Systems modeling reveals that store-operated calcium entry modulates force and fatigue during exercise

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Abstract

The dynamics of calcium ions (Ca^{2+}) in skeletal muscles link electrochemical activation and contractile force generation. An improved quantitative understanding of the mechanisms by which Ca^{2+} dynamics modulate force production is crucial for optimizing muscle performance. Recent experimental data suggest that store-operated Ca^{2+} entry (SOCE), the process of extracellular Ca^{2+} influx upon depletion of Ca^{2+} from the sarcoplasmic reticulum (SR), helps delay the onset of muscle fatigue. However, the mechanistic links between SOCE and sustained force generation in muscle remain unclear. We hypothesize that SOCE regulates force generation during sustained muscle activity by allowing for increased Ca^{2+} release from the SR. We test this hypothesis with a quantitative biophysical model that simulates the biochemical events of muscle contraction, from initial depolarization at the sarcolemma and 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. We estimate the free parameters in the model by fitting them to experiments that measured sarcolemma membrane voltage and myoplasmic Ca^{2+} transients in single muscle fibers in vitro. We then test the effects of SOCE inhibition on Ca^{2+} dynamics and force production and find that the magnitude of myoplasmic Ca^{2+} and force are lower than in wild-type cells over repetitive stimuli. Finally, we predict the effects of varying the degree of SOCE inhibition during patterns of stimulus chosen to mimic those observed during resistance exercise or high-intensity interval training. These simulations predict a context-dependent relationship between force generation and SOCE, wherein increased SOCE is associated with greater force production during resistance exercise, but worsens the effects of fatigue in certain cases of high-intensity training.

Introduction

Skeletal muscle fibers (myofibers) are responsible for generating force and supporting locomotion of the animal body. They are positioned at the mesoscale of the muscle hierarchy, between whole muscle composed of many fiber bundles (fascicles) and subcellular myofibrils consisting of aggregated thin and thick filaments [1, 2]. Contraction is initiated at the myofiber level – each fiber is stimulated by a motor neuron at the neuromuscular junction (NMJ). Understanding how myofibers integrate electrochemical inputs to initiate and sustain contraction is essential to dissecting muscle performance in different contexts, from peak performance to rapid fatigue.

The evolution of contractile force in a myofiber 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 myofiber membrane (sarcolemma) allowing an influx of positively charged ions [3]. The frequency of ACh release events determines the extent of fiber contraction, an example of the phenomenon known as rate encoding [4, 5]. 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 one mechanism for modulating muscle force during different activities. Consequently, different exercises are characterized by different patterns of stimulus at the NMJ; for instance, electromyography (EMG) measurements revealed 10-60 Hz signals during resistance exercise [6] versus

60-180 Hz signals during high-intensity interval training (HIIT) [7].

Following stimulation at the NMJ, force production is largely mediated by Ca^{2+} elevations in the myoplasm. Initial depolarization of the sarcolemma leads to the opening of voltage-gated Na^{+} channels, resulting in a full action potential that is conducted along the length of the fiber via the sarcolemma, and radially inwards through specialized membrane invaginations known as transverse tubules (T-tubules). As voltage increases, voltage-gated Ca^{2+} channels such as the dihydropyridine receptor (DHPR) are activated, which allosterically couple to 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 myofiber contraction [8]. The rate of this contraction differs between fibers, broadly classified as fast-twitch (type II) or slow-twitch (type I) according to their protein composition, metabolism, and speed of contraction [1, 9]. Importantly, fast-twitch fibers are also more easily fatigued, in part due to rapid accumulation of phosphate in the myoplasm, reduced Ca^{2+} release from the SR, and reduced myofibrillar Ca^{2+} sensitivity [10–13].

Efficient contraction of muscle fibers relies on rapid transport of Ca^{2+} into and out of the myoplasm. Accordingly, 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. Due to the constant removal of some Ca^{2+} from the cell, SR Ca^{2+} levels are depleted over time in the absence of additional Ca^{2+} entry through the sarcolemma. The phenomenon of store-operated Ca^{2+} entry (SOCE) provides a natural solution to this problem by coupling SR Ca^{2+} depletion to an additional influx through Ca^{2+} channels in the plasma membrane [14, 15]. In short, stromal interaction molecule 1 (STIM1) in the SR membrane acts as a Ca^{2+} sensor, binding SR Ca^{2+} through its luminal domain [16, 17]. Following Ca^{2+} dissociation from STIM1, it oligomerizes and then binds to the Ca^{2+} channel Orai1 in the T-tubules, allowing Ca^{2+} influx into the myoplasm [17, 18].

Although the importance of SOCE was originally appreciated in nonexcitable cells, many recent studies have uncovered its importance in excitable cells such as neurons, cardiomyocytes, and myofibers [19–24]. For instance, during exercise in wild-type mice, STIM1 and Orai1 channels were found to colocalize at the SR-T-tubule junction, functioning as Ca^{2+} entry units (CEUs) [14, 20, 21]. CEUs have been proposed to facilitate rapid recovery of Ca^{2+} within the fiber and delay the onset of fatigue [20, 21]. Indeed, muscles from dominant-negative Orai1 mice (dnOrai1) were more susceptible to fatigue and produced lower contractile force ex vivo [20]. However, other recent data suggests that SOCE might worsen fatigue in some cases; phosphorylation of STIM1 by AMP-activated protein kinase (AMPK) was found to both decrease SOCE activity and prevent fatigue [25].

Here, we seek to disentangle the effects of SOCE on force production in myofibers using a computational model. Such models have been instrumental in characterizing the complex mechanical, biochemical and electrical processes underlying muscle contraction [26–28]. We present a novel multi-compartment Ca^{2+} signaling model to test the hypothesis that SOCE facilitates prolonged force production and helps mitigate fatigue in fast-twitch skeletal muscle fibers. We first fit our model to experimental measurements of myoplasmic Ca^{2+} and sarcolemma voltage in the literature, and then show that our model recapitulates measurements of Ca^{2+} and force in SOCE-deficient myofibers. We use our model to predict the role of SOCE for different patterns of stimuli chosen to mimic high-intensity exercise and resistance training. Our findings reconcile apparent contradictions in the literature, showing that the role of SOCE is context-dependent, often enhancing force production but, in certain cases, accelerating the onset of fatigue.

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, and force generation in fast-twitch myofibers. In this model, multiple excitation-contraction coupling and Ca^{2+} signaling models were adapted to represent the behavior of the different ion channels and buffers [10, 26–28]. Rather than represent muscle signaling dynamics in full spatial detail, we adopted a compartmental approach, differentiating between junctional and bulk regions of each major compartment, including the myoplasm, SR, and extracellular space (Figure 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 fiber, and 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 clarity; for instance, Ca^{2+} concentration in the junctional myoplasm is written as $[Ca^{2+}]_{myo,junc}$.

Our model is divided into 4 modules, including action potential generation (Module 1, Figure 1B), Ca^{2+} influx and release (Module 2, Figure 1C), Ca^{2+} binding events and cross-bridge cycling (Module 3, Figure 1D), and Ca^{2+} removal and SOCE (Module 4, Figure 1E). The complete model consists of a system of 51 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 more detailed description provided in Methods and Supplementary Information (Tables S3 to S7).

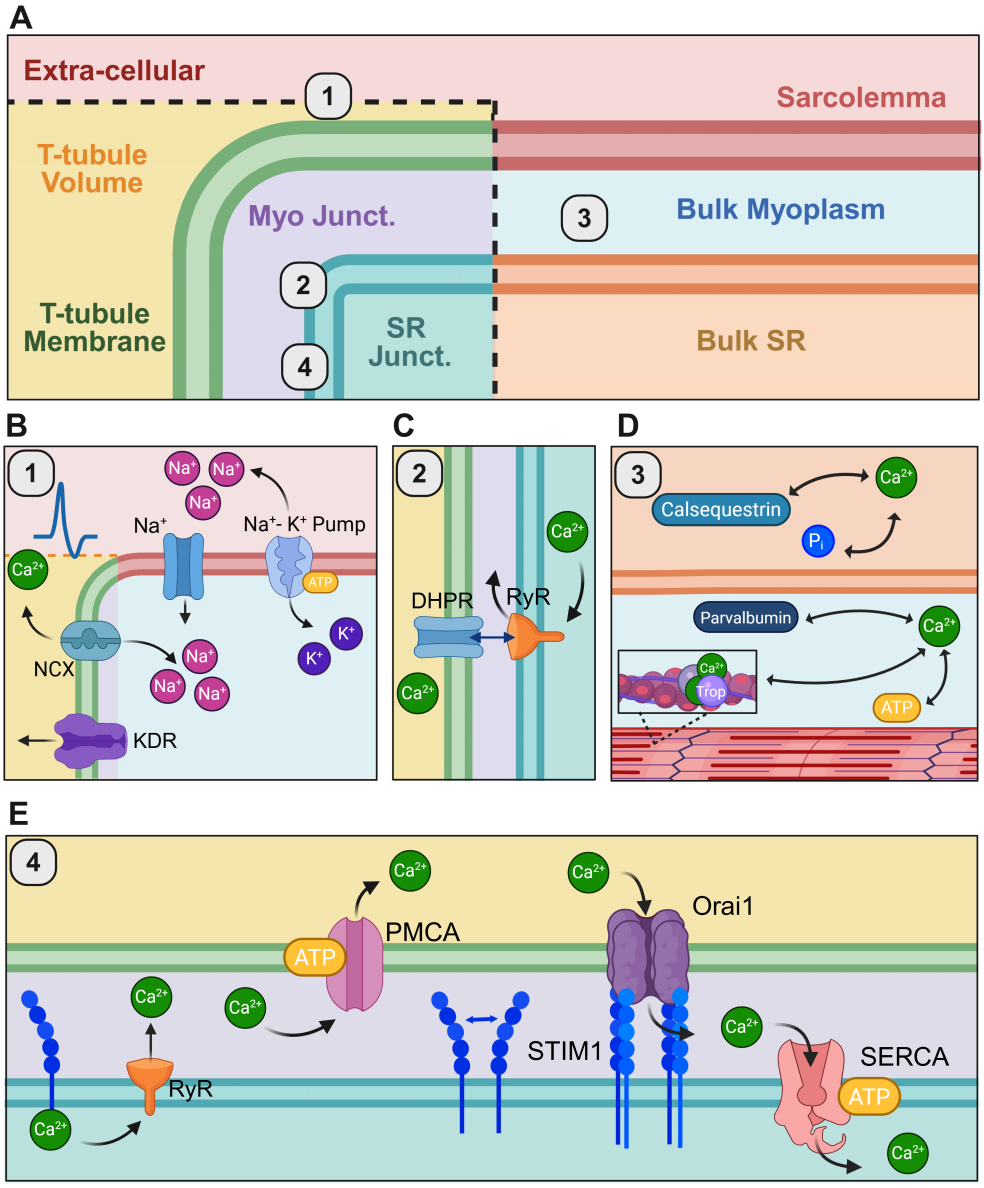

Figure 1: Schematic for model components of myofiber Ca^{2+} signaling. A) A subregion of the cross-section of the muscle fiber is divided into compartments, each labeled with different colors. Ions freely diffuse between bulk and junctional regions of each compartment, separated by black dashed lines here. B-D) The sequence of signaling events directly correlated to force generation are grouped into four 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 DHPR-RyR interactions, triggering Ca^{2+} release from the SR (C). In module 3, Ca^{2+} binds to proteins and induces the cross-bridge cycle, producing contractile force (D). In module 4, Ca^{2+} unbinds from STIM1 during SR depletion, causing STIM1 to oligomerize and form complexes with Orai1, allowing Ca^{2+} influx from the extracellular space into the myoplasm (E). Figure created in BioRender.

Module 1: Action potential generation

Action potentials were modeled using a Hodgkin-Huxley-type formulation, as in previous models developed for mammalian skeletal muscle [10, 26, 27, 29] (Figure 1B). Briefly, 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 [26]. We also included currents through the K^+ inward rectifier (I_{KIR}), which helps to maintain the resting potential of the membrane [30], and the Na^+-K^+ pump ($I_{Na^+\text{-}K^+}$), which restores gradients in Na^+ and K^+ across the sarcolemma [26]. 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:

$$\begin{aligned} I_{\text{ionic,TT}} = \; & 0.1 I_{\text{Na}^+,\text{TT}} + 0.1 I_{\text{Na}^+\text{-K}^+,\text{TT}} + I_{\text{NCX,TT}} + \\ & 0.45 I_{\text{KDR,TT}} + I_{\text{KIR,TT}} + 0.1 I_{\text{Cl}^-,\text{TT}} + I_{\text{Ca}^{2+},\text{TT}}, \end{aligned} \tag{1}$$

$$\begin{aligned} I_{\text{ionic,SL}} = \; & I_{\text{Na}^+,\text{SL}} + I_{\text{Na}^+\text{-K}^+,\text{SL}} + I_{\text{NCX,SL}} + I_{\text{KDR,SL}} + \\ & I_{\text{KIR,SL}} + I_{\text{Cl}^-,\text{SL}} + I_{\text{Ca}^{2+},\text{SL}}, \end{aligned} \tag{2}$$

where $I_{Ca^{2+},TT}$ and $I_{Ca^{2+},SL}$ are additional Ca^{2+} currents defined in Module 4. Full expressions for each current are given in Table S5 and their relative contribution at T-tubules vs. sarcolemma are summarized in Table S3. Balancing these ionic currents with a capacitive current at the sarcolemma and accounting for conduction between the sarcolemma and T-tubules yields:

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

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

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 modeled as square waves with a pulse width of 1 ms. $I_{SL\text{-}TT}$ is the current from the sarcolemma into the T-tubule membrane, calculated as in [26]:

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

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 DHPR, acts as a voltage sensor in the T-tubule membrane, allosterically interacting with the ryanodine receptor (RyR) in the SR membrane and leading to its opening. 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 [31].

The resulting flux through the RyR is given by:

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

where $\nu_{max,RyR}$ is the maximum permeability of the RyR and $P_{O,RyR}$ is the open probability of the RyR governed by Ca^{2+} concentration and TT membrane voltage. The voltage dependence of $P_{O,RyR}$ is dictated by a 10-state Monod, Wyman, and Changeux (MWC)-type model (as in [32]) and the Ca^{2+} dependence captures slow deactivation, as introduced by Senneff and Lowery [27] (see expressions in Table S5).

Module 3: Ca^{2+} binding

Many proteins in the myoplasm and SR act as Ca^{2+} buffers, such that the total Ca^{2+} in either compartment is much higher than the free Ca^{2+} [33–35]. 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 [10, 27, 36, 37] In the SR lumen, we accounted for Ca^{2+} binding to calsequestrin [10, 27, 28] and Ca^{2+} precipitation with inorganic phosphate [10] (see below).

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 [1, 8, 10]. We modeled this using a six-state cross-bridge model, modified from the eight-state model in Shorten et al [10] as detailed in Tables S5 and S6 and depicted in Figure S1. The production of contractile force was assumed to evolve with changes in the density of cross-bridges in a post-power stroke (force-generating) conformation A_{Post}:

$$\frac{d[A_{\text{Post}}]}{dt} = h_0[A_{\text{Pre}}] - g_0[A_{\text{Post}}][\text{ATP}]_{\text{myo,bulk}} - h_p[A_{\text{Post}}][P_i]_{\text{myo,bulk}}, \tag{7}$$

where 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 and inorganic phosphate according to mass action, an effect neglected in some previous models [10, 27]. The dependence of power stroke reversal on phosphate has been suggested as a particularly important regulator of fatigue [12].

We tracked the consumption of ATP and release of phosphate due to cross-bridge cycling and activity of ion pumps and other intracellular processes. To avoid depletion of ATP at steady-state, we introduced an empirical ATP production term (Table S5). Phosphate also moves into the SR and conditionally forms a Ca^{2+}-phosphate precipitate in line with previous work [10, 38, 39]. This reduces the SR Ca^{2+} available for rapid release, providing an additional mechanism for phosphate-mediated fatigue.

Module 4: 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 S5). 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 Equations (1) and (2)).

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 [17, 22, 40, 41] 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 [16]. Upon depletion of SR Ca^{2+}, Ca^{2+} unbinding leads to STIM1 oligomerization and binding to tetrameric Orai1 channels at SR-T-tubule junctions [17], leading to Ca^{2+} influx through Orai1. Previous experimental measurements [16, 18] 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 [22, 24, 41]. Accordingly, we modeled the steady-state open probability of Orai1 channels, $P_{\text{O,Orai1},\infty}$, as:

$$P_{\text{O,Orai1},\infty} = \frac{1}{1 + \left(\frac{[\text{Ca}^{2+}]_{\text{SR,junc}}}{K_{\text{STIM1}}}\right)^4}, \tag{8}$$

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 oligimerization, diffusion, and binding with Orai1 to form CEUs [17]:

$$\frac{dP_{\text{O,Orai1}}}{dt} = \frac{P_{\text{O,Orai1},\infty} - P_{\text{O,Orai1}}}{\tau_{\text{SOCE}}}. \tag{9}$$

This minimal approach allowed us to represent SOCE without introducing a large number of new free parameters. The Ca^{2+} flux due to SOCE was assumed to be restricted to the junctional region and is given by:

$$J_{\mathrm{SOCE}} = P_{\mathrm{O,Orai1}} g_{\mathrm{Orai1}} \frac{N_{\mathrm{A}}}{2F} (E_{\mathrm{Ca^{2+},junc}} - V_{\mathrm{TT}}), \tag{10}$$

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

$$E_{\mathrm{Ca^{2+},junc}} = \frac{RT}{2F} \ln \left(\frac{[\mathrm{Ca^{2+}}]_{\mathrm{TT}}}{[\mathrm{Ca^{2+}}]_{\mathrm{myo,junc}}} \right), \tag{11}$$

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

The fluxes outlined in Modules 2-4 allowed us to write ODEs for Ca^{2+} in each region of the myoplasm and the SR. Furthermore, we computed the additional Ca^{2+} currents through the T-tubule membrane and bulk sarcolemma introduced in Equations (3) and (4):

$$I_{\mathrm{Ca^{2+},TT}} = \frac{2F}{N_A} \left(J_{\mathrm{SOCE}} + J_{\mathrm{Ca^{2+},TT\ leak}} - J_{\mathrm{PMCA}} \right), \tag{12}$$

$$I_{\mathrm{Ca^{2+},SL}} = \frac{2F}{N_A} \left(J_{\mathrm{Ca^{2+},SL\ leak}} - J_{\mathrm{PMCA}} \right), \tag{13}$$

where $J_{\mathrm{Ca^{2+},TT\ leak}}$ and $J_{\mathrm{Ca^{2+},SL\ leak}}$ 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 $P_{\mathrm{O,Orai1}}$ (T-tubules only) and all cross-bridge related variables (bulk myoplasm only). Furthermore, concentrations of ions were tracked within the T-tubule volume, but extracellular ion concentrations were assumed constant (see Figure 1A for clearly annotated regions).

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

Our model utilizes a unique combination of channels and receptors not combined in previous models; accordingly, while we used initial parameter estimates from the literature, it was necessary to formally estimate best-fit values for each parameter. Excluding geometric parameters, there are 106 free parameters Table S7 in the model. We first conducted a global Morris sensitivity analysis to identify the most influential parameters in the model [45]. 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:

$$[\mathrm{Ca^{2+}}]_{\mathrm{avg}} = \frac{[\mathrm{Ca^{2+}}]_{\mathrm{myo,junc}} vol_{\mathrm{myo,junc}} + [\mathrm{Ca^{2+}}]_{\mathrm{myo,bulk}} vol_{\mathrm{myo,bulk}}}{vol_{\mathrm{myo,junc}} + vol_{\mathrm{myo,bulk}}}. \tag{14}$$

Morris sensitivity analysis was used to identify several of the most influential parameters for Ca^{2+} and voltage dynamics (Figure 2A-B, Figure S2). Sarcolemma voltage was most sensitive to parameters associated with the Na^+ channel (V_m, V_h, $V_{S\infty}$; refer to Table S7) and the K^+ delayed rectifier (V_n), in agreement with previous work [10, 26]. Ca^{2+} dynamics were most sensitive to parameters related to Na^+ channel activity (V_m, V_h, Ca^{2+} export through PMCA (g_{PMCA}), and currents through the Na^+-Ca^{2+} exchanger ($k_{\mathrm{sat,NCX}}$).

We proceeded to estimate parameters in two stages, as fully described in Methods. Briefly, we first fit the model to measurements of membrane potential [42], only varying the 28 parameters with greater than 10% relative sensitivity with respect to average and/or peak voltage (Figure S2A-B). Then, starting from these parameters as initial estimates and allowing all other 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 myofibers [41–43]. Overall, the fitting process resulted in a strong match with all the experiments considered (Figure 2C-E).

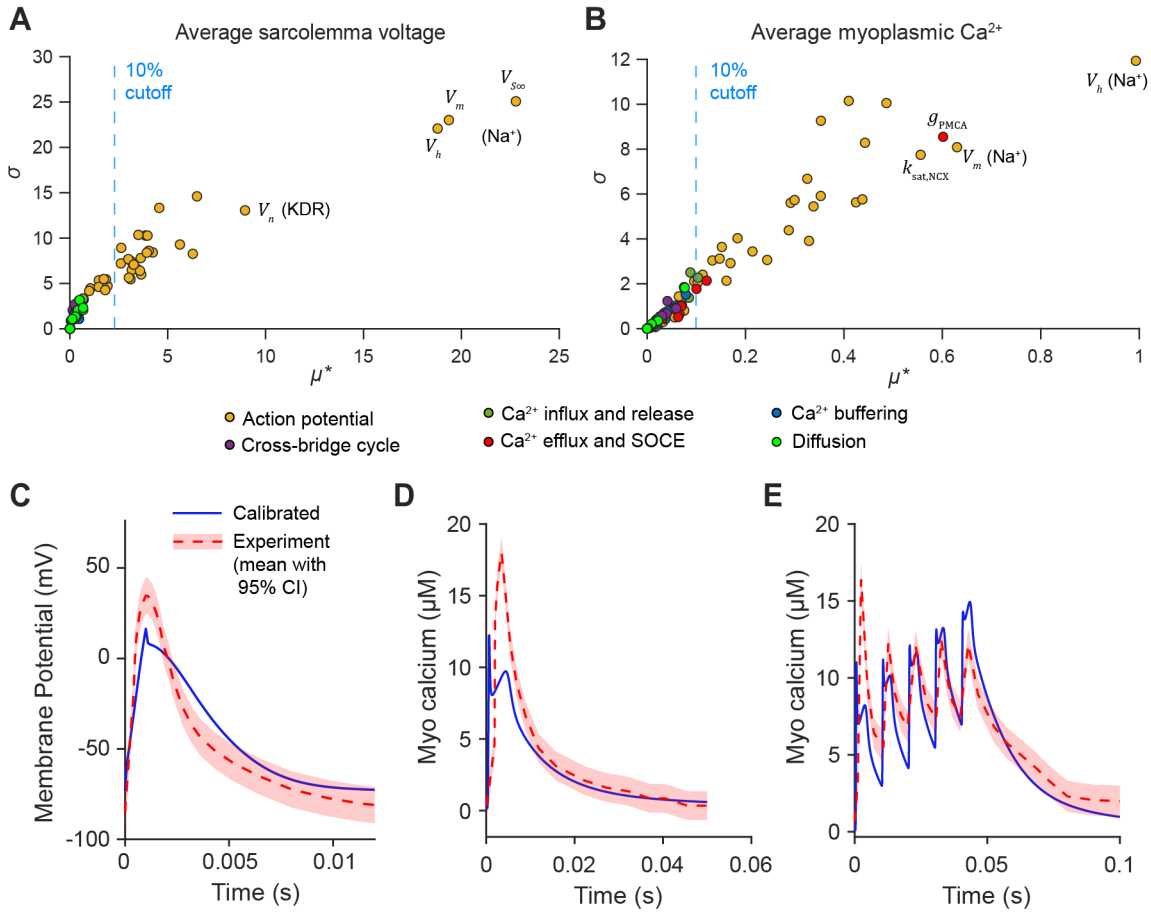

Figure 2: Sensitivity analysis and model calibration. A, B) Results from Morris sensitivity analysis of average sarcolemma voltage (A) and average $[Ca^{2+}]_{myo}$ (B) as quantities of interest (QOIs). The horizontal axis displays the absolute value of the mean, μ^*, and the vertical axis shows the standard deviation, σ, of elementary effects. The parameters with the four highest values of μ^* are labeled individually. C) Results of model calibrated to experimental membrane potential measurements [42] (C) and to $[Ca^{2+}]_{myo}$ dynamics data for a single pulse stimulus (D) [43] or a train of five stimulus pulses at 100 Hz (E) [41]. All experimental data were extracted from source papers using PlotDigitizer [44]; specifically, from Fig 2B (WT curve) in [42], from Fig 2A (fast twitch curve) in [43], and from Fig 1B (IIB curve) in [41]. Conditions were varied according to extracellular buffer and temperature in each experiment. [42]: $[Na^+]_{EC} = 1.18 \times 10^5$ µM, $[K^+]_{EC} = 5.33 \times 10^3$ µM, $[Cl^-]_{EC} = 1.26 \times 10^5$ µM, $[Ca^{2+}]_{EC} = 1.80 \times 10^3$ µM, $T = 295.15$ K; [43]: $[Na^+]_{EC} = 1.50 \times 10^5$ µM, $[K^+]_{EC} = 2.00 \times 10^3$ µM, $[Cl^-]_{EC} = 1.58 \times 10^5$ µM, $[Ca^{2+}]_{EC} = 2.00 \times 10^3$ µM, $T = 293.15$ K; [41]: $[Na^+]_{EC} = 1.38 \times 10^5$ µM, $[K^+]_{EC} = 3.90 \times 10^3$ µM, $[Cl^-]_{EC} = 1.44 \times 10^5$ µM, $[Ca^{2+}]_{EC} = 1.00 \times 10^3$ µM, $T = 295.15$ K.

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

We also dissected these events in terms of their constitutive currents and fluxes (Figure 4). Membrane depolarization leads to Na^+ channel opening in the T-tubules and sarcolemma, followed by opening of the K^+ delayed rectifier during repolarization (Figure 4A). The Na^+-K^+ pump counteracts these fluxes to restore the resting gradients of both ions, while other small currents through the Na^+-Ca^{2+} exchanger and the K^+ inward rectifier play secondary roles (Figure 4A). In parallel, depolarization triggers activation of DHPRs in the T-tubules, which allosterically interact with RyRs in the junctional SR membrane to trigger their opening (Figure 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 (Figure S3). Myoplasmic Ca^{2+} then decreases due to buffering and removal to the extracellular and SR spaces through PMCA and SERCA (Figure 4B). 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.

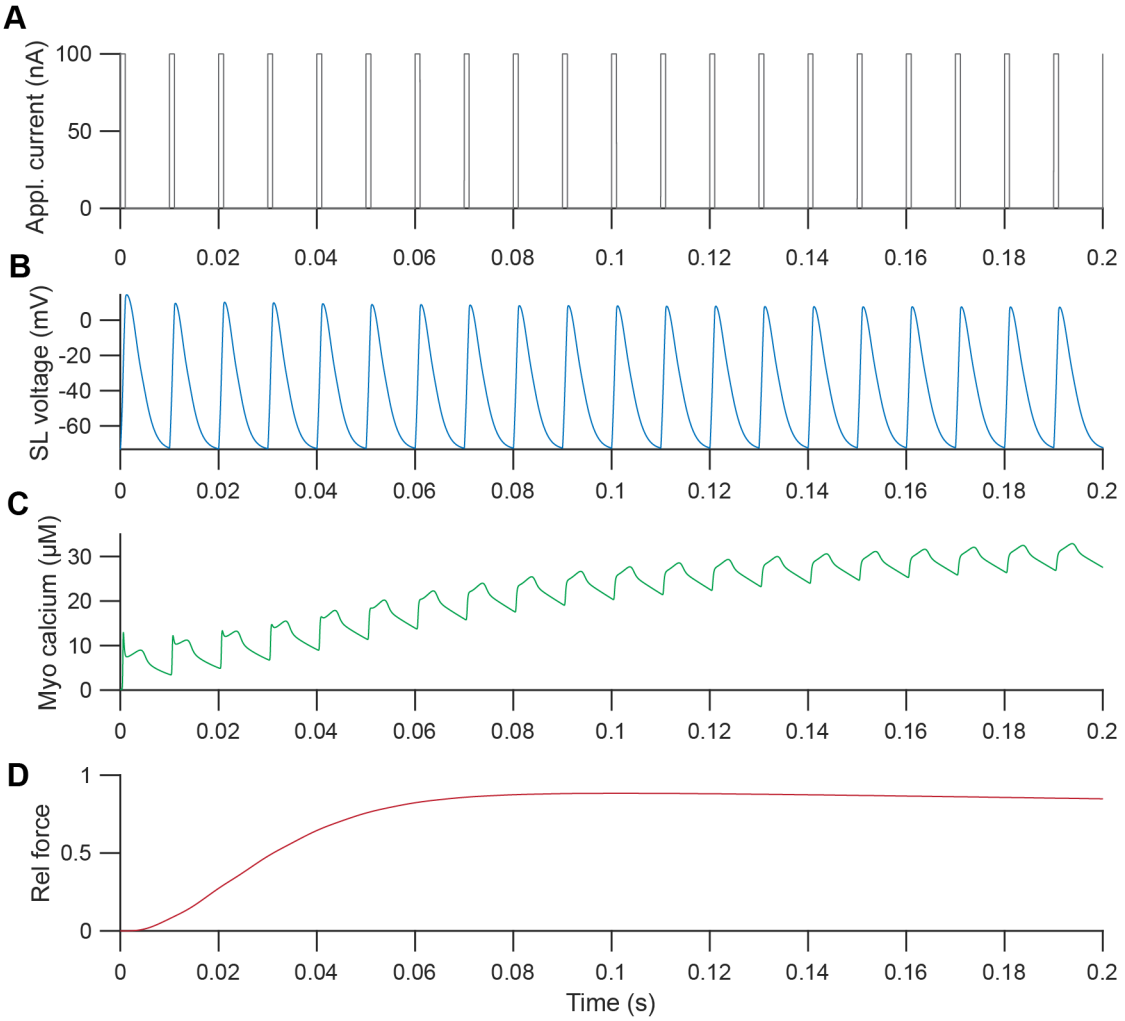

Figure 3: Model output for membrane potential and Ca^{2+} dynamics at 100 Hz stimulus. Input 100 Hz square wave current at the bulk sarcolemma (A) gives rise to action potentials in the sarcolemma (SL) (B) and subsequent increases in myoplasmic Ca^{2+} (C). Binding of Ca^{2+} to troponin leads to cross 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.

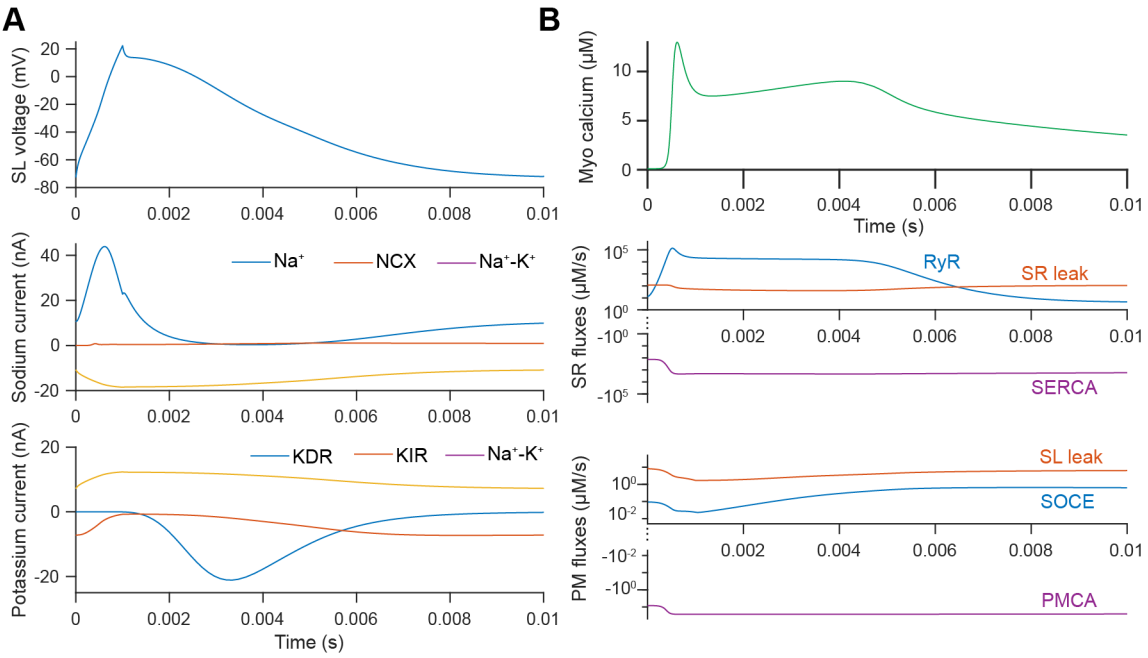

Figure 4: Currents and Ca^{2+} fluxes during the first action potential of a train. A) Sarcolemma depolarization and repolarization (upper) coincide with an inward Na^+ current (middle) and outward K^+ delayed rectifier (KDR) current (lower), respectively. Currents through the Na^+-K^+ pump, Na^+-Ca^{2+} exchanger (NCX), and K^+ inward rectifier (KIR) are also plotted for reference. B) Ca^{2+} transient driven by the initial action potential (upper) is decomposed into fluxes through the SR membrane (middle) and cell membrane (SL and TT; lower). Release through RyR and reuptake into the SR through SERCA are the dominant fluxes.

Although model predictions agree with experimental measurements up to the 100 ms time scale, we found that implausibly high levels of myoplasmic Ca^{2+} (up to 50 μM) were predicted when stimuli continued to 1 s or longer. We attributed this to the simplified, well-mixed assumptions of our model. In reality, additional time is required for Ca^{2+} to diffuse not only from the junctional to bulk myoplasmic regions, but throughout the bulk region itself. We phenomenologically represented this effect by reducing the effective diffusion coefficient of ions between the junctional and bulk regions (D_{ion}) to 10% of its original value in all subsequent simulations, which extend to longer time scales.

SOCE facilitates sustained calcium elevations

Having established the agreement between our baseline model and the physiological signaling events in skeletal muscle fibers, we systematically examined the importance of SOCE for Ca^{2+} dynamics and force production. As initial validation of our simplified mathematical treatment of SOCE, we simulated treatment of cells with the SERCA inhibitor, thapsigargin (TG), commonly used to assess SOCE across cell types [17, 18, 25, 46]. In this experiment, 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. Upon reintroducing Ca^{2+} to the media, a large store-operated Ca^{2+} influx occurs. Predictions from our computational version of this experiment agree well with previous measurements in L6 myoblasts [25] – 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 introduced (Figure 5A). 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 (Figure 5A).

We then considered stimulation of a myofiber at 100 Hz with and without SOCE. In this case, SOCE played a significant role in Ca^{2+} dynamics. Increasing Orai1 conductance by a factor of 20 led to a 21.6% increase in peak myoplasmic Ca^{2+} (Figure 5B). This increased Orai1 conductance remains physiologically relevant, as the assembly of CEUs during exercise is expected to significantly enhance Ca^{2+} flux through Orai1 [21]. We also examined the contractile force exerted by fibers with and without SOCE. The predicted dynamics of contractile force were similar in both cases, but we observed a 4.8% increase in the maximum relative force for cells with strong (20×) Orai1 conductance over cells with no SOCE. Furthermore, in both cases, contractile force declined after less than 1 s of

stimulation. This effect was largely mediated by phosphate accumulation in the myoplasm (Figure S4), in line with the established role of phosphate in muscle fatigue [10–13, 47].

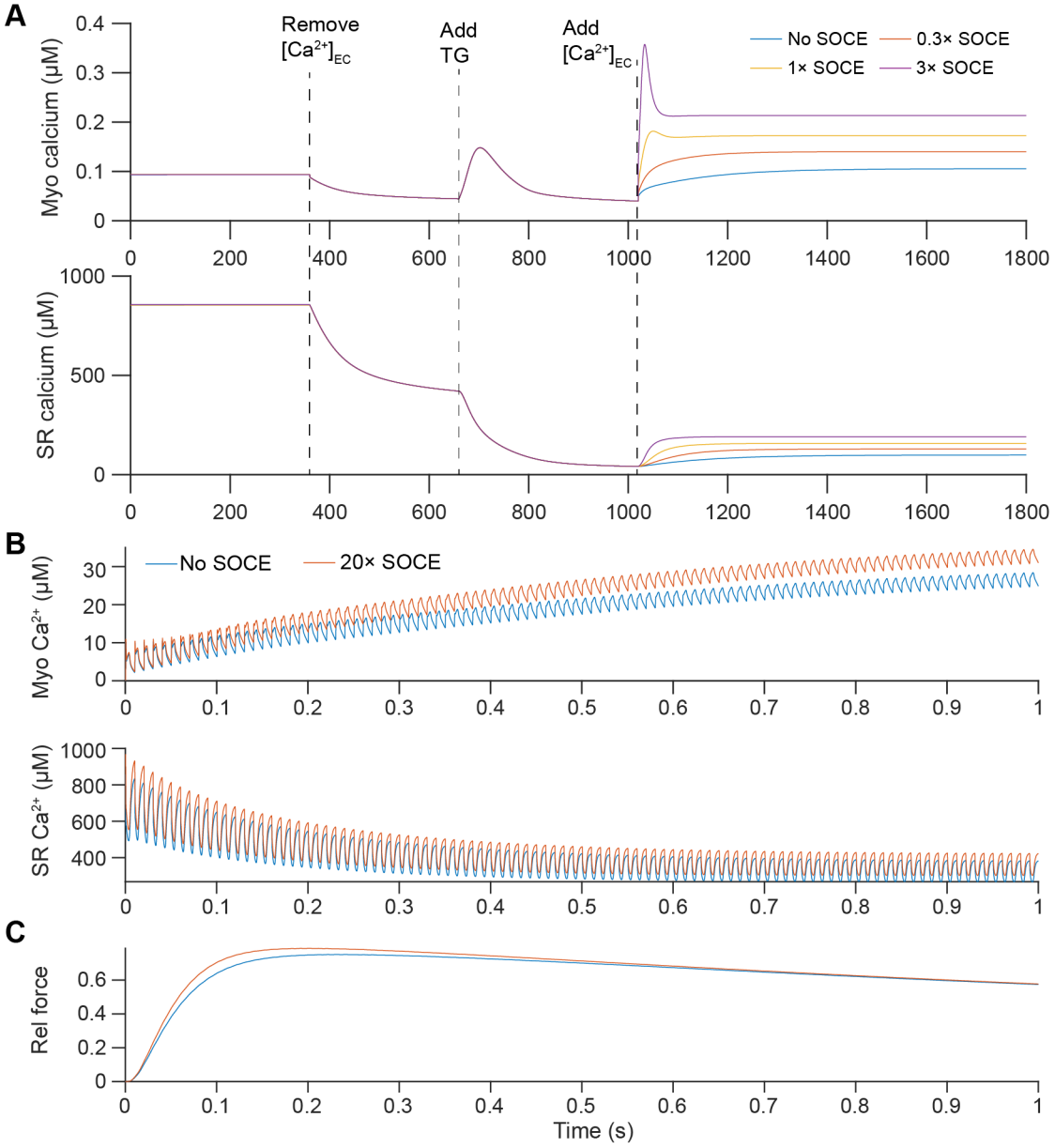

Figure 5: Predicted effects of SOCE in computational experiments. A) Predicted myoplasmic (upper) and SR (lower) Ca^{2+} dynamics in a conventional thapsigargin (TG) assay, modeled after tests in [25]. Extracellular Ca^{2+} is initially 1300 μM, then reduced to 0 μM at $t = 360$ s (e.g., via addition of a Ca^{2+} chelator such as EGTA). Addition of thapsigargin at $t = 660$ s reduces SERCA activity to 10% of its resting activity, inducing Ca^{2+} release from the SR. Extracellular Ca^{2+} (1300 μM) is then reintroduced at $t = 1020$ s, triggering SOCE, with Orai1 conductance varied relative to the estimated value in Table S7. B) Predicted myoplasmic (upper) and SR (lower) Ca^{2+} dynamics in response to 100 Hz stimulus with high SOCE (20 times baseline estimated conductance) vs. no SOCE, $0.1D_{\text{ion}}$. C) Predicted relative contractile force for conditions shown in panel B.

SOCE modulates 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. We next used our model to predict whether this effect depends on stimulus frequency. We considered a case previously tested experimentally, in which dominant-negative Orai1 (dnOrai1) mouse EDL muscle exhibited frequency-dependent inhibition of force generation [20]. As in these experiments, we applied different frequencies of

stimuli consisting of square-wave pulse trains for a total of 0.5 s each.

At low stimulus frequencies (less than 20 Hz), we observed very low force production regardless of SOCE, as tetani remained unfused (Figure 6A). However, SOCE consistently enhanced force production by a small amount at moderate to high stimulus frequencies (Figure 6B-C). Our simulations did not reproduce the fast decline in force over time observed in experiments with dnOrai1 muscle at higher frequencies (greater than 100 Hz), and our simulations generally overestimated contractile force in SOCE knockout fibers at high frequencies (Figure 6C-D). This suggests that additional SOCE-regulated processes not considered in our model may contribute at higher stimulus frequencies. As one possible mechanism, we considered that SOCE might modulate the resting levels of phosphate in the myoplasm, perhaps through regulation of mitochondrial fission and fusion [48] (see Discussion). Upon increasing the resting concentration of myoplasmic phosphate by 50%, we effectively rescued the trend of force vs. frequency measured in experiments (Figure 6C-D).

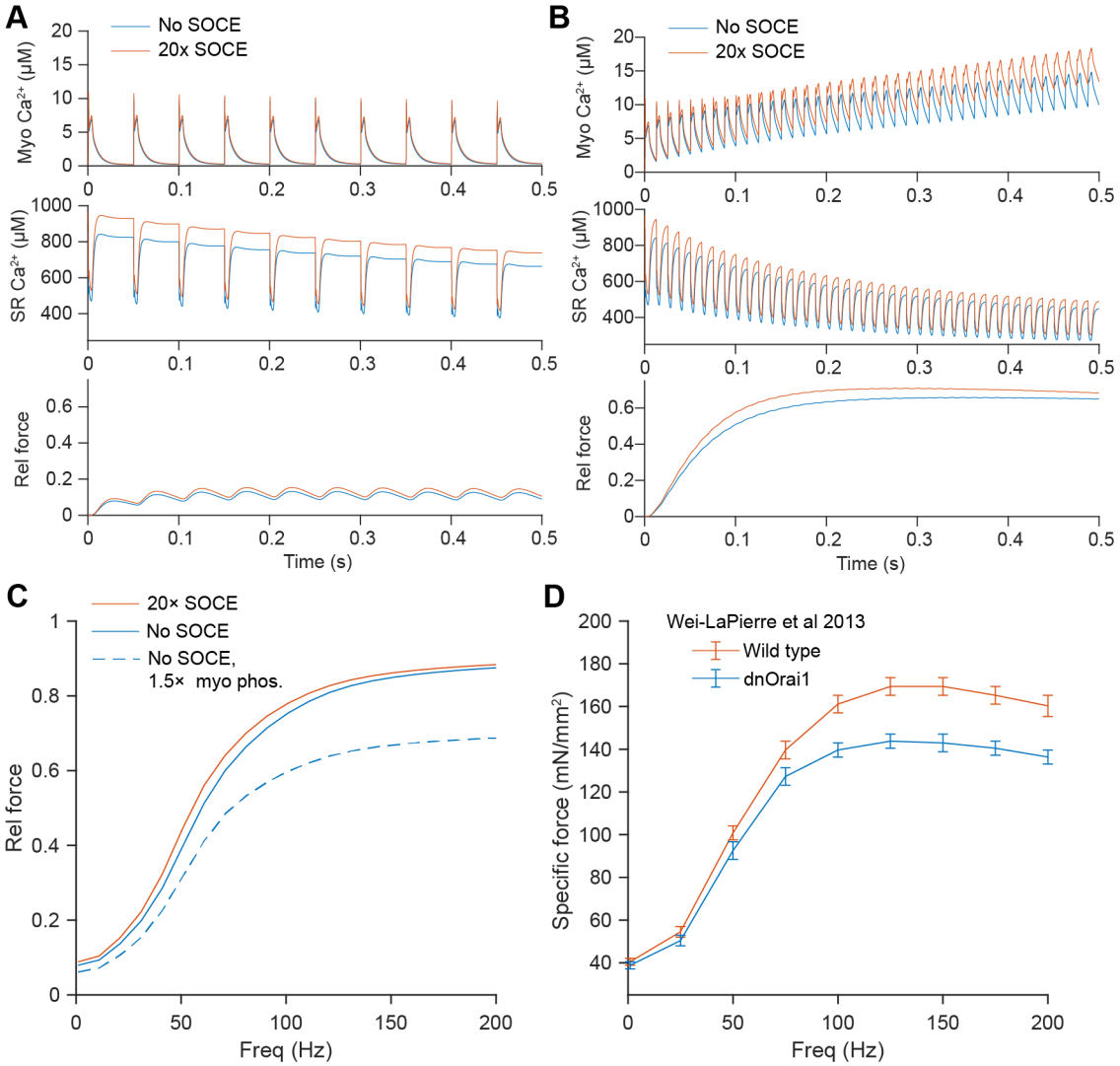

Figure 6: Frequency-dependent force generation with vs. without SOCE. A-B) Predicted effects of SOCE on myoplasmic Ca^{2+}, SR Ca^{2+}, and contractile force are plotted for 20 Hz (A) and 80 Hz (B) stimuli. C) Frequency-dependent maximum contractile force with and without SOCE. Dashed line indicates case without SOCE and with a 50% increase in resting myoplasmic phosphate. D) Experimental measurements of maximum force during in vitro testing of muscle from the EDL of wild type or dominant negative Orai1 (dnOrai) mice [20]. $n = 14$ 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.

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 – from 10-50 Hz for resistance exercise [6] and 60-180 Hz for HIIT [7]. Accordingly, we modeled resistance exercise using 40 Hz stimuli; we further assumed 3 s of contraction followed by 3 s of rest for each repetition, modeled after the isometric contraction measured in the quadriceps in previous work [6]. 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 high abundance of fast twitch fibers (gastrocnemius medialis or the gastrocnemius lateralis) [49], we assumed that stimulus occurs over a 0.1625 s time interval for each 0.65 s stride, with a stimulus frequency of 100 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 potential roles for SOCE in exercise, we varied Orai1 conductance (g_{Orai1}) and STIM1 Ca^{2+} sensitivity (K_{STIM1}) over a wide range of values (0.1 to 100 times baseline for g_{Orai1}, 0.3 to 3 times for K_{STIM1}. Shifts in g_{Orai1} can be attributed changes in SR/T-tubule organization [21], transport of STIM1 [50, 51], or Orai receptor turnover [52]. On the other hand, changes in K_{STIM1} may be associated with biochemical modifications that alter STIM1-Orai1 binding, STIM1 autoinhibition, or STIM1-Ca^{2+} binding. For instance, phosphorylation of STIM1 by AMPK has been shown to bias STIM1 towards a folded, autoinhibited conformation [25]. This is expected to decrease K_{STIM1}; that is, phosphorylated STIM1 requires further depletion of SR Ca^{2+} prior to binding to Orai1 and inducing Ca^{2+} influx. In parallel with these SOCE-related parameters, we varied the troponin-Ca^{2+} binding rate ($k_{\mathrm{on,T1}}$) and phosphate degradation rate ($k_{\mathrm{phos,deg}}$) to dissect how the effects of SOCE depend on other important factors in the model (Figures S5 and S6).

We found that force production was enhanced by elevating Orai1 conductance during resistance exercise (Figure 7A-B). Mechanistically, these higher forces were attributed to a significantly higher fraction of troponin binding to Ca^{2+} in the myoplasm, allowing for faster crossbridge cycling despite increased accumulation of myoplasmic phosphate (Figure 7A). This effect was tuned by STIM1 Ca^{2+} sensitivity and Orai1 conductance, with the largest effects on force observed upon increasing both parameters (Figure 7B). Phosphate degradation rate mainly determined the extent of force reduction (fatigue) during the simulation, whereas troponin Ca^{2+} sensitivity dictated the relative increase in force due to SOCE (Figure S5).

Surprisingly, in certain cases increased SOCE reduced the force produced at the end of a 20-second HIIT stimulus (Figure 7C-D). Specifically, when troponin-Ca^{2+} binding rate was high, elevated SOCE accelerated the onset of fatigue (Figure S6). At this higher stimulus frequency compared to resistance exercise (100 Hz vs. 40 Hz), myoplasmic Ca^{2+} and the resulting fraction of troponin bound to Ca^{2+} was high regardless of SOCE (Figure 7C). Therefore, increased accumulation of myoplasmic phosphate for high SOCE (high conductance or high K_{STIM1}) was detrimental, resulting in lower contractile force compared to low SOCE by the end of the simulation (Figure 7C-D). Thus, while increased SOCE often improves force output, very high rates of Ca^{2+} entry can worsen fatigue in some cases, revealing a nuanced, context-dependent role for SOCE 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. While many recent experimental studies had suggested a central role of SOCE in enhancing force and mitigating fatigue [20, 21], 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+} handling, constrains model parameters to experimental data, and generates testable 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 (Figures 5 and 6). However, in contrast to our initial expectations, we found that this relationship was not consistent across all parameter regimes, which helps resolve an apparent contradiction in the current literature regarding SOCE in skeletal muscle. Multiple studies have measured reduced force and increased susceptibility to fatigue in SOCE-deficient mice [20, 53]. In contrast, recent data suggests that SOCE deficiency

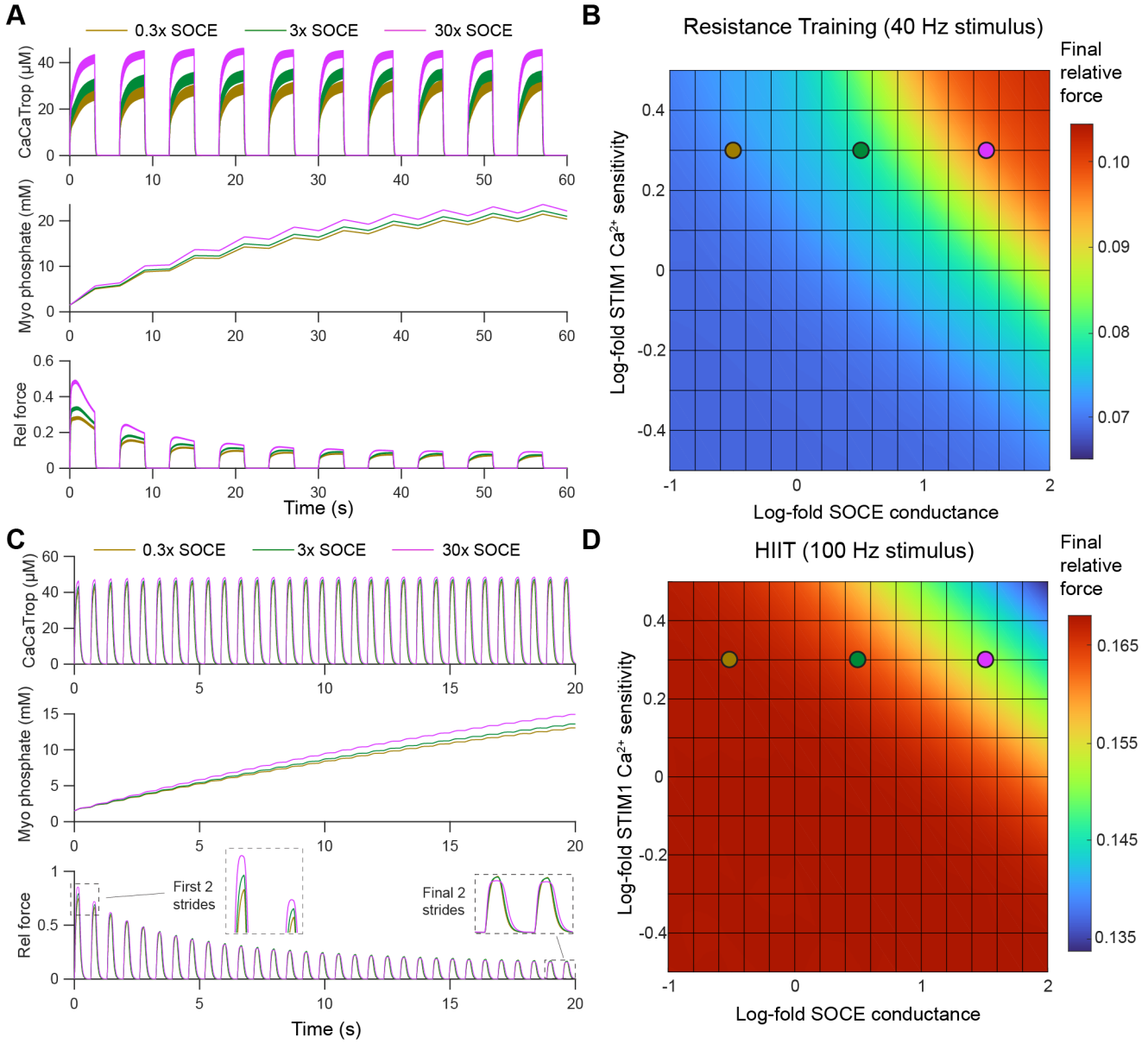

Figure 7: Predicted effects of SOCE in different exercise regimes. A) Predicted dynamics of [Trop-2Ca^{2+}], myoplasmic phosphate, and force over time during patterns of 40 Hz stimuli designed to mimic vastus lateralis activity during resistance training [6]. B) Phase diagram summarizing the maximum contractile force during the final repetition of a 10-repetition resistance training set as a function of K_{STIM1}) and 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 [49]. D) Phase diagram summarizing the maximum contractile force during the final stride of a 20 s running interval as a function of STIM1 Ca^{2+} sensitivity (K_{STIM1}) and Orai1 conductance (g_{Orai1}). Tests in panels A and C are conducted for low (0.3×), moderate (3×), and high (30×) Orai1 conductance at $2\times K_{\mathrm{STIM1}}$, as indicated by points on the phase diagrams in panels B and D. All tests shown here used baseline estimated values for $k_{\mathrm{on,T1}}$ and $k_{\mathrm{phos,deg}}$, with $0.1D_{\mathrm{ion}}$.

might actually mitigate fatigue in certain cases [25]. 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 or higher rates of phosphate clearance, we predict that SOCE enhances Ca^{2+} levels, leading to increased contractile force. However, at high-frequency stimulus and slow phosphate clearance, increased SOCE is associated with higher rates of ATP hydrolysis, leading to 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 [25]. These shifts may also be attributable to organismal differences since some studies consider mice [20, 53], while Nelson et al examine fatigue in Drosophila [25].

While SOCE plays obvious roles in Ca^{2+} handling, it also regulates other intracellular processes. For instance, SOCE has been shown to regulate the fission and fusion dynamics of mitochondria [48]. Additionally, 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 [54, 55]. The multiplicity of roles for SOCE might help explain the disagreement between experimental measurements of force vs. frequency in dnOrai1 cells and predictions of our model Figure 6. For instance, changes in the mitochondrial network associated with SOCE-related mutations [56] might impact the rates of phosphate and ATP cycling in our model as well as Ca^{2+} uptake in the myoplasm. As shown in Figure 6, a 50% increase in resting myoplasmic phosphate would account for the difference between model predictions and experimental measurements.

While 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 may be explored in future models. However, detailed models introduce many free parameters, requiring additional experimental data for robust model calibration [57, 58]. Additionally, all of our parameters are currently estimated using data collected in single mouse myofibers at room temperature [37, 41, 42]; new datasets are needed to reliably calibrate models of human myofibers at physiological temperatures. Finally, future work is required to elucidate the spatial features of SOCE in skeletal muscle. Experimental data show that myofibers undergo dramatic structural reorganization following exercise, including changes in the T-tubule system and assembly of CEUs [21, 59] While our model differentiates between junctional and bulk regions within the myofiber, spatial modeling approaches using software packages such as VCell [60] or SMART [61] are required to properly explore this aspect of Ca^{2+} signaling in skeletal muscle. Such models provide a natural opportunity to include compartmentalization of Ca^{2+} into additional organelles such as mitochondria, which serve as a Ca^{2+} sink and are themselves regulated by Ca^{2+} [62, 63]. 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 [64].

Despite these limitations, our findings may have implications beyond skeletal muscle, including neurons and cardiomyocytes [19, 22, 23, 65], as well as nonexcitable cells such as immune cells [66, 67]. For example, the frequency-dependent role of SOCE explored here may naturally extend to postsynaptic signaling in neuronal dendritic spines [19, 68]. 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

Numerical implementation

The full model is described in Supplementary Information (Tables S1 to S7) and consists of 51 nonlinear ordinary differential equations. Initial conditions were estimated starting from default values of each variable (Table S4) and integrating the equations with no input current to $t = 1000$ s in MATLAB using the stiff ODE solver, ode15s. The initial values were then either defined by the values at $t = 1000$ s or the values when the following condition was met:

$$\frac{dy_i}{dt}\frac{1}{y_{i,\text{norm}}} < 10^{-5} \text{ for } i \text{ from 1 to 51}, \tag{15}$$

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 S4.

In all cases, the steady-state solution with no SOCE ($g_{\text{Orai1}} = 0$) was computed before defining K_{STIM1} as a fraction of the resting SR Ca^{2+} concentration without SOCE (Table S7). This strategy 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 Orai1 conductance ($g_{\text{Orai1}} > 0$) 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 myoplasmic phosphate and SR phosphate, which were fixed to estimated parameter values (Table S7), and SR Ca^{2+}-phosphate precipitate, which was always assumed to start at 0 μM. MATLAB code from this study is freely available on Github and through Zenodo [69].

Sensitivity Analysis

Morris sensitivity analysis was conducted using the UQLab framework in MATLAB [70]. As described in the main text, maximum and average values of myoplasmic Ca^{2+} and sarcolemma voltage were used as quantities of interest (QOIs). All parameters were varied according to a uniform distribution, from 0.5 to 2 times the reference value from literature (Table S7) Morris analysis yielded the absolute value of the mean (μ^*) and the standard deviation (σ) of the elementary effects. A parameter was treated as sensitive if μ^* was greater than 10% of the maximum μ^* over all parameters for that QOI.

Parameter Estimation

To ensure good fits to both sarcolemma voltage and myoplasmic Ca^{2+}, we conducted parameter estimation in two stages, first calibrating the model to match physiological action potentials and then further constraining parameters to simultaneously recapitulate myoplasmic Ca^{2+} and sarcolemma voltage over time. In the first step, the objective function was defined as the normalized sum of square errors between experimentally measured voltage and model predictions for the data from [42]:

$$\mathcal{F}_V = \frac{1}{N}\sum_{j=1}^{N}\left(\frac{V_{\mathrm{SL,model}}(t_j) - V_{\mathrm{SL,exp}}(t_j)}{\sigma_V}\right)^2, \tag{16}$$

where $V_{\mathrm{SL,model}}(t_j)$ and $V_{\mathrm{SL,exp}}(t_j)$ 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 standard deviation of the measurement error (fixed at 5 mV). For this step, only parameters with greater than 10% of the maximum sensitivity index for average or peak voltage (35 parameters total, Figure 2A) were included in the fitting. Fitting was conducted using particle swarm optimization in MATLAB, with a swarm size of 30 particles and maximum stall count of 25.

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

$$\mathcal{F}_{\mathrm{Ca^{2+}}} = \sum_{i=1}^{2}\left[\mathcal{F}_{i,\mathrm{Ca^{2+}}\infty} + \frac{1}{N_i}\sum_{j=1}^{N_i}\left(\frac{[\mathrm{Ca^{2+}}]_{\mathrm{avg,model},i}(t_j) - [\mathrm{Ca^{2+}}]_{\mathrm{avg,exp},i}(t_j)}{\sigma_c}\right)^2\right], \tag{17}$$

where $[\mathrm{Ca^{2+}}]_{\mathrm{avg,model},i}(t_j)$ and $[\mathrm{Ca^{2+}}]_{\mathrm{myo,exp},i}(t_j)$ are the predicted (defined in Equation (14)) and experimentally measured spatially averaged myoplasmic Ca^{2+} concentration at time t_j for experiment i and σ_c is the standard deviation of the measurement error (fixed at 0.5 μM). $\mathcal{F}_{i,\mathrm{Ca^{2+}}\infty}$ represents an additional fitting constraint for each experiment that ensures a good match with experimentally characterized myoplasmic and SR Ca^{2+} at steady-state:

$$\mathcal{F}_{i,\mathrm{Ca^{2+}}\infty} = \left(\frac{[\mathrm{Ca^{2+}}]_{i,\mathrm{myo}}(t=0) - 0.1\ \mu\mathrm{M}}{0.1\ \mu\mathrm{M}}\right)^2 + \left(\frac{[\mathrm{Ca^{2+}}]_{\mathrm{SR},i}(t=0) - 500\ \mu\mathrm{M}}{500\ \mu\mathrm{M}}\right)^2 \tag{18}$$

A total objective function was constructed to ensure good fits with both Ca^{2+} and voltage data simultaneously, $\mathcal{F}_V + \mathcal{F}_{\mathrm{Ca^{2+}}}$. All parameters were included in this fitting step, starting with initial guesses given by the previous estimation for applicable parameters and using the same specifications for particle swarm optimization. The final optimized values for each parameter are reported in Table S7.

Acknowledgments

The authors would like to thank Ingvild Devold for her careful review and helpful feedback on our manuscript. We would also like to thank Profs. Samuel Ward, Andrew McCulloch, and Simon Schenk for insightful conversations on skeletal muscle biology and exercise.

Simulation results presented in this paper benefited from the Triton Shared Computing Cluster at the San Diego Supercomputer Center [71]. This work was supported in part by the Wu Tsai Human Performance Alliance at the University of California, San Diego (to P.R.). E.A.F. is supported by the National Science Foundation under grant EEC-2127509 to the American Society for Engineering Education and by the Wu Tsai Human Performance Alliance.

Declaration of 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.

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