_{IP3}$, $k_{Ca}=0 s^{-1}$, while Ca^{2+} activity was monitored in node 5. (Bottom) Representative Ca^{2+} traces for $d_{\text{shaft}}=d_0$ (red), $\frac{d_0}{2}$ (black) and $\frac{d_0}{3}$ (blue), with $\tau_{IP3}=250\text{ms}$ (left) and 3000ms (right), expressed as SNR (see Methods). (C) (Left) Time to 1st peak in node 5 increases with τ_{IP3} for $d_{\text{shaft}}=d_0$ (***) , $d_{\text{shaft}}=\frac{d_0}{2}$ (***) and $d_{\text{shaft}}=\frac{d_0}{3}$ (***) . Time to 1st peak is higher for $d_{\text{shaft}}=d_0$ compared to $d_{\text{shaft}}=\frac{d_0}{2}$ and $d_{\text{shaft}}=\frac{d_0}{3}$. (Right) Ca^{2+} peak probability in node 5 is lower for $d_{\text{shaft}}=d_0$ compared to $d_{\text{shaft}}=\frac{d_0}{2}$ and $d_{\text{shaft}}=\frac{d_0}{3}$ and decreases as τ_{IP3} increases for $d_{\text{shaft}}=d_0$ (***) and $d_{\text{shaft}}=\frac{d_0}{2}$ (***) but not $d_{\text{shaft}}=\frac{d_0}{3}$ (p-value=0.054). Note that Ca^{2+} peak probability was lower and time to 1st peak higher compared to simulations with reflective boundary conditions. Data are represented as mean \pm STD, $n=20$ for each value of d_{shaft} and of τ_{IP3} . Lines are guides for the eyes. The effect of d_{shaft} on each Ca^{2+} signal characteristic was tested using one-way ANOVA. Significance is assigned by * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$.