Respiratory-Like Rhythmic Activity Can Be Produced by an Excitatory Network of Non-Pacemaker Neuron Models

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Kosmidis, Efstratios K., Olivier Pierrefiche, and Jean-François Vibert. Respiratory-like rhythmic activity can be produced by an excitatory network of non-pacemaker neuron models. J Neurophysiol 92: 686–699, 2004; 10.1152/jn.00046.2004. It is still unclear whether the respiratory-like rhythm observed in slice preparations containing the pre-Bötzinger complex is of pacemaker or network origin. The rhythm persists in the absence of inhibition, but blocking pacemaker activity did not always result in rhythm abolition. We developed a computational model of the slice to show that respiratory-like rhythm can emerge as a network property without pacemakers or synaptic inhibition. The key currents of our model cell are the low- and high-threshold calcium currents and the calcium-dependent potassium current. Depolarization of a single unit by current steps or by raising the external potassium concentration can induce periodic bursting activity. Gaussian stimulation increased the excitability of the model without evoking oscillatory activity, as indicated by autocorrelation analysis. In response to hyperpolarizing pulses, the model produces prolonged relative refractory periods. At the network level, an increase of external potassium concentration triggers rhythmic activity that can be attributed to cellular periodic bursting, network properties, or both, depending on different parameters. Gaussian stimulation also induces rhythmic activity that depends solely on network properties. In all cases, the calcium-dependent potassium current has a central role in burst termination and interburst duration. However, when periodic inhibition is considered, the activation of this current is responsible for the characteristic amplification ramp of the emerged rhythm. Our results may explain controversial results from studies blocking pacemakers in vitro and show a shift in the role of the calcium-dependent potassium current in the presence of network inhibition.

INTRODUCTION

The pre-Bötzinger complex (pBC) within the lower brain stem is suggested to represent a “kernel” of the respiratory rhythm in mammals (Funk and Feldman 1995; Rekling and Feldman 1998; Smith et al. 1991). In vivo, pharmacological lesion of the pBC in the adult cat results in central apnea (Ramirez et al. 1998). Slice preparations from neonatal and juvenile rodents containing this kernel display a respiratory-like rhythm when the external potassium concentration ([K+]ext) is raised (Del Negro et al. 2002b; Johnson et al. 1994; Koshiya and Smith 1999; Lieske et al. 2000; Ramirez et al. 1997; Rybak et al. 2003b; Shao and Feldman 1997; Thoby-Brisson and Ramirez 2001). The rhythm persists despite blockade of synaptic inhibition (Shao and Feldman 1997). Intrinsically bursting pacemaker cells have been experimentally identified in the pBC of neonatal rodent rhythmic slices, and it has been proposed that they provide the cellular mechanism for respiratory frequency control (Del Negro et al. 2002a; Koshiya and Smith 1999; Thoby-Brisson and Ramirez 2001). The central role of pBC in rhythm generation was demonstrated by showing that the rhythm could emerge in isolated pBC “islands” from neonatal rodent slices (Johnson et al. 2001). Again, high extracellular potassium concentrations were needed, inhibition was not necessary, and pacemaker activity was detected with optical recordings.

Mathematical models of excitatory networks incorporating synaptically coupled bursting pacemaker neurons are consistent with these results (Butera et al. 1999a,b; Del Negro et al. 2001). These models suggest that a slowly inactivating persistent sodium current (INaP) is responsible for the pacemaker properties of the cells. Whole cell recordings from pacemaker pBC neurons in vitro revealed that these neurons have a larger ratio of persistent sodium current (INaP) over leak conductances than nonpacemaker pBC cells (Del Negro et al. 2002a; Rybak et al. 2003a). Blocking the persistent sodium conductance and consequently pacemaker activity in pBC slices should stop the rhythm. Indeed, application of riluzole, a semiselective blocker of INaP stopped the rhythm in rat slices (Rybak et al. 2003b). However, it has also been reported that blockade of the persistent sodium channel with riluzole did not affect the rhythm in similar preparations (Del Negro et al. 2002b). Thus rhythmic activity may persist in the absence of pacemaker activity and in the absence of reciprocal inhibition relying on network properties. A demonstration that rhythmic activity may actually emerge from an excitatory network of nonpacemaker cells could support this possibility. Theoretically, emergence of rhythmic activity has been shown in sparsely, randomly connected excitatory networks of spike-response (Pham et al. 1998a,b) and leaky-integrate-and-fire (Brunel 2000; Wiedemann and Lüthi 2003) neuron models.

In this study we show that respiratory-like activity can emerge in an excitatory network of nonpacemaker biophysical model neurons. The model units incorporate a minimum number of ionic currents, all known to exist in respiratory neurons. The calcium-dependent potassium current (INaP) is the key ionic current of the model, and it is triggered by the high- and
low-threshold calcium currents ($I_a$ and $I_t$, respectively). Their combination generates a long relative refractory period in response to increased firing frequency or prolonged inhibition. The $I_{NaP}$ has been excluded from this study because we are interested in rhythm emergence after this current has been blocked. We show at both the single cell and the network levels that $[K^+]_{ext}$ elevation can lead to a bursting oscillatory behavior. Rhythm emergence in the network can be due to either cellular rhythmic activity or network properties and perhaps both. This might be a possible explanation for the controversial results obtained in vitro when the pacemaker activity is pharmacologically blocked. In contrast, Gaussian stimulation evokes oscillatory behavior at the network level but not at the single cell level. In this case, the emergence of rhythmic activity is due to network properties. In both cases, the biophysical mechanism we propose is responsible for burst termination and contributes to the interburst duration. We also test our network model with inhibition determining the interburst duration. Under these conditions, the proposed biophysical mechanism is responsible for the characteristic incrementing pattern of activity at the beginning of each inspiratory phase.

**METHODS**

**Single neuron**

Our model is a single compartment Hodgkin-Huxley-type. The neuron membrane potential $V$ is described by the differential equation

$$C \frac{dV}{dt} = -I_m - I_k - I_l - I_{NaP} - I_{NaLeak} - I_{Kleak} - \sum I_{AMPA} + I_{in} + \xi(t)$$

The capacitance, $C = 0.025$ nF, was calculated on the basis of the average area of respiratory neurons and specific capacitance of 1 μF/cm² (see Rybak et al. 1997). The $I_{NaLeak}$ and $I_{Kleak}$ are the sodium ($Na^+$) and potassium ($K^+$) leak currents. $I_k$ and $I_l$ are the low- and high-threshold calcium ($Ca^{2+}$) currents, $I_{Na}$ is the fast potassium current, $I_{NaP}$ is a $Ca^{2+}$-activated $K^+$ current, and $I_{Na}$ is the fast transient sodium current. The term $\sum I_{AMPA}$ describes the summation of all excitatory synaptic input, mediated by the activation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and $I_{in}$ is the external stimulation. The term $\xi(t)$ represents a Gaussian noise input of 0 mean and (SD) $\sigma$. Control cases with the Ornstein-Uhlenbeck process instead of Gaussian were tested and gave similar results.

All currents were described according to Ohm’s law. For a current $i$

$$i = g_i(V - E_i)$$

where $g_i$ is the current’s conductance. Following the Hodgkin-Huxley formalism conductances of ionic channels are described as

$$g_i = G_m|V|^n h_i$$

$$\frac{dm_i}{dt} = (m_{i,0} - m_i)\tau_{m_i}$$

$$\frac{dh_i}{dt} = (h_{i,0} - h_i)\tau_{h_i}$$

where $G_i$ is the channel’s maximal conductance and $E_i$ is its reversal potential; $m_i$ and $h_i$ are the voltage-dependent probabilities of the activation and inactivation particles, respectively, to be in their permissive modes raised in the power of $a$ or $b$. Their steady-state values, $m_{i,0}$ and $h_{i,0}$ depend on the membrane potential, or for the $I_{NaP}$ current, on the internal calcium concentration. The equations describing the kinetics of each current are the same as in Huguenard and McCormick (1992). Unless otherwise mentioned, the maximum conductance values ($G_m$) for each current used in this study were the following (in $\mu$S):

$G_{Ina} = 3$, $G_{IK} = 0.9$, $G_{IT} = 0.002$, $G_{IL} = 0.0015$, $G_{AHP} = 0.3$, $G_{InaLeak} = 0.00265$, $G_{IKLeak} = 0.0075$, and $G_{AMPA} = 0.0035$.

For simulations where the extracellular potassium concentration ($[K^+]_{ext}$) was varied, the potassium reversal potential was approximated with the Nernst potential for potassium

$$E_k = \frac{RT}{ZF} \ln \frac{[K^+]_{ext}}{[K^+]_0}$$

where $R$ is the gas constant, $T$ is the temperature, $F$ is Faraday’s constant, and $Z$ is the valence of the ion. Internal potassium concentration ($[K^+]_0$) has been considered constant at 155 mM, and the value of the $RT/ZF$ term was 26.54 mV.

**CALCIUM DYNAMICS.** Calcium dynamics were modeled in the same way as in Rybak et al. (1997). Free calcium ions enter the cell through voltage-gated calcium channels. Internal calcium concentration is regulated by calcium buffering, pumps, and transporters. The dynamics of the free intracellular calcium ions are described by the following differential equation

$$\frac{d[Ca^{2+}]_{i}}{dt} = \frac{I_{Ca} + I_{Ca,Ext}}{2F_a} (1 - P_B) + \frac{[Ca^{2+}]_{ext} - [Ca^{2+}]_{i}}{\tau_{pump}(V)}$$

where $a$ is the volume of the space 0.1 μm under the cell membrane. Buffering is modeled as an instantaneous process. The influx is multiplied by the probability of an ion to be free ($1 - P_B$), where $P_B$ is the probability of a $Ca^{2+}$ ion to be buffered. This probability is given by

$$P_B = \frac{[B]_{Total}}{[Ca^{2+}]_{Total} + [B]_{Total} + K}$$

where $[B]_{Total}$ is the sum of concentrations of free and bound buffer, assumed to be constant, and $K$ is the equilibrium constant of the buffering reaction. The following parameters were set for calcium dynamics: $u = 2.5 \times 10^{-5}$ nM, $[Ca^{2+}]_{ext} = 5 \times 10^{-5}$ mM, $[B]_{Total} = 0.030$ mM, and $K = 0.001$ mM.

$[Ca^{2+}]_{i}$ is the equilibrium $Ca^{2+}$ concentration of the pump, and $\tau_{pump}(V)$ is the pump’s time constant, which is given by

$$\tau_{pump}(V) = \frac{17.7}{v^2 \sigma}$$

The calcium reversal potential was estimated from the Nernst equation for calcium ions

$$E_{Ca} = \frac{RT}{ZF} \ln \frac{[Ca^{2+}]_{ext}}{[Ca^{2+}]_{i}}$$

where $[Ca^{2+}]_{ext}$ is the external calcium concentration assumed to be constant at 4 mM. The $RT/ZF$ value used for calcium was 13.27 mV.

**Autocorrelation analysis (correlogram)**

To check for randomness in the discharge patterns of our model neurons, we used the autocorrelation function of their membrane potential traces. This analysis assesses whether successive observations are correlated. The correlation is computed between one time series and the same series lagged by one or more time units. The first-order autocorrelation coefficient is the simple correlation coefficient of the first $n$ observations and the next $n - 1$ observations (Cox and Lewis 1966). For $n$ sufficiently large so that $n/(n - 1) \approx 1$, the correlation coefficient $r_k$ between observations $x_i$ and $x_{i+k}$ separated by $k$ lags is given by

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where $\bar{x}$ is the overall mean.

The plot of the autocorrelation function as a function of lag is called correlogram. For a random series, lagged values of the series are uncorrelated, and we expect correlogram. For a random series, lagged values of the series are further approximated to $\pm 2\sqrt{n}$, which for large $n$’s are barely outside the confidence limits. Also, a large $r_k$ at a low lag is far more likely to represent persistence in most cases than a large $r_k$ isolated at some higher lag.

**Network**

To have reasonable computational times, we simulated networks of $n = 100$ units for a period of 10-20 s of biological time. However, we have tested networks $\leq 400$ units and 100 s of biological time and obtained similar results. The connection between neurons $i$ and $j$ is a binary variable $W_{ij}$. It equals 1 if there is a connection from neuron $j$ to neuron $i$ and 0 otherwise. Cases where self-connections were not allowed and gave the same results as cases with self-exciting neurons, so we decided not to distinguish between these two possibilities. The connections were randomly chosen according to a uniform law which attributes the value 1 to $W_{ij}$ with probability $p$. Each spike released a fixed amount of neurotransmitter in the synaptic cleft and activated AMPA receptors (Destexhe et al. 1994). This activation resulted in an excitatory postsynaptic potential (EPSP) after a transmission delay $d$, set to 5 ms in this study. The effect of this inter-neuronal transmission delay to synchronization of biologically plausible networks has been studied previously (Vibert et al. 1994).

The global network activity was determined as the number of neurons activated in a time window of 5 ms. Initial conditions for each unit were randomly assigned over a physiological relevant range (the membrane potential within the range of $-90$ to $10$ mV and voltage-dependent probabilities between 0 and 1) and typically the first 1,000 ms of each network simulation were truncated as a transition phase.

**Integration methods**

In all simulations, we used the Euler scheme for the integration of the differential equations with a time step of $\Delta t = 0.01$. For the deterministic cases ($\sigma = 0$), simulations using smaller time steps and the Runge-Kutta method of fourth order were used for comparison and yielded similar results with the Euler method. All simulation programs were coded in the C programming language and run on Pentium-based LINUX workstations.

**RESULTS**

We describe the response properties of our model neuron to excitatory and inhibitory stimuli and present simulations at the network level.

**Single cell model**

We tested our model’s response to steady current injection and increases of external potassium concentration. We have also used white Gaussian stimulation to increase the model neuron’s mean firing rate.

EXCITATION WITH STEP CURRENT STIMULATION AND INCREASES IN EXTERNAL POTASSIUM CONCENTRATION. Current step excitation. In the absence of external stimulation, the cell is quiescent (Fig. 1A). As the stimulus amplitude increases, there is a range for which the cell produces bursting oscillations (Fig. 1B), and for higher input amplitudes, the cell displays tonic, or beating, behavior (Fig. 1C). In the bursting regime, the burst duration and frequency increase monotonically with the stimulus amplitude. In our model, bursting behavior emerges through the interplay of the high-threshold calcium current ($I_{\text{Calc}}$) and the calcium-activated potassium current ($I_{\text{KCa}}$). The mechanism is shown in Fig. 1D, where the absolute amplitude values of the $I_L$ (2nd trace) and the $I_{\text{KCa}}$ (3rd trace) currents are plotted during and after a burst of action potentials (voltage, top trace).

The $I_L$ is activated at each spike, and calcium ions enter the cell through $I_L$ channels. During the burst, internal calcium ions accumulation is higher than their removal. The resulting elevated levels of internal calcium concentration ([Ca$^{2+}$]$_{\text{int}}$) activate the $I_{\text{AHP}}$ current, which in turn allows an efflux of potassium ions, hyperpolarizing the membrane potential and terminating the burst. In the absence of spikes, the $I_L$ channels are closed, internal calcium is removed, and the $I_{\text{AHP}}$ slowly decreases in amplitude. The cell depolarizes under the influence of the external stimulus until it starts firing again. For larger stimulation amplitudes, greater concentrations of internal calcium are needed for the $I_{\text{AHP}}$ to prevent firing and the number of spikes in each burst increases. Also, the time needed for the cell to start firing again gets shorter, therefore the frequency increases. Overall, the slow dynamics of the $I_{\text{AHP}}$ and the stimulus amplitude determine the interburst duration. However, further increases in input amplitude are not followed by analogous increases of [Ca$^{2+}$]$_{\text{int}}$. Indeed, high intracellular calcium concentration is prevented in the model by two processes: buffering by calcium binding proteins and removal by specific calcium pumps or transporters. When the maximum internal concentration has been reached and it is not sufficient to prevent firing, the cell fires continuously. In this mechanism, the contribution of calcium ions through low-threshold calcium channels is negligible. As shown in the Fig. 1D (4th trace), the $I_C$ current amplitude is close to zero during and after the burst.

The response profile of the model cell to current step excitation is summarized in Fig. 1E, where the bifurcation diagram of the inverse interspike intervals (ISI) to increasing current step amplitudes is presented. For low stimulus amplitudes, the model remains silent, and no point appears in this regime (S). As the stimulus amplitude increases, the cell enters the bursting regime (B, for $I_{\text{ext}} = 0.127$ nA). In the bursting regime, two groups of points can be distinguished. In the top group (ISI of shorter duration), the points correspond to the ISI of the spikes in each burst; while in the bottom one (ISI of longer duration), they correspond to the ISI between the last spike of a burst and the first spike of the next burst. As the stimulus amplitude increases, there is a point (at $I_{\text{ext}} = 0.143$ nA) where the cell enters the tonic discharge regime. The model responds initially with a high-frequency discharge and accommodates to a lower firing frequency. This is why at the tonic regime (T, tonic discharge), the line gets broader as the stimulus amplitude increases. The key role of the $I_L$ and the
\( I_{\text{AHP}} \) currents in generating the bursting mode can be shown by setting their maximal conductances to zero \((G_L = 0 \, S)\), i.e., removing the currents from the model. When either of these currents is removed, the bursting regime disappears. \( I_L \) removal results in a lower current threshold and a higher frequency for a given \( I_{\text{ext}} \). In the absence of the \( I_{\text{AHP}} \) current, the threshold moves to an even lower value, the frequency further increases, and the accommodation disappears.

External potassium concentration elevation. Another way to increase the cell excitability is to increase the external potassium concentration. This manipulation is often used in neonatal brain stem rhythmic slice preparations to compensate for the loss of synaptic and other inputs to the cells and thus to stabilize rhythmic activity over long recording periods (Ramirez et al. 1997). The increase of external potassium concentration brings the potassium reversal potential to higher values and consequently shifts the resting potential to more depolarized levels. In our model, the effect of external potassium concentration elevation is reminiscent of a steady current injection. In all of the cases described above, a fixed potassium reversal potential was used \((E_K = -105 \, \text{mV})\). Assuming an internal potassium concentration of \([K^+]_{\text{ion}} = 155 \, \text{mM}\), we calculate from the Nernst equation (see METHODS) an external potassium concentration of \([K^+]_{\text{ext}} = 2.42 \, \text{mM}\). At this concentration, the cell is silent. As it was the case for increased step stimulation amplitudes, subsequent elevations of \([K^+]_{\text{ext}} \) evoked bursting oscillations starting at \([K^+]_{\text{ext}} = 4.55 \, \text{mM}\), and later, starting at \([K^+]_{\text{ext}} = 4.78 \, \text{mM}\), tonic discharge. Representative examples from each regime, silent (S), depolarizing (B), and tonic (T), are shown in Fig. 1, F–H, respectively. A monotonic increase of burst frequency and number of spikes in each burst was observed with elevation of external potassium concentration. The biophysical mechanism for burst initiation and termination relies again on the interaction of the \( I_L \) and the \( I_{\text{AHP}} \) currents as described for excitatory step current stimulation.

INHIBITION WITH STEP CURRENT STIMULATION. We show here that the response of the model to inhibitory pulses depends on the interplay between the low-threshold calcium current \((I_T)\) and the \( I_{\text{AHP}} \) current. As it has been experimentally shown, the low-threshold calcium current needs a strong membrane potential hyperpolarization to be activated in respiratory neurons (Pierrefiche et al. 1999). We have thus systematically tested hyperpolarizing pulses of different amplitudes and durations. In Fig. 2A, we show the threshold effect of our model neuron in response to hyperpolarization. On release from a pulse of 1,400-ms duration and of \(-0.40\) and \(-0.41\) nA amplitude \((I_{\text{ext}})\), the neuron responded with a small and transient depo-

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**FIG. 1.** Single cell response to step current excitation and increase of \([K^+]_{\text{ext}}\). Top row: time course of the membrane potential \((V)\) in response to external stimulation. A: at rest. B: \( I_{\text{ext}} = 0.14 \, \text{nA} \). C: \( I_{\text{ext}} = 0.15 \, \text{nA} \). D: time course of the membrane potential \((V)\) and of the absolute amplitudes \((nA)\) of the high-threshold calcium \((I_L)\), the calcium-dependent potassium \((I_{\text{AHP}})\), and the low-threshold calcium \((I_T)\) currents during a burst of action potentials \((I_{\text{ext}} = 0.14 \, \text{nA})\). E: bifurcation diagram of the inverse interspike intervals. S, silent mode; B, bursting mode; T, tonic mode. Bottom row: time course of the membrane potential \((V)\) in response to external potassium concentration elevation \([K^+]_{\text{ext}}\). F: \([K^+]_{\text{ext}} = 3 \, \text{mM}\). G: \([K^+]_{\text{ext}} = 4.6 \, \text{mM}\). H: \([K^+]_{\text{ext}} = 5 \, \text{mM}\).
polarization followed by a rapid return to its resting potential. However, for a slightly larger stimulus amplitude ($I_{ext} = -0.42 \text{nA}$, threshold level), the model produced a rebound spike. In this later case, the $I_T$ current is significantly activated and boosts the membrane potential level to activate the $I_{AHP}$ current. The importance of the $I_T$ current in the model’s response on release from hyperpolarization is shown in Fig. 2B, where the same conditions as in Fig. 2A apply, but the $I_T$ current has been removed. For all three stimulus amplitudes, the membrane potential returned rapidly to its resting potential with no signs of after-depolarization or spike discharge.

The effect of the $I_{AHP}$ activation on a spiking neuron is presented in Fig. 2, C and D. We delivered a hyperpolarizing pulse ($I_{hyp} = -0.4 \text{nA}$), long enough to activate the low-threshold calcium current $I_T$ while the excitatory stimulus ($I_{ext} = I_{dep} + I_{hyp}$, 2nd trace) was maintained at a level where the cell fires tonically ($I_{dep} = 0.15 \text{nA}$). On release of the hyperpolarizing pulse, the cell responds with a rebound burst of a few spikes then goes quiescent for some time, before starting firing again (Fig. 2C, top trace, membrane potential). A stimulus of the same duration but of smaller amplitude ($I_{hyp} = -0.2 \text{nA}$) failed to strongly activate the $I_{AHP}$, and the model cell returned to continuous firing immediately on release (Fig. 2D, top trace, membrane potential). The interplay between the key ionic currents is revealed by presenting their absolute amplitudes in both cases. The stronger inhibition activated the $I_T$ current (Fig. 2C, 3rd trace), which contributed to the calcium influx along with the $I_{AHP}$ current (Fig. 2C, 4th trace). As a result, a strong $I_{AHP}$ (Fig. 2C, 5th trace) current is produced on the inhibition release, and its slow dynamics determine the following quiescent period. On the other hand, the weaker inhibition failed in activating the $I_T$ (Fig. 2D, 3rd trace), and the calcium influx from the $I_T$ current (Fig. 2D, 4th trace) alone is not sufficient to strongly activate the $I_{AHP}$ current (Fig. 2D, 5th trace). As a result, the model cell continues to fire immediately on release.

STIMULATION WITH WHITE GAUSSIAN NOISE. The aim of this study is to investigate the possibility of rhythm emergence from a population of nonpacemaker cells. We have shown that step current stimulation and elevation of external potassium concentration can evoke bursting pacemaker behavior in our model cell. We tested white Gaussian noise as a mean to increase the excitability of our units aiming to avoid periodic firing.

The SD ($\sigma$) of the Gaussian input is referred to as its amplitude. We have varied the Gaussian input amplitude (from $\sigma = 0$ to 0.25 with $\Delta \sigma = 0.01$) as we did for constant stimulation to investigate the response profile of our single unit model. As an example, the responses of the model unit for two different noise amplitudes are shown in Fig. 3, A ($\sigma = 0.13$) and B ($\sigma = 0.18$). An almost linear relationship has been observed between the cell firing frequency and the amplitude.
of the input (data not shown). Periodicity of the responses has been checked by calculating the correlograms for each response. An absolute $r_k$ value close to 1 indicates strong correlation, whereas an absolute value close to 0 indicates no correlation. The autocorrelation coefficients were estimated using $n = 454,500$ values of membrane potential ($V$) for each noise amplitude. The 95% confidence interval bounds exist at approximately ±0.003. To illustrate the ability of this technique to detect periodic activity in membrane potential traces, in Fig. 3F, we present the correlogram of a neuron in the bursting regimen (Fig. 3C, $I_{ext} = 0.13$ nA) and in the absence of Gaussian stimulation ($\sigma = 0$). Periodicity in the response is indicated by the equally periodic appearance of $r_k$ values close to 1 in the correlogram. The presence of large values of $r_k$ and especially at large $k$’s is a strong indication of periodicity (see METHODS). The pattern of large $r_k$ values around the maximum value reflects the fact that the cell undergoes bursting oscillations. On the contrary, and as it can be seen in the correlograms in Fig. 3, D and E, when Gaussian stimulation was used (membrane potential traces in Fig. 3, A for $\sigma = 0.13$ and B for $\sigma = 0.18$, respectively) most of the $r_k$ values lie within or close to the 95% interval, indicating no correlation. The same result holds for all Gaussian stimulus amplitudes tested, and we thus concluded that this type of stimulus does not trigger rhythmic responses in our model neuron per se. We also tested our model in the bursting regimen with noise. The periodic bursting behavior is very sensitive to noise. Relatively small noise amplitudes disrupt periodic behavior and result in a stochastic pattern of activation (results not shown).

In the same model, current step stimulation and external potassium elevation can induce bursting pacemaker activity while a more realistic stimulus does not. The model cell is not an intrinsic pacemaker cell since at rest it is quiescent, but it incorporates the biophysical machinery to display bursting pacemaker activity in response to certain stimuli.

### Sparsely connected excitatory network

We considered a network of $n = 100$ randomly connected units to represent the in vitro slice preparation. Each unit has a certain probability $p$ to be connected to any other neuron, referred to as the network’s connectivity. Sporadic, bursting, and tonic network activities emerge subsequently as the connectivity takes on larger values. In this study, we considered the connectivity of the network to be constant ($p = 0.16$). This specific connectivity value has been chosen on the basis that it provides a broad range for all three types of activity to occur.

#### RAISING THE EXTERNAL POTASSIUM CONCENTRATION

In this section, we describe our results on rhythm emergence after an elevation of external potassium concentration at the network level. We report that the rhythm can be due to network or pacemaker properties or a mixture of the two.

A case where the rhythm relies on network properties. We begin with the case of rhythm emergence as a network property with increases of the external potassium concentration. With the higher [K+]ext any sporadic activity at the cellular level will increase in frequency as the membrane potential of each unit approaches threshold. As neuronal excitability increases, the probability of a given unit to fire and excite other units of the network rises. Subsequently, a large fraction of neurons will be activated and go silent simultaneously as their $I_{AHP}$ currents get activated. The rhythm is thus produced at the network level by an amplification of this activity due to synaptic interactions. No intrinsic pacemaker activity is needed. The above scenario is depicted in Fig. 4, A and B. In Fig. 4A, we start with the external potassium at its normal

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**Fig. 3.** Single cell white Gaussian noise stimulation. Top row: time course of membrane potential ($V$) in response to stimuli with a constant and a fluctuating component. A: constant component is 0 ($I_{ext} = 0$ nA) and the fluctuating component has a SD of $\sigma = 0.13$. B: constant component is 0 ($I_{ext} = 0$ nA) and the fluctuating component has a SD of $\sigma = 0.18$. C: constant component is $I_{ext} = 0.13$ nA and the fluctuating component has a 0 SD ($\sigma = 0$). Bottom row: corresponding correlograms of membrane potential traces presented in A–C in D–F, respectively.
concentration (control) in our study: $[\text{K}^+]_{\text{ext}} = 2.42\, \text{mM}$. As previously shown, at this concentration of external potassium, the single cell is silent. The small uncorrelated activity observed in Fig. 4A is due to low-amplitude intrinsic Gaussian noise ($\sigma = 0.1$), which causes sparse firing at the cellular level and represents the spontaneous activity of neurons observed in experiments. Although some amplification of this activity is performed by synaptic interactions ($G_{\text{AMPA}} = 0.0035\, \mu S$), the unitary firing rate is still low, and no periodic activity is observed. We now increase the $[\text{K}^+]_{\text{ext}}$ to 3 mM (Fig. 4B). At this level, the single cell with no input is still silent (see Fig. 1F). However, this increase in external potassium concentration depolarized the cells and increased their firing activity induced by the intrinsic noise ($\sigma = 0.1$). This activity is now important enough to produce the rhythm seen in Fig. 4B at the network level through synaptic interactions. The rhythm relies on network properties because, for this level of external potassium concentration, no bursting activity has been observed at the single cell level, and Gaussian stimulation does not induce periodic patterns of discharge as shown above in this study.

A case where the rhythm relies on stimulus dependent cellular bursting. As we increase the $[\text{K}^+]_{\text{ext}}$ rhythmic activity at the cellular level will occur when the membrane potential of each unit reaches the threshold for bursting behavior as seen at the single cell level (Fig. 1G). In Fig. 4C, the external potassium concentration induces bursting oscillations at the cellular level ($[\text{K}^+]_{\text{ext}} = 4.6\, \text{mM}$). At the same time, we have blocked synaptic excitation and the Gaussian input in the network ($G_{\text{AMPA}} = 0.0005\, \mu S$). A: $[\text{K}^+]_{\text{ext}} = 2.42\, \text{mM}$ induces phasic or bursting activity. C: $[\text{K}^+]_{\text{ext}} = 4.6\, \text{mM}$ induces bursting oscillations at the cellular level but the absence of synaptic connectivity and the fact that individual neurons have different initial conditions account for low amplitude. A low synaptic strength increases amplitude of bursts in D ($G_{\text{AMPA}} = 0.0005\, \mu S$). E: $[\text{K}^+]_{\text{ext}} = 4.6\, \text{mM}$ induces bursting oscillations at the cellular level, synaptic connectivity synchronizes their activity ($G_{\text{AMPA}} = 0.0005\, \mu S$), and Gaussian input ($\sigma = 0.1$) increases burst frequency and reduces burst amplitude. F: elevated levels of $[\text{K}^+]_{\text{ext}} = 5\, \text{mM}$ and Gaussian input result in network tonic discharge. A–F: network of $n = 100$ units and connectivity $\rho = 0.16$.

**FIG. 4.** Effects of increase of external potassium concentration, connectivity, and Gaussian input to network activity. A: sporadic network activity under control external potassium concentration ($[\text{K}^+]_{\text{ext}} = 2.42\, \text{mM}$) in the presence of Gaussian input ($\sigma = 0.1$). B: an increase in external potassium concentration ($[\text{K}^+]_{\text{ext}} = 3\, \text{mM}$) induces phasic or bursting activity. C: $[\text{K}^+]_{\text{ext}} = 4.6\, \text{mM}$ induces bursting oscillations at the cellular level but the absence of synaptic connectivity and the fact that individual neurons have different initial conditions account for low amplitude. A low synaptic strength increases amplitude of bursts in D ($G_{\text{AMPA}} = 0.0005\, \mu S$). E: $[\text{K}^+]_{\text{ext}} = 4.6\, \text{mM}$ induces bursting oscillations at the cellular level, synaptic connectivity synchronizes their activity ($G_{\text{AMPA}} = 0.0005\, \mu S$), and Gaussian input ($\sigma = 0.1$) increases burst frequency and reduces burst amplitude. F: elevated levels of $[\text{K}^+]_{\text{ext}} = 5\, \text{mM}$ and Gaussian input result in network tonic discharge. A–F: network of $n = 100$ units and connectivity $\rho = 0.16$. 

**Coexistence of the two mechanisms.** We further show that the two above mentioned mechanisms are not exclusive but may co-exist within the same network. This possibility is
shown in Fig. 4E. External potassium concentration ([K$^+$/H$_{ext}$/H]$]_{ext}$ = 4.6 mM) produces bursting oscillations at the cellular level, synaptic connectivity synchronizes them ($G_{AMPA} = 0.0005 \mu$S), but now the addition of Gaussian input ($\sigma = 0.01$) increases the frequency of bursts and causes a decrease of their mean amplitude (compare Fig. 4, D and E). It is no longer possible to attribute rhythm emergence to either pacemaker or network properties since they both contribute to the rhythm’s characteristics. Further increase of any of these parameters can lead to network tonic discharge (Fig. 4F), completing the transition from sporadic to rhythmic and then tonic network discharge as the [K$^+$/H$_{ext}$/H]$]_{ext}$ increases.

**INCREASE OF GAUSSIAN STIMULUS AMPLITUDE.** Next, we tested Gaussian input to stimulate the units of the network and increase their firing frequency. This stimulus does not induce periodic firing at the single cell level as shown previously. Therefore by using Gaussian stimulation we may attribute any periodic activity to network properties.

For low Gaussian input amplitudes, sporadic activity has been observed, and as the input amplitude increased, periodic activity emerged and later on, tonic. In the first row of Fig. 5, the network’s global activity is shown for four different values of input amplitude, and in the second row, the corresponding raster plots are displayed where each dot represents a spike. In all cases, [K$^+$/H$_{ext}$/H]$]_{ext}$ was kept at 2.42 mM and $G_{AMPA}$ at 0.0035 \mu$S. For low Gaussian input amplitude (Fig. 5A, $\sigma = 0.12$), sporadic activity is observed at the network level. Rhythmic activity emerges as individual spiking frequency is increased with an increase of the input amplitude (Fig. 5B, $\sigma = 0.14$). Further increases of the input amplitude result in an increase of the rhythm’s frequency and a decrease in burst amplitude (Fig. 5C, $\sigma = 0.16$). For higher input amplitudes, we get a tonic network activity (Fig. 5D, $\sigma = 0.24$). Inspection of the raster plots reveals that all neurons in the network present similar, yet not identical, patterns of activation during a burst. There is a gradual increase in their firing frequency that comes in contrast to the initial high and later low frequency described at the single cell level with steady depolarization. The biophysical mechanism that terminates the burst relies on the same principles as the one presented at the single cell level, but now burst initiation is caused by the synaptic input and the noise. When more neurons get activated and activate others, individual firing frequency increases, and calcium enters the cell. Accumulation of internal calcium activates the $I_{AHP}$ current, which is responsible for burst termination. As in the case of the single cell model, the $I_L$ current provides the majority of calcium ions influx needed for this activation to occur. The tonic network response occurs in analogy with the single cell level when the
maximum value of $I_{\text{AMP}}$ is not sufficient to abolish the effect of the excitatory input.

The increase in population frequency was followed by a decrease of the mean burst amplitude. This result is quantified in Fig. 5A, where the dotted line shows the increase in frequency as estimated by simulations of 200-s duration. A burst was detected each time the global activity of the network was larger than 20 units in a time window of 5 ms. This threshold value was found to properly discriminate between burst and intermediate sporadic activity of the network’s global activity. In the same panel, each point represents the mean of the maximum values of all amplitudes in the same simulation set, with their respective SE. The decrease in burst amplitude is in contrast with the single unit case where an increase in input strength of the synaptic drive, is an important factor in the rhythmicity of the cellular response.

BURSTING BEHAVIOR AT THE CELLULAR LEVEL DEPENDS ON TOTAL SYNAPTIC INPUT. At the cellular level, within the network, we further show that all units did not respond in the same way. Figure 6A shows the membrane potential of three units along with the global activity of the network. Units 1 and 89 increased their firing rate significantly during the population burst, while unit 9 showed only a slight increase of activation during rhythmic activity. Such differences in neuronal firing behavior come from the variability in the connectivity of the units in the network. An examination of the number of connections ($k$) each of those units received revealed that unit 1 received 16 synaptic drives ($k = 16$, i.e., the mean of a 100 units network with a probability $P = 0.16$), while unit 9 received only 8 and unit 89 received 28 synaptic drives. This figure suggests that the amount of synaptic input, i.e., a network property, has a key role in the emergence of rhythmic behavior. This is further shown in Fig. 6B, where the membrane potential correlograms of the same three units are shown. The correlograms were again constructed from a 454,500 point series, and the 95% confidence limits are represented by the straight lines in each trace. For comparison purposes, the top trace in the figure shows the correlogram of the network’s global activity, constructed from only 10,000 points (a 50-s simulation binned each 5 ms). As it can be seen, the periodicity in the response is more prominent in units 1 and 89, with unit 89 presenting larger absolute values. Unit 9 shows slight if any significant periodicity in its responses. By contrast, the correlograms of single cells stimulated by Gaussian input (Fig. 3, D and E) did not indicate any periodic activity. Thus rhythmic responses emerged because cells were embedded in a network and received both Gaussian stimulation and network synaptic inputs. In addition, the number of synaptic inputs, i.e., the strength of the synaptic drive, is an important factor in the rhythmicity of the cellular response.

PACEMAKER-BASED RHYTHMIC ACTIVITY IS DECREMENTING WHILE NETWORK-BASED ACTIVITY IS INCREMENTING. Whether the network burst displayed a decrementing or an incrementing pattern was consistently found to be dependent on the underlying mechanism. In all the cases we tested when pacemaker activity was generating the rhythm, the burst started with a large value (many neurons simultaneously active) and slowly decayed to lower values. Similar decrementing patterns of population
bursts are usually found in the in vitro slices. The case was different when network properties were giving rise to the rhythm. A small number of active neurons started a cascade of activation to interconnected cells, and a characteristic amplification ramp was generated at the onset of the burst. The activation of the $I_{AHP}$ current in all active neurons terminated the burst in both cases. Examples are presented in Fig. 6, C and D. The top trace in these figures is the global activity of the network during one burst and the lower part is the corresponding raster plot. In Fig. 6C, it is the pacemaker behavior of the cells generating the rhythm ($(K^+)_\text{ext} = 4.6$ mM, $\sigma = 0$, $G_{\text{AMPA}} = 0.0005 \mu$S, see Fig. 4D). The pacemaker cells, once synchronized, all start their burst at about the same time. Thus at the beginning, the synchronization is high. The fact that not all pacemakers have the same synaptic input in our network results in slight differences in their subsequent firing times, reducing the degree of synchronization. Also, firing accommodation due to the $I_{AHP}$ current occurs at the cellular level, reducing the network burst amplitude. The overall pattern of activation in this case reflects the properties of bursting at the single cell level. On the contrary, when network properties underlie the rhythm as in Fig. 6D ($(K^+)_\text{ext} = 2.42$ mM, $\sigma = 0.16$, $G_{\text{AMPA}} = 0.0035 \mu$S, see Fig. 5C), a few neurons are needed initially to activate others and slowly increase the synchronization of the network until the $I_{AHP}$ abruptly terminates the activity.

RESPONSE OF THE NETWORK TO INHIBITION DURING TONIC MODE ACTIVITY. It has been shown that synaptic inhibition is essential for the maintenance of respiratory rhythmic activity in vivo (Pierrefiche et al. 1998; Richter et al. 1991). We wished to investigate the role of the mechanism we proposed when inhibition determines the interburst duration in our network model of the slice. In Fig. 7, A and B, we show examples of a network in its tonic mode ($\sigma = 0.21$, $G_{\text{AMPA}} = 0.0035 \mu$S) receiving periodic inhibitory pulses of large amplitude ($I_{\text{ext}} = -0.5$ nA on each cell). In both cases, the interpulse duration is 1.5 s but the pulse duration is 1.5 s for Fig. 7A and 1 s for Fig. 7B. During the pulse, the network is inhibited, but as soon as the neurons are released from the inhibitory pulse, the network activity undergoes a synchronized rebound activity followed by a slowly amplifying activity, similar to the one observed in vivo at the onset of phrenic nerve discharge. Here, inhibition (pulse duration) determines the frequency of the rhythmic activity. The rebound activity, resulting from $Ca_T$ channels activation immediately on release from synaptic inhibition, is in agreement with experimental observations in respiratory neurons (Pierrefiche et al. 1999; Richter et al. 1993). In Fig. 7C, we show a single population burst preceded by the rebound burst after a 1-s inhibitory pulse. The cellular mechanism responsible for these phenomena is depicted in Fig. 7D and is similar to that described at the single cell level (see Fig. 2C). As soon as the inhibition ceases, the $I_L$ and the $I_{Ca}$ currents are activated and then trigger the $I_{AHP}$ current. During the refractory period due to the $I_{AHP}$ current, neurons are able to restore their firing levels progressively and the characteristic incrementing activity of an inspiratory phase is then produced at the network level. Consequently, the progressive return to physiological firing levels produces the amplification ramp of the inspiratory phase. The long relative refractory period caused by the $I_{AHP}$ is here responsible for the emergence of the amplification ramp and not for the interburst phase, which is now determined by inhibition as showed above.

DISCUSSION

The aim of this study is to show that rhythmic activity can emerge as a network property in a model of the in vitro pBC.
slice preparation in the absence of pacemaker activity and synaptic inhibition.

Hybrid pacemaker/network hypothesis: role of the persistent sodium current

The fact that a respiratory-like rhythm emerges in a slice in the absence of inhibition allowed some authors to propose the pacemaker hypothesis, suggesting that the intrinsic bursting properties of pacemaker neurons (a population of the PBC contained within the slice) formed the “kernel” of the central pattern generator for breathing (Smith et al. 1991). The pacemaker hypothesis evolved into the hybrid pacemaker/network hypothesis since excitatory synaptic interactions are needed to synchronize the pacemakers and nonpacemaker or follower cells contribute in shaping the rhythm (Smith et al. 2000).

The hybrid pacemaker/network hypothesis was further supported by simulation studies (Butera et al. 1999a,b; Del Negro et al. 2001). In these studies, a heterogeneous population of pacemaker cells along with a follower cell population has been developed and was able to reproduce the results obtained experimentally. A persistent sodium current with fast activation and slow inactivation ($I_{\text{NaP}}$) was proposed as the key current underlying the periodic bursting behavior of the pacemaker cells.

All pBC neurons possess the persistent sodium ($I_{\text{NaP}}$) current (Del Negro et al. 2002a; Rybak et al. 2003a), the difference between pacemaker and nonpacemaker neurons being that the prior have a higher ratio of $I_{\text{NaP}}$ over leak current conductions. Biophysical properties of this current were measured from the current-voltage relationship of pacemaker neurons but inactivation constants could not be estimated with this method. In the hybrid pacemaker/network theory, the in vitro rhythm emerges based on the bursting behavior of pacemaker neurons in the pBC, which depends on the $I_{\text{NaP}}$ current. It follows that blocking the $I_{\text{NaP}}$ would disrupt the rhythm. Indeed, application of the semiselective blocker riluzole in the rat slice resulted in rhythm abolition (Rybak et al. 2003b). However, the role of $I_{\text{NaP}}$ in rhythm generation is still unclear. In a recent publication, Del Negro et al. (2002b) did not succeed in disrupting the in vitro rhythmic activity after pharmacological blockade of the $I_{\text{NaP}}$ with riluzole. More specifically, the authors reported that the rhythm persisted for several minutes after the $I_{\text{NaP}}$ current was completely blocked. These results suggest that under certain conditions the in vitro rhythm may emerge as a network property. The fact that the rhythm disappears later may be explained by other actions of riluzole. Riluzole is known to inhibit voltage-dependent sodium channels, but it also inhibits glutamate release and blocks postsynaptic N-methyl-D-aspartate (NMDA)- and kainate-type glutamate receptors (Bryson et al. 1996; Wagner and Landis 1997). If the rhythm is based on network properties, disruption of synaptic excitation would lead in rhythm elimination.

A different alternative for why the rhythm persists when the $I_{\text{NaP}}$ is blocked has been proposed by Thoby-Brisson and Ramirez (2001). According to that study in the pBC there exist two populations of pacemaker cells, one depending on $I_{\text{NaP}}$ and the other one on both $I_{\text{NaP}}$ and calcium-dependent currents. However, it is not yet known what is the percentage of pacemaker cells depending on calcium currents and whether these can support the rhythm once the $I_{\text{NaP}}$ current is blocked.

Model of respiratory rhythmogenesis based on the calcium-dependent potassium current

In this study, we suggest that rhythmic activity may emerge in a slice containing no intrinsically bursting cells and in the absence of inhibition as a network property. Therefore we excluded the $I_{\text{NaP}}$ from our single cell model. The proposed mechanism relies on the calcium-dependent potassium current $I_{\text{AHP}}$. Indirect evidence for the existence of this current in respiratory neurons has been provided in vitro (Rekling et al. 1996), in vivo (Pierrefiche et al. 1995; Richter et al. 1993) and in situ (Büsselberg et al. 2003). Of particular interest to our study is the demonstration that respiratory neurons possessing such currents are connected by excitatory connections (Rekling et al. 2000) as it has been suggested that they form a nonpacemaker-based group of rhythmogenic neurons (Rekling and Feldman 1998). Rhythmic activity in vitro is triggered by raising the external potassium concentration that increases the excitability of the whole network. This type of stimulation can induce periodic bursting firing in neurons with a certain combination of intrinsic currents. In our model, steady depolarization of the cell over a range of amplitudes evoked periodic bursting (see Fig. 1, B and G) through the interplay of $I_{\text{AHP}}$ and the high-threshold calcium current $I_L$, also known to exist in respiratory neurons (Elsen and Ramirez 1998; Mironov and Richter 1998; Pierrefiche et al. 1999). Although our proposal involves a calcium-dependent potassium current for the afterhypermolarization, it has been shown that electrogenic pump currents (i.e., from the Na$^+$/K$^+$-ATPase), mGLU-R currents or Na$^+$-dependent K$^+$ currents may also play a role in the afterhypermolarization (Dong et al. 1996; Manzoni et al. 1997; Safronov and Vogel 1996; Vaillend et al. 2002). Particularly interesting is the demonstration that current from the Na-K-ATPase participates in the autoregulation mechanisms of neuronal network excitability in a model of cultured spinal network (Darbon et al. 2003). However, our model involving synergistic action between $I_L$ and $I_{\text{AHP}}$-induced afterhypermolarization found some support from Bowden et al. (2001), who described a functional coupling between SK (small-conductance calcium-activated potassium channel) and $I_L$ in the generation of afterhypermolarization in rat hippocampal neurons.

Rhythm due to external potassium concentration elevation may be based on different properties, while it has a network origin for Gaussian stimulation. Our simulations suggest that the increase of external potassium concentration at the network level can produce the rhythm based on network properties (Fig. 4B), cellular bursting (Fig. 4D), or a combination of the two (Fig. 4E). In our model, the calcium ions needed for the activation of the $I_{\text{AHP}}$ current were provided by influx from the extracellular space through $I_L$ channels. An interesting alternative source has recently been proposed involving intracellular calcium stores (Dunin-Barkowski et al. 2003). Irrespective of the source of internal free calcium ions, the activation of the $I_{\text{AHP}}$ current is essential for the mechanism proposed in our study.

As discussed above, the increase in $[\text{K}^+]_\text{ext}$ makes it difficult to decide whether the emergent rhythm relies on pacemaker activity or on network properties or both. Therefore we have used a different stimulus to increase neuronal excitability, Gaussian stimulation. Our results showed that this stimulus does not induce periodic bursting in our single cell model (Fig.
3). Bursting behavior in this model emerges through a periodic activation of the $I_{\text{AHP}}$ due to high-frequency discharge. While a steady depolarization can induce such a pattern of activation, it is pretty unlikely that the highly variable form of the white Gaussian input can induce a series of action potentials timed closely together to activate the $I_{\text{AHP}}$. Even if this is true occasionally, the probability of a periodic activation in response to Gaussian input is almost negligible. However, Gaussian input induces bursting at the network level (Fig. 5). We have shown that, at the network level, the rhythmic activity is based on network properties. Autocorrelation analysis of the cellular activity within the network showed that the units receiving larger synaptic input displayed stronger rhythmic activity in terms of respiratory drive potential amplitude (Fig. 6, A and B).

**When Periodic Inhibition Is Present, the $I_{\text{AHP}}$ Is Responsible for the Amplification Ramp.** Inhibition is essential for the maintenance of respiratory rhythmic activity in vivo (Pierrefiche et al. 1998; Richter et al. 1991). In this study, we also considered a tonically active network of highly excited neurons periodically receiving inhibition to investigate the role of our mechanism in such a case. The emerged rhythm shows an augmenting pattern of activation and an abrupt termination (Fig. 7, A and B). The strong periodic inhibition is responsible for the silent phase. Once inhibition terminates, the $I_{\text{T}}$ current gets activated, which causes a calcium ion influx and activates the $I_{\text{AHP}}$ current. The activation of the $I_{\text{AHP}}$ reduces the excitability of the cells, and it is responsible for the amplification ramp during the active phase of rhythmic activity. The simulated units slowly regain their excitability due to both Gaussian and excitatory synaptic inputs. At the network level, this slow recovery generates an amplification ramp, reminiscent of that observed in vivo (Bianchi et al. 1995; Richter and Spyer 2001).

When inhibition is absent, the $I_{\text{AHP}}$ determines the interburst duration, but when inhibition disrupts the rhythm, the $I_{\text{AHP}}$ is responsible for the amplification ramp. This shift in roles of the $I_{\text{AHP}}$ is an exciting idea that recently received experimental verification (Büsselberg et al. 2003). Büsselberg and collaborators have shown that in the nonanesthetized in situ mouse preparation, when glycineric inhibition fails a “big” calcium-dependent potassium conductance, is responsible for the observed persistence of the respiratory rhythm.

**Model Limitations.** The biophysical mechanism we propose is based on a minimal set of intrinsic currents. Beside the currents used in this study, a number of other currents are known to exist in respiratory neurons. This includes the potassium A-current ($I_{\text{A}}$, Champagnat et al. 1986) and the hyperpolarization-activated ionic current ($I_{\text{h}}$, Mironov et al. 2000; Thoby-Brisson et al. 2000), which may alter neuronal dynamics. Most of these currents were incorporated in a computer model of a single medullary respiratory neuron, which showed good agreement with experimental results (Athanasiasides et al. 2000).

Another limitation of our model is that we have a homogenous population of point cells. It is known that respiratory neurons have been classified according to their discharge patterns and their timing of discharge during the respiratory cycle in vivo. They are usually classified as preinspiratory, early-inspiratory, ramp inspiratory, postinspiratory, stage II expiratory, and augmenting expiratory neurons (for review, see Bianchi et al. 1995). This classification reflects differences in the physiology, geometry, and anatomical connections of these neurons. In contrast, our network model is homogeneous in terms of cell population; therefore any variation in the neuronal discharge patterns originates from the variability of the input and the variability of network connections. Both detailed anatomy and physiology are probably important in fine tuning respiratory rhythmic activity. Nevertheless, we showed that with a minimum set of currents in the cell model and a very simple anatomy we were able to reproduce some of the experimental observations.

Finally, the fact that Gaussian input does not induce periodic bursting in our model allowed us to use this input at the network level and attribute the rhythm to network properties. However this input might trigger bursting in other models or neurons. In this case, a different input has to be applied to check for network origin of the rhythm.

**Testable Predictions.** One of the main predictions of our model is that stimulation of the pBC neurons in a nonrhythmic slice (low $[K^+]_\text{ext}$) with Gaussian noise of certain amplitude would generate rhythmic activity. Stimulation with Gaussian noise of single neurons will reveal whether this type of input evokes bursting oscillations in pBC neurons. The rhythm should persist after blocking of inhibition and pacemaker activity. In our model, the activation of $I_{\text{AHP}}$ determines the interburst duration of the rhythm in the absence of inhibition. Application of apamin, a blocker of calcium-activated potassium currents, should trigger rhythmic activity in low $[K^+]_\text{ext}$, increase the frequency in rhythmic slices, and in high concentrations, greatly disturb the rhythm. Also, application of apamin in the pBC in situ or in a slice with tonic activity and external periodic inhibition should result in loss of the amplification ramp.

**Conclusion.** Our model suggests that respiratory-like activity can emerge in vitro in the absence of inhibition and after pacemaker activity has been blocked. A steady depolarization, like the one caused by $[K^+]_\text{ext}$ elevation, may generate the rhythm either by evoking bursting in nonautorhythmic cells (conditional bursters), or based on network properties and perhaps both. Gaussian stimulation in our model induced the rhythm solely on network properties. Although we claim that the rhythm can emerge in the absence of pacemaker activity, the presence of pacemaker neurons in the pBC is not to be excluded at any developmental stage. It is possible that at early developmental stages, when the network has not yet reached full maturity, pacemaker activity could provide a more secure mechanism for the generation and maintenance of the rhythm than a network-based mechanism. Intrinsically bursting cells can be incorporated in our hypothesis, suggesting a mechanism similar to the one described by Butera et al. (1999b). In a previous study, we have shown that, under certain conditions, autorhythmic cells will follow the rhythm imposed by synaptic input and network properties (Kosmidis and Vibert 2001). However, they are able to regain their own rhythmic activity and to drive the network once synaptic activity gets weaker.

We believe that such “switch-security” mechanisms, as the alternation between network and pacemaker origins and the
shift in the roles of $I_{AHP}$ discussed previously, are necessary for the maintenance of such a vital rhythm.

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