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## Dynamics of excitatory networks of bursting pacemaking neurons: Modeling and experimental studies of the respiratory central pattern generator

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### Abstract

We have explored the dynamics of a computational model of an excitatory network of bursting pacemaker neurons with heterogeneous properties. The network generates synchronous bursts of activity, and the frequency of both single cells and the synaptically coupled pacemaker cell population may be controlled by varying the degree of depolarizing input (DI). The dynamic range of DI where stable bursting occurs is significantly larger for the coupled population than that of individual cells, suggesting a functional role of cellular heterogeneity in making biological rhythms more robust. Experimental evidence is presented from the pacemaker-network generating the respiratory rhythm in the mammalian brainstem. © 2000 Elsevier Science B.V. All rights reserved.

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The respiratory rhythm originates in the brainstem [2,17] in a critical region of the ventrolateral medulla called the pre-Bötzinger complex (pre-BötC), discovered by Smith and colleagues [23]. Evidence from in vitro [8,12,15,16,23,25] and in vivo

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studies [11,14,21] suggests that this region contains a locus of rhythm-generating neurons that are necessary and sufficient to generate the inspiratory phase of the respiratory rhythm. Experimentally respiratory rhythm generation can be studied in such reduced preparations as the in vitro *en bloc* brainstem-spinal cord [25,26] and the *transverse slice* preparation [8,10,12,14,15,23], which contains the pre-BötC and a local network transmitting the rhythm to the hypoglossal (XII) motonucleus in a single brainstem slice. The transverse slice generates a stable rhythmic discharge that can be recorded from both the pre-BötC and XII motonucleus as well as the ventral nerve roots of the XII nucleus [23]. Thus, it is possible to perform single-cell recordings from both the *rhythm generator* (pre-BötC) and *transmission* circuitry as well as recordings of the XII ventral root activity, which is a measure of the overall population-wide “output” of the network.

How is the neuronal rhythm in the pre-BötC generated? Networks generate their rhythms through three basic mechanisms: (1) a rhythm that is primarily determined by network connectivity, (2) a rhythm that is generated by intrinsically rhythmic (pacemaker) neurons, or (3) a combination of these mechanisms. Traditionally, the observations of firing times as well as correlation analyses have led to the conclusion that respiratory rhythm generation occurs primarily via complex network of inhibitory and excitatory connections that determines the timing of the firing of all respiratory neurons [2,18]. This view is reflected in virtually all computational models of respiratory rhythm generation published to date [1,3,9,20]. However, in the in vitro *en bloc* and slice preparations, rhythmogenesis persists, when synaptic inhibition is blocked [4,7,13]. Furthermore, it has been shown in the transverse slice that pacemaker neurons exist [23], their activity persists when synaptic coupling is blocked [10], and that excitatory synaptic coupling is responsible for synchronizing pacemaker neurons within the pre-BötC [12]. This has led to the idea that a network of intrinsically bursting pacemaker neurons are the kernel for respiratory rhythmogenesis [22,24], at least in younger mammals and reduced preparations. Thus, in general, rhythm generation in this system must be modeled as a hybrid of cellular pacemaker and network properties [22,24].

We have previously developed computational models of plausible biophysical mechanisms for rhythmic burst generation in the pre-BötC [5] pacemaker cells. These models do not possess the entire range of ionic currents known to exist in respiratory neurons [19], but rather focus on the minimal ionic current mechanisms necessary to generate voltage-controlled oscillatory bursting consistent with experimental recordings. Our primary candidate single-cell model consists solely of action-potential generating currents ( $\text{Na}^+$  and  $\text{K}^+$ ), a  $\text{K}^+$  leakage current, and a persistent  $\text{Na}^+$  current with slow inactivation that is responsible for burst generation. This minimal model reproduces a wide range of experimental findings, such as the control of activity mode (silence, oscillatory bursting or beating) and burst frequency as the cells baseline membrane potential is depolarized, and the range of spike-frequencies during a burst typically recorded in vitro. We then developed a large heterogeneous network of these models [6] that presents a mechanism for rhythm generation in the pre-BötC: neurons with slow adaptation properties due to the persistent  $\text{Na}^+$  current (some in their oscillatory bursting regime), in combination with excitatory synaptic coupling, are

critical for the generation of the network rhythm. This modeled network, which also incorporated follower neurons transmitting the rhythm to output neurons, was capable of generating synchronous population-wide rhythmic bursting, mimicking the population activity in the slices, in a variety of parameter regimes. Emergent rhythms and synchronous bursting could even occur in parameter ranges where none of the neurons were intrinsically bursting (but still adapting) if the synaptic connectivity was made strong enough.

In the computational studies of Ref. [6], two predictions were made regarding the dynamics of the pacemaker-network in the slice preparations for which we now offer direct experimental evidence:

- (1) The bursting frequency of the network increases as the slice is depolarized, accompanied by a decrease in both the burst duration and amplitude of the integrated XII activity (which follows the overall spiking activity of the pre-BötC), as well as a change in the shape of the burst discharge.
- (2) The *dynamic range* of the heterogeneous pacemaker-network (range of depolarizing input where rhythmic bursting occurs) is greater than that of a typical bursting neuron within the pre-BötC.

To model the effect of depolarizing input on the pre-BötC rhythm in the slice, we utilized our previously developed network model [6]. This model consists of 50 heterogeneous cells, some intrinsically bursting (depending on the randomly distributed cell parameters), with excitatory (glutamatergic-like) synaptic coupling among the neurons in the population. The model was depolarized by varying  $E_K$  in an equivalent manner for all of the neurons in the network. The effect of  $E_K$  on network activity is shown in Fig. 1 (left side). Each panel illustrates the number of action potentials occurring across the population summed into 10 ms bins. The network “turns on” at  $-85$  mV, and as the network is depolarized the period decreases, the bursts become shorter, the amplitude of the population burst decreases, and the baseline spike activity increases as more and more cells are recruited into a mode of constant spiking (i.e. beating). At  $E_K = -68$  mV, the population switches to a state of continuous spiking.

The equivalent experimental data is shown in Fig. 1 (right panels). Each panel illustrates a XII ventral root recording AC-rectified with the resulting signal averaged into 100 ms bins. Initially, the network is firing sporadically ( $[K^+]_0 = 5$  mM). As the network is depolarized, a regular rhythm emerges with trends similar to the modeled network: the burst period, amplitude, and burst duration decreases with depolarization. We do not see a shift in baseline XII activity, as in the model, since (1) the signal was AC-coupled and (2) the XII neurons are post-synaptic to the rhythm-generating circuitry in the pre-BötC, thus we presume that a shift in baseline spiking activity at higher values of  $[K^+]_0$  occurs in the pre-BötC but is insufficient to trigger a significant baseline shift in XII motoneuron activity.

One of the major model predictions [6] was that cellular heterogeneity is functionally advantageous in that it makes the rhythm more robust. Fig. 2 (left panel, black) illustrates the range of depolarizing input (via  $E_K$ ) where synchronous bursting occurs and the resulting burst period for a simulated heterogeneous network similar to

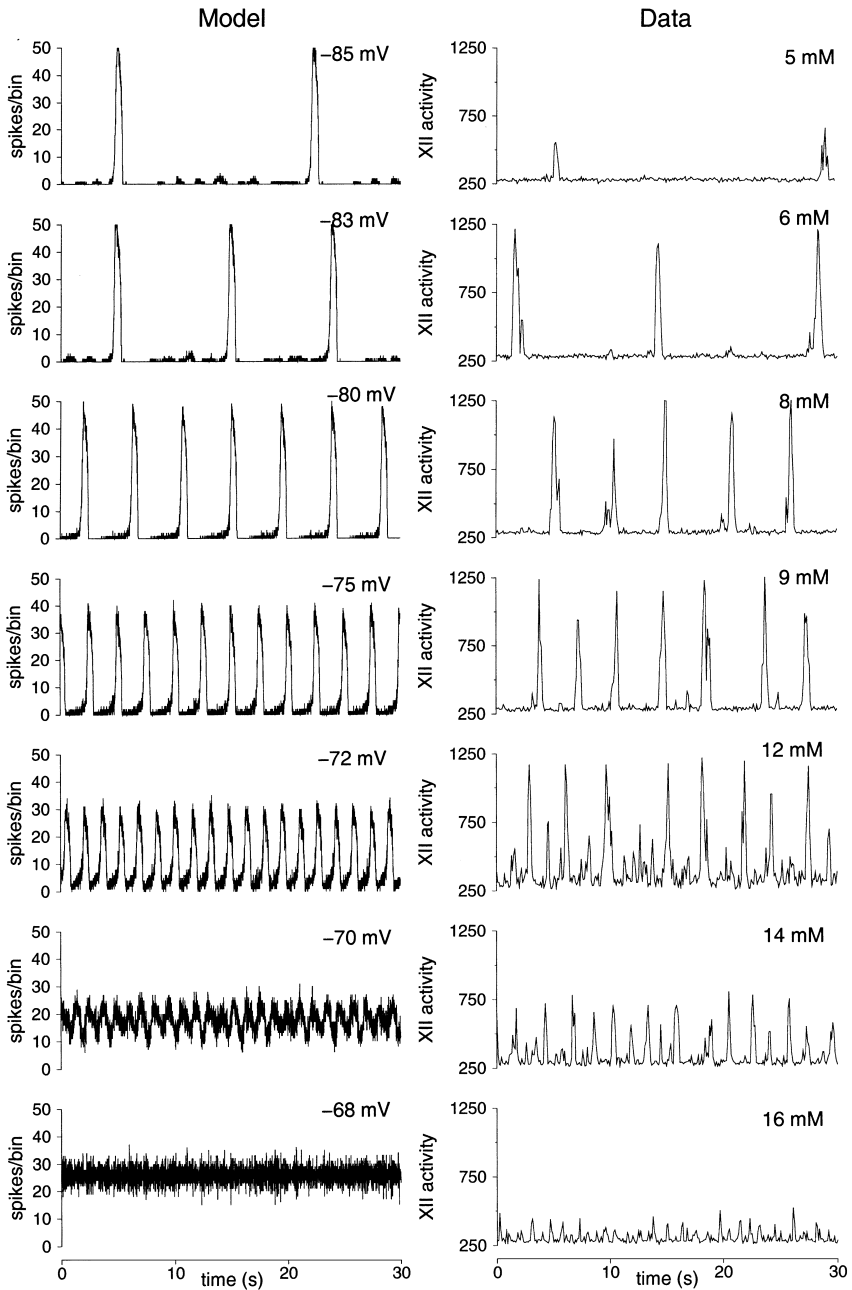


Fig. 1. Voltage-dependent control of the slice rhythm. Left panel shows modeled pre-BötC activity as the network is depolarized via  $E_K$ . Plots show number of spikes firing within the network in 10 ms bins and exhibit rhythmic population-wide bursting; modeled network consists of 50 heterogeneous burst-capable cells as described in Ref. [6]. Right panel shows the XII population activity (AC-rectified nerve activity) as an in vitro transverse slice generating respiratory rhythm is depolarized via  $[K^+]_0$ .

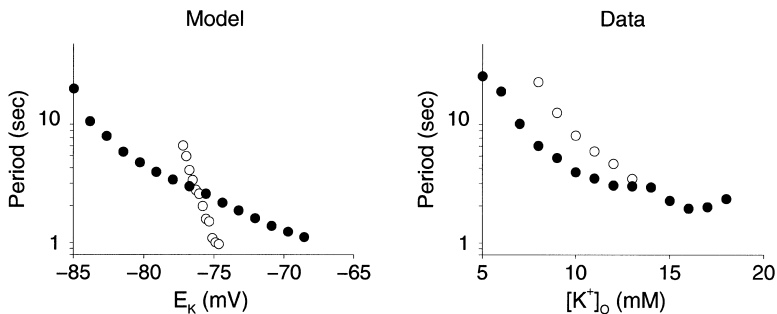


Fig. 2. Range of periods of synchronous bursting for the network (black filled circles) and isolated pacemaker cells (open circles) as depolarizing input is increased. Left panel shows the range of frequencies from a network simulation (black) and an “average” neuron whose parameters are the means for the random distribution of parameters used for the network. Depolarization was accomplished by increasing  $E_K$ . Right panel illustrates data from a single transverse slice experiment. Black circles indicate the period of synchronous bursting for the slice, while the open circles indicate the period of an intrinsically bursting pacemaker neuron recorded within the pre-BötC after network activity and glutamatergic synaptic coupling was blocked with CNQX. Depolarization was accomplished by increasing  $K^+_o$ .

that shown of Fig. 1. The open circles illustrate the behavior an isolated bursting pacemaker neuron whose parameter values are the means used for the random distribution of parameters in the network simulation. The dynamic input range where synchronous bursting is supported is clearly much greater than for the “mean field” neuron. Further analysis in Ref. [6] indicated that this increased dynamic range was due to a synergistic combination of 2 factors: cellular heterogeneity and synaptic coupling. We further found that synaptic coupling alone in a homogeneous network, while increasing the dynamic input range, actually *decreased* the output range of burst periods, and this range was enhanced beyond that of the isolated neuron when heterogeneity was introduced into the network. Most of this increase in dynamic output range occurred at the low end where the burst periods were long (10 s or greater).

We sought to verify if this situation existed within the transverse slice preparation in vitro. Network activity in the slice was measured via the integrated XII nerve recordings as  $[K^+]_o$  was varied. This is illustrated in Fig. 2 (right panel, black circles) for a typical slice. After excitatory coupling was blocked via CNQX, the same protocol was applied using cellular recordings from intrinsically bursting pre-BötC neurons. A typical result is shown in Fig. 2 (right panel, open circles), which demonstrates that the isolated neuron has an intrinsic range of burst periods as well as range of  $[K^+]_o$  where bursting is supported that is less than that of the slice network.

The pooled results are shown in Fig. 3. The model data was based on 3 “experiments” consisting of a given random distribution of parameters in a 50 neuron network and varying  $E_K$  to observe the steady-state rhythmic activity. The single neuron results were obtained by pooling the activity of all the rhythmically active neurons in their isolated state from the entire simulation. The experimental data was obtained as described in the figure caption.

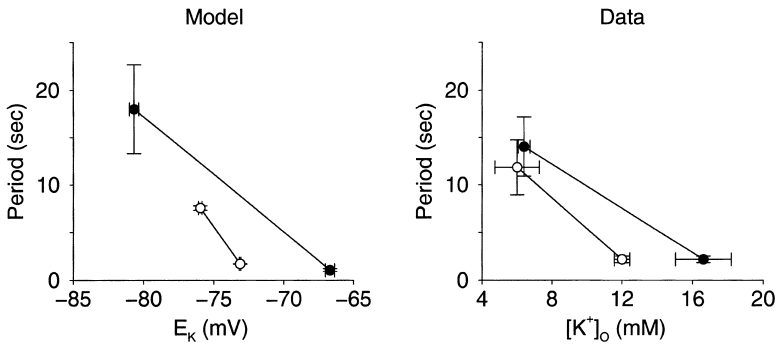


Fig. 3. Dynamic ranges of input and output for single neurons and the network in rhythmic slices. Each curve has two points, showing the minimum and maximum values of the input parameters ( $E_K$  in left panel,  $K^+_o$  in right panel) where bursting was supported. Periods at those parameter values are also shown, and horizontal and vertical error bars indicate SEM. Black circles represent the network response and open circles are the single neuron response. Left panel is modeling results ( $n = 3$  experiments for the network, and  $n = 150$  for isolated bursting neuron), right panel is from *in vitro* transverse slice recordings ( $n = 10$  for the whole slice network and  $n = 5$  for isolated pre-BötC pacemaker neurons).

The experimental data clearly shows an enhanced dynamic input range that is extended (although not as large as the modeling results) for the functional network as opposed to the single pacemaker neuron, with most of this extended dynamic range occurring at the higher range of  $[K^+]_o$  values. The data also shows that on average the slice network is capable of generating stable rhythms at periods slower than that possible from individual neurons within the network.

From these modeling and experimental results, we postulate that cellular heterogeneity plays a functionally advantageous role in extending the dynamic range of a pacemaker network: heterogeneity makes the rhythm more robust and controllable. Our other modeling results [6] also indicate that the dynamic output (frequency) range is broadest when synaptic coupling strength is not strong but sufficient for synchronizing bursting in the network. In the case of the respiratory network, rhythm generation must be a stable, robust process, adaptable over a wide range of frequencies. Neurons are naturally heterogeneous in cellular properties and synaptic coupling and this may allow broader dynamic tuning and control of the respiratory network. We predict that heterogeneity may play an general role in regulating the dynamic behavior of excitatory networks of bursting neurons.

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