Acute Optogenetic Modulation of Cardiac Twitch Dynamics Explored Through Modeling

Yasser Aboelkassem

Institute for Computational Medicine,
Department of Biomedical Engineering,
Johns Hopkins University,
Baltimore, MD 21218
e-mail: yasser@jhu.edu

Stuart G. Campbell

Department of Biomedical Engineering,
Yale University,
New Haven, CT 06511
e-mail: stuart.campbell@yale.edu

Optogenetic approaches allow cellular membrane potentials to be perturbed by light. When applied to muscle cells, mechanical events can be controlled through a process that could be termed “optomechanics.” Besides functioning as an optical on/off switch, we hypothesized that optomechanical control could include the ability to manipulate the strength and duration of contraction events. To explore this possibility, we constructed an electromechanical model of the human ventricular cardiomyocyte while adding a representation of channelrhodopsin-2 (ChR2), a light-activated channel commonly used in optogenetics. Two hybrid stimulus protocols were developed that combined light-based stimuli with traditional electrical current (all-or-none) excitation. The first protocol involved delivery of a subthreshold optical stimulus followed 50–90 ms later by an electrical stimulus. The result was a graded inhibition of peak cellular twitch force in concert with a prolongation of the intracellular Ca^{2+} transient. The second protocol was comprised of an electrical stimulus followed by a long light pulse (250–350 ms) that acted to prolong the cardiac action potential (AP). This created a pulse duration-dependent prolongation of the intracellular Ca^{2+} transient that in turn altered the rate of muscle relaxation without changing peak twitch force. These results illustrate the feasibility of acute, optomechanical manipulation of cardiomyocyte contraction and suggest that this approach could be used to probe the dynamic behavior of the cardiac sarcomere without altering its intrinsic properties. Other experimentally meaningful stimulus protocols could be designed by making use of the optomechanical cardiomyocyte model presented here. [DOI: 10.1115/1.4034655]

1 Introduction

Optogenetic techniques make use of genetically encoded, light-sensitive ion channels to manipulate cellular function with light [1,2]. Optogenetics has made a significant impact in the neuroscience field by enabling complex brain functions to be probed via specific modulation of selected cells within targeted regions of neural tissue [3,4]. More recently, microbial opsins such as the light-gated ion channel channelrhodopsin-2 (ChR2) have been transfected into cardiomyocytes, allowing cardiac muscle contractions to be initiated by pulses of light [5]. Cardiac optogenetics raises numerous interesting possibilities, including optical pacemakers and defibrillation accomplished through precise spatio-temporal application of light [6–8]. Optogenetics in combination with tissue-engineered muscle has also produced light-actuated “biobots” whose movements are directed by optical pulses [9]. The merging of optogenetics with mechanically active cells such as cardiomyocytes could be termed optomechanics.

Although existing efforts have limited the use of optomechanics to binary control (activation or inhibition) of muscle contraction [7,9], we hypothesized that this same approach could also be used to modify the strength and duration of cardiac twitch events using lower intensity light pulses. Such perturbations would be advantageous in that they could be instantaneously and acutely applied, within a single cardiac beat. Acute contractile manipulation in an intact, beating cell is only experimentally feasible at present using a whole-cell patch clamp approach [10]. Although this technique has been used to good effect, it requires direct physical contact between the patch electrode and each cell tested, a process which is labor intensive and may also interfere with contractile motion of the cell. A noncontact, optomechanical approach could be a more tractable tool for studying the dynamic properties of cardiac muscle contraction.

Cardiac muscle contraction is controlled by the concentration of intracellular Ca^{2+} , which is transiently elevated after electrical stimulus through a process known as excitation–contraction coupling [11]. Simultaneous measurement of intracellular Ca^{2+} concentration and contractile force over time therefore yields paired input/output data [12,13] that can be used for quantitative analyses. For instance, models of cardiac myofilament (MF) activation can be optimized to reproduce contraction force in response to a measured Ca^{2+} input, giving insights into the behavior of muscle proteins [14–16]. This technique is also being used to understand the impact of mutant muscle proteins on physiological function [17]. As contraction models become more sophisticated, additional input/output data sets are needed for the purposes of parameter validation and assessment of predictive accuracy. A cardiac specimen electrically paced at a given frequency produces one input/output set unless some type of additional perturbation is applied. The Ca^{2+} transient can be perturbed by changing the pacing interval, altering Ca^{2+} concentration in the bathing medium, or introducing pharmacological agents, but each of these approaches has potential drawbacks. Changes in pacing interval can be easily and acutely applied, but they also impact subsequent beats [18]. The fact that the effects of a single pacing interval change linger into the next beats suggests that this type of perturbation may trigger rapid posttranslational modification of myofilament proteins [19,20]. Extracellular Ca^{2+} or pharmacological agents can be quickly changed, but these maneuvers are technically challenging, and the presence of spatial concentration gradients, particularly in whole-muscle tissue preparations such as trabeculae, could confound results.

Optomechanical manipulation offers an interesting alternative approach to perturb intracellular Ca^{2+} release. The reason is that unlike electrical field stimulus, which is essentially an all-or-nothing event, ion conductance through ChR2 is approximately proportional to the intensity of the light applied [21]. By varying the intensity and timing of a light-based stimulus, the shape of the cell’s action potential (AP) could be altered strategically to impact

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excitation–contraction coupling and Ca^{2+} release. We envision an experimental protocol in which a single cardiomyocyte or tissue preparation expressing ChR2 is electrically paced using field stimulus. Occasionally, the normal stimulus would be modified to include a light pulse that would modify a single beat. Because this perturbation is acutely administered to only a small fraction of the total beats, the system would remain at a dynamic steady state driven by the primary pacing stimulus. The data collected during occasional perturbed beats would expose system responses under a range of different inputs, perhaps without disrupting the phosphorylation status of myofilament proteins. Such carefully controlled manipulations would be extremely useful in cases where cardiomyopathy-linked myofilament protein mutations are being studied, for instance.

Although ChR2 channels should theoretically allow modification of AP morphology and Ca^{2+} release [22], the relationship between light stimulus and muscle contraction involves several complex dynamic systems, and hence the practicality of such an approach is not intuitively clear. For this reason, we have constructed an optomechanical model of a human epicardial ventricular myocyte (Fig. 1). The model was used to evaluate several candidate hybrid stimulus protocols in which a combination of electrical and optical stimuli is applied. The results suggest that acute optogenetic control of cardiac contraction events is feasible. Our efforts contribute to the growing use of computational modeling to understand the behavior of optogenetic cardiac tissue [23].

2 Methods

In order to investigate the effect of using hybrid electric-light stimuli on ChR2-genetically modified human cardiomyocytes, we derived a coupled integrative optogenetics-electromechanical mathematical model. The model is composed of three coupled modules: a light controlled ion channel (ChR2) module [21], an electrical cellular model with enhanced representation of calcium-induced calcium release (CICR) [24], and a myofilament (MF) mechanical component [25]. The cell membrane connects activity of ChR2 with other time- and voltage-dependent ionic currents in the cell. The Ca^{2+} transient produced by CICR then links ChR2

activity to the local cardiac MF dynamics causing muscle to contract. The problem formulation along with the three component mathematical modules is shown schematically in Fig. 1. Details of the mathematical coupling between these three model components are described in Secs. 2.1–2.3.

2.1 Optogenetics Module: Light-Sensitive ChR2 Current.

The ChR2 ionic current (I_{ChR2}) model is based on a Markovian four-state scheme [21,26]. This includes two closed states (C_1 , C_2) and two open states (O_1 , O_2). These states correspond to the light and dark gating functional modes, respectively. The equation that governs I_{ChR2} as a function of the membrane potential V_m can be given as

$$I_{\text{ChR2}} = g_{\text{ChR2}} G(V_m) (O_1 + \gamma O_2) (V_m - E_{\text{ChR2}}) \quad (1)$$

where $g_{\text{ChR2}} = 2$ (ms/cm²) is the maximum conductance, $\gamma = 0.1$ is the conductance ratio between open states (O_2/O_1), and $E_{\text{ChR2}} = 0$ (mV) is the reversal potential. $G(V_m)$ is a voltage-dependent rectification function

$$G(V_m) = \frac{1}{V_m} \left(a_1 - a_2 * \exp\left(\frac{-V_m}{a_3}\right) \right) \quad (2)$$

with $a_1 = 10.6408$, $a_2 = 14.6408$, $a_3 = 42.7671$. These are empirical constants [21] having units of mV. The differential equations that govern the probabilities of light opening–closing modes are given by the following system of equations:

$$\frac{d}{dt} \mathbf{x}_L = \mathbf{A}(t, V_m(O_1, O_2)) \mathbf{x}_L \quad (3)$$

where $\mathbf{x}_L$ is a vector containing all state variables and $\mathbf{A}$ is a matrix of coefficients that control light activation and deactivation transition rates. Full details of these ChR2 model equations, along with a complete list of related parameters, are published elsewhere [21]. All ChR2 parameters used in this study were validated and chosen such that they agree well with published patch-clamp

Fig. 1 Schematic diagram of a human cardiac ventricular myocyte model that includes cellular electrophysiology, myofilament contractile function, and light-activated ion channels. An electrophysiological model representing a human epicardial myocyte [24] was coupled to models of ChR2 [21] and myofilament mechanics [25]. The equations representing each model component were merged into a single system and solved simultaneously to enable simulations of light-activated mechanical events.

recordings [21]. V_m , which appears in each of the Chr2 equations, is determined by the overall membrane dynamics described in the electrophysiology model.

2.2 Electrophysiology Module: Human Left-Ventricular Epicardial Myocyte. The electrophysiology component of the model is the human epicardium cell model developed by Iyer et al. [24]. The Iyer model was selected due to the fact that it represents Ca^{2+} -induced Ca^{2+} release at a level of detail that was sufficiently complex to capture time- and voltage-dependent Ca^{2+} release dynamics, but simple enough to be computationally tractable. The equation that governs the transmembrane potential V_m state variable is the seminal Hodgkin–Huxley equation, which was modified to include the effect of photosensitive ionic current I_{Chr2}

$$\frac{d}{dt}V_m = \frac{-1}{C_m} \left[\sum_{i=1}^N I_{\text{ion},i}(V_m) + I_{\text{stim}}(t) + I_{\text{Chr2}}(O_1, O_2, V_m) \right] \quad (4)$$

where C_m is the membrane capacitance per unit area. Now, one can write the system of equations [24] that governs the whole-cell electrophysiology module in a matrix form as

$$\frac{d}{dt}\mathbf{x}_E = \mathbf{B}(t, O_1(V_m), O_2(V_m))\mathbf{x}_E \quad (5)$$

where $\mathbf{x}_E$ is a vector containing all state variables of the whole-cell electric module including V_m and $[\text{Ca}^{2+}]$ (the intracellular Ca^{2+} concentration). The matrix $\mathbf{B}$ includes coefficients that control electric activation and deactivation of each ionic channels and pumps. In this model, $[\text{Ca}^{2+}](t)$ is described by

$$\frac{d}{dt}\text{Ca}^{2+} = f(t, \text{Ca}^{2+}, V_m, J_{\text{xfer}}, J_{\text{up}}, J_{\text{trpn}}) \quad (6)$$

where J_{xfer} , J_{up} , and J_{trpn} represent intracellular Ca^{2+} fluxes induced by the CICR process. The detailed equations and parameters associated with Ca^{2+} concentration and fluxes are given in Ref. [24].

2.3 Mechanics Module: Cardiac Myofilament Dynamics. With the Chr2 and electrophysiology modes coupled together, Ca^{2+} release becomes dependent on light stimuli. Because Ca^{2+} activates the cardiac thin filament, adding in MF equations to the model ultimately relates light stimuli to muscle contraction. The MF model by Rice et al. [25] is used here to describe the contraction response of a genetically modified cardiomyocyte when

stimulated by engineered (i.e., at specific timing and duty cycles) light and electric pulses. This particular MF model is based on actin–myosin crossbridge cycling and was chosen because it replicates a wide range of experimental data, including the dynamic force and length responses triggered by Ca^{2+} transients. The details of this model can be found in Ref. [25], but they can be compactly represented in a matrix form as

$$\frac{d}{dt}\mathbf{x}_{\text{MF}} = \mathbf{C}(t, \text{Ca}^{2+}(V_m))\mathbf{x}_{\text{MF}} \quad (7)$$

where $\mathbf{x}_{\text{MF}}$ is a vector containing state variables of the cardiac MF module. The entries of the matrix $\mathbf{C}$ represent all the transition rates that control the process of crossbridge actomyosin cycling. The MF model accounts for the sarcomere length changes during contraction–relaxation using an integrodifferential equation. Herein, for convenience and numerical stability, the sarcomere length-dependent equation is rederived using a system of two first-order differential equations as follows.

Recalling the free-body diagram of Rice and co-workers (Fig. 1(c) in Ref. [25]), their model includes both active and passive elements of the sarcomere in addition to viscous damping and inertial effects. Based on the momentum balance along the active direction, the equation that governs the dynamical motion of the sarcomere length can be given as

$$m \frac{d^2}{dt^2} \text{SL} + \mu \frac{d}{dt} \text{SL} = F(t, \text{SL}) \quad (8)$$

where SL is sarcomere length, m is the MF equivalent mass, μ is viscosity, and F is the total force acting on the MF. This includes active, passive, preload, and afterload components. Since Eq. (8) is a second-order and linear ordinary differential equation (ODE), it can be decomposed into two first-order equations that describe the time rate of change of both the sarcomere length and velocity

$$\frac{d}{dt}v = \frac{1}{m}(F(t, \text{SL}) - \mu v) \quad (9)$$

$$\frac{d}{dt}\text{SL} = v \quad (10)$$

In order to clearly show the coupling between the three presented model components (Chr2, electrophysiology, and MF), the above set of Eqs. (3), (5), and (7) can be assembled in a single matrix form as

Fig. 2 Hybrid stimulation protocols involving a combination of light and electrical current. (a) In protocol 1, an initial light pulse is followed by electrical current stimulus. The delay between light initiation and electrical stimulus was set to $t_{\text{SE}1} = 50, 70, \text{ or } 90 \text{ ms}$. The voltage traces in this panel show how the cell responds to each of the stimuli when they are applied separately. (b) In protocol 2, a depolarizing electric current stimulus was followed by light pulses of varying duration, including $t_{\text{DL}} = 250, 300, \text{ or } 350 \text{ ms}$. As in panel A, the voltage traces illustrate responses that occur when the two stimuli are applied on their own.

Fig. 3 Simulation of protocol 1 within a train of normally stimulated beats. A series of five beats was simulated. Beats 1, 2, 4, and 5 were elicited by a 3 ms, 10 mW/mm² light pulse. Protocol 1 was executed only on beat 3 (indicated by the shaded events), consisting of a 3 ms subthreshold optical stimulus (intensity 0.111 mW/mm²) followed 70 ms later by an electrical current pulse. Membrane voltage (a), intracellular Ca²⁺ (b), and relative contractile force (c) are plotted over time.

$$\frac{d}{dt}\mathbf{x} = \mathbf{M}\mathbf{x} \quad (11)$$

where $\mathbf{x} = [\mathbf{x}_L \ \mathbf{x}_E \ \mathbf{x}_{MF}]^T$ is a vector that combines state variables from light, electric, and mechanics modules. Similarly, the matrix $\mathbf{M}$ represents a global coefficient matrix that combines transition rates from each module

$$\mathbf{M} = \begin{bmatrix} \mathbf{A}(t, V_m(O_1, O_2)) & 0 & 0 \\ 0 & \mathbf{B}(t, O_1(V_m), O_2(V_m)) & 0 \\ 0 & 0 & \mathbf{C}(t, \text{Ca}^{2+}(V_m)) \end{bmatrix} \quad (12)$$

It should be noted that the interaction between the abovementioned modules is achieved because the opening (O_1, O_2) ChR2 gating kinetics are explicitly dependent on the membrane potential V_m (connecting light to electrophysiology) and because muscle contraction depends on the Ca²⁺ transient connecting electrophysiology to mechanics. To clearly show these coupling interactions, one can represent the above system in terms of state flow diagram as

$$\text{OM} \xrightleftharpoons[V_m(O_1, O_2)]{O_1(V_m), O_2(V_m)} \text{EPM} \xrightarrow{\text{Ca}^{2+}(V_m)} \text{MM} \quad (13)$$

Fig. 4 Effect of varying the delay between optical and electrical current stimuli under protocol 1. The delay interval used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Membrane voltage (a), intracellular Ca²⁺ (b), and relative contractile force (c) are plotted over time for each condition. Ca²⁺-force phase loops indicate the ability of delay intervals to probe Ca²⁺-contraction dynamics (d). The time course of sarcomere shortening under each delay interval is also shown (e).

where OM, EPM, and MM stand for optogenetics, electrophysiology, and mechanics modules, respectively. Finally, it should be noted that, in the above formulations, the overall system input is light (L) and/or electrical (E) current stimuli. The output is contractile force and sarcomere length. Other outputs, including ionic currents, were computed in order to draw mechanistic insights from simulation results.

2.4 Hybrid Stimulus Protocols. Using the model, we examined two hybrid stimulus protocols, consisting of pulses of light and electrical current, administered as step changes with finite magnitude and duration (Fig. 2). Stimulus parameters were determined through iterative experimentation until desired results were achieved. Protocol 1 began with a light pulse 3 ms in duration, with a magnitude of 0.111 mW/mm^2 . After a delay of t_{SE} ms, an electrical current stimulus 0.5 ms in duration and $-100 \mu\text{A}/\mu\text{F}$ in magnitude was administered. Values of $t_{SE} = 50, 70,$ and 90 ms were examined in simulations. Protocol 2 consisted of an electrical current stimulus 0.5 ms in duration and $-100 \mu\text{A}/\mu\text{F}$ in magnitude at time zero. A light pulse was also added, starting at $t = 0.25 \text{ ms}$ and continuing for a set duration given by the variable t_{DL} . Magnitude of the light pulse in each case was $10/t_{DL}$ such that the integrated light intensity was constant for all values of t_{DL} . Simulations were performed for t_{DL} values of 250, 300, and 350 ms.

3 Results

Preliminary experimentation with the coupled optomechanical model allowed us to establish electrical current stimulus

parameters that elicited a full AP (Fig. 2(b)). We also determined optical pulse parameters that initiated both full APs (Fig. 2(b)) and subthreshold depolarizations (Fig. 2(a)). Using these as basic building blocks, two protocols for optomechanical control of contraction were formulated.

3.1 Protocol 1: Light Stimulus Followed by Electrical Stimulus ($L + E$). In protocol 1, a prepulse of subthreshold light is administered prior to electrical stimulus that ultimately prolongs the membrane action potential, attenuates intracellular Ca^{2+} release, and reduces the peak cellular twitch force. An illustration of this protocol at work among normal, electrically stimulated events is shown in Fig. 3. This series of beats illustrates the ability of protocol 1 to acutely modulate Ca^{2+} release and twitch force with no apparent effect on the following contractions. We further determined that by varying t_{SE} (the interval between the light prepulse and the electrical stimulus) between 50 and 90 ms, the degree of attenuation could be controlled (Fig. 4). In effect, protocol 1 allows the magnitude and duration of intracellular Ca^{2+} to be acutely perturbed (Fig. 4(b)). The fact that this enables meaningful exploration of dynamic Ca^{2+} -contraction coupling space is evident in plots of force- Ca^{2+} loops in Fig. 4(d). Brief light prepulses, when properly timed, move these loops progressively inward. The relevance of this technique to isolated cardiomyocyte measurements is demonstrated in Fig. 4(e), which shows unloaded sarcomere shortening transients. These too are successfully perturbed through the application of protocol 1. In summary, these mixed electromechanical results indicate that protocol 1 has

Fig. 5 Ion currents predicted by the model in response to varying delay intervals under protocol 1. The delay interval used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Note that varying time and current scales are used in each panel to better view the details of each current type. I_{Na} (a), I_{Ca} (b), I_{to1} (c), I_{NaCa} (d), and I_{NaK} (e) are shown.

Fig. 6 Additional ion currents predicted by the model in response to varying delay intervals under protocol 1. The delay interval used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Note that varying time and current scales are used in each panel to better view the details of each current type. I_{Kr} (a), I_{Ks} (b), $I_{Ca,K}$ (c), I_{K1} (d), and I_{ChR2} (e) are shown.

Fig. 7 Simulation of protocol 2 within a train of normally stimulated beats. A series of five beats was simulated: beats 1, 2, 4, and 5 were elicited by a 0.5 ms, $-100 \mu A/\mu F$ pulse; protocol 2 was executed only on beat 3 (indicated by the shaded events), consisting of the same electrical current stimulus followed 0.25 ms later by a 300 ms light pulse with an intensity of 0.033 mW/mm². Membrane voltage (a), intracellular Ca^{2+} (b), and relative contractile force (c) are plotted over time.

a negative inotropic effect on the mechanical response of genetically modified cardiomyocytes. These effects are a result of altered ionic currents, whose time- and voltage-dependencies render them sensitive to induced ChR2 currents.

Examining the ion currents predicted by the model under protocol 1 reveals the mechanism of Ca^{2+} -transient attenuation under these circumstances (Figs. 5 and 6). The ChR2 current triggered by the light pulse is not sufficient to trigger full opening of voltage-gated Na^+ channels (Fig. 5(a)). Instead, the ChR2 current raises the membrane potential to a level that nears (but does not cross) the Na^+ channel opening threshold. As a consequence, I_{Na} experiences a slow, partial opening that decays by the 50 ms time point. When the electrical stimulus occurs, raising membrane potential above the Na^+ channel threshold, the partial pre-activation by ChR2 has rendered these channels somewhat refractory, and subsequently the rapid upstroke of the action potential (phase 0) is attenuated (Fig. 4(a)).

Although there was no immediate Ca^{2+} channel opening, the light pulse does result in slow, partial opening of L-type Ca channels and a small increase in intracellular Ca^{2+} (see trace in Fig. 4(b)). When the delayed electrical current stimulus arrives, it triggers a full AP (Fig. 4(a)). However, the light-based prepulse has conditioned the cell such that the responses differ from a typical AP. This is mainly due to voltage- and time-dependent inactivation of ion channels, which are prematurely initiated by the subthreshold stimulus provided by ChR2 current. For instance, the L-type Ca^{2+} channel ion current I_{Ca} undergoes a subthreshold opening coincident with light stimulus. This event causes reduced

peak current later on when the electrical stimulus is delivered (Fig. 5(b)). These reductions in peak current are significant because they result in attenuated Ca^{2+} release.

The other current that was markedly affected by the light pre-pulse is the transient outward K^+ current, I_{to1} (Fig. 5(c)). The partial premature activation of this current lowers its magnitude more than fourfold by the time electrical stimulus is delivered. The drastic reduction in this current has far-reaching effects, beginning with the absence of a “notch” (phase 1) in the hybrid stimulus action potentials (Fig. 4(a)). Without a full expression of the transient outward K^+ current, the cell achieves a greater degree of depolarization. This ultimately prolongs the action potential plateau, which in turn drives prolongation or delay in the other currents (Figs. 5(d) and 5(e) and 6(a)–6(d)). The ChR2 current initiated by the light pulse (I_{ChR2}) is shown in Fig. 6(e).

Note that the final intracellular Ca^{2+} transients shown in Fig. 4(b) are the temporal summation of two separate release events occurring closely in time. The first is the result of the light stimulus; the second occurs with electrical current stimulus. As t_{SE} is increased, a notch appears (green trace), demarcating the two release events and leading to a further reduction in Ca^{2+} transient amplitude. The increasing delay between the two events also leads to a prolongation of the Ca^{2+} transient and the duration of twitch contraction.

3.2 Second Protocol: Electrical Stimulus Followed by Light Stimulus (E + L). A distinct strategy was employed in the design of protocol 2. In protocol 2, the AP is initiated with a conventional electrical stimulus. During the application of electrical

current, an optical stimulus is also initiated. That second stimulus continues for an extended period (t_{DL}), ranging from 250 to 350 ms. The response of the model to protocol 2 ($t_{\text{DL}} = 300$ ms) is shown in Fig. 7. Protocol 2 is applied to the third beat of the five-beat series, and has the effect of prolonging the AP, Ca^{2+} transient, and twitch force while preserving the overall amplitude of each. As with protocol 1, the simulations indicate that the effects of protocol 2 are acute in nature, with no obvious effects on the following beats. Repeating the simulation of protocol 2 with $t_{\text{DL}} = 250$, 300, and 350 ms reveals that the duration of the light pulse impacts the relaxation of the cell, essentially independent of other effects (Figs. 8(a)–8(c)). These effects are shown clearly in the force- Ca^{2+} loops and cell shortening traces of Figs. 8(d) and 8(e).

An examination of the corresponding membrane currents (Figs. 9 and 10) reveals that protocol 2 works by maintaining ChR2 in an open state throughout phase 2 (plateau) of the AP. During this phase, membrane voltage hovers just above zero, very near the reported reversal potential of ChR2 [21]. The model's other ionic currents are plotted in Figs. 9 and 10 in order to show the full effects of protocol 2. As expected, since the light pulse is administered later in time and because the Na^+ channels have fast opening–closing kinetics, the fast inward Na^+ current (I_{Na}) is insensitive to this protocol (Fig. 9(a)). Although there were no distinct changes in phase 0 or phase 1 of the AP traces, the light pulse does result in a prolongation of phase 2 (Fig. 8(a)). This is mainly due to the fact that the slow inactivation process of the L-type Ca^{2+} channel is influenced by the light pulse duty cycle and its induced ChR2 current. For instance, the late decaying phase of the I_{Ca} traces (Fig. 9(b)) is slowed down as a result of alterations in

Fig. 8 Effect of varying the duration of the light pulse following electrical current stimulus under protocol 2. The light pulse duration used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Membrane voltage (a), intracellular Ca^{2+} (b), and relative contractile force (c) are plotted over time for each condition. Ca^{2+} -force phase loops indicate the ability of the light pulse duration to probe Ca^{2+} -contraction dynamics (d). The time course of sarcomere shortening under each pulse duration is also shown (e).

Ca^{2+} flux imposed by using variable light duty cycles $t_{\text{DL}} = 250, 300, 350$ ms. Interestingly, the Ca^{2+} -induced K^+ current I_{CaK} exhibits a slight change in the decaying phase, when compared with the E -only trace (Fig. 9(c)). Changes in the membrane transporter ion currents, such as I_{NaCa} and I_{NaK} , are shown in Figs. 9(d) and 9(e), respectively. Once again, changes induced by the light pulse occur only in the late decaying phase of these currents.

The components of the outward K^+ currents (I_{Kr} , I_{Ks} , I_{to1} , I_{K1}), in addition to the ChR2 current (I_{ChR2}) induced by using protocol 2, are shown in Figs. 10(a)–10(e), respectively. The changes in these currents, when compared with the E -only case, also contribute to explaining the observed rate of relaxation changes in both Ca^{2+} transients and twitch force traces. I_{to1} (Fig. 10(c)) did not change at all when using this protocol because its rapid kinetics give no chance for the light impulse to alter its behavior. On the other hand, the rapidly activating delayed rectifier K^+ current I_{Kr} and rectified current I_{K1} were prolonged as t_{DL} is increased (Figs. 10(a) and 10(e)). Similarly, the slowly activated delayed rectifier K^+ current I_{Ks} (Fig. 10(b)) is prolonged. In each of these cases, the outward K^+ currents are counteracted by the effect of modulated L -type Ca^{2+} channel ion currents and the strong inward ChR2 current during this time. In other words, as the rectifying K^+ currents begin to repolarize the membrane (Figs. 10(a) and 10(b)), ChR2 admits inward current with increasing magnitude (Fig. 10(e)). This inward current counteracts the outward K^+ currents and slows repolarization. This in turn delays the final inactivation of I_{Ca} (Fig. 9(b)) and prolongs the Ca^{2+} transient. Under Protocol 2, only events late in the AP are affected. Hence, it is the rate of twitch force relaxation that is manipulated, not the peak twitch tension.

3.3 Protocol 1 and 2 Effects on Property of Cardiac Twitch Dynamics.

The effects of hybrid stimulation protocols on cardiac twitch characteristics are summarized as changes to peak force, time from peak force to 50% relaxation, and time from peak to 75% relaxation (T_{50} and T_{75} , respectively). Figures 11(a) and 11(b) show a summary of the results obtained when protocol 1 ($L + E$) is administered. A significant loss of function is observed in the form of reduced peak force in a nonlinear fashion with the t_{SE} values. In fact, a more than 25% force reduction can actually be obtained if the electric pulse is applied at $t_{\text{SE}} = 90$ ms. This peak force reduction is in general associated with a slower relaxation time, which depends on exactly when the electrical stimulus is introduced. For instance, for a range of $40 \leq t_{\text{SE}} \leq 70$ ms, both T_{50} and T_{75} increased linearly. For $70 \leq t_{\text{SE}}$, T_{50} exhibited a nonlinear recovery back to the baseline value, but T_{75} continued to increase linearly. Similarly, Fig. 11(c) shows the results obtained when protocol 2 ($E + L$) is used. This protocol primarily manipulates the relaxation phase of twitches without affecting the strength of the contraction. An increase of T_{50} has been observed for a range of $200 \leq t_{\text{DL}} \leq 350$ ms. This increase levels off and remains constant for $350 \leq t_{\text{DL}}$. T_{75} increases as well, but in a nonlinear fashion for a range of $200 \leq t_{\text{DL}} \leq 400$ ms. For $450 \leq t_{\text{DL}}$, T_{75} starts to decrease.

4 Discussion

Although acute optomechanical manipulation of cardiomyocyte contraction is clearly feasible from a theoretical standpoint, we have used detailed modeling to test specific stimulus protocols and estimate the size and character of their effects on contraction. These predictions provide a solid basis for future experiments.

Fig. 9 Ion currents predicted by the model in response to varying the duration of the light pulse following electrical current stimulus under protocol 2. The light pulse duration used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Note that varying time and current scales are used in each panel to better view the details of each current type. I_{Na} (a), I_{Ca} (b), I_{to1} (c), I_{NaCa} (d), and I_{NaK} (e) are shown.

Fig. 10 Additional ion currents predicted by the model in response to varying the duration of the light pulse following electrical current stimulus under protocol 2. The light pulse duration used in each simulation is indicated by the trace color, as shown in the legend. Each perturbation is compared to a baseline electrically stimulated event. Note that varying time and current scales are used in each panel to better view the details of each current type. I_{Kr} (a), I_{Ks} (b), I_{CaK} (c), I_{K1} (d), and I_{ChR2} (e) are shown.

Fig. 11 Summary of protocol 1 and 2 effects on properties of the twitch force. By altering the interval between stimuli (t_{SE} under protocol 1), it was possible to alter peak twitch force (a) and the time from peak force to 50 and 75% relaxation (T_{50} and T_{75} , respectively) (b). Under protocol 2, the duration of the light pulse after electrical current stimulus (t_{DL}) also yielded targeted alterations in T_{50} and T_{75} .

Simulations support the idea that by interspersing occasional manipulated beats among many otherwise consistent events (e.g., Figs. 3 and 7), underlying properties of the myofilaments can be expected to remain constant while a collection of perturbed system responses is gradually accumulated. The range of these responses is summarized in Fig. 11, and includes manipulations of peak twitch force and the relaxation rate. Therefore, if proven in practice, this new “optomechanical” approach would have important implications for computational modeling and for the study of cardiac muscle pathologies.

Data collected using the proposed protocols would improve the accuracy of muscle contraction models. In most cases, model parameters are determined by inputting a measured Ca^{2+} transient and adjusting values until model-generated force matches observed twitch force. Once this fitting process is complete, the predictive validity of the model is difficult to test because the data available for validation have been exhausted. That limitation would be removed by optogenetic manipulation, through the generation of many additional myofilament input/output responses. The challenge of reproducing comprehensive data sets would act to improve and validate these models to a much greater degree than currently possible.

In a similar way, the ability to elicit a broad variety of responses from intact cardiac cells has the potential to provide much more accurate characterizations of disease-causing mutations to myofilament proteins. These mutations, occurring most frequently in myosin heavy chain and cardiac myosin binding protein C [27], are linked to inherited disorders known as familial hypertrophic cardiomyopathies [28]. Although mutant proteins can be purified and studied in vitro [29], it is often difficult to translate such findings into meaningful physiological effects. Better physiological relevance can be attained through studies of intact cardiomyocytes that express mutant proteins (e.g., Ref. [30]), but working with intact cells also poses limitations. In terms of characterizing the cell’s Ca^{2+} -contraction dynamics, only one response to a given pacing frequency can be obtained. If other pacing frequencies are employed, evidence suggests that myofilament proteins are modified as a consequence, causing the mutation to operate in a different context [19]. Hence, Ca^{2+} -contraction data collected under different pacing rates may not reflect mutation effects alone, but could be tainted by secondary modifications mediated by Ca^{2+} -dependent phosphorylation events [31,32]. Once again, optomechanical perturbations may provide a way to obtain a more comprehensive data set in the presence of cardiomyopathy mutations by measuring their responses to multiple Ca^{2+} inputs without altering the intrinsic pacing rate.

The approach detailed here is not without limitations. ChR2 does not provide straightforward light-based control of AP morphology. The proportionality between light intensity and ChR2 current is advantageous, but ChR2 is voltage- and time-dependent as well [21]. It is also a nonspecific cation channel, meaning that the current produced upon activating ChR2 depends heavily on context [22]. The complex interplay between this channel and the others present in cardiomyocytes necessitates the simulation approach we have employed here in an attempt to design useful optomechanical manipulations. It is worth noting that some groups have been successful at modulating excitation-contraction coupling in cardiac myocytes using whole-cell AP clamp. Cordeiro et al. [10] manipulated the AP of canine ventricular myocytes while simultaneously recording Ca^{2+} release and cell shortening. They were able to observe AP-driven alterations in these parameters. This technical feat allows exquisite control of membrane potential, but ultimately this too is an indirect approach to perturbing Ca^{2+} release. We believe that transfection of cardiomyocytes with ChR2 and the application of hybrid stimuli is an attractive alternative to AP clamp, as this approach is noncontact and most likely higher-throughput. The theoretical perturbations that are possible with optomechanical control appear sufficiently large to provide useful data for modeling and analysis (Fig. 11).

Having predicted the feasibility of meaningful optomechanical control of cardiomyocytes, the next step is to implement model-designed protocols in a real experimental system. Aside from confirming that the simulated protocols work as predicted, experiments must also be done to determine whether optogenetic manipulations are truly acute. It is possible, for instance, that a single altered beat, even with the same cycle length as surrounding beats, could trigger some type of myofilament posttranslational modification. This would be manifested as a difference between the twitch forces measured in beats immediately prior to and following the perturbed beat. Even more fundamental is the question of whether transfection of cells with ChR2 causes intrinsic changes to myofilament biology. Careful comparison of the Ca^{2+} -contraction coupling behavior of ChR2 and non-ChR2 cardiomyocytes would have to be made.

We are also conducting further simulation-based exploration with the optomechanical cardiomyocyte model in order to identify alternative protocols and other light-sensitive channels (e.g., halorhodopsins [7]) that may yield other useful manipulations of contractile function. Desirable manipulations are those which allow the Ca^{2+} -contraction dynamic space to be widely explored. The two protocols discovered so far succeed in doing this (Figs. 4(d) and 8(d)), but many more possibilities could be tested. For instance, the impact of light stimulus magnitude, particularly in relation to Na^+ and Ca^{2+} channel opening thresholds, was not studied here in detail. Manipulating the intensity of the light pulse may offer other distinct perturbations of the intracellular Ca^{2+} transient. The inclusion of halorhodopsins could open the possibility of increasing Ca^{2+} release, instead of blunting it as in the case of ChR2. Systematic exploration of these additional parameters and their effect on Ca^{2+} -contraction coupling should ultimately yield a large family of diverse and useful acute perturbations to cardiac muscle contraction.

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