INTRODUCTION

Ultimately the output of a given neuron type is most directly determined by the makeup and characteristics of voltage-gated ion channels inserted in the membrane at a given time. Yet neurons exhibit remarkably robust electrical behavior even when underlying biological parameters of the same cells in different individuals show a great deal of variability (Golowasch et al. 1992; Khorkova and Golowasch 2007; Schulz et al. 2006; Swensen and Bean 2005). A compelling question with respect to this phenomenon is how the individual components of biological cells possess variable underlying mechanisms and yet converge to generate highly similar functional output. One possibility is that different ionic conductances compensate for each other, and potentially are coregulated, to maintain a particular output. For example, at the level of mRNA, quantitative single-cell polymerase chain reaction analyses on individual neurons of the stomatogastric ganglion (STG) revealed different sets of correlated ion channel mRNAs in each class of identified neuron (Schulz et al. 2007), some of which have been demonstrated to hold true at the level of membrane conductance (Khorkova and Golowasch 2007), suggesting that cellular output in the STG may be determined in part by characteristic sets of correlated expression of ion channel genes and their subsequent ionic currents. This hypothesis is strongly supported by the fact that overexpression of a hyperpolarizing conductance ($G_A$) in the pyloric dilator neuron of the STG leads to very little or no change in its activity via a compensatory up-regulation of a depolarizing conductance ($G_T$) (MacLean et al. 2003, 2005). These results suggest that positive coregulation of $G_A$ and $G_T$ exerts a stabilizing force on bursting output in these cells, thus maintaining the cells in a parameter space that allows for appropriate activity.

Alternatively, there is evidence that when faced with more radical alterations to their output, cells can decouple these stabilizing mechanisms to regain, de novo, their patterns of activity. For example, when STG neurons are cultured in isolation, they undergo dramatic changes in their firing patterns, from silent to tonic spiking until finally they recover a bursting phenotype (Haedo and Golowasch 2006; Turrigiano et al. 1995). Underlying these changes in firing patterns is a negative relationship between depolarizing ($G_{Na}$) and hyperpolarizing ($G_K$) conductances (Olypher and Prinz 2010); moreover, $G_{Ca}$ and $G_{Na}$ (depolarizing conductances) are up-regulated, whereas $G_K$ (hyperpolarizing conductances) are down-regulated (Haedo and Golowasch 2006; Turrigiano et al. 1995). These results suggest that when forced into a parameter space that does not produce appropriate output, there is a decoupling of stabilizing coregulation that allows cells to travel across parameter space to achieve bursting output.

Motivated by these biological phenomena, the present study investigates common mechanisms for robustness of a particular characteristic of a class of slow-wave bursting cells, the “underlying membrane potential oscillation.” The underlying oscillation, which is revealed in the membrane potential trace if the spikes are removed (e.g., see Fig. 1), determines the number of spikes per burst for such cells, which in turn affects outputs downstream, such as muscle actuation. We use a biophysical modeling approach to examine the parameter space that allows for such underlying oscillations, to determine not only the potential coregulations among currents that might preserve this oscillation for slow-wave bursting cells, but also how discrete boundaries may exist between conductances that shift a cell from oscillatory to other forms of firing.

Underlying oscillation controls burst characteristics

Underlying oscillations can lead to bursting in both forced and endogenous bursters. In forced bursters, such oscillations are typically elicited in response to a synaptic stimulus and are also referred to as “driver potentials” by some authors (e.g., Tazaki and Cooke 1979a). An underlying oscillation in such a cell is a graded depolarization from rest and repolarization to rest of the cell on the order of a few hundred milliseconds. The depolarization is typically 20–30 mV above the resting state. There are numerous mechanisms that cells use to generate a bursting phenotype (for comprehensive surveys see Coombes
and Bressloff 2005; Izhikevich 2003, 2007). The classification of bursters using phenomenological models has been well studied. Izhikevich (2007) lists various types of fast-slow bursters for a class of such mathematical model systems. However, such a classification of bursters from an electrophysiology perspective is presently lacking.

Our primary test case in this modeling study—the large cell (LC) of the crab cardiac ganglion—is a motor neuron characterized as a forced burster that exhibits a typical driver potential profile. An underlying oscillation in the LC occurs with a depolarization driven mainly by Ca\(^{2+}\) currents and with repolarization driven by K\(^+\) currents (Berlind 1982; Cooke 2002; Tazaki and Cooke 1979b, 1986, 1990). Similar calcium currents have also been implicated in depolarization during bursting in lamprey spinal motoneurons (Grillner et al. 2001) and low-threshold calcium currents have been shown to be involved in the rhythmic activity of leech heart interneurons (Ivanov and Calabrese 2000). Bursts can be initiated by increased calcium or persistent sodium currents, or by decreased potassium currents, and a combination of these mechanisms is typically used (Harris-Warrick 2002). An initial study of the relationships of ion channel mRNA copy numbers revealed strong correlations in channel expression in LCs. Among these was a robust relationship between cacophony, which encodes a voltage-gated calcium channel, and shab, which encodes a delayed rectifier conductance (Tobin et al. 2009). Based on

![Diagram](https://example.com/diagram.png)

**FIG. 1.** The underlying membrane potential oscillations and corresponding currents for the 3 example cases. A: large cell (LC) of the crustacean cardiac ganglion. B: anterior burster (AB) cell of the crustacean stomatogastric ganglion. C: R15 cell of the *Aplysia* abdominal ganglion. The voltage trace has been cut off at \(-30\) mV for the *Aplysia* R15 cell to magnify the underlying oscillation. The current trace has also been cut off at \(-50\) and \(50\) nA for the *Aplysia* R15 cell to magnify the slow currents.
these observations, Ball et al. (2010) used a computational model to show that covariations in a potentially related set of ionic conductances (\(G_{CaT}\) and \(G_{Kd}\)) preserves the peak and duration of driver potentials of LCs in a crab cardiac ganglion. In our study, in addition to extending the investigation of covariation to other conductances in the LC, we generalize this concept to two other types of slow-wave bursting neurons with distinct intrinsic underlying oscillations. The biophysical models reported here elucidate the development of the underlying membrane potential oscillation for such cells from a biologically realistic, i.e., electrophysiology, perspective.

Hypothesis about phases of the underlying oscillation

The sum total of these experimental and modeling studies led us to hypothesize that the underlying oscillation for a slow-wave bursting cell has three phases: generation, maintenance, and termination and that different currents or "modules" of currents are coregulated to preserve the characteristics of each phase. **Generation** is the phase during which the underlying oscillation is initiated, by a synaptic pulse for the forced case and intrinsically for the endogenous case. The peak and duration of the oscillation are controlled during the **maintenance** phase. **Termination** (or repolarization) is the phase during which the underlying oscillation ends and the membrane potential is brought back to its rest value.

We investigated whether distinct modules of ionic currents were primarily responsible for the different phases of **generation**, **maintenance** (of peak value and duration), and **termination** of the underlying oscillation and, if so, whether they covaried to preserve the peak and duration of the underlying oscillation. We investigated these hypotheses using the LC model studied by our group (Ball et al. 2010): a forced burster cell that has six active currents \(I_B, I_A, I_{Na}, I_{CaT}, I_{Kd}\), and \(I_{KCa}\) and a passive leak current. We then investigated whether the correlations were preserved for randomly selected points in the LC maximal conductance parameter space and then devised yet another strategy to test their validity. We considered the question: "Would the correlations hold for other types of cells reported in the literature that expressed the same set of conductances?" This led us to consider example cases 2 and 3, the anterior burster (AB) neuron of the STG (Soto-Treviño et al. 2005) and the *Aplysia* R15 neuron (Bower and Beeman 1998), using models reported in the literature.

**Methods**

We considered two properties to characterize the slow underlying membrane potential oscillation, its peak and duration. The highest value of the membrane potential was defined as the **peak** of the slow underlying membrane potential oscillation. **Duration** of the oscillation was calculated, as in Tazaki and Cooke (1979b); by extending lines down from the points of maximum rates of rise and fall of the oscillation, in the membrane potential versus time plot. Duration was the time between the intersections of these two lines with the resting potential of the cell (Ball et al. 2010; Supplemental Fig. SM1). In the endogenous cases, resting potential was taken to be the value of the membrane potential immediately prior to the upswing of an underlying oscillation.

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1 The online version of this article contains supplemental data.
and Cooke 1990) revealed that calcium current inactivation occurs with two apparent time constants, a shorter time constant of 40 ms, followed by a longer time constant of 180 ms. Two types of calcium currents were implemented in the model to reproduce this behavior: a persistent calcium current $I_{Ca,p}$ and a transient calcium current $I_{Ca,t}$. Three outward potassium currents have been found in LCs (Tazaki and Cooke 1979a, 1986): an early outward current $I_{K}$, a delayed outward current $I_{K,d}$, and a calcium-dependent potassium current $I_{Ca,K}$. The soma was modeled with these five active currents and a leak current. The axonal compartment was modeled with transient sodium current $I_{Na,A}$, $I_{Ca,T}$, and a leak current to produce action potentials in response to depolarizing currents.

The following equations represent the membrane voltage equations for the two compartments of the LC:

$$C_s \frac{dV_s}{dt} = -g_{La}(V_s - E_{La}) - g_{C}(V_s - V_A) - \sum g_{int}^{int}$$

(1)

$$C_a \frac{dV_a}{dt} = -g_{La}(V_a - E_{La}) - g_{C}(V_a - V_A) - \sum g_{int}^{int}$$

(2)

where $V_s/V_a$ are the somatic/axonal membrane potentials; $g_{int}^{int}$ are the intrinsic currents in the soma/axon compartments; $C_s/C_a$ are the membrane capacitances of the soma/axon compartments; $g_{La}/g_{C}$ represent, respectively, the leak conductance and reversal potential for the soma/axon compartments; and $g_{C}$ is the coupling conductance between the soma and the axon. The passive properties of the model were adjusted to reproduce the input resistance and resting potential of LCs recorded in the axon. The values for the leak conductance, membrane capacitance, and cytoplasmic (axial) resistance are listed in Table 1.

CURRENT KINETICS. The cells in the crustacean cardiac ganglion share many similarities in form and function with those in the well-studied STG (Buchholtz et al. 1992; Golowasch et al. 1992; Prinz et al. 2003, 2004; Turrigiano et al. 1995). Accordingly, kinetics of the currents in the LC model and the ranges for maximal conductances were based on the current models in a database of model STG neurons (Prinz et al. 2003). The ionic current for channel $i$ was modeled as $I_i = g_i m^p h^q(V - E_i)$, where $g_i$ is its maximal conductance, $m$ is its activation variable (with exponent $p$), $h$ is its inactivation variable (with exponent $q$), and $E_i$ is its reversal potential. The kinetic equation for each of the gating variables $x$ (m or h) takes the form

$$\frac{dx}{dt} = x_a(V, [Ca^{2+}]) - x$$

(3)

where $x_a$ is the voltage- and/or calcium-dependent steady state and $\tau_x$ is the voltage-dependent time constant. The maximal conductances for all ionic currents and the expressions for the gating variables $x_a$ and $\tau_x$ were largely the same as those in the STG database (Prinz et al. 2003) and are listed in Table 2.

CALCIUM DYNAMICS. Intracellular calcium modulates the conductance of the calcium-activated potassium current and influences the magnitude of the inward calcium current in the LC (Tazaki and Cooke 1990). A calcium pool was modeled in the LC with its concentration governed by the first-order dynamics of the following equation (Prinz et al. 2003; Soto-Treviño et al. 2005)

$$\frac{d[Ca^{2+}]}{dt} = -F \times I_{Ca} - [Ca^{2+}]_{pool}$$

(4)

where $F = 0.256 \mu M/\mu A$ is the constant specifying the amount of calcium influx that results per unit (nA) inward calcium current and $[Ca^{2+}]_{pool}$ represents the calcium removal time constant from the pool. Voltage-clamp experiments of the calcium current in the LC (Tazaki and Cooke 1990) showed the intracellular calcium buffering time constant to be 640 ms and so this value was used for $\tau_{Ca}$. This calcium concentration was also used in the Nernst equation to determine the reversal potential for calcium currents, assuming an extracellular calcium concentration of 13 mM, at a temperature of 25°C, as used in electrophysiological experiments by Tazaki and Cooke (1979a,b,c).

As mentioned, example cases 2 and 3 also had the same currents as those in example case 1, resulting in the same set of Eqs. 1–4, albeit with different parameters and activation/inactivation functions (see Supplemental Tables SM1 and SM2). The activation values for the Aplysia R15 model were used in table form from experimental values (Bower and Beeman 1998). All the models have been validated by their authors (Ball et al. 2010; Bower and Beeman 1998; Soto-Treviño et al. 2005). For the present study, the models for all the example cases were developed using the General Neural Simulation System (GENESIS; Bower and Beeman 1998), with an integration time step of 10 μs.

RESULTS

Slow underlying oscillations in the nominal model

The nominal models of the three example cases produced underlying membrane potential wave forms with the following

### Table 1. Model parameters of example case 1 (cardiac ganglion LC)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soma</th>
<th>Axon</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{max}$, mS/cm²</td>
<td>$E_{rev}$, mV</td>
<td>$G_{max}$, mS/cm²</td>
</tr>
<tr>
<td>$I_{Na}$</td>
<td>—</td>
<td>600</td>
</tr>
<tr>
<td>$I_{Kd}$</td>
<td>190.00</td>
<td>−73</td>
</tr>
<tr>
<td>$I_{K}$</td>
<td>90.25</td>
<td>−73</td>
</tr>
<tr>
<td>$I_{Ca,s}$</td>
<td>40.00</td>
<td>−73</td>
</tr>
<tr>
<td>$I_{Na}$</td>
<td>6.83</td>
<td>Nernst</td>
</tr>
<tr>
<td>$I_{Ca,T}$</td>
<td>2.40</td>
<td>Nernst</td>
</tr>
<tr>
<td>$I_{Leak}$</td>
<td>0.04</td>
<td>−55</td>
</tr>
</tbody>
</table>

A. Current parameters

B. Other parameters

- Surface area $8.88 \times 10^{-3}$ cm²
- Capacitance 20.84 nF
- Base $[Ca^{2+}]_{in}$ 0.5 μM
- $\tau_{Ca^{2+}}$ 640 ms
- $F_{Ca^{2+}}$ 0.256 μM/μA
- $R_{axial}$ 1.5 MΩ

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The LC model had a peak of $-31$ mV and a duration of $283$ ms; the AB model had a peak of $-27$ mV and duration of $151$ ms; and the Aplysia R15 model had a peak of $-38$ mV and duration of $10.4$ s. Figure 1A shows the plots of the LC currents that made up the underlying oscillation initiated after a current pulse of $40$ nA for $20$ ms (see Ball et al. 2010).
The initial spike in the voltage and currents in Fig. 1A was a result of this current pulse. Note that the other two example cases are endogenous bursters and so do not require such input pulses. The underlying membrane potential oscillations and the corresponding currents for the AB and Aplysia R15 cells are shown in Fig. 1. B and C, respectively. The membrane potential in Fig. 1C exceeded 0 mV at the peak of action potentials, but was cut off at −30 mV to focus on the underlying slow-wave oscillation. The current plot in Fig. 1A was also zoomed in to the −50 to 50 nA scale to focus on the slow currents.

**EFFECTS OF MAXIMAL CONDUCTANCE VARIATIONS OF INDIVIDUAL CURRENTS.** We began our investigation of the effects of altering maximal conductances with the LC model. All five maximal conductances were varied individually to 0.5-, 3-, and 5-fold their nominal value, to investigate the effect on duration and peak of the underlying membrane potential oscillation. Representative results for LC are shown in Fig. 2, where the small arrows indicate traces that represent silent and endogenous behaviors. Increasing \( G_B \) from 0.5- to 5-fold its nominal value resulted in moving the cell progressively from not producing an underlying oscillation at 0.5-fold the nominal value, to a forced burster at the nominal value, and then to an endogenous burster at 3- and 5-fold nominal value (Fig. 2A). Changing only \( G_A \) across the same range had exactly the opposite effect, i.e., increasing \( G_A \) over this range moved the cell progressively from being a forced burster to not producing an underlying oscillation (Fig. 2B). Increasing only \( G_{CaT} \) from 0.5- to 5-fold its nominal value caused the peak to increase by 18% and the duration to decrease by 64%, as shown in Fig. 2C. Actual values for peak and duration for the plots in Fig. 2, A–E are given in Fig. 2F. Similar changes to \( G_{Kd} \) had exactly the opposite effects to those of \( G_{CaT} \), i.e., increasing \( G_{Kd} \) from 0.5- to 3-fold its nominal value decreased the peak by 46% and increased duration by 175% (Fig. 2D). Increasing the conductance to 5-fold its nominal value caused the cell to become silent. A cell was considered silent if the membrane potential did not rise >10 mV above its “rest” membrane potential. Finally, increases in only \( G_{KCa} \) from 0.5- to 5-fold the nominal value had little effect on the peak, reducing it by only 3%, whereas it caused a decrease in duration of 34% (Fig. 2E). The results in Fig. 2 suggested pairs of currents that might oppose each other in their effects on membrane potential characteristics, which we then investigated as possible “modules” subject to coregulation.

The currents in the other example cases, the AB cell and the Aplysia R15 cell, exhibited similar characteristics, but both were endogenous oscillators at nominal conductance values. Peak and duration values for individual current conductance variations for the AB cell and the Aplysia R15 cell are shown in Supplemental Tables SM3A and SM3B, respectively. In both cells, increasing only \( G_A \) (to 1.5-fold the nominal value
for AB and to 4-fold for Aplysia R15) caused the cell to cease endogenous oscillation. Decreasing only $G_B$ (to 0.5-fold the nominal value for AB and to 0.2-fold for Aplysia R15) also gave the same result. Increasing only $G_{CaT}$ across the same range (0.5- to 5-fold the nominal) caused an increase in peak (AB: 29.6%; R15: 8%) and a simultaneous decrease in duration (AB: 60.9%; R15: 46.5%) for both AB and Aplysia R15 models. Increasing only $G_{Kd}$ over the range caused both lower peaks (AB: 25.9%; R15: 2.9%) and shorter durations (AB: 45.7%; R15: 60.9%). In both models, increasing only $G_{KCa}$ reduced the duration (AB: 18%; R15: 51.2%) with a saturation effect, but with little effect on peak height (AB: 3.7%, R15: 5%). Decreasing only $G_{KCa}$ below nominal values lengthened the duration. These trends were similar to those seen for example case 1.

Role of current modules in various phases of the underlying oscillation of the LC

We first set out to exhaustively examine the role of the ionic conductances as combined units, or “modules,” in the generation, maintenance, and termination of bursting activity using one of our model cells, the LC of the cardiac ganglion.

Generation of the underlying oscillation. Increases in only $G_B$ from 0.5- to 5-fold its nominal value caused the LC to move from silent, to forced bursting, to endogenous bursting. The exact opposite behavior was observed when increases in only $G_A$ were considered, raising the question whether $G_B$ and $G_A$ might coregulate to preserve the generation of the slow underlying oscillation. We hypothesized that the pair $G_B$ and $G_A$ act as the “generation module” and thus we varied both the conductances proportionally (i.e., covaried them), to investigate the effects on the underlying oscillation.

For the LC, which is a forced burster, the underlying oscillation had to be initiated by an input pulse that was sufficiently strong to activate $I_B$, which then began the generation of an oscillation. For the LC, a 40- nA, 20-ms pulse was chosen for the plots in Fig. 3. Ball et al. (2010) showed that the underlying oscillation had similar peaks and durations even with variations in input stimulus amplitude. Since $I_A$ countered $I_B$ during the generation of the underlying oscillation, we investigated whether there was a boundary in the $G_W$-$G_A$ parameter space to separate a forced burster from an endogenous one and, similarly, separated a forced burster from a “silent” cell (i.e., a cell that does not produce an underlying oscillation in response to a given input stimulus). Our investigation led to the finding of two characteristic boundary lines for the LC, as shown in Fig. 3A1 (note that the nominal model has a multiplier of 1 for $G_A$ and $G_B$). Data points in Fig. 3A1 were generated by specifying fixed values of either $G_A$ or $G_B$, then increasing or decreasing the other conductance incrementally until the generation dynamics was changed. All other conductances were kept at nominal values. The solid upper line delineates the boundary between forced and endogenous oscillations and the lower dashed line separates the forced bursters from the silent cells. For instance, if $G_B$ is increased from a point below this boundary to one above it, keeping $G_A$ constant, the cell will transition from a forced burster to an endogenous one. The upper boundary is not affected by the strength of the stimulus since the entire line is a bifurcation line (Izhikevich 2007) for the system as $G_B$ and $G_A$ are varied. The line separates endogenous from nonendogenous behavior and no stimulus is needed to generate oscillations above the line. If, on the other hand, the cell is located in the middle of the forced burster (meaning it is silent without an input stimulus) region and $G_A$ is increased, keeping $G_B$ constant, the cell moves below the lower boundary line into the silent region where no oscillation can be initiated with the given input stimulus.

Increasing the input stimulus beyond the nominal value lowered the dashed line (and vice versa), as expected, since a stronger input pulse requires less $G_B$ to initiate the oscillation. Conversely, a larger $G_A$ is required to prevent the oscillation with a stronger pulse. Since $I_B$ is activated during the pulse and is responsible for initiating the underlying oscillation, stronger pulses increasingly activated $I_B$. If the input pulse is stronger, the underlying oscillation can be initiated with a smaller $G_B$ but more activated $I_B$. Therefore stronger input pulses lowered the boundary for initiation along the $G_B$ axis. Doubling the length of the input stimulus lowered the initiation boundary line by 14%, whereas a doubling of both length and magnitude of the stimulus lowered it by 36%.

Examination of the effects of varying the other conductances revealed that $G_{Kd}$ could be viewed as having a role in the “generation module”; higher multiples of $G_{Kd}$ resulted in shifting the LC from a forced burster to a silent cell (Fig. 2D). To investigate the role of $G_{Kd}$ in the generation of bursting, we added this conductance to the covariance experiments with $G_B$ and $G_A$. Although $G_{Kd}$ was able to suppress bursting activity at high conductance values ($>4\times$ multiplier), this conductance made no substantial impact on the boundary lines that separated the regions of generation (Fig. 3A2) and thus was not considered to be part of the generation module. The effect of $G_{CaT}$ on the generation boundaries was also studied. As shown in Fig. 3A2, increasing $G_{CaT}$ from 1- to 5-fold the nominal value did lower the endogenous boundary, but not substantially, and so this current was not considered to be part of the generation module.

Maintenance of the underlying oscillation. Previous modeling experiments in the LC have shown that the peak and duration of the underlying oscillation can be preserved by $I_{CaT}$ and $I_{Kd}$ (Ball et al. 2010). Our nonintuitive finding from examining the individual current conductance variations in Fig. 2, C and D (see also Fig. 2F) indicated, in addition, that increasing only $G_{CaT}$ decreased the duration of the oscillation and increasing only $G_{Kd}$ increased it. To study the potential relationship between $G_{CaT}$ and $G_{Kd}$ in preserving peak and duration of the oscillation, each was varied from 0.5- to 5-fold its nominal values across the two-dimensional space.

As cited, the nominal LC model produced an underlying membrane potential oscillation with a peak of $-31$ mV and duration of 283 ms. For comparison with biological ranges, in biological recordings from 79 cells, the oscillation duration had a mean of 250 ms (SD 50 ms) and the peak had a mean of $-32$ mV (SD 3 mV) (Tazaki and Cooke 1979b). This represents an SD of 20% of the mean for duration values. Figure 3, B1 and B2 shows the percentage changes in peak and duration values, respectively, as $G_{CaT}$ and $G_{Kd}$ are varied from 0.5- to 5-fold their nominal values (with all other $G_i$ values constant). The figures were generated using peak and duration data at every 0.5 multiple of $G_{CaT}$ and $G_{Kd}$ in the two-dimensional space.
MATLAB was then provided the data at the grid points (shown by dots in the figures) and its contour plot utility generated the trends shown. The positive (negative) directional arrows indicate increasing (decreasing) peaks and durations. They show that a 1:1 variation might help preserve both features, with a deviation of 13% in peak and 7% in duration for a 5-fold increase in nominal values for both conductances.

How should the ratio of $G_{CaT}$:$G_{Kd}$ vary to best maintain the peak and duration of the oscillation? Our model experiments showed that this was dependent on the nominal biological values of the conductances. In the nominal LC cell, equal increases in both $G_{CaT}$ and $G_{Kd}$ disproportionately strengthened $I_{CaT}$, yielding larger peaks and shorter durations for the underlying oscillation. Using this insight, we were able to...
determine that a 1:1.1 ratio in variations of \( G_{\text{CaT}}:G_{\text{Kd}} \) best preserved the features (for numerical estimates see Supplemental Table SM4). The ratio for coregulation will thus depend on the relative strengths of \( G_{\text{CaT}} \) and \( G_{\text{Kd}} \) at nominal values for different types of cells (see other example cases in the following text).

We then investigated the relationship between peak and duration in a given maintenance phase of the underlying oscillation by performing a regression analysis on the outputs for the entire range of \( G_{\text{CaT}} \) and \( G_{\text{Kd}} \) values. Figure 3C shows that peak and duration had an approximately linear inverse relationship when only \( G_{\text{CaT}} \) and \( G_{\text{Kd}} \) were permitted to vary over the two-dimensional space, i.e., higher peaks resulted in shorter durations and vice versa \((R^2 = 0.89)\). This relationship can be explained by noting that higher peaks provided more \( \text{Ca}^{2+} \) influx, which led to faster and stronger termination via \( I_{\text{KCa}} \), as well as to faster inactivation of the depolarizing \( \text{Ca}^{2+} \) currents. This relationship allows for a prediction of the peak based solely on duration, or vice versa, assuming \( G_{\text{KCa}} \) to be fixed. This also shows how \( G_{\text{CaT}} \) and \( G_{\text{Kd}} \) can maintain the duration of the oscillation by controlling the level of depolarization (i.e., peak), for fixed values of \( G_{\text{KCa}} \), supporting our counterintuitive prediction that higher peaks result in shorter durations.

**Termination of the underlying oscillation.** Model experiments showed that \( I_{\text{KCa}} \) was the main current in controlling termination of the oscillation for the LC, due to its dependence on calcium for activation. Without \( I_{\text{KCa}} \), the oscillation returned to the resting potential only after 603 ms, primarily due to the slow inactivation of \( I_{\text{K}} \). Although the \( G_{\text{CaT}}:G_{\text{Kd}} \) module controlled both peak and duration, as discussed in the previous section, \( G_{\text{KCa}} \) was found to modulate the duration of the oscillation with little effect on peak (Fig. 3D). Larger \( G_{\text{KCa}} \) promoted shortening of the duration, but with a saturation effect. Varying \( G_{\text{KCa}} \) from 0.5 to 5-fold the nominal value resulted in the duration values changing from +16% to −24% of the nominal duration. Importantly, for any duration set by a fixed value of \( G_{\text{KCa}} \), the \( G_{\text{CaT}}:G_{\text{Kd}} \) module could maintain that duration and peak if they covaried in a particular proportion.

Although interburst interval is not considered in this study due to the fact that it depends additionally on synaptic inputs, it is noted that increasing \( G_{\text{KCa}} \) may increase the refractory period, i.e., it may extend the time elapsed before another successful oscillation can be generated with the same input stimulus.

*Conservation of generation, maintenance, and termination modules in other model bursting cells*

After establishing the role of these ionic conductances in all phases of bursting output, we sought out, as cited, two other independent model neurons from the literature, the STG AB cell (Soto-Treviño et al. 2005) and the *Aplysia* R15 cell (Bower and Beeman 1998), to determine whether these findings from the LC model applied to other types of bursting cells that had the same currents.

*Generation of the underlying oscillation.** Our initial LC results indicated that \( G_{\text{B}} \) and \( G_{\text{A}} \) may form a module controlling the generation of the underlying observation. Further, we were able to establish boundary lines across conductance levels for \( G_{\text{B}} \) and \( G_{\text{A}} \) that governed the transition from silent cells to forced bursters to endogenous oscillators for the LC. Our results for AB and R15 were consistent with and extended these observations.

*Example case 2: AB cell.* For the AB cell, the nominal parameter values placed the endogenous burster in the upper region of the parameter space in Fig. 4A. A 5-nA pulse for 10 ms was used to generate the boundaries below the “nominal” point (large filled circle) in the figure. The amplitude of this stimulus was sufficient to initiate the oscillation and the duration of the pulse was 5–10% of the oscillation duration, similar to example case 1. As shown in the figure, we were able to replicate the finding of these boundary lines for the AB cell. In addition, the boundaries were steepest for this example case. Increasing the input stimulus also altered the lower boundary in AB; doubling the duration of the input stimulus lowered the

**FIG. 4. Relationships among conductances in the proposed current modules for the AB cell and *Aplysia* R15 cell also follow the same pattern as found for the LC. A: characteristic \( G_{\text{B}}:G_{\text{A}} \) boundaries. Upper boundary (solid line) represents the transition from endogenous oscillation to forced burster, and lower boundary (dashed line) is the transition from a forced burster to a silent cell (i.e., does not produce an underlying oscillation). \( x \)-axis is the conductance multiplier for variation from the nominal values of \( G_{\text{A}} \); \( y \)-axis is the conductance multiplier for variation from nominal values of \( G_{\text{B}} \). Nominal model values are at location (1, 1). B: as in Fig. 3C, the peak of the underlying oscillation varied inversely with duration when \( G_{\text{CaT}} \) and \( G_{\text{Kd}} \) were varied individually across a 0.5- to 5-fold range of their nominal (in bold) values, for the AB and *Aplysia* R15 example cells also. C: the variation of duration with \( G_{\text{KCa}} \), with all other \( G \) held at nominal (in bold) values, followed a similar pattern to that for the LC (Fig. 3D).
boundary line by 14%, whereas doubling both duration and magnitude lowered it by 28%.

**Example case 3: Aplysia R15 cell.** For the R15 neuron, as expected, the parameter values also placed this endogenous burster in the upper endogenous region in the parameter space of Fig. 4A. Using the same logic as that in example cases 1 and 2, a 40-nA pulse for 250 ms was used to generate the boundaries. The slopes of the boundaries were smallest in this case, indicating that a comparatively larger change in $G_A$ was needed to counter an increase in $G_B$. Doubling the duration of the input stimulus lowered the lower boundary line by 21%, whereas doubling both duration and magnitude of the stimulus lowered it by 28%.

All three example cases had boundary lines with different slopes due to the differences in the maximal current conductances and kinetics for each of the models. Furthermore, individual adjustments to the other current conductances ($G_{CaT}$, $G_{Kd}$ and $G_{KCa}$) did have some effect on the endogenous boundaries. At nominal conductance values for the other currents, increasing $G_{KCa}$ to fivefold its nominal value raised the endogenous boundary line by only 3.3 and 5%, respectively, for example cases 1 (LC) and 2 (AB). In example case 3 (R15), increasing it to fivefold its nominal value raised the endogenous boundary by 445%. Increasing only $G_{CaT}$ to fivefold the nominal value shifted the endogenous boundaries down by only 23 and 9%, respectively, for LC and R15. For AB, an increase of $G_{CaT}$ to fivefold the nominal value caused the cell to become endogenous even with $G_{KCa}$ set to 0. Increasing only $G_{Kd}$ to fivefold the nominal value shifted the endogenous boundaries up by 33% in LC, 289% in AB, and 9% in R15. Thus although $G_B$ and $G_A$ appear to form a module to conserve the generation of bursting, the unique output of a cell is, as always, a mix of the total ionic conductances present at any given time.

**MAINTENANCE OF THE UNDERLYING OSCILLATION.** Our initial LC analysis identified a relationship between $G_{CaT}$ and $G_{Kd}$ that maintained the peak and duration of the underlying oscillation. Our model experiments with the AB and R15 cells also yielded consistent findings.

**Example case 2: AB cell.** The nominal AB model produced an underlying membrane potential oscillation with a peak of $-27$ mV and duration of 151 ms. For comparison with biological ranges, in biological recordings from isolated AB neurons, the oscillation duration had a mean of roughly 170 ms (SD $\sim 20$ ms) and the peak amplitude had a mean of about 9 mV (SD $\sim 0.7$ mV) (Bal et al. 1988). This represents an SD of 12% of the mean for duration values. Figure 5, A1 and A2 show plots of the percentage changes in peak and duration values, respectively, as $G_{CaT}$ and $G_{Kd}$ were varied from 0.5- to 5-fold their nominal values (with all other $G_i$ values constant) for AB. These figures were generated in the same manner as that in Fig. 3, B1 and B2. Positive (negative) directional arrows indicated increasing (decreasing) peak heights and durations. Gray areas in Fig. 5, A1 and A2 show cases where $G_{Kd}$ was too large to allow normal endogenous oscillations. A 1:1 ratio of covariation for $G_{CaT}$ and $G_{Kd}$ was found to result in a deviation of 65% in peak and 160% in duration, with a 5-fold increase in nominal values for both conductances. In the nominal AB cell, equal increases in both $G_{CaT}$ and $G_{Kd}$ disproportionately strengthened $I_{Kd}$. We found that a 1:0.5 ratio for $G_{CaT}$/$G_{Kd}$ best preserved the features of the underlying oscillation for the model AB cell (for numerical estimates see Supplemental Table SM5).

**Example case 3: Aplysia R15 cell.** The nominal Aplysia R15 cell model had an underlying membrane potential oscillation with a peak of $-38$ mV and duration of 10.4 s. For comparison with biological ranges, in biological recordings from $n = 7$ R15 neurons, the oscillation duration had a mean of about 8.5
s (SD ~1.875 s) and the peak amplitude had a mean of about 8.5 mV (SD ~0.6 mV) (Coyer 1986). This represents an SD of 22% of the mean for duration values. Figure 5, B1 and B2 shows plots of the percentage changes in peak and duration values, respectively, as $G_{\text{CaT}}$ and $G_{\text{Kd}}$ were varied from 0.5- to 5-fold their nominal values (with all other $G_i$ values constant). These figures were generated in the same manner as that in Fig. 3, B1 and B2, i.e., positive (negative) directional arrows indicated increasing (decreasing) peak heights and durations. A 1:1 variation was found to result in a change of 8% in peak and 92% in duration for a 5-fold increase from nominal values of both conductances. Again, careful analysis revealed that a ratio of 1:0.35 in covariations for $G_{\text{CaT}}$:$G_{\text{Kd}}$ best preserved the output figures (for numerical estimates see Supplemental Table SM6) for the model R15 cell.

As in the LC example, we performed regression analyses on height versus duration for the entire range of $G_{\text{CaT}}$ and $G_{\text{Kd}}$ values, for AB and R15 model cells. For the Aplysia R15 cell, we removed values where $G_{\text{Kd}}$ was too strong because this prevented action potentials and so affected the activation of $G_{\text{CaT}}$. Figure 4B shows that for AB and R15 cells, as with the LC, oscillation height and duration had an inverse relationship when only $G_{\text{CaT}}$ and $G_{\text{Kd}}$ were permitted to vary independently across the two-dimensional space, i.e., higher peaks resulted in shorter durations and vice versa. $R^2$ values were 0.71 and 0.87, respectively, for the AB and Aplysia R15 model cells.

Termination of the underlying oscillation. $G_{\text{KCa}}$ was found to modulate the duration of the oscillation without affecting peak in AB and R15 cells, as it did in the case of the LC. Larger $G_{\text{KCa}}$ promoted shortening of the duration, but with a saturation effect (Fig. 4C). Varying $G_{\text{KCa}}$ from 0.5- to 5-fold the nominal value resulted in the duration values changing from +7 to −13% and +62 to −21% of nominal duration values for the AB and R15 cells, respectively. As in the LC case, for any duration set by a fixed value of $G_{\text{KCa}}$, the $G_{\text{CaT}}$-$G_{\text{Kd}}$ module could maintain that duration and peak if they covaried in the right proportion.

Discussion

Slow-wave bursting cells exhibit a characteristic underlying oscillation in their membrane potential. We investigated how maximal conductances of the active ionic currents might interact to preserve the peak and duration of such oscillations, for both forced and endogenous bursters. This led to the several findings related to the role of individual currents and current groups (modules) in shaping the characteristics of the phases of the oscillation. The LC and AB models are likely fold/homoclinic or “square-wave” bursters with the intracellular Ca$^{2+}$ concentration as the slow resonant variable. Ca$^{2+}$ builds up during the oscillation and the cell transitions back to resting through the Ca$^{2+}$-gated activation of $I_{\text{KCa}}$ and the Ca$^{2+}$-gated inactivation of $I_B$. The time constant for $I_B$ is not “slow” for these two examples. However, for the R15 model the time constant of $I_B$ is 10-fold higher than that of the fast currents and so $I_B$ acts as an additional slow variable, leading to circle/circle or “parabolic” bursting (Izhikevich 2007). Since this study focuses on insights from an electrophysiology perspective, these analytical issues have not been emphasized here.

Characteristics of the slow-wave underlying oscillation

Role of individual currents in shaping the underlying oscillation. Analysis of the effects of individual variations in maximal current conductances (e.g., Fig. 2, where the effects of varying individual current conductances are shown) showed that the peak and duration are not robust to changes in each conductance. However, coregulation of certain current “module” conductances was discovered to largely preserve the features of generation and to preserve its peak and duration. Based on an analysis of the individual currents during the slow-wave underlying oscillation and the effects of individual conductance variations as shown in Fig. 2, the role of the active currents involved in the underlying oscillation can be summarized as follows: $I_A$ countered $I_B$ during the generation of the oscillation, with $I_{\text{CaT}}$ taking over for the majority of the faster depolarizing dynamics of the oscillation. Depolarization activated $I_{\text{Kd}}$, which then countered $I_{\text{CaT}}$ to control the peak of the oscillation. $I_{\text{CaT}}$ and $I_{\text{Kd}}$ had similar effects but with opposite signs. Ca$^{2+}$ buildup within the cell strengthened $I_{\text{KCa}}$, which terminated the oscillation by repolarizing the membrane potential. $I_{\text{Kd}}$ decreased as the oscillation went down, whereas $I_{\text{CaT}}$ increased and $I_B$ inactivated. This implies that $I_{\text{KCa}}$ contributed to termination (together with inactivation of $I_{\text{CaT}}$ and $I_B$) of the oscillation. It is noted that because $I_{\text{CaT}}$ may be involved in generation of the oscillation in some cells, it could be included in the term burst current $I_B$ in such an analysis. Individual current variations thus affect the dynamics of the underlying oscillation in different, yet somewhat related ways.

Peak and duration of an underlying oscillation are strongly related. Counter to intuition, it was found that increasing the hyperpolarizing current $I_{\text{Kd}}$ lengthened the duration of the oscillation. It was also found that increasing the depolarizing current $I_{\text{CaT}}$ shortened the duration. Two reasons account for these phenomena. First, higher peaks occurred when the depolarizing current $I_{\text{CaT}}$ was stronger. This caused greater activation of Ca$^{2+}$ currents leading to a larger buildup of calcium. This buildup, along with a higher voltage, led to stronger and quicker activation of $I_{\text{KCa}}$, resulting in a faster termination of the oscillation. Second, higher peaks (voltages) also caused the depolarizing Ca$^{2+}$ currents to inactivate earlier and cause quicker termination. On the other hand, increases in $G_{\text{Kd}}$ caused lower peaks. Lower peaks caused less activation of the Ca$^{2+}$ currents and less buildup of calcium, resulting in a later onset of the terminating $I_{\text{KCa}}$ current and slower inactivation of the depolarizing Ca$^{2+}$ currents. Thus counter to intuition, stronger $I_{\text{Kd}}$ lengthened the duration of the oscillation and stronger $I_{\text{CaT}}$ shortened it. Also, higher peaks were found to correspond to shorter durations and vice versa.

Separate current modules control different phases of the underlying oscillation

An underlying oscillation of a slow-wave bursting cell can be divided into three phases: generation, maintenance of peak and duration, and termination. Opposing currents with similar activations seem to pair together to control these separate phases. $I_B$ and $I_A$ form the generation module since, when varied individually, they act in opposite ways to control the generation of the oscillation. It is noted that the other currents do have an effect on the boundary lines as quantified earlier,
but a key observation is that for fixed values of those currents, $I_B$ and $I_A$ could always be covaried to control generation of the underlying oscillation.

Similarly, $I_{CaT}$ and $I_{kd}$ form the maintenance module, i.e., they can be appropriately covaried to preserve the peak and duration of the oscillation. These currents have faster dynamics and higher voltage activations, providing them with finer control for shaping the peak and duration of the oscillation, with $I_{CaT}$ promoting higher peaks and shorter durations and $I_{kd}$ doing the opposite. Again, the other currents did affect peak and duration values, but with those fixed, covariation of only $I_{CaT}$ and $I_{kd}$ was found to preserve peak and duration of the oscillation.

$I_{KCa}$ was found to act as a termination module due to its calcium-dependent activation. The activation of $I_{KCa}$ occurred only after calcium build-up during the depolarized phase of the oscillation. Thus it became active only during the termination phase and had little effect on the early dynamics. Although $I_{KCa}$ did affect the duration of the maintenance module to a limited extent, it had little effect on peak. It is not considered a part of the maintenance “module” since it cannot be covaried with either $G_{CaT}$ or $G_{kd}$ to maintain both duration and peak height, has a saturation effect in its limited ability to shorten the duration, and alters other properties such as afterhyperpolarization (AHP). Moreover, as cited, it was found that for any fixed $G_{KCa}$, $G_{CaT}$ and $G_{kd}$ can be covaried appropriately to maintain the peak and duration of that oscillation, indicating that $G_{KCa}$ plays only a secondary role during the maintenance phase. In summary, $I_{KCa}$ can be viewed as semi-independent of the other modules with its primary role being termination of the oscillation.

Once the correlations were determined in the LC model for the baseline case, we questioned whether the correlations were general and held throughout the conductance space. As cited earlier, in addition to investigating whether the correlations were preserved for randomly selected points in the parameter space, we devised a different strategy to test their validity. We considered example cases 2 and 3, the AB cell and the R15 cell, both of which had the same set of conductances.

To investigate whether the correlations would hold at other locations in the parameter space for the LC model, we considered several widely separated points in the maximal conductance space. For the $G_B-G_A$ generation boundary, four points were considered: (1$\times$ $G_{CaT}$, 1$\times$ $G_{kd}$, 1$\times$ $G_{KCa}$), (5$\times$ $G_{CaT}$, 1$\times$ $G_{kd}$, 1$\times$ $G_{KCa}$), (1$\times$ $G_{CaT}$, 5$\times$ $G_{kd}$, 1$\times$ $G_{KCa}$), and (5$\times$ $G_{CaT}$, 5$\times$ $G_{kd}$, 1$\times$ $G_{KCa}$). Here $G_i$ represents the nominal value of the conductance $i$. The correlation did hold at each point and also the boundary did not deviate by $>33\%$ for any of these tested points. This boundary was also found to deviate very little for maximal changes in $G_{KCa}$, $<4\%$ difference for 5$\times$ $G_{KCa}$. Since the boundary was found to exist for maximal changes in each of the conductances, as well as in the maximal two-dimensional space of $G_{CaT}$ and $G_{kd}$, without drastic changes to the boundaries for any of these conditions, we feel that the $G_B-G_A$ module will hold for any set of $G_{CaT}$, $G_{kd}$, and $G_{KCa}$.

Further, the coregulation of $G_{CaT}$ with $G_{kd}$ to preserve the duration and peak was also found to hold for variations in the other three parameters. Three random points from the parameter space were selected: (1.25$\times$ $G_B$, 1$\times$ $G_A$, 1$\times$ $G_{CaT}$, 0.8$\times$ $G_{kd}$, 1.5$\times$ $G_{KCa}$), (1.75$\times$ $G_B$, 2$\times$ $G_A$, 1$\times$ $G_{CaT}$, 0.75$\times$ $G_{kd}$, 0.5$\times$ $G_{KCa}$), and (2.5$\times$ $G_B$, 3.5$\times$ $G_A$, 1.6$\times$ $G_{CaT}$, 2.5$\times$ $G_{kd}$, 5$\times$ $G_{KCa}$). Our finding is that a ratio between $G_{CaT}$ and $G_{kd}$, albeit different for different sets of parameters, can be found that preserves the duration and peak of the underlying oscillations. For all three random points in the parameter space, we were able to find appropriate ratios that preserved the characteristics. Since the insight is also based on the similarities of the current kinetics (shapes of the activation/inactivation curves and time constants), we believe it is general enough and should hold for other points in the conductance space.

The current $I_{KCa}$ does not activate until the end of the oscillation after enough calcium has accumulated inside the cell. For each of the points chosen, we consistently found that $I_{KCa}$ shortened the duration without any significant effect on the peak of the underlying oscillation.

Random sampling of points in the multidimensional space for the LC model thus indicates that the $G_B-G_A$ generation boundary is present for other points in the ($G_B$, $G_{CaT}$, $G_{KCa}$) space and that it is possible to find a ratio of $G_{kd}$ to $G_{CaT}$ for other points in the ($G_B$, $G_A$, $G_{KCa}$) space that preserves duration and peak of the oscillations. The generation boundaries and the $G_{kd}$ to $G_{CaT}$ ratios themselves do vary, depending on the other parameters, but they have been shown to exist for all points where the cell is not silent. As cited, the kinetics of the activation and inactivation curves for the currents involved also support the existence of these correlations. These correlations were then also found to hold for example cases 2 and 3. Thus we conclude that distinct currents or modules of currents largely control the different phases of the underlying oscillation for such slow-wave bursters, with $G_B$ and $G_A$ controlling generation, $G_{CaT}$ and $G_{kd}$ controlling maintenance of peak and duration, and $G_{KCa}$ primarily controlling termination.

$G_B$-$G_A$ parameter subspace has characteristic “functional” boundary lines

Model experiments revealed that the $G_B$-$G_A$ parameter subspace was divided into three separate functional regions, by two characteristic boundaries: an upper region where the parameter values led to an endogenous oscillation, a middle region where forced oscillations occurred such that the cell was silent (i.e., does not produce an underlying oscillation) until an appropriate input stimulus caused an oscillation, and a lower region where the cell did not produce an oscillation, irrespective of the stimulus strength. Changes in the other conductances ($G_{CaT}$, $G_{kd}$, and $G_{KCa}$) had an effect, albeit minor, on either the location or the overall shape of the characteristic boundaries. The leak current may appear to play a role similar to that of the burst current in the generation module, in some cases. However, we feel that it should not be grouped with $I_A$ in the generation module for several reasons. First $I_{leak}$ switches between being an inward or outward current due to its reversal potential being close to the “rest” for both AB and R15 cells, whereas $I_A$ is always an outward current. Thus in the AB and R15 models, $I_{leak}$ assists $I_B$ with depolarization during the early part of the “rest” phase but then assists $I_A$ in hyperpolarizing the cell prior to the upswing of the membrane potential. Kuznetsova et al. (2010) reported that in dopaminergic neurons, A-type potassium currents can increase the firing frequency of pacemaking neurons, if the conductance is reduced. We find a similar result for all three model cases in that
the interburst interval decreases as $G_A$ is reduced. However, reducing $G_{\text{leak}}$ actually prolongs the amount of time until the initiation of an oscillation in the AB and R15 models, playing an exactly opposite role. Second, $I_{\text{leak}}$ is not an active current. For that reason $I_{\text{leak}}$ can increase only linearly with membrane potential changes. $I_{\text{leak}}$ is thus not capable of counteracting the rapidly increasing nonlinear activation of $I_B$ during the generation phase. However, $I_A$ also has a nonlinear activation curve that increases rapidly around “rest,” making it an ideal candidate to counter $I_B$. To prevent a forced oscillation by an input current injection in the LC, it was found that the percentage increase in $I_{\text{leak}}$ had to be 2.3-fold that of the percentage increase in $G_A$. To prevent endogenous oscillation in the other models, it was found that the percentage increase in $I_{\text{leak}}$ had to be 10-fold that of the percentage increase in $G_A$ for the AB model (1.25-fold for the R15 cell), showing that $I_A$ correlates much more strongly with $I_B$, compared with $I_{\text{leak}}$. Third, $I_{\text{leak}}$ is persistent and so affects other phases of the oscillation more compared with the rapidly inactivating $I_A$. This means that increases to $G_A$ will primarily alter the generation module, but increases to $G_{\text{leak}}$ can affect the entire course of the oscillation, including the AHP.

We thus find that the mechanism delineating endogenous oscillating, forced bursting, and nonbursting is most directly attributable to the relative contributions of $G_B$ and $G_A$. Furthermore, these conductances can covary to maintain generation of the underlying oscillation, a feature that may explain similar variability in conductances reported in recent studies (Ball et al. 2010).

$G_{Kd}$ and $G_{CaT}$ can coregulate to maintain peak and duration

For slow-wave bursting cells with the active currents cited, a proportional ratio could always be found for $G_{CaT}$ and $G_{Kd}$ such that the peak and duration of the underlying oscillation were preserved by coregulating the two conductances in that ratio. For example case 1 (LC), a $G_{CaT}$:$G_{Kd}$ ratio of 1:1.1 best preserved the peak and duration values, indicating that the strength of $I_{CaT}$ was comparable to that of $I_{Kd}$. Remarkably, this ratio is consistent with the relationship in mRNA copy number for two channels that may contribute to this relationship, cacophony and shab (Tobin et al. 2009). However, the precise relationship between mRNA copy number and conductance is unknown for these channels. For example cases 2 and 3 (AB cell and the Aplysia R15 cell), $I_{Kd}$ was stronger compared with $I_{CaT}$ when each conductance was varied in the similar proportion from its nominal value. So, a near 1:1 coregulation did not preserve the peak and duration in this case, with positive changes resulting in lower peaks and longer durations. However, coregulation in $G_{CaT}$:$G_{Kd}$ in ratios of 1:0.5 and 1:0.35, respectively, for the AB cell and Aplysia R15 cell, ensured that the peak and duration were preserved. These results are consistent with those reported for channel mRNA; different cell types of the STG often have similar correlations among channel mRNA levels that differ in the slope of their relationship (Schulz et al. 2007). Therefore even with different activation curves and conductances for the currents in the three example cases, coregulation of $G_{CaT}$ and $G_{Kd}$ can preserve the peak height and duration of the underlying oscillation in all the cases.

Conclusions

If compensatory mechanisms exist to stabilize or regenerate the output of neurons, then a balance must be struck between stabilizing mechanisms and those that initiate processes of plasticity for recovery of output. Evidence of stabilizing mechanisms has been documented at multiple levels. For example, STG neurons collected from intact networks with ongoing activity show distinct correlations at the level of both ion channel expression (Schulz et al. 2007) and membrane conductance (Khorkova and Golowasch 2007). Furthermore, when an STG cell with intact ongoing activity is challenged with an overexpression of a hyperpolarizing conductance ($G_{\lambda}$), it is able to maintain its output by a complementary up-regulation of a depolarizing conductance $G_H$ (MacLean et al. 2003, 2005).Thus evidence for coregulation of conductances to stabilize or maintain output exists to justify this line of thought. However, when the output of a cell is changed dramatically, there appear to be plasticity mechanisms that override these stabilizing forces to recover rhythmic activity. For example, when an STG neuron is isolated in cell culture, it undergoes a series of changes in firing pattern ranging from tonic spiking to silent before recovery of bursting is obtained (Haedo and Golowasch 2006; Turrigiano et al. 1995). These mechanisms include an increase in inward currents such as calcium currents and a decrease in outward potassium currents (Haedo and Golowasch 2006; Turrigiano et al. 1995). In a modeling study of the functional recovery of STG neurons, a full account of the regulation was found in the case of correlated or anticorrelated changes of the maximal conductances of the calcium and potassium currents (Olypher and Prinz 2010). Thus these two classes of maintenance of output—stabilization and recovery—may represent changes in the relationship among currents from covariation to independent or inverse regulation.

Our study identifies potentially conserved “modules” involved in different phases of output conservation. In addition, we identify boundaries among these relationships that divide the output of these cells into endogenous oscillating, forced bursting, and silent (not producing underlying oscillations). These modules allow for targeted hypothesis generation for examining potential regulatory mechanisms. For example, if a cell loses its ability to generate bursting, this may represent disruption in the relationship of $G_B$ and $G_A$. In this case, as with the cultured STG neurons, the cells seem to shift into the “silent” phase of the generation module boundary. One possible mechanism toward recovery of bursting activity could be an uncoupling of compensatory coregulation of the generation module, so that the cell can once again cross boundaries (by up-regulating $G_B$ and/or down-regulating $G_{\lambda}$) and reenter the oscillating zone (see Fig. 3A1 for example boundary regions).

Limitations. The present study has some limitations that should be noted. First, preservation of the underlying oscillation in a biological cell may not simply entail balancing maximal conductances, but may also result from posttranslational mechanisms modifying channel kinetics. Although compensatory changes that stabilize neuronal output in the absence of changes in channel kinetics have been demonstrated in Drosophila neurons (Peng and Wu 2007), homeostatic regulation may occur at multiple levels of processing. Second, our model assumes colocalization for all conductances known to generate the underlying oscillation. However, little is known...
about functional compartmentalization of channel types in these cells. The study considers a class of bursters with six active currents and future studies could explore coregulation features in other types of bursting cells. Moreover, the reported models and analyses can be extended to include posttransla-
tional and other relevant mechanisms as they become better understood biologically and can then be used to study their effect on preserving output function.

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DISCLOSURES

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