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Review

## Electron-conformational model of ryanodine receptor lattice dynamics

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### Abstract

We propose a simple, physically reasonable electron-conformational model for the ryanodine receptor (RyR) and, on that basis, present a theory to describe RyR lattice responses to L-type channel triggering as an induced non-equilibrium phase transition. Each RyR is modelled with a single open and a single closed (electronic) state only, described utilizing a  $s = \frac{1}{2}$  pseudospin approach. In addition to the fast electronic degree of freedom, the RyR channel is characterized by a slow classical conformational coordinate,  $Q$ , which specifies the RyR channel calcium conductance and provides a multimodal continuum of possible RyR states. The cooperativity in the RyR lattice is assumed to be determined by inter-channel conformational coupling. Given a threshold sarcoplasmic reticulum (SR) calcium load, the RyR lattice fires due to a nucleation process with a step-by-step domino-like opening of a fraction of lattice channels, providing for a sufficient release to generate calcium sparks. The optimal mode of RyR lattice functioning during calcium-induced calcium release implies a fractional release with a robust termination due to a decrease in SR calcium load, accompanied by a respective change in effective conformational strain of the lattice. SR calcium overload is shown to result in excitation of RyR lattice auto-oscillations with spontaneous RyR channel opening and closure.

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**Keywords:** Calcium-induced calcium release; Ryanodine receptor/channel; Electron-conformational coupling; Auto-oscillation

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## 1. Introduction

In mammalian cardiac muscle, a large fraction of the intracellular activator calcium ( $\text{Ca}^{2+}$ ) is released from  $\text{Ca}^{2+}$  stores in the sarcoplasmic reticulum (SR) via the so-called  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) through ryanodine receptors (RyR; Bers, 2002). Ultra-structural studies suggest that the junctional SR contains nearly crystalline arrays of RyR, organized in clusters with several hundred (100–300) RyR (Franzini-Armstrong et al., 1999). These form a nearly square lattice with  $\sim 30$  nm inter-spacing of RyR. RyR channels appear to be homotetrameric units, each touching four neighbours with an FK-binding protein (FKBP) at, or near, the point of contact. These may promote co-ordinated activation of neighbouring channels (Marx et al., 2001). It is understood that the key initiator of CICR is  $\text{Ca}^{2+}$  entry via voltage-dependent sarcolemmal dihydropyridine (DHP)-sensitive L-type  $\text{Ca}^{2+}$  channels. Opposite to RyR, the DHP channels are located more randomly and in the T-tubular membrane at an average distance of 10 nm from RyR channels. The RyR:DHP ratio within T-tubules varies from 7:1 in rat, to 5.6:1 in human, and 5:1 in guinea pig (Bers, 2002), and may be variable even within a single species, cell type, cell, or T-tubule.

The main features of CICR that are usually characterized include stability, high gain, gradedness, adaptation, and termination of  $\text{Ca}^{2+}$  release. Two broad classes of models, termed by Stern (1992) ‘common-pool’ and ‘local control’ models, have been developed and used to study the fundamental properties of CICR. Most existing models use an effective medium theory, where  $\text{Ca}^{2+}$  concentrations in the sub-sarcolemmal space (or subspace,  $\text{Ca}_{\text{SS}}$ ) and in the SR lumen ( $\text{Ca}_{\text{lum}}$ ) are the main governing parameters that obey standard reaction–diffusion equations, while RyR gating is usually considered in a simplified manner through a dependence of the release on  $\text{Ca}^{2+}$  concentrations. Nevertheless, we are still far from a comprehensive understanding of the underlying mechanisms. Stern and Cheng (2004) point out that  $\text{Ca}^{2+}$  release via CICR “poses a *paradox of control*”, including a number of unresolved problems. The *paradox of termination* is one of the outstanding problems in the field of EC coupling. Some researchers point to inter-RyR communication and cooperativity to be key factors determining CICR features. At the supramolecular level, two mechanisms have been proposed for such a cooperativity: CICR and FKBP-coupled gating of RyR (Bers, 2002). The role of coupled gating in spark termination is still somewhat speculative, and it is by no means obvious how to represent it in biophysical terms. Moreover, some authors (Stern and Cheng, 2004) suggest that there is a missing qualitative aspect

of the problem that so far escaped identification. Thus, despite the success of local-control models in explaining many features of cardiac EC coupling, more work will be needed to provide a sound theoretical basis.

Our work was stimulated by a series of recent papers by Stern et al. (1999), Marx et al. (2001), Sobie et al. (2002), Soeller and Cannell (2004), Stern and Cheng (2004), and others, developing the local control concept and revealing the specific role of inter-channel coupling. The approach is based on a biophysical adaptation of the well-established theory of photo-induced structural phase transitions, which has been applied to different solids (Nagaosa and Ogawa, 1989). Here, we adopt the idea of conformational cooperativity (for review see Bray and Duke, 2004). A new, physically reasonable model of RyR channel and of the coupled RyR lattice is proposed, with a possible scenario of RyR gating dynamics (Moskvina et al., 2005). The proposed model captures several important features of the individual and cooperative behaviour of RyR.

## 2. Electron-conformational model for a single RyR channel

Our knowledge of molecular mechanisms of RyR channel functioning is limited; hence we are forced to start with the most general approach, which is typical for protein biophysics. Such an approach requires a reduction of the large variety of RyR degrees of freedom to only two: a fast and a slow one, conditionally termed electronic and conformational, respectively. Bearing in mind the main function of RyR channels, we assume only two actual electronic RyR states: “open” and “closed”, and a single conformational degree of freedom,  $Q$ , to be described by a classical continuous variable. We shall assume a simple harmonic approximation of the conformational energy and use a formula similar to Hooke's law for potential energy:  $E = \frac{1}{2}KQ^2$ , where  $K$  is the effective “elastic” constant and  $Q = 0$  relates to a base state. The electronic degree of freedom for RyR as nanoscopic species is assumed to be generally described quantum-mechanically, though the approach applied allows us to readily reduce the description to a classical one. The two electronic RyR states, open and closed, form a doublet and can be properly described within the framework of the so-called  $s = \frac{1}{2}$  pseudospin formalism, with “up” ( $\uparrow$ ) and “down” ( $\downarrow$ ) pseudospin states. All the processes involving these two states can be described by the energy operator, or (pseudospin) Hamiltonian, which can be expressed in terms of Pauli matrices (Moskvina et al., 2005).

Hereafter we assume that the conformational variable  $Q$  specifies the RyR channel “cross-section” or, more precisely, a permeability for  $\text{Ca}^{2+}$ , while the dichotomic electronic variable determines its opening and closure. This allows us to describe the  $\text{Ca}^{2+}$  flux through RyR as follows:

$$J_{\text{RyR}} = D(Q)(\text{Ca}_{\text{lum}} - \text{Ca}_{\text{ss}}) \quad (1)$$

if the channel is open, and  $J_{\text{RyR}} = 0$ , if it is closed. Here, the permeability coefficient  $D(Q)$  reflects the ease with which  $\text{Ca}^{2+}$  passes through an open RyR. Its functional dependence on the conformational coordinate should be one of essential model assumptions. Below we shall assume that the  $D(Q)$  is an increasing function of conformational coordinate, varying from zero to some saturated value  $D_0$  ( $0 < D(Q) < D_0$ ), where  $Q$  “oscillates” from large negative to large positive magnitudes, passing through some sub-conductive state at  $Q = 0$  with a conductance  $D(0) \approx \frac{1}{2}D_0$ .

As the simplest limiting case, we may consider the step-like dependence  $D(Q < 0) = 0$ ,  $D(Q \geq 0) = D_0$ .

Despite the above simplifications and obvious limitations of this approach, the model is very instructive in elucidating probable modes of behaviour both for a single RyR channel and a RyR lattice. It may even advance our understanding of ‘design principles’ which might have been adopted by cells during evolution.

As a starting point of the model we introduce the effective electron-conformational Hamiltonian for a single RyR channel as follows

$$H_{\text{RyR}} = -h_z \hat{s}_z - h_x \hat{s}_x - pQ + \frac{K}{2} Q^2 + aQ \hat{s}_z, \quad (2)$$

where  $\hat{s}_z = \frac{1}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}$ ,  $\hat{s}_x = \frac{1}{2} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}$  are Pauli matrices, and the first term describes the bare energy splitting of up and down (open and closed) states with an energy gap  $\Delta = h_z$ , while the second term describes the mixing of up and down pseudospin states. In terms of a pseudospin analogy, the  $h_{x,z}$  parameters may be associated with effective fields. It is worth noting that the quantum approach implies a nonzero  $h_x$  parameter, while given  $h_x = 0$  we arrive at a classical approach with a dichotomic electron variable. The third and fourth terms in (2) describe the linear and quadratic contributions to the conformational energy. Here, the linear term formally corresponds to the energy of an external conformational stress, described by an effective stress parameter  $p$ , while the quadratic term with the ‘‘elastic’’ constant  $K$  is associated with a conventional harmonic approximation for the RyR channel conformational energy. The last term describes the electron–conformational interaction where the parameter  $a$  is an electron–conformational coupling constant. Hereafter we make use of the dimensionless conformational variable  $Q$ ; therefore all of the model parameters ( $h_{x,z}$ ,  $p$ ,  $K$ ,  $a$ ) are assigned energy units. Our model Hamiltonian has the most general form, except the simplified expression for electron–conformational coupling, where we omit the term  $\propto Q \hat{s}_x$ . In the frame of adiabatic approximation, the eigenfunctions for the Hamiltonian can be written as follows:

$$\Psi_+ = \cos \alpha \cdot \Psi_{\uparrow} + \sin \alpha \cdot \Psi_{\downarrow}, \quad \Psi_- = \sin \alpha \cdot \Psi_{\uparrow} - \cos \alpha \cdot \Psi_{\downarrow},$$

where  $\Psi_{\uparrow, \downarrow}$  are open and closed electronic states and  $\tan 2\alpha = h_x/h_z - aQ$ . The corresponding eigenvalues

$$E_{\pm}(Q) = \frac{K}{2} Q^2 - pQ \pm \frac{1}{2} [(h_z - aQ)^2 + h_x^2]^{1/2} \quad (3)$$

define the upper and lower branches of the conformational potential (CP), respectively (see Fig. 1). These potential curves describe the energy of  $\Psi_{\pm}$  states as functions of conformational coordinate  $Q$ . What is the physical meaning of quantum superpositions  $\Psi_{\pm}$ ? Quantum mechanics states that the coefficients of these superposition yield the probability amplitudes to find the system in the bare ‘‘up’’ or ‘‘down’’ states. In other words, we may interpret that quantum superpositions  $\Psi_{\pm}$  define the specific mixed states of the RyR channels when they appear to be open with a probability  $P_{\text{open}}^+ = P_{\uparrow}^+ = |\cos \alpha|^2$  for upper branch of the CP, and  $P_{\text{open}}^- = P_{\uparrow}^- = |\sin \alpha|^2$  for lower branch of the CP, respectively.

The effect of different model parameters on the shape of the CP can be illustrated, if we neglect the quantum effects ( $h_x = 0$ ) and deal with two ideal parabolas, describing the conformational

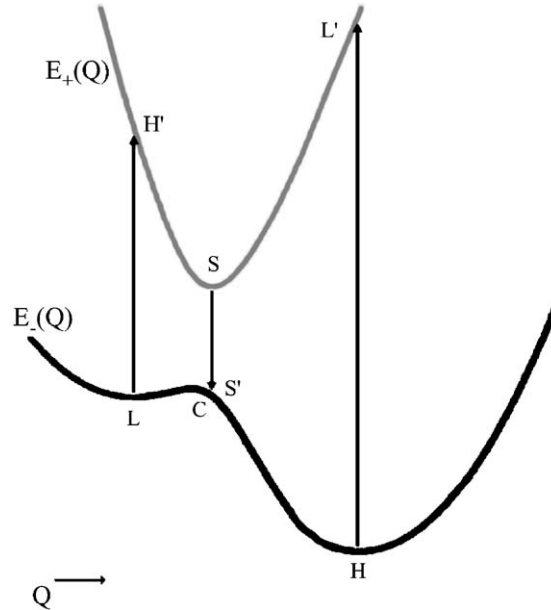


Fig. 1. Example of the conformational potential of a RyR channel (see text for details).

energy for open and closed states,  $\Psi_{\uparrow,\downarrow}$ , respectively. These identical parabolas with minima at  $Q_{\pm}^{(0)} = (p \pm (a/2))/K$  are shifted relative to each other. The shift in the conformational coordinate is  $\Delta Q = Q_{+}^{(0)} - Q_{-}^{(0)} = a/K$ , while the shift in energy is  $\Delta E^{(0)} = E_{+}^{(0)} - E_{-}^{(0)} = -pa/K + h_z$ , where  $E_{\pm}^{(0)} = E(Q_{\pm}^{(0)})$  or the energy of the minima of the respective parabolas. Interestingly, the energy shift is determined by both “mechanic” and “electronic” forces. It is worth noting that the  $Q$ -shift of the “center of gravity” of CP is determined by the effective stress,  $p$ , that in the framework of our model means the appropriate shift of the RyR channel conductance. The electron–conformational coupling leads to a pulling-apart of the parabolas for open and closed electronic states, thus separating the appropriate RyR conductances. The condition:  $|\Delta E^{(0)}| = |-pa/K + h_z| = a^2/2K$  defines two specific points, where the minimum of one of the branches crosses another branch, thus specifying the parameters range permissive of bistability effects.

In the quantum case with  $h_x \neq 0$  we arrive at a crucial rearrangement of CP near the cross-section point of two branches, with a number of new effects of principal importance. We obtain two isolated branches of CP (upper and lower), with quantum superpositions  $\Psi_{\pm}$ , which describe unconventional RyR channel states (see Fig. 1). The upper branch of CP has a single minimum point  $S$  with an approximate “fifty-fifty” of composition  $\Psi_{\uparrow,\downarrow}$  in the respective superposition state  $\Psi_{+}$ . In other words, this represents the  $P_{\text{open}}^{+} \approx 0.5$  “semiconductive” state. The  $S$  point separates the upper branch of the CP into two halves with a high- $P_{\text{open}}$  (left) and a low- $P_{\text{open}}$  (right). In contrast, the  $C$  point, which is opposite to point  $S$  in the lower branch of the CP, separates the branch into a low- $P_{\text{open}}$  half on the left and a high- $P_{\text{open}}$  half on the right. The lower branch of the CP has either a single minimum, or a two-well structure, the latter leading to bistability (see Fig. 1). Moreover, the latter leads to the formation of quantum tunnelling states as a result of the tunnelling between two wells of the CP. The two wells of the CP may be associated with two

important conformations of RyR channel: closed and open. In this case, our analysis shows the origin of these conformations and its subtle structure as electron–conformational entities. The extreme points  $L$  (low  $P_{\text{open}}$ ),  $H$  (high  $P_{\text{open}}$ ),  $C$ , and  $S$  are of primary importance for our analysis. The system is locally stable in  $L$ ,  $S$ , and  $H$ , though the  $L$ -mode is metastable and the  $H$ -mode is globally stable. The  $L$ - and  $H$ -modes are theoretically stable at zero temperature, while the transiently refractory  $S$ -mode is unstable—even at zero temperature—because of the spontaneous decay to the  $S'$ -mode. In fact, given large values of the effective fields  $h_{x,z}$ , the system does not manifest bistability, i.e. the lower branch of conformational potential has only a single minimum,  $L$ , or  $H$ . The points  $L$ ,  $H$ , and  $S$  correspond to the three stationary states of the RyR channel, with distinct properties. The stationary transient “half-open” state  $S$  of the upper CP branch seems to play an essential role as an intermediate semiconductive state in the electronic RyR triggering via the L-type channels, while the two stationary states of the lower branch would be identified with the so-called high and low open probability  $P_{\text{open}}$  modes ( $H$ - and  $L$ -modes). Depending on parameter values, the respective modes are expected to be locally or globally stable, though one cannot exclude the possibility of degeneracy when both modes correspond to the same energy, however with different conformational coordinates.

The results of numerous experiments suggest that changes in  $\text{Ca}^{2+}$  concentration on either side of the RyR receptor can modulate  $\text{Ca}^{2+}$  release (Bers, 2002). Up to this point, the proposed model does not capture all subtleties of  $\text{Ca}^{2+}$ –RyR interaction from both the subspace and the luminal sides. Hereafter, we assume a purely electronic character of the interaction between  $\text{Ca}^{2+}$  stimulus via L-type channel, and, in turn,  $\text{Ca}_{\text{SS}}$  and RyR-channel gating. The most general form of such an interaction (L–RyR) is described by the effective pseudospin Hamiltonian

$$H_{\text{L-RyR}} = -h_z(\text{Ca}_{\text{SS}}(t))\hat{s}_z - h_x(\text{Ca}_{\text{SS}}(t))\hat{s}_x \quad (4)$$

with two time-dependent parameters that are assumed to comprise all the information about the  $\text{Ca}_{\text{SS}}$  binding to RyR. The interaction of L-type channel and RyR, or of  $\text{Ca}_{\text{SS}}$  and RyR, can be incorporated to the total Hamiltonian by a simple renormalization of the respective electronic (pseudo-spin) parameters  $h_z$  and  $h_x$ . This consideration offers a first attempt to describe the L–RyR coupling due to  $\text{Ca}^{2+}$  binding in terms of a time-dependent perturbation scheme. The first, or “diagonal” term of this interaction is responsible only for the energy splitting effects, and does not yield “up–down” electronic transitions, while the second “off-diagonal” term can provide both the energy splitting and “up–down” mixing, or transition effects. At present we cannot distinctly specify the relative roles of diagonal and off-diagonal terms in effective L–RyR coupling. However, it seems likely that the off-diagonal term plays a key role in the  $\text{Ca}^{2+}$  regulation on the subspace-side of the RyR channel. A simple picture of  $\text{Ca}^{2+}$  stimuli implies the presence of a fast component, or  $\text{Ca}^{2+}$  spike, and a slow background component. The former is believed to be responsible for the up–down excitation, or electronic transition effects, while the latter may underlie the energy splitting and mixing effects, providing some adaptation phenomena. Interestingly, the steep rise of the off-diagonal  $h_x$  term with  $\text{Ca}_{\text{SS}}$  levels contributes to the lowering the energy barrier between  $L$ - and  $H$ -modes, thus promoting RyR channel transitions from the metastable to the globally stable state.

In contrast with purely electronic L–RyR coupling, the effect of relatively slowly varying  $\text{Ca}_{\text{lum}}$  on the RyR channels will be assumed to be purely “mechanical”, through the respective

conformational stress applied to RyR channels. The effect can be incorporated to the total Hamiltonian, if we assume the stress parameter,  $p$ , to be a function of  $\text{Ca}_{\text{lum}}$  or the lumen-to-subspace  $\text{Ca}^{2+}$  gradient ( $\text{Ca}_{\text{lum}} - \text{Ca}_{\text{SS}}$ ). Such a concept is based on evidence that RyR gating might be modulated by the  $\text{Ca}^{2+}$  level on the luminal side of the channel, so that RyR opening is favoured when  $\text{Ca}_{\text{lum}}$  is high (Bers, 2002). It should be noted that in the framework of an electron–conformational model, the  $\text{Ca}_{\text{lum}}$ -dependence of effective stress parameter,  $p(\text{Ca}_{\text{lum}})$ , specifies the  $\text{Ca}_{\text{lum}}$  dependence of  $Q_{L,H}$ , and, respectively, the magnitude of the RyR conductance  $D(Q_{L,H})$  at extreme points of the CP.

Under the adiabatic approximation, the dynamics of a single RyR channel consist of the adiabatic motion of the conformational subsystem and electronic up–down transitions. The classical dynamics of the conformational mode for RyR channel is assumed to obey the conventional Langevin equation of motion

$$\ddot{Q} = - \frac{\partial}{\partial Q} E_{\pm}(Q) - \Gamma_{\pm} \dot{Q} + \eta(t), \quad (5)$$

where  $\Gamma$  is the effective dimensionless friction constant, and  $\eta$  is the thermal fluctuation force (Gaussian–Markovian noise). The last two terms reflect the coupling to an external environment of channels. We believe that the effect of thermal fluctuations for a “heavy” conformational variable may be negligibly small, hence the thermal and quantum probabilistic effects are significant only for electronic degree of freedom. It should be noted that in general, the relaxation dynamics within the upper and lower branch of CP can differ substantially, with fast processes for the former and slow ones for the later.

Our model implies three types of transitions in a single RyR channel: (i) direct electronic transitions; (ii) indirect transitions; and (iii) thermal transitions. The direct electronic transitions imply rapid Franck–Condon *electronic* transitions  $L \rightarrow H'$   $H \rightarrow L'$  see (Fig. 1) with RyR channel activation (inactivation) due to the binding (dissociation) of  $\text{Ca}^{2+}$  ions to (from) the protein. According to the Franck–Condon principle, the motion of the lattice is negligible during such an electronic transition, so the transition is represented by the vertical arrows in Fig. 1. Along with these induced transitions, we introduce spontaneous Franck–Condon transitions between the upper and lower branches of the conformational potential. It should be noted that the probability of a non-resonant electronic transition under the step-like L-type channel signal is expected to depend on the value of conformational coordinate  $Q$ , and to obey the power law:  $P \propto (E_+ - E_-)^{-2}$ . Hereafter, we shall assume that L-type channel stimuli can trigger Franck–Condon transitions between the lower and upper branches of the conformational potential. The direct electronic  $L \rightarrow H'$  ( $H \rightarrow L'$ ) excitation shifts the RyR from one of the local minima of the lower branch to the upper branch. The subsequent relaxation proceeds in three steps. First, the system quickly relaxes according to Eq. (5) with  $E_+$  from Eq. (3) to its minimum within the time of the order of  $\Gamma_+^{-1}$  ( $\Gamma_+$ ) when  $\Gamma_+$  is large (small). The next step implies a spontaneous  $S \rightarrow S'$  transition, after which the system starts to slowly relax at the lower branch  $E_-$  of CP (see Eq. (3)). The inequality  $\left. \frac{\partial}{\partial Q} E_-(Q) \right|_{S'} < 0$  ( $> 0$ ) defines the condition that after spontaneous transition the channel ‘finds itself’ positioned to left (to right) of the maximum ( $C$ ) of the lower CP branch moving to an  $L$  ( $H$ ) state, respectively. The *indirect* transitions  $L \rightarrow H$  imply induced quantum tunnelling through the energy barrier. The respective probability is expected to be strongly reduced due to the overlap of respective vibronic functions. Its magnitude strongly depends on the  $L$ – $H$ -energy

separation; maximal probability is expected for resonance conditions. The relative role of different mechanisms of  $L \rightarrow H$  (closed-open) switching, whether due to a complex pathway  $L \rightarrow H' \rightarrow S \rightarrow S' \rightarrow H$  through two *direct* electronic transitions or apparently shorter indirect transition  $L \rightarrow H$ , will depend on the time course of the L-type channel trigger signal. In any case, one has to bear in mind that an apparently more complex  $L \rightarrow H' \rightarrow S \rightarrow S' \rightarrow H$  four-step transition is able to provide the fastest RyR channel triggering due to ‘assistance’ by the upper CP branch, characterized by very short relaxation times. In general, the classical equations of motion for conformational modes should be complemented by the equation which describes transitions between different branches of a CP.

The *thermal* transitions are caused by the thermal fluctuating force and free from the Franck–Condon principle. They occur only on the lower branch of the conformational potential with the probability proportional to the thermal activation factor:  $P = \tau^{-1} e^{(-\Delta E/kT)}$ , where  $\Delta E$  is an activation energy, and  $\tau^{-1}$  is an attempt frequency. The whole system will finally relax to the thermal equilibrium.

### 3. Cooperative dynamics of the RyR lattice

The effective Hamiltonian of the RyR lattice can be written as a superposition of single channel Hamiltonians (2), and an inter-channel conformational coupling, which can be described as follows:

$$H_{\text{RyR-RyR}} = -\frac{1}{2} \sum_{m,n} K_{mn} Q_m Q_n, \quad (6)$$

where  $m, n$  labels different RyR channels, and  $K_{mn}$  is an inter-channel conformational coupling constant. One of the most familiar theoretical approaches to treat the lattice Hamiltonian implies making use of the mean-field approximation (MFA). However, MFA implies uniform transformations in a system, and neglects any fluctuations, which are important for the short-range conformational coupling  $K_{mn}$ . At the same time, there are some hints to the spatially non-uniform RyR gating which resembles the behaviour of systems near the first-order phase transition with a nucleation of droplets of a new phase and a phase separation. Nucleation of critical droplets in a metastable phase has been attracting much attention in both physics and chemistry. As a remarkable example of such a crucial manifestation of strong correlations we refer to the photo-induced nucleation theory (Nagaosa and Ogawa, 1989), developed to describe the structural phase transition between the two locally stable structural phases, where the structural change is brought about by the relaxation after photo-excitation of the system. We believe that such a model approach could be instructive for the description of cooperative RyR dynamics.

To illustrate the cooperative dynamics of a RyR lattice in the framework of electron–conformational models, we simulated dynamics within  $11 \times 11$  square RyR lattice after excitation of a single, centrally located RyR channel ( $m = 0$ ). The channels dynamics have been simplified (no transfer between CP branches). Their in-branch dynamics are assumed to be governed by eq. (5) with no temperature fluctuations ( $\eta = 0$ ) and equal relaxation rates:  $\Gamma_+ = \Gamma_- = \Gamma$ . We studied such simplified Newtonian dynamics of the RyR-lattice as a function of two parameters: effective stress  $p$  and conformational cooperativity constants  $k$ , specifying nearest neighbour inter-channel

coupling ( $K_{mn} = k$ , if  $m, n$  are nearest neighbours, otherwise  $K_{mn} = 0$ ). The remaining parameters were as follows:  $h_x = 0.1$ ,  $h_z = 0$ ,  $a = 2.5$ ,  $K = 2$ ,  $\Gamma = 5$ . Free boundary conditions have been applied. At an initial point  $t_0 = 0$  all channels (excluding the central one) were closed (or, more precisely, located at point  $L$  of the lower CP branch, thus having the conformational coordinate  $Q_m = Q_L$ ). The central channel  $m = 0$  was at the point  $H'$  on the upper CP branch to imitate opening due to a  $\text{Ca}^{2+}$  stimulus from an opposing L-type channel. After the relaxation to point  $S$  at the instant  $t = 20$  (all time steps in [ms]), the central channel was instantly moved to the  $S'$  point on the lower CP branch that imitated spontaneous  $S-S'$  transition. Subsequently, all channels relaxed within the lower CP branch. The lattice dynamics are described by means of a system of 121 ordinary differential equations

$$\ddot{Q}_m = - \frac{\partial}{\partial Q_m} E_*(\{Q\}) - \Gamma \dot{Q}_m, \quad (7)$$

where

$$E_*(\{Q\}) = \sum_m E(Q_m) + \sum_{(n,m)} K_{nm} (Q_m - Q_n)^2 \quad (8)$$

given

$$E(Q_m) = \frac{K}{2} Q_m^2 - p Q_m \pm \frac{1}{2} [(h_z - a Q_m)^2 + h_x^2]^{1/2} \quad (9)$$

with initial conditions:  $Q_m(0) = Q_L$ ,  $\dot{Q}_m(0) = 0$ . The “+” sign in (9) is used for the only case where  $m = 0$  and  $t \in [0, 20]$ . The system was then represented as the system of 242 first order differential equations and was solved by the Euler method with an integration time step  $\Delta t = 0.01$ .

## 4. Different modes of a RyR lattice behaviour

### 4.1. Fixed external conditions

Behaviour of the RyR lattice turns out to be crucially governed by the strength of the inter-channel conformational coupling  $k$ , and the effective stress  $p$ . Depending on their values, we arrive at three qualitatively different modes of RyR lattice dynamics after triggered opening of the central channel: (a) every channel goes back to the low- $P_{\text{open}}$  state (phase I); (b) only the excited central channel transfers to high- $P_{\text{open}}$  state (phase II); and (c) all the channels transfer to high- $P_{\text{open}}$  state (phase III; see Fig. 2 for an appropriate  $(p, k)$ -phase diagram). Phase I is equivalent to the inactivated phase, phase II is a single-channel opening, and phase III is a high gain multiple opening phase with a high degree of cooperativity. The curve separating the phases I and II, III is defined by the condition  $\left. \frac{\partial}{\partial Q} E_-(Q) \right|_S = 0$ , meaning that after spontaneous transition the central channel is located at the maximum of the lower CP branch.

In phase I (II, III) the central channel is positioned to left (to right) of the maximum, moving to  $L$  ( $H$ ) states, respectively. However, in phase II the effect of the central channel on its neighbours appears to be insufficient to make them overcome the energy barrier so they remain at the left of the  $C$  point in the low- $P_{\text{open}}$  state. In contrast, in phase III the effect of the central channel on its nearest neighbours appears to be large enough, to make them overcome the energy barrier and

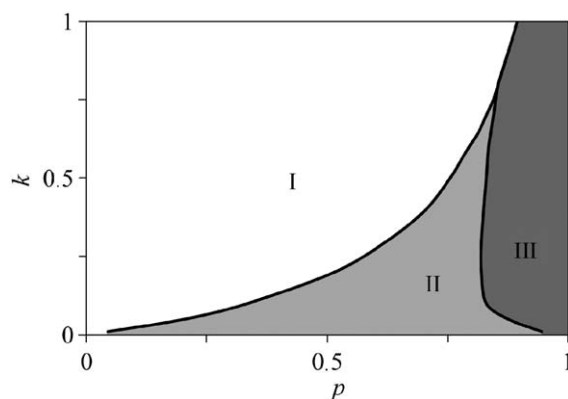


Fig. 2. The  $(p, k)$ -phase diagram, showing three phases of  $11 \times 11$  square RyR lattice dynamics: inactivation (phase I), single RyR channel activation (phase II), domino-type lattice activation (phase III).

move to the  $H$  point, or high- $P_{\text{open}}$  state, inducing such a transfer in their neighbours and so on. In other words, in phase III one deals with an avalanche-like process of a step-by-step opening of all the RyR channels triggered by a stimulus via the L-type channel. Such a domino effect is illustrated in Fig. 3, where we show step-by-step opening of RyR channels, induced by the opening of the central channel. The domino effect and phase III appear only with parameters that render the system close to bistability. It takes place where the potential barrier  $\Delta E_{LC}$  is small, and the metastable mode is fragile. It is worth noting that in the given case this is true at rather large effective stress  $p > p^* \approx 0.83$ . Finally, one may conclude that mode III is associated with L-type channel induced non-equilibrium phase transitions from closed to open RyR channels, proceeding through the nucleation phenomenon.

A closer analysis of  $(p, k)$ -phase diagram illustrates that the rise of stress can effectively shift the system from phase I to phase II, and finally to phase III. Thus, variations in effective stress can govern the RyR lattice gating (both opening and closure). Below we show the effective stress  $p$  to be a main factor through which the luminal  $\text{Ca}^{2+}$  governs the RyR channel gating, in particular, the termination effect. The *paradox of termination* of  $\text{Ca}^{2+}$  sparks due to the closure of the RyR continues to be one of the controversial problems in CICR. Simulating RyR lattice firing termination (closure), all the channels were located in a high- $P_{\text{open}}$   $H$ -mode with an initial value of the effective stress of  $p_0 = 0.85$  and a conformational cooperativity constant of  $k = 0.4$  (corresponding to phase III). Then the effective stress decreased linearly with time according to the relation:  $p = p_0 - 0.1(p_0 - p_1)t$  when  $t \in [0, 10]$ , and was held constant with  $p = p_1$  at  $t > 10$  to imitate a threshold to which the stress goes down in a process of  $\text{Ca}^{2+}$  release from SR. Solving the system (7)–(9) with initial conditions  $Q_m(0) = Q_H$ ,  $\dot{Q}_m(0) = 0$ , we obtain two characteristic modes of RyR lattice dynamics. If  $p_1 > p_1^* \approx -0.96$ , all channels change their conformational coordinate  $Q$ , however stay open (not overcoming the energy barrier between open and closed states). With  $p_1 < p_1^*$  the energy barrier disappears, and the  $H$  point collapses, which results in a simultaneous transition of all channels to a low- $P_{\text{open}}$  mode and sharp closure of the RyR lattice. This demonstrates a possible mechanism of termination of RyR excitation, due to functional coupling between luminal  $\text{Ca}^{2+}$  levels and effective stress. In the framework of our model, the

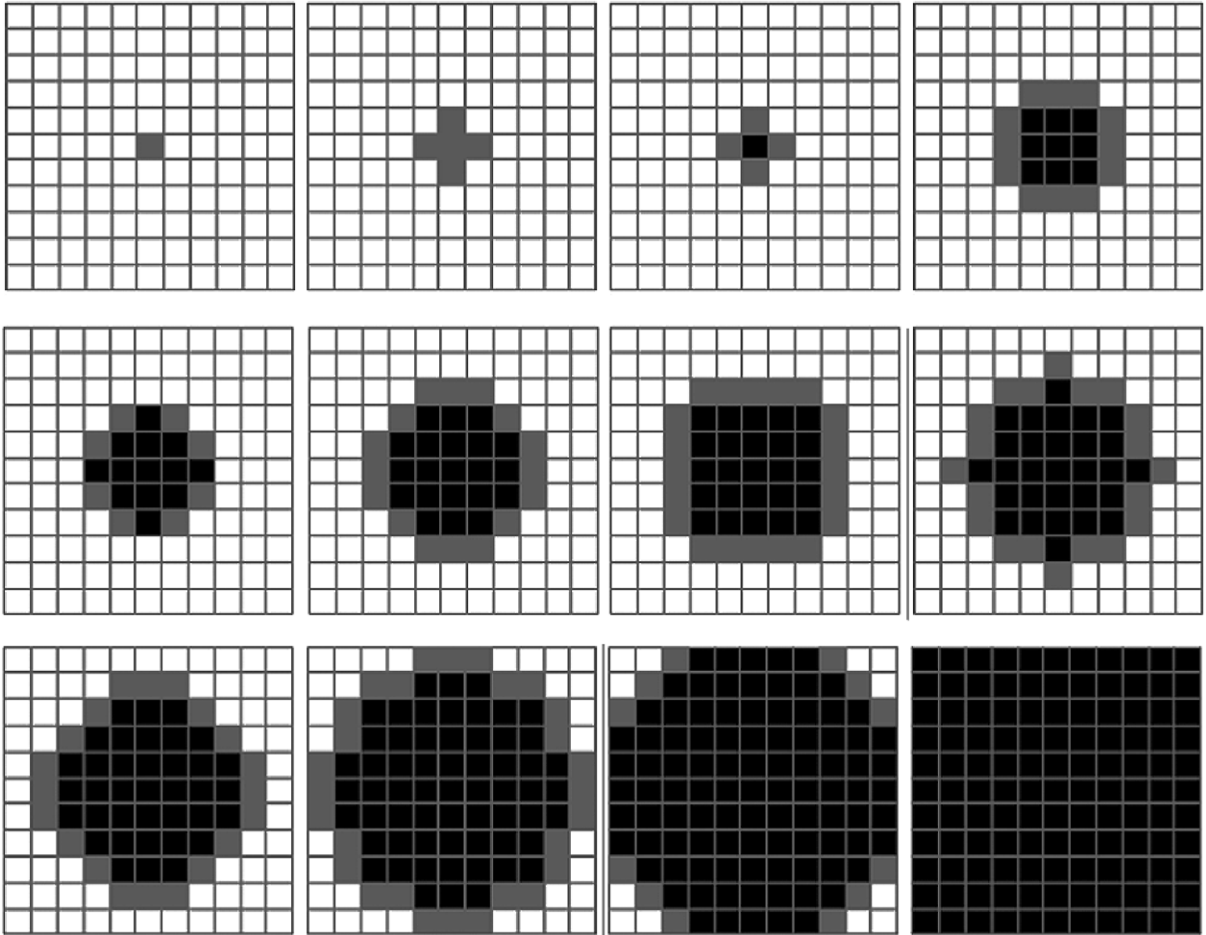


Fig. 3. Illustration of the step-by-step ( $t = 10, 25, 41, 45, 47, 48, 49, 51, 52, 55, 58, 65$  ms) domino-like opening of RyR channels. Black and white filling points to open and closed RyR channels, respectively, grey marks RyR channels which are transferring to the open state.

termination is determined by a stabilization of the closed state due to the stress decrease-induced shift of CP parabolas. It is worth noting that the shift is accompanied by an increase in  $L-H'$  excitation energy and, thus, leads to diminution of the excitation probability.

#### 4.2. RyR gating under CICR conditions

The above simulations demonstrate in vitro-like dynamics of RyR lattice when the surrounding  $Ca^{2+}$  is assumed to be a source of effective external fields (effective stress). Hereafter we address the in vivo-like situation where the RyR lattice dynamics are accompanied by a complex process of CICR. In this case, we deal with  $Ca^{2+}$ -dependent field parameters and positive/negative feedback effects.

Electron–conformational dynamics of the RyR lattice are assumed to specify the flux from the SR to the subspace through RyR channels as a sum of individual channel fluxes:  $J_{\text{rel}} = \sum_m D(Q_m) \cdot (Ca_{\text{lum}} - Ca_{\text{ss}})$ . For simplicity, we assume a step-like  $Q$ -dependence of the RyR channel conductivity, so that the channel is open (closed) being located to right (left) of the  $C$  point on the lower CP branch. Then we can introduce an average conductivity for open channels,  $D_0 = 1 \text{ ms}^{-1}$ , and obtain  $\sum_m D(Q_m) = D_0 N_{\text{open}}$ , where  $N_{\text{open}}$  is a number of open RyR channels.

Because of our main goal is to uncover the role of RyR lattice gating in CICR, we use a simplified model of  $Ca^{2+}$  dynamics (e.g. Sobie et al., 2002). The  $Ca^{2+}$  fluxes were assumed to obey a standard system of two differential equations with typical parameters used for  $Ca^{2+}$  dynamics modelling (e.g. Bers, 2002).

$$\frac{dCa_{\text{ss}}}{dt} = \left[ 1 + \frac{K_{dB} \cdot B_{\text{tot}}}{(K_{dB} + Ca_{\text{ss}})^2} \right]^{-1} (-J_{\text{diff}} + J_{\text{rel}}), \quad (10)$$

$$\frac{dCa_{\text{lum}}}{dt} = \left[ 1 + \frac{K_{dC} \cdot C_{\text{tot}}}{(K_{dC} + Ca_{\text{lum}})^2} \right]^{-1} (J_{\text{refill}} - \delta \cdot J_{\text{rel}}), \quad (11)$$

where  $J_{\text{diff}} = k_{\text{diff}}(Ca_{\text{ss}} - Ca_c)$  and  $J_{\text{refill}} = k_{\text{refill}}(Ca_{\text{SR}} - Ca_{\text{lum}})$  are diffusion fluxes from subspace to cytosol, and between the SR net and the SR lumen;  $k_{\text{diff}} = 100 \text{ ms}^{-1}$  and  $k_{\text{refill}} = 0.05 \text{ ms}^{-1}$ ;  $\delta = 0.02$  is a relative volume of subspace with regard to the luminal one,  $K_{dC} = 630 \mu\text{M}$ ,  $C_{\text{tot}} = 30000 \mu\text{M}$ ,  $K_{dB} = 0.7 \mu\text{M}$ ,  $B_{\text{tot}} = 1200 \mu\text{M}$  are model parameters specifying the processes of  $Ca^{2+}$  binding to calsequestrin in the lumen and with  $Ca^{2+}$ -ligands in subspace. The cytosolic  $Ca^{2+}$  concentration ( $Ca_c$ ) was held constant  $Ca_c = 0.1 \mu\text{M}$ , while  $Ca^{2+}$  concentration in the SR net ( $Ca_{\text{SR}}$ ) was varied in a range of 500–2000  $\mu\text{M}$ . Initial values of subspace and luminal  $Ca^{2+}$  concentrations were  $Ca_{\text{ss}}(0) = Ca_c$  and  $Ca_{\text{lum}}(0) = Ca_{\text{SR}}$ , respectively.

The effective stress  $p$  was assumed to rise with the luminal  $Ca^{2+}$  concentration in accordance with the Hill curve:

$$p = 2 \frac{[Ca_{\text{lum}}]^n}{[Ca_{\text{lum}}]^n + K_{Ca}^n} - 1, \quad (12)$$

where  $K_{Ca} = 600 \mu\text{M}$  is the half maximal value,  $n = 6$  is a Hill coefficient.

The system demonstrated four different modes of behaviour, depending on  $Ca_{\text{SR}}$ , or SR load. If  $Ca_{\text{SR}} \leq 800 \mu\text{M}$  (or at rather small SR load), it arrived at an *inactivation* mode I, where the central channel opened after stimulation and then closed with no noteworthy effect on neighbouring closed channels. With further increase in the SR load, up to a  $Ca_{\text{SR}} \approx 900 \mu\text{M}$ , the system turns to a *single channel activated* mode II, where stimulation leads to stationary opening of the central channel only. In this case the dynamic balance between  $J_{\text{rel}}$  and  $J_{\text{refill}}$  is maintained and the effective stress lowers insufficiently to cause closure of the central channel. With further increase in the SR load, up to  $Ca_{\text{SR}} \approx 1175 \mu\text{M}$ , the system turns to a *domino-like firing–termination* mode III with high degree of cooperativity, where stimulation causes step-by-step opening of the central channel and its neighbours, with formation of a cluster composed of  $\leq 40\%$  open RyR. The process results in an effective high-gain  $Ca^{2+}$  release from the SR lumen (Fig. 4a). However, the decrease in SR load leads to a lowering of the effective stress, accompanied by the shift of the

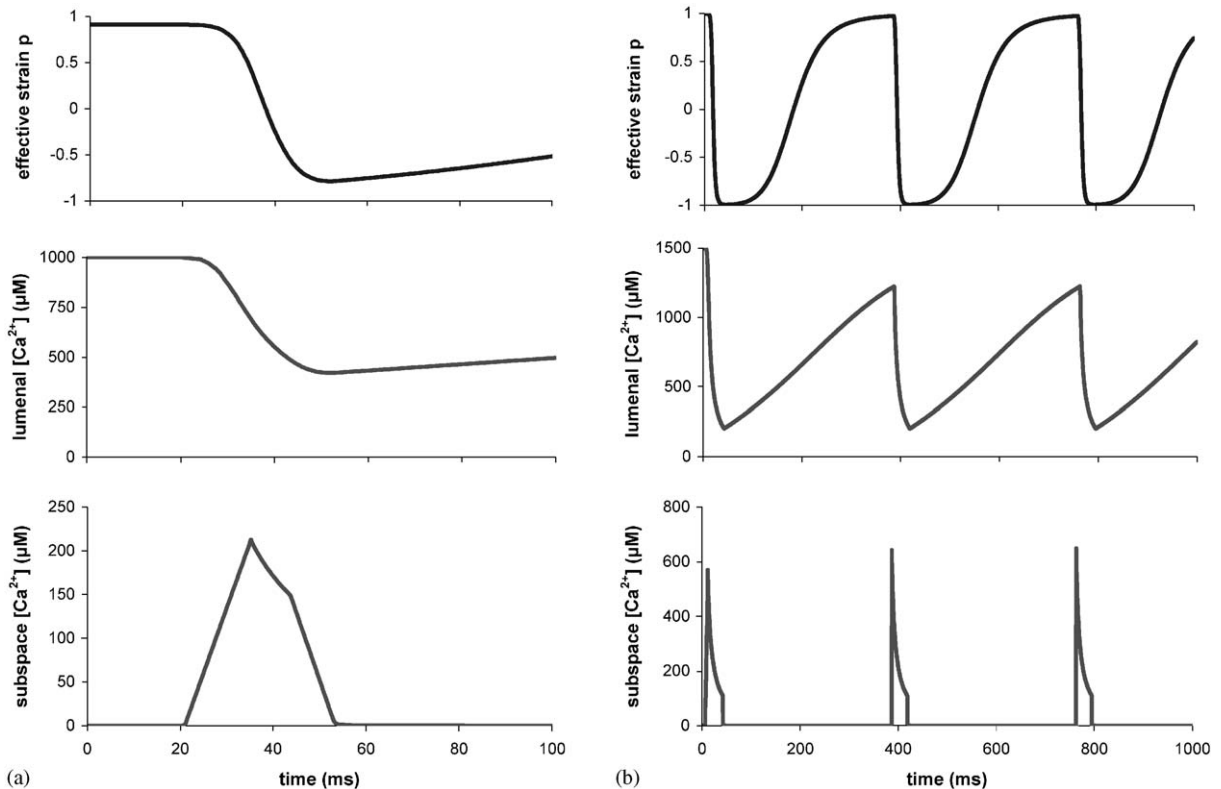


Fig. 4. Simulation of the time course of effective stress  $p$  (top panel),  $Ca_{lum}$  (middle panel), and  $Ca_{SS}$  (bottom panel) after single RyR channel triggering for two different  $Ca_{SR}$  load: a—Mode III ( $Ca_{SR} = 1000 \mu M$ ); b—Mode IV, or auto-oscillation regime ( $Ca_{SR} = 1500 \mu M$ ).

system to preferable channel closure. This negative feedback effect results, first, in a slowing down of the nucleation process and, second, in an evolution of an *inverse domino-like* effect with full collapse of the cluster of open RyR and termination of  $Ca^{2+}$  release. Such a fractional release seems to be more preferable compared to an all-or-none response, because it affords both stability and adaptation.

Our model simulation offers an explanation for the *termination paradox* and explains several other features of CICR, in particular, the delay between the  $Ca^{2+}$  stimulus and SR release (Zahradnikova et al., 1999) as a result of a finite velocity of a conformational spread in the RyR lattice (see Fig. 4a), the partial SR depletion during release (Shannon et al., 2003) (see Fig. 4a), the influence of SR load on the amount of  $Ca^{2+}$  released with a threshold load required for a nonlinear high-gain  $Ca^{2+}$  release in mode III (Shannon et al., 2000). It appears that, within the framework of electron–conformational models, RyR regulation by luminal  $Ca^{2+}$  provides optimal CICR only within a certain range of SR loads.

SR overload can result in the excitation of RyR lattice auto-oscillations (Fig. 4b). Indeed, if  $Ca_{SR} \geq 1175 \mu M$ , stimulation of the central channel causes a domino-like opening of a cluster of RyR channels, and effective  $Ca^{2+}$  release that, in turn, causes a lowering of the effective stress and

simultaneous closure of channels before the luminal concentration starts rising. However, until  $Ca_{lum}$  approaches the initial value, at a critical concentration all channels re-open simultaneously, and  $Ca^{2+}$  release is repeated spontaneously. This behaviour is repetitive, i.e. the system turned out to behave in an *auto-oscillation mode IV* with a spontaneous SR  $Ca^{2+}$  release which has been observed experimentally (see, e.g. Shannon et al., 2000).

## 5. Discussion

We propose a simple electron–conformational model and a theory to describe RyR lattice responses to L-type channel triggering as an induced non-equilibrium phase transition. The main feature of the model consists of the assumption of only two essential degrees of freedom for a RyR channel, fast electronic and slow conformational. The former is governed predominantly by  $Ca^{2+}$  entering through L-type channels ( $Ca_{SS}$ ), and the latter—by luminal  $Ca^{2+}$  or lumen-subspace  $Ca^{2+}$  gradients. In contrast to conventional models, however, we deal with a *continuum* of possible RyR channel states. We illustrate the model’s ability to resolve the *paradox of local control* and to reproduce several features of CICR, including gradedness, coupled gating, irreversibility, inactivation/ adaptation, and spark termination. The model predictions further suggest that intra-channel electron–conformational and inter-channel conformational coupling (rather than simply  $Ca^{2+}$  regulation) are key mechanisms of multistability, cooperativity and large-scale integrative behaviour of RyR lattices. We have shown that the SR load may strongly affect RyR lattice gating and that it may play a crucial role in regulating the SR  $Ca^{2+}$  release process. Given a threshold SR load, the RyR lattice fires due to a nucleation process, or a step-by-step domino-like opening of RyR channels, providing the extremely large amplification, or gain, of the cluster release. The typical mode III of RyR lattice functioning in a CICR process implies fractional release with a robust termination, due to the essential decrease in the local SR level and respective change in the effective stress of the RyR lattice. SR overload leads to an unconventional auto-oscillation regime with periodic spontaneous  $Ca^{2+}$  release.

Our physical modelling approach does not contradict the more usual one based on Markov chain (kinetic) models, which imply a discrete set of RyR states (open, closed, etc.) with a transition probabilities matrix. Strictly speaking, Markov chain models merely imply specific approximations for transition probabilities between different states of a system, rather than a physical model which starts from simplifying predictions regarding the structure of a system, the most important degrees of freedom (variables), the interactions between components, etc. Any physical model is primarily focused on understanding/revealing the spectrum of possible states for a system, while Markov models usually pre-assume states and addresses their coupling to an external source of stochasticity. Once a physical model is formulated, and possible states are specified, one may relate this information to simple Markov models (e.g. to approximate transition probabilities), or turn to potentially more appropriate non-Markovian models. However, current insight into the ultra-structure of ion channels is limited. For this reason, one is usually prone to start straight off from kinetic Markov model with a set of predetermined states. In contrast to simple kinetic Markov models, our physical model holds the potential of providing a deeper understanding of mechanisms involved in RyR functioning. In fact, it could provide a better rationale for conventional Markovian models and the magnitudes of transition

probabilities. In simple cases, one may obtain an analytical link between both types of approaches (see, e.g. Rice et al., 2003). As in conventional models of CICR, we use a number of phenomenological parameters, which should be fitted in comparison with experimental data, and predictions need to be experimentally validated. This is an aim of ongoing work; the number of model parameters that needs to be defined would appear to be reasonably small for such a complicated system, and they may be open to straightforward *physical* interpretation.

A primary goal of the paper was to formulate an electron–conformational model of RyR lattice dynamics, and to explore whether the model, in its simplest form, could reproduce principal features of a single RyR and RyR lattice function. While the model has shown the potential of a physical approach, several questions remain unanswered. In particular, this concerns the role of quantum effects, mainly a quantum probabilistic viewpoint on the RyR gating. Our assumption of the “electronic” role of  $Ca_{SS}$  against the “conformational” role of  $Ca_{lum}$  may only be resolved by further modelling in conjunction with careful experimental work. Other open questions relate to the number and relative gating behaviour of DHPR and RyR. The model simulations presented here naturally involved a number of simplifications and limitations. First, the model simulations lack random forces in the Langevin conformational dynamics, thus reducing it to a Newtonian one. We believe that the effect of thermal fluctuations for a probably “heavy” conformational variable may be negligibly small, hence the thermal and quantum probabilistic effects are assumed to be significant only for electronic degree of freedom. Another limitation is the simplified  $Ca^{2+}$  handling. In addition, we did not conduct a systematic assessment of the magnitude and relative role of different model parameters. However, although the present model is intentionally simplistic (no faithful description of all the subtle interactions of L-type and RyR channels within the diadic space), it offers novel insight into potential mechanisms of CICR and may thus provide a starting point for further exploration of physical principles guiding cardiac CICR in vitro and in vivo.

### Editors note

Please see also related communications in this volume by Richard et al. (2005) and Sobie et al. (2005). For further downloadable content please see <http://www.physiome.org.nz/publications/PBMB-2005-89/Moskvina/>

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