

Interplay between store-operated calcium entry and mitochondrial phosphate handling modulates force and fatigue during exercise

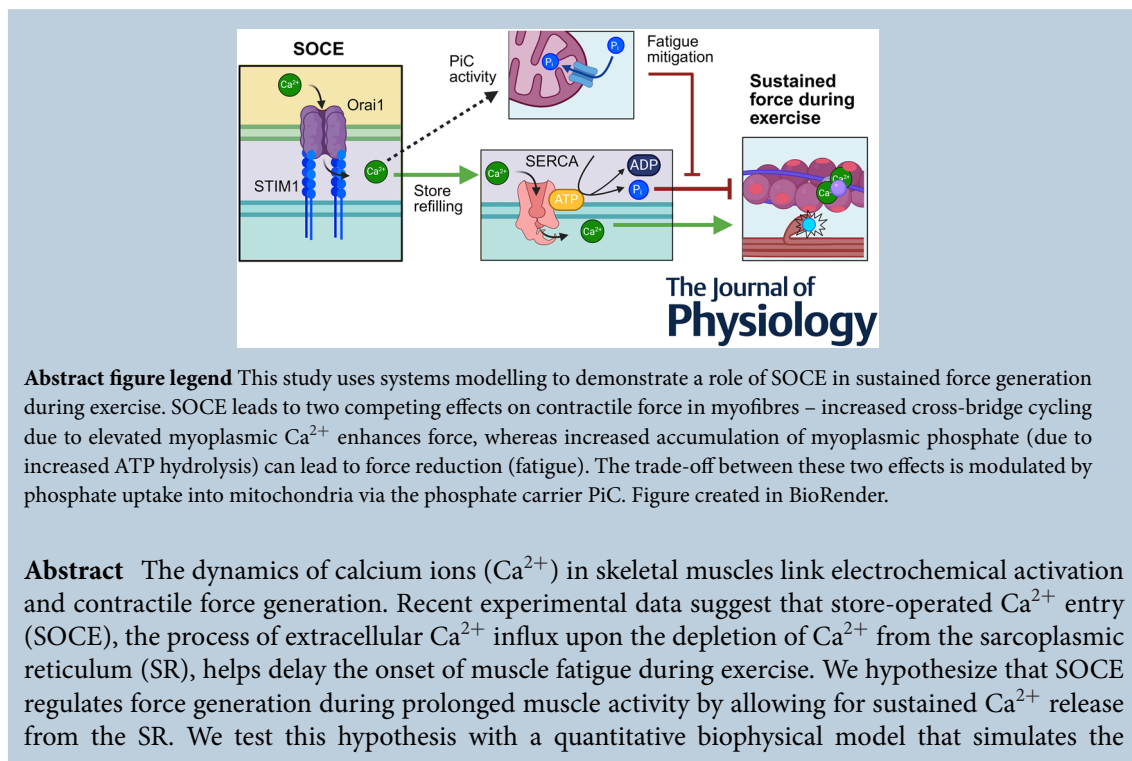
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biochemical events of muscle contraction, from depolarization at the T-tubules to Ca^{2+} release from the SR to Ca^{2+} binding and force generation throughout the myoplasm. We also consider the balance between Ca^{2+} removal from the myoplasm and SOCE through the T-tubule membrane, along with mitochondrial uptake of free Ca^{2+} and phosphate. We use the model to test the effects of SOCE inhibition on force production. The magnitude of myoplasmic Ca^{2+} and force is lower in SOCE knockout cells, especially when SOCE reduction is combined with impaired uptake of phosphate by mitochondria. We then test the effects of SOCE during resistance exercise or high-intensity interval training. These simulations predict a context-dependent relationship between force generation and SOCE – increased SOCE is associated with greater force production during resistance exercise but worsens the effects of fatigue in certain cases of high-intensity training. Such SOCE-induced fatigue is attributed to phosphate accumulation in the myoplasm and can be mitigated by increased rates of mitochondrial phosphate uptake.

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Key points

- Store-operated calcium entry (SOCE) provides a mechanism for calcium ion (Ca^{2+}) influx following the depletion of Ca^{2+} from intracellular stores such as the sarcoplasmic reticulum (SR).
- Recent experiments suggest that SOCE is an important modulator of contractile force generation in skeletal muscle.
- Here we develop a computational model of Ca^{2+} handling in the myoplasm, SR, and mitochondria and the resulting effects on force generation in skeletal muscle fibres to examine the role of SOCE during extended periods of activity.
- Our model predicts that increasing SOCE leads to enhanced force over periods of repeated stimuli during resistance exercise due to sustained Ca^{2+} release.
- Our simulations show a complex relationship between SOCE and force production during high-intensity interval training, with exacerbated phosphate accumulation in the myoplasm leading to force reduction for very high levels of SOCE. This effect can be mitigated by enhanced mitochondrial phosphate uptake.

Introduction

Skeletal muscle fibres (myofibres) are responsible for generating force to support locomotion of the animal body. They are positioned on the mesoscale of the muscle hierarchy, between the whole muscle composed of many bundles of fibres (fascicles) and subcellular myofibrils consisting of thick and thin filaments (Mukund & Subramaniam, 2020; Uchida & Delp, 2021). Contraction is initiated at the myofibre level where each fibre is stimulated by a motor neuron at the neuromuscular junction (NMJ). Understanding how myofibres integrate electrochemical inputs to initiate and sustain contraction is essential to dissect muscle performance in different contexts, from peak performance to rapid fatigue.

The evolution of contractile force in a myofibre is largely governed by the pattern of input stimulus received from its motor neuron. This stimulus is provided

mainly through acetylcholine (ACh) released into the synaptic cleft, which binds to its receptor in the myofibre membrane (sarcolemma) allowing an influx of positively charged ions (Rodríguez Cruz et al., 2020). The frequency of ACh release events determines the extent of fibre contraction via rate encoding (Enoka & Duchateau, 2017; Fuglevand et al., 1993). At low frequencies individual contractions (twitches) relax prior to the next stimulus, resulting in low force generation. In contrast, at higher stimulus frequencies, twitches fuse into a single tetanic contraction, producing much higher forces. This provides a mechanism for modulating muscle force during different activities. Consequently, different exercises are characterized by different patterns of stimulus at the NMJ. Force production itself is mediated by Ca^{2+} elevation in the myoplasm, which is initiated by voltage changes along specialized membrane invaginations known as transverse tubules (T-tubules).

As voltage increases, voltage-gated Ca^{2+} channels are activated, triggering the opening of ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) membrane to facilitate Ca^{2+} release from the SR. Myoplasmic Ca^{2+} then binds troponin on thin filaments, uncovering myosin binding sites to initiate cross-bridge cycling leading to myofibre contraction (Calderón et al., 2014).

The efficient contraction of muscle fibres relies on rapid transport of Ca^{2+} into and out of the myoplasm. Certain ATP-dependent membrane channels such as the plasma membrane Ca^{2+} ATPase (PMCA) in the sarcolemma and T-tubules and sarco/endoplasmic reticulum ATPase (SERCA) in the SR membrane pump Ca^{2+} back into the extracellular space or SR lumen, respectively. Additionally, excess free Ca^{2+} is absorbed by mitochondria via transport through the mitochondrial Ca^{2+} uniporter (MCU) (Dong & Tsai, 2023). Due to the constant removal of some Ca^{2+} from the cell through PMCA, SR Ca^{2+} levels are depleted over time in absence of additional Ca^{2+} entry through the sarcolemma. The phenomenon of store-operated Ca^{2+} entry (SOCE) compensates for this Ca^{2+} loss by coupling SR Ca^{2+} depletion to an additional influx through Ca^{2+} channels in the plasma membrane (Hogan & Rao, 2015; Protasi et al., 2021). SOCE relies on stromal interaction molecule 1 (STIM1) in the SR membrane, which acts as a Ca^{2+} sensor by binding SR Ca^{2+} through its luminal domain (Furukawa et al., 2014; Hoover & Lewis, 2011). Following Ca^{2+} dissociation from this binding site, STIM1 oligomerizes and binds to the Ca^{2+} channel Orai1 in the T-tubules, allowing Ca^{2+} influx into the myoplasm (Hoover & Lewis, 2011; Luik et al., 2008).

The importance of SOCE was originally appreciated in nonexcitable cells, but many recent studies have uncovered its importance in excitable cells such as neurons, cardiomyocytes and myofibres (Basnayake et al., 2021; Boncompagni et al., 2017; Hermes et al., 2023; Koenig et al., 2019; Saftenku, 2022; Wei-LaPierre et al., 2013). For instance, during exercise in mice, STIM1 and Orai1 channels were found to colocalize at the SR-T-tubule junction, functioning as Ca^{2+} entry units (CEUs) (Boncompagni et al., 2017; Protasi et al., 2021; Wei-LaPierre et al., 2013). STIM-Orai assembly was shown to facilitate SOCE in the junctional region during each action potential, a phenomenon referred to as phasic SOCE (pSOCE) (Koenig et al., 2018, 2019; Lilliu et al., 2020, 2021, 2025). CEUs have been proposed to facilitate rapid recovery of Ca^{2+} within the fibre and delay the onset of fatigue (Boncompagni et al., 2017; Wei-LaPierre et al., 2013). Indeed, muscles from dominant-negative Orai1 mice (dnOrai1) were more susceptible to fatigue and produced lower contractile force *ex vivo* (Wei-LaPierre et al., 2013). However, other recent data suggest that SOCE can worsen fatigue in some cases; phosphorylation of STIM1 by AMP-activated protein kinase (AMPK) was

found to both decrease SOCE activity and prevent fatigue (Nelson et al., 2020).

Understanding the role of SOCE in muscle fatigue requires a comprehensive understanding of the molecular mechanisms underlying fatigue in single muscle fibres, including rapid accumulation of phosphate in the myoplasm, reduced Ca^{2+} release from the SR, and reduced myofibrillar Ca^{2+} sensitivity (Allen & Trajanovska, 2012; Cheng et al., 2018; Hendry et al., 2025; Shorten et al., 2007). On the one hand, SOCE allows for increased store refilling and might be expected to alleviate fatigue. However, high levels of SOCE can also lead to Ca^{2+} overload, causing the accumulation of phosphate in the myoplasm due to increased rates of cross-bridge cycling and increased activity of SERCA and PMCA. Such phosphate accumulation causes a reduction in contractile force due to the enhanced reversal of the power stroke (Allen & Trajanovska, 2012; Allen & Westerblad, 2001). Removal of phosphate from the myoplasm relies, in part, on transport into the SR (Shorten et al., 2007) and into the mitochondrial matrix through the mitochondrial phosphate carrier (PiC) (Dong & Tsai, 2023; Seifert et al., 2015). It is crucial to understand how the dynamics of phosphate uptake by mitochondria affects fatigue onset in cells with elevated SOCE.

Here we use a computational model to predict when SOCE enhances force production and when it accelerates the onset of fatigue in myofibres. We first fit our model to experimental measurements of myoplasmic Ca^{2+} and sarcolemma voltage in the literature and then show that this model recapitulates measurements of Ca^{2+} and force in SOCE-deficient myofibres. We furthermore consider how the effects of SOCE deficiency in cells depend on phosphate uptake by mitochondria. Finally we predict the role of SOCE for different patterns of stimuli chosen to mimic high-intensity interval training and resistance exercise. Our findings reconcile apparent contradictions in the literature, showing that the role of SOCE is context-dependent – force production is generally enhanced by SOCE at lower stimulus frequencies but can worsen fatigue during extended, high-frequency activation due to phosphate accumulation in the myoplasm. This effect is tuned by the rates of phosphate uptake by mitochondria in the myoplasm.

Results

Model development

To investigate the impact of SOCE on Ca^{2+} dynamics in skeletal muscle, we developed a well-mixed multi-compartment model describing action potential generation, Ca^{2+} handling, phosphate handling and force generation in fast-twitch myofibres. We included junctional and bulk regions of the myoplasm, SR,

mitochondria, and extracellular space (Fig. 1A). Junctional regions correspond to the volumes and surfaces at the triad, which consists of a T-tubule surrounded by two sections of the SR on either side (terminal cisternae). We did not consider propagation of electrochemical signals along the length of the fibre,

instead choosing to focus on a small representative region of the myofibre over which molecules remain relatively well mixed. Furthermore, we assumed that the action potential propagates into the T-tubules much faster than other events in our model. Throughout, we differentiate between each subcompartment using subscripts for

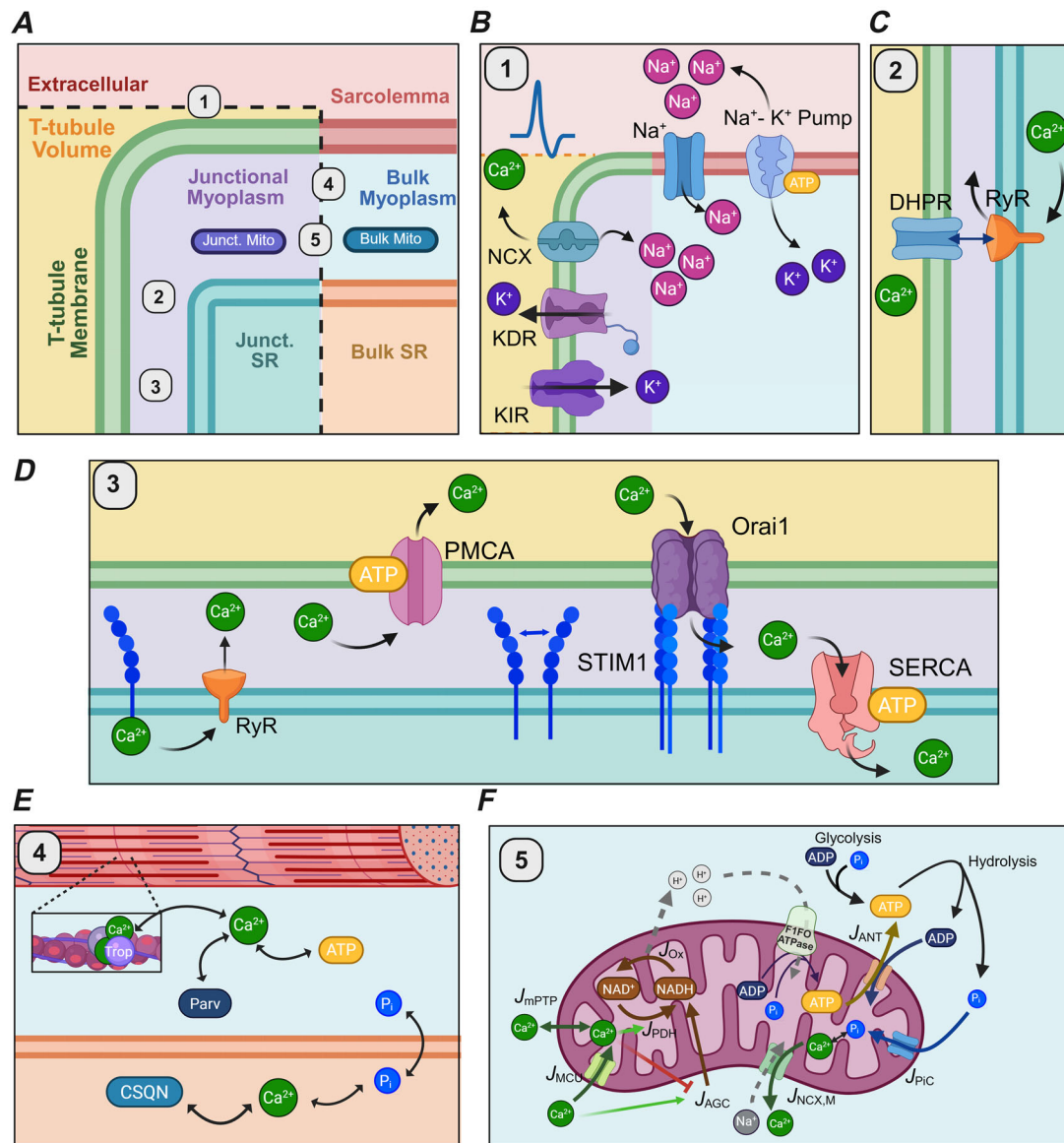


Figure 1. Schematic for model components of myofibre Ca^{2+} signalling

A, a subregion of the cross-section of the muscle fibre is divided into compartments, each labelled with different colours. Ions freely diffuse between bulk and junctional regions of each non-mitochondrial compartment, separated by black dashed lines here. B–D, the sequence of signalling events directly correlated to force generation are grouped into five modules. In module 1, the applied current triggers an action potential through Na^+ and K^+ fluxes (B). In module 2, action potential propagation leads to dihydropyridine receptor-ryanodine receptor (DHPR-RyR) allosteric interactions, triggering Ca^{2+} release from the SR (C). In module 3, Ca^{2+} unbinds from STIM1 during SR depletion, causing stromal interaction molecule 1 (STIM1) to oligomerize and form complexes with Orai1 and allowing Ca^{2+} influx from the extracellular space into the myoplasm (D). In module 4, Ca^{2+} binds to proteins in the sarcoplasmic reticulum (SR) and myoplasm and induces the cross-bridge cycle, producing contractile force (E). In module 5, Ca^{2+} and phosphate are taken up by the mitochondria, leading to enhanced ATP production via oxidative phosphorylation (F). Figure created in BioRender.

clarity; for instance, Ca^{2+} concentration in the junctional myoplasm is written as $[\text{Ca}^{2+}]_{\text{myo,junc}}$ (all species are included in Table A4). Species in the myoplasm and SR are allowed to diffuse between the bulk and junctional regions, whereas mitochondrial species are confined to either junctional or bulk regions.

Our model is divided into five modules, including action potential generation (module 1, Fig. 1B), Ca^{2+} influx and release (module 2, Fig. 1C), Ca^{2+} removal and SOCE (module 3, Fig. 1D), Ca^{2+} buffering and cross-bridge cycling (module 4, Fig. 1E) and Ca^{2+} and phosphate handling by mitochondria (module 5, Fig. 1F). The complete model consists of a system of 65 ordinary differential equations (ODEs), including flux balance equations and equations governing changes in receptor or channel state. We briefly summarize each module below, with a full description of the model given in Appendix A (Table A4 to A7).

Module 1: Action potential generation. Action potentials were modelled using a Hodgkin-Huxley-type formulation, as in previous models developed for mammalian skeletal muscle (Cannon et al., 1993; Senneff & Lowery, 2021; Shorten et al., 2007; Wallinga et al., 1999) (Fig. 1B). We considered a Na^+ current through a voltage-gated Na^+ channel (I_{Na^+}), followed by K^+ efflux through the K^+ delayed rectifier (I_{KDR}), governed by equations from Wallinga et al. (1999). We also included currents through the K^+ inward rectifier (I_{KIR}), which helps to maintain the resting potential of the membrane (Standen & Stanfield, 1978), and the Na^+ - K^+ pump ($I_{\text{Na}^+-\text{K}^+}$), which restores gradients in Na^+ and K^+ across the sarcolemma (Wallinga et al., 1999). Other secondary currents regulate the resting membrane potential, including Na^+ import through the Na^+ - Ca^{2+} exchanger (I_{NCX}) and a Cl^- current (I_{Cl^-}). Altogether the total ionic currents through the T-tubule membrane (subscript TT) and bulk sarcolemma (subscript SL) are given by

$$I_{\text{ionic,TT}} = 0.1 I_{\text{Na}^+, \text{TT}} + 0.1 I_{\text{Na}^+-\text{K}^+, \text{TT}} + I_{\text{NCX,TT}} + 0.45 I_{\text{KDR,TT}} + I_{\text{KIR,TT}} + 0.1 I_{\text{Cl}^-, \text{TT}} + I_{\text{Ca}^{2+}, \text{TT}}, \quad (1)$$

$$I_{\text{ionic,SL}} = I_{\text{Na}^+, \text{SL}} + I_{\text{Na}^+-\text{K}^+, \text{SL}} + I_{\text{NCX,SL}} + I_{\text{KDR,SL}} + I_{\text{KIR,SL}} + I_{\text{Cl}^-, \text{SL}} + I_{\text{Ca}^{2+}, \text{SL}}, \quad (2)$$

where $I_{\text{Ca}^{2+}, \text{TT}}$ and $I_{\text{Ca}^{2+}, \text{SL}}$ are additional Ca^{2+} currents defined in Module 3. Full expressions for each current are given in Table A5, and their relative contribution at T-tubules vs. sarcolemma are summarized in Table A3. Balancing these ionic currents with a capacitive current at

the sarcolemma and accounting for conduction between the sarcolemma and T-tubules yields

$$\frac{dV_{\text{SL}}}{dt} = \frac{SA_{\text{SL}} I_{\text{ionic,SL}} + I_{\text{SL}} - I_{\text{SL-TT}}}{SA_{\text{SL}} C_{\text{M}}}, \quad (3)$$

$$\frac{dV_{\text{TT}}}{dt} = \frac{SA_{\text{TT}} I_{\text{ionic,TT}} + I_{\text{SL-TT}}}{SA_{\text{TT}} C_{\text{M}}}, \quad (4)$$

where SA_{SL} is the surface area of the sarcolemma, SA_{TT} is the surface area of the T-tubules, C_{M} is the specific membrane capacitance and I_{SL} is the stimulus current through the sarcolemma. This stimulus was modelled as square waves with a pulse width of 1 ms. $I_{\text{SL-TT}}$ is the current from the sarcolemma into the T-tubule membrane, calculated as in Wallinga et al. (1999):

$$I_{\text{SL-TT}} = SA_{\text{SL}} \frac{V_{\text{TT}} - V_{\text{SL}}}{R_{\text{a}}}, \quad (5)$$

where R_{a} is the access resistance at the entrance to the T-tubules.

Module 2: Ca^{2+} release. Ca^{2+} release from the SR occurs in the junctional region upon a sufficient increase in T-tubule membrane voltage. The L-type Ca^{2+} channel, the dihydropyridine receptor (DHPR), acts as a voltage sensor in the T-tubule membrane, allosterically interacting with the ryanodine receptor (RyR) in the SR membrane to facilitate RyR opening (Ríos et al., 1993). In line with previous literature we assumed that the Ca^{2+} flux through DHPR itself is negligible and it mainly serves as a voltage sensor to gate the RyR (Dayal et al., 2017).

The resulting flux through the RyR is given by:

$$J_{\text{RyR}} = \nu_{\text{max,RyR}} P_{\text{O,RyR}} ([\text{Ca}^{2+}]_{\text{SR,junc}} - [\text{Ca}^{2+}]_{\text{myo,junc}}), \quad (6)$$

where $\nu_{\text{max,RyR}}$ is the maximum permeability of the RyR and $P_{\text{O,RyR}}$ is the open probability of the RyR governed by Ca^{2+} concentration and T-tubule membrane voltage. The voltage dependence of $P_{\text{O,RyR}}$ is dictated by a 10-state Monod, Wyman and Changeux (MWC)-type model (as in Ríos et al., 1993), and the Ca^{2+} dependence captures slow deactivation, as introduced by Senneff and Lowery (2021) (see expressions in Table A5).

Module 3: Ca^{2+} removal and SOCE. Ca^{2+} homeostasis relies on rapid removal from the myoplasm via ATP-dependent fluxes through PMCA into the extracellular space and through SERCA into the SR lumen. We represented these fluxes phenomenologically using first-order Hill equations that depend on both Ca^{2+} concentration and ATP concentration (Table A5). Ca^{2+} is also passively transported from the myoplasm to the extracellular space through NCX, as dictated by J_{NCX} (proportional to I_{NCX} in eqns 1 and 2) and

into mitochondria via the mitochondria Ca^{2+} uniporter (MCU), described in module 5 below.

Given that not all Ca^{2+} released from the SR is pumped back in through SERCA, SOCE is required to restore SR Ca^{2+} levels following rapid depletion. We drew from previous work across several cell types (Dolan & Diamond, 2014; Hoover & Lewis, 2011; Rincón et al., 2021; Saftenku, 2022) to mathematically describe SOCE in skeletal muscle. The SR luminal domain of STIM1 binds SR Ca^{2+} , allowing it to act as a Ca^{2+} sensor (Furukawa et al., 2014). Upon the depletion of SR Ca^{2+} , Ca^{2+} unbinding leads to STIM1 oligomerization and binding to tetrameric Orai1 channels at SR-T-tubule junctions (Hoover & Lewis, 2011), leading to Ca^{2+} influx through Orai1. Previous experimental measurements (Furukawa et al., 2014; Luik et al., 2008) demonstrate that the relationship between Orai1 conductance and SR Ca^{2+} is well represented by a Hill equation with a coefficient close to 4, as utilized by multiple previous models (Koenig et al., 2019; Mansour et al., 2026; Rincón et al., 2021; Saftenku, 2022). Accordingly, we modelled the steady-state open probability of Orai1 channels, $P_{\text{O,Orai1},\infty}$, as:

$$P_{\text{O,Orai1},\infty} = \frac{1}{1 + \left(\frac{[\text{Ca}^{2+}]_{\text{SR,junc}}}{K_{\text{STIM1}}} \right)^4}, \quad (7)$$

where K_{STIM1} is an equilibrium constant dictating the junctional SR Ca^{2+} concentration at which SOCE is at half maximum activation. We expected the system to approach steady state over some characteristic time τ_{SOCE} required for STIM1 oligomerization, diffusion and binding with Orai1 to form CEUs (Hoover & Lewis, 2011):

$$\frac{dP_{\text{O,Orai1}}}{dt} = \frac{P_{\text{O,Orai1},\infty} - P_{\text{O,Orai1}}}{\tau_{\text{SOCE}}}. \quad (8)$$

This approach allowed us to represent SOCE without introducing a large number of additional free parameters. The Ca^{2+} flux due to SOCE was localized to the T-tubules and was given by

$$J_{\text{SOCE}} = P_{\text{O,Orai1}} g_{\text{Orai1}} \frac{N_{\text{A}}}{2F} (E_{\text{Ca}^{2+},\text{junc}} - V_{\text{TT}}), \quad (9)$$

where g_{Orai1} is the Orai1 conductance, N_{A} is Avogadro's number and F is Faraday's constant. $E_{\text{Ca}^{2+},\text{junc}}$ is the Nernst potential for Ca^{2+} across the T-tubule membrane:

$$E_{\text{Ca}^{2+},\text{junc}} = \frac{RT}{2F} \ln \left(\frac{[\text{Ca}^{2+}]_{\text{TT}}}{[\text{Ca}^{2+}]_{\text{myo,junc}}} \right), \quad (10)$$

where R is the gas constant and T is the temperature.

Module 4: Ca^{2+} binding and cross-bridge cycling. Many proteins in the myoplasm and SR act as Ca^{2+} buffers, such that the total Ca^{2+} concentration in either compartment

is much higher than the free Ca^{2+} concentration (Butera et al., 2021; Murphy et al., 2009; Nogueira et al., 2022). In the myoplasm we considered Ca^{2+} binding to parvalbumin, ATP and troponin, in addition to competitive binding of Mg^{2+} to parvalbumin and ATP (Baylor & Hollingworth, 2012, 2007; Senneff & Lowery, 2021; Shorten et al., 2007). In the SR lumen we accounted for Ca^{2+} binding to calsequestrin (Cannell & Allen, 1984; Senneff & Lowery, 2021; Shorten et al., 2007) and Ca^{2+} precipitation with inorganic phosphate (Shorten et al., 2007).

Ca^{2+} binding to troponin on the thin filament in the bulk myoplasm leads to cross-bridge cycling. In short, two Ca^{2+} ions bind sequentially to a single troponin complex, causing tropomyosin to change shape and expose myosin binding sites on the thin filament (Calderón et al., 2014; Shorten et al., 2007; Uchida & Delp, 2021). We modelled this using a six-state cross-bridge model, modified from the eight-state model in Shorten et al. (2007) as detailed in Table A5 and A6 and depicted in Fig. A1. The production of contractile force was assumed to be proportional to the density of cross-bridges in a post-power stroke (force-generating) conformation A_{Post} :

$$\begin{aligned} \frac{d[A_{\text{Post}}]}{dt} = & h_0[A_{\text{Pre}}] - g_0[A_{\text{Post}}][\text{ATP}]_{\text{myo,bulk}} \\ & - h_p[A_{\text{Post}}][\text{Pi}]_{\text{myo,bulk}}, \end{aligned} \quad (11)$$

where $[A_{\text{Pre}}]$ is the density of pre-power stroke cross-bridges, h_0 is the power stroke rate, g_0 is the rate of myosin head detachment post-power stroke and h_p is the reverse rate to the pre-power stroke conformation. In the above relationship we explicitly assumed that reactions depended on concentrations of ATP ($[\text{ATP}]_{\text{myo,bulk}}$) and inorganic phosphate ($[\text{Pi}]_{\text{myo,bulk}}$) based on mass action, an effect neglected in some previous models (Senneff & Lowery, 2021; Shorten et al., 2007). This is an important consideration, as the dependence of power stroke reversal on phosphate has been suggested as a mechanism for fatigue (Allen & Trajanovska, 2012).

Module 5: Adenine nucleotide, phosphate and Ca^{2+} handling by mitochondria. In the final module, we include the effects of mitochondrial phosphate uptake, Ca^{2+} uptake, and ATP generation via oxidative phosphorylation. ATP hydrolysis due to cross-bridge cycling and activity of ion pumps and other intracellular processes in the above modules was explicitly tracked in the myoplasm. In line with the dominant role of glycolytic fluxes in fast-twitch muscle fibres (Murgia et al., 2021; Schiaffino and Reggiani, 2011), we assumed that ATP was generated within the myoplasm via glycolysis to restore ATP concentrations to a setpoint during simulations of extended exercise. This mathematical description of glycolysis was not meant to capture the full

complexity of glycolytic flux regulation in skeletal muscle, but to implicitly represent the established dependence of glycolysis rates on muscle activation (Conley et al., 1998) and concentrations of phosphate, ATP and ADP (Crowther et al., 2002).

We adopted models of Ca^{2+} handling and ATP generation in mitochondria from several previous studies (Bertram et al., 2006; Leung et al., 2021; Wacquier et al., 2016), assuming that ATP generation is positively regulated by mitochondrial Ca^{2+} uptake due to enhanced production of NADH required for oxidative phosphorylation (Fig. 1F). We accounted for several Ca^{2+} fluxes across the mitochondrial membranes due to the mitochondrial Ca^{2+} uniporter (MCU), mitochondrial permeability transition pore (mPTP) and mitochondrial NCX (Fig. 1F). In addition phosphate is transported into the mitochondria via the mitochondrial phosphate carrier (PiC) (Seifert et al., 2015). We simulated this flux using a reversible Hill equation:

$$J_{\text{PiC}} = v_{\text{PiC}} \frac{\frac{[\text{Pi}]_{\text{myo}}}{K_{\text{Pi}}} - \frac{[\text{Pi}]_{\text{Mito}}}{K_{\text{Pi,M}}}}{1 + \frac{[\text{Pi}]_{\text{myo}}}{K_{\text{Pi}}} + \frac{[\text{Pi}]_{\text{Mito}}}{K_{\text{Pi,M}}}}, \quad (12)$$

where $[\text{Pi}]_{\text{myo}}$ and $[\text{Pi}]_{\text{Mito}}$ are the free phosphate concentrations in the myoplasm and in the mitochondrial matrix, respectively, K_{Pi} and $K_{\text{Pi,M}}$ are the associated Hill constants and v_{PiC} is the maximal phosphate transport rate through PiC. This expression carries the same dependence of PiC on mitochondrial and extra-mitochondrial phosphate as in previous models (Bazil et al., 2010; Nguyen et al., 2007). We also accounted for retention of Ca^{2+} in mitochondria due to Ca^{2+} -phosphate formation (Seifert et al., 2015; Wei et al., 2015), which increases the mitochondrial capacity for Ca^{2+} uptake.

Assembly of the full model. The fluxes outlined in modules 2–5 were assembled into systems of ODEs for Ca^{2+} in each region of the myoplasm, SR and mitochondria. Furthermore we computed the additional Ca^{2+} currents through the T-tubule membrane and bulk sarcolemma introduced in eqn (3) and (4):

$$I_{\text{Ca}^{2+},\text{TT}} = \frac{2F}{N_A} (J_{\text{SOCE}} + J_{\text{Ca}^{2+},\text{TT leak}} - J_{\text{PMCA}}), \quad (13)$$

$$I_{\text{Ca}^{2+},\text{SL}} = \frac{2F}{N_A} (J_{\text{Ca}^{2+},\text{SL leak}} - J_{\text{PMCA}}), \quad (14)$$

where $J_{\text{Ca}^{2+},\text{TT leak}}$ and $J_{\text{Ca}^{2+},\text{SL leak}}$ are leak fluxes through the T-tubule membrane and sarcolemma, respectively.

Most species in the model were assumed to exist in both junctional and bulk regions, with the exception of Orail (T-tubules only) and all cross-bridge-related variables (bulk myoplasm only). Furthermore concentrations of

ions were tracked within the T-tubule volume, but bulk extracellular ion concentrations were assumed to be constant.

Calibrated model recapitulates dynamics of membrane potential and myoplasmic Ca^{2+}

Our model utilizes a collection of channels and receptors not combined in previous models. Accordingly although we used initial parameter estimates from the literature, it was necessary to formally estimate best-fit values for each parameter. This allowed us to reliably consider the importance of SOCE relative to other Ca^{2+} fluxes throughout the myofibre. Excluding geometric parameters there were 142 free parameters (Table A7) in the model. Forty of these parameters were specific to mitochondria (module 5) and were calibrated independently to data from Wei et al. (2015) as described in Methods and shown in Fig. A3. After fixing these parameters we conducted a global Morris sensitivity analysis to identify the most influential parameters in the model (Morris, 1991) (Fig. 2A,B, Fig. A2). As quantities of interest (QOIs) we considered the maximum and time-averaged values of the myoplasmic Ca^{2+} concentration and sarcolemma voltage over a single excitation event. Myoplasmic Ca^{2+} was defined as a weighted average by volume:

$$[\text{Ca}^{2+}]_{\text{avg}} = \frac{[\text{Ca}^{2+}]_{\text{myo,junc}} \text{vol}_{\text{myo,junc}} + [\text{Ca}^{2+}]_{\text{myo,bulk}} \text{vol}_{\text{myo,bulk}}}{\text{vol}_{\text{myo,junc}} + \text{vol}_{\text{myo,bulk}}}. \quad (15)$$

Average sarcolemma voltage was most sensitive to parameters associated with the Na^+ channel (V_m , V_h , $V_{S\infty}$, V_{α_m} and V_{β_m} ; defined in Table A7) and with the KDR channel (V_n), in agreement with previous work (Shorten et al., 2007; Wallinga et al., 1999) (Fig. 2A). Ca^{2+} dynamics were most sensitive to Ca^{2+} export through PMCA (g_{PMCA}) and efflux through the Na^+ - Ca^{2+} exchanger (g_{NCX} and $K_{\text{Ca,i}}$), validating the central importance of Ca^{2+} efflux mechanisms (Fig. 2B).

We proceeded to estimate parameters in two additional stages, as fully described in Methods. We first fit the model to measurements of membrane potential (Miranda et al., 2020), only varying the parameters with greater than 20 % relative sensitivity with respect to average and/or peak voltage (Fig. A2A,B). Then starting from these parameter values as new initial estimates and allowing all parameters to vary, we simultaneously fit our model to voltage and to myoplasmic Ca^{2+} data collected from multiple papers that conducted fluorescence or voltage measurements in single mouse fast-twitch myofibres (Baylor & Hollingworth, 2003; Miranda et al., 2020; Rincón et al., 2021). In general,

the fitting process resulted in a strong fit with all the experiments considered (Fig. 2C-E).

We then examined the baseline dynamics of our model in terms of membrane voltage, Ca^{2+} and contractile force (Fig. 3). In response to a 100 Hz stimulus (Fig. 3A), we observed consistent action potentials that match the overall magnitude of a typical skeletal muscle action potential (Fig. 3B). Simultaneously Ca^{2+} accumulates within the myoplasm (Fig. 3C), leading to increases in contractile force as indicated by the relative density of post-power stroke cross-bridges (Fig. 3D). As expected for this high-frequency stimulus, individual contractile

events (twitches) fuse and rapidly approach maximum activation, corresponding to tetanic contraction.

We then analysed these events in terms of their constitutive currents and fluxes to determine the relative contributions of the various channels (Fig. 4). Membrane depolarization leads to Na^+ channel opening in the T-tubules and sarcolemma, followed by the opening of the K^+ delayed rectifier during repolarization (Fig. 4B). The Na^+ - K^+ pump counteracts these fluxes to restore the resting gradients of both ions, whereas other currents through the Na^+ - Ca^{2+} exchanger and the K^+ inward rectifier play secondary roles (Fig. 4B). In parallel

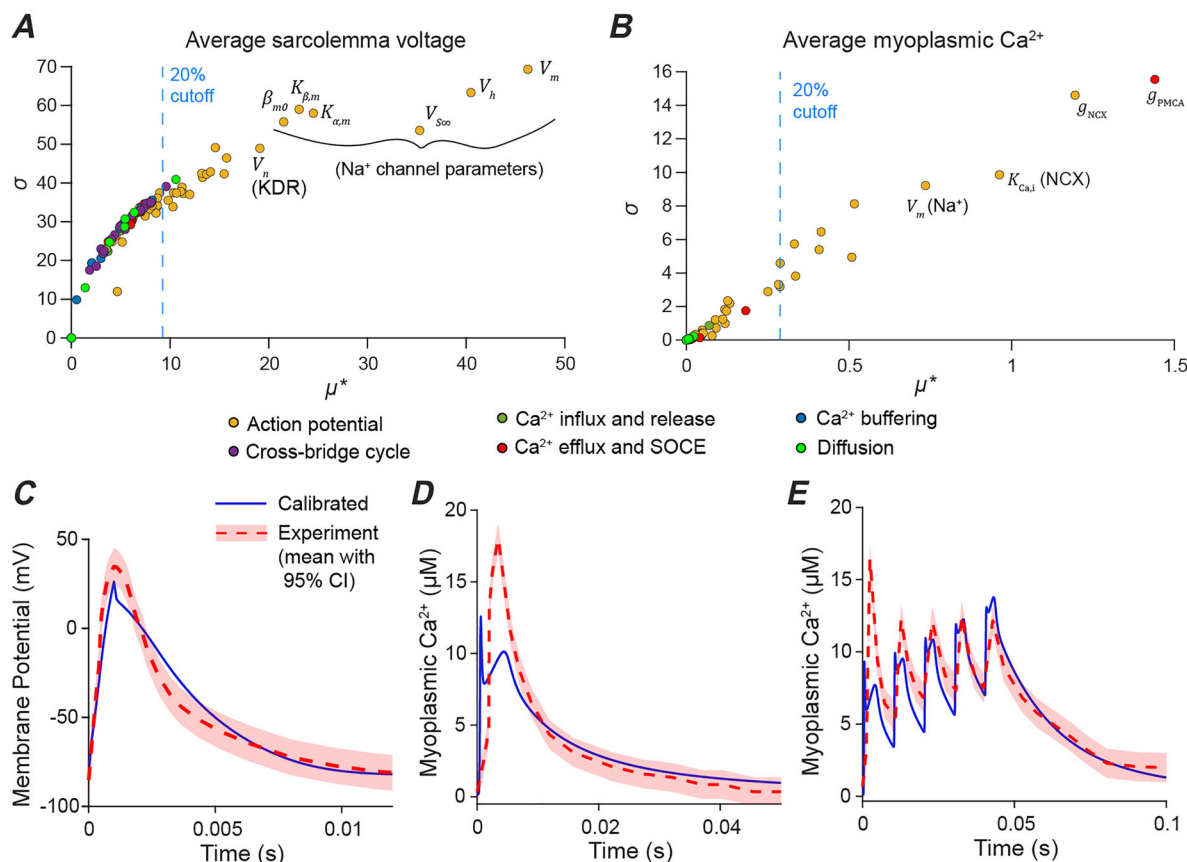


Figure 2. Sensitivity analysis and model calibration

A, B, results from Morris sensitivity analysis of average sarcolemma voltage (A) and average $[\text{Ca}^{2+}]_{\text{myo}}$ (B) as quantities of interest (QOIs). The horizontal axis displays the absolute value of the mean, μ^* , and the vertical axis shows the standard deviation, σ , of elementary effects. The parameters with the highest values of μ^* are labelled individually. C, results of model calibrated to experimental membrane potential measurements (Miranda et al., 2020) (C) and to $[\text{Ca}^{2+}]_{\text{myo}}$ dynamics data for a single pulse stimulus (D) (Baylor & Hollingworth, 2003) or a train of five stimulus pulses at 100 Hz (E) (Rincón et al., 2021). All experimental data were extracted from source papers using PlotDigitizer; specifically, from Fig. 2B (WT curve) in Miranda et al. (2020), from Fig. 2A (fast-twitch curve) in Baylor and Hollingworth (2003) and from Fig. 1B (IIB curve) in Rincón et al. (2021). The minimized weighted sum of square errors (defined in eqns 21–22–23) was 32.375. Conditions were varied based on extracellular buffer and temperature in each experiment. (Miranda et al., 2020): $[\text{Na}^+]_{\text{EC}} = 1.18 \times 10^5 \mu\text{M}$, $[\text{K}^+]_{\text{EC}} = 5.33 \times 10^3 \mu\text{M}$, $[\text{Cl}^-]_{\text{EC}} = 1.26 \times 10^5 \mu\text{M}$, $[\text{Ca}^{2+}]_{\text{EC}} = 1.80 \times 10^3 \mu\text{M}$, $T = 295.15 \text{ K}$; (Baylor & Hollingworth, 2003): $[\text{Na}^+]_{\text{EC}} = 1.50 \times 10^5 \mu\text{M}$, $[\text{K}^+]_{\text{EC}} = 2.00 \times 10^3 \mu\text{M}$, $[\text{Cl}^-]_{\text{EC}} = 1.58 \times 10^5 \mu\text{M}$, $[\text{Ca}^{2+}]_{\text{EC}} = 2.00 \times 10^3 \mu\text{M}$, $T = 293.15 \text{ K}$; (Rincón et al., 2021): $[\text{Na}^+]_{\text{EC}} = 1.38 \times 10^5 \mu\text{M}$, $[\text{K}^+]_{\text{EC}} = 3.90 \times 10^3 \mu\text{M}$, $[\text{Cl}^-]_{\text{EC}} = 1.44 \times 10^5 \mu\text{M}$, $[\text{Ca}^{2+}]_{\text{EC}} = 1.00 \times 10^3 \mu\text{M}$, $T = 295.15 \text{ K}$.

depolarization triggers the activation of DHPRs in the T-tubules, which allosterically interact with RyRs in the junctional SR membrane to facilitate their opening (Fig. 4B). This large Ca^{2+} release through RyRs is the dominant source for Ca^{2+} elevations in the myoplasm and results in higher concentrations in the junctional compartment compared to the bulk myoplasm (Fig. A4). The features of the RyR flux itself lead to the shape of Ca^{2+} concentration curve observed here and throughout, with one initial peak followed by a secondary phase of more gradual Ca^{2+} release (Fig. 4C). Myoplasmic Ca^{2+} then decreases due to buffering and removal to the extracellular, SR and mitochondrial spaces through PMCA, SERCA and MCU/mPTP, respectively (Fig. 4C,D). During this phase of store refilling, we observed a concomitant increase in SOCE, but J_{SOCE} remained small compared to other Ca^{2+} fluxes in the model (Fig. 4D).

We then considered the predictions of our model out to longer time points, maintaining 100 Hz stimulus for 10

s (Fig. 5). During this period as SR calcium was further depleted, SOCE continued to increase gradually (Fig. 5B), suggesting that SOCE may play an increasingly important role over extended periods of stimulus. Furthermore SOCE is activated separately in the junctional region during each action potential, in agreement with previous measurements of phasic SOCE in skeletal muscle (Koenig et al., 2018, 2019; Lilliu et al., 2020, 2021, 2025) (Fig. 5C).

SOCE facilitates sustained calcium elevations

Having established an agreement between our baseline model and the physiological signalling events in skeletal muscle fibres, we systematically examined the importance of SOCE for Ca^{2+} dynamics and force production. We first considered the treatment of cells with the SERCA inhibitor, thapsigargin (TG), commonly used to assess SOCE across cell types (Hoover & Lewis, 2011; Luik et al., 2008; Nelson et al., 2020; Rabesahala de Meritens et al.,

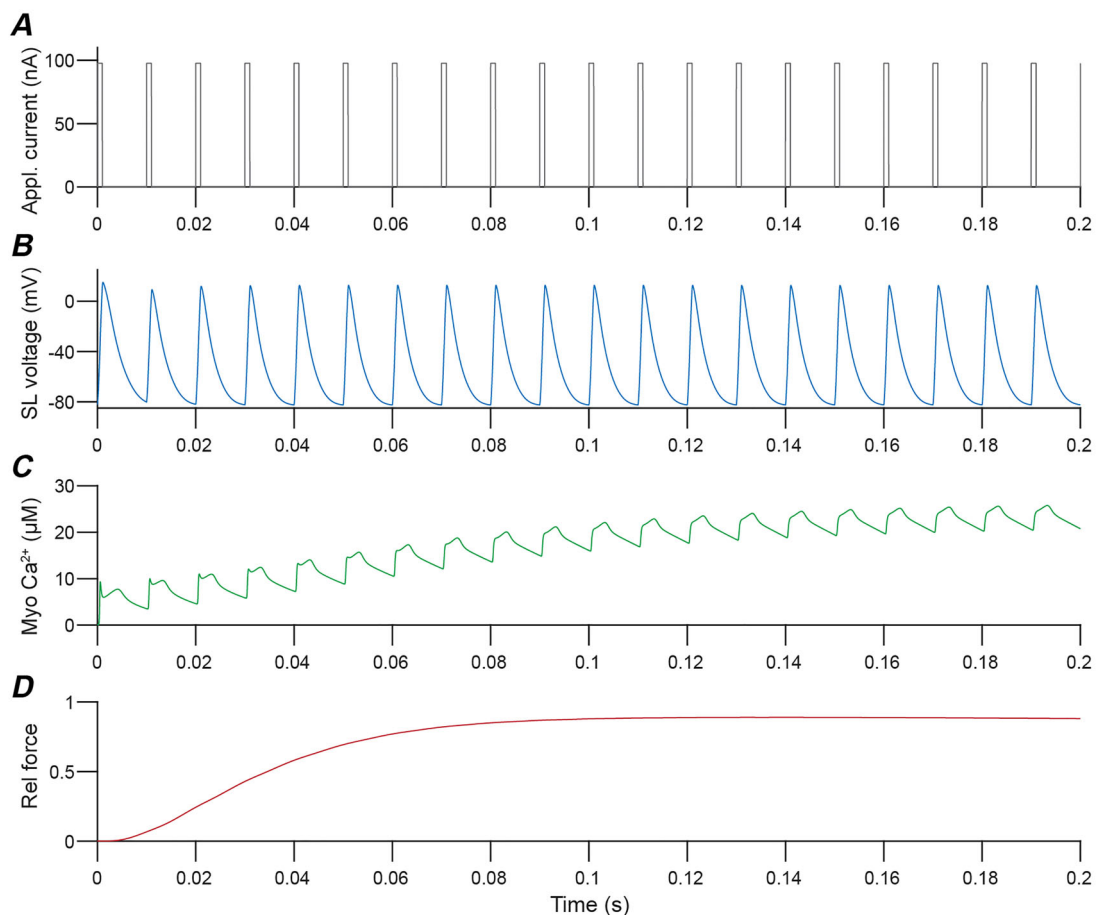


Figure 3. Model output for membrane potential and Ca^{2+} dynamics at 100 Hz stimulus

Input 100 Hz square wave current at the bulk sarcolemma (A) gives rise to action potentials in the sarcolemma (SL) (B) and subsequent increases in myoplasmic Ca^{2+} (C). Binding of Ca^{2+} to troponin leads to cross-bridge cycling and the associated increase in contractile force (D). Here and throughout, relative force is defined as the concentration of post-power stroke cross-bridges relative to their concentration at saturating Ca^{2+} concentrations.

2025). In such experiments cells are placed in Ca^{2+} -free media and then treated with TG, causing Ca^{2+} to leak from the SR into the myoplasm and slowly exit the cell due to efflux through PMCA. Ca^{2+} is then reintroduced into the media, leading to a large store-operated Ca^{2+} influx. This experiment was previously conducted in L6 myoblasts, (Nelson et al., 2020), revealing that the increase in myoplasmic Ca^{2+} following TG treatment was smaller and more short-lived than the elevation due to store-operated Ca^{2+} influx once extracellular Ca^{2+} was reintroduced (see Figure 5 in Nelson et al. 2020). We recapitulated this experiment in our simulations by altering extracellular Ca^{2+} and SERCA activity in a time-dependent manner as indicated in Fig. 6A. Our simulations show agreement with the measurements by Nelson et al. (2020), with a

smaller TG-induced flux followed by extended SOCE, with the peak Ca^{2+} concentration determined by the strength of SOCE. Notably our model allowed us to predict the dynamics of other quantities not readily accessible in experiments, such as concentration of Ca^{2+} in the SR (Fig. 6A).

We then considered stimulation of a myofibre at 100 Hz with and without SOCE. We increased Orai1 conductance to represent enhanced SOCE activity in skeletal muscle. This increase can be attributed to channel aggregation, or to formation of CEUs outside the triadic junction, as suggested by recent experiments (Boncompagni et al., 2017; Michelucci et al., 2019; Protasi et al., 2021). We determined a reasonable value for increased conductance by assuming a total density of

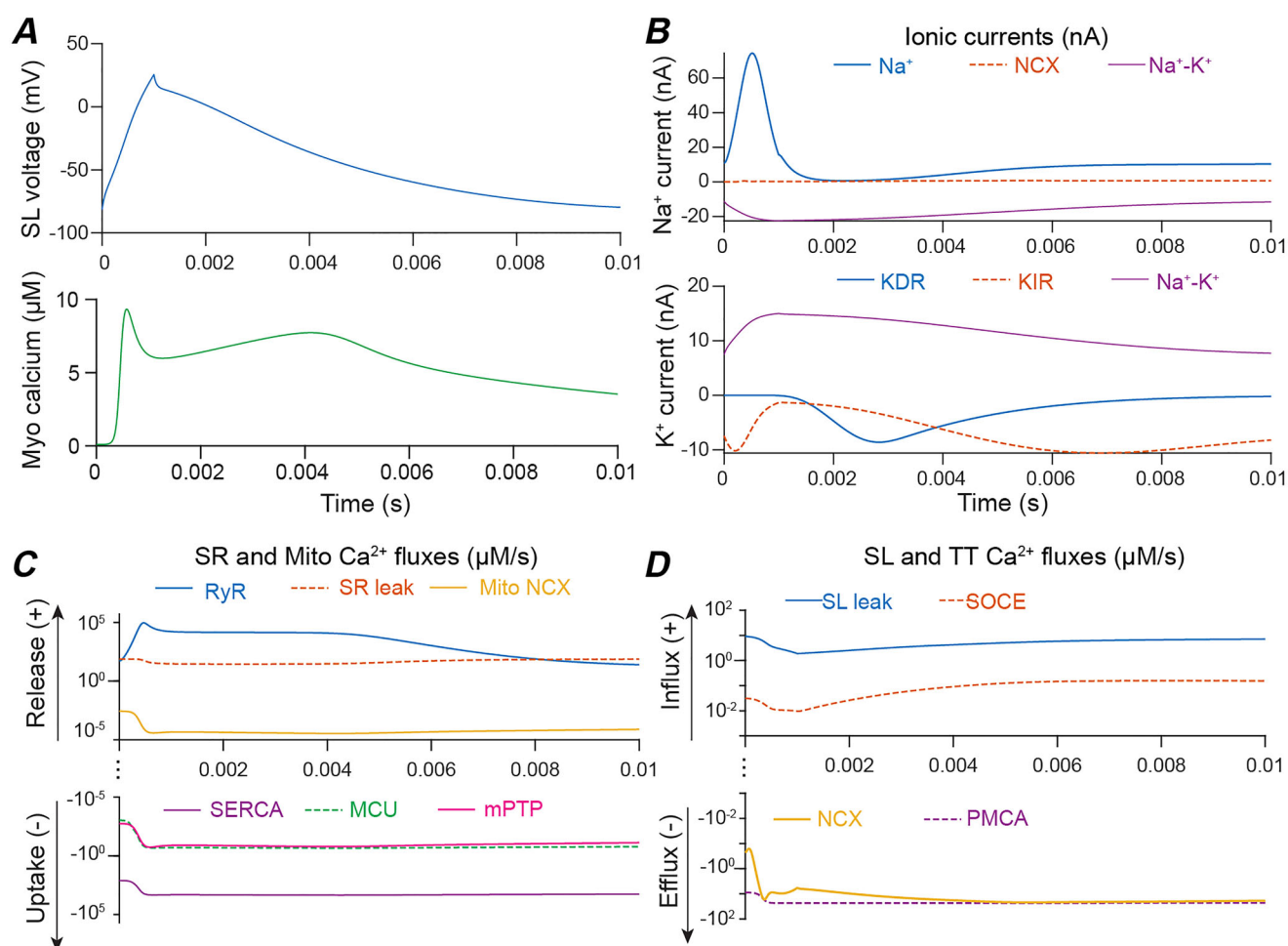


Figure 4. Currents and Ca^{2+} fluxes during the first action potential of a train

A, sarcolemma voltage and Ca^{2+} concentration plotted over a single action potential, with the stimulus occurring from 0 to 0.001 s. B, sarcolemma depolarization and repolarization coincide with an inward Na^+ current (upper plot) and outward K^+ delayed rectifier (KDR) current (lower plot), respectively. Currents through the Na^+-K^+ pump, $\text{Na}^+-\text{Ca}^{2+}$ exchanger (NCX) and K^+ inward rectifier (KIR) are also plotted for reference. C, fluxes through the sarcoplasmic reticulum (SR) and mitochondrial membranes, with the upper axes showing release from organelles and the lower axes showing uptake into organelles. D, fluxes through the cell membrane (SL and TT), with the upper axes showing influxes and the lower axes showing effluxes.

about 200 receptors per square micron (within the range of measurements of Orai1 density in STIM1-Orai1 puncta (Peckys et al., 2021)) and single channel conductance of 10 fS (Amcheslavsky et al., 2015), resulting in a total conductance of 2000 fS per square micron in the T-tubules. In this test SOCE played a significant role in Ca^{2+} dynamics – the increased Orai1 conductance led to a 21.3% increase in peak myoplasmic Ca^{2+} (Fig. 6B). We also examined the contractile force exerted by fibres with and without SOCE. The predicted dynamics of contractile force were similar in both cases, with only a minor (1.98%) increase in the maximum relative force for cells with strong Orai1 conductance over cells with no SOCE.

With or without SOCE, contractile force declined after less than 1 s of stimulation. This effect was largely attributed to phosphate accumulation in the myoplasm, as fixing the myoplasmic phosphate to a constant value almost entirely eliminated the observed force reduction (Fig. 6C, Fig. A5). This agrees well with the established role of phosphate in muscle fatigue (Allen & Trajanovska, 2012; Allen et al., 2008; Cheng et al., 2018; Hendry et al., 2025; Shorten et al., 2007). In our model one of the main mechanisms for phosphate clearance from the myoplasm is transport through the mitochondria phosphate carrier (PiC). We hypothesized that changes in

the rate of phosphate uptake by mitochondria (dictated by v_{PiC} , the permeability of mitochondria PiC) could provide an important factor modulating fatigue in our model. To test this we increased v_{PiC} by a factor of 10. Without altering Ca^{2+} dynamics, this led to much less accumulation of phosphate in the myoplasm (Fig. A5), and, consequently, partially mitigated the decrease in force (Fig. 6C). Given the established interplay between SOCE and mitochondrial network morphology (Nan et al., 2021), changes in phosphate transport through PiC may provide another mechanism for SOCE-mediated modulation of fatigue, as explored in further detail below.

SOCE and phosphate transport modulate contractile force in a frequency-dependent manner

Our initial simulations suggested that SOCE is an important regulator of Ca^{2+} dynamics and force production in skeletal muscle, in conjunction with changes in mitochondrial phosphate uptake. We next used our model to predict whether the effects of SOCE depend on stimulus frequency. We considered a case previously tested experimentally, in which dominant-negative Orai1 (dnOrai1) mouse extensor digitorum longus (EDL) muscle exhibited frequency-dependent inhibition

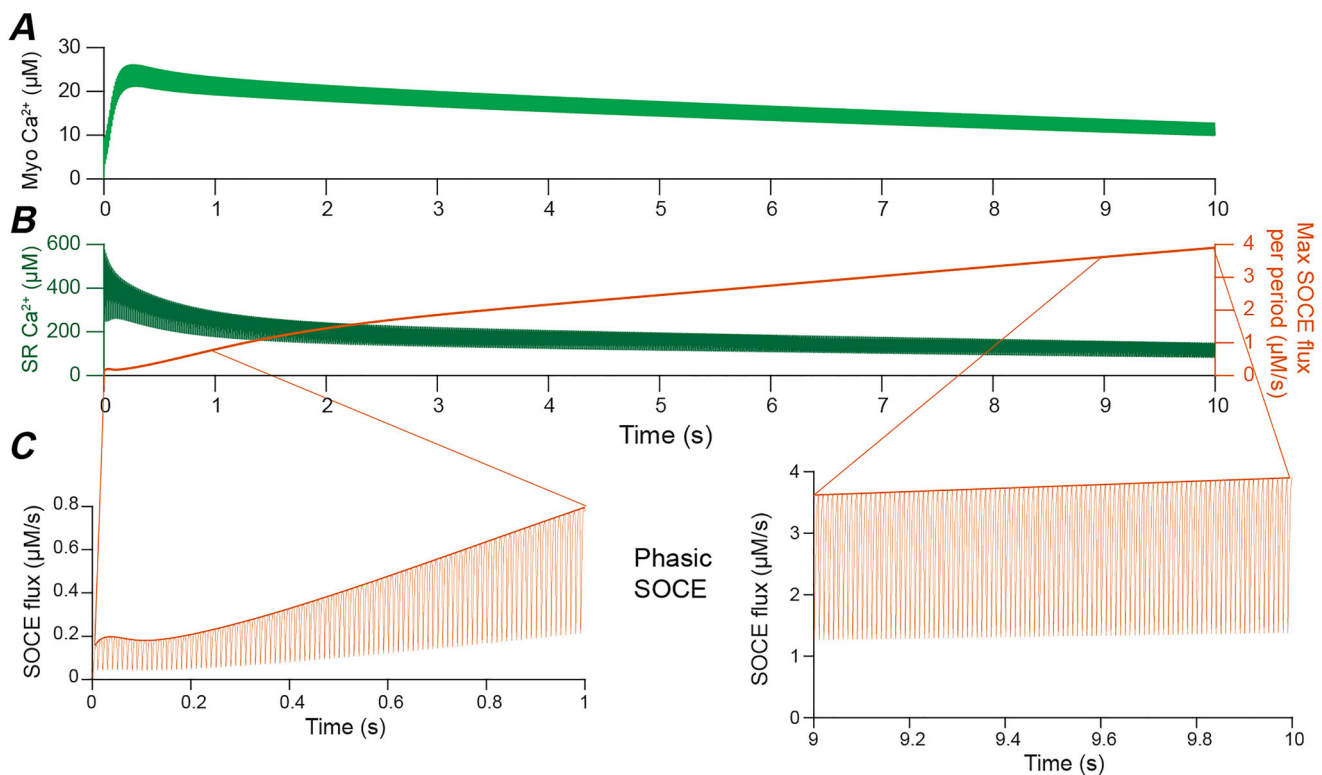


Figure 5. Ca^{2+} concentrations and store-operated Ca^{2+} entry (SOCE) flux during 10 s of 100 Hz stimulus (A),(B), myoplasmic Ca^{2+} (A) and SR Ca^{2+} (B) are plotted over time, with the maximum SOCE flux over each stimulus period plotted on the right y-axis in panel B. C, insets showing the full dynamics of SOCE within the first 1 s of simulation and the final 1 s of simulation.

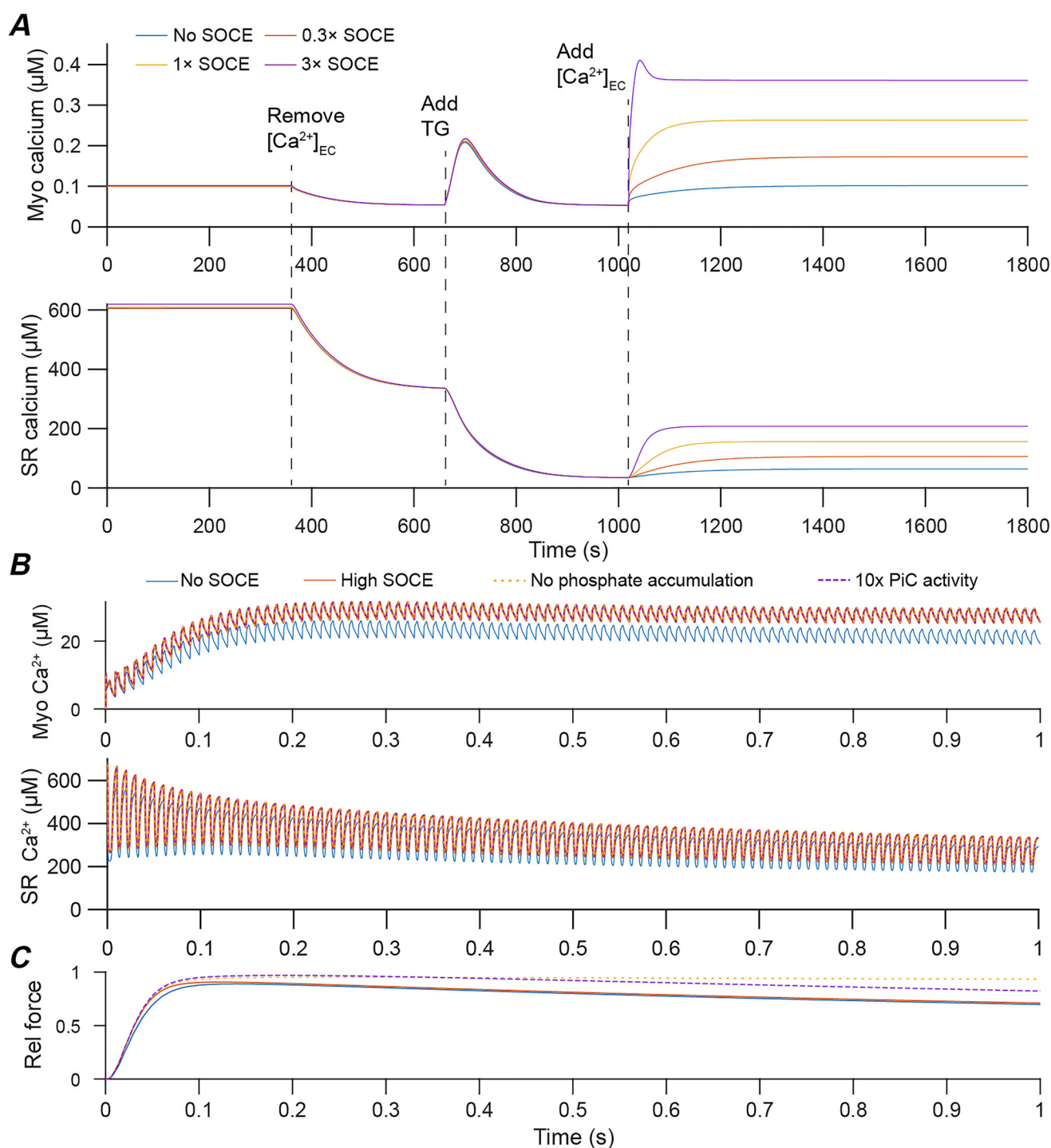


Figure 6. Crosstalk between store-operated Ca^{2+} entry (SOCE) and phosphate handling in computational experiments

A, predicted myoplasmic (upper) and sarcoplasmic reticulum (SR) (lower) Ca^{2+} dynamics in a conventional thapsigargin (TG) assay, modelled after tests in Nelson et al. (2020). Extracellular Ca^{2+} is initially $1300 \mu\text{M}$, then reduced to $0 \mu\text{M}$ at $t = 360 \text{ s}$ (e.g. via addition of a Ca^{2+} chelator such as EGTA). Addition of thapsigargin at $t = 660 \text{ s}$ reduces sarco/endoplasmic reticulum ATPase (SERCA) activity to 10% of its resting activity, inducing Ca^{2+} release from the SR. Extracellular Ca^{2+} ($1300 \mu\text{M}$) is then reintroduced at $t = 1020 \text{ s}$, triggering SOCE, with Orai1 conductance varied relative to the baseline (uncalibrated) value in Table A7. **B**, predicted myoplasmic (upper) and SR (lower) Ca^{2+} dynamics in response to 100 Hz stimulus with high SOCE ($g_{\text{SOCE}} = 2000 \text{ fS per square micron}$) vs. no SOCE. Results are also shown for high SOCE and either no phosphate accumulation or 10 times baseline PiC transport rate (these conditions overlap with the high SOCE curves here). **C**, predicted relative contractile force for conditions shown in panel **B**.

of force generation (Wei-LaPierre et al., 2013). As in these experiments, we applied different frequencies of stimuli consisting of square-wave pulse trains for a total of 0.5 s each. In each test we allowed basal levels of phosphate accumulation by running the simulation for an initialization period of 10 s without stimulus, as shown in Fig. A6.

At low stimulus frequencies (less than 20 Hz), we observed low force production regardless of SOCE, as tetani remained unfused (Fig. 7A). SOCE did enhance force production by a small amount at moderate stimulus frequencies (Fig. 7B), but had almost no effect on force production at higher (> 80 Hz) frequencies (Fig. 7C). Compared to the experimental data, these simulations

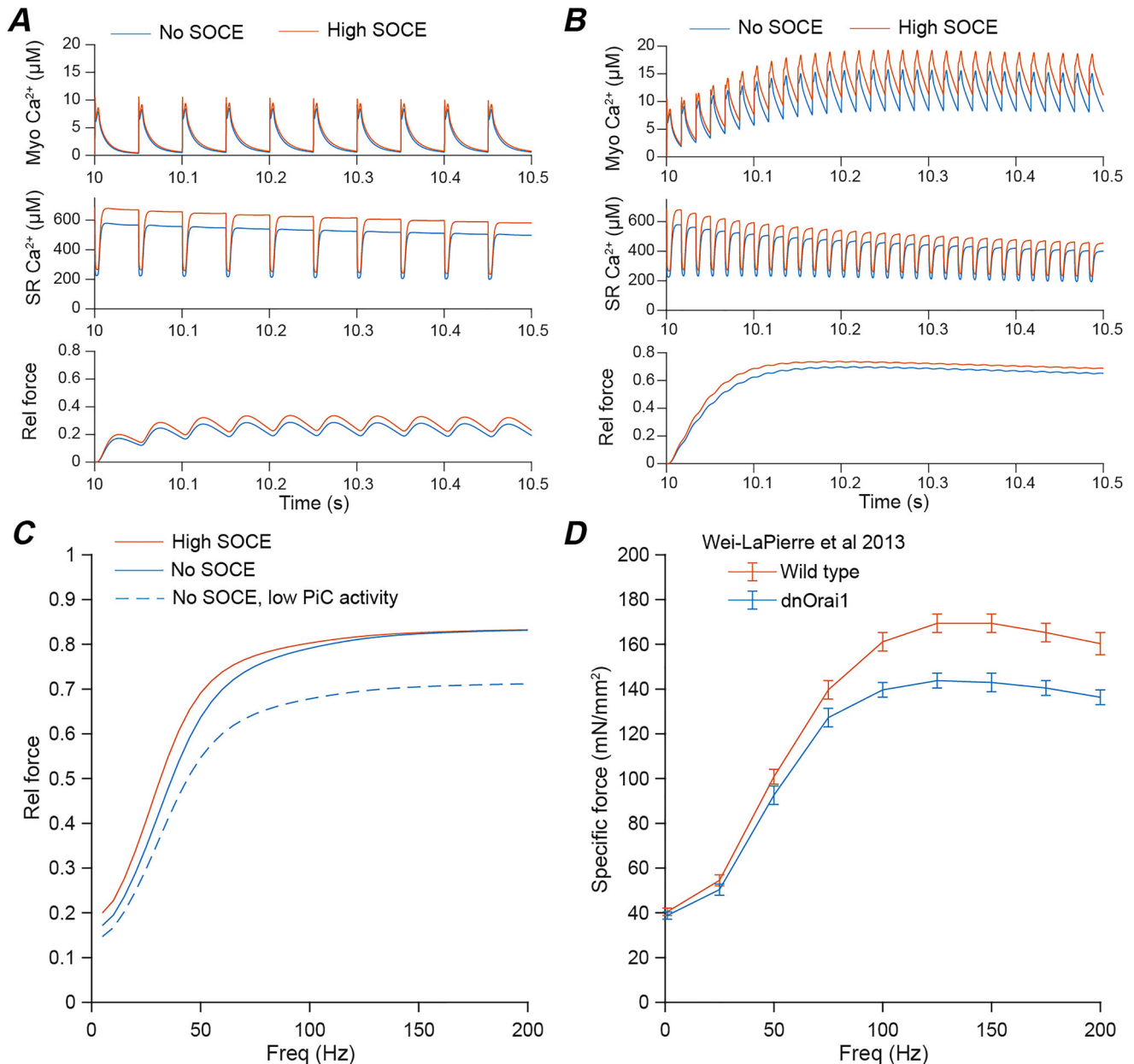


Figure 7. Frequency-dependent force generation with vs. without store-operated Ca^{2+} entry (SOCE) (A),(B), predicted effects of SOCE on myoplasmic Ca^{2+} , SR Ca^{2+} and contractile force are plotted for 20 Hz (A) and 60 Hz (B) stimuli. (C), frequency-dependent maximum contractile force with SOCE (2000 fS conductance) and without SOCE. The dashed line indicates case without SOCE and with 10% PiC activity. (D), experimental measurements of maximum force during *in vitro* testing of muscle from the EDL of wild type or dominant negative Orai1 (dnOrai) mice (Wei-LaPierre et al., 2013). $n = 14$ for both wild type and dnOrai1 conditions and error bars denote standard error of the mean. Data were extracted from the original figure using manual analysis in ImageJ.

generally overestimated contractile force in SOCE knockout fibres at high frequencies (Fig. 7C–D). We reasoned that additional SOCE-regulated processes not considered in our model might contribute increasingly at higher stimulus frequencies. SOCE mutants have been known to exhibit increased mitochondrial fragmentation and volume loss (Choi et al., 2019; Collins et al., 2014; Maus et al., 2017), which can disrupt the normal proton gradient (Alan & Scorrano, 2022) and accordingly reduce phosphate transport through PiC. We therefore reduced the PiC activity by a factor of 10 in SOCE-deficient cells, resulting in a significant reduction in force at higher stimulus frequencies in agreement with experiments (Fig. 7C,D). This reinforces findings from our initial simulations in Fig. 6, suggesting that SOCE and PiC activity co-modulate force reduction in myofibres.

Computational predictions suggest context-dependent role of SOCE in different forms of exercise

Finally, we considered the predictions of our model for different frequencies and patterns of stimuli relevant to resistance exercise and high-intensity interval training (HIIT). EMG measurements suggest that these exercises are marked by different stimulus frequencies at the NMJ – about 10–50 Hz for resistance exercise (Bellemare et al., 1983; Pucci et al., 2006) and 60–180 Hz for HIIT (Ament et al., 1993). Some studies indicate that firing frequencies can increase up to about 70–100 Hz in certain cases of rapid maximal volumetric contraction (MVC) (Škarabot et al., 2024). Accordingly we modelled high-load (approaching 100% MVC) resistance exercise using either 40 Hz or 70 Hz stimuli, with 3 s of contraction followed by 3 s of rest for each repetition, modelled after the isometric contraction measured in the quadriceps in previous work (Pucci et al., 2006). During high-intensity exercise such as sprinting, stimulus of a given muscle occurs in discrete phases during each stride. Considering running data collected in muscles that have a high abundance of fast-twitch fibres (gastrocnemius medialis or the gastrocnemius lateralis) (Hamner & Delp, 2013), we assumed that stimulus occurs over a 0.1625 s time interval for each 0.65 s stride, with stimulus frequencies of either 70 or 120 Hz. In the following tests we considered stimulus trains designed to mimic 20 s of running or 10 repetitions (60 s total) of resistance exercise.

To examine the role of SOCE in force production during exercise, we varied Orai1 conductance (g_{Orai1}) and increased STIM1 Ca^{2+} sensitivity ($K_{\text{STIM1}} = 0.5[\text{Ca}^{2+}]_{\text{SR},\infty}$, where $[\text{Ca}^{2+}]_{\text{SR},\infty}$ is the steady-state SR Ca^{2+} concentration). Shifts in g_{Orai1} can be attributed changes in SR/T-tubule organization (Boncompagni et al., 2017), transport of STIM1 (Grigoriev et al., 2008; Wu et al.,

2007) or Orai receptor turnover (Yeh et al., 2020). Changes in K_{STIM1} are associated with biochemical modifications (e.g. phosphorylation) that alter STIM1-Orai1 binding or STIM1 autoinhibition (Nelson et al., 2020; Pozo-Guisado & Martin-Romero, 2013). To examine the role of SOCE in fatigue, we simultaneously varied other parameters that were important determinants of fatigue – the troponin- Ca^{2+} binding rate ($k_{\text{on,T1}}$, determining the cross-bridge Ca^{2+} sensitivity) and the permeability of mitochondrial PiC (ν_{PiC}).

In the case of resistance exercise, we found that force production was enhanced by elevating Orai1 conductance (Fig. 8A,B). This effect held at both stimulus frequencies tested and regardless of the values for $k_{\text{on,T1}}$ and ν_{PiC} (Fig. A7). Mechanistically, these higher forces were attributed to a significantly higher fraction of troponin binding to Ca^{2+} in the myoplasm, allowing for faster cross-bridge cycling despite increased accumulation of myoplasmic phosphate (Fig. 8A). The rate of phosphate uptake by mitochondria did not influence the final force output for low levels of SOCE, but at higher Orai1 conductance, increased rates of PiC activity were required for optimal force production (Fig. 8B, Fig. A7).

Surprisingly, increasing levels of SOCE did not always increase the final force in HIIT (Fig. 8C,D). Rather, at higher rates of phosphate uptake, increasing Orai1 conductance actually reduced the force at the final stride (Fig. 8C,D). At very high levels of SOCE, the fraction of troponin bound to Ca^{2+} was already saturated, and so further increases in SOCE reduced force production due to phosphate accumulation (Fig. 8C). This effect was particularly apparent at the highest stimulus frequency (120 Hz) and highest Ca^{2+} -troponin binding rate (Fig. A8). In these simulations, increasing PiC activity had a crucial modulatory effect – fatigue was minimized only when the rates of phosphate clearance were sufficiently high (Fig. 8D). Thus, although increased SOCE often enhances force output, very high rates of Ca^{2+} entry can worsen fatigue. This reveals an emergent interplay between SOCE and mitochondrial phosphate handling in excitation-contraction coupling in skeletal muscle.

Discussion

Given the well-established role of Ca^{2+} release in skeletal muscle contraction, understanding conditions that optimize Ca^{2+} release and contractile force generation in exercise, and how such factors contribute to muscle failure in fatigue, is important for establishing predictive relationships between Ca^{2+} dynamics and athletic performance. Here we investigate the particular role of store-operated Ca^{2+} entry in excitation-contraction coupling in skeletal muscle. Although many recent experimental studies had suggested a central role of SOCE

in enhancing force and mitigating fatigue (Boncompagni et al., 2017; Wei-LaPierre et al., 2013), a majority of the existing computational models of excitation-contraction coupling in skeletal muscle did not include this important

pathway. Our model not only addresses this gap in the literature but combines action potential machinery with detailed Ca^{2+} and phosphate handling, constrains model parameters to experimental data and generates testable

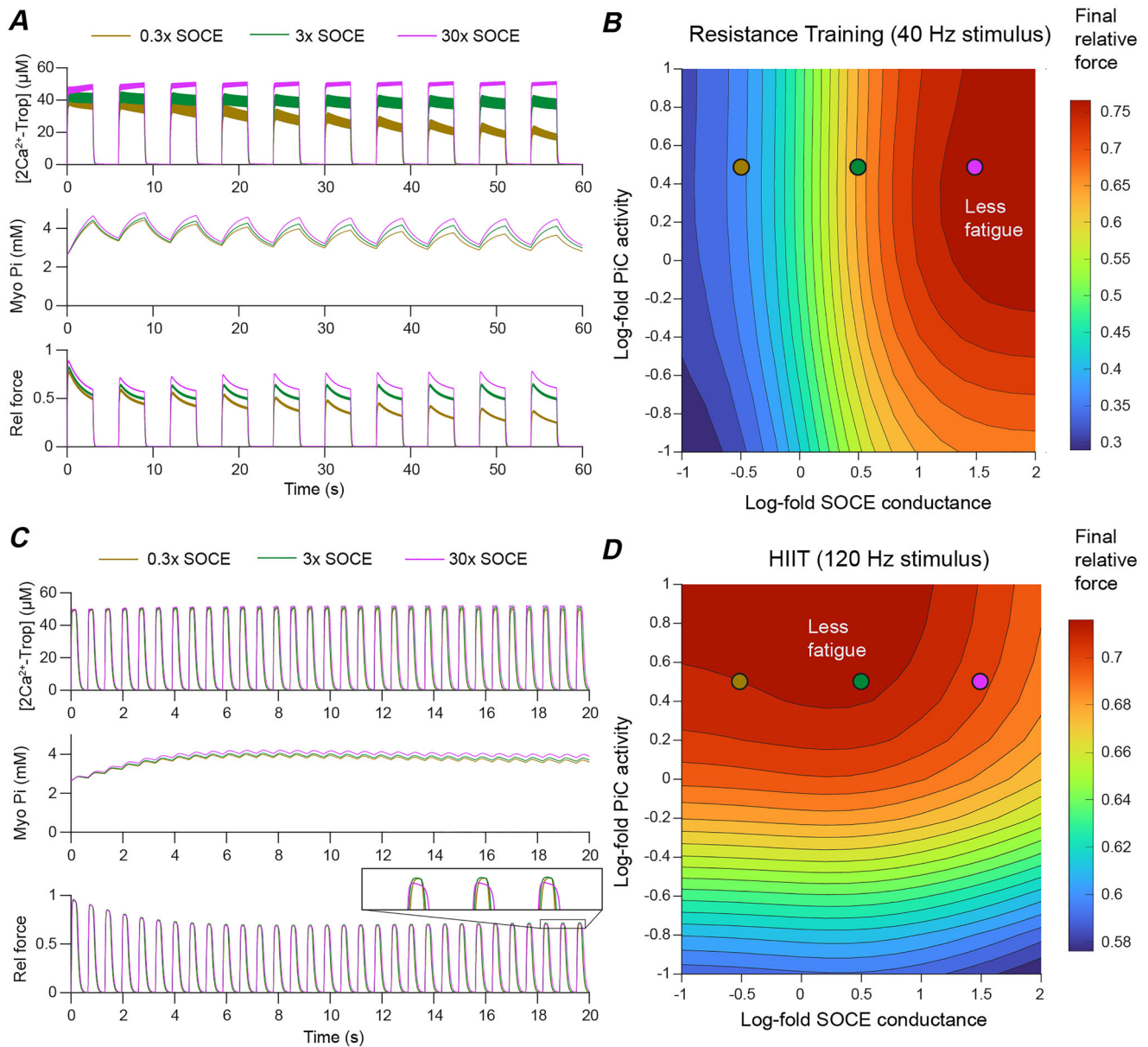


Figure 8. Predicted effects of altering store-operated Ca^{2+} entry (SOCE) and phosphate uptake rate in different exercise regimes

A, predicted dynamics of [Trop-2Ca^{2+}], myoplasmic phosphate and force over time during patterns of 40 Hz stimuli designed to mimic vastus lateralis activity during resistance training (Pucci et al., 2006). B, phase diagram summarizing the maximum contractile force during the final repetition of a 10-repetition resistance training set as a function of PiC permeability (v_{PiC}) and SOCE conductance (g_{Orai1}). C, predicted dynamics of [Trop-2Ca^{2+}], myoplasmic phosphate and force over time during patterns of 100 Hz stimuli designed to mimic gastrocnemius medialis activity during running (Hamner & Delp, 2013). D, phase diagram summarizing the maximum contractile force during the final stride of a 20 s running interval as a function of v_{PiC} and g_{Orai1} . Tests in panels A and C are conducted for low (0.3 $\times$), moderate (3 $\times$) and high (30 $\times$) the original (uncalibrated) Orai1 conductance at $3 \times v_{\text{PiC}}$, as indicated by points on the phase diagrams in panels B and D. All tests shown here used $K_{\text{STIM1}} = 0.5[\text{Ca}^{2+}]_{\text{SR},\infty}$ and 3 times the baseline estimated value for $k_{\text{on},\text{T1}}$.

predictions about the impact of SOCE on fatigue in skeletal muscle.

Our model predicts that SOCE can significantly enhance elevations in myoplasmic Ca^{2+} and contractile force, in line with our original hypothesis (Fig. 6 7). However, in contrast to our initial expectations, we found that this relationship was not consistent across all parameter regimes, which helps resolve an apparent discrepancy in the current literature regarding SOCE in skeletal muscle. Multiple studies have measured reduced force and increased susceptibility to fatigue in SOCE-deficient mice (Pan et al., 2002; Wei-LaPierre et al., 2013). In contrast, recent data suggest that SOCE deficiency might actually mitigate fatigue in certain cases (Nelson et al., 2020). Here we show that these two effects can be understood as the outcomes of the same biophysical system operating in different regimes. At lower stimulus frequencies and higher rates of phosphate uptake by mitochondria, we predict that SOCE enhances Ca^{2+} levels, leading to increased contractile force. However, for high-frequency stimulus, increasing SOCE can lead to reduced force due to the accumulation of phosphate in the myoplasm. In this case reducing SOCE mitigates fatigue, in agreement with experiments that show silencing STIM1 or inhibiting it via phosphorylation results in less susceptibility to fatigue (Nelson et al., 2020). These shifts may also be attributable to organismal differences as some studies consider mice (Pan et al., 2002; Wei-LaPierre et al., 2013), whereas Nelson et al. examine fatigue in *Drosophila* (Nelson et al., 2020).

The interplay between SOCE and phosphate dynamics within myofibres played an important role throughout our simulations. Previous models included phosphate cycling in the myoplasm and SR (Senneff & Lowery, 2021, 2022; Shorten et al., 2007), but we additionally account for a major mode of phosphate uptake through the phosphate transporter in the mitochondrial inner membrane (Seifert et al., 2015). Not only does this provide a natural mechanism to mitigate fatigue in our model, but phosphate also serves as a buffer for mitochondrial Ca^{2+} , allowing for increased Ca^{2+} uptake through the MCU. Increased levels of mitochondrial Ca^{2+} lead to increased rates of ATP production via oxidative phosphorylation. Our work suggests that mitochondrial phosphate handling is a key determinant of whether SOCE alleviates or worsens fatigue in myofibres. This connection is especially relevant given the established regulatory role of SOCE in mitochondrial fission and fusion (Nan et al., 2021). High levels of SOCE activity lead to increased mitochondria Ca^{2+} levels, which can induce fission (Han et al., 2008) or disruptions to mitochondrial morphology (Walkon et al., 2022). Such effects may perturb phosphate transport and increase the occurrence of SOCE-induced fatigue.

The control mechanisms that determine the relationship between SOCE and phosphate transport in myofibres require detailed considerations of metabolic regulation in skeletal muscle, necessitating models that extend beyond the time range considered here. Many such responses rely on metabolic regulation by AMP-activated protein kinase (AMPK), which previous models have shown to be modulated by the duration of stimulus during exercise (Fowler et al., 2024; Linden-Santangeli et al., 2025b). One recent computational study considered the resulting changes in mitochondrial fission and fusion downstream of different types of exercise, including inputs similar to those tested for resistance and HIIT here (Khalilimeybodi et al., 2026). This model predicted the enhanced activation of mitochondrial fusion downstream from HIIT, suggesting a possible compensatory mechanism for the effects of fatigue observed in this work. Long-term responses can also involve volumetric growth of the muscle itself, as considered in other recent modelling work (Devold et al., 2025; Villota-Narvaez et al., 2021). Bridging the time and length scales between the single-fibre, short timescale considered here to models of long-term remodelling and growth at the whole muscle scale presents a distinct challenge for future multiscale modelling work.

In addition to their important roles in Ca^{2+} handling and mitochondrial fission and fusion, STIM and Orai regulate other intracellular processes. STIM1 has been shown to engage a variety of other binding partners in addition to Orai1, including POST (partner of STIM1), which facilitates its interactions with SERCA, PMCA, the Na^+ - K^+ pump and nuclear transporters (Hooper et al., 2013; Krapivinsky et al., 2011). The multiplicity of roles for SOCE likely has many implications for the development of STIM and Orai germline knockout animals. Accordingly the experimental data considered in Fig. 7 must be interpreted carefully. Indeed, inducible Orai1 knockout mice do not exhibit the same reduction in maximal force during repeated stimulus but still exhibit increased fatigability (Carrell et al., 2016; Michelucci et al., 2019). Additional work is required to untangle the effects of acute SOCE dysfunction *versus* effects observed due to lack of SOCE during development.

Although our model incorporates many of the important players in excitation and contraction in skeletal muscle, we note some opportunities for future developments. We acknowledge that our treatment of SOCE is simplified, consisting of a single ODE governing Orai1 open probability; the detailed dynamics of STIM1 oligomerization, diffusion and association with Orai1 would require a more detailed biophysical model. Furthermore, other channels such as transient receptor potential canonical channels (TRPCs) can contribute to SOCE in skeletal muscle through interactions with STIM1 (Choi et al., 2020) and may be included in future

models. However detailed models introduce many free parameters, requiring additional experimental data for robust model calibration (Linden et al., 2022; Qiao et al., 2025). All of our parameters are currently estimated using data collected in single mouse myofibres at room temperature over a maximum of five action potentials (Baylor & Hollingworth, 2007; Miranda et al., 2020; Rincón et al., 2021); new datasets are needed to reliably calibrate models of human myofibres at physiological temperatures over extended times. Such data will better constrain many of the parameters to which the model was not currently sensitive, and may present opportunities for other approaches from uncertainty quantification such as Bayesian parameter estimation (Linden et al., 2022; Qiao et al., 2025) or Bayesian model selection (Linden-Santangeli et al., 2025b, 2025a). Finally, future work is required to elucidate the spatial features of SOCE in skeletal muscle. Experimental data show that myofibres undergo dramatic structural reorganization following exercise, including changes in the T-tubule system and assembly of CEUs (Boncompagni et al., 2017; Cully et al., 2017). Although our model differentiates between junctional and bulk regions within the myofibre, spatial modelling approaches using software packages such as VCell (Cowan et al., 2012) or SMART (Francis et al., 2025) are required to properly explore this aspect of Ca^{2+} signalling in skeletal muscle. Such models provide a natural opportunity to further explore how the transport of Ca^{2+} and phosphate into mitochondria depends on features of their morphology altered by fission or fusion. Additionally spatial models can help elucidate the importance of structural features of the NMJ, which have been shown to significantly impact the strength of signal transduction (Santoso et al., 2021).

Our findings may have implications beyond skeletal muscle, including neurons and cardiomyocytes (Basnayake et al., 2021; Benedetti et al., 2025; Hermes et al., 2023; Saftenku, 2022), as well as nonexcitable cells such as immune cells (Clemens & Lowell, 2015; Vig & Kinet, 2009). For example, the frequency-dependent role of SOCE explored here may naturally extend to postsynaptic signalling in neuronal dendritic spines (Basnayake et al., 2021; Dembrow & Spain, 2022). Thus our study positions SOCE as a key regulator of SR and myoplasmic Ca^{2+} , particularly important for cellular events that trigger rapid store depletion.

Materials and methods

Ethical approval

No human or animal data were collected for this work; all experimental data shown were from previous studies as indicated.

Numerical implementation

The full model is described in Appendix (Tables A1–A7) and consists of 65 nonlinear ordinary differential equations (including eqn 3, eqn 4, and eqn 8). Initial conditions were estimated by starting from default values of each variable (Table A4) and integrating the equations with no input current to $t = 5000$ s in MATLAB using the stiff ODE solver, ode15s. The initial values were then either defined by the values at $t = 5000$ s or the values when the following condition was met:

$$\frac{dy_i}{dt} \frac{1}{y_{i,\text{norm}}} < 10^{-5} \text{ for } i \text{ from 1 to 65,} \quad (16)$$

where y_i is the state variable associated with index i and $y_{i,\text{norm}}$ is a normalizing factor for y_i , defined in Table A4.

In all cases the steady-state solution with no SOCE ($g_{\text{Orail}} = 0$) was computed before defining K_{STIM1} as a fraction of the resting SR Ca^{2+} concentration without SOCE (Table A7). This approach was adopted to avoid cases with unrealistic levels of SOCE under resting conditions. Steady-state values for each state variable were then estimated for the desired Orail conductance ($g_{\text{Orail}} > 0$) at the assigned value of K_{STIM1} , before solving the dynamics with various stimuli using ode15s. This strategy was used to compute steady state for all variables except concentrations of phosphate, ADP and ATP in each compartment, which were fixed to estimated parameter values (for myoplasmic ATP, myoplasmic phosphate and SR phosphate) or to default values (for myoplasmic ADP and for mitochondrial ATP and phosphate) before starting the simulation (Table A7). SR Ca^{2+} -phosphate precipitate was always assumed to start at 0 μM . MATLAB code from this study is freely available on Github and through Zenodo (Kumar et al., 2026).

Sensitivity analysis

Morris sensitivity analysis was conducted using the UQLab framework in MATLAB (Marelli & Sudret, 2014). As described in the main text, maximum and average values of myoplasmic Ca^{2+} and sarcolemma voltage were used as QOIs. All non-mitochondrial parameters were varied according to a uniform distribution, from 0.5 to 2 times the reference value from literature (Table A7) Morris analysis yielded the absolute value of the mean (μ^*) and the SD (σ) of the elementary effects. A parameter was treated as sensitive if μ^* was greater than 20% of the maximum μ^* over all parameters for that QOI.

Parameter estimation

To ensure good fits to sarcolemma voltage, myoplasmic Ca^{2+} and mitochondrial Ca^{2+} , parameter

estimation was conducted in three stages. First, mitochondria-related parameters were independently calibrated in a mitochondria-exclusive test described below. We carried these parameters to the full model and then fit all the voltage-related parameters to match physiological action potentials. In the final step, model parameters were further constrained by fitting the full model to both myoplasmic Ca^{2+} and sarcolemma voltage over time.

Mitochondrial parameter estimation. The model of mitochondrial Ca^{2+} and phosphate handling was first fit separately from the rest of the model. We considered experimental data from Wei et al. (2015), in which the authors measured mitochondrial Ca^{2+} (both free and bound) and mitochondrial membrane voltage over time in vitro. In these experiments guinea pig heart mitochondria were isolated and initially depleted of phosphate and placed in a phosphate-free, Ca^{2+} -low solution. They then used fluorescence microscopy to simultaneously measure free matrix Ca^{2+} , external Ca^{2+} and membrane voltage. Monitoring external Ca^{2+} together with free matrix Ca^{2+} allowed them to monitor the ratio of bound-to-free Ca^{2+} in the mitochondrial matrix. Each experiment started with adding Ca^{2+} to the external solution (shown at $t = 110$ s in Fig. A3A), leading to Ca^{2+} uptake by mitochondria and an associated reduction in the mitochondrial membrane voltage. They then added free phosphate (either 1, 10, 100 or 1000 μM) to the solution (at $t = 240$ in Fig. A3A), leading to further Ca^{2+} uptake and a dramatic increase in the ratio of free to bound matrix Ca^{2+} , illustrating the role of matrix phosphate as a Ca^{2+} buffer. We used our model to simulate these experiments, applying external Ca^{2+} and phosphate as known quantities. The system was initialized by starting with $[\text{Ca}^{2+}]_{\text{Mito}} = 0.1 \mu\text{M}$, $[\text{ATP}]_{\text{Mito}} = 10 \times 10^3 \mu\text{M}$, $[\text{NADH}]_{\text{Mito}} = 50 \mu\text{M}$, $V_{\text{Mito}} = 150 \text{ mV}$, and no mitochondrial phosphate or Ca^{2+} -phosphate, then running the system for 400 s with no applied phosphate or Ca^{2+} in the extra-mitochondrial solution. Model predictions could then be directly leveraged against their data (Fig. A3C,D), with the overall error computed by the following weighted sum of square errors:

$$\mathcal{F}_{\text{Mito}} = \sum_{i=1}^4 \frac{1}{N_i} \sum_{j=1}^{N_i} \left[\left(\frac{[\text{Ca}^{2+}]_{\text{mito,model},i}(t_j) - [\text{Ca}^{2+}]_{\text{mito,exp},i}(t_j)}{\sigma_{c,\text{mito}}} \right)^2 + \left(\frac{c_{\text{ratio,model},i}(t_j) - c_{\text{ratio,exp},i}(t_j)}{\sigma_{\text{ratio}}} \right)^2 + \left(\frac{V_{\text{mito,model}}(t_j) - V_{\text{mito,exp}}(t_j)}{\sigma_{V,\text{mito}}} \right)^2 \right], \quad (17)$$

where N_i is the number of time points considered for experiment i and the weighting factors

($\sigma_{c,\text{mito}}$, σ_{ratio} , $\sigma_{V,\text{mito}}$ were chosen to represent measurement error (we chose $\sigma_{c,\text{mito}} = 5 \mu\text{M}$, $\sigma_{\text{ratio}} = 100$ and $\sigma_{V,\text{mito}} = 10 \text{ mV}$, based on the magnitude of the SEM from Wei et al. (2015)).

To compute c_{ratio} from our model, we accounted for both the rapid buffering and Ca^{2+} binding to phosphate in the matrix. The rapid-buffering approximation here assumes that, of the Ca^{2+} not bound to phosphate, a fraction $1 - b_M$ is bound to other buffers; that is:

$$[\text{Ca}^{2+}]_{\text{bound,rapid}} = (1 - b_M) ([\text{Ca}^{2+}]_{\text{bound,rapid}} + [\text{Ca}^{2+}]_{\text{Mito}}), \quad (18)$$

where $[\text{Ca}^{2+}]_{\text{bound,rapid}}$ is the concentration of Ca^{2+} bound to buffers other than phosphate.

The ratio of bound-to-free Ca^{2+} is

$$c_{\text{ratio,model}} = \frac{[\text{Ca}^{2+}]_{\text{bound,rapid}} + [\text{Ca}^{2+} - \text{Pi}]_{\text{Mito}}}{[\text{Ca}^{2+}]_{\text{Mito}}}, \quad (19)$$

which, upon substituting eqn 18, yields the expression used to compute the bound-to-free ratio in our model:

$$c_{\text{ratio,model}} = \frac{(1 - b_M)[\text{Ca}^{2+}]_{\text{Mito}} + b_M[\text{Ca}^{2+} - \text{Pi}]_{\text{Mito}}}{b_M[\text{Ca}^{2+}]_{\text{Mito}}}. \quad (20)$$

This fit was conducted using particle swarm optimization (PSO) in MATLAB with a swarm size of 30 and maximum stall iterations of 20. We allowed each parameter to vary from 0.5 to 2 times its baseline value, except for voltage-shift parameters (parameters M17 and M23 in Table A7), which could only vary $\pm 10 \text{ mV}$. The fit reproduced key qualitative features of the experimental measurements (Fig. A3C,D) with the exception of the mitochondrial membrane voltage, which decreased at the end of simulations with higher added amounts of phosphate. We attribute this to negative ion fluxes across the mitochondrial membrane not accounted for in our model, which are outside the scope of this study.

Membrane voltage parameter estimation. In the following step, the objective function was defined as the normalized sum of square errors between experimentally measured voltage and model predictions for the data from Miranda et al. (2020):

$$\mathcal{F}_V = \frac{1}{N} \sum_{j=1}^N \left(\frac{V_{\text{SL,model}}(t_j) - V_{\text{SL,exp}}(t_j)}{\sigma_V} \right)^2, \quad (21)$$

where $V_{\text{SL,model}}(t_j)$ and $V_{\text{SL,exp}}(t_j)$ are, respectively, the predicted and experimentally measured sarcolemma voltage at time t_j . N is the number of time points considered, and σ_V is the SD of the measurement error (fixed at 5 mV). For this step only the 27 parameters with greater than 20% of the maximum sensitivity index for average or peak voltage (Fig. A2A,B) were included in the fitting.

Each of these parameters was allowed to vary between 0.5 and 2 times its baseline value, except for voltage-shift parameters (parameters 76–83 in Table A7), which could only vary ± 10 mV. Fitting was conducted using particle swarm optimization in MATLAB, with a swarm size of 30 particles and maximum stall count of 20.

Full model parameter estimation. In the final step a new objective function was defined, starting with the weighted sum of square errors between experimental and simulated myoplasmic Ca^{2+} :

$$\mathcal{F}_{\text{Ca}^{2+}} = \sum_{i=1}^2 \left[\mathcal{F}_{i,\text{Ca}^{2+}\infty} + \frac{1}{N_i} \sum_{j=1}^{N_i} \left(\frac{[\text{Ca}^{2+}]_{\text{avg,model},i}(t_j) - [\text{Ca}^{2+}]_{\text{avg,exp},i}(t_j)}{\sigma_c} \right)^2 \right], \quad (22)$$

where $[\text{Ca}^{2+}]_{\text{avg,model},i}(t_j)$ and $[\text{Ca}^{2+}]_{\text{myo,exp},i}(t_j)$ are the predicted (defined in eqn 15) and experimentally measured spatially averaged myoplasmic Ca^{2+} concentration at time t_j for experiment i and σ_c is the SD of the measurement error (fixed at $0.5 \mu\text{M}$). $\mathcal{F}_{i,\text{Ca}^{2+}\infty}$ represents an additional fitting constraint for each experiment that ensures a good match with experimentally characterized myoplasmic and SR Ca^{2+} at steady state:

$$\mathcal{F}_{i,\text{Ca}^{2+}\infty} = \left(\frac{[\text{Ca}^{2+}]_{i,\text{myo}}(t=0) - 0.1 \mu\text{M}}{0.1 \mu\text{M}} \right)^2 + \left(\frac{[\text{Ca}^{2+}]_{\text{SR},i}(t=0) - 500 \mu\text{M}}{500 \mu\text{M}} \right)^2 \quad (23)$$

A total objective function was constructed to ensure good fits with both Ca^{2+} and voltage data simultaneously, $\mathcal{F}_V + \mathcal{F}_{\text{Ca}^{2+}}$. All parameters were included in this fitting step, starting with initial guesses given by the previous estimation for applicable parameters. We used the calibrated values from the previous step as new baseline values for applicable parameters here. All parameters were allowed to vary between 0.5 and 2 times their baseline values, except for voltage-shift parameters (parameters 76–83 in Table A7), which could only vary ± 10 mV. The same specifications for particle swarm optimization were used as in the previous step. The final optimized values for each parameter are reported in Table A7.

Appendix A

Full model description

In our model four major compartments (extracellular space, myoplasm, mitochondria, and SR lumen) are each

divided into two well-mixed regions – one ‘junctional’ region and one ‘bulk’ region. Each junctional region is adjacent to the SR-T-tubule junction (either T-tubule volume, terminal myoplasm, junctional mitochondria, or terminal SR) and is treated as a separate compartment due to the highly localized ionic fluxes in the region. The myoplasm and extracellular space are separated by the plasma membrane (consisting of T-tubules and sarcolemma), whereas the myoplasm and SR lumen are separated by the SR membrane. In contrast the junctional and bulk regions of each major compartment other than mitochondria are not separated by membranes. Accordingly we assume that fluxes across the associated interface (denoted by dashed lines in Fig. 1) are purely diffusive. That is for a given species c :

$$\frac{dc_{\text{bulk}}}{dt} = J_{c,\text{bulk}} + D_c \frac{SA_{\text{bulk-junc}}(c_{\text{junc}} - c_{\text{bulk}})}{\text{vol}_{\text{bulk}} \Delta d_{\text{diff}}}, \quad (A1)$$

$$\frac{dc_{\text{junc}}}{dt} = J_{c,\text{junc}} + D_c \frac{SA_{\text{bulk-junc}}(c_{\text{bulk}} - c_{\text{junc}})}{\text{vol}_{\text{junc}} \Delta d_{\text{diff}}}, \quad (A2)$$

where Δd_{diff} is the characteristic length for diffusion, vol_{bulk} and vol_{junc} are the volumes of the bulk and junctional regions, respectively and $SA_{\text{bulk-junc}}$ is the area of the interface between the bulk and junctional regions. $J_{c,\text{bulk}}$ and $J_{c,\text{junc}}$ are the sum of reaction rates and other nondiffusive fluxes of species c in the bulk and junctional spaces, respectively. The above relation only applies to species in a volumetric compartment (SR lumen, myoplasm, or T-tubule volume). Variables associated with the state of surface species (e.g. ion channels in the sarcolemma) are assumed to be non-diffusive, with the exception of membrane voltage, which diffuses rapidly between the sarcolemma and T-tubules (eqn 3, 4).

To calculate the volume and surface area of each compartment (sarcolemma, T-tubule, myoplasm, SR membrane, SR lumen and mitochondrial volume), we approximate the muscle fibre as a cylinder of radius $20 \mu\text{m}$, matching the size of mouse fast-twitch fibres (Luff & Atwood, 1972; Wallinga et al., 1999). The total volume and sarcolemma surface area are given by the volume and surface area of a cylindrical cross-section. We assume the fibre is homogeneous along the longitudinal direction, such that the length of our cylindrical volume of interest is arbitrary. The subcellular volumes (myoplasm, mitochondria and SR lumen) are then calculated from previously determined volume fractions (Rincón et al., 2021; Senneff & Lowery, 2021), whereas the T-tubule and SR surface area are given by the surface area to volume ratios measured in Peachey (1965) and Mobley and Eisenberg (1975). The junctional myoplasmic volume is defined as the region within Δd_{diff} of the T-tubule surface, and, similarly, the junctional SR volume is defined as the volume within Δd_{diff} of the junctional

Table A1. Geometric parameters

Name	Description	Value	Reference
R_{fibre}	Fibre radius	20 μm	Wallinga et al. (1999)
L_{fibre}	Fibre length	100 μm	(arbitrary)
ϕ_{myo}	Myoplasm volume fraction	0.947	Shorten et al. (2007)
ϕ_{SR}	SR volume fraction	0.05	Shorten et al. (2007)
ϕ_{TT}	T-tubule volume fraction	0.003	Mathias et al. (1977)
θ_{TT}	T-tubule volume-to-surface-area ratio	0.01 μm	Peachey (1965)
θ_{SR}	SR volume-to-surface-area ratio	0.1 μm	Mobley and Eisenberg (1975)
$\phi_{\text{SRM, TT-SR}}$	Area fraction of SRM at SR-TT junction	0.5	Mobley and Eisenberg (1975)
ϕ_{Mito}	Mitochondria volume fraction (per myoplasm)	0.05	Chen et al. (2001)

Table A2. Equations for sub-compartment volumes and surface areas

Major compartment	Subregion	Equation
Extracellular	Total	$\text{vol}_{\text{EC,tot}} \rightarrow \infty$
	Junctional	$\text{vol}_{\text{TT}} = \phi_{\text{TT}} \pi R_{\text{fibre}}^2 L_{\text{fibre}}$
	Bulk	$\text{vol}_{\text{EC}} \rightarrow \infty$
	Bulk-junc interface	$\text{SA}_{\text{EC,bulk-junc}} = \phi_{\text{TT}} 2\pi R_{\text{fibre}} L_{\text{fibre}}$
Plasma membrane	Total	$\text{SA}_{\text{PM}} = \text{SA}_{\text{SL}} + \text{SA}_{\text{TT}}$
	Junctional	$\text{SA}_{\text{TT}} = \frac{\phi_{\text{TT}} \pi R_{\text{fibre}}^2 L_{\text{fibre}}}{\theta_{\text{TT}}}$
	Bulk	$\text{SA}_{\text{SL}} = 2\pi R_{\text{fibre}} L_{\text{fibre}}$
	Bulk-junc interface	$\text{SA}_{\text{myo,bulk-junc}} = \text{SA}_{\text{TT}} (1 - \phi_{\text{SRM,TT-SR}})$
Myoplasm	Total	$\text{vol}_{\text{myo}} = \phi_{\text{myo}} (1 - \phi_{\text{Mito}}) \pi R_{\text{fibre}}^2 L_{\text{fibre}}$
	Junctional	$\text{vol}_{\text{myo,junc}} = \text{SA}_{\text{TT}} \Delta d_{\text{diff}} (1 - \phi_{\text{Mito}})$
	Bulk	$\text{vol}_{\text{myo,bulk}} = \text{vol}_{\text{myo}} - \text{vol}_{\text{myo,junc}}$
	Bulk-junc interface	$\text{SA}_{\text{myo,bulk-junc}} = \text{SA}_{\text{TT}} (1 - \phi_{\text{SRM,TT-SR}})$
SR membrane	Total	$\text{SA}_{\text{SRM}} = \frac{\phi_{\text{SR}} \pi R_{\text{fibre}}^2 L_{\text{fibre}}}{\theta_{\text{SR}}}$
	Junctional	$\text{SA}_{\text{SRM,junc}} = \text{SA}_{\text{TT}} \phi_{\text{SRM,TT-SR}}$
	Bulk	$\text{SA}_{\text{SRM,bulk}} = \text{SA}_{\text{SRM}} - \text{SA}_{\text{SRM,junc}}$
	Bulk-junc interface	$\text{SA}_{\text{SR,bulk-junc}} = \text{SA}_{\text{SRM,junc}}$
SR	Total	$\text{vol}_{\text{SR}} = \phi_{\text{SR}} \pi R_{\text{fibre}}^2 L_{\text{fibre}}$
	Junctional	$\text{vol}_{\text{SR,junc}} = \text{SA}_{\text{SRM,junc}} \Delta d_{\text{diff}}$
	Bulk	$\text{vol}_{\text{SR,bulk}} = \text{vol}_{\text{SR}} - \text{vol}_{\text{SR,junc}}$
	Bulk-junc interface	$\text{vol}_{\text{Mito,bulk}} = \text{vol}_{\text{Mito}} - \text{vol}_{\text{Mito,junc}}$
Mito matrix	Total	$\text{vol}_{\text{Mito}} = \phi_{\text{myo}} \phi_{\text{Mito}} \pi R_{\text{fibre}}^2 L_{\text{fibre}}$
	Junctional	$\text{vol}_{\text{Mito,junc}} = \text{SA}_{\text{TT}} \Delta d_{\text{diff}} \phi_{\text{Mito}}$
	Bulk	$\text{vol}_{\text{Mito,bulk}} = \text{vol}_{\text{Mito}} - \text{vol}_{\text{Mito,junc}}$
	Bulk-junc interface	0

SR membrane. The interface between the junctional SR and T-tubule membrane is assumed to occupy about 50% of the T-tubule surface, in good agreement with the 67% occupancy measured in frog skeletal muscle (Mobley & Eisenberg, 1975). Finally the surface area of the junction between T-tubules and the extracellular space ($\text{SA}_{\text{EC,bulk-junc}}$) is computed assuming the volume fraction of T-tubules is constant throughout the fibre. All geometric parameters are summarized in Table A1, and expressions for individual subvolumes and surface areas are given in Table A2.

The equations used in our system of ordinary differential equations (ODEs) were adapted from different papers that incorporated Ca^{2+} signalling and action potential generation within mammalian muscle fibres. Reactions/fluxes are given for each module in Table A5. Throughout whenever a given flux or variable is associated with both bulk and junctional regions, we denote the intracellular region with subscript i, the extracellular region with subscript o and the associated region of the plasma membrane (either T-tubules or bulk

Table A3. Ionic fluxes summarized by region

Flux	Ions (stoich. *)	TT	SL	Junctional SRM	Bulk SRM	Reference
J_{Na}	Na^+ (+1)	0.1	1	0	0	Cannon et al. (1993)
J_{NaK}	Na^+ (-3), K^+ (+2)	0.1	1	0	0	Wallinga et al. (1999)
J_{NCX}	Na^+ (+3), Ca^{2+} (-1)	1	1	0	0	This study
J_{KIR}	K^+ (+1)	1	1	0	0	Ashcroft et al. (1985)
J_{KDR}	K^+ (-1)	0.45	1	0	0	Wallinga et al. (1999)
J_{Cl^-}	Cl^- (+1)	0.1	1	0	0	Wallinga et al. (1999)
$J_{Ca^{2+} leak,M}$	Ca^{2+} (+1)	1	1	0	0	This study
J_{SOCE}	Ca^{2+} (+1)	1	0	0	0	Protasi et al. (2021)
J_{PMCA}	Ca^{2+} (-1)	1	1	0	0	This study
J_{RyR}	Ca^{2+} (+1)	0	0	1	0	Senneff and Lowery (2021)
J_{SERCA}	Ca^{2+} (-1)	0	0	0.1	1	Scheibe et al. (2008)
$J_{Ca^{2+} leak,SR}$	Ca^{2+} (+1)	0	0	1	1	Wallinga et al. (1999)

References provided motivate the distribution of channels between junctional and bulk regions; in absence of additional evidence, we assume equal distribution across surface compartments. * Stoichiometric coefficient is positive for fluxes into the myoplasm and negative for fluxes out of the myoplasm.

Table A4. All state variables and their default values

Name	Description	Default value	Normalization const
Junctional variables			
V_{TT}	TT membrane voltage	-88 mV	100 mV
$P_{O,Orai1}$	open probability of Orai1	0.0122	1
$[Na^+]_{TT}$	Na^+ in TT volume	$1.47 \times 10^5 \mu M$	$1.47 \times 10^5 \mu M$
$[Na^+]_{myo,junc}$	Na^+ in junctional myoplasm	$1.47 \times 10^4 \mu M$	$1 \times 10^3 \mu M$
m_{TT}	Na^+ channel activation variable in TT	0.0128	1
h_{TT}	Na^+ channel inactivation variable in TT	0.8051	1
S_{TT}	Na^+ channel slow inactivation variable in TT	0.8487	1
$[K^+]_{TT}$	K^+ in T-tubule volume	$4 \times 10^3 \mu M$	$4 \times 10^3 \mu M$
$[K^+]_{myo,junc}$	K^+ in junctional myoplasm	$1.545 \times 10^5 \mu M$	$1 \times 10^5 \mu M$
n_{TT}	KDR activation variable in TT	0.003	1
$h_{K,TT}$	KDR inactivation variable in TT	0.9983	1
$[Cl^-]_{TT}$	Cl^- in T-tubule volume	$1.28 \times 10^5 \mu M$	$1.28 \times 10^5 \mu M$
$[Cl^-]_{myo,junc}$	Cl^- in junctional myoplasm	$5.83 \times 10^3 \mu M$	$1 \times 10^3 \mu M$
$[Ca^{2+}]_{TT}$	Ca^{2+} in T-tubule volume	$1.3 \times 10^3 \mu M$	$1.3 \times 10^3 \mu M$
$[Ca^{2+}]_{myo,junc}$	Ca^{2+} in junctional myoplasm	0.1 μM	0.1 μM
$[Ca^{2+}]_{SR,junc}$	Ca^{2+} in junctional SR	$1.5 \times 10^3 \mu M$	$1.5 \times 10^3 \mu M$
w_{RyR}	Slow inactivation variable for RyR	0.9091	1
$[Ca^{2+}-Parv]_{myo,junc}$	Ca^{2+} -Parv in junctional myoplasm	0 μM	500 μM
$[Mg^{2+}-Parv]_{myo,junc}$	Mg^{2+} -Parv in junctional myoplasm	0 μM	$1 \times 10^3 \mu M$
$[ATP]_{myo,junc}$	Free ATP in junctional myoplasm	$8 \times 10^3 \mu M$	1 μM
$[Ca^{2+}-ATP]_{myo,junc}$	Ca^{2+} -ATP in junctional myoplasm	0.3632 μM	1 μM
$[Mg^{2+}-ATP]_{myo,junc}$	Mg^{2+} -ATP in junctional myoplasm	0 μM	1 μM
$[Pi]_{myo,junc}$	Inorganic phosphate in junctional myoplasm	$4 \times 10^3 \mu M$	1 μM
$[Pi]_{SR,junc}$	Inorganic phosphate in junctional SR	$1.6 \times 10^3 \mu M$	1 μM
$[Ca^{2+}-Pi]_{SR,junc}$	Ca^{2+} -phosphate precipitate in junctional SR	0 μM	1 μM
$[CSQN]_{SR,junc}$	Free calsequestrin in junctional SR	$1.5 \times 10^4 \mu M$	$1 \times 10^3 \mu M$
$[ADP]_{myo,junc}$	ADP in the junctional myoplasm	100 μM	100 μM
$[Ca^{2+}]_{Mito,junc}$	Free Ca^{2+} in junctional mitochondria	0.1 μM	1 μM
$[Pi]_{Mito,junc}$	Phosphate in junctional mitochondria	$5 \times 10^3 \mu M$	$1 \times 10^3 \mu M$

(Continued)

Table A4. (Continued)

Name	Description	Default value	Normalization const
[Ca ²⁺ -Pi] _{Mito,junc}	Ca ²⁺ -phosphate in junctional mitochondria	0 μM	100 μM
[ATP] _{Mito,junc}	ATP in junctional mitochondria	5 × 10 ³ μM	1 × 10 ³ μM
[NADH] _{Mito,junc}	NADH in junctional mitochondria	50 μM	100 μM
V _{Mito,junc}	Junctional mitochondrial membrane potential	150 mV	100 mV
Bulk variables			
V _{SL}	SL membrane voltage	−88 mV	100 mV
[Na ⁺] _{myo,bulk}	Na ⁺ in bulk myoplasm	1.47 × 10 ⁴ μM	1 × 10 ³ μM
m _{SL}	Na ⁺ channel activation variable in SL	0.0128	1
h _{SL}	Na ⁺ channel inactivation variable in SL	0.8051	1
S _{SL}	Na ⁺ channel slow inactivation variable in SL	0.8487	1
[K ⁺] _{myo,bulk}	K ⁺ in bulk myoplasm	1.545 × 10 ⁵ μM	1 × 10 ⁵ μM
n _{SL}	KDR activation variable in SL	0.003	1
h _{K,SL}	KDR inactivation variable in SL	0.9983	1
[Cl [−]] _{myo,bulk}	Cl [−] in bulk myoplasm	5.83 × 10 ³ μM	1 × 10 ³ μM
[Ca ²⁺] _{myo,bulk}	Ca ²⁺ in bulk myoplasm	0.1 μM	0.1 μM
[Ca ²⁺] _{SR,bulk}	Ca ²⁺ in bulk SR	1.5 × 10 ³ μM	1.5 × 10 ³ μM
[Ca ²⁺ -Parv] _{myo,bulk}	Ca ²⁺ -Parv in bulk myoplasm	0 μM	500 μM
[Mg ²⁺ -Parv] _{myo,bulk}	Mg ²⁺ -Parv in bulk myoplasm	0 μM	1 × 10 ³ μM
[ATP] _{myo,bulk}	Free ATP in bulk myoplasm	8 × 10 ³ μM	1 μM
[Ca ²⁺ -ATP] _{myo,bulk}	Ca ²⁺ -ATP in bulk myoplasm	0.3632 μM	1 μM
[Mg ²⁺ -ATP] _{myo,bulk}	Mg ²⁺ -ATP in bulk myoplasm	0 μM	1 μM
[Pi] _{myo,bulk}	Inorganic phosphate in bulk myoplasm	4 × 10 ³ μM	1 μM
[Pi] _{SR,bulk}	Inorganic phosphate in bulk SR	1.6 × 10 ³ μM	1 μM
[Ca ²⁺ -Pi] _{SR,bulk}	Ca ²⁺ -phosphate precipitate in bulk SR	0 μM	1 μM
[CSQN] _{SR,bulk}	Free calsequestrin in bulk SR	1.5 × 10 ⁴ μM	1 × 10 ³ μM
[Trop-Ca ²⁺]	Troponin- Ca ²⁺ in bulk myoplasm	0 μM	1 μM
[Trop-2Ca ²⁺]	Troponin-2 Ca ²⁺ in bulk myoplasm	0 μM	1 μM
[D]	Unblocked, detached cross-bridge density	0 μM	1 μM
[A _{pre}]	Attached cross-bridge density, pre-power stroke	0 μM	1 μM
[A _{post}]	Attached cross-bridge density, post-power stroke	0 μM	1 μM
[ADP] _{myo,bulk}	ADP in the bulk myoplasm	100 μM	100 μM
[Ca ²⁺] _{Mito,bulk}	Free Ca ²⁺ in bulk mitochondria	0.1 μM	1 μM
[Pi] _{Mito,bulk}	Phosphate in bulk mitochondria	5 × 10 ³ μM	1 × 10 ³ μM
[Ca ²⁺ -Pi] _{Mito,bulk}	Ca ²⁺ -phosphate in bulk mitochondria	0 μM	100 μM
[ATP] _{Mito,bulk}	ATP in bulk mitochondria	5 × 10 ³ μM	1 × 10 ³ μM
[NADH] _{Mito,bulk}	NADH in bulk mitochondria	50 μM	100 μM
V _{Mito,bulk}	Bulk mitochondrial membrane potential	150 mV	100 mV

sarcolemma) with subscript M. For instance we write all Nernst potentials as:

$$E_{\text{Na}^+, \text{M}} = \frac{RT}{F} \ln \left(\frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right) \quad (\text{A3})$$

$$E_{\text{K}^+, \text{M}} = \frac{RT}{F} \ln \left(\frac{[\text{K}^+]_o}{[\text{K}^+]_i} \right) \quad (\text{A4})$$

$$E_{\text{Cl}^-, \text{M}} = -\frac{RT}{F} \ln \left(\frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i} \right) \quad (\text{A5})$$

$$E_{\text{Ca}^{2+}, \text{M}} = \frac{RT}{2F} \ln \left(\frac{[\text{Ca}^{2+}]_o}{[\text{Ca}^{2+}]_i} \right), \quad (\text{A6})$$

where [M, i, o] can either be ['TT', 'myo, junc', 'TT volume'] or ['SL', 'myo, bulk', 'EC']. Similarly 'SR' and 'Mito' are used to refer to the associated junctional compartment ('SR, junc', 'Mito, junc') or bulk compartment ('SR, bulk', 'Mito, junc') where applicable. The resulting ODEs are summarized in Table A6, and all associated parameters are provided in Table A7.

Table A5. Reactions and fluxes

Name	Description	Equation
Module 1		
$I_{KIR,M}$	Inward rectifier current	$G_K Q_{KIR} \left(\frac{K_R^2}{K_K + K_R^2} \right) \left(1 - \left(1 + \frac{K_S \left(1 + \frac{K_R^2}{K_K} \right)}{S_K^2 \exp \left(\frac{2(1-\delta)V_M F}{RT} \right)} \right)^{-1} \right) (E_{K^+,M} - V_M),$ where $K_R = [K^+]_o \exp \left(-\frac{\delta E_{K^+,M} F}{RT} \right)$
$I_{KDR,M}$	Delayed rectifier current	$g_K Q_{KDR} n_M^4 h_{K,M} (E_{K^+,M} - V_M)$
$I_{NaK,M}$	Na ⁺ -K ⁺ pump current	$\frac{F f_{NaK} Q_{NaK} J_{NaK}}{\left(1 + \frac{K_{mK}}{[K^+]_o} \right)^2 \left(1 + \frac{K_{mNa}}{[Na^+]_i} \right)^3} \left(\frac{[ATP]_i}{K_{ATP} + [ATP]_i} \right),$ where $f_{NaK} = \left(1 + 0.12 \exp \left(-\frac{V_M F}{10RT} \right) + 0.04 \sigma_M \exp \left(-\frac{V_M F}{RT} \right) \right)^{-1}$ and $\sigma_M = \frac{1}{7} \left(\exp \left(\frac{[Na^+]_o}{67300 \mu M} \right) - 1 \right)$
$I_{Na^+,M}$	Na ⁺ channel current	$g_{Na} Q_{Na} m_M^3 h_{M,SM} (E_{Na^+,M} - V_M)$
$I_{NCX,M}$	Na ⁺ -Ca ²⁺ exchanger current	$g_{NCX} Q_{NCX} K_{a,NCX} \frac{s_1 - s_2}{s_3 \left(1 + k_{sat} \exp \left(\frac{(V_{NCX} - 1)V_M F}{RT} \right) \right)},$ where $s_1 = \exp \left(\nu_{NCX} V_M \frac{F}{RT} \right) [Na^+]_i^3 [Ca^{2+}]_o,$ $s_2 = \exp \left((\nu_{NCX} - 1.0) \frac{V_M F}{RT} \right) [Na^+]_o^3 [Ca^{2+}]_i,$ $s_3 = K_{Ca,i} [Na^+]_o^3 \left(1 + \left(\frac{[Na^+]_i}{K_{Na,i}} \right)^3 \right) + K_{Na,o}^3 [Ca^{2+}]_i \left(1 + \frac{[Ca^{2+}]_i}{K_{Ca,i}} \right) +$ $K_{Ca,o} [Na^+]_i^3 + [Na^+]_i^3 [Ca^{2+}]_o + [Na^+]_o^3 [Ca^{2+}]_i,$ and $K_{a,NCX} = \frac{1.0}{1.0 + \left(\frac{K_{dAct}}{[Ca^{2+}]_i} \right)^2}$
$I_{Cl^-,M}$	Cl ⁻ channel current	$g_{Cl} Q_{Cl} A^4 (E_{Cl^-,M} - V_M),$ where $A = \frac{1}{1 + \exp \left(\frac{V_M - V_a}{A_a} \right)}$
Module 2		
J_{RyR}	Ca ²⁺ release through RyR	$\nu_{max,RyR} P_{O,MWC} W_{RyR} \left([Ca^{2+}]_{SR,junc} - [Ca^{2+}]_{myo,junc} \right)$ where $P_{O,MWC} = \frac{(1 + \varepsilon_V f_{RyR}^{-2})^4}{(1 + \varepsilon_V f_{RyR}^{-2})^4 + L_{RyR} (1 + \varepsilon_V)^4}$ and $\varepsilon_V = \exp \left(\frac{V_M - V_{RyR}}{4K_{RyR}} \right)$
Module 3		
$J_{PMCA,M}$	Ca ²⁺ efflux through PMCA	$J_{PMCA,0} Q_{PMCA} \left(\frac{[Ca^{2+}]_i}{K_{PMCA} + [Ca^{2+}]_i} \right) \left(\frac{[ATP]_i}{K_{ATP} + [ATP]_i} \right)$
$I_{Ca^{2+}leak,M}$	Ca ²⁺ leak through SRM	$g_{leak,M} ([Ca^{2+}]_o - [Ca^{2+}]_i)$
J_{SERCA}	SERCA Ca ²⁺ flux into SR	$J_{SERCA,0} Q_{SERCA} \left(\frac{[Ca^{2+}]_i}{K_{SERCA} + [Ca^{2+}]_i} \right) \left(\frac{[ATP]_i}{K_{ATP} + [ATP]_i} \right)$
$J_{Ca^{2+}leak,SR}$	Ca ²⁺ leak through SRM	$\nu_{leak,SR} ([Ca^{2+}]_{SR} - [Ca^{2+}]_i)$
J_{SOCE}	Ca ²⁺ influx due to SOCE	$P_{O,Orai1} g_{Orai1} \frac{N_A}{2F} (E_{Ca^{2+},junc} - V_{TT})$
Module 4		
$J_{Ca^{2+}-Parv,i}$	Ca ²⁺ binding to parvalbumin	$k_{on,CP} [Parv]_i [Ca^{2+}]_i - k_{off,CP} [Ca^{2+}-Parv]_i$
$J_{Mg^{2+}-Parv,i}$	Mg ²⁺ binding to parvalbumin	$k_{on,MP} [Parv]_i [Mg^{2+}]_i - k_{off,MP} [Mg^{2+}-Parv]_i$
$J_{Ca^{2+}-ATP,i}$	Ca ²⁺ binding to ATP	$k_{on,CA} [ATP]_i [Ca^{2+}]_i - k_{off,CA} [Ca^{2+}-ATP]_i$
$J_{Mg^{2+}-ATP,i}$	Mg ²⁺ binding to ATP	$k_{on,MA} [ATP]_i [Mg^{2+}]_i - k_{off,MA} [ATP-Mg^{2+}]_i$
$J_{Trop-Ca^{2+},i}$	Ca ²⁺ binding to troponin	$k_{on,T1} [Ca^{2+}]_i [Trop]_i - k_{off,T1} [Trop-Ca^{2+}]_i$

(Continued)

Table A5. (Continued)

Name	Description	Equation
$J_{\text{Trop-2Ca}^{2+},i}$	Ca^{2+} binding to Ca^{2+} -troponin	$k_{\text{on},T2}[\text{Ca}^{2+}]_i[\text{Trop-Ca}^{2+}]_i - k_{\text{off},T2}[\text{Trop-2Ca}^{2+}]_i$
$J_{\text{Ca}^{2+}\text{-CSQN}}$	Ca^{2+} binding to calsequestrin in SR	$\frac{k_{\text{off},\text{CSQN}}}{K_{\text{CSQN}}}[\text{Ca}^{2+}]_{\text{SR}}[\text{CSQN}]_{\text{SR}} - k_{\text{off},\text{CSQN}}([\text{CSQN}]_{\text{tot}} - [\text{CSQN}]_{\text{SR}})$
$J_{\text{Ca}^{2+}\text{-phos}}$	Ca^{2+} precipitation with phosphate in SR	$\mathcal{H}([\text{Pi}]_{\text{SR}}[\text{Ca}^{2+}]_{\text{SR}} - \text{PP})([\text{Pi}]_{\text{SR}}[\text{Ca}^{2+}]_{\text{SR}} - \text{PP})A_{\text{P}}[\text{Pi}]_{\text{SR}}[\text{Ca}^{2+}]_{\text{SR}} - \mathcal{H}(\text{PP} - [\text{Pi}]_{\text{SR}}[\text{Ca}^{2+}]_{\text{SR}})B_{\text{P}}(\text{PP} - [\text{Pi}]_{\text{SR}}[\text{Ca}^{2+}]_{\text{SR}})[\text{Ca}^{2+}\text{-Pi}]_{\text{SR}}$ where $\mathcal{H}$ is the Heaviside function
$J_{\text{hyd},i}$	Total ATP hydrolysis rate	$\frac{N_{\text{A}}N_{\text{AK},\text{M}}}{F} + J_{\text{PMCA},\text{M}} + \frac{J_{\text{SERCA},\text{SR}}S_{\text{A},\text{SR}}}{2\text{vol}_i} + k_{\text{hyd}}\frac{[\text{ATP}]_i}{[\text{ATP}]_i + K_{\text{H}}}$
$J_{\text{phos},\text{SR}}$	Transport of phosphate into SR	$k_{\text{P}}([\text{Pi}]_i - [\text{Pi}]_{\text{SR}})$
J_{D}	Rate of tropomyosin unblocking	$k_{\text{unblock}}[\text{Trop-2Ca}^{2+}][\text{ATP}]_i - k_{\text{block}}[D]$
J_{pre}	Rate of myosin head binding	$f_0[D] - f_{\text{p}}[A_{\text{Pre}}]$
J_{post}	Rate of power stroke	$h_0[A_{\text{Pre}}] - h_{\text{p}}[A_{\text{Post}}][\text{Pi}]_i$
J_{detach}	Rate of myosin head detaching post-power stroke	$g_0[A_{\text{Post}}][\text{ATP}]_i$
Module 5		
J_{MCU}	Rate of mitochondrial Ca^{2+} import through MCU	$\nu_{\text{MCU}} \frac{\frac{[\text{Ca}^{2+}]_i}{K_{\text{trans}}} \left(1 + \frac{[\text{Ca}^{2+}]_i}{K_{\text{trans}}}\right)^3}{\left(1 + \frac{[\text{Ca}^{2+}]_i}{K_{\text{trans}}}\right)^4 + L_{\text{MCU}} \left(1 + \frac{[\text{Ca}^{2+}]_i}{K_{\text{act}}}\right)^{-2.3}} \exp(p_1 V_{\text{Mito}})$
$J_{\text{NCX},\text{M}}$	Rate of mitochondrial Ca^{2+} export through NCX	$\nu_{\text{NCX},\text{Mito}} \left(\frac{[\text{Ca}^{2+}]_{\text{Mito}}}{[\text{Ca}^{2+}]_i}\right) \exp(p_2 V_{\text{Mito}})$
J_{mPTP}	Ca^{2+} flux through mitochondrial Permeability Transition Pore	$k_{\text{mPTP}}([\text{Ca}^{2+}]_i - [\text{Ca}^{2+}]_{\text{Mito}}) \exp(p_3 V_{\text{Mito}})$
J_{AGC}	Rate of NADH shuttling due to the aspartate-glutamate carrier	$\nu_{\text{AGC}} \left(\frac{[\text{Ca}^{2+}]_i}{K_{\text{AGC}} + [\text{Ca}^{2+}]_i}\right) \left(\frac{K_{\text{Ca}}}{K_{\text{Ca}} + [\text{Ca}^{2+}]_{\text{Mito}}}\right)$
J_{Gly}	NADH production rate due to glycolysis in the cytosol	$k_{\text{Gly}} \frac{\text{vol}_{\text{Mito}}}{\text{vol}_{\text{myo}}} \frac{[\text{ATP}]_{\text{set}} - [\text{ATP}]_i}{10 \mu\text{M}} \frac{[\text{Pi}]_i}{1000 \mu\text{M}}$
J_{PDH}	NADH production rate due to glycolysis, pyruvate dehydrogenase and Krebs cycle	$J_{\text{Gly}} \left(\frac{\text{vol}_{\text{myo}}}{\text{vol}_{\text{Mito}}}\right) \left(\frac{[\text{Ca}^{2+}]_{\text{Mito}}}{K_{\text{mCa}} + [\text{Ca}^{2+}]_{\text{Mito}}}\right) \left(\frac{1}{K_{\text{mNAD}} + \frac{[\text{NADH}]_{\text{Mito}}}{[\text{NAD}]_{\text{tot}} - [\text{NADH}]_{\text{Mito}}}}\right)$
J_{Ox}	NADH oxidation rate coupled to proton extrusion from mitochondria	$\nu_{\text{Ox}} \left(\frac{[\text{NADH}]_{\text{Mito}}}{K_{\text{Ox}} + [\text{NADH}]_{\text{Mito}}}\right) \frac{1}{1 + \exp\left(\frac{V_{\text{Mito}} - p_4}{p_5}\right)}$
J_{F1FO}	ADP phosphorylation rate by the F1FO ATPases	$\nu_{\text{F1FO}} \frac{1}{1 + \exp\left(\frac{p_6 - V_{\text{Mito}}}{p_7}\right)} \left(\frac{K_{\text{iATP}}}{K_{\text{iATP}} + [\text{ATP}]_{\text{Mito}}}\right) \left(\frac{[\text{Pi}]_{\text{Mito}}}{1000 \mu\text{M}}\right)$
J_{ANT}	Rate of mitochondrial ADP import / ATP export	$\nu_{\text{ANT}} \frac{1 - \left(\frac{\alpha_{\text{c}}[\text{ATP}]_{\text{i,tot}}}{[\text{ADP}]_i}\right) \left(\frac{[\text{ANP}]_{\text{tot}} - [\text{ATP}]_{\text{Mito}}}{\alpha_{\text{Mito}}[\text{ATP}]_{\text{Mito}}}\right) \exp\left(-\frac{FV_{\text{Mito}}}{RT}\right)}{\left(1 + \frac{\alpha_{\text{c}}[\text{ATP}]_{\text{i,tot}}}{[\text{ADP}]_i} \exp\left(-f_{\text{ANT}} \frac{FV_{\text{Mito}}}{RT}\right)\right) \left(1 + \frac{[\text{ANP}]_{\text{tot}} - [\text{ATP}]_{\text{Mito}}}{\alpha_{\text{Mito}}[\text{ATP}]_{\text{Mito}}}\right)}$ where $[\text{ATP}]_{\text{i,tot}} = [\text{ATP}]_i + [\text{Ca}^{2+}\text{-ATP}]_{\text{i,tot}} + [\text{Mg}^{2+}\text{-ATP}]_i$
$J_{\text{Hleak},\text{M}}$	Rate of mitochondrial proton leak	$\frac{p_8 V_{\text{Mito}} + p_9}{\frac{[\text{Pi}]_{\text{myo}}}{K_{\text{Pi}}} - \frac{[\text{Pi}]_{\text{Mito}}}{K_{\text{Pi},\text{M}}}}$
J_{PiC}	Rate of phosphate transport into mitochondria	$\nu_{\text{PiC}} \frac{\frac{[\text{Pi}]_{\text{myo}}}{K_{\text{Pi}}} - \frac{[\text{Pi}]_{\text{Mito}}}{K_{\text{Pi},\text{M}}}}{1 + \frac{[\text{Pi}]_{\text{myo}}}{K_{\text{Pi}}} + \frac{[\text{Pi}]_{\text{Mito}}}{K_{\text{Pi},\text{M}}}}$
$J_{\text{Ca}^{2+}\text{-phos},\text{M}}$	Rate of Ca^{2+} -phosphate binding in the mitochondria	$\frac{k_{\text{off},\text{B}}}{K_{\text{dB}}}[\text{Pi}]_{\text{Mito}}[\text{Ca}^{2+}]_{\text{Mito}} - k_{\text{off},\text{B}}[\text{Ca}^{2+}\text{-Pi}]_{\text{M}}$

Table A6. System of ordinary differential equations (ODEs)

Variable	Equation
Junctional variables	
$\frac{d[\text{Na}^+]_{\text{TT}}}{dt}$	$\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{TT}}F} \left(-0.1I_{\text{Na}^+,\text{TT}} + 3 \times 0.1I_{\text{NaK},\text{TT}} - I_{\text{NCX},\text{TT}} \right) + \frac{D_{\text{ion,EC}}SA_{\text{EC,bulk-junc}}}{\text{vol}_{\text{TT}}\Delta d_{\text{diff}}} ([\text{Na}^+]_{\text{EC}} - [\text{Na}^+]_{\text{TT}})$
$\frac{d[\text{Na}^+]_{\text{myo,junc}}}{dt}$	$\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{myo,junc}}F} \left(0.1I_{\text{Na}^+,\text{TT}} - 3 \times 0.1I_{\text{NaK},\text{TT}} + I_{\text{NCX},\text{TT}} \right) + \frac{D_{\text{ion}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Na}^+]_{\text{myo,bulk}} - [\text{Na}^+]_{\text{myo,junc}})$
$\frac{dm_{\text{TT}}}{dt}$	$\alpha_m (1 - m_{\text{TT}}) - \beta_m m_{\text{TT}}$, where $\beta_m = \beta_{m0} Q_{\beta_m} \exp\left(-\frac{V_{\text{TT}} - V_m}{K_{\beta_m}}\right)$ and $\alpha_m = \alpha_{m0} Q_{\alpha_m} \frac{V_{\text{TT}} - V_m}{1 - \exp\left(\frac{V_m - V_{\text{TT}}}{K_{\alpha_m}}\right)}$
$\frac{dh_{\text{TT}}}{dt}$	$\alpha_h (1 - h_{\text{TT}}) - (\beta_h h_{\text{TT}})$, where $\beta_h = Q_{\beta_h} \frac{\beta_{h0}}{1 + \exp\left(-\frac{V_{\text{TT}} - V_h}{K_{\beta_h}}\right)}$ and $\alpha_h = \alpha_{h0} Q_{\alpha_h} \exp\left(-\frac{V_{\text{TT}} - V_h}{K_{\alpha_h}}\right)$
$\frac{dS_{\text{TT}}}{dt}$	$\frac{S_{\infty} - S_{\text{TT}}}{\tau_S}$, where $S_{\infty} = \frac{1}{1 + \exp\left(\frac{V_{\text{TT}} - V_{S_{\infty}}}{A_{S_{\infty}}}\right)}$ and $\tau_S = \frac{60}{0.2 + 5.65\left(\frac{V_{\text{TT}} + V_{\tau}}{100\text{mV}}\right)^2}$
$\frac{d[\text{K}^+]_{\text{TT}}}{dt}$	$\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{TT}}F} \left(-I_{\text{KIR},\text{TT}} + 0.45I_{\text{KDR},\text{TT}} - 2 \times 0.1I_{\text{NaK},\text{TT}} \right) + \frac{D_{\text{ion,EC}}SA_{\text{EC,bulk-junc}}}{\text{vol}_{\text{TT}}\Delta d_{\text{diff}}} ([\text{K}^+]_{\text{EC}} - [\text{K}^+]_{\text{TT}})$
$\frac{d[\text{K}^+]_{\text{myo,junc}}}{dt}$	$\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{myo,junc}}F} \left(I_{\text{KIR},\text{TT}} - 0.45I_{\text{KDR},\text{TT}} + 2 \times 0.1I_{\text{NaK},\text{TT}} \right) + \frac{D_{\text{ion}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{K}^+]_{\text{myo,bulk}} - [\text{K}^+]_{\text{myo,junc}})$
$\frac{dn_{\text{TT}}}{dt}$	$\alpha_n (1 - n_{\text{TT}}) - \beta_n n_{\text{TT}}$, where $\beta_n = \beta_{n0} Q_{\beta_n} \exp\left(-\frac{V_{\text{TT}} - V_n}{K_{\beta_n}}\right)$ and $\alpha_n = \alpha_{n0} Q_{\alpha_n} \frac{V_{\text{TT}} - V_n}{1 - \exp\left(\frac{V_n - V_{\text{TT}}}{K_{\alpha_n}}\right)}$
$\frac{dh_{\text{K},\text{TT}}}{dt}$	$\frac{h_{\text{K}\infty} - h_{\text{K},\text{TT}}}{\tau_{h_{\text{K}}}}$, where $\tau_{h_{\text{K}}} = \exp\left(-\frac{V_{\text{TT}} + 40}{25.75\text{mV}}\right)$ and $h_{\text{K}\infty} = \left(1 + \exp\left(\frac{V_{\text{TT}} - V_{h_{\text{K}\infty}}}{A_{h_{\text{K}\infty}}}\right)\right)^{-1}$
$\frac{d[\text{Cl}^-]_{\text{TT}}}{dt}$	$-\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{TT}}F} \left(-0.1I_{\text{Cl}^-, \text{TT}} \right) + \frac{D_{\text{ion,EC}}SA_{\text{EC,bulk-junc}}}{\text{vol}_{\text{TT}}\Delta d_{\text{diff}}} ([\text{Cl}^-]_{\text{EC}} - [\text{Cl}^-]_{\text{TT}})$
$\frac{d[\text{Cl}^-]_{\text{myo,junc}}}{dt}$	$-\frac{SA_{\text{TT}}\eta_{\text{conv},1}}{\text{vol}_{\text{myo,junc}}F} \left(0.1I_{\text{Cl}^-, \text{TT}} \right) + \frac{D_{\text{ion}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Cl}^-]_{\text{myo,bulk}} - [\text{Cl}^-]_{\text{myo,junc}})$
$\frac{d[\text{Ca}^{2+}]_{\text{TT}}}{dt}$	$\frac{SA_{\text{TT}}}{\text{vol}_{\text{TT}}\eta_{\text{conv},2}} \left(-J_{\text{Ca}^{2+} \text{ leak},\text{TT}} - J_{\text{SOCE},\text{TT}} + \frac{N_A}{F} I_{\text{NCX},\text{TT}} \right) + J_{\text{PMCA},\text{TT}} \frac{\text{vol}_{\text{myo,junc}}}{\text{vol}_{\text{TT}}} + \frac{D_{\text{ion,EC}}SA_{\text{EC,bulk-junc}}}{\text{vol}_{\text{TT}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+}]_{\text{EC}} - [\text{Ca}^{2+}]_{\text{TT}})$
$\frac{d[\text{Ca}^{2+}]_{\text{myo,junc}}}{dt}$	$\frac{SA_{\text{TT}}}{\text{vol}_{\text{myo,junc}}\eta_{\text{conv},2}} \left(J_{\text{Ca}^{2+} \text{ leak},\text{TT}} + J_{\text{SOCE},\text{TT}} - \frac{N_A}{F} I_{\text{NCX},\text{TT}} \right) - J_{\text{PMCA},\text{TT}} + \frac{SA_{\text{SRM,junc}}}{\text{vol}_{\text{SR,junc}}} \left(J_{\text{RyR,junc}} + J_{\text{Ca}^{2+} \text{ leak SR,junc}} - 0.1J_{\text{SERCA,junc}} \right) - J_{\text{Ca}^{2+} \text{ -Parv,junc}} - J_{\text{Ca}^{2+} \text{ -ATP,junc}} - \frac{\text{vol}_{\text{Mito,junc}}}{\text{vol}_{\text{myo,junc}}} (J_{\text{MCU,junc}} - J_{\text{NCX,Mito,junc}} + J_{\text{mPTP,junc}}) + \frac{D_{\text{ion}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+}]_{\text{myo,bulk}} - [\text{Ca}^{2+}]_{\text{myo,junc}})$
$\frac{d[\text{Ca}^{2+}]_{\text{SR,junc}}}{dt}$	$\frac{SA_{\text{SRM,junc}}}{\text{vol}_{\text{SR,junc}}} \left(-J_{\text{Ca}^{2+} \text{ leak SR,junc}} - J_{\text{RyR,junc}} + 0.1J_{\text{SERCA,junc}} \right) - J_{\text{Ca}^{2+} \text{ -CSQN,junc}} + \frac{D_{\text{ion}}SA_{\text{SR,bulk-junc}}}{\text{vol}_{\text{SR,junc}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+}]_{\text{SR,bulk}} - [\text{Ca}^{2+}]_{\text{SR,junc}})$
$\frac{dw_{\text{RyR}}}{dt}$	$\alpha_w \left(1 - w_{\text{RyR}} \left(1 + \frac{[\text{Ca}^{2+}]_{\text{myo,junc}}}{K_w} \right) \right)$
$\frac{d[\text{Ca}^{2+} \text{ -Parv}]_{\text{myo,junc}}}{dt}$	$J_{\text{Ca}^{2+} \text{ -Parv,junc}} + \frac{D_{\text{Parv}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+} \text{ -Parv}]_{\text{myo,bulk}} - [\text{Ca}^{2+} \text{ -Parv}]_{\text{myo,junc}})$
$\frac{d[\text{Mg}^{2+} \text{ -Parv}]_{\text{myo,junc}}}{dt}$	$J_{\text{Mg}^{2+} \text{ -Parv,junc}} + \frac{D_{\text{Parv}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Mg}^{2+} \text{ -Parv}]_{\text{myo,bulk}} - [\text{Mg}^{2+} \text{ -Parv}]_{\text{myo,junc}})$
$\frac{d[\text{ATP}]_{\text{myo,junc}}}{dt}$	$-J_{\text{Ca}^{2+} \text{ -ATP,junc}} - J_{\text{Mg}^{2+} \text{ -ATP,junc}} - J_{\text{hydro,junc}} + J_{\text{Gly,junc}} N_{\text{ATP-per-NADH}} + J_{\text{ANT,junc}} \frac{\text{vol}_{\text{Mito,junc}}}{\text{vol}_{\text{myo,junc}}} + \frac{D_{\text{ATP}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{ATP}]_{\text{myo,bulk}} - [\text{ATP}]_{\text{myo,junc}})$
$\frac{d[\text{Ca}^{2+} \text{ -ATP}]_{\text{myo,junc}}}{dt}$	$J_{\text{Ca}^{2+} \text{ -ATP,junc}} + \frac{D_{\text{ATP}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+} \text{ -ATP}]_{\text{myo,bulk}} - [\text{Ca}^{2+} \text{ -ATP}]_{\text{myo,junc}})$
$\frac{d[\text{Mg}^{2+} \text{ -ATP}]_{\text{myo,junc}}}{dt}$	$J_{\text{Mg}^{2+} \text{ -ATP,junc}} + \frac{D_{\text{ATP}}SA_{\text{myo,bulk-junc}}}{\text{vol}_{\text{myo,junc}}\Delta d_{\text{diff}}} ([\text{Mg}^{2+} \text{ -ATP}]_{\text{myo,bulk}} - [\text{Mg}^{2+} \text{ -ATP}]_{\text{myo,junc}})$

(Continued)

Table A6. (Continued)

Variable	Equation
$\frac{d[\text{Pi}]_{\text{myo,junc}}}{dt}$	$J_{\text{hydro,junc}} - J_{\text{Gly,junc}} N_{\text{ATP-per-NADH}} - J_{\text{phos,SR,junc}} - J_{\text{PiC,junc}} \frac{\text{vol}_{\text{Mito,junc}}}{\text{vol}_{\text{myo,junc}}} + \frac{D_{\text{Pi}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,junc}} \Delta d_{\text{diff}}} ([\text{Pi}]_{\text{myo,bulk}} - [\text{Pi}]_{\text{myo,junc}})$
$\frac{d[\text{Pi}]_{\text{SR,junc}}}{dt}$	$J_{\text{phos,SR,junc}} - J_{\text{Ca}^{2+}\text{-phos,junc}} + \frac{D_{\text{Pi}} S_{\text{ASR,bulk-junc}}}{\text{vol}_{\text{SR,junc}} \Delta d_{\text{diff}}} ([\text{Pi}]_{\text{SR,bulk}} - [\text{Pi}]_{\text{SR,junc}})$
$\frac{d[\text{Ca}^{2+}\text{-Pi}]_{\text{SR,junc}}}{dt}$	$J_{\text{Ca}^{2+}\text{-phos,junc}} + \frac{D_{\text{Pi}} S_{\text{ASR,bulk-junc}}}{\text{vol}_{\text{SR,junc}} \Delta d_{\text{diff}}} ([\text{Ca}^{2+}\text{-Pi}]_{\text{SR,bulk}} - [\text{Ca}^{2+}\text{-Pi}]_{\text{SR,junc}})$
$\frac{d[\text{CSQN}]_{\text{SR,junc}}}{dt}$	$-J_{\text{Ca}^{2+}\text{-CSQN,junc}} + \frac{D_{\text{CSQN}} S_{\text{ASR,bulk-junc}}}{\text{vol}_{\text{SR,junc}} \Delta d_{\text{diff}}} ([\text{CSQN}]_{\text{SR,bulk}} - [\text{CSQN}]_{\text{SR,junc}})$
$\frac{d[\text{ADP}]_{\text{myo,junc}}}{dt}$	$J_{\text{hydro,junc}} - J_{\text{Gly,junc}} N_{\text{ATP-per-NADH}} - J_{\text{ANT,junc}} \frac{\text{vol}_{\text{Mito,junc}}}{\text{vol}_{\text{myo,junc}}} + \frac{D_{\text{ATP}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,junc}} \Delta d_{\text{diff}}} ([\text{ADP}]_{\text{myo,bulk}} - [\text{ADP}]_{\text{myo,junc}})$
$\frac{d[\text{Ca}^{2+}]_{\text{Mito,junc}}}{dt}$	$b_{\text{M}} (J_{\text{MCU,junc}} - J_{\text{NCX,Mito,junc}} + J_{\text{mPTP,junc}} - J_{\text{Ca}^{2+}\text{-phos,Mito,junc}})$
$\frac{d[\text{Pi}]_{\text{Mito,junc}}}{dt}$	$J_{\text{PiC,junc}} - J_{\text{Ca}^{2+}\text{-phos,Mito,junc}}$
$\frac{d[\text{Ca}^{2+}\text{-Pi}]_{\text{Mito,junc}}}{dt}$	$J_{\text{Ca}^{2+}\text{-phos,Mito,junc}}$
$\frac{d[\text{ATP}]_{\text{Mito,junc}}}{dt}$	$J_{\text{F1FO,junc}} - J_{\text{ANT,junc}}$
$\frac{d[\text{NADH}]_{\text{Mito,junc}}}{dt}$	$J_{\text{PDH,junc}} + J_{\text{AGC,junc}} - J_{\text{Ox,junc}}$
$\frac{dV_{\text{Mito,junc}}}{dt}$	$(a_1 J_{\text{Ox,junc}} - a_2 J_{\text{F1FO,junc}} - J_{\text{Hleak,Mito,junc}} - J_{\text{NCX,Mito,junc}} - 2J_{\text{MCU,junc}} - 2J_{\text{mPTP,junc}} - J_{\text{ANT,junc}} - J_{\text{AGC,junc}}) / C_{\text{Eff,Mito}}$
Bulk variables	
$\frac{d[\text{Na}^+]_{\text{myo,bulk}}}{dt}$	$\frac{S_{\text{ASL}} \eta_{\text{conv},1}}{\text{vol}_{\text{myo,bulk}} F} (I_{\text{Na}^+,\text{SL}} - 3I_{\text{NaK,SL}} + I_{\text{NCX,SL}}) + \frac{D_{\text{ion}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{Na}^+]_{\text{myo,junc}} - [\text{Na}^+]_{\text{myo,bulk}})$
$\frac{dm_{\text{SL}}}{dt}$	$\alpha_m (1 - m_{\text{SL}}) - \beta_m m_{\text{SL}}, \text{ where } \beta_m = \beta_{m0} Q_{\beta m} \exp\left(-\frac{V_{\text{SL}} - V_m}{K_{\beta m}}\right) \text{ and } \alpha_m = \alpha_{m0} Q_{\alpha m} \frac{V_{\text{SL}} - V_m}{1 - \exp\left(-\frac{V_m - V_{\text{SL}}}{K_{\alpha m}}\right)}$
$\frac{dh_{\text{SL}}}{dt}$	$\alpha_h (1 - h_{\text{SL}}) - (\beta_h h_{\text{SL}}), \text{ where } \beta_h = Q_{\beta h} \frac{\beta_{h0}}{1 + \exp\left(-\frac{V_{\text{SL}} - V_h}{K_{\beta h}}\right)} \text{ and } \alpha_h = \alpha_{h0} Q_{\alpha h} \exp\left(-\frac{V_{\text{SL}} - V_h}{K_{\alpha h}}\right)$
$\frac{dS_{\text{SL}}}{dt}$	$\frac{S_{\infty} - S_{\text{SL}}}{\tau_S}, \text{ where } S_{\infty} = \frac{1}{1 + \exp\left(\frac{V_{\text{SL}} - V_{S\infty}}{A_{S\infty}}\right)} \text{ and } \tau_S = \frac{60}{0.2 + 5.65 \left(\frac{V_{\text{SL}} + V_T}{100 \text{ mV}}\right)^2}$
$\frac{d[\text{K}^+]_{\text{myo,bulk}}}{dt}$	$\frac{S_{\text{ASL}} \eta_{\text{conv},1}}{\text{vol}_{\text{myo,bulk}} F} (I_{\text{KIR,SL}} - I_{\text{KDR,SL}} + 2I_{\text{NaK,SL}}) + \frac{D_{\text{ion}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{K}^+]_{\text{myo,junc}} - [\text{K}^+]_{\text{myo,bulk}})$
$\frac{dn_{\text{SL}}}{dt}$	$\alpha_n (1 - n_{\text{SL}}) - \beta_n n_{\text{SL}}, \text{ where } \beta_n = \beta_{n0} Q_{\beta n} \exp\left(-\frac{V_{\text{SL}} - V_n}{K_{\beta n}}\right) \text{ and } \alpha_n = \alpha_{n0} Q_{\alpha n} \frac{V_{\text{SL}} - V_n}{1 - \exp\left(-\frac{V_n - V_{\text{SL}}}{K_{\alpha n}}\right)}$
$\frac{dh_{\text{K,SL}}}{dt}$	$\frac{h_{\text{K}\infty} - h_{\text{K,SL}}}{\tau_{h_{\text{K}}}}, \text{ where } \tau_{h_{\text{K}}} = \exp\left(-\frac{V_{\text{SL}} + 40}{25.75 \text{ mV}}\right) \text{ and } h_{\text{K}\infty} = \left(1 + \exp\left(\frac{V_{\text{SL}} - V_{h_{\text{K}\infty}}}{A_{h_{\text{K}\infty}}}\right)\right)^{-1}$
$\frac{d[\text{Cl}^-]_{\text{myo,bulk}}}{dt}$	$-\frac{S_{\text{ASL}} \eta_{\text{conv},1}}{\text{vol}_{\text{myo,bulk}} F} (I_{\text{Cl}^-, \text{SL}}) + \frac{D_{\text{ion}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{Cl}^-]_{\text{myo,junc}} - [\text{Cl}^-]_{\text{myo,bulk}})$
$\frac{d[\text{Ca}^{2+}]_{\text{myo,bulk}}}{dt}$	$\frac{S_{\text{ASL}}}{\text{vol}_{\text{myo,bulk}} \eta_{\text{conv},2}} (J_{\text{Ca}^{2+}\text{ leak,SL}} - \frac{N_A}{F} I_{\text{NCX,SL}}) - J_{\text{PMCA,SL}} + \frac{S_{\text{ASRM,bulk}}}{\text{vol}_{\text{myo,bulk}}} (J_{\text{Ca}^{2+}\text{ leak SR,bulk}} - J_{\text{SERCA,bulk}}) - J_{\text{Ca}^{2+}\text{-Parv,bulk}} - J_{\text{Ca}^{2+}\text{-ATP,bulk}} - \frac{\text{vol}_{\text{Mito,bulk}}}{\text{vol}_{\text{myo,bulk}}} (J_{\text{MCU,bulk}} - J_{\text{NCX,bulk}} + J_{\text{mPTP,bulk}}) + \frac{D_{\text{ion}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{Ca}^{2+}]_{\text{myo,junc}} - [\text{Ca}^{2+}]_{\text{myo,bulk}})$
$\frac{d[\text{Ca}^{2+}]_{\text{SR,bulk}}}{dt}$	$\frac{S_{\text{ASRM,bulk}}}{\text{vol}_{\text{SR,bulk}}} (-J_{\text{Ca}^{2+}\text{ leak SR,bulk}} + J_{\text{SERCA,bulk}}) - J_{\text{Ca}^{2+}\text{-CSQN,bulk}} + \frac{D_{\text{ion}} S_{\text{ASR,bulk-junc}}}{\text{vol}_{\text{SR,bulk}} \Delta d_{\text{diff}}} ([\text{Ca}^{2+}]_{\text{SR,junc}} - [\text{Ca}^{2+}]_{\text{SR,bulk}})$
$\frac{d[\text{Ca}^{2+}\text{-Parv}]_{\text{myo,bulk}}}{dt}$	$J_{\text{Ca}^{2+}\text{-Parv,bulk}} + \frac{D_{\text{Parv}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{Ca}^{2+}\text{-Parv}]_{\text{myo,junc}} - [\text{Ca}^{2+}\text{-Parv}]_{\text{myo,bulk}})$
$\frac{d[\text{Mg}^{2+}\text{-Parv}]_{\text{myo,bulk}}}{dt}$	$J_{\text{Mg}^{2+}\text{-Parv,bulk}} + \frac{D_{\text{Parv}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{Mg}^{2+}\text{-Parv}]_{\text{myo,junc}} - [\text{Mg}^{2+}\text{-Parv}]_{\text{myo,bulk}})$
$\frac{d[\text{ATP}]_{\text{myo,bulk}}}{dt}$	$-J_{\text{Ca}^{2+}\text{-ATP,bulk}} - J_{\text{Mg}^{2+}\text{-ATP,bulk}} - J_{\text{hydro,bulk}} + J_{\text{Gly,bulk}} N_{\text{ATP-per-NADH}} + J_{\text{ANT,bulk}} \frac{\text{vol}_{\text{Mito,bulk}}}{\text{vol}_{\text{myo,bulk}}} - J_{\text{D}} - J_{\text{detach}} + \frac{D_{\text{ATP}} S_{\text{Amyo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}} \Delta d_{\text{diff}}} ([\text{ATP}]_{\text{myo,junc}} - [\text{ATP}]_{\text{myo,bulk}})$

(Continued)

Table A6. (Continued)

Variable	Equation
$\frac{d[\text{Ca}^{2+}\text{-ATP}]_{\text{myo,bulk}}}{dt}$	$J_{\text{Ca}^{2+}\text{-ATP,bulk}} + \frac{D_{\text{ATP}}S_{\text{A,myo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+}\text{-ATP}]_{\text{myo,junc}} - [\text{Ca}^{2+}\text{-ATP}]_{\text{myo,bulk}})$
$\frac{d[\text{Mg}^{2+}\text{-ATP}]_{\text{myo,bulk}}}{dt}$	$J_{\text{Mg}^{2+}\text{-ATP,bulk}} + \frac{D_{\text{ATP}}S_{\text{A,myo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}}\Delta d_{\text{diff}}} ([\text{Mg}^{2+}\text{-ATP}]_{\text{myo,junc}} - [\text{Mg}^{2+}\text{-ATP}]_{\text{myo,bulk}})$
$\frac{d[\text{Pi}]_{\text{myo,bulk}}}{dt}$	$J_{\text{hydro,bulk}} - J_{\text{phos,SR,bulk}} + J_{\text{post}} - J_{\text{PiC,bulk}} \frac{\text{vol}_{\text{Mito,bulk}}}{\text{vol}_{\text{myo,bulk}}} + \frac{D_{\text{Pi}}S_{\text{A,myo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}}\Delta d_{\text{diff}}} ([\text{Pi}]_{\text{myo,junc}} - [\text{Pi}]_{\text{myo,bulk}})$
$\frac{d[\text{Pi}]_{\text{SR,bulk}}}{dt}$	$J_{\text{phos,SR,bulk}} - J_{\text{Ca}^{2+}\text{-phos,bulk}} + \frac{D_{\text{Pi}}S_{\text{A,SR,bulk-junc}}}{\text{vol}_{\text{SR,bulk}}\Delta d_{\text{diff}}} ([\text{Pi}]_{\text{SR,junc}} - [\text{Pi}]_{\text{SR,bulk}})$
$\frac{d[\text{Ca}^{2+}\text{-Pi}]_{\text{SR,bulk}}}{dt}$	$J_{\text{Ca}^{2+}\text{-phos,bulk}} + \frac{D_{\text{Pi}}S_{\text{A,SR,bulk-junc}}}{\text{vol}_{\text{SR,bulk}}\Delta d_{\text{diff}}} ([\text{Ca}^{2+}\text{-Pi}]_{\text{SR,junc}} - [\text{Ca}^{2+}\text{-Pi}]_{\text{SR,bulk}})$
$\frac{d[\text{CSQN}]_{\text{SR,bulk}}}{dt}$	$-J_{\text{Ca}^{2+}\text{-CSQN,bulk}} + \frac{D_{\text{CSQN}}S_{\text{A,SR,bulk-junc}}}{\text{vol}_{\text{SR,bulk}}\Delta d_{\text{diff}}} ([\text{CSQN}]_{\text{SR,junc}} - [\text{CSQN}]_{\text{SR,bulk}})$
$\frac{d[\text{Ca}^{2+}\text{Trop}]}{dt}$	$J_{\text{Trop-Ca}^{2+}} - J_{\text{Trop-2Ca}^{2+}}$
$\frac{d[\text{Trop-2Ca}^{2+}]}{dt}$	$J_{\text{Trop-2Ca}^{2+}} - J_D$
$\frac{d[D]}{dt}$	$J_D - J_{\text{pre}} + J_{\text{detach}}$
$\frac{d[A_{\text{pre}}]}{dt}$	$J_{\text{pre}} - J_{\text{post}}$
$\frac{d[A_{\text{post}}]}{dt}$	$J_{\text{post}} - J_{\text{detach}}$
$\frac{d[\text{ADP}]_{\text{myo,bulk}}}{dt}$	$J_{\text{hydro,bulk}} - J_{\text{Gly,bulk}}N_{\text{ATP-per-NADH}} - J_{\text{ANT,bulk}} \frac{\text{vol}_{\text{Mito,bulk}}}{\text{vol}_{\text{myo,bulk}}} + J_{\text{detach}} + \frac{D_{\text{ATP}}S_{\text{A,myo,bulk-junc}}}{\text{vol}_{\text{myo,bulk}}\Delta d_{\text{diff}}} ([\text{ADP}]_{\text{myo,junc}} - [\text{ADP}]_{\text{myo,bulk}})$
$\frac{d[\text{Ca}^{2+}]_{\text{Mito,bulk}}}{dt}$	$b_M (J_{\text{MCU,bulk}} - J_{\text{NCX,Mito,bulk}} + J_{\text{mPTP,bulk}} - J_{\text{Ca}^{2+}\text{-phos,Mito,bulk}})$
$\frac{d[\text{Pi}]_{\text{Mito,bulk}}}{dt}$	$J_{\text{PiC,bulk}} - J_{\text{Ca}^{2+}\text{-phos,Mito,bulk}}$
$\frac{d[\text{Ca}^{2+}\text{-Pi}]_{\text{Mito,bulk}}}{dt}$	$J_{\text{Ca}^{2+}\text{-phos,Mito,bulk}}$
$\frac{d[\text{ATP}]_{\text{Mito,bulk}}}{dt}$	$J_{\text{F1FO,bulk}} - J_{\text{ANT,bulk}}$
$\frac{d[\text{NADH}]_{\text{Mito,bulk}}}{dt}$	$J_{\text{PDH,bulk}} + J_{\text{AGC,bulk}} - J_{\text{Ox,bulk}}$
$\frac{dV_{\text{Mito,bulk}}}{dt}$	$(a_1J_{\text{Ox,bulk}} - a_2J_{\text{F1FO,bulk}} - J_{\text{Hleak,Mito,bulk}} - J_{\text{NCX,Mito,bulk}} - 2J_{\text{MCU,bulk}} - 2J_{\text{mPTP,bulk}} - J_{\text{ANT,bulk}} - J_{\text{AGC,bulk}})/C_{\text{Eff,Mito}}$

Table A7. Model parameters

Index	Name	Description	Value	Reference
Free parameters				
1	A_a	Steepness factor for A	$1.500 \times 10^2 \text{ mV}$ $5.017 \times 10^2 \text{ mV}$	Wallinga et al. (1999) Calibrated
2	$A_{h_{K\infty}}$	Steepness factor for h_K	7.500 mV 13.65 mV	Adrian et al. (1970) Calibrated
3	$A_{S\infty}$	Steepness factor for S	5.800 mV 4.205 mV	Wallinga et al. (1999) Calibrated
4	α_{h0}	Rate constant for h	8.100 s^{-1} 16.2 s^{-1}	(Wallinga et al. 1999) Calibrated
5	α_{m0}	Rate constant for m	$2.880 \times 10^2 \text{ s}^{-1} \text{ mV}^{-1}$ $1.402 \times 10^2 \text{ s}^{-1} \text{ mV}^{-1}$	Wallinga et al. (1999) Calibrated
6	α_{n0}	Rate constant for n	$13.10 \text{ s}^{-1} \text{ mV}^{-1}$ $6.55 \text{ s}^{-1} \text{ mV}^{-1}$	Wallinga et al. (1999) Calibrated
7	α_w	Rate constant for w_{RyR}	$1 \times 10^2 \text{ s}^{-1}$ $1.998 \times 10^2 \text{ s}^{-1}$	Standen and Stanfield (1978) Calibrated
8	β_{h0}	Rate constant for h	$4.380 \times 10^3 \text{ s}^{-1}$ $4.10 \times 10^3 \text{ s}^{-1}$	Wallinga et al. (1999) Calibrated
9	β_{m0}	Rate constant for m	$1.380 \times 10^3 \text{ s}^{-1}$ $4.051 \times 10^3 \text{ s}^{-1}$	Wallinga et al. (1999) Calibrated
10	β_{n0}	Rate constant for n	67.00 s^{-1} 33.61 s^{-1}	Wallinga et al. (1999) Calibrated
11	C_{PM}	Specific membrane capacitance	$1.000 \times 10^{-5} \text{ pC mV}^{-1} \mu\text{m}^{-2}$ $2.51 \times 10^{-5} \text{ pC mV}^{-1} \mu\text{m}^{-2}$	Cannon et al. (1993) Calibrated
12	K_{STIM1}	STIM1 Ca^{2+} sensitivity	$0.2500[\text{Ca}^{2+}]_{\text{SR},\infty}$ $0.2500[\text{Ca}^{2+}]_{\text{SR},\infty}$	Luik et al. (2008) Varied ^a
13	I_{SL}	Amplitude of stimulus current	$1.989 \times 10^{-1} \text{ pA } \mu\text{m}^{-2}$ $7.769 \times 10^{-1} \text{ pA } \mu\text{m}^{-2}$	Wallinga et al. (1999) ^b Calibrated
14	δ	Electrical distance	0.4000 0.4448	Standen and Stanfield (1978) Calibrated
15	f_{RyR}	Allosteric factor for RyR-DHPR coupling	0.2000 0.100	Ríos et al. (1993) Calibrated
16	g_{Cl^-}	Cl^- conductance	$0.1000 \text{ pA mV}^{-1} \mu\text{m}^{-2}$ $0.0730 \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Wallinga et al. (1999) ^b Calibrated
17	g_K	KDR conductance	$0.6480 \text{ pA mV}^{-1} \mu\text{m}^{-2}$ $0.3250 \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Wallinga et al. (1999) Calibrated
18	G_K	KIR conductance	$0.1110 \text{ pA mV}^{-1} \mu\text{m}^{-2}$ $0.3328 \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Wallinga et al. (1999) Calibrated
19	g_{Na}	Na^+ conductance	$8.040 \text{ pA mV}^{-1} \mu\text{m}^{-2}$ $6.755 \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Wallinga et al. (1999) Calibrated
20	g_{NCX}	NCX conductance	$0.1290 \text{ pA } \mu\text{m}^{-2}$ $0.0645 \text{ pA } \mu\text{m}^{-2}$	Grandi et al. (2010) ^b Calibrated
21	J_{NaK}	Na^+ - K^+ pump conductance	$3.542 \times 10^{-5} \text{ pmol } \mu\text{m}^{-2} \text{ s}^{-1}$ $5.608 \times 10^{-5} \text{ pmol } \mu\text{m}^{-2} \text{ s}^{-1}$	Wallinga et al. (1999) ^b Calibrated
22	K_{α_h}	Steepness factor for h	14.70 mV 14.59 mV	Cannon et al. (1993) Calibrated
23	K_{α_m}	Steepness factor for m	10.00 mV 31.86 mV	Cannon et al. (1993) Calibrated
24	K_{α_n}	Steepness factor for n	7.000 mV 1.755 mV	Cannon et al. (1993) Calibrated
25	K_{β_h}	Slope factor for h	9.000 mV 35.10 mV	Cannon et al. (1993) Calibrated

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
26	$K_{\beta m}$	Slope factor for m	18.00 mV 40.79 mV	Cannon et al. (1993) Calibrated
27	$K_{\beta n}$	Slope factor for n	40.00 mV 79.96 mV	Cannon et al. (1993) Calibrated
28	K_K	Dissociation constant for K^+	$9.500 \times 10^8 \mu\text{M}^2$ $4.420 \times 10^8 \mu\text{M}^2$	Standen and Stanfield (1978) Calibrated
29	K_{mK}	Affinity constant of the Na^+ - K^+ pump for K^+	$1.000 \times 10^3 \mu\text{M}$	Wallinga et al. (1999)
30	K_{mNa}	Affinity constant of the Na^+ - K^+ pump for Na^+	$1.999 \times 10^3 \mu\text{M}$ $1.300 \times 10^4 \mu\text{M}$	Calibrated Wallinga et al. (1999)
31	K_{PMCA}	Michaelis constant	$1.253 \times 10^4 \mu\text{M}$ 0.17 μM 0.1593 μM	Calibrated Kapela et al. (2008) Calibrated
32	K_{RyR}	Association rate constant for RyR	4.500 mV 3.358 mV	Ríos et al. (1993) Calibrated
33	K_S	Dissociation constant for blocking S	$1.000 \times 10^6 \mu\text{M}^2$	Standen and Stanfield (1978)
34	K_{SERCA}	SERCA Michaelis constant	$1.551 \times 10^6 \mu\text{M}^2$ 1.000 μM 1.999 μM	Calibrated Baylor and Hollingworth (1998) Calibrated
35	$K_{w,r0}$	Ca^{2+} sensitivity of w	1.000 μM 1.859 μM	Standen and Stanfield (1978) Calibrated
36	K_{dAct}	NCX Ca^{2+} dissociation constant	0.0500 μM 0.1 μM	Grandi et al. (2010) Calibrated
37	$K_{Ca,i}$	NCX Ca^{2+} Michaelis constant	6.590 μM 10.06 μM	Grandi et al. (2010) Calibrated
38	k_{sat}	NCX constant	0.3200 0.64	Grandi et al. (2010) Calibrated
39	v_{NCX}	NCX constant	0.2700 0.5390	Grandi et al. (2010) Calibrated
40	S_i	Intracellular blocking concentration	$1 \times 10^4 \mu\text{M}$	Standen and Stanfield (1978)
41	τ_{SOCE}	Activation time for SOCE	$3.336 \times 10^4 \mu\text{M}$ 0.0100 s 0.020 s	Calibrated This study Calibrated
42	v_{SERCA}	Maximum SERCA uptake rate	$1.134 \times 10^4 \mu\text{M} \mu\text{M} \text{ s}^{-1}$ $5.670 \times 10^3 \mu\text{M} \mu\text{M} \text{ s}^{-1}$	(Shorten et al., 2007) ^c Calibrated
43	$J_{PMCA,0}$	Max PMCA flux	39.75 $\mu\text{M} \text{ s}^{-1}$ 20.00 $\mu\text{M} \text{ s}^{-1}$	Kapela et al. (2008) ^c Calibrated
44	$v_{leak,SR}$	SR leak permeability	0.4652 $\mu\text{M} \text{ s}^{-1}$ 0.2326 $\mu\text{M} \text{ s}^{-1}$	This study Calibrated
46	$k_{on,CA}$	Ca^{2+} -ATP binding rate	15.00 $\mu\text{M}^{-1} \text{ s}^{-1}$ 7.500 $\mu\text{M}^{-1} \text{ s}^{-1}$	Baylor and Hollingworth (1998) ^b Calibrated
47	$k_{off,CA}$	Ca^{2+} -ATP unbinding rate	$3.000 \times 10^2 \text{ s}^{-1}$ $6.0 \times 10^2 \text{ s}^{-1}$	Baylor and Hollingworth (1998) ^b Calibrated
48	$k_{on,CP}$	Ca^{2+} -parvalbumin binding rate	41.70 $\mu\text{M}^{-1} \text{ s}^{-1}$ 20.86 $\mu\text{M}^{-1} \text{ s}^{-1}$	Baylor and Hollingworth (1998) Calibrated
49	$k_{off,CP}$	Ca^{2+} -parvalbumin unbinding rate	0.5000 s^{-1}	Baylor and Hollingworth (1998)
50	$k_{on,MP}$	Mg^{2+} -parvalbumin binding rate	0.2658 s^{-1} 0.0330 $\mu\text{M}^{-1} \text{ s}^{-1}$ 0.0660 $\mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated Baylor and Hollingworth (1998) Calibrated

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
51	$k_{\text{off,MP}}$	Mg^{2+} -parvalbumin unbinding rate	3.000 s^{-1}	Baylor and Hollingworth (1998)
			1.515 s^{-1}	Calibrated
52	$[\text{Parv}]_{\text{tot}}$	Total parvalbumin concentration	$1.500 \times 10^3 \text{ }\mu\text{M}$	Baylor and Hollingworth (1998)
			$7.506 \times 10^2 \text{ }\mu\text{M}$	Calibrated
53	$k_{\text{on,MA}}$	$[\text{Mg}^{2+}]$ -ATP binding rate	$1.500 \text{ }\mu\text{M s}^{-1}$	Baylor and Hollingworth (1998)
			$2.960 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated
54	$k_{\text{off,MA}}$	$[\text{Mg}^{2+}]$ -ATP unbinding rate	150.0 s^{-1}	(Baylor and Hollingworth, 1998)
			76.10 s^{-1}	Calibrated
55	k_{hyd}	Maximal rate of ATP hydrolysis	$100.0 \text{ }\mu\text{M s}^{-1}$	Wacquier et al. (2016)
			$68.95 \text{ }\mu\text{M s}^{-1}$	Calibrated
56	k_{H}	Michaelis constant for ATP hydrolysis	$1.000 \times 10^3 \text{ }\mu\text{M}$	Wacquier et al. (2016)
			$5.00 \times 10^2 \text{ }\mu\text{M}$	Calibrated
57	k_{prod}	ATP production rate	100 s^{-1}	This study
			51.44 s^{-1}	Calibrated
58	$[\text{Mg}^{2+}]$	Myoplasmic $[\text{Mg}^{2+}]$	$1.000 \times 10^3 \text{ }\mu\text{M}$	Baylor and Hollingworth (1998)
			$2.00 \times 10^3 \text{ }\mu\text{M}$	Calibrated
59	$k_{\text{on,T1}}$	Ca^{2+} -troponin binding rate	$177.0 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Baylor and Hollingworth (2007)
			$88.50 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated
60	$k_{\text{off,T1}}$	Ca^{2+} -troponin unbinding rate	$1.544 \times 10^3 \text{ s}^{-1}$	Baylor and Hollingworth (2007)
			$3.086 \times 10^3 \text{ s}^{-1}$	Calibrated
61	k_{unblock}	Ca^{2+} -RU activation rate	150.0 s^{-1}	Razumova et al. (2000) ^c
			75.06 s^{-1}	Calibrated
62	k_{block}	Ca^{2+} -RU deactivation rate	50.00 s^{-1}	Razumova et al. (2000)
			99.98 s^{-1}	Calibrated
63	f_0	Rate of cross-bridge attachment	$1.500 \times 10^3 \text{ s}^{-1}$	Shorten et al. (2007)
			$7.822 \times 10^2 \text{ s}^{-1}$	Calibrated
64	f_p	Rate of cross-bridge detachment	$1.500 \times 10^4 \text{ s}^{-1}$	Shorten et al. (2007)
			$2.999 \times 10^4 \text{ s}^{-1}$	Calibrated
65	h_0	Forward rate of the power stroke	240 s^{-1}	Shorten et al. (2007)
			120.1 s^{-1}	Calibrated
66	h_p	Backward rate of the power stroke	$0.0600 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Senneff and Lowery (2021)
			$0.0709 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated
67	g_0	Rate of myosin head unbinding	$0.1714 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Senneff and Lowery (2021)
			$0.3428 \text{ }\mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated
68	PP	Phosphate solubility product	$6.000 \times 10^6 \text{ }\mu\text{M}^2$	Allen and Westerblad (2001)
			$1.117 \times 10^7 \text{ }\mu\text{M}^2$	Calibrated
69	k_p	Rate of phosphate transport into the SR	$8.421 \times 10^{-3} \text{ }\mu\text{M s}^{-1}$	Shorten et al. (2007) ^c
			$1.625 \times 10^{-2} \text{ }\mu\text{M s}^{-1}$	Calibrated
70	A_p	Rate of phosphate precipitation from the SR	$1.000 \times 10^{-6} \text{ }\mu\text{M}^{-3} \text{ s}^{-1}$	Shorten et al. (2007)
			$2.00 \times 10^{-6} \text{ }\mu\text{M}^{-3} \text{ s}^{-1}$	Calibrated
71	B_p	Rate of phosphate precipitate solubilization	$1.000 \times 10^{-7} \text{ }\mu\text{M}^{-2} \text{ s}^{-1}$	Shorten et al. (2007)
			$1.995 \times 10^{-7} \text{ }\mu\text{M}^{-2} \text{ s}^{-1}$	Calibrated
73	$[\text{Trop}]_{\text{tot}}$	Total troponin concentration	$140 \text{ }\mu\text{M}$	Baylor and Hollingworth (1998)
			$70.05 \text{ }\mu\text{M}$	Calibrated
74	$[\text{Pi}]_{\text{SR,init}}$	Initial SR phosphate concentration	$3.000 \times 10^3 \text{ }\mu\text{M}$	This study
			$1.504 \times 10^3 \text{ }\mu\text{M}$	Calibrated

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
75	[Pi] _{myo,init}	Initial myoplasmic phosphate concentration	$3.000 \times 10^3 \mu\text{M}$	This study
76	V_a	Half-maximal voltage for A	$2.635 \times 10^3 \mu\text{M}$	Calibrated
			70.00 mV	Wallinga et al. (1999)
77	V_h	Half-maximal voltage for h	80.00 mV	Calibrated
			−45.00 mV	Cannon et al. (1993)
78	$V_{hK\infty}$	Half-maximal voltage for h_K	−47.99 mV	Calibrated
			−40.00 mV	Adrian et al. (1970)
79	V_m	Half-maximal voltage for m	−30.22 mV	Calibrated
			−46.00 mV	Cannon et al. (1993)
80	V_n	Half-maximal voltage for n	−45.40 mV	Calibrated
			−40.00 mV	Cannon et al. (1993)
81	$V_{S\infty}$	Half-maximal voltage for S	−20.00 mV	Calibrated
			−78.00 mV	Wallinga et al. (1999)
82	V_τ	Voltage parameter for τ_5	−81.38 mV	Calibrated
			90.00 mV	Ruff and Whittlesey (1992)
83	V_{RyR}	RyR Activation rate constant	83.12 mV	Calibrated
			−20.00 mV	Ríos et al. (1993)
84	k_{ATP}	ATP dependence of SR calcium pump	−30.00 mV	Calibrated
			$0.04000 \mu\text{M}$	Lytton et al. (1992)
85	$K_{\text{Na},i}$	Myoplasmic Na^+ Michaelis constant	$0.07978 \mu\text{M}$	Calibrated
			$1.229 \times 10^4 \mu\text{M}$	Grandi et al. (2010)
86	$K_{\text{Na},o}$	Extracellular Na^+ Michaelis constant	$2.400 \times 10^4 \mu\text{M}$	Calibrated
			$8.750 \times 10^4 \mu\text{M}$	Grandi et al. (2010)
87	$K_{\text{Ca},o}$	Extracellular Ca^{2+} Michaelis constant	$1.739 \times 10^5 \mu\text{M}$	Calibrated
			$1.300 \times 10^3 \mu\text{M}$	Grandi et al. (2010)
89	$g_{\text{leak},\text{SL}}$	Ca^{2+} leak conductance	$1.477 \times 10^3 \mu\text{M}$	Calibrated
			$1 \times 10^{-5} \text{ pA mV}^{-1} \mu\text{m}^{-2}$	This study
90	L_{RyR}	Baseline release of Ca^{2+}	$1.999 \times 10^{-5} \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Calibrated
			5×10^5	Ríos et al. (1993)
92	$v_{\text{max},\text{RyR}}$	Maximum release rate of Ca^{2+} from RyR	6.226×10^5	Calibrated
			$1.02 \times 10^8 \mu\text{m s}^{-1}$	Ríos et al. (1993) ^d
93	$k_{\text{on},\text{T2}}$	Regulatory unit activation rate	$2.022 \times 10^8 \mu\text{m s}^{-1}$	Calibrated
			$88.5 \mu\text{M}^{-1} \text{ s}^{-1}$	Baylor and Hollingworth (2007)
94	$k_{\text{off},\text{T2}}$	Regulatory unit deactivation rate	$44.25 \mu\text{M}^{-1} \text{ s}^{-1}$	Calibrated
			17.1 s^{-1}	Baylor and Hollingworth (2007)
95	g_{Orai1}	SOCE conductance	34.15 s^{-1}	Calibrated
			$4.752 \times 10^{-5} \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Luik et al. (2008) ^e
96	[CSQN] _{tot}	Total Calsequestrin concentration	$2.393 \times 10^{-5} \text{ pA mV}^{-1} \mu\text{m}^{-2}$	Calibrated
			$3.100 \times 10^4 \mu\text{M}$	Cannell and Allen (1984)
97	K_{CSQN}	CSQN- Ca^{2+} dissociation constant	$6.200 \times 10^4 \mu\text{M}$	Calibrated
			800 μM	Ostwald and MacLennan (1974)
98	$k_{\text{off},\text{CSQN}}$	CSQN- Ca^{2+} unbinding rate in SR	400.0 μM	Calibrated
			5 s^{-1}	Cannell and Allen (1984)
99	d_{diff}	characteristic diffusion length	9.999 s^{-1}	Calibrated
			0.1 μm	This study
100	D_{ion}	Ion diffusion coefficient	0.1999 μm	Calibrated
			$1000 \mu\text{m}^2 \text{ s}^{-1}$	This study ^f
			$1062 \mu\text{m}^2 \text{ s}^{-1}$	Calibrated

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
101	D_{ATP}	ATP diffusion coefficient	500 $\mu\text{m}^2 \text{s}^{-1}$ 992.6 $\mu\text{m}^2 \text{s}^{-1}$	This study ^f Calibrated
102	D_{Parv}	Parv diffusion coefficient	1 $\mu\text{m}^2 \text{s}^{-1}$ 0.5120 $\mu\text{m}^2 \text{s}^{-1}$	This study ^f Calibrated
103	D_{CSQN}	CSQN diffusion coefficient	0.1 $\mu\text{m}^2 \text{s}^{-1}$ 0.1769 $\mu\text{m}^2 \text{s}^{-1}$	This study ^f Calibrated
104	D_{Pi}	Phosphate diffusion coefficient	500 $\mu\text{m}^2 \text{s}^{-1}$ 640.7 $\mu\text{m}^2 \text{s}^{-1}$	This study ^f Calibrated
105	R_a	Access resistance for current from SL to TT	$1.500 \times 10^{-2} \mu\text{m}^2 \text{mV pA}^{-1}$	Cannon et al. (1993)
106	$[ATP]_{set}$	Myoplasmic ATP set-point	$3.00 \times 10^{-2} \mu\text{m}^2 \text{mV pA}^{-1}$ 700 μM 350.5 μM	Calibrated This study Calibrated
Mitochondria-related parameters (separate estimation)				
M1	K_{db}	Ca^{2+} -phosphate dissociation constant	$5 \times 10^2 \mu\text{M}$	This study ⁹
M2	b_M	Rapid-buffering free Ca^{2+} fraction	$2.5 \times 10^2 \mu\text{M}$ 0.01	Calibrated This study ⁹
M3	v_{MCU}	MCU rate constant	0.005 $6.0 \times 10^{-4} \mu\text{M s}^{-1}$ $1.20 \times 10^{-3} \mu\text{M s}^{-1}$	Calibrated Wacquier et al. (2016) Calibrated
M4	K_{trans}	Dissoc. constant for Ca^{2+} translocation by MCU	6.0 μM	Wacquier et al. (2016)
M5	K_{act}	Dissoc. constant for Ca^{2+} -mediated MCU activation	3.0 μM 0.38 μM	Calibrated Magnus and Keizer (1997)
M6	L_{MCU}	Allosteric equilibrium constant for MCU	0.1900 μM 50	Calibrated Magnus and Keizer (1997)
M7	p_1	Voltage dependence coefficient for MCU	25.93 0.1 mV^{-1}	Calibrated Wacquier et al. (2016)
M8	$V_{NCX,Mito}$	Mitochondrial NCX rate constant	0.06716 mV^{-1} 0.35 $\mu\text{M s}^{-1}$ 0.1751 $\mu\text{M s}^{-1}$	Calibrated Wacquier et al. (2016) Calibrated
M9	p_2	Mito NCX voltage dependence coefficient	$1.6 \times 10^{-2} \text{mV}^{-1}$	Wacquier et al. (2016)
M10	k_{mPTP}	Rate constant for mPTP	$8.0 \times 10^{-3} \text{mV}^{-1}$ $8.0 \times 10^{-3} \text{s}^{-1}$ $4.000 \times 10^{-3} \text{s}^{-1}$	Calibrated Wacquier et al. (2016) Calibrated
M11	p_3	Voltage dependence coefficient for mPTP	$5.0 \times 10^{-2} \text{mV}^{-1}$	Wacquier et al. (2016)
M12	k_{Gly}	Glycolysis rate	$3.655 \times 10^{-2} \text{mV}^{-1}$ $4.5 \times 10^2 \text{s}^{-1}$ $8.996 \times 10^2 \text{s}^{-1}$	Calibrated Bertram et al. (2006)* Calibrated
M13	K_{Mca}	MM constant for Ca^{2+} -dependent KC activation	0.1 μM	Wacquier et al. (2016)
M14	K_{mNAD}	MM constant for NAD ⁺ consumption by the KC	0.0500 μM 1.0 2.000	Calibrated Bertram et al. (2006) Calibrated

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Table A7. (Continued)

Index	Name	Description	Value	Reference
M15	ν_{Ox}	NADH oxidation rate constant (ETC)	$6.0 \times 10^2 \mu\text{M s}^{-1}$	Bertram et al. (2006)
M16	K_{Ox}	MM constant for NADH oxidation (ETC)	$3.005 \times 10^2 \mu\text{M s}^{-1}$ $1.0 \times 10^2 \mu\text{M}$	Calibrated Bertram et al. (2006)
M17	p_4	Voltage-dependence coefficient for ETC (shift)	$2.000 \times 10^2 \mu\text{M}$ $1.77 \times 10^2 \text{ mV}$	Calibrated Bertram et al. (2006)
M18	p_5	Voltage-dependence coefficient for ETC (sensitivity)	$1.870 \times 10^2 \text{ mV}$ 5.0 mV	Calibrated Bertram et al. (2006)
M19	ν_{AGC}	Rate constant for NADH production via MAS	2.5 mV $25.0 \mu\text{M s}^{-1}$	Calibrated Wacquier et al. (2016)
M20	K_{AGC}	Dissociation constant of Ca^{2+} from AGC	$12.65 \mu\text{M s}^{-1}$ $0.14 \mu\text{M}$	Calibrated Contreras et al. (2007)
M21	K_{iCa}	Constant for inhibition of AGC by cytosolic Ca^{2+}	$0.07 \mu\text{M}$ $0.1 \mu\text{M}$	Calibrated Wacquier et al. (2016)
M22	ν_{F1FO}	Rate constant for F1FO ATPase	$0.0500 \mu\text{M}$ $3.5 \times 10^4 \mu\text{M s}^{-1}$ $1.750 \times 10^4 \mu\text{M s}^{-1}$	Calibrated Bertram et al. (2006)
M23	p_6	Voltage dependence coefficient for ATPase (shift)	$1.9 \times 10^2 \text{ mV}$	Calibrated Bertram et al. (2006)
M24	p_7	Voltage dependence coefficient for ATPase (sensitivity)	$2.0 \times 10^2 \text{ mV}$ 8.5 mV	Calibrated Bertram et al. (2006)
M25	K_{iATP}	Constant for inhibition of ATPase activity by ATP	4.25 mV $1.0 \times 10^4 \mu\text{M}$	Calibrated Bertram et al. (2006)
M26	ν_{ANT}	Rate constant for ANTs	$5.0 \times 10^3 \mu\text{M}$ $5.0 \times 10^3 \mu\text{M s}^{-1}$ $2.5 \times 10^3 \mu\text{M s}^{-1}$	Calibrated Bertram et al. (2006)
M27	α_{c}	Factor for cytosolic ADP and ATP buffering	0.111	Calibrated Fall and Keizer (2001)
M28	α_{Mito}	Factor for mitochondrial ADP and ATP buffering	0.2216 0.139	Calibrated Fall and Keizer (2001)
M29	f_{ANT}	Controls sensitivity of ANTs to voltage	0.06950 0.5	Calibrated Wacquier et al. (2016)
M30	p_8	Voltage-dependent proton leak parameter	0.25 $2.0 \mu\text{M s}^{-1} \text{ mV}^{-1}$	Calibrated Bertram et al. (2006)
M31	p_9	Voltage-independent proton leak parameter	$4.0 \mu\text{M s}^{-1} \text{ mV}^{-1}$ $-30 \mu\text{M s}^{-1}$	Calibrated Bertram et al. (2006)
M32	ν_{PiC}	Rate constant for PiC	$-15.09 \mu\text{M s}^{-1}$ $5.0 \times 10^3 \mu\text{M s}^{-1}$ $1.0 \times 10^4 \mu\text{M s}^{-1}$	Calibrated This study ^h Calibrated
M33	C_{pEff}	Effective mitochondrial membrane capacitance	$1.8 \mu\text{M mV}^{-1}$ $3.383 \mu\text{M mV}^{-1}$	Bertram et al. (2006) Calibrated

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
M34	a_1	Contribution of NADH consumption to voltage change	20	Wacquier et al. (2016)
			21.56	Calibrated
M35	a_2	Contribution of ATPase activity to voltage change	3.43	Bertram et al. (2006)
M36	K_{pi}	Hill constant for PiC activity	1.715 $1 \times 10^3 \mu\text{M}$	Calibrated This study ^h
M37	$k_{off,B}$	Ca^{2+} -phosphate unbinding rate in mitochondria	$5.000 \times 10^2 \mu\text{M}$ 1.0 s^{-1}	Calibrated This study ^g
M38	$[\text{ANP}]_{\text{tot}}$	Total mitochondrial conc. of adenine nucleotides	1.988 s^{-1} $1.5 \times 10^4 \mu\text{M}$	Calibrated Bertram et al. (2006)
M39	$[\text{NAD}]_{\text{tot}}$	Total mitochondrial conc. of NAD and NADH	$3.0 \times 10^4 \mu\text{M}$ $2.5 \times 10^2 \mu\text{M}$	Calibrated Wacquier et al. (2016)
M40	$K_{Pi,M}$	PiC Hill constant for mitochondrial phosphate	$4.167 \times 10^2 \mu\text{M}$ $1.0 \times 10^4 \mu\text{M}$	Calibrated Wacquier et al. (2016)
			$5.000 \times 10^3 \mu\text{M}$	Calibrated
Fixed parameters				
Temperature and extracellular ion concentrations are fixed except in cases tested for parameter estimation (see Fig. 2 caption).				
	$[\text{Ca}^{2+}]_{\text{EC}}$	Bulk extracellular Ca^{2+} concentration	$1.3 \times 10^3 \mu\text{M}$	Senneff and Lowery (2021)
	$[\text{Cl}^-]_{\text{EC}}$	Bulk extracellular Cl^- concentration	$1.280 \times 10^5 \mu\text{M}$	Senneff and Lowery (2021)
	$[\text{K}^+]_{\text{EC}}$	Bulk extracellular K^+ concentration	$4.000 \times 10^3 \mu\text{M}$	Senneff and Lowery (2021)
	$[\text{Na}^+]_{\text{EC}}$	Bulk extracellular Na^+ concentration	$1.470 \times 10^5 \mu\text{M}$	Senneff and Lowery (2021)
	$N_{\text{ATP-per-NADH}}$	Ratio of ATP per NADH produced in glycolysis	1	
	$D_{\text{ion,EC}}$	Diffusion coefficient for extracellular ions	$1.000 \times 10^3 \mu\text{m}^2 \text{ s}^{-1}$	This study
	T	Temperature	295.15 K	
	F	Faraday's constant	$9.6485 \times 10^4 \text{ pC pmol}^{-1}$	
	N_A	Avogadro's number	$6.022 \times 10^{11} \text{ molecules pmol}^{-1}$	
	R	Gas constant	$8.3145 \times 10^3 \text{ fJ pmol}^{-1} \text{ K}^{-1}$	
	$\eta_{\text{conv},1}$	Unit conversion factor 1	$1 \times 10^9 \mu\text{M} \mu\text{m}^3 \text{ pmol}^{-1}$	
	$\eta_{\text{conv},2}$	Unit conversion factor 2	$602.2 \text{ molecules} \mu\text{m}^{-3} \mu\text{M}^{-1}$	
Temperature coefficients. In all cases $\Delta T = T - 293.15$				
	Q_{NCX}		$1.57 \frac{\Delta T}{10}$	Grandi et al. (2010)
	Q_{KDR}		$1.5 \frac{\Delta T}{10}$	Standen and Stanfield (1978)
	Q_{Na}		$1.5 \frac{\Delta T}{10}$	Stephanova and Dimitrov (1982)
	Q_{KIR}		$1.55 \frac{\Delta T}{10}$	Caffier and Shvinka (1986)
	Q_{NaK}		1	Senneff and Lowery (2021)
	Q_{Cl}		$1.5 \frac{\Delta T}{10}$	Fortune and Lowery (2009)
	Q_{CSL}		$1.02 \frac{\Delta T}{10}$	Fortune and Lowery (2009)
	Q_{α_n}		$2.5 \frac{\Delta T}{10}$	Standen and Stanfield (1978)
	Q_{α_m}		$2.3 \frac{\Delta T}{10}$	Fortune and Lowery (2009)
	Q_{α_h}		$2.5 \frac{\Delta T}{10}$	Standen and Stanfield (1978)

(Continued)

Table A7. (Continued)

Index	Name	Description	Value	Reference
	$Q_{\beta n}$		$2.5 \frac{\Delta T}{10}$	Standen and Stanfield (1978)
	$Q_{\beta m}$		$2.3 \frac{\Delta T}{10}$	Fortune and Lowery (2009)
	Q_{β_h}		$2.3 \frac{\Delta T}{10}$	Standen and Stanfield (1978)
	Q_{SERCA}		$2.6 \frac{\Delta T}{10}$	Shannon et al. (2004)
	Q_{PMCA}		$2.35 \frac{\Delta T}{10}$	Shannon et al. (2004)

^aFixed to 0.25 times the resting SR Ca^{2+} without SOCE in baseline simulations, varied in Figs. 8, A7 and A8.

^bOrder-of-magnitude estimate from source studies.

^cFlux magnitude corrected based on the ratio of volumes of cells (or volume of compartments) in the referenced studies vs. myofibre volume here.

^dAssuming an SR Ca^{2+} concentration of 500 μM corresponds to a total flux (by volume) of 150 $\mu\text{M/s}$, close to the measured values in Ríos et al. (1993).

^eConverted from the 1 A/F current density measured in Luik et al. (2008), assuming specific capacitance of 1.0 $\mu\text{F/cm}^2$, and $E_{\text{Ca}^{2+}} - V_{\text{rest}} \approx 200 \text{ mV}$.

^fOrder-of-magnitude estimate based on molecular size.

^gInitial estimates for phosphate-independent Ca^{2+} buffering and Ca^{2+} -phosphate dissociation coefficient informed by data from Wei et al. (2015), given initial (phosphate-free) fraction of free Ca^{2+} and concentrations of phosphate tested. The Ca^{2+} -phosphate off-rate was fixed at 1.0 s^{-1} and did not affect the fit.

^hThe order of magnitude for maximum transport rate and Hill constant for PiC were converted from previous measurements (maximum rate of 100-1000 $\text{nmol mg}^{-1} \text{ min}^{-1}$ in terms of mitochondrial protein content and 50% maximum flux at 1-2 mM phosphate (Coty and Pedersen, 1974; Seifert et al., 2015; Wohlrab, 1980)), assuming total mitochondrial density of 1.2 g/mL (Glas and Bahr, 1966) and mitochondrial protein content of about 25% (Fuchs et al., 2020).

Acronyms used here and not previously defined – AGC: aspartate/glutamate carrier, ETC: electron transport chain, KC: Krebs cycle, MAS: malate-aspartate shuttle, MM: Michaelis-Menten.

Appendix B

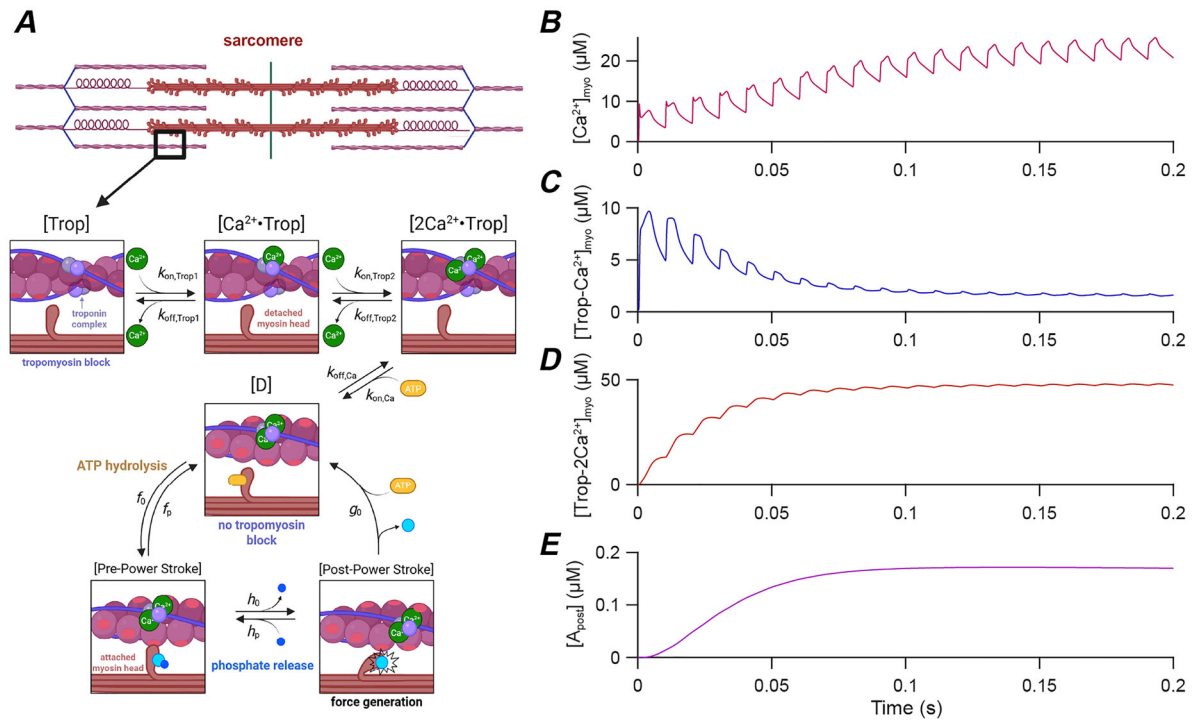
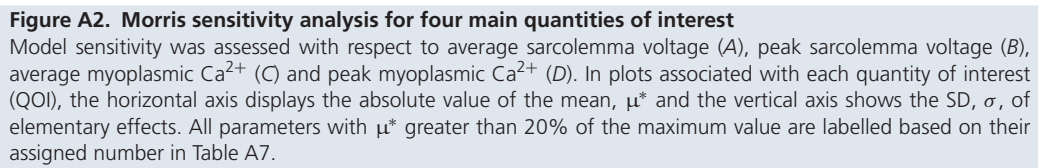


Figure A1. Summary of six-state cross-bridge model

A, schematic of cross-bridge model. Two calcium ions bind to troponin, facilitating the unblocking of the myosin-head binding sites on actin. ATP hydrolysis enables myosin-head binding to actin, and phosphate release drives the power stroke. Figure created in BioRender. B–E, model predictions of myoplasmic Ca^{2+} (B) and different cross-bridge states during repetitive stimuli, including troponin bound to one Ca^{2+} (C), troponin bound to two Ca^{2+} (D) and cross-bridges post-power stroke (E).



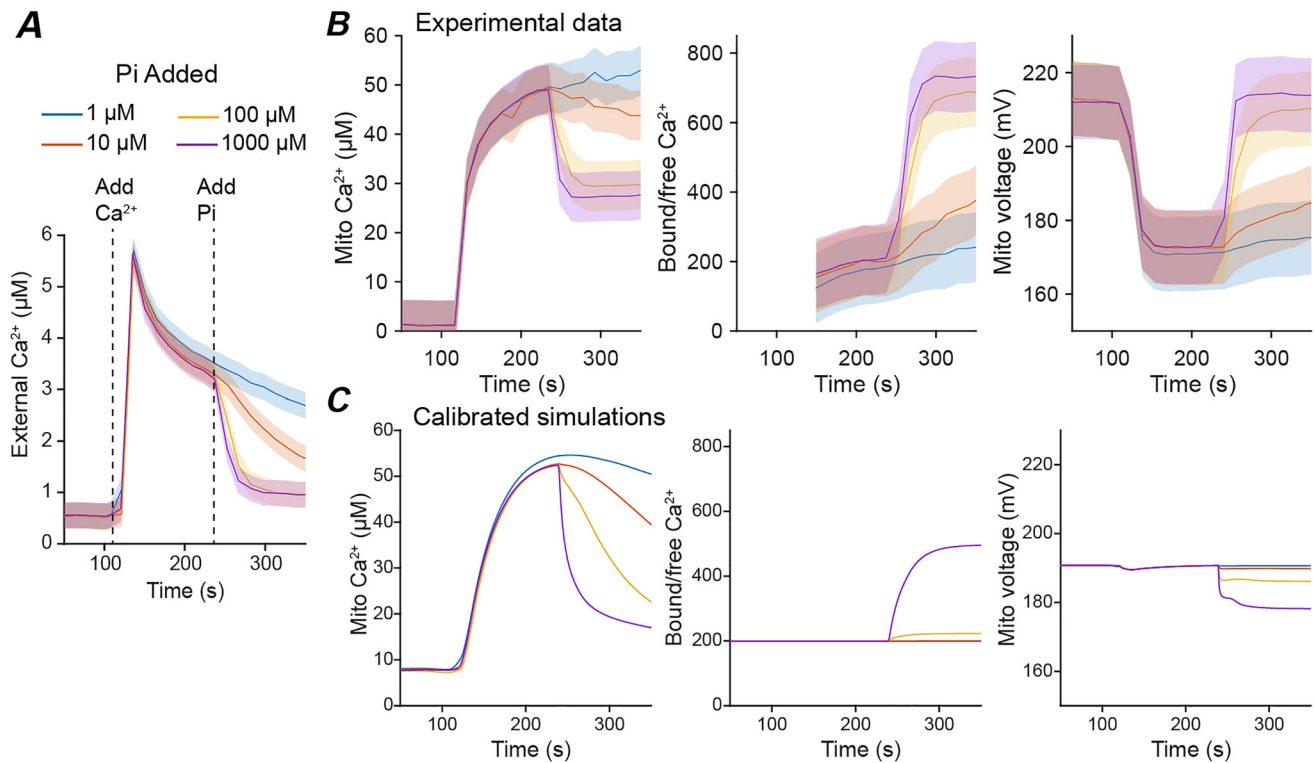


Figure A3. Calibration of mitochondrial Ca^{2+} and phosphate handling for module 5

A, experimental measurements of Ca^{2+} in the extra-mitochondrial solution from Wei et al. (2015). The mean curve is used as a driving function in the simulations shown in panel C. B, experimental measurements from Wei et al. (2015) ($n = 3$ experiments in each case). Error bars in A and B indicate the assumed SEM in our calibration procedure (see eqn 17). C, predictions of our model with calibrated parameters as reported in Table A7, assuming time-dependent Ca^{2+} from panel A and time-dependent phosphate as a Heaviside function with the indicated concentration, starting at time $t = 240$ s. The minimized weighted sum of square errors (defined in eqn 17) was 38.518.

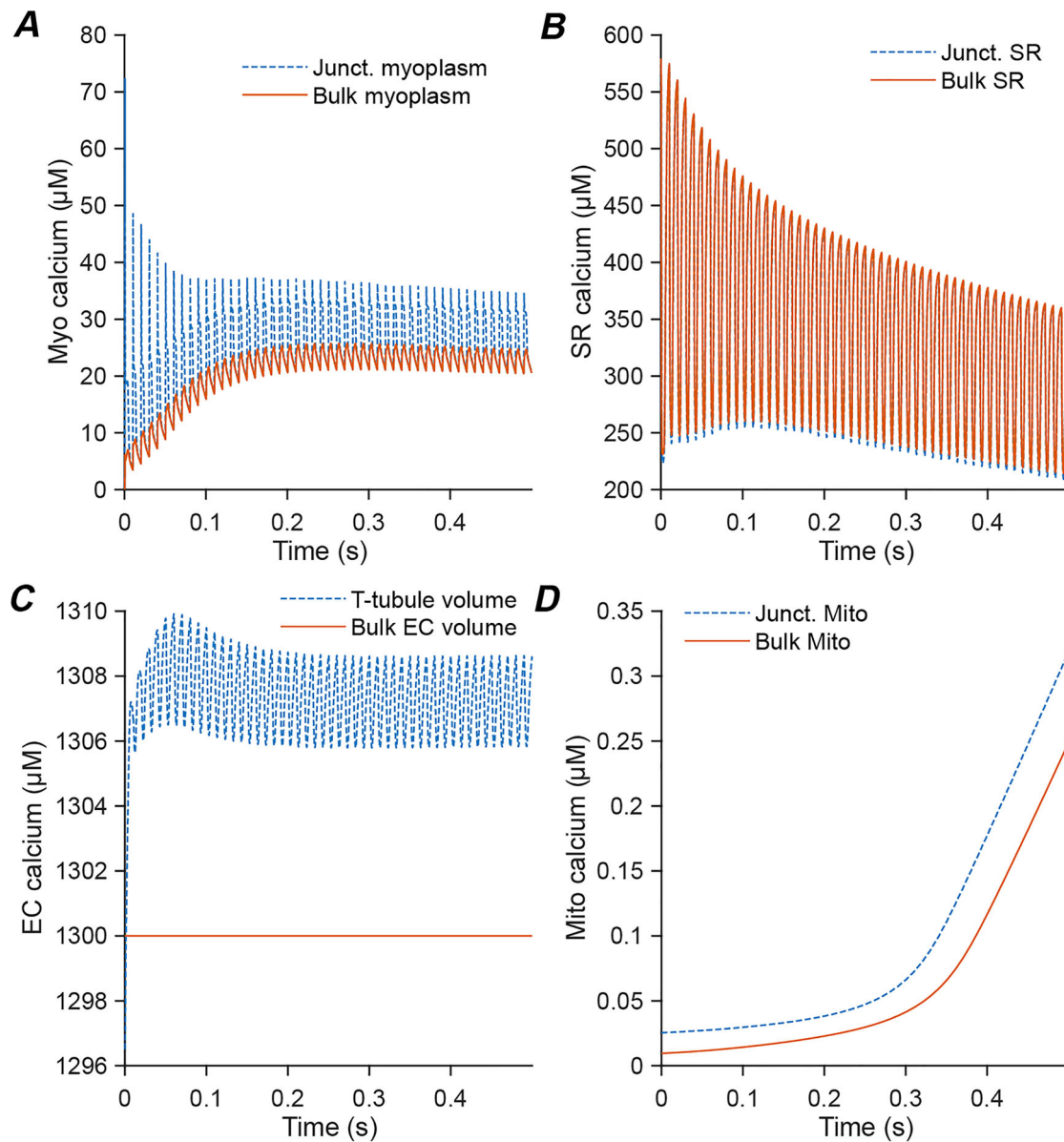


Figure A4. Calcium concentrations in different subregions of the compartmental model

A, schematic summarizing the subregions of extracellular, myoplasmic and sarcoplasmic reticulum (SR) compartments. B, myoplasmic Ca^{2+} in the bulk vs. junctional myoplasm. B, SR Ca^{2+} in the bulk vs. junctional SR. C, Ca^{2+} in the bulk extracellular volume vs. the T-tubule volume. D, Ca^{2+} in bulk mitochondria vs. junctional mitochondria. All concentrations were computed from the same test conditions shown in Fig. 3.

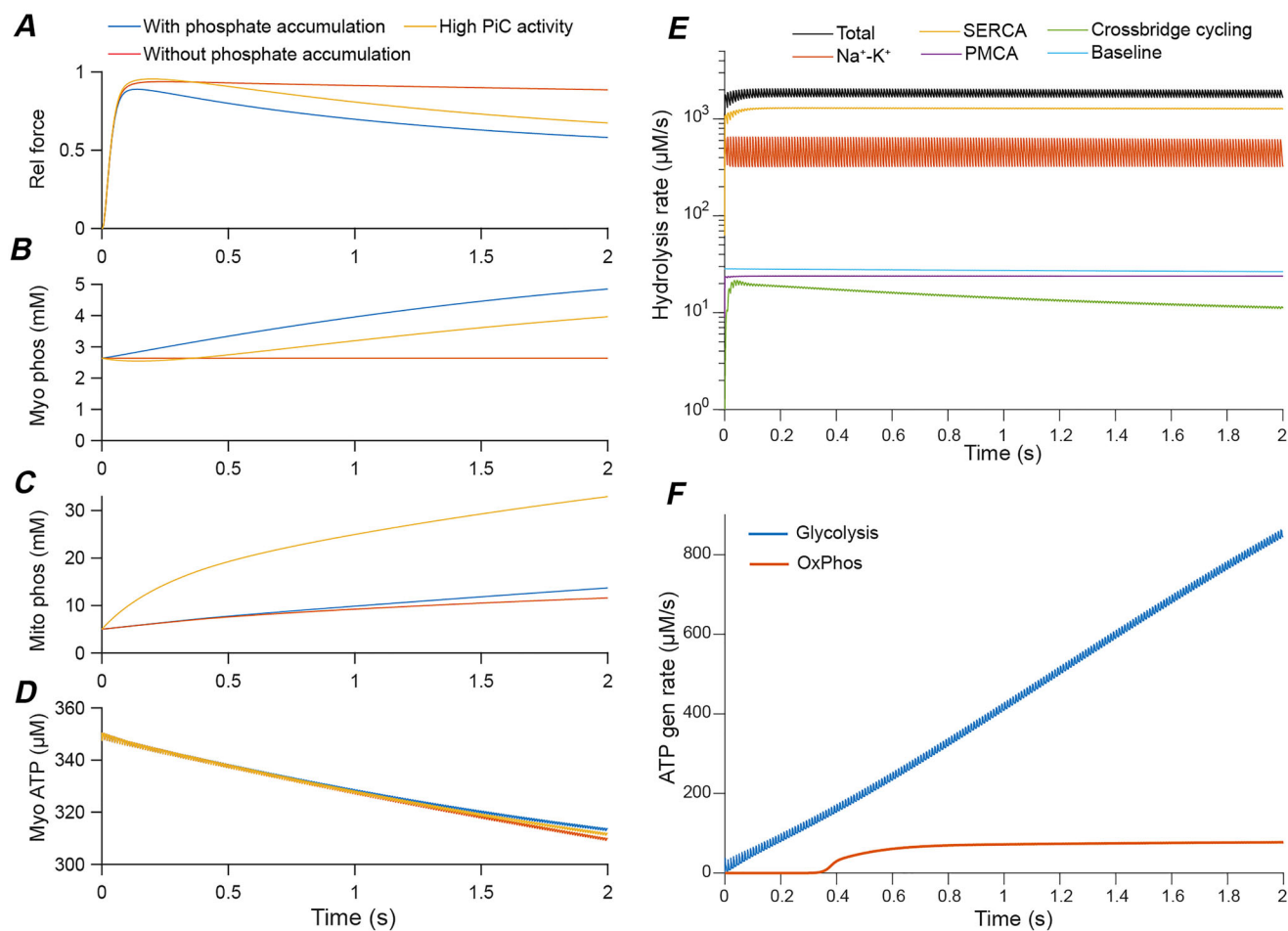


Figure A5. Effects of phosphate accumulation due to ATP hydrolysis in the myoplasm

A–D, model predictions with and without phosphate accumulation, or with 10 times the baseline value for PiC permeability. A, Plots show relative force, B, myoplasmic phosphate, C, mitochondrial phosphate and D, myoplasmic ATP. E, Contributions of each ATP-consuming process in the model for a simulation with normal phosphate accumulation. F, Contribution of glycolysis vs. oxidative phosphorylation to ATP generation over time for a simulation with normal phosphate accumulation.

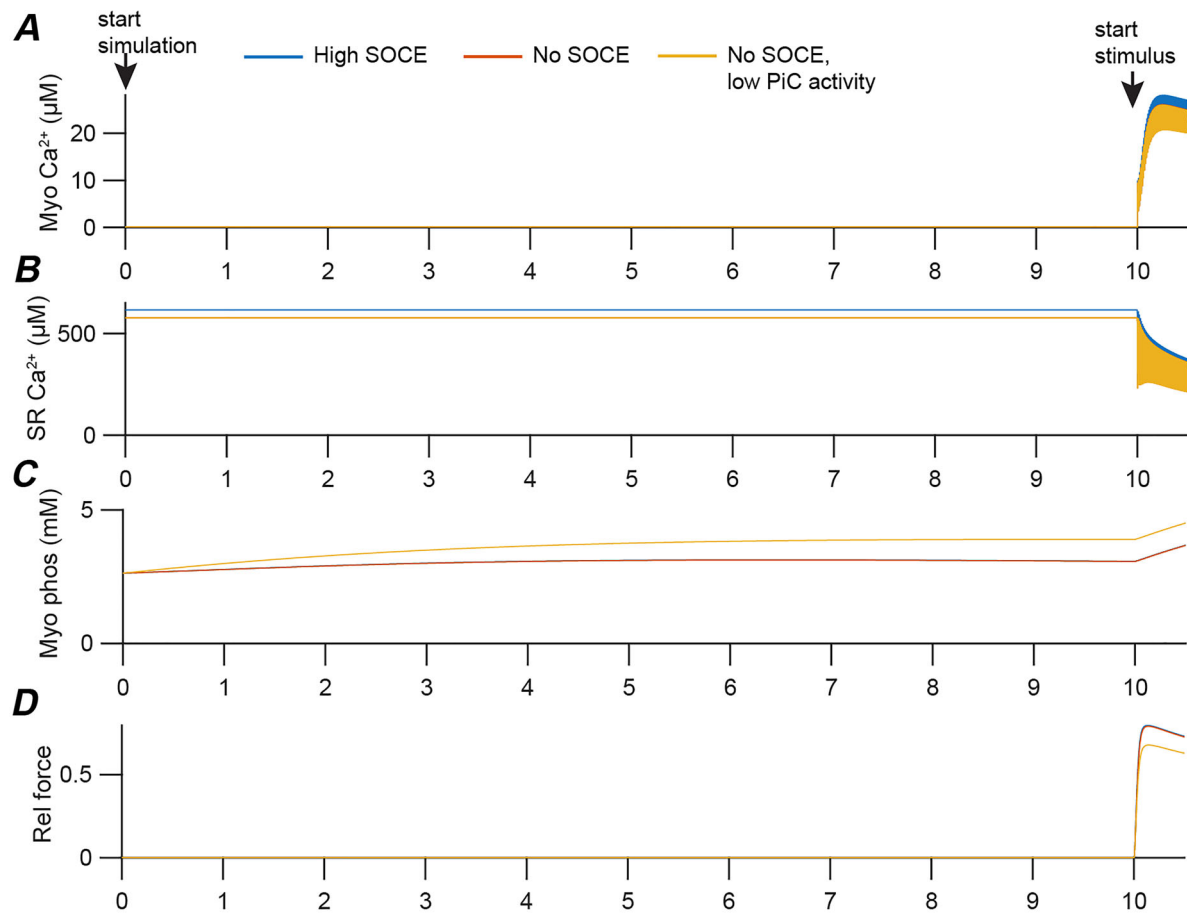


Figure A6. Initialization protocol for simulations shown in Fig. 7

After normal initialization of the system (as described in Methods), these tests were run for 10 s before the stimulus occurred. As shown, myoplasmic (A) and sarcoplasmic reticulum (SR) (B) Ca^{2+} concentrations remain mainly constant prior to stimulus, whereas myoplasmic phosphate gradually accumulates due to resting ATP hydrolysis (C). The resulting predictions for relative force are shown in panel D.

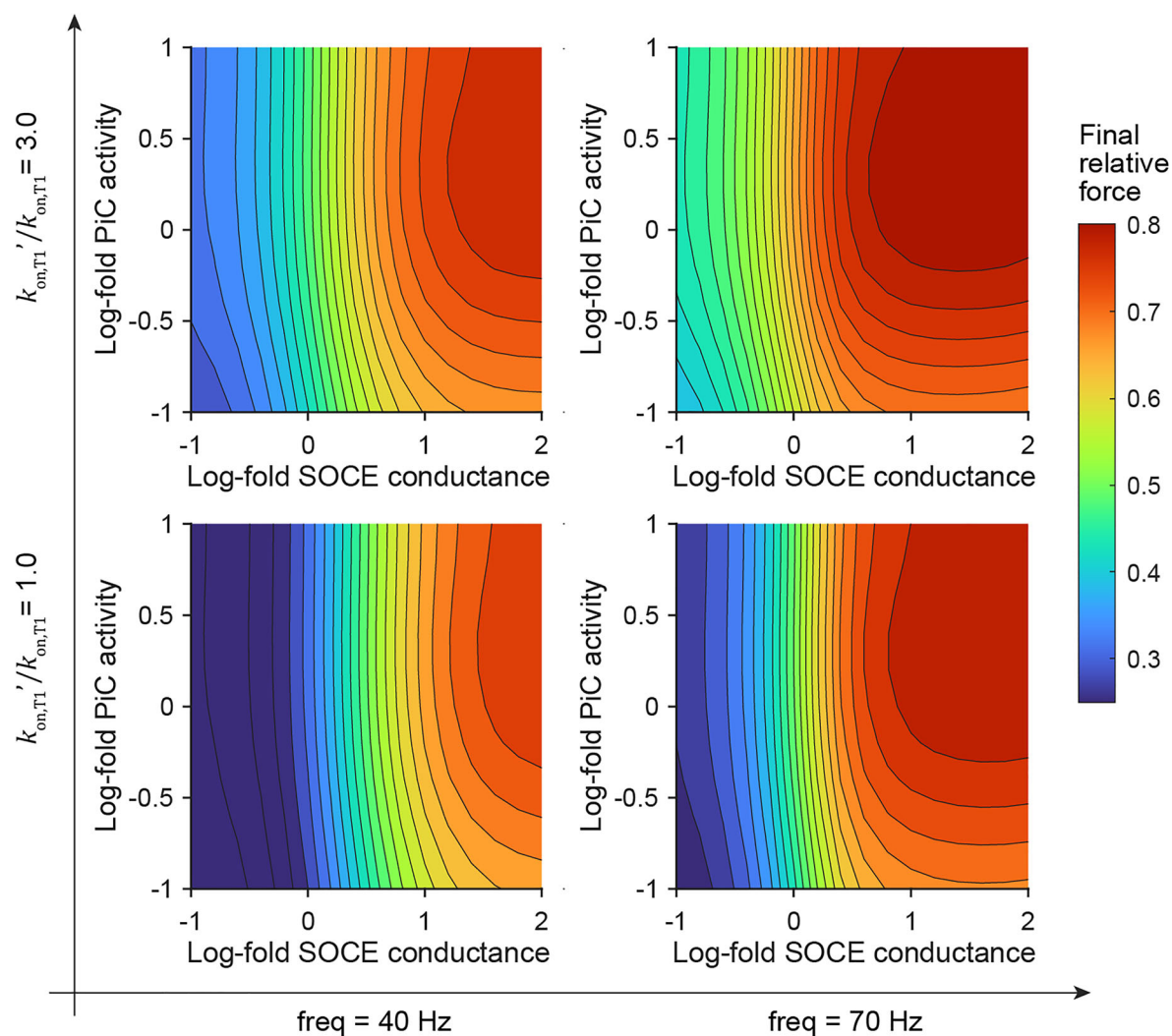


Figure A7. Effect of store-operated Ca^{2+} entry (SOCE) on force during resistance exercise, tested over different parameter regimes

Force as a function of Orai1 conductance and PiC activity in the final repetition of a 60 s resistance exercise simulation for stimulus frequencies of 40 Hz (left) or 70 Hz (right) and for troponin Ca^{2+} sensitivity at 100% (lower) or 300% (upper) of the estimated value.

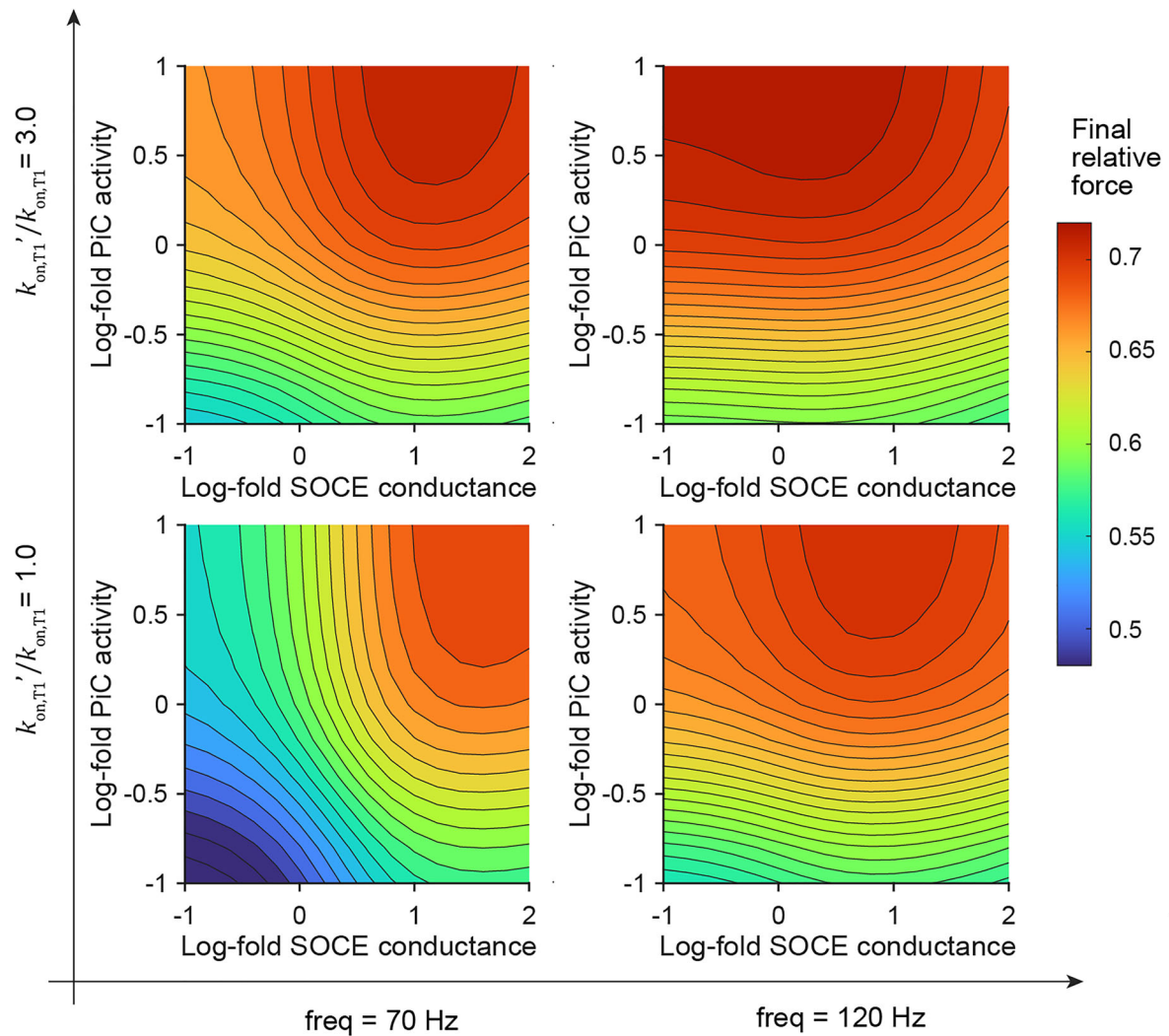


Figure A8. Effect of store-operated Ca^{2+} entry (SOCE) on force during high-intensity interval training (HIIT), tested over different parameter regimes

Force as a function of Orai1 conductance and PiC activity in the final stride of a 20 s HIIT simulation for stimulus frequencies of 70 Hz (left) or 120 Hz (right) and for troponin Ca^{2+} sensitivity at 100% (lower) or 300% (upper) of the estimated value.

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Additional information

Data availability statement

Our code, with all data necessary to reproduce figures in this manuscript, is freely available on Github and is deposited on Zenodo (Kumar et al., 2026).

Competing interests

P.R. is a consultant for Simula Research Laboratory in Oslo, Norway, and receives income. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict-of-interest policies.

Author contributions

E.A.F.: Conceptualization, funding acquisition, supervision, data curation, formal analysis, investigation, methodology, software, visualization, writing original draft, review and editing; J.H.: conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, review and editing; A.K.: conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, writing original draft, review and editing; P.R.: Conceptualization, funding acquisition, resources, project administration, supervision, review and editing.

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Generative AI statement

Claude (Sonnet 5) was used to check the manuscript for mathematical consistency.

Keywords

calcium release, excitation-contraction coupling, exercise physiology, mitochondria, phosphate transport, skeletal muscle, store-operated calcium entry, systems biophysics

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History