Estimating the biophysical properties of neurons with intracellular calcium dynamics

Jingxin Ye, Paul J. Rozdeba, and Uriel I. Morone

Department of Physics, University of California, San Diego, La Jolla, California 92093-0374, USA

Arij Daou

Department of Organismal Biology and Anatomy, University of Chicago, Chicago, Illinois 60647, USA

Henry D. I. Abarbanel*

Department of Physics and Marine Physical Laboratory (Scripps Institution of Oceanography) University of California, San Diego La Jolla, California 92093-0374, USA

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We investigate the dynamics of a conductance-based neuron model coupled to a model of intracellular calcium uptake and release by the endoplasmic reticulum. The intracellular calcium dynamics occur on a time scale that is orders of magnitude slower than voltage spiking behavior. Coupling these mechanisms sets the stage for the appearance of chaotic dynamics, which we observe within certain ranges of model parameter values. We then explore the question of whether one can, using observed voltage data alone, estimate the states and parameters of the voltage plus calcium (V+Ca) dynamics model. We find the answer is negative. Indeed, we show that voltage plus another observed quantity must be known to allow the estimation to be accurate. We show that observing both the voltage time course V(t) and the intracellular Ca time course will permit accurate estimation, and from the estimated model state, accurate prediction after observations are completed. This sets the stage for how one will be able to use a more detailed model of V+Ca dynamics in neuron activity in the analysis of experimental data on individual neurons as well as functional networks in which the nodes (neurons) have these biophysical properties.

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I. INTRODUCTION

A. Dynamical model completion

In developing and using methods of statistical data assimilation to characterize the biophysical properties of functional networks of neurons, we have previously built a Hodgkin-Huxley-type model (HH model) of the dynamics of individual neurons [1–3]. This model, as all such models, has numerous unknown fixed parameters that must be determined for each class of neuron under consideration. We used well-designed stimulating currents for individual neurons in the avian song system nucleus high vocal center (HVC) and measured the response of the membrane voltage to estimate the many biophysical parameters in the voltage and kinetic equations of such a model. This estimation “completes” the model in the sense that once the fixed parameters are established, then given initial conditions for the state variables, observed and unobserved, we can predict the response of the model neuron to a new stimulus [3].

We tested and validated the biophysical HH model by showing that with the estimated parameters it could reliably predict the observed response to new stimulating currents. The required initial conditions for prediction in the completed model were established by using a very short (100 ms) segment of the data set for times beyond the observation window.

The methods we utilize here were quite instrumental in designing the large collection of data sets analyzed in Ref. [3], and we expect that to be the case again when we move from simulations of neurons with important Ca^{2+} dynamics to the design of experiments to explore those dynamics.

\*habarbanel@ucsd.edu
be those where chaotic solutions to the dynamical equations are possible for some biophysically plausible set of stimuli and model parameters. We found that $Ca^{2+}$ dynamics could cause failure in determining properties of neurons with voltage measurement only.

Many examples are known of the necessity for more measurements at each measurement time to remove impediments associated with the instability of the manifold in state space where the data and the model output are synchronized [3]. When synchronization fails, the synchronization error Eq. (1) has multiple local minima as a function of the parameter or state value sought through the minimization. This impediment to estimation must be regulated to provide a smooth surface on which one implements a search procedure for the minimum of the cost function Eq. (1).

There is now substantial evidence of the role of $Ca^{2+}$ dynamics in the neurons of HVC [4–8]. This adds a set of rather slow dynamical processes to the much faster voltage-gated processes involving $Na^+$ and $K^+$ ions. When we extended these voltage dynamics models to include the important biophysical processes of $Ca^{2+}$ uptake and release from internal stores, the question of how many measurements are required changes. Mixing slow and fast dynamical processes, coupled nonlinearly to each other, can be a setting for the appearance of chaotic behavior. In the case of $V+Ca$ dynamics chaos does appear.

The appearance of chaos in calcium oscillations is quite natural. Calcium bursting behavior characterized by an irregular number of secondary spikes and irregular spacing between the initial spikes and nonperiodic oscillations with varying amplitudes are observed widely under different stimuli [9–11]. Seemingly erratic, or irregular, time-series behavior with little apparent structure in the time course can be generated by a deterministic system operating in chaotic regime. This inspired many analyses of chaotic calcium models [12–14]. Houart et al. produced chaos by extending the simpler Dupont-Goldbeter [15] model to account for fluctuating IP$_3$ concentrations [13]. The IP$_3$ concentration is synthesized by the introduction of $Ca^{2+}$ ions, and in turn prompts $Ca^{2+}$ release from internal stores. This simple feedback leads to oscillatory behavior that results in chaos. Borghans et al. found similar chaotic behavior when accounting for a variable IP$_3$ concentration [12]. Feedback dynamics can easily result in systems with chaotic regimes, so it is instructive to analyze the difficulties of estimating such a system.

This leads to a situation where more than voltage measurements alone are required

1. to synchronize data with model output,
2. accurately estimate fixed parameters and unobserved state variables, and
3. provide accurate predictions as validation of the consistency of the model with the observations.

The goal of this paper is to determine the difficulties of estimating model parameters and state variables in a model that exhibits chaotic oscillations. The model presented is not intended to be a quantitatively accurate description of real neurons. Rather, our more modest aim is to present a system which replicates qualitative features that we anticipate in the HVC neurons of the avian song system. HVC and the other nuclei of the song system are our target neurons for the experiments we plan [3], so our focus on their properties will appear several times within the general issues in this paper.

In particular, our model exhibits spiking bursts and has regimes of chaotic behavior. These features make it challenging to correctly estimate parameters and unmeasured state variables, as was described in Ref. [3]. Indeed, we show that the model in this paper requires more than just voltage measurements to completely estimate the full state of the model and use those estimates to make accurate predictions as a test of model validity.

### B. The biophysical role of $Ca^{2+}$ dynamics

Calcium ions play an important role in regulating a great variety of neuronal processes. Calcium can act in signal transduction resulting from activation of ion channels or as a second messenger caused by indirect signal transduction pathways. Intracellular calcium signals regulate processes that operate over a wide range of time scales, from neurotransmitter release at the microsecond scale to gene transcription, which lasts for minutes and hours [16]. In the HVC of the songbird, the contributions of calcium channels and calcium-mediated events to spiking and bursting have been observed. In vivo, L-type $Ca^{2+}$-bursting activity in HVC$_{RA}$ neurons [8], and calcium transients show strong preference for the presentation of the bird’s own song in identified HVC neurons where a strong correspondence between calcium signals and juxtacellular electrical activity is exhibited [5]. In vitro, T-type low-voltage-activated Ca channels are expressed in HVC neurons contributing to their postinhibitory rebound firing [7]. Moreover, the spike frequency adaptation seen in HVC projection neurons is largely due to a $Ca^{2+}$-induced K channel [7,17]. In addition, multiple calcium-binding proteins that determine the dynamics of free calcium inside neurons [18] are enriched in the HVC of songbirds [4,6]. All these studies motivate an investigation of the roles that $Ca^{2+}$ ions play in the electrical activity of HVC neuronal subpopulations in vitro and during singing.

Calcium is a crucial intracellular messenger in mammalian neurons where the final transduction of any neuronal signal involves the movement of calcium ions. At rest, the intracellular calcium concentration of most neurons is about 50–100 nM, and that can rise to levels that are 10 to 100 times higher during electrical activity [19]. The cytosolic calcium concentration is determined by the balance between calcium influx and efflux as well as the exchange of calcium with internal stores. In addition, calcium-binding proteins such as parvalbumin or calretinin, acting as calcium buffers, determine the dynamics of free calcium inside neurons. Most importantly, only the free calcium ions inside the cytosol are biologically active.

Calcium influx from the extracellular space is controlled by various mechanisms, including voltage-gated calcium channels, ionotropic glutamate receptors, nicotinic acetylcholine receptors, and transient receptor potential type C channels [20–23]. The extrusion of calcium ions from the cytosol is done via the plasma membrane calcium ATPase and the sodium-calcium exchanger [16]. The release of messenger calcium ions from internal stores, mostly the endoplasmic reticulum (ER), is controlled by the inositol trisphosphate
receptors and ryanodine receptors [24]. The high calcium level inside the ER is controlled by the sarcoplasmic reticulum calcium ATPase pump that transports calcium ions from the cytosol to the ER.

In addition to the ER, mitochondria can also play the role of calcium buffers by absorbing calcium ions during cytosolic calcium elevations via the calcium uniporter and then releasing the calcium ions back to the cytosol slowly via the sodium-calcium exchange [25].

Variation in the intracellular concentration of Ca$^{2+}$ ions, $[\text{Ca}^{2+}]_i(t) = Z_{\text{Ca}}(t)$, is governed by the flow of these ions through the cell membrane via voltage-gated channels, as well as by uptake and release by the ER as a storage device. These properties and more are discussed in many research papers [16,26], including a very informative review and summary by Falcke and colleagues [27].

Earlier work on coupling voltage and calcium dynamics [28,29], as well as the dynamics of Ca$^{2+}$ uptake and release [13], independent of its connection to voltage dynamics of Na$^+$ and K$^+$ ion flow, provide the foundation for the model we discuss here. The presence of Ca$^{2+}$ channels and their interaction with voltage dynamics in HVC neuron cells has been established by Ref. [7]. These results have strongly motivated us to explore the inclusion of Ca$^{2+}$ dynamics in the model utilized in conjunction with our analysis of experiments on HVC neurons [3].

Ca$^{2+}$ ions are released and stored in the ER predominately through the mediation of inositol 1,4,5-triphosphate (IP$_3$) in many cell types [27], and we will incorporate a model of these processes in our overall model of the cellular dynamics. This uptake and release via IP$_3$ mediation has been suggested as a source of Ca$^{2+}$ oscillations in the work of Houart et al. [13], and we adopt their model, with a faster time scale, compared to their original idea of its role in more global rhythms of animals. This is not the only candidate for incorporating calcium dynamics into neuronal processes; we have analyzed two other models of Ca$^{2+}$ dynamics with some care [28,29], but we do not report on them here.

Examples of the important role of calcium in intracellular dynamics abound. For instance, the permeability of some potassium channels in the cellular membrane are affected by the presence of calcium, and membrane calcium channels themselves may be voltage-gated [2,30]. The interplay between these two mechanisms acts to regulate firing patterns [31]. Additionally, calcium is known to be a major determinant in the potentiation and depression of excitatory synaptic strength, and thus is expected to play an important role in memory and learning. This is widely thought to underly how networks of neurons “rewire” and learn. While these processes and unobserved state variables using time series of observed quantities from experiments.

We do not yet use any of these models in the analysis of experiments in this paper, but we perform “twin experiments.” In these we generate data by solving the model with known parameters, and then by presenting observables such as membrane voltage $V(t)$ or intracellular Ca$^{2+}$ concentration $Z_{\text{Ca}}(t)$ to the model, we are able to examine our estimation methods in a controlled context.

When additional complex dynamics is introduced into a neuron model, such as the intracellular calcium dynamics we present here, the synchronization manifold of the model may become unstable [32]. This can make it impossible to estimate unknown parameters and unobserved states with voltage measurements alone, raising the following interesting questions:

1. How do calcium dynamics effect neuron behavior?
2. How many measurements are needed to synchronize such a neuron model with observed data after the introduction of calcium dynamics?
3. If other measurements are required in addition to membrane voltage, what can play that role?

We do not have full answers to these questions. However, in this paper, we construct a conductance-based neuron model, into which we couple a detailed model of intracellular calcium dynamics. Using this model, we investigate the estimation problems through numerical experiments to give a clear image of the biophysical issues raised via calcium dynamics. We will show that voltage measurements are not enough to “cure” the instabilities just noted, and we will address a solution to this.

When we move from simulations to experimental data, we expect to encounter the same issues in a richer context.

II. MODELS OF V+Ca DYNAMICS

A. Dynamics of voltage-dependent channels

We now describe the coupled model of voltage and Ca$^{2+}$ dynamics, beginning with the membrane voltage and currents. This is a single-compartment point neuron model, with ion channels comprising standard Na$^+$, K$^+$, and leak channels coupled to additional currents seen in the work of Ref. [7]: two voltage-gated Ca$^{2+}$ channels $I_{\text{Ca}_2}(t)$ and $I_{\text{Ca}_{\text{L}}}(t)$; an $h$ channel $I_h(t)$ with a long time constant and operating primarily in the hyperpolarized regime; and, crucially, a potassium channel $I_{K_{\text{Ca}}}$ whose conductance depends on the intracellular calcium concentration $Z_{\text{Ca}}(t)$. The consequences of the presence of $I_{K_{\text{Ca}}}$ are outlined in Sec. II B.

With these currents, the HH equation for the voltage dynamics has the form

$$\frac{d V(t)}{dt} = I_{\text{Na}}(t) + I_{K}(t) + I_{\text{leak}}(t) + I_{\text{Ca}_{\text{L}}}(t) + I_{\text{Ca}_2}(t) + I_{h}(t) + I_{\text{app}}(t),$$

where $C_m$ is the membrane capacitance and $I_{\text{app}}(t)$ is an externally applied current selected by us. The ion currents themselves are given by

$$I_{\text{Na}}(t) = g_{\text{Na}} [m(t)^3 h(t)] E_{\text{Na}} - V(t),$$

$$I_{K}(t) = g_{\text{K}} n(t)^4 [E_{\text{K}} - V(t)],$$

$$I_{L}(t) = g_{L} [E_{L} - V(t)],$$

$$I_{\text{Ca}_{\text{L}}}(t) = I_{\text{Ca}_2}(t) = I_{h}(t) = I_{\text{app}}(t).$$

$$I_{K_{\text{Ca}}}(t) = g_{K_{\text{Ca}}}[Z_{\text{Ca}}(t)] [E_{\text{K}} - V(t)],$$
TABLE I. Parameter values in the voltage dynamics.

| \( g_{Na} \) | 450 nS | \( E_{Na} \) | 45 mV | \( g_k \) | 50 nS | \( E_K \) | -90 mV | \( g_L \) | 2 nS | \( E_L \) | -70 mV | \( \kappa_{K/Ca} \) | 0.35 \( \mu \)M | \( C_m \) | 100 pF |

\[
I_{CaL}(t) = g_{CaL}V_0[V(t)][E_{Ca} - V(t)].
\]

\[
I_{CaT}(t) = g_{CaT}(a_{T0}[V(t)] b_{T0}[r_T[V(t)]] )[E_{Ca} - V(t)].
\]

\[
I_{K/Ca}(t) = g_{K/Ca} \frac{Z_{Ca}(t)^4}{Z_{Ca}(t)^4 + \kappa_{K/Ca}^4}[E_K - V(t)],
\]

\[
I_K(t) = g_K[0.3 r_T(t) + 0.7 r_T(t)][E_K - V(t)],
\]

where the values of parameters are given in Table I.

The gating variables \( X(t) = [h(t), n(t), r_T(t), r_T(t), r_L(t)] \) obey first-order kinetics according to the equations

\[
\frac{dX(t)}{dt} = \frac{X_0[V(t)] - X(t)}{\tau_X[V(t)]},
\]

where the voltage dependence of the \( X_0 \) and the \( \tau_X \)'s are given in Table II. The other four gating variables \( \{m, s, a_T, b_T\} \) are assumed to have fast time constants, so they do not have their own dynamics and in Eqs. (3) they are set to \( X_0(V) \).

As it stands so far, \( Z_{Ca}(t) \) only plays the role of an external time-dependent forcing of the neuron through the Hill function kinetics in \( I_{K/Ca}(t) \), as we have not yet introduced a dynamical equation for \( Z_{Ca}(t) \) itself. Note that \( Z_{Ca}(t) \) would have also entered into the equations for \( I_{CaL}(t) \) and \( I_{CaT}(t) \) had we used the Goldman-Hodgkin-Katz (GHK) form for the voltage-current relation. We did examine the effects of the change from the ohmic current dependence in \( I_{CaL}(t) \) and \( I_{CaT}(t) \) we use here to the full GHK form of these currents arising because of the substantial difference in \( \text{Ca}^{2+} \) concentration within and without the cell. There was no visible difference in the results we present relevant to our main question of the ability to estimate the states and parameters of the model neuron using voltage measurements alone. We retain the ohmic form here recognizing that use of the GHK formulation may be important elsewhere.

The model we used in Ref. [3] for describing the experimental data on stimulus and voltage response experiments we have conducted on HVC neurons comprised these currents, absent the \( I_{K/Ca}(t) \) current, along with additional Na and K currents. The manner in which some voltage-dependent conductances, especially \( I_h(t) \), was represented there is different in that model, and the Ca currents used GHK [2] voltage-current relations reflective of the 10 000:1 ratio of extracellular to intracellular \( \text{Ca}^{2+} \) concentrations. As we are concentrating here on the role of the added slow \( \text{Ca}^{2+} \) dynamics to be described in a moment, we adopted a subset of the full model used earlier.

When we utilize the lessons from the “twin experiment” analysis of this \( V + Ca \) model and select models with which to analyze the observed laboratory data, we will examine several variants, all with the same core issue as explored here, but with somewhat different realizations of the Calcium dynamics. To proceed, we select one version of Calcium dynamics: the model of Houart et al. [13].

B. Coupling \( \text{Ca}^{2+} \) into voltage dynamics

We introduce a calcium-dependent potassium current \( I_{K/Ca} \) as the first ingredient in coupling the voltage and calcium dynamics. The conductance of this channel depends on \( Z_{Ca}(t) \) through a Hill function, which has the generic form

\[
\mathcal{H}(x, \kappa, n) = \frac{x^n}{x^n + \kappa^n},
\]

where the Hill coefficient \( n \) is a positive integer. This type of \( I_{K/Ca} \) is also referred to as a small-potassium (SK) current, distinct from a so-called big-potassium (BK) current, whose conductance has dependence on both voltage and intracellular calcium [30]. This gives rise to a \( K/Ca \) current of the form

\[
I_{K/Ca}(t) = \frac{g_{K/Ca} Z_{Ca}(t)^4}{Z_{Ca}(t)^4 + \kappa_{K/Ca}^4}[E_K - V(t)]
\]

\[
I_{K/Ca}(t) = \frac{g_{K/Ca} \mathcal{H}(Z_{Ca}(t), \kappa_{K/Ca}, 4)[E_K - V(t)]}{Z_{Ca}(t)^4 + \kappa_{K/Ca}^4}
\]

The choice \( n = 4 \) gives it a high sensitivity to \( Z_{Ca}(t) \) and is widely used in similar analyses [28,33]. Additionally, we selected \( \kappa_{K/Ca} = 0.35 \mu \text{M} \) since intracellular calcium levels are normally about 0.1 \( \mu \text{M} \). This means only a slight rise in internal calcium levels are required to activate \( I_{K/Ca} \), which suppresses spiking behavior and “turns off” bursts. Since calcium levels are generally about 10\(^4\) times larger in the extracellular medium than in the cytoplasm, this yields a relatively small influx of \( \text{Ca}^{2+} \) ions.

TABLE II. Contributions to the kinetics of the voltage-gated channels. \( \sigma(x, y, z, V) \equiv 0.5[1 + \tanh(z^{-1})] \). \( E(x, y, z) \equiv \exp(z^{-1}) \).

<table>
<thead>
<tr>
<th>( I_{channel}(t) )/pA</th>
<th>( X(t) )</th>
<th>( X_0(V) )</th>
<th>( \tau_X(V) )/ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{Na}(t) )</td>
<td>( m(t) )</td>
<td>( \sigma(V(t), -35, -5) )</td>
<td>—</td>
</tr>
<tr>
<td>( I_{CaL}(t) )</td>
<td>( h(t) )</td>
<td>( \sigma(V(t), -37.4, 4.3) )</td>
<td>1</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( n(t) )</td>
<td>( \sigma(V(t), -30, -5) )</td>
<td>( 10 \lceil \cosh \left( \frac{V(t) + 30}{30} \right) \rceil^{-1} )</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( a_T[V(t)] )</td>
<td>( \sigma(V(t), -65, -7.8) )</td>
<td>—</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( r_T(t) )</td>
<td>( \sigma(V(t), -67) )</td>
<td>200 + 87.5( \sigma(V(t), 68, 2.2) )</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( b_T[r_T(t)] )</td>
<td>( \sigma(r_T(t), 0.4, -0.1) - \sigma(0.4, 0.4, -0.1) )</td>
<td>—</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( n(t) )</td>
<td>( \sigma(V(t), -40, -5) )</td>
<td>—</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( r_T(t) )</td>
<td>( \sigma(V(t), -105, 5) )</td>
<td>( 100 \left[ \frac{7.4(V(t) + 70)}{E[V(t), 30] - 0.8} \right]^{-1} + 65 E[V(t), 56, 23] )</td>
</tr>
<tr>
<td>( I_{CaT}(t) )</td>
<td>( r_T(t) )</td>
<td>( \sigma(V(t), -105, 25) )</td>
<td>1500</td>
</tr>
</tbody>
</table>
With an appropriate stimulus, a model containing only $I_{Na}$ and $I_K$ would produce a continuously repeating spike train. The introduction of $I_{K(Ca)}$ means the neuron model intermittently activates another K current, which drives the voltage response of the neuron towards $E_K = -90$ mV and turns off spiking behavior of the neuron. Combined with the hyperpolarization-activated $I_h$ and $I_{CaL}$, the neuron model’s subthreshold behavior is greatly enriched, as will be seen in the simulations to follow.

### C. Modeling intracellular Ca$^{2+}$ uptake and release

To fully model the complexity of calcium dynamics, we focus on one model for which exhibits various complex behaviors. Dupont and Goldbeter proposed a model to study complex Ca$^{2+}$ oscillations [15], later studied in detail by Refs. [12,13]. They demonstrated that the model shows complex oscillatory phenomena such as limit cycle oscillations, bursting, quasiperiodic oscillations, and deterministic chaos [13]. In many cell types, the uptake and release of Ca$^{2+}$ ions by the ER is mediated predominantly by IP$_3$ [27]. This uptake and release via IP$_3$ mediation has been suggested as a source of intracellular Ca$^{2+}$ oscillations. The complex $Z_{Cal}(t)$ oscillations arising in this model are due to the release of Ca$^{2+}$ from internal stores, with dynamics based mainly on mechanisms of Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) that take into account the Ca$^{2+}$-stimulated degradation of IP$_3$ by a 3-kinase [13].

CICR was originally found to occur in muscle and cardiac cells, and was later found in a variety of other cells including neurons [34]. Soloyvoya et al. have observed CICR in cultured rat dorsal root ganglia neurons [35]. However, they also observed CICR triggered by Ca$^{2+}$ entry through voltage-gated Ca$^{2+}$ channels. The sum of the two voltage-gated calcium currents $I_{CaL}$ and $I_{CaT}$, may, therefore, act as a calcium-release stimulus. We thus used the intracellular calcium dynamics model of Houart et al. [13], but with the replacement $\beta_{input} \rightarrow \beta_{input}[I_C(t) + I_T(t)]$ for the (previously constant) external stimulus. This change now acts to couple the calcium dynamics to the membrane voltage.

There are three dynamical quantities of interest in the intracellular medium. The first is the cytosolic calcium concentration $Z_{Ca}(t)$, the second is the calcium concentration $Z_{ER}(t)$ in the endoplasmic reticulum, and the third is the concentration of catalytic IP$_3$, $Z_{IP3}(t)$. Following Ref. [13], we adopt

$$
\frac{dZ_{Ca}(t)}{dt} = v_0 + \beta_{input}[I_{CaL}(t) + I_{CaT}(t)] - \gamma_{leak}Z_{Ca}(t) + \gamma_{CICR}\mathcal{H}(Z_{Ca}(t), \kappa_{Ca}, 2)\mathcal{H}(Z_{Ca}(t), \kappa_{Ca}, 2) + \gamma_{ERleak}Z_{ER}(t) - \gamma_{pump}\mathcal{H}(Z_{Ca}(t), \kappa_{p}, 2),
$$

$$
\frac{dZ_{ER}(t)}{dt} = -\gamma_{CICR}\mathcal{H}(Z_{Ca}(t), \kappa_{Ca}, 2)\mathcal{H}(Z_{ER}(t), \kappa_{ER}, 2) - \gamma_{ERleak}Z_{ER}(t) + \gamma_{pump}\mathcal{H}(Z_{Ca}(t), \kappa_{p}, 2),
$$

$$
\frac{dZ_{IP3}(t)}{dt} = v_{synthesis} + \alpha_0 \beta_{input}[I_C(t) + I_T(t)] - \gamma_{IP3leak}Z_{IP3}(t) - \gamma_{degradation}\mathcal{H}(Z_{IP3}(t), \kappa_{IP3}, 1)\mathcal{H}(Z_{Ca}(t), \kappa_{pCa}, 4),
$$

where the form of $\mathcal{H}(x, \kappa, n)$ is given in Eq. (5). Values of parameters are listed in Table III.

In this model we assume free calcium ions are uniformly distributed across the cytosol. This avoids the additional complication of introducing a partial differential equation to model the spatial dependence of the $Z_{Ca}(t)$ dynamics. This simplification is consistent with experimental results [35].

### III. DATA GENERATION FOR TWIN EXPERIMENTS

As with previous V + Ca models [28,29], we focus our attention in this paper on chaotic oscillations. This requires the parametrization of the calcium dynamics to lie within a particular parameter range specified in Ref. [13]. We adopted the intracellular calcium model of Refs. [13,36] with the exception that all time constants were reduced by a factor of 60, so that the calcium oscillations would occur on the scale of seconds rather than minutes. Since the time between voltage spikes is on the order of milliseconds, this rescaling makes the voltage behavior switch between a resting state with no spiking and a firing state with repeated production of action potentials lasting about ten spikes. There is no loss in generality or information here, since the length of a burst is still much longer than that of a single spike. This also allows the model to undergo several bursts within a reasonable timescale for numerical integration. Finally, the scaling coefficient ($\beta_{input}$ in the model) of the calcium currents was adjusted to limit the stimulus to the chaotic regime according to the stability diagram in Ref. [13].

The model was integrated with an adaptive Runge-Kutta fourth-order scheme (see Ref. [37], Sec. 16.2), using a maximum integration time step $\Delta t = 0.02$ ms and an error tolerance $\epsilon = 10^{-8}$. Figure 1 shows the resulting time course of the membrane voltage $V(t)$ and cytosolic Ca$^{2+}$ concentration $Z_{Ca}(t)$ with a constant applied stimulating current $I_{app} = 600$ pA. The main characteristic in the behavior of the model neuron is the production of action potential bursts with varying durations controlled by the variation of $Z_{Ca}(t)$.

As in the other models of V+Ca dynamics, we find irregular bursting of the action potential. The model neuron enters into a bursting period of action potentials when $Z_{Ca}(t)$ decreases. Low $Z_{Ca}(t)$ cuts off the current $I_{K(Ca)}$, allowing the neuron to depolarize countering the tendency of $I_{K(Ca)}$ to drive the neuron to $E_K \approx -90$ mV, which involves deep hyperpolarization. Within a burst the spiking frequency is sensitive to the change of $Z_{Ca}(t)$ through the current $I_{K(Ca)}$. This is called spike frequency accommodation; however, it
is not visible in Fig. 1. During a burst, calcium ions flood into the cytosol through voltage-gated calcium channels. The free calcium ions in the cytosol induce release of Ca\(^{2+}\) into the cytosol through voltage-gated calcium channels. The competition between polarizing and depolarizing processes results in chaotic oscillations in the parameter range we have selected.

To determine whether the irregular behavior visible in Fig. 1 is chaotic, we evaluated the largest Lyapunov exponent for the observed time series, and we display that in Fig. 2. We see that for \(\beta_{\text{input}}\), which sets the scale of the driving force from voltage-gated calcium channels into the ER dynamics, just above zero to about 4 \(\times 10^{-5} \mu \text{M/(ms pA)}\), a positive Lyapunov exponent appears. This is similar to the behavior of the other two V+Ca dynamical models we analyzed [28,29].

Lyapunov exponents are obtained by a recursive QR decomposition of the Oseledec matrix [38]. For each \(\beta_{\text{input}}\), a trajectory of \(T = 600 \text{ s} \) is generated. To avoid overflow, the Jacobian matrices, \(DF(x, n\Delta t)\) \(n = 1, 2, \ldots\) are calculated by integrating the variational equations with \(DF[x,(n - 1)\Delta t] = \text{identity matrix}\). If \(\Delta t\) is too small, of the order of the spiking period (1 ms), the Lyapunov exponents exhibit spiking behavior. They can be made smoother by averaging over the time of voltage bursts, namely over \(\Delta t = 1500 \text{ ms}\).

IV. ESTIMATION OF MODEL STATES AND PARAMETERS FROM SPARSE DATA

The central question we address in this paper has to do with the number of measurements required to permit accurate estimation of the fixed parameters and unobserved state variables of the model using time series of the data. There is a direct connection between the ability to synchronize the data with the model output and the ability to accurately estimate unknown parameters and unobserved state variables in the model [3]. If this synchronization is absent, then the surface over which one searches in the estimation procedure has many local minima. When synchronization occurs, this surface becomes quite smooth and estimation proceeds with accuracy. This connection is present here as well, as we shall show.

We introduce information about the data into the model dynamics through terms in the dynamical equation for state variables \(x_i(t)\), which are observed. These have the form \(g_{sl} \{y_l(t) - x_i(t)\}; l = 1, 2, \ldots, L\), where the \(g_{sl}\) are constant, nonzero, and positive coupling strengths for the measured components. We will consider two kinds of data within the V + Ca models: measurements of the membrane voltage \(V(t)\); \(y_1(t) = V_{\text{data}}(t)\) and measurements of the cytosolic calcium concentration \(Z_{\text{Ca}}(t)\); \(y_2(t) = Z_{\text{Ca data}}(t)\). Accordingly, we modify the equations in the model to be

\[
\frac{dV(t)}{dt} = F_V[V(t), Z_{\text{Ca}}(t), Y(t)] + g_V [y_1(t) - V(t)] \tag{7a}
\]

\[
\frac{dZ_{\text{Ca}}(t)}{dt} = F_{\text{Ca}}[V(t), Z_{\text{Ca}}(t), Y(t)] + g_{\text{Ca}} [y_2(t) - Z_{\text{Ca}}(t)], \tag{7b}
\]

where \(F_V[V(t), Z_{\text{Ca}}(t), Y(t)]\) and \(F_{\text{Ca}}[V(t), Z_{\text{Ca}}(t), Y(t)]\) are the vector fields in the model as outlined in Sec. II. The dependence on \(V(t)\) and \(Z_{\text{Ca}}(t)\) is written explicitly for clarity. The rest of the dynamical variables, whose equations remain unchanged, are collected in \(Y(t)\).

Our overall goal is to provide a method for accurately estimating the parameters and state variables of a model of neuron dynamics when we observe a sparse subset of the state variables in the model. The general formulation of this is given in Ref. [3], and here we use the variational approach, which minimizes a cost function comparing the model output with the data that has been collected, subject to the dynamical equations of the model. The model thus acts as a nonlinear filter that passes information in the data along to the unobserved state variables and constrains the parameters.
In the present case, we have a model with many gating variables, fixed parameters, and dynamical variables, and we seek to determine all of these parameters and all states from observations over a temporal window $[0, T]$. If we have good estimations of the parameters from data in this window and we have accurate estimations of the states at $t = T$, we can use the model differential equations to predict observed behavior for $t > T$. We consider here the possibility of measuring two state variables, the membrane voltage $V(t)$ and the intracellular calcium concentration $Z_{Ca}(t)$. From one or both of these time series observations during $[0, T]$, we want to estimate the fixed parameters and all the gating variables and other state variables at $T$.

A. Estimation with $V(t)$ measurements only

We first ask if we can estimate parameters and states of the $V + Ca$ system with voltage measurements alone using a coupling term as in Eq. (7). To this end, we look at the synchronization error as a function of the selected value of the initial condition of calcium concentration for the model $Z_{Ca}(t = 0)_{\text{Model}}$ minus the known initial value for the calcium concentration in the data $Z_{Ca}(t = 0)_{\text{Data}}$ when all other parameters fixed to their known values in generating the data, one must have a zero when $Z_{Ca}(t = 0)_{\text{Model}}$ is chosen correctly. We evaluate this synchronization error, or cost function, with $g_V$ fixed at $g_V = 2.0$ ms$^{-1}$,

$$ C_{\text{SE}}[Z_{Ca}(t = 0)_{\text{Model}} - Z_{Ca}(t = 0)_{\text{Data}}, g_{Ca}] = \frac{2}{N} \sum_{n=N/2}^{N} (y_1(t_n) - V(t_n))^2, \quad (8) $$

and for various values of $g_{Ca}$. This is displayed in Fig. 3. $V(t_n)$ is a time series of the model output obtained by forward integration of Eq. (7) starting with a different initial condition than that used to generate the data. The first half of the integration data points containing the initial transient behavior is discarded. The synchronization error is evaluated using the model output voltage only, even though in this twin experiment we know all of the other state variables because we generated the data. In a laboratory experiment, we would only know the observed and model output voltages, and, perhaps, the observed and model output $Z_{Ca}(t)$.

In generating the model trajectories, we fixed all parameters and initial state values at the values used in generating data except for the initial condition $Z_{Ca}(t = 0)$. To estimate this initial condition, we should minimize the synchronization error over a range of values of $Z_{Ca}(t = 0)_{\text{Model}}$. In Fig. 3 we display $C_{\text{SE}}[Z_{Ca}(t = 0)_{\text{Model}} - Z_{Ca}(t = 0)_{\text{Data}}, g_{Ca}]$ versus the difference $Z_{Ca}(t = 0)_{\text{Model}} - Z_{Ca}(t = 0)_{\text{Data}}$ for various values of the calcium coupling $g_{Ca}$ and a fixed value of the voltage coupling $g_V$. To estimate $Z_{Ca}(t = 0)_{\text{Model}}$, we could search over the surface shown by some form of iterative procedure. However, with $g_{Ca} = 0$ s$^{-1}$, which means no calcium data are presented, the surface is peppered by local minima, and local optimization procedures will fail to find the correct $Z_{Ca}(t = 0)_{\text{Model}}$ in general.

With $g_{Ca} = 0$, then, namely no information about $Z_{Ca}(t)_{\text{Data}}$ passed to the model, the surface over which we must search for $Z_{Ca}(t = 0)_{\text{Model}}$ has multiple local minima, and this impedes accurate estimation.

B. Estimation with $V(t)$ and $Z_{Ca}(t)$ measurements

We next increased $g_{Ca}$ to 2.0 s$^{-1}$ and again computed the synchronization error. Note that in the figure, this synchronization error is multiplied by a factor of 5 so that it is clearly seen on the same scale as the surface for $g_{Ca} = 0$ s$^{-1}$. While the surface is smoother, there remains a visible local minimum away from zero, and again local optimization procedures are not suitable, especially if there are more local minima not shown in the plot.

The final curve in Fig. 3 was computed with $g_{Ca} = 5.0$ s$^{-1}$. The search surface is smoothed out enough so that essentially any search algorithm will result in the correct answer. This display has the synchronization error multiplied by a factor of 10 so that it is clearly seen on the same scale as the surface for $g_{Ca} = 0$ s$^{-1}$. Thus, increasing $g_{Ca}$ with $g_V$ fixed and large enough results in the synchronization of the data and the model output, both of which are chaotic, as well as smoothing the surface over which a search must proceed.

We can examine the effect of varying both $g_V$ and $g_{Ca}$ using the joint synchronization error in $V(t)$ and $Z_{Ca}(t)$ measurements:

$$ C_{\text{SE}}(g_V, g_{Ca})_{V\&Ca} = \frac{2}{N} \sum_{n=N/2}^{N} \left\{ \frac{[V(t_n)_{\text{Data}} - V(t_n)]^2}{S_v^2} + \frac{[Z_{Ca}(t_n)_{\text{Data}} - Z_{Ca}(t_n)]^2}{S_{Ca}^2} \right\}. \quad (9) $$

The constants $S_v$ and $S_{Ca}$ are selected to scale the $V(t)$ and $Z_{Ca}(t)$ dynamical ranges to be nearly equal. For this purpose
when only $V$ coupling strengths, $g$ synchronization of the model output with the data. However, this we set $gV$ and $YE, ROZDEBA, MORONE, DAOU, AND ABARBANEL PHYSICAL REVIEW E 89$. This last step is the validation criterion for the model itself.

$t > T$ initial conditions at we are then able to use the model with estimated parameters and in the $V$ will allow accurate estimation of the parameters and unobserved states in the $V + Ca$ model. At the end of the observation window, $t = T$, we are then able to use the model with estimated parameters and initial conditions at $t = T$ to predict the response of the neuron for $t > T$. This last step is the validation criterion for the model itself.

we set $S_k = |\max x_k - \min x_k|$ for the $k$th contribution to SE, where the minimum and the maximum are taken over the observation window. The result is shown in Fig. 4 with $S_V = 82.5 \text{ mV}$ and $S_{Ca} = 0.236 \mu \text{M}$.

This figure demonstrates that when either $gV$ or $gCa$ is zero, the synchronization error does not go to zero, leading to inaccurate estimations of the parameters and state values determining the model output.

V. STATE AND PARAMETER ESTIMATION; PREDICTION AFTER ESTIMATION

So far we have established that in coupling measurements of $V(t)$ and $Z_{Ca}(t)$ into the overall cellular dynamics via Eq. (7), we are able to transmit enough information to the model to smooth out the search surface of the synchronization error. The couplings $gV$ and $gCa$ are not part of the biophysics of the model, however. After they are used to synchronize the model output and the data, they should be disposed of when predicting data beyond the observation window.

To allow these couplings to regulate the instabilities on the synchronization manifold, and then send them to zero when the estimation procedure is completed, we now explicitly make the coupling strengths $gV$ and $gCa$ functions of time and treat $gV(t_n)$ and $gCa(t_n)$ as additional parameters to be estimated according to our estimation protocol. In principle, this means they will be small at times along the orbit when the synchronization manifold is nearly stable and larger in unstable regions. More precisely, we introduce the coupling strengths as additional penalties into the cost function Eq. (9) as

$$C_{SE} = \frac{2}{N} \sum_{\frac{N}{2}}^{N} \left\{ \frac{|V(t_n)_{data} - V(t_n)|^2}{S_V^2} + \frac{|Z_{Ca}(t_n)_{data} - Z_{Ca}(t_n)|^2}{S_{Ca}^2} + gV(t_n)^2 + gCa(t_n)^2 \right\},$$

(10)

which is to be minimized subject to Eq. (7). The minimization was done using the publicly available nonlinear optimization software IPOPT [39–41]. This estimates all the model parameters, all the model state variables at time within the observation window, and estimates $g_{Ca}(t_n)$ and $g_{V}(t_n)$.

A. Prediction with only $V(t)$ measurements

First, we performed the state and parameter estimation for the full model but with voltage measurements alone. This was done to verify the conclusion made above that it is not feasible to estimate unobserved states with voltage data only. We performed the optimization with 80 000 voltage data points spaced in model time by $\Delta t = 0.02 \text{ ms}$, with all parameters fixed and an external stimulus $I_{app} = 600 \text{ pA}$. The estimated states were then used to continue integrating the model forward with the same $I_{app}$ but with $gV = 0 \text{ ms}^{-1}$, $gCa = 0 \text{ ms}^{-1}$.

FIG. 4. (Color online) Synchronization error as a function of two coupling strengths, $g_{Ca}$ and $g_V$; $C_{SE}$ [Eq. (9)] remains at nonzero values when either $g_{Ca}$ or $g_V$ is equal to zero. This indicates that when only $V(t)$ or only $Z_{Ca}(t)$ are observed, it does not allow synchronization of the model output with the data. However, this figure shows that the synchronization error does go to zero when $g_{Ca}$ and $g_V$ are both large enough. This indicates that two measurements will allow accurate estimation of the parameters and unobserved states in the $V + Ca$ model. To allow these couplings to regulate the instabilities on the synchronization manifold, we set $S_k = |\max x_k - \min x_k|$ for the $k$th contribution to SE, where the minimum and the maximum are taken over the observation window. The result is shown in Fig. 4 with $S_V = 82.5 \text{ mV}$ and $S_{Ca} = 0.236 \mu \text{M}$.

FIG. 5. (Color online) Results from dynamical state estimation for the $V + Ca$ model with only voltage data presented ($g_{Ca} = 0 \text{ s}^{-1}$). 80 000 voltage data points are used for estimation; this is an assimilation window of 1600 ms. All parameters of the model are fixed, and all the states are to be estimated. The result shown is the integration based on using the estimated state variables at $t = 0 \text{ ms}$ as initial conditions then integrating the dynamical equations with $g_V = 0 \text{ ms}^{-1}$ using the estimated initial conditions. The disagreement between estimate and data shows that the information from voltage observations alone is not enough to accurately estimate all the unobserved states. The known data are in black. Estimates are shown in red.
were very accurately estimated except for estimation; this is an assimilation window of 1600 ms. All the states except \( V(t) \) and \( Z_{Ca}(t) \) are to be estimated. In this case we also estimated the parameters listed in Table IV. Other parameters in the model are fixed. Prediction is made by integrating the model forward using the estimated parameters and estimated state variables in the model are fixed. Prediction is made by integrating the model forward using the estimated parameters and estimated state variables at \( T = 1600 \) ms as initial conditions. A vertical line indicates where estimations terminate and prediction begins. The known data are in black. Estimates are in red, and predictions are shown in blue.

Since, when only \( V(t) \) is observed, the SE surface should be riddled with local minima, any “optimal” solution found by the estimation should make the model predict future solutions poorly. Indeed, this was the case, and an example inaccurate estimation should make the model predict future solutions poorly. Indeed, this was the case, and an example inaccurate prediction is shown in Fig. 5.

**B. Prediction with both \( V(t) \) and \( Z_{Ca}(t) \) measurements**

When we presented both voltage and calcium measurements to the model, the estimation using IPOPT returned accurate state and parameter values, which gave the model strong predictive behavior (Fig. 6). The resulting estimates are listed in Table IV. Note that all of the conductances were very accurately estimated except \( g_{CaT} \) and \( g_h \). This is probably because \( I_{CaT} \) and \( I_h \) are triggered by large hyperpolarizing currents [7], but we stimulated the neuron with a contant depolarizing current. In our model, however, \( I_{K/Ca} \) can hyperpolarize the neuron below threshold and thus activate \( I_{CaT} \) and \( I_h \) to some extent, so inaccurate estimates for these two maximal conductances appear not to effect the value of SE very much. If the neuron were driven by a large hyperpolarized step current, the estimates of these quantities might be improved significantly.

**VI. DISCUSSION AND SUMMARY**

We have shown that the use of a neuron model that couples membrane voltage dynamics to the relatively slow dynamics of intracellular calcium uptake and release requires more than a measurement of the membrane voltage trace alone to estimate model parameters and initial conditions when the parameter values give the model chaotic behavior. Specifically, we have shown that simultaneous measurements of voltage \( V(t) \) and cytosolic calcium concentration \( Z_{Ca}(t) \) are sufficient to regularize this system and estimate the maximal conductances of the membrane currents.

The calcium oscillations in our \( V + Ca \) model are intrinsically chaotic. Complex forms of calcium oscillations are typically observed in biological phenomena; oscillations express both periodic as well as nonperiodic behavior [12,42–51]. The erratic behavior is often thought to be the consequence of stochastic effects; however, the same qualitative effects can be generated by a chaotic deterministic system. It is important to consider what effects of deterministic chaos are when trying to replicate experimental observations. The model we used here that was found to exhibit both aperiodic oscillation and bursting behaviors in its chaotic regime (Fig. 7 in Ref. [14]). Similar calcium-bursting behavior characterized by irregular number of secondary spikes and an irregular spacing between main spike and aperiodic oscillation with varying amplitudes are observed in hepatocytes under different stimuli [9–11]. Hazledine et al. found the largest Lyapunov exponent is positive for calcium oscillation data obtained from certain legume plants, which directly identifies that calcium oscillation is chaotic [51,52].

The nonperiodic nature of calcium oscillations is related to the concentration of extracellular agonists such as hormones and neurotransmitters, thereby dictating that the external signal is encoded in terms of the temporal pattern of calcium oscillations, the so-called frequency encoding calcium signals [42,43]. It is not yet known to what extent this chaotic nature of oscillations is essential to the cell function (muscle, neurons, etc.); however, it had been hypothesized that chaotic oscillations is likely a common feature of calcium signal transductions where calcium permits greater flexibility in the regulation of protein activity than either stochastic or stable systems, allowing differential responses in multifunctional signaling pathways [44,51].

We think it is reasonable, therefore, to expect chaotic dynamics to appear in the biological neuron systems we wish to study. Chaos should produce unique challenges to the practice of state and parameter estimation, because even very similar trajectories will rapidly diverge. With this in mind, our model is intended to serve as a test bed to study and refine our techniques.
in a chaotic context. It is a toy model, which retains what we consider the most distinctive and challenging features of a neuron system (bursting, dynamics at different time scales, and chaos), without adding excessive complications by demanding strict biophysical accuracy.

Our focus here is on properties of coupled voltage and calcium dynamics as it relates to our ability to use observed time courses in individual cells, as well as in networks, to determine the biophysical cellular processes and the network connectivity in networks of neurons.

We used “twin experiments” in which model output trajectories with known initial conditions and parameter values were used as sources of experimental “data” in the dynamical synchronization procedure. This is to be compared to a realistic experimental setting in which only a limited number of physical quantities can be measured and in general with a nonnegligible level of noise [3]. The advantage of using twin experiments is that they provide a controlled setting in which we have exact knowledge of the entire state of the system. While the true metric of success is the estimation and accurate prediction of the measurable quantities, twin experiments give one the opportunity to investigate the efficacy of the data assimilation methods on the entire model space and to possibly eliminate unnecessary or degenerate degrees of freedom.

When such a $V + \text{Ca}$ model is applied to analyze experimental data, knowledge of the neuron’s membrane potentials and the intracellular $\text{Ca}^{2+}$ concentration is needed to interpret $\text{Ca}^{2+}$ signaling. Loading calcium indicators into neurons has been widely used in recent years. Fluorescent $\text{Ca}^{2+}$ indicators can be loaded into biological neurons through the same microelectrodes used for recording the intracellular voltage, thereby allowing simultaneous electrophysiological recordings and ratiometric calcium imaging. In the early years, calcium indicators were delivered through sharp microelectrodes both in vitro [53] and in vivo [54]. In more recent years, calcium dyes are delivered through whole-cell patch-clamp micropipettes [55–58] and the whole-cell recordings are generally performed under visual guidance using two-photon imaging [59–61].

When using the kind of model explored in this paper to analyze data from individual neurons [3] it may suffice to use the single compartment approach described here. However, we recognize that when neurons with voltage and $\text{Ca}^{2+}$ dynamics are to be used in biophysical realizations of networks, we will need to introduce spatial representations (compartments) for dendritic processes where the $\text{Ca}^{2+}$ dynamics resides, and axonal processes to facilitate communication of neural activity within the network.

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