Spatiotemporal intracellular calcium dynamics during cardiac alternans

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Cellular calcium transient alternans are beat-to-beat alternations in the peak cytosolic calcium concentration exhibited by cardiac cells during rapid electrical stimulation or under pathological conditions. Calcium transient alternans promote action potential duration alternans, which have been linked to the onset of life-threatening ventricular arrhythmias. Here we use a recently developed physiologically detailed mathematical model of ventricular myocytes to investigate both stochastic and deterministic aspects of intracellular calcium dynamics during alternans. The model combines a spatially distributed description of intracellular calcium cycling, where a large number of calcium release units are spatially distributed throughout the cell, with a full set of ionic membrane currents. The results demonstrate that ion channel stochasticity at the level of single calcium release units can influence the whole-cell alternans dynamics by causing phase reversals over many beats during fixed frequency pacing close to the alternans bifurcation. They also demonstrate the existence of a wide range of dynamical states. Depending on the sign and magnitude of calciumvoltage coupling, calcium alternans can be spatially synchronized or desynchronized, in or out of phase with action potential duration alternans, and the node separating out-of-phase regions of calcium alternans can be expelled from or trapped inside the cell. This range of states is found to be larger than previously anticipated by including a robust global attractor where calcium alternans can be spatially synchronized but out of phase with action potential duration alternans. The results are explained by a combined theoretical analysis of alternans stability and node motion using general iterative maps of the beat-to-beat dynamics and amplitude equations. © 2009 American Institute of *Physics*. [DOI: 10.1063/1.3207835]

The contractile machinery of a heart cell is activated by the copious release of calcium from intracellular stores. This release causes the calcium concentration to rise transiently and then decrease as calcium is pumped back into the stores to be available for release at the next beat. This "calcium cycling" in and out of the stores can become dynamically unstable, with the net result that the peak calcium concentration alternates from beat to beat. These period-two oscillations, known as "calcium transient alternans," have been linked to the onset of lifethreatening heart rhythm disorders.^{1,2} The spatiotemporal dynamics of intracellular calcium during alternans, however, is still far from being fully explored. This dynamics is made especially rich by the fact that calcium and membrane voltage are bidirectionally coupled.³ Calcium entry into the cell via voltage gated L-type calcium channel (LCC) triggers calcium release, and the transient rise in calcium affects calcium-dependent membrane currents and hence the time course of membrane voltage during the action potential. Experiments⁴⁻⁶ have shown the existence of dynamical states where, subcellularly, calcium alternans can be spatially synchronized or desynchronized, and calcium alternans at the whole cell level are more widely known to be either in or out of phase with action potential duration alternans depending on physiological conditions.^{1,2} Here we use a physiologically detailed model of bidirectionally coupled intracellular calcium dynamics⁷ and membrane voltage dynamics to shed light on the emergence of different dynamical states. Numerical simulations of the model allow us to test existing theoretical predictions of deterministic models⁸ and to explore the effect of ion channel stochasticity on whole cell dynamics. The results highlight the importance of stochastic effects and reveal a richer dynamical behavior than previously anticipated.

I. INTRODUCTION

The main cellular signals responsible for the contraction of cardiac myocytes are the transmembrane voltage and the intracellular calcium concentration. Membrane depolarization propagates as a wave across the atria and ventricles and regulates the entry of calcium into the cell, triggering further intracellular calcium release and the subsequent activation of the contractile machinery. Under rapid stimulation or pharmacological stress, the normally periodic transmembrane voltage or calcium concentration can undergo a perioddoubling bifurcation. The resulting beat-to-beat alternations in the action potential duration are known as action potential duration alternans (*voltage alternans*), and beat-to-beat alternations in the peak calcium concentration are known as calcium transient alternans (*calcium alternans*). This pathologi-

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FIG. 1. Schematic representation of EMC (left) and EMD (right) alternans.

cal behavior is associated with arrhythmias and sudden cardiac death.^{1,2} An understanding of the mechanisms by which alternans are generated in a single cell could potentially have impact in the prevention and treatment of the diseases associated with this abnormal condition.

While voltage alternans are uniform in the cell due to fast voltage diffusion, slow calcium diffusion allows, in some cases, the development of calcium alternans with opposite phase in different regions of the cell (i.e., a smalllarge-small-large calcium pattern in one region of the cell and a large-small-large-small pattern in another region). This type of calcium alternans is known as spatially discordant alternans (SDA) and has been observed in confocal microscopy images of cat atrial cells⁴ and in the intact rat heart.^{5,6} Calcium alternans with the same phase over the whole cell are called spatially concordant alternans (SCA). (We note that the same terminology is applied to alternans patterns in cardiac tissue;² in this paper we will always refer to subcellular scales.) An amplitude equation describing the coupled dynamics of intracellular calcium and voltage was proposed by Shiferaw and Karma.⁸ It leads to the prediction that the formation of SDA can be described by a Turing instability of the spatially extended coupled calcium-voltage dynamics. The conditions for the formation of SDA were found to depend on the bidirectional coupling between calcium and voltage alternans, and to be the same as those for which calcium and voltage alternans are out of phase with each other (i.e., a long-short-long-short action potential duration pattern occurring with a small-large-small-large calcium pattern). In this paper we will extend the results of Ref. 8 and show numerically and theoretically that it is possible to have calcium and voltage alternans that are out of phase and spatially synchronized over the whole cell as a global attractor of the dynamics. The situation where voltage and calcium alternans are in phase (i.e., a long-short-long-short action potential duration pattern and a large-small-large-small calcium pattern) is the most common and is referred to as electromechanically con*cordant* (EMC) alternans. The opposite situation is observed under some conditions^{9,10} and is known as *electromechani*cally discordant (EMD) alternans. In Fig. 1 we show schematically the two different cases.

There have been recent experimental, theoretical, and numerical studies of SDA. Movement of nodes separating two regions with out-of-phase calcium alternans has been

observed using confocal microscopy in intact rat heart myocytes.⁶ Reference 8 derived an amplitude equation from iterative map dynamics of coupled voltage and calcium describing the evolution of intracellular calcium alternans on slow time scales. Numerical studies have modeled myocytes deterministically as a one-dimensional (1D) array of coupled sarcomeres.^{6,8} Reference 6 has found that SDA can form upon a change in stimulation period, and that nodes separating two out-of-phase regions exhibit rich dynamics. The numerical studies in Ref. 6, however, considered deterministic dynamics and a voltage signal uncoupled from calcium. Here we explore coupled voltage and calcium dynamics using a model of intracellular calcium dynamics which fully accounts for the local nature of calcium release.⁷ We compare the numerical simulations of this detailed model with an analysis of iterative maps describing the coupled dynamics of voltage and calcium. Reference 6 finds there is no node movement with a periodic voltage signal. Our numerical and theoretical results agree with this result, with the caveat that stochasticity of calcium release results in drifting node motion when the voltage is periodic. When the voltage signal is coupled to calcium through the calcium-dependent membrane currents, we find that the node motion depends on the form of the calcium-voltage coupling. We predict the conditions when alternans are spatially concordant or discordant and electromechanically concordant or discordant in terms of a general two-dimensional (2D) iterative map of the beat-tobeat dynamics. In addition, we make predictions for the motion of the node separating spatially out-of-phase regions using an amplitude equation approach, and we qualitatively validate these theoretical results by studying the movement of the node separating out-of-phase regions when alternans are initiated with opposite phase in two regions of the cell.

We investigate the effects of local fluctuations on experimentally observable quantities such as the cytosolic calcium concentration. We address a fundamental problem of any model of calcium release that includes stochasticity and spatial calcium release: how do individual calcium release units (CRUs) maintain the coherent pattern of release to produce macroscopic alternations of calcium release stable against stochastic dephasing? We find that coherence is maintained by local coupling through calcium diffusion or globally by interactions through the membrane voltage.

This paper is organized as follows. In Sec. II A we describe the essential elements of voltage and calcium dynamics and in Sec. II B we summarize the basic features of cardiac cellular alternans. In Sec. III we briefly describe the physiologically detailed model used for the simulations of alternans dynamics. The results are then presented in Sec. IV. We explore numerically the effects of spatial structure and fluctuations on the genesis of alternans. We also investigate the relationship between calcium-voltage coupling and the spatial synchronization of calcium alternans. In Sec. V we interpret our results in terms of an iterative map of the beatto-beat dynamics and predictions for node motion and stability that are derived from an amplitude equation in appendices. In Sec. VI we summarize our results and give concluding remarks.



FIG. 2. (Color online) Top: a myocyte consists of $\sim 20~000$ CRUs arranged in a 3D grid. Bottom left: in the resting state, calcium (circles) concentration is high outside the cell and inside the SR, which has terminal cisternae in each CRU. Membrane depolarization triggers calcium influx (arrow) through the LCC channels. Bottom right: the increase in calcium concentration triggers further localized calcium release from the SR (a calcium spark). The released calcium can diffuse to neighboring CRUs and trigger more sparks.

II. BACKGROUND

A. Excitation-contraction coupling

Here we briefly summarize the dynamical processes by which the membrane depolarization signal is relayed to the contractile machinery of the cell, named *excitationcontraction coupling*. For a thorough review of excitationcontraction coupling, see, for example, Ref. 3.

When a myocyte is electrically excited by neighboring cells, voltage sensitive ion channels open, triggering a temporary (~ 200 ms) depolarization of the membrane from its resting potential, about -80 to 20 mV. This action potential is the result of the activation and subsequent inactivation of various ionic currents which transport mostly calcium, potassium, and sodium across the membrane. The depolarization of the membrane triggers influx of calcium into the cell through the LCCs. These channels are localized close to the terminal compartments of an internal saclike structure [sarcoplasmic reticulum (SR)] that stores calcium inside the cell. These terminal compartments have a cluster of about 100 calcium sensitive channels [ryanodine receptors (RyRs)] which open when the nearby calcium concentration increases. They, in turn, release even more calcium from the SR, raising the calcium concentration inside the cell and activating its contractile machinery. Subsequently, calcium is both reuptaken into the SR and extruded from the cell in preparation for the next stimulus.

A few LCC channels and ~ 100 RyRs are clustered in CRUs that are distributed in a three-dimensional (3D) grid across the myocyte, so as to guarantee a uniform calcium release across the volume of the cell (see Fig. 2). The cell membrane has invaginations (T tubules) that form a dense

network inside the cell, thus allowing close proximity to the membrane for even the innermost CRUs. The number of CRUs in a typical myocyte is estimated to be around 20 000.³ Inter-CRU distances have been measured to be $\sim 1 \ \mu m$ in the transversal directions and $\sim 2 \ \mu m$ in the longitudinal direction.^{11,12} Figure 2 shows a schematic representation of excitation-contraction coupling.

The elementary process in excitation-contraction coupling is the release of calcium at a single CRU, known as a *calcium spark*.³ Calcium sparks are highly localized both in time (~30 ms) and in space (~2 μ m). The cellular calcium concentration measured in experiments is the result of the aggregate calcium released from a large number of calcium sparks. The amount of calcium released by a CRU in a single spark depends on the amount of calcium in the local portion of the SR prior to release, and on the probability of the RyR channels to open in response to calcium in the vicinity of the RyR cluster. Importantly, after a spark is elicited in a CRU, the probability of firing a subsequent spark in the same CRU immediately decreases and slowly recovers after \sim 400 ms.¹³ Thus, CRUs act as stochastic excitable elements with a refractory period.

Under normal conditions, calcium sparks occur independently of each other, in such a way that the number of sparks is roughly proportional to the number of opening LCC channels. However, under certain conditions, a calcium spark can trigger a spark in a neighboring CRU through calcium diffusion and produce calcium waves.^{14,15} Diffusive coupling between CRUs can also play a role in subcellular SDA.^{4–6} These phenomena interfere with normal calcium signaling and are potentially arrhythmogenic.

A summary of the features of intracellular calcium release that will be essential is as follows:

- A single myocyte consists of a very large number of CRUs arranged in a 3D grid.
- Individual CRU calcium release dynamics is stochastic.
- As the stimulation period is decreased, due to the refractoriness of RyR channels there is a decreased probability to produce a calcium spark in the beat after a spark is produced.
- A spark in a CRU increases the probability of sparks occurring in the neighboring CRUs in the same beat.

B. Alternans

When a myocyte is periodically stimulated, excitationcontraction coupling normally results in periodic membrane voltage and whole-cell calcium concentration signals. However, for rapid stimulation, or under other abnormal conditions,^{1,2} this periodic behavior can degenerate into period-two dynamics. In voltage alternans, the action potential duration alternates in a long-short-long-short pattern. In calcium alternans, the peak cytosolic calcium concentration alternates in a large-small-large-small pattern.

A mechanism leading to voltage alternans is a steep restitution function, which determines the duration of the action potential at beat n, A_n , from the duration of the action potential at beat n-1, A_{n-1} . Assuming no other dynamics such as memory effects or calcium coupling, voltage alternans develop if the slope of the restitution function is steep enough, $\partial A_n / \partial A_{n-1} < -1$.¹⁶ Since calcium and membrane voltage dynamics are bidirectionally coupled through calcium and calcium-dependent ionic currents, it was originally thought that calcium alternans were just a consequence of restitution-caused voltage alternans. Experiments where the membrane voltage is enforced to be periodic, but calcium nevertheless develops alternans, demonstrated that the calcium system can be unstable by itself.¹⁷ In the last decade, experiments and theoretical analyses^{2,7,18–22} have suggested that the origin of the calcium instability is related to the process of calcium release from and reuptake into the SR.

Today it is recognized that both voltage and calcium instabilities can independently produce cellular cardiac alternans and that due to their bidirectional coupling,^{21,22} alternans caused by either mechanism might ultimately be observed as both calcium and voltage alternans. Experimentally determining the origin of the period-two instability in a particular system can be nontrivial.²³ Pinning down the cause of alternans in a particular setting might be useful to determine possible drug interventions or, potentially, to select appropriate protocols for spatial alternans control.^{24,25} Depending on the form of the coupling between calcium and voltage alternans, they can be in phase or out of phase with each other,² and we refer to these cases as EMC or EMD, respectively.

As we described in Sec. II A, calcium concentration is the result of calcium release from a large number of CRUs spatially distributed across the cell. Various spatiotemporal dynamics have been observed during calcium alternans. These include calcium alternans with opposite phase in different regions of the cell,^{4–6} the movement of nodes separating such regions,⁶ and more complex spatiotemporal behavior, including calcium waves.²⁶ We refer to spatially synchronized calcium alternans as SCA and to calcium alternans with opposite phase in opposite regions as SDA.

III. METHODS

We study the intracellular dynamics of calcium during alternans using a detailed model of calcium cycling developed recently by the authors.⁷ This model consists of a 3D grid of $\sim 20\ 000\ CRUs$, each one consisting of various compartments depicted in Fig. 3. The dynamics of the calcium concentration in each compartment is described by differential equations that include diffusive currents between the different compartments and Markov models for individual LCC and RyR channels. (The reason for modeling the CRUs in detail is to make contact with experiments.) Neighboring CRUs are coupled by diffusive currents, and calcium released by a spark in one CRU may trigger sparks in neighboring CRUs. We couple this model of calcium dynamics with membrane currents for rabbit ventricular cells.²⁷ More details about the model can be found in Appendix A and Refs. 7 and 27.

The release of calcium from the SR compartment in each CRU occurs through a cluster of ~ 100 stochastic RyR channels. The opening probability of these channels increases with the calcium concentration near the RyR cluster. Recent experiments²⁸ indicate that, in addition, the opening prob-



FIG. 3. (Color online) Schematic representation of the compartments that constitute a single CRU. The arrows indicate the average direction of calcium flux during one beat. The three lower arrows indicate diffusive coupling between the compartments of adjacent CRUs. For details, see Appendix A and Ref. 7.

ability is controlled by the calcium concentration inside the SR compartment. This dependence is such that after the SR releases calcium, there is a period of recovery during which the opening probability of the RyR channels is lower. Our model includes a description of this so-called "luminal gating" of calcium release and we have found that the recovery time after calcium release is partly responsible for the instability in the calcium cycling.⁷ By adjusting a parameter that determines the time scale of recovery we can control the degree of calcium instability. We incorporate the observed heterogeneity in the properties of CRUs (Ref. 11) by considering the proximal volume of each CRU to be randomly drawn from a Gaussian distribution (see Appendix A). It has been shown that heterogeneity in CRU properties accounts for the characteristic smooth shape of the graded-release (SR release versus I_{Ca} current) curve.⁷

We emphasize that this model is the first combining a physiologically detailed CRU structure with diffusive interaction of CRUs, and it combines features that allow us to realistically investigate the effect of stochasticity and spatial structure on the genesis of alternans: (i) the release of calcium from the CRUs is stochastic, since individual RyR and LCC channels are described with Markov models; (ii) the model incorporates spatial structure and diffusive coupling between neighboring CRUs; and (iii) the model includes physiologically detailed currents that allow us to explore the effect of pharmacological or genetic interventions on alternans dynamics.

The variables of interest for the study of spatially distributed alternans are the calcium concentration in the cytosolic compartment of each CRU (see Fig. 3), which we will denote as $c^{(m)}$, with m=1,2,...,N indicating the different CRUs, the whole-cell cytosolic calcium concentration $c=(1/N)\sum_{m=1}^{N}c^{(m)}$, and the transmembrane voltage V. For convenience, we will focus on the peak calcium concentration on a given beat c_n , where n indicates the beat number.



FIG. 4. Averaged peak calcium concentration in steady state for T=340 ms and the parameters of case A (see Table I) as a function of a parameter ξ that rescales the calcium diffusion time scales. Larger ξ corresponds to weaker diffusive coupling between CRUs. Stable alternans are sustained only for stronger local diffusive coupling.

The model can be simulated using two different methods which correspond to commonly used experimental protocols.¹⁷ In the *unclamped* protocol, a small stimulus current is periodically applied, initiating an action potential by the activation of inward ionic currents. In the *clamped* protocol, the membrane potential is forced to be a prescribed periodic signal, the *voltage clamp*. In the simulations, we obtain this signal by averaging the unclamped case membrane potential over many beats, so that the voltage clamp closely resembles the unclamped action potential.

IV. RESULTS

A. Diffusive coupling

First, we establish that stable calcium alternans in the clamped case are sustained by the local diffusive coupling of neighboring CRUs, a result first reported in Ref. 7. In Fig. 4 we plot the time-averaged peak calcium concentration for T=340 ms for even and odd beats (squares) as a function of a parameter ξ which scales the calcium diffusion coefficients (i.e., transversal, longitudinal, cytosolic, and intra-SR). More precisely, for a given value of ξ , we divide all the calcium diffusion coefficients by ξ . Thus, larger ξ corresponds to weaker diffusive coupling, and vice versa. The results in Fig. 4 show that alternans are sustained only for diffusive coupling above a certain threshold.

This result demonstrates that the emergence of calcium alternans at the whole cell level is a strongly cooperative phenomenon mediated by the diffusive coupling of a large number of CRUs. The whole cell dynamics behaves almost deterministically in a nonlinear regime of well-developed alternans, albeit not close to the bifurcation as described in Sec. IV B. The dynamics of each CRU, in contrast, is always strongly stochastic in any regime. Release events do not exhibit simple period two dynamics and it is impossible to conclude that whole cell alternans are formed from the examination of the dynamics of a single CRU.

B. Fluctuations

In order to investigate the effects of fluctuations and spatial structure on the transition to calcium alternans, we simulate a myocyte for different pacing periods. For each period T, we simulate the cell for 4000 beats. In the clamped case,



FIG. 5. (Color online) Peak calcium concentration at even (red) and odd (blue) beats as a function of the beat number *n* for the clamped (left panel) and unclamped (right panel) cases for values of *T* ranging from T=350 ms (top) to T=300 ms (bottom). The peak calcium concentration undergoes a period-doubling bifurcation as *T* is decreased. Close to the bifurcation, there are extremely long periods of stable alternans that eventually reverse phase (e.g., horizontal bar in the clamped, T=325 ms panel). The simulation corresponds to a cell with normal parameters (case A in Table I).

we first average the unclamped membrane voltage for 100 beats after steady state has been reached, and subsequently use this average signal as the voltage clamp. That is, the voltage clamp is $V_c(t) = \sum_{j=1}^{100} V(t+jT)/100$, where V is the steady-state unclamped voltage. In Fig. 5 we show the peak calcium concentration at even (red) and odd (blue) beats as a function of the beat number n for the clamped (left panel) and unclamped (right panel) cases for values of T ranging from T=350 ms (top) to T=300 ms (bottom). The peak calcium concentration undergoes a period-doubling bifurcation as T is decreased. This bifurcation occurs at a lower value of T for the clamped case. The reason for the different bifurcation thresholds is that when the voltage is unclamped, calcium and voltage alternans interact synergistically to promote calcium alternans (see theory in Sec. V).

Remarkably, there are long periods of stable alternans that eventually reverse phase. For example, for the clamped case at T=325 and T=315 ms, and for the unclamped case at T=340 and T=345 ms. Periods of well defined alternans are separated by phase reversals, changes in sign of the difference between the peak calcium transient in consecutive



FIG. 6. (Color online) Action potential duration (thin lines) and peak calcium concentration (thick lines) at even and odd beats for simulations corresponding to the unclamped, T=345 ms panel in Fig. 5. Fluctuations in the action potential duration track the fluctuations in peak calcium concentration during a phase reversal.

beats $c_{2n}-c_{2n-1}$, measured on even beats. The time between consecutive phase reversals can be as large as ~ 900 beats (e.g., horizontal bar in the clamped, T=325 ms panel of Fig. 5). At a critical period T, the time between phase reversals diverges and a well defined phase for alternans prevails. These results are in contrast to those in previous models of calcium alternans, where the peak calcium concentration undergoes a period doubling bifurcation in a deterministic way.²⁰ A signature of calcium fluctuations that could be detected experimentally is that due to calcium-voltage coupling, the action potential duration exhibits fluctuations that track those in the calcium concentration. In Fig. 6 we show the action potential duration (thin lines) and peak calcium concentration (thick lines) at even and odd beats for simulations corresponding to the unclamped, T=345 ms panel in Fig. 5, showing how fluctuations in the action potential duration track the fluctuations in peak calcium concentration during a phase reversal. If the alternans are voltage driven, we expect that there would be almost no fluctuations in the action potential duration alternations, since fluctuations in membrane ion channels are averaged out by the long range voltage coupling of these channels. Even though there is still a feedback from the calcium dynamics which could introduce fluctuations in action potential duration alternations, the dominant component of the alternans is almost deterministic and thus we expect fluctuations to be much smaller.

Of particular interest is the calcium dynamics that mediates the phase reversals. In the spatial model, a phase reversal occurs as domains with opposite phase to the whole-cell alternans phase grow due to fluctuations and eventually become dominant. Similar spatiotemporally complex subcellular discordant alternans have been observed experimentally,⁴ and we find that they are common during the spontaneous phase reversals of calcium alternans.

Because CRUs are heterogeneous (in the model, as in real myocytes, they have heterogeneous volumes), we normalize the calcium release at individual CRUs with respect to their long term average release. We define, for the m^{th} CRU and beat n, the quantity $\Delta c_n^{(m)} = (c_n^{(m)} - \overline{c}^{(m)}) / \sigma_m$, where $\overline{c}^{(m)}$ indicates an average of $c_n^{(m)}$ over the beat number n, and σ_m^2 is the average of $(c_n^{(m)} - \overline{c}^{(m)})^2$ over n. Positive (negative) values of $\Delta c_n^{(m)}$ indicate a larger (smaller) release in CRU m



FIG. 7. (Color online) Normalized calcium release $\Delta c_m^{(n)}$ during a phase reversal on a 2D slice of a simulated cell. Top: during the phase reversal, CRUs have a complex spatial release pattern. Bottom: after the phase reversal, a majority of CRUs have a large release in the even beat.

and beat *n* than the long term average release at CRU *m*. In Fig. 7 we show $\Delta c_n^{(m)}$ at two consecutive beats in a 2D slice of a simulated cell showing the transversal and longitudinal directions during (top) and after (bottom) a phase reversal. The sequence shows that the phase reversal is characterized by complex spatial dynamics. In Fig. 8 we show the spatial average of $\Delta c_n^{(m)}$ as a function of the beat number *n*. This is a measure of the calcium alternans amplitude and is zero when the phase reverses around n=370. In the same figure we show the standard deviation of $\Delta c_n^{(m)}$ (squares), which measures the strength of the local period two dynamics, independent of their phase. We observe that this variable is always of order 1, indicating that the period two dynamics at the level of CRUs persists during the phase reversal.

C. Voltage-calcium coupling

We now turn to explore the genesis of spatially concordant or discordant alternans by studying the dynamics of the node separating out-of-phase regions within the cell. If the node is expelled, spatially synchronized alternans form, with only one phase of calcium alternans in the cell. If, on the other hand, the node stabilizes in the middle of the cell, SDA form, with the two halves of the cell alternating with opposite phase. In order to create an initial condition with two regions with opposite phases of calcium alternans, we temporarily change after steady state is reached a parameter that increases the open probability of the RyR channels for those



FIG. 8. Two measures of alternans strength during a phase reversal: spatial average (circles) and standard deviation (squares) of $\Delta c_n^{(m)}$ every four beats (see text). The average of $\Delta c_n^{(m)}$ measures the global phase of cellular calcium alternans and changes sign during a phase reversal, while the standard deviation of $\Delta c_n^{(m)}$ measures the strength of the alternans at the CRU level irrespective of their phase and remains positive during a phase reversal.



FIG. 9. (Color online) Determination of the node position. The transversal calcium average $c_T(j,n)$ averages peak calcium $c_n^{(m)}$ in beat *n* over all the CRUs *m*, which are in the *j*th longitudinal position. The node position *x* is determined by interpolation as the value *x* such that $c_T(x,n)=c_T(n)$ for even beats, where $c_T(n)$ is the spatial average of $c_T(j,n)$.

CRUs with longitudinal coordinate smaller than 3/5 of the longitudinal extension of the myocyte. This induces larger release in those CRUs during one beat, creating two regions with calcium alternans of opposite phase. We then track the movement of the node separating these regions by calculating the transversal calcium average $c_T(j,n)$, defined as the average of peak calcium $c_n^{(m)}$ in beat *n* over all the CRUs *m* which share the same *j*th longitudinal coordinate (see Fig. 9). The position of the node along the longitudinal direction of the cell at beat *n* is then defined as the coordinate *x* such that $c_T(x,n)=c_T(n)$, where $c_T(n)$ is the spatial average of $c_T(j,n)$ and *x* is found by interpolation of the function $c_T(j,n)$ evaluated at discrete values of *j*.

In order to explore different dynamical regimes, we vary some of the parameters of the cellular model. In Appendix A we introduce parameters γ_{NCX} and γ_{Ca} that control the strength of the calcium current (I_{Ca}) and the sodium-calcium exchanger current (I_{NCX}) . In addition, in Appendix A we also introduce two parameters $\alpha_{\rm NCX}$ and $\alpha_{\rm Ca}$ representing the ratio of effective membrane capacitance for the NCX and I_{ca} currents to that of the other membrane currents, which depends on the area distribution of ion channels in the external membrane and the T tubules²⁹ (see Appendix A). These parameters modify the effect of I_{Ca} and I_{NCX} on the membrane voltage without directly affecting calcium dynamics, and their values were chosen so that the action potential shape is physiological. We will show unclamped simulations for normal parameters ($\gamma_{NCX}=1$, $\gamma_{Ca}=1$, $\alpha_{NCX}=2$, $\alpha_{Ca}=1$, case "A"), reduced NCX with normal conductance (γ_{NCX} =0.2, $\gamma_{Ca}=1$, $\alpha_{NCX}=2$, $\alpha_{Ca}=1$, case "B"), normal NCX with higher I_{Ca} conductance ($\gamma_{NCX}=1$, $\gamma_{Ca}=1$, $\alpha_{NCX}=2$, $\alpha_{Ca}=2$, case "C"), and reduced NCX with higher I_{Ca} conductance $(\gamma_{\text{NCX}}=0.2, \gamma_{\text{Ca}}=1, \alpha_{\text{NCX}}=2, \alpha_{\text{Ca}}=2, \text{ case "D"})$. The results are described below and summarized in Table I.

First, we consider normal conductance with normal NCX current, case A. In Fig. 10 we show the node position versus the beat number for the parameters corresponding to a normal myocyte for both the unclamped and clamped protocols. SDA were induced using the method described above, with the position of the node initially at the longitudinal coordinate of \sim 36 out of a total of 60 CRUs in the longitudinal direction. The node position drifts for the clamped simulations (thick solid line). On the other hand, the node is

TABLE I. Simulation parameters and alternans states. Results for four different sets of simulation parameters corresponding to different magnitude of LCC and/or NCX currents and/or membrane areas associated with those currents (see text). Cases A, B, and C show SCA that are EMC or EMD. Those states are global attractors of the dynamics owing to the property that nodes of SDA are expelled from the cell. In case D, the node of SDA is stable and the dynamically selected state depends on initial conditions. The SDA state, which produces no APD alternans, is selected when a node is present anywhere along the length of the cell except close to the boundaries. The EMD/SCA state, in turn, is selected when the initial condition is spatially concordant, as observed during a change in pacing cycle length.

Case	$\alpha_{\rm NCX}$	$\alpha_{\rm Ca}$	$\gamma_{ m NCX}$	γ_{Ca}	State
A	2	1	1	1	EMC/SCA only
В	2	1	0.2	1	EMD/SCA only
С	2	2	1	1	EMC/SCA only
D	2	2	0.2	1	SDA or EMD/SCA

expelled in about 70 beats for the unclamped protocol (thin solid line), indicating the formation of SCA. For these parameters, alternans are EMC.

In order to induce EMD alternans, we reduce the strength of the NCX current to 20% of its standard value (case B), as has been demonstrated experimentally.¹⁰ When intracellular calcium concentration is high, the NCX current extrudes calcium and brings sodium in, the net effect being an inward sodium flux and an increase in the action potential duration. Thus, the NCX current promotes EMC alternans, and by reducing its strength EMD alternans can be produced. In Fig. 10 we also show the node position as a function of the beat number for unclamped simulations with 20% NCX (case B, dot-dashed line). In this case alternans are EMD and spatially concordant.

As will be shown in Sec. V, an important parameter for node dynamics is the coupling between calcium and voltage alternans. In order to explore a broader range of possible phenomena, we modify our model to have a weaker calciumvoltage coupling by increasing the effective membrane capacitance of the calcium current by a factor of 2 (α_{Ca} =4, cases C and D), decreasing the effect of the calcium current on the action potential duration and thereby decreasing calcium-voltage coupling. It is known that different currents might act over different effective areas in the complex sur-



FIG. 10. Longitudinal node position at even beats vs the number of beats for the clamped case (thick black line), the unclamped case A (thin line), and the reduced NCX case B (dot-dashed line) with T=250 ms. Details about the different cases are in the text and Table I. The node drifts for the clamped case and is expelled in the unclamped cases A and B. Alternans are EMC in case A and EMD in case B.



FIG. 11. Longitudinal node position at even beats vs the number of beats for case D (thick black line) and three simulations of case C with the same parameters (thin lines) with T=200 ms. Details about the different cases are in the text and Table I. The node is expelled in case C and attracted to the center in case D. Alternans are EMC in case C and EMD in case D.

face of the cell membrane, and thus might have different effective total capacitances.²⁹ Here, this modification is an effective way to assess the effect of decreased calcium-voltage coupling. Figure 11 shows the result of three simulations with the same parameters (thin solid lines, case C) and a simulation with 20% NCX reduction in addition to the capacitance modification (thick solid line, case D). While the normal NCX simulations result in spatially concordant and EMC alternans, in the reduced NCX simulation the node is not expelled but, instead, is stabilized in the center of the myocyte. More information in addition to the node position is presented in Fig. 12, where we show the transversal calcium average $c_T(j,2n)$ as a function of the longitudinal coordinate *j* and the beat number 2n for case D (a) and case C



FIG. 12. (Color online) Transversal calcium average $c_T(j, 2n)$ as a function of the longitudinal coordinate *j* and the beat number 2n for (a) case D and (b) case C with T=200 ms, as in Fig. 11. The node is expelled in case C and attracted to the center in case D.



FIG. 13. (Color online) Calcium concentration (bottom) and voltage (top) signals during alternans for cases C (left) and D (right) for even (solid lines) and odd (dashed lines) beats for simulations with spatially concordant initial conditions. The period is T=200 ms as in Fig. 11.

(b). In case D, alternans are spatially discordant, while SCA are EMD (see Fig. 13), consistent with the predictions in Ref. 8.

Simulations where the node was initiated in other positions along the cell also show that the node becomes attracted to the midpoint of the cell. However, for simulations that are started in the spatially concordant state or with the node very close to the boundary, alternans remain spatially concordant. This shows that for these parameters there are two stable states: the EMD/spatially concordant state and the spatially discordant state. In Sec. V we will interpret these results by studying the stability of these nonlinear states.

The results in Fig. 11 show, in addition, that stochastic effects may result in slightly different outcomes for simulations with identical parameters. However, we have not found qualitatively different behavior for simulations with the same parameters. In Fig. 13 we show the calcium concentration (bottom) and voltage (top) signals during alternans for cases C (left) and D (right) for even (solid lines) and odd (dashed lines) beats, for simulations with spatially concordant initial conditions. Case C has EMC alternans and case D has EMD alternans.

In summary, under various conditions we have observed EMC with SCA, EMD with SCA, and EMD with SDA.

V. THEORETICAL ANALYSIS

In this section, we analyze our simulation results in the general theoretical framework of iterative maps of the beatto-beat dynamics and amplitude equations derived from those maps, which has been used previously to shed light on basic aspects of the spatiotemporal dynamics of alternans on both tissue³⁰ and subcellular scales.⁸ In the latter context, this approach was used to explain the spontaneous formation of subcellular spatially discordant calcium alternans in terms of a Turing-like instability (see also Gierer and Meinhardt³¹) mediated by voltage and calcium diffusion.⁸ In this analogy with a Turing instability, the amplitude of calcium alternans plays the role of a local slow diffusing activator, while the amplitude of voltage alternans plays the role of a fast diffusing inhibitor. Since voltage diffuses essentially instantaneously across a myocyte on the time scale of one beat, this

instability causes period doubling oscillations of the calcium concentration to develop out of phase in two halves of a cell. In Ref. 8, the existence of this instability was demonstrated using a physiologically detailed 1D model of bidirectionally coupled voltage and calcium dynamics, where CRUs with deterministic dynamics are spatially distributed along the length of the myocyte.

Our present simulations confirm the existence of a Turing instability for fast pacing rate and parameters where SCA are EMD, as predicted in Ref. 8. Stochasticity provides a natural mechanism to trigger the instability but does not alter fundamentally the development of the instability in a strongly nonlinear regime when alternans have a large enough amplitude. One new finding, however, is that EMD alternans can also occur for parameters where the Turing instability is absent. In contrast, in Ref. 8, it was concluded that the condition for the occurrence of a Turing instability was the same as the one for the occurrence of EMD alternans, in apparent disagreement with our finding. Here we revisit the iterative map based stability analysis of alternans of Ref. 8 to resolve this disagreement. To help us interpret our simulation results, we also derive predictions for the motion of the node separating out-of-phase regions and characterize the nonlinear character of the alternans bifurcation in the case of negative voltage-calcium coupling. We conclude at the end of this section that this disagreement is a consequence of a simplifying assumption made in Ref. 8. Relaxing this assumption leads to the prediction that the condition for the occurrence of EMD alternans is distinct from the one for the occurrence of a Turing instability, and hence EMD alternans can exist as a global attractor of the dynamics.

A. Stability of period one state against spatially concordant and discordant perturbations

The starting point of our analysis is the general map of the beat-to-beat dynamics describing the spatially concordant state where calcium alternans have the same amplitude and phase across the myocyte,

$$A_n = f_1(A_{n-1}, c_{n-1}), \tag{1}$$

$$c_n = f_2(A_{n-1}, c_{n-1}), \tag{2}$$

where A_n and c_n are the action potential duration and the peak value of the calcium concentration at the n^{th} beat. The stability of this map is governed by the Jacobian

$$\mathbf{J} = \begin{pmatrix} J_{11} & J_{12} \\ J_{21} & J_{22} \end{pmatrix} = \begin{pmatrix} \frac{\partial A_n}{\partial A_{n-1}} & \frac{\partial A_n}{\partial c_{n-1}} \\ \frac{\partial c_n}{\partial A_{n-1}} & \frac{\partial c_n}{\partial c_{n-1}} \end{pmatrix},$$
(3)

where the diagonal elements J_{11} and J_{22} control the stability of the isolated voltage and calcium systems, respectively, and the off-diagonal terms J_{12} and J_{21} control the bidirectional coupling between those two systems. These matrix elements are evaluated at the fixed point of the map corresponding to the period one state, defined by $A_n=A_0$ and $c_n=c_0$. Without coupling between the voltage and calcium systems, $-J_{11}$ simply measures the slope of the action potential duration restitution curve, which relates the action potential duration on a given beat with the preceding diastolic interval (i.e., the interval between the end of the preceding action potential and the start of the next one). Voltage alternans are unstable for a slope larger than unity, which corresponds here to the condition $J_{11} < -1$. In turn, J_{22} controls the stability of the calcium system. The latter can be studied experimentally or numerically by stimulating a cell periodically with a clamped action potential waveform. Under such a protocol where the voltage dynamics is enforced to be periodic, calcium transient alternans occur when $J_{22} < -1$.

The stability of the coupled voltage and calcium systems under general unclamped conditions is governed by the eigenvalues of the Jacobian matrix that are obtained from the vanishing determinant condition $|\mathbf{J}-\lambda_c\mathbf{I}|=0$, where \mathbf{I} is the identity matrix. This condition yields at once

$$\lambda_c^{\pm} = \frac{1}{2} (J_{11} + J_{22} \pm \sqrt{(J_{11} - J_{22})^2 + 4J_{12}J_{21}}), \qquad (4)$$

where the subscript of the eigenvalues has been introduced as a reminder that λ_c^{\pm} controls the stability of the spatially "concordant" state. To simplify the analysis, we assume that the off-diagonal terms of the Jacobian matrix are much smaller in magnitude than the diagonal terms, or

$$\left|\frac{J_{12}J_{21}}{J_{11}-J_{22}}\right| \ll 1.$$
(5)

As we show below, this assumption turns out to be quantitatively justified for the present simulations since the velocity of node separating out-of-phase regions is proportional to this ratio. Thus, the observation that the node velocity is very small in the simulations (i.e., that the node moves a distance comparable to the length of the myocyte over tens to hundreds of beats) can be used to infer that this condition is satisfied. In addition, we focus on the case where the calcium system is unstable but the voltage system is stable, or

$$J_{22} < -1,$$
 (6)

$$J_{11} > -1,$$
 (7)

where the second condition corresponds to an action potential slope less than unity. In order to estimate J_{11} , we decrease the recovery time of the RyR channels,³² thereby suppressing the calcium instability. In this case, with assumption (5), the eigenvalue with largest magnitude is J_{11} and voltage alternans decay after a perturbation as $A_n \sim (J_{11})^n$. In these conditions, we find that voltage alternans typically decay in four to five beats, from which we conclude that the magnitude of the restitution slope is substantially different than unity, or $J_{11}+1 \sim O(1)$. This estimation assumes that $J_{22} \sim 0$, but relaxing this assumption leads to the same conclusion.

Using condition (5), the eigenvalues (4) simplify to

$$\lambda_c^+ \approx J_{11} + \frac{J_{12}J_{21}}{J_{11} - J_{22}},\tag{8}$$

$$\lambda_c^- \approx J_{22} - \frac{J_{12}J_{21}}{J_{11} - J_{22}}.$$
(9)

Since $A_n \sim (\lambda_c^{\pm})^n \sim (-1)^n e^{n \ln(-\lambda_c^{\pm})}$, and similarly for c_n , alternans forms when $\lambda_c^{\pm} < -1$, such that the exponential amplification factor $\ln(-\lambda_c^{\pm})$ is positive. Conditions (6) and (7), together with the fact that $J_{11}+1 \sim O(1)$, imply that $\lambda_c^+ > -1$. Thus SCA form when $\lambda_c^- < -1$.

Next, to find out if alternans develop concordantly or discordantly, we need to compare λ_c^- to the eigenvalue that controls the stability of the spatially discordant state with a node in the middle of the cell. Because voltage diffuses instantaneously on the scale of the myocyte, voltage dynamics is only influenced by the spatial average of calcium-dependent membrane currents, which include the L-type calcium current (LCC) and the NCX current. Since in the spatially discordant state two halves of the cell alternate out of phase, this spatial average, and hence the action potential duration, is the same at even and odd beats, as seen in the simulations. Thus the eigenvalue that controls the stability of the spatially discordant state is the same as the one controlling the calcium system paced with a periodic action potential clamped waveform, or simply

$$\lambda_d = J_{22}.\tag{10}$$

Consequently, SDA will have a larger exponential amplification rate than SCA (Turing instability) if $\lambda_d < \lambda_c^-$ or, using Eqs. (8) and (9), if $J_{12}J_{21} < 0$, and vice versa for $J_{12}J_{21} > 0$. When calcium alternans develop spatially discordantly, they produce no voltage alternans because of the aforementioned spatial averaging of membrane currents. In contrast, when they develop spatially concordantly, calcium alternans produce voltage alternans that can be temporally in phase or out of phase with calcium alternans (i.e., EMC or EMD). To determine if calcium and voltage oscillations are in phase, we need to examine the linear eigenvector (δA , δc) corresponding to λ_c^- , which governs the dynamics near the fixed point: $A_n = A_0 + \delta A(\lambda_c^-)^n$ and $c_n = c_0 + \delta c(\lambda_c^-)^n$. To dominant order, this eigenvector is given by

$$\delta A = -\left(\frac{J_{12}}{J_{11} - J_{22}}\right) \delta c. \tag{11}$$

Since $J_{11}-J_{22}$ is always positive from Eqs. (6) and (7), alternans are EMD if $J_{12}>0$, in which case δA has the opposite sign as δc , and, in addition, $J_{12}J_{21}>0$, such that calcium alternans are spatially concordant and generate voltage alternans. If $J_{12}>0$ and $J_{12}J_{21}<0$, SCA are EMD but have a smaller amplification rate than SDA that are preferred and do not generate voltage alternans. Thus, in this case, SDA should be observed at the whole cell level.

B. Node motion for arbitrary weak coupling

So far, we have examined the relative stability of the spatially concordant and discordant states. In order to interpret our simulation results, it is also useful to predict the motion of the node separating spatially out-of-phase regions. This is possible using an amplitude equation approach⁸ as detailed in Appendix B. The main result is an expression for the velocity of the node

$$\frac{dx_N}{dt} = 6\frac{x_N(t)}{L} \left(\frac{J_{12}J_{21}}{J_{11} - J_{22}}\right) \sqrt{\frac{D}{2T|1 + J_{22}|}},$$
(12)

where $x_N(t)$ denotes the deviation of the node from the midpoint of the cell along the long axis of the cell, L is the cell length, T is the pacing period, D is the diffusion coefficient of free Ca^{2+} in the cytosol, and this expression is strictly valid for weak voltage-calcium coupling $|J_{12}J_{21}/(J_{11}-J_{22})|$ $\ll 1$ and close to the calcium alternans bifurcation $|1+J_{22}|$ $\ll 1$. Since $J_{11} - J_{22}$ is always positive from Eqs. (6) and (7), the above expression implies that if $J_{12}J_{21} > 0$, the node is unstable and moves exponentially away from the midpoint of the cell, while if $J_{12}J_{21} < 0$, it is stable and returns to the midpoint if perturbed slightly. Thus the condition governing node stability turns out to be identical to the one governing the relative stability of the spatially concordant and discordant states derived above, with the node being unstable (stable) in the case where the spatially concordant (discordant) state has a faster exponential amplification rate. While physically intuitive, the coincidence of those two stability conditions is nonetheless nontrivial since node motion measures the stability of a fully developed nonlinear state of SDA, while the relative stability condition compares the amplification rate of linear perturbations of the spatially homogeneous periodic state without alternans.

It is important to emphasize that Eq. (12) allows us to deduce the sign and magnitude of the voltage-calcium coupling parameter $J_{12}J_{21}$ from the observation of node motion. The sign of the coupling can be deduced from whether the node is trapped or expelled. Furthermore, a magnitude of coupling much smaller than unity implies that the node moves slowly across the cell over many beats as observed in the present simulations. One can further deduce the relative magnitudes of J_{12} and J_{21} from the relative amplitudes of voltage and calcium alternans using Eq. (11). In the present simulations (see Fig. 12), voltage and calcium alternans have comparable amplitudes. We deduce that $|J_{12}|$ is of order unity and thus that $|J_{21}| \ll 1$ since $|J_{12}J_{21}| \ll 1$ from the slow node motion. Physically this means that changes in the calcium transient amplitude have a strong effect on action potential duration $(|J_{12}| \sim O(1))$ but that changes in action potential duration have a weak effect on calcium transient amplitude $(|J_{21}| \ll 1).$

C. Bistability of spatially concordant and discordant alternans for weak negative coupling

For weak negative coupling, the above analysis predicts that the node of SDA is attracted to the center of the cell. Using the results of Sec. V A, only the spatially discordant state can form if $0 < |1+J_{22}| < |J_{12}J_{21}/(J_{11}-J_{22})|$, where $|1+J_{22}|$ measures the degree of instability of the calcium cycling system; the first inequality reflects that the instability of the spatially discordant state is not influenced by voltage and the second inequality follows from Eq. (9). For $|1+J_{22}| > |J_{12}J_{21}/(J_{11}-J_{22})|$, in contrast, the period one state without

alternans is unstable to both SDA and SCA perturbations, and the question arises as to what nonlinear state is dynamically selected. As shown in Appendix C, the nonlinearly saturated SCA state is unstable for $|J_{12}J_{21}/(J_{11}-J_{22})| < |1+J_{22}| < (3/2)|J_{12}J_{21}/(J_{11}-J_{22})|$ and stable for

$$|1 + J_{22}| > (3/2)|J_{12}J_{21}/(J_{11} - J_{22})|.$$
(13)

The nonlinearly saturated SDA state, in turn, is stable for $|1+J_{22}|>0$. Thus if condition (13) is satisfied, and $J_{12}J_{21} < 0$, both SDA and SCA are stable nonlinear states. Since the voltage-calcium coupling $|J_{12}J_{21}/(J_{11}-J_{22})| \le 1$ in our simulations, these two states are always bistable except in a negligible parameter range very close to the alternans bifurcation. This bistability leads to a nonuniqueness of the dynamically selected state for negative coupling that has been noted in previous studies of coupled cells and tissue.³³⁻³⁵

The analysis of the weak negative coupling limit makes it possible here to obtain a simple analytical prediction of the condition for bistability and the results will also hold for two coupled cells in this limit.

D. Interpretation of numerical results

Let us now turn to the interpretation of our simulation results. First, for the same parameters of case A which resulted in node motion away from the midpoint when the initial conditions were a spatially discordant state (thin solid line in Fig. 10), we found that a spatially concordant state developed as the instability threshold was crossed by increasing the recovery time for the RyR channels in small steps. Similarly, for case D that resulted in node motion toward the midpoint when the initial conditions were a spatially discordant state (thick solid line in Fig. 11), we found that a spatially discordant state developed as the instability threshold was crossed by increasing the recovery time for the RyR channels in small steps. Thus, in the simulations, the development of a spatially concordant or discordant state at the whole cell level is intimately linked to node motion, as predicted by the theory. The reason to use the recovery time of the RyR channels as the controlling parameter for the instability rather than the stimulation period is that, for the present parameters, SDA form spontaneously only at fast pacing. Thus, slowly decreasing the period would lock alternans in the concordant state before the discordant regime was reached. In practice, there are various parameters, other than the period, that could slowly change promoting alternans (e.g., changes associated with heart failure^{3b}).

Second, for case D, we observed that both the SDA and SCA states could be obtained from different initial conditions. This observation is consistent with the prediction of Eq. (13) that both states are stable attractors of the dynamics for weak negative coupling in a regime of well-developed alternans. We also found that both SDA and SCA have essentially the same amplitude, as shown in Fig. 14, consistent with theoretical predictions of the nonlinear amplitudes of those states in Appendix C.

Third, we observed that node motion was faster when the magnitude or effect of the calcium-dependent membrane



FIG. 14. (Color online) Steady-state amplitude of the transversal calcium average in even beats minus its time average for case D in the spatially concordant state, for case D in the spatially discordant state, and for case C which is only spatially concordant in steady state. Consistent with the theory in Appendix B and the very slow node motion observed in the simulations, the amplitudes for the spatially concordant and discordant states in case D are very close. The dashed lines are fits to constants for the spatially concordant states and a hyperbolic tangent as in Eq. (B8) for the spatially discordant state. The period is T=200 ms.

currents (LCC and/or NCX) was increased in the simulations. The magnitude of those currents determines the strength of the bidirectional coupling between membrane voltage dynamics and calcium cycling measured by $|J_{12}J_{21}|$. Thus, an increase in the magnitude of those currents should translate into an increase in $|J_{12}J_{21}|$ and hence a faster node motion according to Eq. (12) as seen in the simulations. For example, a reduction in the NCX current by a factor of 5 (case B) decreases node motion velocity by roughly a factor of 2 (compare cases A and B in Fig. 10). An increment in the effective membrane capacitance for the calcium current by a factor of 2, reducing the effect of calcium on the action potential, but not the calcium dynamics, results also in slower node motion (cases C and D in Fig. 11). From the comparison of theory and simulations, it can also be inferred that the relationship between the magnitude of calcium-dependent membrane currents and $|J_{12}J_{21}|$ must be highly nonlinear since a fivefold decrease in the NCX current resulted in a node velocity only a factor of 2 slower. In addition to the nonlinear dependence of $|J_{12}J_{21}|$ on these currents, J_{22} and J_{11} might also change. While a nonlinear relationship between those membrane currents and $|J_{12}J_{21}|$ is to be expected since the voltage and calcium systems are nonlinearly coupled, the precise form of this relationship is generally very difficult to determine since the form of the iterative maps cannot be easily derived quantitatively from the underlying physiologically detailed ionic model. From this standpoint, Eq. (12) provides a useful means to extract the sign and the strength of the voltage-calcium coupling, as measured by $J_{12}J_{21}$, from the observation of node motion, with the sign and magnitude of this coupling determined by node stability and velocity, respectively.

Fourth, we found that although the deterministic dynamics of the coupled calcium-voltage system predicts the basic features of node motion (i.e., the node being expelled or attracted to the center), stochastic effects result in somewhat different node trajectories for simulations with identical parameters (see the three top curves in Fig. 11). Moreover, stochastic effects account for the drifting node motion in the unclamped case. The clamped simulations have a perfectly periodic voltage, which implies $J_{12} \equiv 0$. Thus, Eq. (12) predicts no node motion in the clamped case, in agreement with the observations in Ref. 6. However, fluctuations result in drifting motion of the node as observed in Fig. 10. This drifting motion might eventually lead to SCA if the node is expelled due to its drifting motion.

Fifth, we have found that SCA can develop with voltage alternans in or out of phase with calcium alternans. As we have seen, this is consistent with the prediction that spatial synchronization requires $J_{12}J_{21} > 0$, while the relative phase of voltage and calcium alternans is governed by the sign of J_{12} in Eq. (11). This prediction is simplest to understand by noting that the peak calcium transient amplitude is determined predominantly by the peak amplitude at the previous beat, and more weakly by the voltage history. Thus J_{12} can be approximated using the chain rule as $J_{12} \approx (\partial A_n / \partial c_n)$ $\times (\partial c_n / \partial c_{n-1})$. Since $\partial c_n / \partial c_{n-1} = J_{22} < 0$, J_{12} is negative only if $\partial A_n / \partial c_n < 0$, or when a larger peak calcium transient amplitude produces a shorter action potential duration. This is expected to occur when the balance between LCC and NCX is shifted toward LCC, consistent with the observation that voltage and calcium alternans are out of phase in our simulations when NCX is reduced by 80% of its normal value (cases B and D).

Let us contrast our present results with those of Ref. 8. In Ref. 8, it was concluded that SDA can only develop under conditions where voltage alternans are out phase with calcium alternans, in apparent disagreement with the present analysis. The analysis of Ref. 8 used a semi-implicit formulation of the maps of the form $A_n = F_1(A_{n-1}, c_n)$ and c_n $=F_2(A_{n-1}, c_{n-1})$. This form is equally general than the form the identification $f_1(A_{n-1}, c_{n-1})$ used here with $=F_1(A_{n-1},F_2(A_{n-1},c_{n-1}))$ and $f_2(A_{n-1},c_{n-1})=F_2(A_{n-1},c_{n-1}).$ In this semi-implicit map formulation, the voltage-calcium coupling is given by $J_{12}J_{21} = (\partial A_n / \partial c_n)(\partial c_n / \partial c_{n-1})$ $\times (\partial c_n / \partial A_{n-1})$ and, like here, $J_{12}J_{21}$ must be negative for SDA to have a faster exponential amplification rate of linear perturbations. In Ref. 8, however, it was assumed that $\partial c_n / \partial A_{n-1}$ must be negative because the amount of SR calcium release increases with the amount of calcium entry into the cell via LCC (the so-called graded release property). Since a shorter action potential duration at the previous beat $(\partial A_{n-1} < 0)$ provides more time for LCC to recover from inactivation after repolarization, it may be expected for a larger number of LCC channel openings to trigger a larger release at the next beat $(\partial c_n > 0)$. Based on this reasoning, it was assumed in Ref. 8 that $\partial c_n / \partial A_{n-1} < 0$. Since $\partial c_n / \partial c_{n-1} < 0$ when alternans are calcium driven, this assumption implies that $J_{12}J_{21} < 0$ only if $\partial A_n / \partial c_n < 0$, which is also the condition for alternans to be EMD. This assumption held true for the simulations of the ionic model of Ref. 8 but not for the present model. In general, a shorter action potential duration at the previous beat also influences the calcium transient during that beat in a way that can influence the amount of release at the next beat independently of LCC magnitude. Thus we conclude that the sign of $\partial c_n / \partial A_{n-1}$ is controlled by a subtle balance of different effects and can generally be positive or negative.

VI. SUMMARY AND FUTURE PROSPECTS

In summary, we have investigated the spatiotemporal dynamics of intracellular calcium under conditions relevant for heart rhythm disorders where the calcium concentration exhibits period doubling oscillations. This work distinguishes itself from previous studies by the use of a spatially distributed model of calcium dynamics with a large number of diffusively coupled CRUs interacting with the membrane voltage system. This model allowed us to investigate the effect of stochasticity of LCC and RyR channel opening and closing in each nanoscopic unit on whole cell macroscopic dynamical behavior on a tenth of a millimeter scale.

The main conclusion is that stochasticity can alter the whole cell dynamics noticeably in the vicinity of the alternans bifurcation. The main effect is to spontaneously reverse the phase of alternans over a time scale of several hundred beats that is roughly comparable to the time necessary for free calcium to diffuse across the length of the cell. These phase reversal events should be experimentally measurable and distinguishable from phase reversal events occurring in one beat due to spontaneous calcium release in one region of the cell during calcium alternans. Such measurements could potentially be used to infer whether alternans are predominantly calcium or voltage driven in a given experiment since membrane ion channel stochasticity is typically completely averaged out at the whole cell level. This averaging-out process traditionally justifies the use of a "deterministic" Hodgkin-Huxley description for the action potential. In contrast, here, the action potential dynamics cannot be described purely deterministically because of its coupling to calcium dynamics where fluctuations remain important at the whole cell level. This is so because the coupling between CRUs mediated by calcium diffusion is short ranged, i.e., calcium only diffuses a short distance on the time scale of one beat, while the coupling between membrane ion channels mediated by voltage diffusion is long ranged due to the fact that voltage diffuses quasi-instantaneously across the cell in one beat.

Since the phase of alternans is discrete (i.e., degenerate by π), the amplitude of alternans has the same symmetry as the coarse-grained magnetization in an Ising model of a ferromagnetic phase transition. This analogy suggests that the fluctuations of alternans phase and amplitude near the alternans bifurcation are a form of Ising-like critical behavior where channel stochasticity and diffusive coupling of CRUs play analogous roles as temperature and ferromagnetic coupling of spins. Exploring this analogy is worth further investigation.

The model was also used to investigate a different alternans behavior by varying physiological parameters that alter the sign and/or magnitude of the bidirectional coupling between calcium and voltage by changing the balance between different calcium-dependent membrane currents (LCC and NCX). Physically, and quite generally, for positive coupling, the voltage alternans generated by calcium alternans tend to increase the amplitude of calcium alternans, thereby mediating a positive feedback of calcium alternans on itself. In contrast, for negative coupling, voltage alternans tend to suppress calcium alternans, thereby mediating a negative feedback of calcium alternans on itself. Mathematically, the coupling is determined by the product of the off-diagonal terms $J_{12}J_{21}$ in a general 2D map of the beat-to-beat dynamics.

We have shown that the sign and magnitude of this coupling can be inferred directly from the motion of the node separating spatially out-of-phase regions of alternans in a simulation seeded with SDA. For positive coupling, the node is expelled from the cell and calcium alternans synchronize in the whole cell, while for negative coupling, the node is attracted to the midpoint of the cell and the SDA state is dynamically stable. In addition, the magnitude of the coupling, determined by the magnitude of calcium-dependent membrane currents and pacing cycle length, determines how fast the node moves. It may be possible to test these predictions experimentally by seeding a SDA state, e.g., by reversing the phase of alternans locally with a calcium wave induced by a localized caffeine pulse or by local photorelease of caged calcium.

As previously predicted in Ref. 8, negative coupling produces a Turing-like instability that favors SDA, while positive coupling favors SCA. Our results validate this prediction but also show that positive coupling does not necessarily imply that action potential duration and calcium alternans are EMC. For one set of parameters (case B), we find that alternans can be EMD for positive coupling, with the sign of the coupling deduced from node motion. For another set of parameters that corresponds to the Turing instability (case D), EMD alternans can also form because of the bistability of the spatially concordant and discordant nonlinear states in this case. Since the concordant state is easier to attain from a change in condition corresponding to reducing the pacing cycle length, we conclude that the observation of EMD alternans at the whole cell level does not suffice to determine the sign of the voltage-calcium coupling. Electromechanical discordance alone implies that calcium alternans are spatially synchronized over a large enough fraction of the cell to produce voltage alternans. It does not, however, predict whether a spatially discordant state obtained from a different set of initial condition would be stable or transient.

These results highlight the difficulty of trying to deduce the sign of the voltage-calcium coupling from physiological considerations. In previous work, it was argued that this coupling is determined by the balance of calcium-dependent membrane current (LCC and NCX),^{21,33} which determines generally if a larger calcium transient prolongs or shortens the action potential duration (calcium effect on voltage). This determination, however, rests on the assumption that the effect of voltage on calcium is positive. Namely, a longer diastolic interval after one beat helps to produce a larger calcium transient at the next beat. Clearly, our results show that the effect of voltage on calcium can also be negative since we observe well-formed EMD alternans for negative overall coupling that combines the effects of calcium on voltage and voltage on calcium. The physiological origin of a negative effect of voltage on calcium, whereby a longer diastolic interval helps to promote a shorter calcium transient at the next beat, remains to be elucidated. In addition, the present study did not systematically survey a large range of physiological parameters given the length of computer time required for each simulation in this spatially distributed model. Therefore it largely remains to be determined what sign of coupling underlies EMD alternans in experimental situations under different pathological conditions.

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APPENDIX A: SIMULATION OF COUPLED CALCIUM AND VOLTAGE

The basis for our simulations is the model for intracellular calcium dynamics developed in Ref. 7 coupled with the set of membrane currents in Ref. 27. Although we will not give a complete description of these models here, we will describe the basic features and the way they the two models are coupled. For the equations that model the currents and functions presented below, see Refs. 7 and 27.

The calcium model is based on the calcium dynamics of a single CRU, with the full model consisting of a 62×32 $\times 12$ 3D grid of diffusively coupled CRUs. Below we describe calcium dynamics in the m^{th} CRU. To simplify the notation, we will omit here the superscript (m) with the understanding that the currents and concentrations refer to those at the m^{th} CRU. The time evolution of the calcium concentration in the cytosolic, submembrane, proximal, network SR, and junctional SR compartments of the m^{th} CRU (see Fig. 3) is governed, respectively, by the differential equations

$$\begin{split} \dot{c}_{i} &= \beta_{i}(c_{i}) \left(I_{\rm dsi} \frac{v_{s}}{v_{i}} - I_{\rm up} + I_{\rm leak} - I_{\rm TCi} + I_{ci} \right), \\ \dot{c}_{s} &= \beta_{s}(c_{s}) \left(I_{\rm dps} \frac{v_{p}}{v_{s}} + \gamma_{\rm NCX} I_{\rm NCX} - I_{\rm dsi} - I_{\rm TCs} + I_{cs} \right), \\ \dot{c}_{p} &= \beta_{p}(c_{p}) (I_{r} + \gamma_{\rm Ca} I_{\rm Ca} - I_{\rm dps}), \\ \dot{c}_{\rm NSR} &= \left((I_{\rm up} - I_{\rm leak}) \frac{v_{i}}{v_{\rm NSR}} - I_{\rm tr} \frac{v_{\rm JSR}}{v_{\rm NSR}} + I_{c\rm NSR} \right), \\ \dot{c}_{\rm JSR} &= \beta_{\rm JSR}(c_{\rm JSR}) \left(I_{\rm tr} - I_{r} \frac{v_{p}}{v_{\rm ISR}} \right), \end{split}$$
(A1)

where the β factors account for instantaneous calcium buffering, and v_i , v_s , v_{NSR} , v_{JSR} , and v_p are the volumes of the cytosolic, submembrane, network SR, junctional SR, and proximal compartments, respectively. $I_{\text{TC}i}$ and $I_{\text{TC}s}$ account for time-dependent buffering to Troponin-C. I_{dsi} and I_{dps} are diffusive currents between the submembrane and cytosolic and between the proximal and submembrane compartments of the CRU. I_{up} , I_{leak} , I_r , and I_{tr} are the uptake, leak, release, and transfer currents, describing calcium cycling into, out of, and within the compartments of the SR. I_{ci} , I_{cs} , and I_{cNSR} are diffusive currents coupling the cytosolic, submembrane, and network SR compartments of adjacent CRUs (three lower arrows in Fig. 3). The calcium current I_{Ca} and the sodium-exchanger current I_{NCX} are calcium transmembrane currents for a single CRU and provide the coupling between the calcium and voltage systems. The factors γ_{NCX} and γ_{Ca} allow us to scale the calcium currents to simulate pharmacological or genetic interventions. Normally, $\gamma_{NCX}=1$ and $\gamma_{Ca}=1$, but we also consider the case $\gamma_{NCX}=0.2$.

In the unclamped simulations, the transmembrane voltage, assumed to be constant throughout the cell, evolves according to

$$\frac{dV}{dt} = \frac{1}{C_M} \left(I_{\text{Na}} + I_{\text{K1}} + I_{\text{Kr}} + I_{\text{Ks}} + I_{to} + \frac{\overline{I}_{\text{NCX}}}{\alpha_{\text{NCX}}} + \frac{\overline{I}_{\text{Ca}}}{\alpha_{\text{Ca}}} + I_{\text{stim}} \right),$$
(A2)

where I_{Na} is the sodium current, and I_{K1} , I_{Kr} , I_{Ks} , and I_{to} are repolarizing potassium currents which we take from the UCLA model.²⁷ I_{stim} is a stimulus current applied periodically to stimulate an action potential. $\overline{I}_{\text{NCX}}$ and \overline{I}_{Ca} are the whole-cell NCX and calcium currents, obtained from those for a single CRU as

$$\overline{I}_{\text{NCX}} = \sum_{m} \gamma_{\text{NCX}} I_{\text{NCX}}^{(m)}, \quad \overline{I}_{\text{Ca}} = \sum_{m} \gamma_{\text{Ca}} I_{\text{Ca}}^{(m)}.$$
(A3)

The constant C_M in Eq. (A2) is the membrane capacitance, $C_M=1 \ \mu F/cm^2 \times A$, where the cell membrane area A was estimated as $2 \times 10^4 \ \mu m^2$. The parameters $\alpha_{\rm NCX}$ and $\alpha_{\rm Ca}$ are factors that take into account the fact that the NCX and calcium currents might have different effective areas of effect²⁹ $A_{\rm NCX}$ and $A_{\rm Ca}$, so that $\alpha_{\rm NCX}=A/A_{\rm NCX}$ and $\alpha_{\rm Ca}=A/A_{\rm Ca}$. We choose the values of these parameters so that the action potential shape is physiological. Normally, we take $\alpha_{\rm NCX}=2$ and $\alpha_{\rm Ca}=1$, but we also consider the case $\alpha_{\rm NCX}=2$ and $\alpha_{\rm Ca}=2$.

Each CRU has four LCC channels described by the seven-state Markov model of Ref. 27 that contribute to the I_{Ca} current of the CRU as described in Ref. 7. In addition, each CRU has 100 Markov RyR channels, simulated efficiently as described in Ref. 7. Following Ref. 20 we adopt a phenomenological expression for the internal sodium concentration $[Na^+]_i$ as a function of the pacing period *T*, taking $[Na^+]_i = a(1+bT^{1/2})^{-1/2}$, where a=78 m*M*, b=10 s^{-1/2}, and *T* is in seconds. The proximal volume of each CRU is drawn from a truncated Gaussian distribution, $v_p^{(n)} = \bar{v}_p(1+r)$, where $\bar{v}_p = 0.001 26 \ \mu m^3$ and *r* is a Gaussian distribution with standard deviation 0.3 restricted to the interval (-0.8,0.8).

Lastly, we note that in Ref. 7, $c_{\rm JSR}$ in Eq. (40) should read $c_{\rm NSR}$. The term $(\tau_{ps}^{-1} + \tau_{si}^{-1} + \tau_{nm}^{-1})$ in Eq. (54) should read $(v_p \tau_{ps}^{-1} / v_s + \tau_{si}^{-1} + \Sigma_m \tau_{nm}^{-1})$, and $v_{\rm NaCa}$ in Table (7) should be 21.0.

APPENDIX B: ANALYSIS OF NODE MOTION

To derive an equation of motion for the node, we use the amplitude equation framework, where the peak calcium concentration at beat n and position x is written in the form

$$c_n(x) = c_0 + (-1)^n c(x, n), \tag{B1}$$

where c(x,n) is the calcium alternans amplitude that evolves slowly from beat to beat close to the alternans bifurcation, $|J_{22}+1| \le 1$. This slow evolution allows us to treat the beat number *n*, or equivalently the time t = nT, as a continuous variable and to write

$$(-1)^n 2T \frac{\partial c(x,t)}{\partial t} = c_{n+2}(x) - c_n(x).$$
 (B2)

At the end of this calculation, we shall find that the node speed $\sim |J_{12}J_{21}/(J_{11}-J_{22})|$. Hence, when $|J_{12}J_{21}/(J_{11}-J_{22})| \leq 1$, voltage alternans relax rapidly on the time scale of motion of the node as long as the restitution slope is not too close to unity, or $J_{11}+1 \sim O(1)$. In this limit, the rapidly diffusing voltage acts as a global coupling of calcium alternans in different regions of the cell. Accordingly, it is natural to assume phenomenologically the equation governing the evolution of calcium alternans to have the form

$$\frac{\partial c(x,t)}{\partial t} = \delta c(x,t) + \kappa \overline{c}(t) - \chi c(x,t)^3 + D \frac{\partial^2 c(x,t)}{\partial x^2}, \quad (B3)$$

where \overline{c} denotes the spatial average of c over the length of the cell with the origin of x at the midpoint of the cell,

$$\overline{c}(t) \equiv \frac{1}{L} \int_{-L/2}^{L/2} c(x,t) \tag{B4}$$

and *D* is the diffusion coefficient of free calcium inside the cell. The linear coefficients $\delta \ll 1$ and $\kappa \ll 1$ can be determined by matching the exponential amplification rates of spatially concordant and discordant perturbations of the c=0 state to those obtained from the iterative maps, which, using the fact that $|J_{22}+1| \ll 1$, yields at once

$$\delta \approx -\frac{(1+\lambda_d)}{T} = -\frac{(1+J_{22})}{T}$$
(B5)

for the discordant mode and

$$\delta + \kappa \approx -\frac{(1+\lambda_c)}{T} \approx -\frac{1}{T} \left(1 + J_{22} - \frac{J_{12}J_{21}}{J_{11} - J_{22}} \right)$$
(B6)

for the concordant mode, from which we obtain that

$$\kappa \approx \frac{1}{T} \left(\frac{J_{12} J_{21}}{J_{11} - J_{22}} \right). \tag{B7}$$

Equation (B3) has a stationary solution

$$c_s(x) = \sqrt{\frac{\delta}{\chi}} \tanh \frac{x}{\sqrt{2D/\delta}},\tag{B8}$$

corresponding to a node at the midpoint of the cell. Since the node moves slowly, we can compute its velocity $v = dx_N(t)/dt$, where $x_N(t)$ is the position of the node at time *t*, by looking perturbatively for solutions of the form $c(x,t) \approx c_s(x-x_N(t)) + \tilde{c}(x-x_N(t),t)$, which is equivalent to transforming Eq. (B3) into a moving frame. Substituting this ansatz into Eq. (B3) we obtain after linearization

$$\left(\delta - 3\chi c_s^2 + D\frac{\partial^2}{\partial x^2}\right)\tilde{c} \equiv \mathcal{L}\tilde{c} = -v\frac{\partial c_s}{\partial x} - \kappa\bar{c}_s,\tag{B9}$$

where the terms on the right-hand side (rhs) can be assumed to be small and

$$\bar{c}_s \approx -\frac{2}{L} x_N(t) \sqrt{\frac{\delta}{\chi}} \tag{B10}$$

follows from Eq. (B8). For a nontrivial solution of Eq. (B9) to exist, the rhs must be orthogonal to the null space of the adjoint of the linear operator \mathcal{L} . Since \mathcal{L} is self-adjoint and the derivative of the stationary solution is a zero mode of \mathcal{L} (i.e., $\mathcal{L}\partial_x c_s = 0$), this yields the solvability condition

$$\int dx \left(-v \frac{\partial c_s}{\partial x} - \kappa \overline{c}_s \right) \frac{\partial c_s}{\partial x} = 0.$$
(B11)

Using the fact that $\int dx (\partial_x c_s)^2 = (2/3)(\delta/\chi)\sqrt{2\delta/D}$ and that $\int dx \partial_x c_s = 2\sqrt{\delta/\chi}$, we obtain

$$v = \frac{dx_N(t)}{dt} = -3\kappa \overline{c}_s \sqrt{\chi/\delta} \sqrt{D/\delta} = 6\kappa \frac{x_N(x)}{L} \sqrt{D/2\delta}, \quad (B12)$$

which is equivalent to Eq. (12) after substituting the expressions for δ and κ .

APPENDIX C: STABILITY ANALYSIS OF SPATIALLY CONCORDANT ALTERNANS

For negative coupling, the node is stable at the midpoint of the cell. The SDA and SCA nonlinear states have amplitudes $\sqrt{\delta/\chi}$ and $\sqrt{(\delta+\kappa)/\chi}$, respectively, where the SCA state bifurcates for $\delta > |\kappa|$. It follows that the difference between those amplitudes is essentially negligible in the weak negative coupling limit ($|\kappa| \le 1, \kappa < 0$) as long as $\delta \ge |\kappa|$. The latter condition is satisfied in the present simulations since calcium alternans develop over a few beats while the node motion takes place on a number of beats at least an order of magnitude larger. The linear stability of the nonlinear SCA state can be readily analyzed by substituting c(x,t) $= \sqrt{(\delta+\kappa)/\chi} + c_1(x,t)$ in the amplitude equation, which yields the evolution equation (B3) for the perturbation $c_1(x,t)$,

$$\partial_t c_1 = (-2\delta - 3\kappa)c_1 + \kappa \overline{c}_1 + D\partial_x^2 c_1.$$
(C1)

Substituting $c_1(x,t) \sim \exp(ikx + \omega_k t)$ in the above equation, we obtain that

$$\omega_k = -2\delta - 3\kappa - Dk^2 (k \neq 0), \tag{C2}$$

and the k=0 mode, which needs to be treated separately, is stable for $\delta > |\kappa|$. Neglecting diffusion effects for the smallest *k*-mode that can be fitted in a cell, $k=\pi/L$, we find that this mode is unstable if $-2\delta - 3\kappa > 0$ or $\delta < (3/2)|\kappa|$. In contrast, all modes are stable for $\delta > (3/2)|\kappa|$, which is the result of Eq. (13). Since SDA are stable for $\delta > 0$, both SCA and SDA are stable for $\delta > (3/2)|\kappa|$ and the state selected SCA on initial condition as seen in the simulations.

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