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Two types of independent bursting mechanisms in inspiratory neurons: an integrative model

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Abstract

The network of coupled neurons in the pre-Bötzinger complex (pBC) of the medulla generates a bursting rhythm, which underlies the inspiratory phase of respiration. In some of these neurons, bursting persists even when synaptic coupling in the network is blocked and respiratory rhythmic discharge stops. Bursting in inspiratory neurons has been extensively studied, and two classes of bursting neurons have been identified, with bursting mechanism depends on either persistent sodium current or changes in intracellular Ca^{2+} , respectively. Motivated by experimental evidence from these intrinsically bursting neurons, we present a two-compartment mathematical model of an isolated pBC neuron with two independent bursting mechanisms. Bursting in the somatic compartment is modeled via inactivation of a persistent sodium current, whereas bursting in the dendritic compartment relies on Ca^{2+} oscillations, which are determined by the neuromodulatory tone. The model explains a number of conflicting experimental results and is able to generate a robust bursting rhythm, over a large range of parameters, with a frequency adjusted by neuromodulators.

Keywords

Respiratory rhythm; Dendritic bursting; Ca^{2+} oscillations; Pre-Bötzinger complex; Intrinsic burster; Endogenous neurotransmitters

1 Introduction

Neuromodulators, such as amines and neuropeptides, alter the intrinsic properties of neurons. Depending on the neuromodulatory tone, the same neurons and neuronal networks can produce dramatically different activity patterns (Marder 1988; Brown et al. 1998; Czarnecki et al. 2009). In this work, we investigate how neuromodulators, which activate G-proteins and second messenger systems, can produce dynamically different bursting patterns in a critical portion of the respiratory neural network, the pre-Bötzinger complex (pBC).

Inspiratory neurons in the pBC produce a regular bursting rhythm in phase with the activity of inspiratory muscles in the diaphragm. These neurons are a vital part of the ponto-medullary neuronal network, which generates a stable respiratory rhythm (Smith et al. 1991; Johnson et al. 1994), whose amplitude and frequency depends on the level of various neurotransmitters (Johnson et al. 1998; Onimaru et al. 1998; Herlenius and Lagercrantz 1999; Ptak and Hilaire 1999; Fujii et al. 2004; Llona and Eugenin 2005).

If excitatory synaptic connectivity in the pBC is blocked, rhythmic population discharge stops, but bursting still persists in a small subpopulation of these neurons (Funk et al. 1993; Johnson et al. 1994). Experiments on isolated inspiratory bursters have reported that these cells have two different intrinsic bursting mechanisms, one which depends on a persistent sodium current, and another which is Ca^{2+} -dependent (Thoby-Brisson and Ramirez 2001; Pena et al. 2004; Del Negro et al. 2005; Pena and Aguileta 2007; Koizumi and Smith 2008). Individual cells that possess one or the other mechanism have been identified, though no experiments had been performed to test for both mechanisms in a single cell.

The role of intrinsic bursters in the respiratory rhythm remains largely unknown. Depending on postnatal age, only 5–15% of neurons have intrinsic bursting properties (Pena et al. 2004; Del Negro et al. 2005), and their activity alone is not enough to drive the respiratory rhythm in the absence of excitatory synaptic transmission. On the other hand, the currents underlying intrinsic bursting activity are essential for the generation of the respiratory rhythm, since coapplication of persistent sodium and Ca^{2+} blockers stops the respiratory rhythm *in vivo* and *in vitro*. Blocking either the persistent sodium or the Ca^{2+} component alone does not abolish the respiratory rhythm (Del Negro et al. 2002a; Pena and Aguileta 2007).

We propose a model of an inspiratory pBC neuron that has the ability to produce both types of intrinsic bursting activity: somatic bursting, which is dependent upon the persistent sodium current, and dendritic bursting, which relies on Ca^{2+} oscillations in the dendrites. Our model explains a number of conflicting experimental results and can produce robust bursting over a large range of input currents. The model also explains how neurotransmitters can affect the burst frequency in pBC inspiratory neurons.

2 Materials and methods

2.1 Mathematical model

We have developed a two-compartment model of an inspiratory pBC neuron, consisting of six nonlinear differential equations. The model has somatic and dendritic compartments, connected through an axial resistance of $1/g_c$ (where g_c is the conductance). The membrane potentials at the soma (V_s) and the dendrite (V_D) are determined using a Hodgkin–Huxley like formalism (Hodgkin and Huxley 1952). For the somatic compartment we used a previously described model of the pBC neuron (Butera et al. 1999), modified to better fit experimental data (Purvis et al. 2007). The somatic compartment contains the essential spiking currents: fast inward Na^+ (I_{Na}), outward delayed rectifying K^+ (I_K), persistent Na^+ current (I_{NaP}) and passive leak (I_{leak}). The dendritic compartment contains only a calcium-activated nonspecific cation current (I_{CaN}). The voltages in the soma and dendrite are described by the following equations:

$$\begin{aligned} C_{ms} \frac{dV_s}{dt} &= -I_{\text{app}} - I_{\text{Na}} - I_{\text{NaP}} - I_K - \frac{g_c}{1-k} (V_s - V_D) - g_L (V_s - V_L) \\ C_{md} \frac{dV_D}{dt} &= -I_{\text{CaN}} - \frac{g_c}{k} (V_D - V_s), \end{aligned}$$

where the currents are given by:

$$\begin{aligned} I_{\text{Na}} &= g_{\text{Na}} m_{\infty}^3 (1-n) (V_s - V_{\text{Na}}) \\ I_K &= g_K n^4 (V_s - V_K) \\ I_{\text{NaP}} &= g_{\text{NaP}} p_{\infty} h (V_s - V_{\text{Na}}) \\ I_{\text{CaN}} &= g_{\text{CaN}} f([Ca]_i) (V_D - V_{\text{Na}}). \end{aligned}$$

Here the parameter g represents the maximal conductance of the appropriate current (Na , K , NaP or CaN), while n and h are activation and inactivation variables, respectively. Each, n and h , is described by the following equation:

$$\frac{dx}{dt} = \frac{x_{\infty}(V) - x}{\tau_x(V)}$$

where $x_{\infty}(V)$ is the steady state activation/inactivation curve and τ_x is the voltage-dependent time constant. The steady state activation/inactivation curve is modeled as a sigmoid, with x representing m, n, h , or p :

$$x_{\infty}(V) = \frac{1}{1 + \exp\left(\frac{V_S - V_x}{s_x}\right)}$$

while the time constant is modeled as follows

$$\tau_x(V) = \frac{\bar{\tau}_x}{\cosh\left(\frac{V_S - V_x}{s_x}\right)}$$

V_x , s_x , $\bar{\tau}_x$ and g_{max} for each current are as follows: $V_m = -34$ mV, $s_m = -5$ mV, $V_n = -29$ mV, $s_n = -4$ mV, $V_p = -40$ mV, $s_p = -6$ mV, $V_h = -48$ mV, $s_h = 5$ mV, $\bar{\tau}_n = 10$ ms, $\bar{\tau}_h = 10000$ ms, $g_k = 11.2$ nS, $g_{Na} = 28$ nS, $g_{NaP} = 1.5$ or 2 nS (See Table 1), $g_{CaN} = 1.5$ nS.

The Ca^{2+} kinetics are described by two equations representing the intracellular Ca^{2+} balance ($[Ca]_i$) and IP_3 channel gating variable (l) (Li and Rinzel 1994).

$$\begin{aligned} \frac{d[Ca]_i}{dt} &= f_i (J_{ERIN} - J_{EROUT}) \\ \frac{dl}{dt} &= A (K_d - l ([Ca]_i + K_d)) \end{aligned}$$

where f_i is a constant reflecting the fraction of bound to free Ca^{2+} concentration in the cytosol (Wagner and Keizer 1994), $f_i = 0.000025$; A is a scaling constant, $A = 0.005$; K_d is the dissociation constant for IP_3 receptor inactivation by Ca^{2+} , $K_d = 0.4$. Since experiments show that Ca^{2+} oscillations originate in the dendrites of pBC neurons (Mironov 2008) we include intracellular Ca^{2+} kinetics only in the dendritic compartment. Parameter values for the dendritic compartment were chosen to fit the voltage profiles measured in experimental recordings from pBC inspiratory intrinsic bursters (Thoby-Brisson and Ramirez 2001; Pena et al. 2004; Del Negro et al. 2005).

The flux into the cytosol from the ER (J_{ERIN}) is regulated by the activity of IP_3 receptors:

$$J_{ERIN} = \left(L_{IP_3R} + P_{IP_3R} \left[\frac{[IP_3][Ca]_i l}{([IP_3] + K_I)([Ca]_i + K_d)} \right]^3 \right) ([Ca]_{ER} - [Ca]_i),$$

where P_{IP_3R} is the maximum total permeability of IP_3 channels, $P_{IP_3R} = 31000$ pL/s; L_{IP_3R} is the ER leak permeability, $L_{IP_3R} = 0.37$ pL/s; $[IP_3]$ is the IP_3 concentration, $[IP_3] = 1$ μ M (unless specified otherwise); $K_I = 1$ μ M and $K_d = 0.4$ μ M are the dissociation constants for IP_3 receptor activation by IP_3 and Ca^{2+} respectively.

The flux from the cytosol back to the ER (J_{EROUT}) is controlled by the activity of SERCA pumps:

$$J_{EROUT} = V_{SERCA} \frac{[Ca]_i^2}{K_{SERCA}^2 + [Ca]_i^2},$$

where V_{SERCA} is the maximal SERCA pump rate, $V_{SERCA} = 400$ aMol/s; K_{SERCA} is the coefficient for the SERCA pumps, $K_{SERCA} = 0.2$.

To simplify the model we assume the total amount of Ca^{2+} within the cell to be constant, neglecting the flux of Ca^{2+} into and out of the cell. With this assumption, the ER Ca^{2+} concentration can be calculated as:

$$[Ca]_{ER} = \frac{[Ca]_{tot} - [Ca]_i}{\sigma}$$

where $[Ca]_{tot} = 1.25$ μ M and σ is the ratio of cytosolic to ER volume, $\sigma = 0.185$.

The function $f([Ca])$ describes the activation of the I_{CaN} by Ca^{2+} . It is fitted to experimental measurements from calcium-activated cationic channels in rat sensory neurons (Cho et al. 2003).

$$f([Ca]) = \frac{1}{1 + \left(\frac{K_{CaN}}{[Ca]} \right)^{nc}}$$

with $K_{CaN} = 0.74$ μ M and $nc = 0.97$.

A voltage-gated Ca^{2+} current participates in action potential generation in pBC inspiratory neurons (Onimaru et al. 1996; Elsen and Ramirez 1998, 2005), producing a Ca^{2+} influx during each spike (Morgado-Valle et al. 2008). Since this current is primarily active at the membrane potentials achieved during spiking, it is unlikely that it affects the bursting mechanism. To verify that somatic Ca^{2+} current does not affect bursting mechanism, we tested our model with additional L-type Ca^{2+} current in the somatic compartment (Rybak et al. 1997), and adjusted leak current. The model with somatic Ca^{2+} current reproduced all results presented in this work. We have therefore omitted this voltage-gated Ca^{2+} current, to simplify our model.

Other parameter values include: the ratio of somatic to total area, $k = 0.3$; the reversal potentials, $V_{Na} = 50$ mV, $V_K = -85$ mV, $V_{leak} = -57$ mV; the membrane capacitances, $C_{mS} = 21$ μ F, $C_{mD} = 5$ μ F; the conductances $g_{leak} = 2.3$ nS, $g_c = 1$ nS; the applied current $I_{app} = 0$ pA (unless specified otherwise). The model equations were integrated with the XPPAUTO software package (Ermentrout 2002) using a 4-th order Runge-Kutta method with a time step 0.1 ms.

3 Results

3.1 Bursting driven by Ca^{2+} oscillations

Our model was inspired by experimental evidence for two types of intrinsic bursting in inspiratory pBC neurons. One type of bursting depends on persistent sodium current

inactivation, whereas the other type relies on intracellular Ca^{2+} . We present our results by focusing on these two types of bursting and varying two critical parameters to switch the bursting mechanism from one type to the other. In the two extreme cases, bursting is driven by either somatic membrane properties or dendritic Ca^{2+} oscillations alone. However, taking into account the heterogeneity of the persistent sodium current (Del Negro et al. 2002b; Purvis et al. 2007), and the large number of endogenous neurotransmitters converging to the same G_q -protein cascade, which result in IP_3 release (Doi and Ramirez 2008), it is conceivable that these two mechanisms could coexist within the same neuron.

Our model of the inspiratory neuron (Fig. 1(a)) has separate somatic and dendritic compartments. Dendritic compartment can produce endogenous Ca^{2+} oscillations which translated into depolarizing potential through activation of I_{CaN} current. Somatic compartment produce firing in response to depolarizing potential.

For the somatic compartment, we used a previously developed minimal model of an inspiratory pBC neuron (Butera et al. 1999; Purvis et al. 2007). The bursting mechanism in this compartment is based on persistent sodium current inactivation (Butera et al. 1999; Del Negro et al. 2001, 2002b), which can be described briefly as follows. When the membrane voltage reaches its threshold, a series of action potentials are generated by a fast sodium current (I_{Na}) and a delayed rectifier potassium current (I_{K}). The burst is then terminated by the slow inactivation of the persistent sodium current (I_{NaP}).

The dendritic compartment does not have its own spike generating currents. Instead, it produces a depolarizing potential following intracellular Ca^{2+} oscillations. More specifically, a calcium-activated nonspecific cation current (I_{CaN}) depolarizes the cell in response to an increase in the intracellular Ca^{2+} concentration. When the Ca^{2+} concentration decreases, I_{CaN} inactivates and the cell repolarizes to its resting potential.

Calcium oscillations in our model are dependent on IP_3 concentration ($[\text{IP}_3]$). The activation of G_q -protein coupled receptors (labeled as R in Fig. 1(a)) leads to an increase in $[\text{IP}_3]$. In an intact respiratory network, $[\text{IP}_3]$ likely changes dynamically, since the activation of synaptic glutamate receptors leads to changes in $[\text{IP}_3]$ (Pace et al. 2007). However, our work is based mostly on experiments performed in synaptically isolated cells, where glutamate receptors are blocked and therefore cannot be a source of IP_3 . Therefore, we assume a constant IP_3 concentration, reflecting constant neuromodulatory tone. There are several neurotransmitters which are released within the pBC and which activate the G_q -protein pathway, thus contributing to the constant basal level of IP_3 . Since IP_3 is a very common second messenger, there are a number of endogenous released neurotransmitters which can stimulate its release. The most probable candidates are serotonin (5-HT), Substance P (SP) and Thyrotropin-releasing hormone (TRH) (Doi and Ramirez 2008; Ptak et al. 2009).

IP_3 binds to its receptor (IP_3R) on the surface of the endoplasmic reticulum (ER) and initiates a Ca^{2+} flux into the cytosol (Fig. 1(d)). This Ca^{2+} flux is terminated by the slow inactivation of IP_3R by Ca^{2+} . As a result, intracellular Ca^{2+} decreases as the SERCA pumps bring Ca^{2+} back to the ER (Fig. 1(e)). This cycle repeats as IP_3R inactivation is removed at low Ca^{2+} concentration.

The depolarizing potential spreads to the soma and activates action potential generating currents (I_{Na} and I_{K}), initiating the burst (Fig. 1(b)). Since the soma is electrotonically connected to the dendrite, the action potential propagates to the dendrite, producing a dendritic burst of smaller amplitude (Fig. 1(c)). The burst is terminated when the depolarizing potential ceases.

3.2 Classification of bursting types

Our model can produce dynamically different bursting behaviors, depending on chosen parameter values. The bursting mechanism in the soma depends on the combination of parameter values for the persistent sodium and leak currents (Koizumi and Smith 2008). Here, we have varied the parameter g_{NaP} to explore the dynamics in the somatic compartment, while keeping the parameter g_{leak} constant. The Ca^{2+} oscillations in the dendritic compartment reflect the neuromodulatory tone, so we choose the parameter $[IP_3]$ to explore its dynamics. There are number of other parameters that can produce similar effects (see Discussion).

To compare the effects of different bursting mechanisms on model dynamics, we introduce a classification of bursting types in Table 1. The bursting types are characterized by different values of the parameters $[IP_3]$ and g_{NaP} (Table 1, Fig. 2(a)). If there are no Ca^{2+} oscillations ($[IP_3] < 0.955 \mu M$), and persistent sodium is in the bursting range ($1.89 < g_{NaP} < 3.4 nS$), we refer to the bursting as *somatic* (Fig. 2(a), dark grey shaded area on the left). When Ca^{2+} oscillates ($0.955 < [IP_3] < 1.4 \mu M$), but g_{NaP} is in the sub-oscillatory range ($g_{NaP} < 1.89 nS$), we refer to the bursting as *dendritic* (Fig. 2(a), dark grey shaded area in the lower right corner). Lastly, when both g_{NaP} and $[IP_3]$ are in the bursting regime, we call the resulting bursting pattern *somato-dendritic* (Fig. 2(a), dark shaded area in the upper right corner).

3.3 Ca^{2+} oscillations increase the dynamic range of bursting in the model

Both experimental and modeling studies of persistent sodium bursting show that an increase in g_{NaP} switches the cell from silence to bursting to tonic spiking, with higher burst frequency for larger values of g_{NaP} (Butera et al. 1999; Del Negro et al. 2002b). In our model, this result is reproduced if $[IP_3]$ is in the sub-oscillatory regime (Fig. 2(b)). The activity pattern in this regime changes from silence to bursting and tonic firing when g_{NaP} increases (Fig. 2(b)). In addition, the burst period decreases with g_{NaP} .

When dendritic Ca^{2+} oscillations occur in addition to the persistent sodium current ($[IP_3] = 1$, $g_{NaP} = 2$, Fig. 2(c)), the silent and tonic cells are converted into bursters. In this Ca^{2+} -driven regime, increase in g_{NaP} does not lead to decrease in burst period, but to an increase in a burst duration (Fig. 2(c)). Therefore, adding dendritic Ca^{2+} oscillations to a *somatic* burster creates a distinct dynamic behavior, in which persistent sodium current controls burst duration, rather than the burst period.

3.4 Effects of pharmacological blockers

Inspiratory intrinsic bursters in the mouse brainstem have been classified by their response to several pharmacological blockers, which affect either persistent sodium current or Ca^{2+} currents. Cadmium, a non-specific blocker of Ca^{2+} channels, was the first pharmacological agent used to identify two subpopulations of intrinsic bursters in the pBC (Thoby-Brisson and Ramirez 2001). Following cadmium application, one subpopulation was suppressed, while the other subpopulation remained active, leading to the names of intrinsic bursters “cadmium sensitive” and “cadmium insensitive”. Further experiments with flufenamic acid (FFA), a specific blocker of I_{CaN} , identified this current as the main source of cadmium sensitivity (Pena et al. 2004; Del Negro et al. 2005). Although most investigators now use FFA to classify intrinsic bursters, the cells are still classified as cadmium sensitive/insensitive. *Dendritic* bursters in our model would be classified as cadmium-sensitive, since bursting stops if the I_{CaN} current is blocked (Fig 3(c), middle).

The persistent sodium current was theoretically predicted to be a burst-driving current in pBC neurons (Butera et al. 1999). Later it was found that all intrinsic bursters in the rat brain, as well as a subpopulation of the intrinsic bursters in the mouse brain, are sensitive to

the I_{NaP} blocker, Riluzole (RIL) (Del Negro et al. 2001; Pena et al. 2004; Del Negro et al. 2005). *Somatic* bursters in our model would be classified as RIL-sensitive, since bursting activity stops if I_{NaP} is blocked (Fig. 3(a), right).

Therefore, there are four possible pharmacologically identifiable types of intrinsic bursters: cadmium-sensitive/insensitive and RIL-sensitive/insensitive. Unfortunately, it is not clear if these types of bursting mechanisms can coexist in the same cell. There are at least two studies available which compare the effect of the I_{NaP} blocker Riluzole (RIL) on cadmium-insensitive bursters (Pena et al. 2004; Del Negro et al. 2005). One study (Del Negro et al. 2005) found that all cadmium-insensitive cells were disrupted by RIL. Another study (Pena et al. 2004) found that the response of cadmium-insensitive bursters to RIL was heterogeneous: some cells stopped bursting in the presence of RIL, whereas others remained rhythmic, but fired less spikes per burst.

Since both *somatic* and *somato-dendritic* bursters remain rhythmic when the I_{CaN} current is blocked (similar to the cadmium-insensitive class of Pena et al.), our model provides a possible explanation for the heterogeneous response of cadmium-insensitive neurons to RIL. In response to RIL, *somatic* bursting stops (Fig. 3(a), right), but *somato-dendritic* bursters remain rhythmic but fire less spikes (Fig. 3(b), middle). Thus, in a mixed population of *somatic* and *somato-dendritic* bursters response to blocking I_{NaP} would be mixed, with some bursters (*somatic*) stopping, but others (*somato-dendritic*) continuing to burst with less spikes, the result similar to experiment of Pena et al.

Therefore, our model suggests that cadmium-insensitive intrinsic bursters may consist of two types, RIL-sensitive and RIL-insensitive. The presence of these types of intrinsic bursters may explain why the inspiratory rhythm still persists if either FFA or RIL is applied alone, but coapplication of these blockers is able to stop the rhythm, both *in vitro* (Pena et al. 2004) and *in-vivo* (Pena and Aguileta 2007).

Both studies mentioned above (Del Negro et al. 2002b; Pena et al. 2004) found that cadmium-sensitive intrinsic bursting persisted in the presence of RIL. Likewise, in our model *dendritic* bursting continues if I_{NaP} is blocked (Fig. 3(c), right), but only for a sufficiently large value of I_{CaN} . We have, therefore, chosen a value for g_{CaN} in our model ($g_{CaN}=1.5$ nS) to fit this condition. With this choice of parameter, both *dendritic* and *somato-dendritic* bursters remain active when I_{NaP} is blocked, therefore reproducing the results from both experimental studies mentioned above.

3.5 Ca^{2+} oscillations change sensitivity to applied current

What physiological advantages could a pBC neuron gain by having two bursting mechanisms? To maintain a persistent respiratory rhythm, an inspiratory neuron should sustain its rhythmic activity through a wide range of neuronal inputs. Any mechanism promoting this ability will benefit the stability of the respiratory rhythm. Figure 2(c) suggests that Ca^{2+} oscillations promote bursting activity, since both tonic and silent cells switch to bursting for larger values of $[IP_3]$. The neurons in the pBC receive a large amount of excitatory and inhibitory input from populations of tonically firing cells and therefore have to sustain bursting activity under the wide range of neuronal input currents. We next test this hypothesis by varying the applied current for different bursting types in our model, and comparing the ranges of input current which result in bursting dynamics (Fig. 4).

The input range of a *somatic* burster was explored both theoretically (Butera et al. 1999) and experimentally (Del Negro et al. 2002b; Koizumi and Smith 2008). An increase in the applied current produces an almost linear decrease in the burst period of cadmium-insensitive (i.e. *somatic*) intrinsic bursters (Del Negro et al. 2005). Our model reproduces

these experimental findings: the *somatic* bursters period depends almost linearly on the applied current (Fig. 4(a), dashed), with larger applied current reducing the number of spikes per burst (Fig. 4(b), dashed). Only a very small range of currents produces bursting behavior.

In contrast, cadmium-sensitive intrinsic bursters (i.e. *dendritic*, *somato-dendritic*) are relatively current-independent in experiment (Del Negro et al. 2005, Fig. 2(e)), and maintain the same frequency over a large interval of applied current. However, the number of spikes per burst increases with an increase in current. In our model the frequency of *somato-dendritic* and *dendritic* bursting changes only for a very small range of injected current (Fig. 4(a), solid, dotted), while the number of spikes per burst increases with applied current (Fig. 4(b), solid, dotted). The range of applied current that produces bursting in *somato-dendritic* and *dendritic* bursters is significantly larger than the corresponding range of applied current in the *somatic* model.

Thus, our model is consistent with the experimental findings, that cadmium-insensitive (*somatic*) intrinsic bursters are current-sensitive, but cadmium-sensitive (*dendritic*) intrinsic bursters are nearly current-independent, with a larger range of input current over which bursting is supported. The model also predicts that, if found experimentally, cadmium-insensitive, RIL-insensitive (*somato-dendritic*) intrinsic bursters are relatively current-independent with a wider range of bursting frequency and a burst duration which increases with the applied current. These findings indicate that Ca^{2+} oscillations contribute to the ability of respiratory neurons to maintain rhythmic activity under a wide range of neuronal input.

3.6 Ca^{2+} oscillations control burst frequency in the dendritic and somato-dendritic model

Another important characteristic of the breathing rhythm is that it is highly adaptable, capable of producing a wide range of frequencies in response to constantly changing metabolic demands and environmental conditions. Thus, an inspiratory neuron should have the ability to produce a wide range of bursting frequencies.

In our model, the frequency of somatic bursting depends on the value of g_{NaP} , as described previously (Butera et al. 1999). However, the frequency of *dendritic* and *somato-dendritic* bursters in our model are determined by Ca^{2+} oscillations, since the depolarizing potential produced by I_{CaN} reflects the changes in intracellular Ca^{2+} .

In an intact network, IP_3 concentration depends on the basal level of endogenously released neurotransmitters and glutamatergic input, both of which likely change dynamically. This work mostly involves experiments performed in synaptically isolated cells, where glutamatergic activation is absent, and therefore only the neuromodulatory tone determines $[\text{IP}_3]$ (see Discussion). Note that although we have chosen $[\text{IP}_3]$ as the control parameter for Ca^{2+} oscillations, similar results would be observed if the $[\text{Ca}]_{\text{tot}}$ value is varied (See Discussion).

To examine the effect of Ca^{2+} oscillations, we manipulated $[\text{IP}_3]$ in the *somato-dendritic* model. If $[\text{IP}_3]$ is small there are no oscillations, and the cell produces purely *somatic* bursting (Fig. 5(a), top), with constant low levels of intracellular Ca^{2+} (Fig. 5(a), bottom). A small increase in $[\text{IP}_3]$ creates low frequency Ca^{2+} oscillations (Fig. 5(b), bottom). Since the oscillation period is very long in this regime, it is possible to have a mixed bursting pattern, with I_{NaP} -driven bursts embedded into the Ca^{2+} oscillations (Fig. 5(b), top). Further increase in $[\text{IP}_3]$ increases the frequency of the Ca^{2+} oscillations (Fig. 5(c,d)). For larger values of $[\text{IP}_3]$, the model switches to tonic spiking (not shown). The amplitude of Ca^{2+} oscillations slightly increases with $[\text{IP}_3]$, but the basal Ca^{2+} level remains the same.

The increase in $[IP_3]$ causes the transition from *somatic* to *somato-dendritic* bursting. Thus, adding Ca^{2+} oscillations to a *somatic* burster produces a bursting regime with a larger range of frequencies. In a network of such *somato-dendritic* cells, the glutamatergic input would influence the intrinsic burster frequency, providing a possible mechanism for frequency modulation.

3.7 Effect of Ca^{2+} modulation

Evidence for dendritic Ca^{2+} oscillations in inspiratory bursters has been found experimentally (Mironov 2008). Calcium imaging of mice medullary slices and respiratory neurons in primary cell cultures shows that spontaneous rhythmic activity depends on slow Ca^{2+} oscillations in the dendrites (Mironov 2008). These oscillations appear at the branching point of the dendritic tree and propagate as Ca^{2+} waves to the soma, where their arrival coincides with the start of the inspiratory burst.

It also has been shown that Ca^{2+} oscillations in the dendrites are the result of Ca^{2+} release from intracellular stores, since the application of the SERCA pump inhibitor, Thapsigargin (Tg), transiently increases the frequency of oscillations while rising the basal level of Ca^{2+} in the dendrites (Mironov 2008). Although the experiments mentioned above were conducted in an intact inspiratory network, our single cell model is able to reproduce these experimental findings. When the maximum activity of the SERCA pump in our model is decreased ($V_{SERCA}=280$), both the burst frequency (Fig. 6(b), top) and the basal Ca^{2+} level (Fig. 6(b), bottom) increase, which is consistent with the experiment of Tg application in Mironov (2008). Interestingly, in contrast to frequency modulation caused by $[IP_3]$, SERCA pump activity also affects the basal level of intracellular Ca^{2+} .

3.8 Responses to Norepinephrine

Neurotransmitters have multiple effects on respiratory rhythmic activity, adjusting the respiratory frequency and amplitude to varying metabolic demands. Norepinephrine (NE), which is released during arousal from noradrenergic neurons in the ventrolateral pons (A5) and locus ceruleus (A6), affects inspiratory activity (Saito et al. 2002) and has a dramatic effect on intrinsic bursting neurons (Hilaire et al. 2004; Viemari and Ramirez 2006; Doi and Ramirez 2008).

It has been shown that NE has differing effects on cadmium-insensitive and cadmium-sensitive inspiratory bursters in mice medullar brain slices (Viemari and Ramirez 2006). In cadmium-insensitive bursters, NE increases the burst frequency, but does not change the burst duration. In contrast, in cadmium-sensitive bursters, NE does not change the burst frequency, but does increase the burst duration.

In the respiratory neurons of newborn mice, the effect of NE is excitatory and is mediated by the α_1 receptor (Arata et al. 1998; Viemari et al. 2004). Since α_1 receptor activation increases nonspecific cationic conductances in a number of different cell types (Helliwell and Large 1997; Hill et al. 2006), and the effect of NE in inspiratory bursters is eliminated by the I_{CaN} antagonist (Viemari and Ramirez 2006), we have simulated the effect of NE by increasing the parameter g_{CaN} (control: $g_{CaN}=1.5$ nS, NE: $g_{CaN}=6$ nS).

We have observed two distinct responses to NE (i.e., increase in g_{CaN}) in our model. In *somatic* bursters, an increase in g_{CaN} increases the burst frequency, but the burst duration remains similar to the control (Fig. 7(a)). Given that in *somatic* bursters Ca^{2+} does not oscillate, the effect of the increase in g_{CaN} in *somatic* bursters is effectively an increase in the depolarizing bias current, which increases burst frequency, but does not affect burst duration. In *dendritic* bursters, however, the frequency depends on Ca^{2+} oscillations and does not change with g_{CaN} (Fig. 7(b)). Instead, an increase in g_{CaN} increases the

depolarizing potential and makes the bursts longer. Thus, g_{CaN} increases the duration of the *dendritic* burst, without changing its frequency. These results are quite similar to the experimentally observed effects of NE on the cadmium-insensitive and cadmium-sensitive intrinsic bursters, respectively.

In *somato-dendritic* bursters, an increase in g_{CaN} produces an effect similar to that observed in *dendritic* bursters, namely an increase in the burst duration but not in the frequency (Fig. 7(c)). Since bursting of cadmium-insensitive neurons was eliminated by 20 μ M RIL (Viemari and Ramirez 2006), we hypothesize that the sampling population in that experiment contained only *somatic* and *dendritic* intrinsic bursters, with no *somato-dendritic* bursters present in the experimental pool. Our model predicts that for this type of intrinsic burster (cadmium-insensitive, RIL-insensitive), NE will increase the burst duration, but leave the frequency unchanged.

3.9 Hypoxia can affect the sources of IP_3

Hypoxia is an oxygen deficiency in the body's tissues. It can be caused by a reduction in oxygen in the environment, inadequate oxygen transport, or an inability of tissues to use oxygen. In spite of a large number of studies of cellular and molecular events caused by hypoxia (Krnjevic 1999; Nieber 1999; Xia and Haddad 1999; Lopez-Barneo et al. 2008), it is still unclear how hypoxia affects respiratory neurons. In an intact respiratory network, inspiratory neurons respond to hypoxia in a biphasic manner (Richter et al. 1991; Ramirez et al. 1997, 1998; Pena and Ramirez 2005), with an initial frequency increase followed by secondary decrease. Upon isolation from the network, the response of inspiratory bursters to hypoxia depends on their type: cadmium-insensitive bursters remain rhythmic and maintain the same frequency, but cadmium-sensitive bursters progressively decrease their frequency, until they finally lose their intrinsic bursting properties (Pena et al. 2004).

We simulated the hypoxic response in our model by qualitative reproducing the experiments of Pena et al. Since hypoxia only affects cadmium-sensitive neurons, we reasoned that hypoxia affects the dendritic compartment. To reproduce the progressive decrease in frequency of cadmium-sensitive bursters, we vary the value of crucial parameters in our model. We found that a progressive decrease in $[IP_3]$ produces a response similar to experimental data of Pena and colleagues. *Somatic* bursters were not affected by decrease in $[IP_3]$ and continued to burst with the same frequency (Fig. 8(a), dashed), similar to the cadmium-insensitive intrinsic bursters. In contrast, the frequency of *dendritic* bursters decreased and bursting stopped when the decrease in $[IP_3]$ reached 5% (Fig. 8(a), dotted). Since all cadmium-sensitive intrinsic bursters in the experimental study were blocked by RIL (Pena et al. 2004), the study only tested the subset of cadmium-sensitive intrinsic bursters which rely on the persistent sodium mechanism (cadmium-insensitive, RIL-sensitive). Our model predicts that for *somato-dendritic* bursters, the response to hypoxia will be mixed. In these simulations, *somato-dendritic* bursters initially slowly decrease their frequency, but, in contrast to *dendritic* bursters, they later switch to the persistent sodium mechanism, and suddenly increase their frequency (Fig. 8(a), solid). The presence of *somato-dendritic* bursters may account for an increase in the average frequency of inspiratory neurons in an intact network under hypoxic conditions (Thoby-Brisson and Ramirez 2000). However, it is important to bear in mind that in addition to the intrinsic properties of individual neurons, hypoxic conditions affect the synaptic connections within the respiratory network (Pena and Ramirez 2005; Bell et al. 2007; Martinet al. 2007), so additional study will be needed to evaluate the hypoxic response in an intact respiratory network.

Pena et al. (2004) did not report changes in the number of spikes per burst in hypoxic conditions. Our model predicts that the number of spikes per burst will remain constant in

case of *somatic* bursters (Fig. 8(b), solid), but will increase until the bursting stops in case of *dendritic* bursters (Fig. 8(b), blue).

Therefore, the model strongly suggests that the hypoxic effect on pBC inspiratory bursters is mediated by the decreasing activity of neurons projecting to the pBC, which is at least partially due to lowering the basal level of IP_3 . This prediction could be tested experimentally by pharmacologically blocking IP_3R in the dendrites. If our model's prediction is correct, the blocker should create a response similar to hypoxia, in which RIL-sensitive intrinsic bursters remain rhythmic, but cadmium-sensitive intrinsic bursters decrease in frequency until bursting stops.

4 Discussion

We propose, to our knowledge, the first model of a pBC inspiratory intrinsic burster with separate dendritic and somatic compartments, each associated with a distinct bursting mechanism. The model has a Ca^{2+} -dependent bursting mechanism in the dendrite, as well as persistent sodium-dependent bursting mechanism in the soma. The somatic model of bursting is similar to previously published studies. The dendritic bursting mechanism depends on Ca oscillations evoked by endogenously released neuromodulators, which provide the source of IP_3 . The concentration of IP_3 controls the frequency of the resulting compound burst, while the persistent sodium current controls the burst duration. This dual component model is capable of explaining how the same neuromodulator, Norepinephrine, can affect the frequency in some neurons and the burst duration in others.

4.1 Our model proposes a novel mechanism for inspiratory pBC rhythm generation

In spite of a number of theoretical studies of respiratory rhythm generation, until recently the dynamics of intracellular Ca^{2+} and I_{CaN} have not been considered important factors in inspiratory bursting. A recent model (Rubin et al. 2009) produces electrical activity similar to recordings from inspiratory neurons and shares some similarities with our work. The model incorporates an intracellular Ca^{2+} store, which accounts for a passive Ca^{2+} leak to the cytosol and for activation of I_{CaN} . However, the model of Rubin et al. does not incorporate a separate dendritic compartment with detailed IP_3R activation and rhythmic Ca^{2+} release from intracellular stores, resulting in Ca^{2+} oscillations. The main advantage of our model is that it is not only able to reproduce the overall activity pattern recorded in pBC inspiratory neurons, but that it is also able to explain several seemingly contradictory experimental results found in cells isolated from the network.

In fairness, it is hard to directly compare our model to that of Rubin et al. (2009) since their model includes explicit activation of glutamate receptors by synaptic input and thus does not require an intrinsic bursting mechanism. Since the major goal of our work is to explain experimental results in cells isolated from the network, we have concentrated on the intrinsic bursting properties only. The role of synaptic coupling in a network of neurons, each with our proposed bursting mechanism, is beyond the scope of this work and will require further modeling effort.

4.2 The heterogeneity of intrinsic burster properties within a neuronal population

Only a small percentage of inspiratory neurons have intrinsic bursting properties (Pena et al. 2004; Del Negro et al. 2005). When tested *in vitro*, neurons in the respiratory network exhibit a continuous distribution of firing properties. This raises an important question: what is the significance of intrinsic bursting properties for the generation of the breathing rhythm? Theoretical studies have shown that the presence of intrinsic bursters is not required to

generate network-wide bursting (Rubin 2006, 2008), but that intrinsic bursters can create a more robust network with a wider range of output frequencies (Purvis et al. 2007).

In our model, the existence of Ca^{2+} oscillations depends on the neuromodulatory tone, which would be lower in synaptically isolated cells. It is not, therefore, surprising that only a few cells maintain regular bursting activity when isolated from the network. In an intact network, the activation of glutamate receptors would provide a significant source of IP_3 , resulting in a much greater proportion of neurons capable of sustaining Ca^{2+} oscillations.

We have shown that in our model the dendritic bursting mechanism is able to convert tonically spiking or silent neurons into bursters (Fig. 2(b,c)). Thus, cells which tonically spike in a low neuromodulatory tone can switch to bursting if synaptically connected (Fig. 2(c), top), providing a mechanism for robust network bursting. Similar reasoning can be applied to the silent neurons, which can be pushed above the spiking threshold by the driving potential from the dendrite (Fig. 2(c), bottom).

The role of the persistent sodium bursting mechanism in inspiratory neurons was addressed in a number of experimental and theoretical studies (Rybak et al. 1997; Butera et al. 1999; Del Negro et al. 2002b; Koizumi and Smith 2008), in which it was shown that the activity pattern is determined by the persistent sodium and leak currents (Del Negro et al. 2002b; Purvis et al. 2007; Koizumi and Smith 2008). In our model, the persistent sodium current (I_{NaP}) is not essential for bursting to occur, but it acts to control the burst duration. This new function of I_{NaP} could conceivably affect the synchronization of the intrinsic bursters within the network, thereby changing the regularity of the breathing rhythm. In addition, the persistent sodium mechanism may be essential to maintaining the breathing rhythm when the neuromodulatory tone is decreased, as in hypoxia (Fig. 8). A clear understanding of the effect of the distribution of intrinsic bursting properties would require building a detailed network model of the pBC region. Such a model would likely be extremely useful in understanding the effect of neurotransmitters on the regularity of the respiratory rhythm.

4.3 Cell morphology can play an important role in bursting of pBC neurons

In this work, we have only considered the effect of neuromodulators on intrinsic bursting, and have therefore varied the IP_3 concentration. Similar results would be obtained if, instead, we were to manipulate the total concentration of intracellular Ca^{2+} ($[Ca^{2+}]_{tot}$). In physiological terms, neurons that don't have enough Ca^{2+} in their dendrites cannot produce Ca^{2+} oscillations, and, therefore, bursting in these neurons can be only somatic. In contrast, neurons with a large Ca^{2+} supply in the dendrites are able to produce Ca^{2+} oscillations, and their bursting type would be *dendritic* or *somato-dendritic*.

The total Ca^{2+} concentration in the dendrite might depend on dendritic morphology. Studies in different neuronal types have been shown that a large fraction of dendritic Ca^{2+} is enclosed in the ER of the dendritic spine (Andrews et al. 1988; Villa et al. 1992; Martone et al. 1993; Spacek and Harris 1997). Thus, it seems plausible that $[Ca^{2+}]_{tot}$ in our model reflects the extent of the dendritic tree. It is plausible to suggest that neurons with small (or partially cut) dendritic trees have a small $[Ca^{2+}]_{tot}$, whereas cells with extensive, spiny dendritic trees have a significant amount of Ca^{2+} locked in the ER in their dendritic spines. Unfortunately, there are few experimental studies available on the dendritic morphology of inspiratory neurons. An experimental study comparing the distribution of dendritic spines in cadmium-sensitive and cadmium-insensitive intrinsic bursters would also be very useful in evaluating this hypothesis.

Differences in dendritic morphology may also help to explain the inter-species differences in burst mechanisms. Experiments in rat brain slices have failed to reveal any cadmium-

sensitive intrinsic bursting activity (Del Negro et al. 2002a). It is possible that the dendritic morphology and/or dendritic Ca^{2+} supply are different in the rat brain. If rat inspiratory neurons have insufficient Ca^{2+} in their dendrites, they could produce only *somatic* (persistent sodium-dependent) bursting. Another factor contributing to the absence of cadmium-sensitive bursting in the rat brain might be a different rate of brain maturation, since the proportion of cadmium-sensitive bursters has been observed to depend on postnatal age in newborn mice (Pena et al. 2004; Del Negro et al. 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Illustration of the dynamics of the model with parameters $[IP_3]_{tot}=1$, $g_{NaP}=2$ nS, $g_{CaN}=1.5$ nS. **(a)** Sketch of the model. **(b)** Voltage in soma. **(c)** Voltage in the dendrite. **(d)** Intracellular Ca^{2+} concentration in the dendrite. **(e)** Dendritic ER Ca^{2+} concentration

Fig. 2.

The type of bursting activity is determined by parameter values. **(a)** The range of parameters g_{NaP} and $[IP_3]$ for different types of electrical activity in the model. White color represents tonic spiking, light gray is silence and dark grey is bursting. The bursting regime is divided into somatic, dendritic and somato-dendritic. Stars shows representative values chosen for simulations (See Table 1). **(b)** Sub-oscillatory Ca^{2+} regime ($[IP_3]=0.9 \mu M$). The model cell is silent if g_{NaP} is low ($g_{NaP}=1.5$ nS, bottom). An increase in g_{NaP} produces somatic bursting ($g_{NaP}=2$ nS, middle), and a further increase yields tonic firing ($g_{NaP}=3.5$ nS, top). **(c)** When Ca^{2+} is in the oscillatory regime ($[IP_3]=1 \mu M$), the cell produces bursting for all three values of g_{NaP} . The frequency of bursting stays the same, but the burst duration increases with increasing g_{NaP} .

Fig. 3.

Effect of I_{CaN} and I_{NaP} blockers on different types of bursters. **(a)** A *somatic* burster (*S*) is blocked by the I_{NaP} blocker RIL (*right*), but persists under the I_{CaN} blocker FFA (*middle*). **(b)** A *somato-dendritic* burster (*S-D*) persists under the both I_{NaP} and I_{CaN} blockers. **(c)** A *dendritic* burster (*D*) is blocked by the I_{CaN} blocker FFA (*middle*), but persists under the I_{NaP} blocker RIL (*right*)

Fig. 4.

Current injection has different effects on *somatic* (dashed), *dendritic* (dotted), and *somato-dendritic* (solid) bursters. **(a)** Current injection into the *somatic* model (dashed curve) decreases the burst period. In the *dendritic* model (dotted curve), the burst period is constant, except at low injected current. In the *somato-dendritic* model (solid curve), current injection produces a response similar to that of the *dendritic* model, with a slightly larger range. **(b)** Current injection into the *somatic* model (dashed curve) decreases the number of spikes per burst. In the *dendritic* model (dotted curve), the number of spikes per burst increases with applied current. In the *somato-dendritic* model (solid curve), the response is similar to that of the *dendritic* model, though over a slightly larger range of applied current

Fig. 5.

The IP_3 concentration determines the frequency of the oscillations. **(a)** When $[IP_3]$ is small ($[IP_3] = 0.9$ μ M) there are no Ca^{2+} oscillations and bursting is *somatic*, due to persistent sodium current. **(b)** A small increase in $[IP_3]$ to 0.95 μ M is enough to start Ca^{2+} oscillations with very long periods. Persistent sodium bursts appear within Ca^{2+} oscillations. **(c)** With further $[IP_3]$ increase to 1 μ M, the bursting is tuned to the frequency of Ca^{2+} oscillations. **(d)** The burst frequency increases further with an increase in $[IP_3]$ to 1.2 μ M. For all panels $g_{NaP} = 2$ nS

Fig. 6.

The model reproduces the results of experiments in intracellular Ca^{2+} modulation. **(a)** Somatic voltage (*top*) and intracellular Ca^{2+} (*bottom*) for a control simulation ($V_{SERCA}=400$, $[IP3]=1$). **(b)** Somatic voltage (*top*) and intracellular Ca^{2+} (*bottom*) for parameters representing the SERCA pump inhibitor, Tg ($V_{SERCA}=280$, $[IP3]=1$). When the activity of the SERCA pumps decreases, the burst frequency increases (*top*) because of faster Ca^{2+} oscillations (*bottom*). The basal level of intracellular Ca^{2+} increases with Tg application

Fig. 7.

The model can explain the difference in the response to NE observed in two different types of intrinsic bursters. We model NE application as increase in I_{CaN} , from $g_{CaN}=1.5$ nS (control; *left column*) to $g_{CaN}=6$ nS (NE; *right column*). (a) NE increases the frequency of *somatic* bursters, but the burst duration stays the same. (b) NE increases the burst duration in *dendritic* bursters, but the burst frequency stays the same. (c) In *somato-dendritic* bursters, the effect of NE is similar to (b), but the change in burst duration is less pronounced. For all panels, $V_{leak}=-59$ mV

Fig. 8.

A decrease in $[IP_3]$ in the model can reproduce the experimentally observed response to hypoxia. **(a)** For *somatic* bursters (*dashed curve*), a decrease in $[IP_3]$ does not change the burst frequency. For *dendritic* bursters (*dotted curve*) the frequency decreases with decreasing $[IP_3]$ until bursting activity stops. For *somato-dendritic* bursters (*solid curve*) the frequency first decreases, then abruptly increases and remains relatively constant. **(b)** A decrease in $[IP_3]$ does not change the number of spikes per burst in *somatic* bursters (*dashed curve*). In *dendritic* bursters (*dotted curve*), the number of spikes per burst increases until bursting ceases. In *somato-dendritic* bursters (*solid curve*), the number of spikes per burst first increases, then abruptly decreases and finally remains relatively constant.

Table 1

Summary of bursting classification

Intrinsic burster type	Somatic compartment behavior (representative parameter value)	Dendritic compartment behavior (representative parameter value)
Somatic	Bursting ($g_{NaP} = 2 \text{ nS}$)	Non-oscillatory ($[IP_3] = 0.9$)
Somato-dendritic	Bursting ($g_{NaP} = 2 \text{ nS}$)	Oscillatory ($[IP_3] = 1$)
Dendritic	Non-Bursting ($g_{NaP} = 1.5 \text{ nS}$)	Oscillatory ($[IP_3] = 1$)