Modeling Recovery of Rhythmic Activity: Hypothesis for the role of a calcium pump

Yili Zhang
Federated Dept. Biological Sciences
Rutgers University, Newark
(973) 353-5080, yilizh@yahoo.com

Jorge Golowasch
Dept. Mathematical Sciences
NJIT
(973) 353-1267, golowasc@adm.njit.edu

CAMS Report 0506-38, Fall 2005/Spring 2006
Center for Applied Mathematics and Statistics
Modeling Recovery of Rhythmic Activity: Hypothesis for the role of a calcium pump

Yili Zhang\textsuperscript{1} and Jorge Golowasch\textsuperscript{1,2}
\textsuperscript{1}Dept. Biological Sciences, Rutgers University and \textsuperscript{2}Dept. Mathematical Sciences, NJIT

Abstract:

The pyloric network of crustaceans is a model system for the study of the recovery of function after perturbation/injury of a central pattern generating network. The network is well characterized anatomically and functionally, yet the cellular mechanism underlying the stabilization or recovery of its activity is not known. In a previous theoretical study long-term activity-dependent regulation of ionic conductances was shown to be sufficient to explain the recovery of rhythmic activity after it is lost due to removal of central input. This model, however, did not capture the complex temporal activity dynamics (bouting) that follows decentralization and that precedes the final stable recovery. Here we build a model of a conditional pacemaker neuron whose ionic conductance levels depend on activity as before, but also includes a slow activity-dependent regulation of the Ca\textsuperscript{2+} uptake (and release). Intracellular Ca\textsuperscript{2+} sensors, representing enzymatic pathways, regulate the Ca\textsuperscript{2+} pump activity as well as Ca\textsuperscript{2+} and K\textsuperscript{+} conductances. Our model suggests that the activity-dependent regulation of Ca\textsuperscript{2+} uptake as well as ionic currents interact to generate the complex changes in pyloric activity that follows decentralization. Supported by NIMH 64711 and NSF IBN-0090250.

Keywords: Bursting, intracellular signaling, activity-dependent regulation, feedback, decentralization, stomatogastric

Introduction

Networks that produce rhythmic patterns of activity are commonly involved in behaviors that serve basic biological functions (e.g. breathing, heartbeat, mastication, etc). The existence of mechanisms of recovery of function may therefore be essential for survival. One example of such a system is the pyloric network of crustaceans. The pyloric network generates a rhythmic activity pattern that normally depends obligatorily on the actions of neuro-modulatory substances released by axon terminals from adjacent ganglia onto the neurons of the network: after action potential transmission along these axons is blocked or destroyed (decentralization) rhythmic activity ceases [2]. However, activity recovers spontaneously within several hours [3-5] following a very complex temporal dynamical process that involves the alternating turning on and off of the rhythm (‘bouting’) [5]. This bouting activity can last several hours, after which a stable pyloric rhythm emerges that is characterized by a lower frequency than control but otherwise similar properties (Fig.1, [4, 5]). Experimental evidence indicates that STG neurons [6, 7], crayfish leg axons [8] and other neuronal types [9] possess feedback mechanisms that sense neuronal activity and can regulate specific ionic currents and pumps. Intracellular Ca\textsuperscript{2+}, [Ca\textsubscript{in}]\textsuperscript{2+}, is a likely second messenger that can act as a feedback element for conductance regulation because [Ca\textsubscript{in}]\textsuperscript{2+} changes appear correlated with neuronal activity changes [9] and are involved in many intracellular signaling pathways. The recovery process of rhythmic pyloric activity has previously been accounted for theoretically in a simplified pyloric network model in terms of activity-dependent regulation of ionic currents [4]. However, the transition between the quiescent and the stable recovery states in this model was monotonic and it failed to explain the complex bouting dynamics that precedes
full recovery. Here we reevaluate the mechanism of long-term activity-dependent regulation of conductances by introducing an intracellular molecular network of [Ca] in regulation that involves active pumping of Ca++ into intracellular Ca++ stores (e.g. endoplasmic reticulum, ER) and inositol 1,4,5-trisphosphate (IP3) receptor-dependent (IP3RCa) Ca++ release from the ER. Our model suggests that slow activity-dependent regulation of an intracellular Ca++ pump is key to generating the complex temporal dynamics of recovery observed during the boutting period.

Methods

For simplicity we build a single isolated neuron using XPP [10], based on experiments showing that rhythmic activity recovery takes place in isolated neurons of the STG [5]. The model neuron consists of two compartments with a soma/neurite (S/N) compartment generating slow-wave oscillations and an axonal compartment generating Hodgkin & Huxley (H&H)-type action potentials (e.g. Fig. 2C). Ionic currents are modeled closely following those described by Golowasch et al. [4], with only two modifications: \( V_{1/2} \) of \( m_{Ca} = -60.6 \text{ mV} \), and the Ca++ equilibrium potential is updated at every integration cycle according to \( E_{Ca} = \frac{RT}{2F} \ln\left(\frac{[Ca]_{out}}{[Ca]_{in}}\right) \). Temperature=10°C and [Ca]out=13 mM. In summary, the S/N compartment is composed of a leakage current, a voltage-gated Ca++ current \( (I_{Ca}) \), a voltage-gated K current \( (I_K) \), an A type K current \( (I_A) \), a voltage-dependent peptide-activate current found in STG neurons \( (I_{P}) \), see [11] and a current flowing symmetrically between the S/N and axonal compartments:

\[
\frac{dV}{dt} = -(I_{Ca} + I_K + I_A) + \frac{m_t}{[Ca]_{in} + [Ca]_{ER}}
\]

\( [Ca]_{in} \) is used as a feedback element whose concentration is related to neuronal activity: as activity increases, \( [Ca]_{in} \) increases due to raised Ca++ influx through \( I_{Ca} \). \( [Ca]_{in} \) depends on four terms: calcium influx via \( I_{Ca} \), calcium diffusion, and intracellular calcium pump and IP3-sensitive calcium receptor-channel (IP3R), both on an intracellular compartment (ER):

\[
d[Ca]_{in} / dt = -\gamma I_{Ca} - D[Ca]_{in} - F_{pump}([Ca]_{in}) + F_{IP3R}([Ca]_{in})
\]

\( [Ca]_{in} \) is calculated in a thin cytoplasmic shell adjacent to the plasma membrane. Diffusion removes Ca++ out of this shell \( (D=0.00017 \text{ msec}^{-1}) \) and diffusion within the shell is ignored. An intracellular Ca++ storage compartment (ER) is evenly distributed and adjacent to the cytoplasmic shell [12]. The charge-to-concentration conversion factor \( \gamma (0.00678 \text{ µM nA}^{-1}\text{msec}^{-1}) = f(zF/V_{shell}) \), where \( f \) is the cytoplasmic buffering coefficient (0.05), \( z \) is the valence (+2), \( F \) is Faraday’s constant, and \( V_{shell} \) is the volume of a 2 µm thick shell and 80 µm neuron diameter (3.8216*10^{-11} lt). \( F_{IP3R}([Ca]_{in}) \) represents the calcium release from the ER into the cytoplasm through IP3Rs [13]:

\[
F_{IP3R}([Ca]_{in}) = r(g_0 + g_1[IP3RCa]^4)([Ca]_{ER} - [Ca]_{in})
\]

IP3R is a tetramer and activation requires binding of one calcium ion to each subunit. IP3RCa is the open state. Collectively IP3R channels will release calcium out of ER store with rate \( g_1 (0.00357 \text{ msec}^{-1}) \) but, even if all channels close, a small calcium leak conductance \( g_0 (0.000286 \text{ msec}^{-1}) \) remains. \( r (0.6) \) is the ratio of ER volume to cytoplasmic volume, \( [Ca]_{ER} \) is
assumed to be constant and ~10 times larger than the total intracellular Ca concentration (=3.56 µM). The term \([\text{Ca}]_{\text{ER}} - [\text{Ca}]_{\text{in}}\) means that if \([\text{Ca}]_{\text{ER}} > [\text{Ca}]_{\text{in}}\) \(\text{Ca}^{2+}\) will flow out of the ER through activated IP\(_3\)R channels, otherwise the channels are closed. \([\text{IP3RCa}]\) represents the fraction of active IP\(_3\) receptors, and the details of channel opening rate \(d[\text{IP3RCa}] / dt\) is exactly as given in [13].

\[ F_{\text{pump}}([\text{Ca}]_{\text{in}}) \text{ represents calcium uptake by a Ca}^{2+} \text{ pump into the ER [14]:} \]

\[ F_{\text{pump}}([\text{Ca}]_{\text{in}}) = R_{\text{pump}} \frac{[\text{Ca}]_{\text{in}}^2}{[\text{Ca}]_{\text{in}}^2 + \alpha_{\text{pump}}^2} \quad [4] \]

\(R_{\text{pump}}\) is the calcium pump rate (in nMsec\(^{-1}\)) and is regulated by activity (see Fig. 2A and below). \(\alpha_{\text{pump}}\) is the Ca\(^{2+}\) concentration for half maximal pump rate (0.2 µM).

Intracellular Ca\(^{2+}\) regulates 3 sensors, \(S_{1g}, S_{2g}\) and \(S_p\), which represent Ca-sensitive signaling pathways. \(S_{1g}, S_{2g}\) regulate the maximum conductances of \(I_{Ca}\) and \(I_K\), \(\overline{G}_{Ca}\) and \(\overline{G}_K\), and \(S_p\) regulates the Ca\(^{2+}\) pump activity (\(R_{\text{pump}}\)) (Fig. 2A). These sensors are modified from Liu et al. [15] and described by:

\[ S_{1g} = \frac{S_{1g}}{M_{1g}} \overline{H}_{1g}, \quad S_{2g} = \frac{S_{2g}}{M_{2g}} \overline{H}_{2g}, \quad S_p = \frac{S_p}{M_p} \alpha_{p} \]

The terms \(\overline{S}_{1g,p}\) are maximum values (\(S_{1g} = 20.5, S_{2g} = S_p = 1\)), and \(M\) are sigmoidal functions of \([\text{Ca}]_{\text{in}}\): \(M_x = 1/(1 + e^{M_{thr-x} - [\text{Ca}]_{\text{in}}})\), \(H_x = 1/(1 + e^{H_{thr-x} - [\text{Ca}]_{\text{in}}})\). Because of the term \(H_{thr-1,2g}\), \(S_{1g}\) is sensitive to a \([\text{Ca}]_{\text{in}}\) range with a maximum at \([\text{Ca}]_{\text{in}} = 0.685\) µM. \(\overline{G}_{Ca}\) and \(\overline{G}_K\) are regulated by sensors \(S_{1g}\) and \(S_{2g}\), but in opposite directions to allow a homeostatic regulation of \(I_{Ca}\) and \(I_K\), implying that when \([\text{Ca}]_{\text{in}}\) increases \(\overline{G}_{Ca}\) will tend to decrease and \(\overline{G}_K\) will tend to increase, and vice-versa:

\[ \tau_g \frac{d\overline{G}_{Ca}}{dt} = \overline{G}_{Ca} \{S_{1g-eq} - S_{1g} + (S_{2g-eq} - S_{2g})\} \]
\[ \tau_g \frac{d\overline{G}_K}{dt} = -\overline{G}_K \{S_{1g-eq} - S_{1g} + (S_{2g-eq} - S_{2g})\} \]

\(\overline{G}_{Ca}\) and \(\overline{G}_K\) vary slowly (\(\tau_g = 100000\)) while both sensors are away from equilibrium with their time averaged steady-state equilibrium values (\(S_{1g-eq} = S_{2g-eq} = 0.2\)). The sensors used here differ from those used in the previous model [4] in that they allow the values of \(\overline{G}_{Ca}\) and \(\overline{G}_K\) to vary without bounds. This method is more consistent with observations of the biological STG neurons, which show large variability of conductances while activity remains largely preserved [15].

\(R_{\text{pump}}\) is regulated by \(S_p\) according to:

\[ \tau_p \frac{dR_{\text{pump}}}{dt} = R_{\text{pump}} (S_{p-eq} - S_p), \quad \text{and} \quad S_{p-eq} (R_{\text{pump}}) = S + \frac{2(\alpha_{p} R_{\text{pump}} - S)}{1 + e^{(R_{\text{pump,Thr}} - R_{\text{pump}})/\alpha}} \quad [7] \]

where \(\tau_p\) is the time constant of this process (the slowest time constant in the system, \(\tau_p = 500000\)). The time courses of change of \(S_{1g}, S_{2g}\) and \(S_p\) as functions of \([\text{Ca}]_{\text{in}}\) are much faster than those of either \(\overline{G}_{Ca}\) and \(\overline{G}_K\) or \(R_{\text{pump}}\) and are assumed to be instantaneous. While \(S_{1g-eq}\) and \(S_{2g-eq}\) are assumed to be constant, we have found that \(S_{p-eq}\) is required to depend on \(R_{\text{pump}}\). When \(R_{\text{pump}}\) is much lower than the threshold pump rate value (\(R_{\text{pump,Thr}} = 4.5\) nMsec\(^{-1}\)) \(S_{p-eq}\) approaches its maximum value \(S = 0.353\). However, when \(R_{\text{pump}}\) approaches \(R_{\text{pump,Thr}}\), \(S_{p-eq}\) tends to its lower bound value \(\alpha R_{\text{pump}} = 0.303\) (\(\alpha = 0.0001\) µMsec\(^{-1}\)). Time has arbitrary time units, hence is unitless.
Figure 2. Model properties. A. Schematic diagram of intracellular Ca++- dependent conductance and Ca++ pump regulation. $G_K$ and $G_{Ca}$ are the conductances of $I_{Ca}$ and $I_K$, respectively. $S_1$, $S_2$, and $S_3$ are the Ca++ sensors, IP$_3$RCa is the activated IP$_3$ receptor/channel, and Ca pump is the sole intracellular Ca++ uptake process. ER represents a generic intracellular Ca++ store. The shaded arrow labeled Diff represents Ca++ diffusion. B. Time course of model’s membrane potential with definitions of measured properties. Decentralization: $I_P=0$. Bottom panels show voltage traces from A at higher magnification. Left: Control trace before decentralization. Middle: During bouting. Right: During stable recovery. C. Magnified trace during control, bouting and stable recovery.

Results

The single-cell model, with all ionic currents activated, including the neuromodulator-activated current $I_P$, generates stable bursting activity (Fig. 2B, bottom left and 2C, top trace).

There are several signature features of the process of pyloric activity recovery after decentralization [5]: 1) The time required to produce the first bout after decentralization on average takes >3hrs; 2) The average period of bouting activity before stable recovery takes >52hrs; 3) The average bout duration is $104 \pm 63$ sec; 4) The average time between individual bouts (i.e. interbout period) is $23.2 \pm 38.1$ min. Consequently, we used the following criteria to develop a model that correctly reproduced the process of activity recovery were: 1) the period of bouting lasted $\geq 10$ times the duration of the silent period immediately after decentralization; 2) the bout duration was shorter than the interbout duration; 3) the average pyloric frequency of the stable recovered rhythm is lower than the control frequency.

Derivation of successful model

Golowasch et al [4] used a single sensor of activity in the form of a sigmoidal function of the difference between Ca++ influx and a set cytoplasmic Ca++ equilibrium level. This proved enough to reproduce the recovery of pyloric activity after decentralization but no bouting activity could be observed before the activity stably recovered. We reasoned that a second slower intracellular process had to take place that interacted intermittently with the regulation of ionic currents. The operation of a Ca++ pump and of an IP$_3$- and Ca++-activated Ca++ channel in intracellular compartments provides a mechanism that explains observed slow [Ca]$_{in}$ oscillations in several systems [13, 16]. Because the original model of activity recovery [4] relied on the regulation of ionic conductances by a slow Ca++-dependent mechanism, [Ca]$_{in}$ oscillations may be sufficient to generate bouting of activity. We incorporated Othmer and Tang’s [13] model of [Ca]$_{in}$ oscillations (described by $F_{pump}([Ca]_{in})$ and $F_{IP3RI}([Ca]_{in})$ in Methods, eqs. 2-4, Fig. 2A) to the original model and we did indeed observe bouts, however they had durations $>10$ times longer than the average interbouts, bouts were $\sim 2$ times longer that the initial silent period before bouting began, and no stable recovery of activity could be produced in spite of extensive parameter search.

In a second approach we replaced the simple sigmoidal function of Ca++ flux of the original model [4] with two sensors of [Ca]$_{in}$, $S_1$ and $S_2$, representing Ca++-dependent enzymatic pathways that feed back and regulate exclusively the ionic currents $I_{Ca}$ and $I_K$ in the manner described in Methods and represented in Fig. 2A but without sensor $S_p$. This form al-
lows the maximal conductances of these currents to vary without bounds [15]. Thus, we reasoned, overshoots and undershoots of these conductances as $[\text{Ca}]_{\text{in}}$ oscillates may generate correct bouting activity. This model indeed generates bouting activity, with bout durations now similar (but not shorter) than interbout durations, and much shorter than the silent period immediately following decentralization. Nevertheless, no recovery of stable bursting activity followed the bouting period. Stable activity could be obtained by either a) increasing the calcium pump rate, $R_{\text{pump}}$, b) decreasing the IP$_3$ receptor/channel conductance, $g_1$, c) decreasing the rate constants leading to, or d) increasing the rate constants leaving, the conducting state of the IP$_3$ receptor/channel. However, all these modifications lead to bout/interbout duration ratios >1. Conversely, changes in the opposite sense to the parameters a)-d) just mentioned resulted in a lowering of the bout/interbout duration ratio to values <1 but stable recovery is lost.

In summary, the analysis described above for our second model shows that the calcium pump rate $R_{\text{pump}}$ is a sensitive parameter in regulating recovery of rhythmic activity. Small increases of $R_{\text{pump}}$ result in a monotonic increase of rhythmic activity until stable activity is recovered with no intervening bouting activity, while small decreases of $R_{\text{pump}}$ restores bouting activity that qualitatively matched the temporal properties of experimentally recorded bouts but destroys stable activity recovery.

**Successful model**

The general features of the final model’s activity as described by Eqs. 1-7 is shown in Fig. 2B and incorporates an intracellular Ca$^{++}$ storage/release mechanism in which the uptake is regulated by activity through a Ca-dependent sensor, and realistic activity sensors [15]. The regular bursting activity observed before decentralization (Fig. 2B, lower left panel, and Fig. 2C, top trace) rapidly ceases after the maximum conductance of $I_p$ is set to zero (decentralization). After a silent period in which the membrane potential gradually increases (“time to first bout”) bouting activity ensues, which lasts for a period significantly longer than the initial silent period, satisfying Criterion 1 (Fig 2B, top and lower middle panel). During this bouting period, bursting activity transiently resumes (Fig. 2B, bottom middle panel, and Fig. 2C, middle trace) and bouts are initially significantly shorter than the interbouts, satisfying Criterion 2 (bout duration=0.25 ± 0.02; interbout duration 0.49 ± 0.01; bout/interbout ratio=0.51), but later the ratio increases to 1.45 before stable activity resumes. Finally, after stable recovery of rhythmic activity, the average oscillation period (0.027) is slightly longer than the control period (0.023), satisfying Criterion 3.

We performed a sensitivity analysis of the different parameters in regulatory paths of the model. We excluded from this analysis the ionic currents. Large variations of the conductance parameters ($g_0$ and $g_1$, eq. 3) have little effect on recovery. $\overline{S_{ig,p}}$ values (eq. 5) can only be varied approximately ±5% without completely disrupting the bouting properties; within ±5% only bout and interbout durations are slightly affected. The sensor terms $M_{\text{thr-xg}}$ and $S_{\text{xg-eq}}$ (eqs. 5-6) are coupled: modification of one must accompany opposite changes of the other. With changes larger than ±10% from the listed values (Methods) stable recovery is never obtained. The sensitivity of the recovery process to the properties of the Ca$^{++}$ pump mentioned before dictated the complex relationship between $[\text{Ca}]_{\text{in}}$ and $R_{\text{pump}}$ (Eqs. 7). The $S_{\text{p-eq}}(R_{\text{pump}})$ value varies between $S$, immediately after decentralization (when the pump activity is low), and $\alpha R_{\text{pump}}$ shortly before stable recovery (when the pump activity is high). Consequently, the duration of the bouting period (but not of either bout or interbout duration, or the time to first bout) is sensitively and inversely related to the value of $S$. 


Aside from $S$, the only other parameters that sensitively regulate the temporal properties of activity recovery after decentralization are $\tau_g$ and $\tau_p$ (Fig. 4). The time constant of conductance regulation, $\tau_g$, strongly affects the duration of the bouting period (inversely, Fig. 4A), and the individual bout and interbout durations (Fig. 4B). The time constant of the pump regulation process, $\tau_p$, directly affects the duration of bouting (Fig. 4C) and the interbout duration (Fig. 4D), but inversely affects the individual bout duration (Fig. 4D). While $\tau_p$ has no effect on the time to first bout (Fig. 4C), $\tau_g$ slightly and directly affects this duration (Fig. 4A).

Discussion

We have developed a model to explain the complex temporal dynamics of the pyloric network activity following the removal of central input to the STG. We assume that STG neurons recover rhythmic activity as they sense changes in their activity [4, 7, 9]. $[\text{Ca}]_{in}$ changes regulate three $\text{Ca}^{++}$ sensors acting as activity transducers (representing Ca-dependent metabolic pathways) to regulate the maximal conductances of $\text{Ca}^{++}$ and $\text{K}^{+}$ currents, and the $\text{Ca}^{++}$ pump activity. Parameter sensitivity analysis indicates that the kinetics of regulation of both ionic conductances and $\text{Ca}^{++}$ pump activity, and not the ionic conductance values and the $\text{Ca}^{++}$ pump rate themselves, are the most critical elements in determining the dynamic temporal properties of rhythmic pyloric activity recovery.

The mechanism of activity recovery can be explained as follows: decentralization removes an inward current that helps sustain membrane potential oscillations, which are partly due to activation of $I_{Ca}$. With $I_{Ca}$ now inactive $[\text{Ca}]_{in}$ drops and rapid activation of all three sensors occurs. As a consequence $\overline{G}_{Ca}$ increases (and $\overline{G}_K$ decreases) with time constant $\tau_g$ until the new balance of $I_{Ca}$ and $I_K$ transiently enables the generation of bursting activity and bouting en-
sues (Fig. 3C, arrows). $R_{pump}$ also increases with similar rate until a point when this increase decelerates (Fig. 3A, arrow). This point corresponds to the beginning of bouting activity, which is marked by an overshoot of $G_{Ca}$ (Fig. 3C, arrow) and of $[Ca]_{in}$ (which before decentralization is on average $<1\, \mu M$). The increase in $[Ca]_{in}$ that results from $I_{Ca}$ activation leads to enhanced sensor activity (Fig. 3B, middle) and consequent decrease in $I_{Ca}$ conductance (Fig 4B, bottom), which terminates the oscillations after a few cycles (Fig. 2B and 4B). During the interbout $[Ca]_{in}$ remain low and both $R_{pump}$ and $G_{Ca}$ increase (and $G_{K}$ decreases) again until a new bout begins. Thus, the key to the generation of bouting activity is the nature of the activity sensors we use, which are based on biologically more realistic assumptions [15] than used previously [4]. However, the key to a recovery of stable rhythmic activity lies in the gradual increase of $R_{pump}$ (Fig. 3A), which by the end of the bouting period has increased enough to effectively control the changes in $[Ca]_{in}$ on a cycle-by-cycle basis, preventing the overshoots that characterize the bouting period (Fig. 3C, allowing stable periodic oscillations of the membrane potential.

Our model predicts that decentralization leads to 1) a significant increase in intracellular Ca$^{2+}$ pump activity that plays a crucial role in the generation of bouting activity; 2) a rapid increase in $I_{Ca}$ and intermittent down- and up-regulation of $G_{Ca}$ as the Ca$^{2+}$ pump activity gradually increases (simultaneously $I_{K}$ shows an opposite trend to $I_{Ca}$), which accounts for the intermittent activation and inactivation of bursting (i.e. bouting activity). These are testable predictions that need to be evaluated experimentally.
References