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A mathematical calcium-induced calcium-release model

G. Queisser

Exzellenzcluster CellNetworks, University of Heidelberg, BioQuant
Im Neuenheimer Feld 267, 69120 Heidelberg

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Summary. — Calcium plays a key role in neurons in the regulation of subcellular processes and links the electrophysiological scale with biochemical processes taking place in the cell. In this paper we present a mathematical model for neuronal calcium induced calcium release (CICR), taking into account synaptic calcium uptake through the plasma membrane, the cytosol and its interaction with the endoplasmic reticulum through channels called *Inositol-3-phosphate* (IP3) and *Ryanodine Receptors* (RYR) that are embedded in the endoplasmic membrane. For this model study we defined a two-dimensional model environment that represents a neuronal spine including the components mentioned above. A reaction-diffusion process is coupled with a transport term on the endoplasmic membrane to regulate endoplasmic calcium sequestration and release. The model was implemented in the simulation environment UG and employs finite-volume discretization together with multigrid solvers for the numerical solution of the underlying problem. This study shows that, depending on the behavior of the endoplasmic reticulum, calcium signals are fairly restricted to the spinal area or can extend further into the dendritic branches. Furthermore we observe signal transduction faster than by passive diffusion, giving rise to the hypothesis that, under certain cytosolic-endoplasmic configurations, CICR-signals can travel faster than by passive calcium diffusion.

PACS 87.19.ll – Models of single neurons and networks.
PACS 87.19.ls – Encoding, decoding, and transformation.
PACS 87.19.lv – Learning and memory.
PACS 87.85.dm – Physical models of neurophysiological processes.

1. – Introduction

A neuron operates on two separate time scales. On the electrophysiological level, the cell receives and propagates electrical signals along its plasma membrane. The interplay between neighboring cells and networks are regulated by different types of coupling, such as chemical or electrical synapses. Furthermore each neuron, on an independent scale, is responsible for transforming its electrical activity into a subcellular response, which is relevant for learning and survival of the cell [1-3].

Calcium plays a key role as a signal encoder in neurons [4-8]. A finely tuned system of calcium-regulated processes, sensitive to calcium concentration levels, duration and frequency of calcium signals as well as entry-sites for calcium, operates within cytoplasm and interacts with the endoplasmic reticulum (ER) [9-13]. The neuron's nucleus finally receives these cytoplasmic calcium signals through its nuclear pores [14], and activates calcium-regulated biochemical cascades which result in the expression of specific genes [4, 6, 15, 16].

Great effort has been undertaken in the last decades to develop experimental tools and mathematical models to investigate signal processing along the plasma membrane of cells. Recent work has advanced common one-dimensional models based on the cable equation [17, 18], by combining Hodgkin-Huxley like equations with a passive three-dimensional (3D) model [19].

In addition to the electrophysiological signaling scale, a model for nuclear calcium signaling on reconstructed nuclear morphologies has shed more light on calcium signaling in the nucleus, the site for survival-relevant biochemical processes [20]. Here, we propose a mathematical model that links both models accounting for the electrical signal transduction on the plasma membrane [19] and the nuclear calcium code [20].

In this model we focus on three calcium regulating components: uptake of intracellular calcium through synaptic activity, buffering of calcium in the cytosol and the sequestration/release of calcium through the ER. Calcium entry into the cell is regulated by depolarization of the plasma membrane [21-25]. Therefore the uptake of calcium is defined by the 3D model of active signal processing mentioned above [19]. In the cytoplasm buffers are present that inactivate free calcium [26-29]. Calcium in the cytosol propagates by a gradient-driven diffusion process (and is damped by buffers), we therefore propose diffusion-reaction equations for cytosolic calcium. The ER is included in the model based on the neuron-within-neuron proposition in ref. [30]. Depending on its state the ER functions as a source or sink. Having set up these calcium induced calcium release (CICR) dynamics [9, 31-33], which induce calcium signals that propagate to the nucleus, we are able to connect two very important signaling scales. The proposed model can be applied in areas involving the ER-dynamics, such as Alzheimer's disease, where the sequestration and release of calcium from ER-stores are strongly affected [34].

A numerical treatment of the model with a finite-volume discretization approach and numerical solvers based on the multigrid theory, all implemented in the simulation platform UG [35, 36], allows for two- or three-dimensional simulations which can be defined on reconstructed morphologies as presented in refs. [37, 19].

2. – The neuronal CICR model

Cellular Calcium Signals are delicately regulated by different factors, such as *entry sites, channel gates, cytosolic buffers* and the interaction with the *endoplasmic reticulum*. The model presented here is meant to link activity on the plasma membrane (and the related calcium entry at synaptic sites) with nuclear calcium. For the model we consider a domain sketched in fig. 1. The plasma membrane of the cell defines the outer boundary of the exterior domain, the *cytosol*, which surrounds the *endoplasmic reticulum* (ER). The ER-membrane continuously extends into the outer of the two nuclear membranes. The *nucleus* can communicate with the cytosol directly through its nuclear pore complexes in a passive manner [14]. The ER contains active transporter complexes, most importantly *Inositol-3-phosphate* (IP3) [38-41], and *Ryanodine receptors* (RYR) [32, 42-45]. Through these transporters the ER can actively sequester calcium from the cytosol or

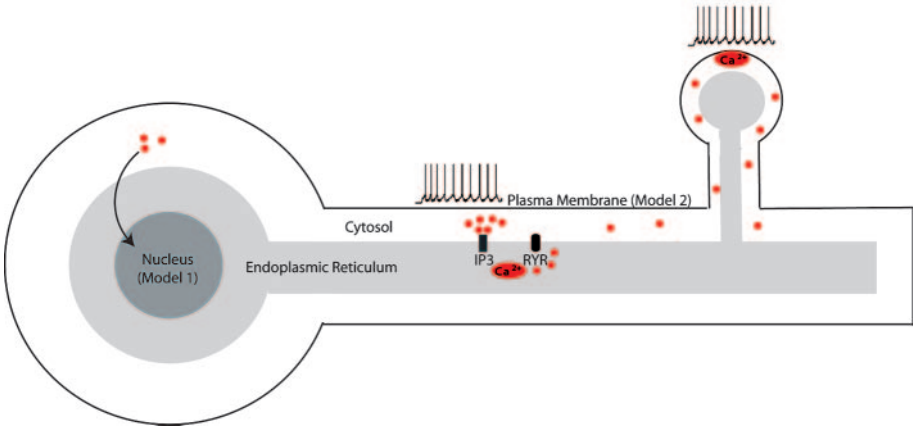

Fig. 1. – Sketch of the model neuron: The neuron consists of the components *plasma membrane, cytosol, endoplasmic reticulum*, and *nucleus*. Interaction between the cytosol and endoplasmic reticulum takes place through the endoplasmic membrane-interface in which biological receptors are integrated. The nucleus is directly connected with the cytosol, not the endoplasmic reticulum, through its nuclear pore complexes.

release calcium back into the cytosol. Both IP3 and RYR are triggered by calcium ions, therefore the process under investigation can be described as calcium induced calcium release (CICR).

In this section we will address the two domains, cytosol and ER, and their coupling by IP3 and RYR. Thus, the presented model describes active calcium signaling originating at the plasma membrane (Model 2 [19], fig. 1), propagating through cytosol and ER down to the soma and into the nucleus (Model 1 [20], fig. 1). First we will introduce the equations in cytosol and ER separately and then focus on their coupling by the ER-membrane.

2˙1. *Cytosolic calcium.* – As mentioned earlier, cytosolic calcium propagates through a passive diffusion process, dampened by cytosolic calcium buffers. We assume these buffers to be stationary, and thus model them by a reaction term that inactivates calcium ions. This yields

$$\frac{\partial C_{\mathrm{cyt}}}{\partial t} = \mathrm{div}(D_{\mathrm{cyt}} \cdot \nabla C_{\mathrm{cyt}}) + \kappa_{BC} \cdot (C_{\mathrm{cyt}} - C^0_{\mathrm{cyt}}), \tag{1}$$

C_{cyt} being the cytosolic calcium concentration, C^0_{cyt} the initial cytosolic calcium concentration, D_{cyt} the cytosolic calcium diffusion coefficient and κ_{BC} the buffering rate for cytosolic calcium. If we consider D_{cyt} as concentration-independent, then eq. (1) is linear and one can rescale the equation additively to resting state zero. Furthermore one can consider base-level cytosolic calcium at a zero-state from a biological standpoint. Therefore, if in future models we consider eq. (1) as non-linear, the assumption of zero-initial condition still holds. Boundary conditions for calcium at the plasma membrane's non-entry sites are considered as Neumann Zero, since the focus here lies in the sequestration effects of the ER, not the calcium uptake by the extracellular space. We set time-dependent Dirichlet-conditions at the calcium entry sites, such as synapses. As

initial conditions for the basal calcium level we can, from a biological standpoint, assume homogeneous distribution of calcium in the cytosol and set $C_{\text{cyt}}|_{t=0} \equiv C^0_{\text{cyt}} = 0$. This defines an initial-boundary value problem of the following type:

$$\frac{\partial C_{\text{cyt}}}{\partial t} = \operatorname{div}(D_{\text{cyt}} \cdot \nabla C_{\text{cyt}}) + \kappa_{BC} \cdot (C_{\text{cyt}} - C^0_{\text{cyt}}), \quad \text{in } \Omega_{\text{cyt}} \tag{2}$$

$$C_{\text{cyt}}|_{t=0} \equiv C^0_{\text{cyt}} = 0, \quad \text{in } \bar{\Omega}_{\text{cyt}} \tag{3}$$

$$\frac{\partial C_{\text{cyt}}}{\partial \vec{n}} = 0, \quad \text{on } \Gamma^{(1)}_{\text{cyt}} \tag{4}$$

$$C_{\text{cyt}} = g(t), \quad \text{on } \Gamma^{(2)}_{\text{cyt}}, \tag{5}$$

where Ω_{cyt} denotes the cytosolic interior, $\bar{\Omega}_{\text{cyt}}$ the cytosol including the plasma membrane, $\Gamma^{(1)}_{\text{cyt}}$ the plasma membrane parts with no calcium uptake and $\Gamma^{(2)}_{\text{cyt}}$ the calcium gating sites on the membrane, *e.g.*, synaptic sites. Since calcium, that is gated through plasma membrane channels (*e.g.*, N-methyl-D-aspartate (NMDA) receptors), can be measured experimentally space- and time-dependently, this data can be used to define space- and time-dependent sources for calcium on the plasma membrane. This justifies the definition of Dirichlet boundary conditions for the plasma membrane.

2˙2. *Endoplasmic sequestration and release.* – The ER functions as a store for calcium that, depending on its state, will sequester cytosolic calcium or release a significant amount of calcium into the cytosol in order to "refuel" the cytosolic calcium transient towards the nucleus. Uptake of calcium into distinct ER-stores can be described by a diffusive flux coupled with directed flux into or out of the store driven by a potential gradient. We denote this behavior by

$$\frac{\partial C_{\text{ER}}}{\partial t} = \operatorname{div}(D_{\text{ER}} \nabla C_{\text{ER}}) + \operatorname{div}(f(C_{\text{ER}}, t)\vec{v} \cdot C_{\text{ER}}), \quad \text{in } \Omega_{\text{ER}}, \tag{6}$$

where C_{ER} denotes the endoplasmic calcium concentration, D_{ER} the diffusion coefficient, $f(C_{\text{ER}}, t)$ a function that controls ER behavior, *i.e.* sequestration or release, that stands in relation to a potential gradient $-\nabla\Phi = \operatorname{div}(f \cdot \vec{v})$, $\vec{v}$ a directed velocity towards local C_{ER}-stores and Ω_{ER} the endoplasmic interior.

The ER-membrane containing IP3 and RYR is considered as an inner boundary coupling the two domains Ω_{cyt} and Ω_{ER}. Cytosolic calcium near the membrane is channeled actively by IP3 and RYR into ER-calcium stores. Active transport of calcium across the membrane is numerically treated by integrative terms across membrane-near areas which function as sinks on the one side and sources on the other. Finally we can present the following system:

$$\frac{\partial C_{\text{cyt}}}{\partial t} = \operatorname{div}(D_{\text{cyt}} \cdot \nabla C_{\text{cyt}}) + \kappa_{BC} \cdot (C_{\text{cyt}} - C^0_{\text{cyt}}), \quad \text{in } \Omega_{\text{cyt}} \tag{7}$$

$$C_{\text{cyt}}|_{t=0} = 0, \quad \text{in } \bar{\Omega}_{\text{cyt}} \tag{8}$$

$$\frac{\partial C_{\text{cyt}}}{\partial \vec{n}} = 0, \quad \text{on } \Gamma^{(1)}_{\text{cyt}} \tag{9}$$

$$C_{\text{cyt}} = g(t), \quad \text{on } \Gamma^{(2)}_{\text{cyt}}, \tag{10}$$

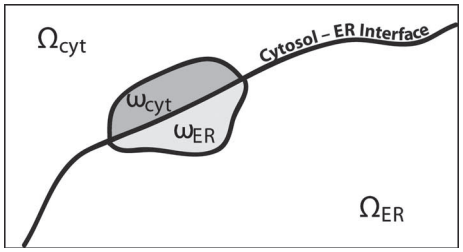

Fig. 2. – Omega domains: in the model defined ω_x-domains can be set, which define the transport of calcium from ω_{cyt} into ω_{ER} and vice versa.

$$\frac{\partial C_{\mathrm{ER}}}{\partial t} = \mathrm{div}(D_{\mathrm{ER}} \nabla C_{\mathrm{ER}}) + \mathrm{div}(f(C_{\mathrm{ER}}, t)\vec{v} \cdot C_{\mathrm{ER}}), \qquad \text{in } \Omega_{\mathrm{ER}}, \tag{11}$$

$$C_{\mathrm{ER}}|_{t=0} = C^0_{\mathrm{ER}}, \qquad \text{in } \Omega_{\mathrm{ER}} \tag{12}$$

$$\int_{\omega_{\mathrm{cyt}}} C_{\mathrm{cyt}} = \int_{\omega_{\mathrm{ER}}} C_{\mathrm{ER}}, \qquad \text{in } \omega_{\mathrm{cyt}} \cup \omega_{\mathrm{ER}} \tag{13}$$

where ω_{cyt} and ω_{ER} denote the membrane transport areas, depicted in fig. 2, which can be defined according to the underlying biological system. The Cytosol-ER interface is a biologically active (ATP consuming) membrane. The model assumption therefore is that cytosolic calcium close to the membrane is transported to membrane-near areas within the ER. This accounts for the integral equality in eq. (13). In addition, since we are focussing on a small spinal area with its ER and experimental data to define f in eq. (11) has yet to be produced, we simplify the right-hand side of eq. (11) by the contraint given in eq. (13).

2˙3. *Solving the equations numerically.* – Solving the above model numerically, independent of space dimensionality and neuronal morphology, we make use of finite-volume space discretization and multigrid solving techniques on a test morphology depicted in fig. 3 [35]. The numerical implementation of the model is realized within the simulation environment UG [36], where we can make direct use of multigrid solvers. For the sake of clearly demonstrating these techniques applied to a PDE-based model, we consider a simplified version of the above model and focus on the diffusive component of eq. (7).

Fig. 3. – Computational domain. Left and middle: simulation domains for cytosolic calcium and ER-calcium, respectively. Right: coarse grid for finite-volume discretization of the underlying PDEs. In the multigrid method this grid functions as the coarsest grid on which a hierarchy of refined, interleaving grids is computed.

The weak approximation yields

$$\int_{\Omega_{\mathrm{cyt}}} \frac{\partial C_{\mathrm{cyt}}}{\partial t} = \int_{\Omega_{\mathrm{cyt}}} \mathrm{div}(D_{\mathrm{cyt}} \cdot \nabla C_{\mathrm{cyt}}). \tag{14}$$

Applying Gauss' theorem we retrieve

$$\frac{\partial}{\partial t} \int_{\Omega_{\mathrm{cyt}}} C_{\mathrm{cyt}} = \int_{\Gamma_{\mathrm{cyt}}^{(1)\cup(2)}} D_{\mathrm{cyt}} \cdot \nabla u \cdot \vec{n}. \tag{15}$$

The finite-volume discretization defines a dual grid $B_i = \bigcup_i b_i$ such that

$$\int_{\Gamma_{\mathrm{cyt}}^{(1)\cup(2)}} D_{\mathrm{cyt}} \cdot \nabla u \cdot \vec{n} = \sum_{i=1}^{m} \int_{\partial b_i} D_{\mathrm{cyt}} \cdot \nabla u \cdot \vec{n}. \tag{16}$$

Applying implicit time stepping, the discrete equation—which defines the system of equations to be solved—leaves us with

$$\begin{aligned} &\int_{b_i} C_{\mathrm{cyt}}(t_{k+1}, x_i)\mathrm{d}x - \int_{b_i} C_{\mathrm{cyt}}(t_k, x_i)\mathrm{d}x = \\ &\Delta t \int_{\partial b_i} \sum_j C_{\mathrm{cyt}}(t_{k+1}, x_j) D_{\mathrm{cyt}} \nabla \xi_j(\gamma) \vec{n}_i \mathrm{d}\gamma, \end{aligned} \tag{17}$$

where ξ_j are defined numerical ansatzfunctions for a surface area γ. Applying this discretization scheme to the entire set of equations, we retrieve a full numerical implementation, applicable to 2D or 3D domains, as well as random neuronal, ER- and nuclear morphologies (which can be reconstructed from microscopy data using the methods presented in ref. [37]).

3. – Results

As stated earlier, one of the ERs very important functions is calcium sequestration and release as a form of cellular memory. In this section we will apply the CICR model to the spine-area of fig. 1, which is shown in fig. 3. The computational domain and example calcium entry time courses are shown in figs. 4 and 5. The top segment of the plasma membrane is defined as a synaptic site, the spherical part in the center will serve as the ER, and at the same time is a local calcium store. Therefore ω_{cyt} is defined by the inner ring and $\omega_{\mathrm{ER}} = \Omega_{\mathrm{ER}}$ in this study. Simulations show calcium release from a local source, *i.e.* a synapse, diffusion and sequestration/release from the ER. Endoplasmic behavior is a concentration regulated process. Therefore calcium is stored in the ER until a certain threshold is reached, then is freed into the cytoplasm until the ER is depleted to a lower concentration threshold. All following evaluations are measurements carried out in the spinal neck.

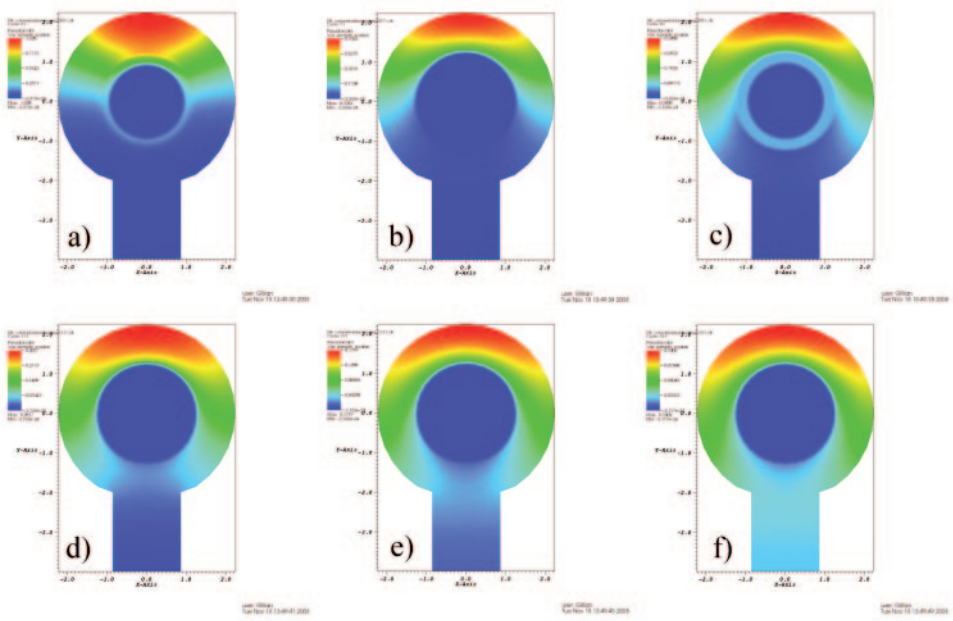

Fig. 4. – Simulation time course of cytsolic calcium: on the very top, a calcium source is defined by synaptic test pulses (fig. 5). Calcium then diffuses in the cytosol and is simultaneously sequestered or released from the ER (ER concentration changes not depicted).

3˙1. *Calcium signaling is regulated by the ER.* – If the ER acts as a strong calcium sink, fig. 6, signals originating in dendritic spines are strongly confined to the spinal area. We compare ER sequestration under different settings (*i.e.* application of test pulses, see fig. 5 and different thresholds) depicted in fig. 7, with calcium signal propagation without any ER influence. A strong shift in concentration levels shows that without ER interaction, calcium waves spill into the dendrites for a more global influence of synaptic activity.

Furthermore, the activity of the ER *locally* increases or decreases calcium concentration levels. In fig. 7 sequestration and release is regulated by the ERs concentration levels, and the rate of ER calcium release can be varied (see different time courses for calcium signals). Instead of a diffusive increase of calcium concentration in the spine neck, we observe local maxima which reflect the state of the ER due to synaptic activity. Therefore the ER can serve as a "memory-storage" for past synaptic events that affected the concentration level of the ER.

3˙2. *ER dynamics enhance signal propagation speed.* – In ref. [46] an electrotonic model for communication between spines and nucleus, based on the idea that the ER-membrane acts as a passive signal transmitter, is proposed. Reason was that certain biological phenomena occur on a time scale that should exclude mere "diffusion-communication". The model proposed here is based on the cytosolic and ER-calcium interaction through IP3 and RYR, which is present in neuronal systems. This type of signaling in our model shows, that indeed the speed of signal propagation lies above the speed of calcium diffusion due to local calcium uptake at the ER membrane, fig. 7. Therefore the neuronal calcium signaling system might be optimized in such a way that the ER uptake of calcium strongly affects signaling speed between spinal dendritic sites and the cell's nucleus.

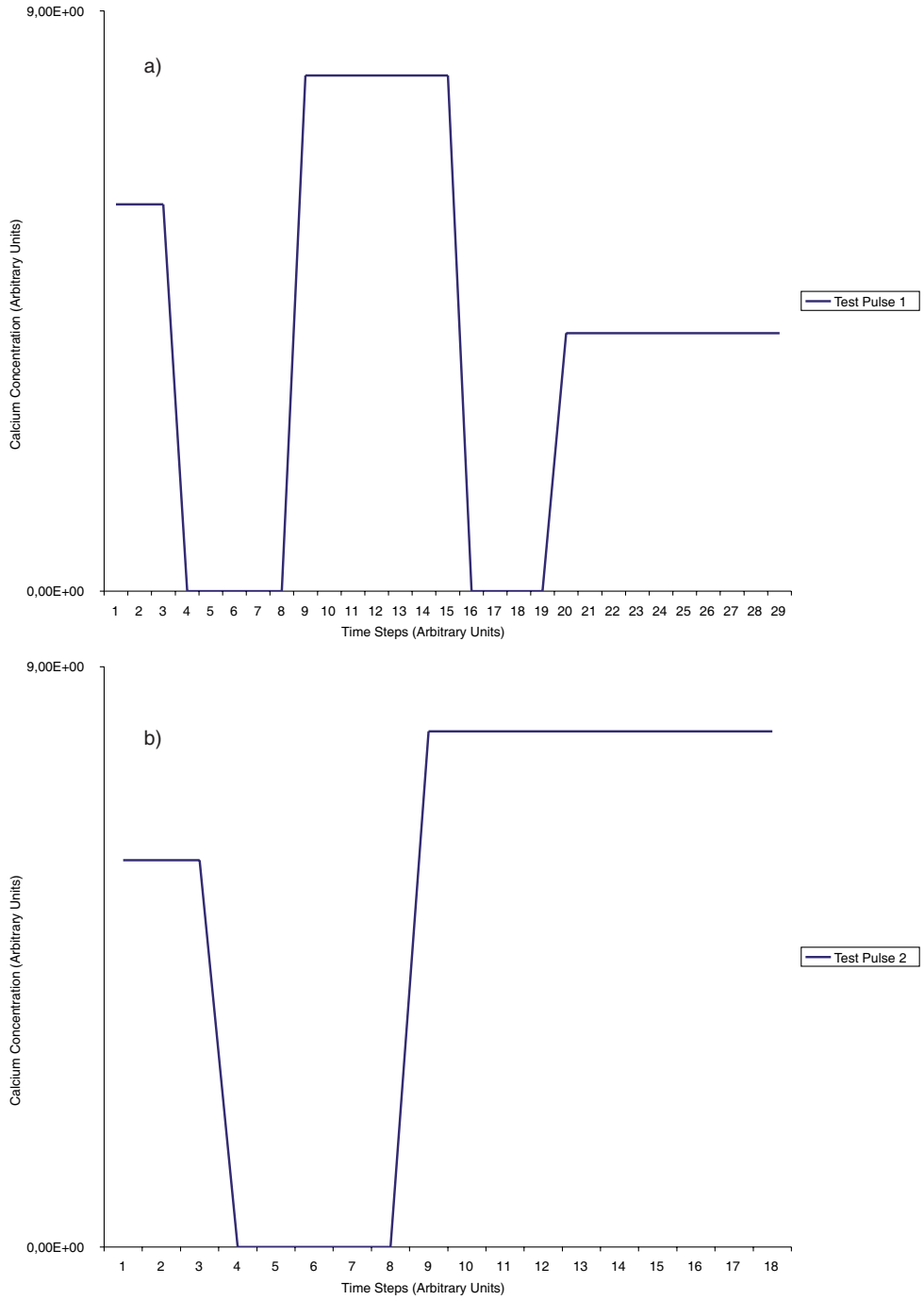

Fig. 5. – Synaptic test pulses: depending on the action potential dynamics in the neuron calcium enters the cell through the synaptic membrane channels. In our test environment we defined to simple pulses to demonstrate the cellular calcium dynamics. a) Test pulse 1. b) Test pulse 2.

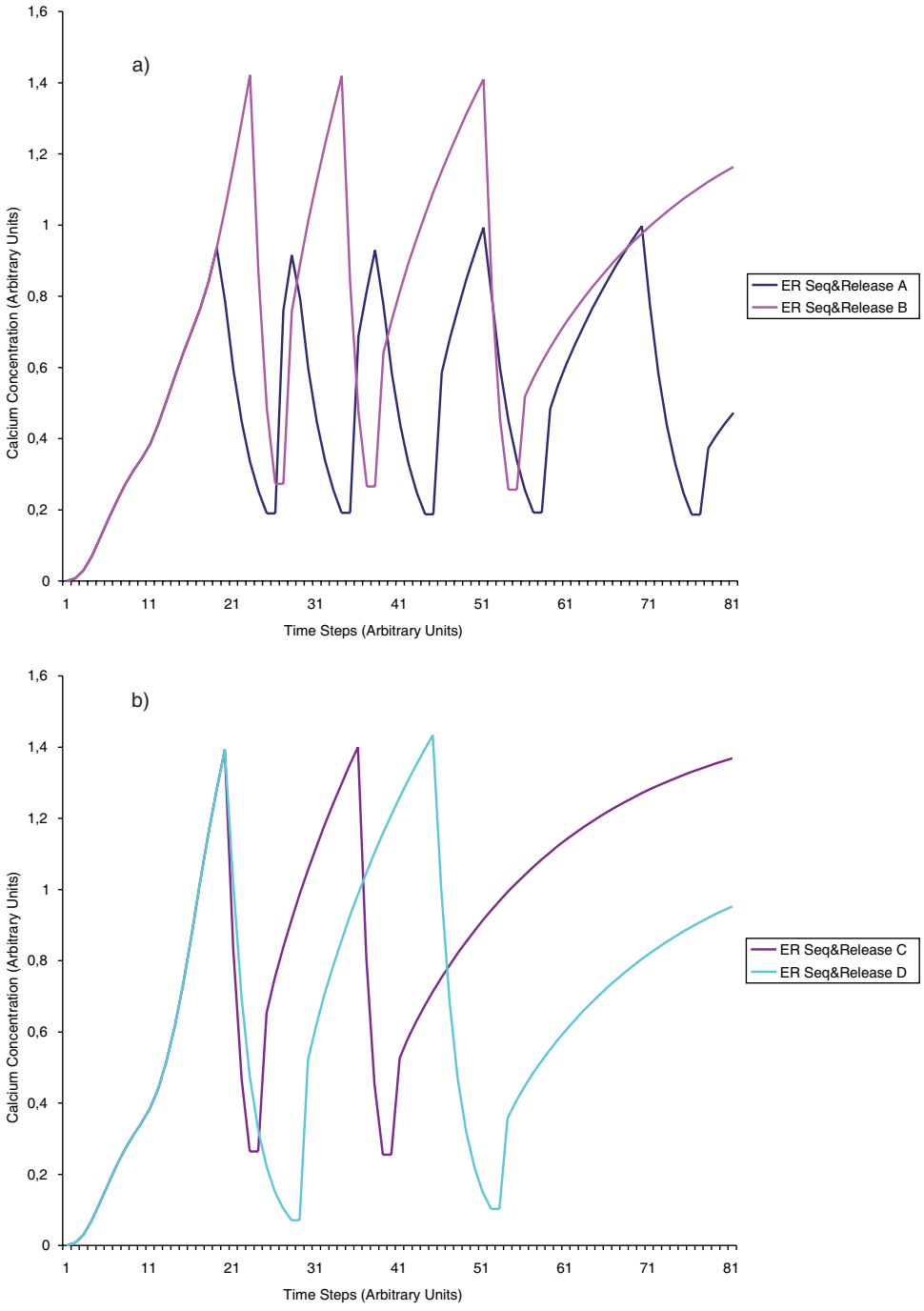

Fig. 6. – ER sequestration and release dynamics: a) ER test release-threshold set to 1.4 and 1.0, respectively, and combined with test pulse 1. b) Release-threshold set to 1.4 and combined with test pulse 2.

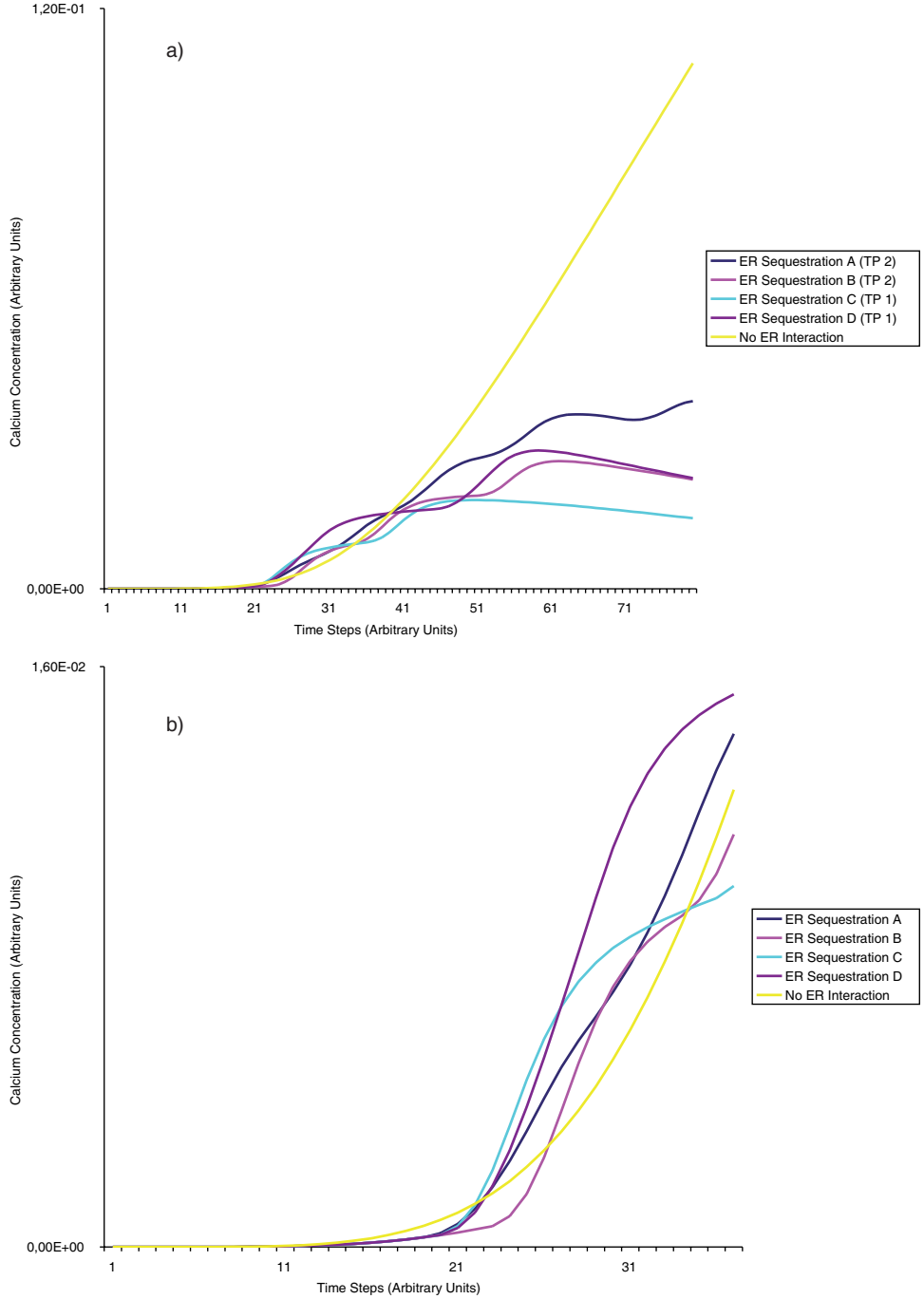

Fig. 7. – Time courses of cytosolic calcium: calcium is evaluated at the spine neck in all simulation runs. a) Depending on ER behavior and test pulse, the course of cytosolic calcium reacts to both these parameters. If the ER sequestration and release is completely inactive, we observe typical diffusion curves. b) In early ER release stages, the calcium signal propagates faster that in mere diffusion processes. This can be observed by the left-shift of the concentration time course.

4. – Discussion

The subcellular answer to synaptic activity triggered by action potentials is dominantly encoded in calcium waves. The propagation of calcium through the cell connects the synaptic activity with the cell nucleus where DNA is transcribed into specific genes. This communication pathway is regulated by the cytosolic and endoplasmic interaction through IP3 and ryanodine receptors.

In this paper we propose a mathematical model based on density-defined partial differential equations that take into account the cytosolic space and the endoplasmic reticulum as a calcium sink or source. The mathematical theory used in this model is highly independent of the imposed computational domain morphology and can easily be applied to three-dimensional geometries, although for a model study and proof of concept we restricted ourselves to two-dimensional testgeometries.

This model completes the gap between two models, one for the investigation of nuclear calcium signaling and one for three-dimensional signal propagation on the neuronal plasma membrane, and therefore links the electrophysiological scale with the subcellular signaling scale involved in learning, development and survival. We have taken into account the biological phenomenon of calcium-induced calcium release (CICR) which plays an important role in subcellular signaling.

From the proposed model we can investigate the effect of the ER on local calcium signals where it functions as an integrator of repetitive stimuli. Therefore the ER can store information of previous synaptic events, and can relay this information, encoded in the calcium release dynamics, to the nucleus. Furthermore, we see that the ER produces super-diffusion speeds which might account for fast communication between synapse and nucleus. These propositions will further be investigated in a three-dimensional setting and can include reconstructed cellular morphologies from microscopy data. The model presented here can eventually bring new insight into the physical properties of neurons and their components *dendrites, soma* and *axon*. Due to the combination of continuum mechanics theory and its implementation into state-of-the-art numerical methods for solving large systems of equations resulting from partial differential equations, we present a way to advance research in an interdisciplinary context.

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