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Dynamics of neuromodulatory feedback determines frequency modulation in a reduced respiratory network: A computational study

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Abstract

Neuromodulators, such as amines and neuropeptides, alter the activity of neurons and neuronal networks. In this work, we investigate how neuromodulators, which activate G_q -protein second messenger systems, can modulate the bursting frequency of neurons in a critical portion of the respiratory neural network, the pre-Bötzinger complex (preBötC). These neurons are a vital part of the ponto-medullary neuronal network, which generates a stable respiratory rhythm whose frequency is regulated by neuromodulator release from the nearby Raphe nucleus. Using a simulated 50-cell network of excitatory preBötC neurons with a heterogeneous distribution of persistent sodium conductance and Ca^{2+} , we determined conditions for frequency modulation in such a network by simulating interaction between Raphe and preBötC nuclei. We found that the positive feedback between the Raphe excitability and preBötC activity induces frequency modulation in the preBötC neurons. In addition, the frequency of the respiratory rhythm can be regulated *via* phasic release of excitatory neuromodulators from the Raphe nucleus. We predict that the application of a G_q antagonist will eliminate this frequency modulation by the Raphe and keep the network frequency constant and low. In contrast, application of a G_q agonist will result in a high frequency for all levels of Raphe stimulation. Our modeling results also suggest that high $[K^+]$ requirement in respiratory brain slice experiments may serve as a compensatory mechanism for low neuromodulatory tone.

Keywords

Central pattern generator; Endogenous bursting; Pre-Bötzinger complex; preBötC

1. Introduction

In spite of constantly changing environmental conditions and metabolic demands, blood oxygen concentration remains relatively constant throughout life. To keep up with oxygen demands, the central nervous system regulates frequency and duration of respiratory rhythm. The inspiratory phase of the respiratory rhythm originates in the pre-Bötzinger complex (preBötC), a region of the ventrolateral medulla (Smith et al., 1991; Schwarzacher et al.,

1995; Wenninger et al., 2004). preBötC neurons generate stable rhythmic output when isolated from the rest of respiratory column. The respiratory rhythm observed in the preBötC depends on excitatory neurotransmission between neurons in the population, since blocking excitatory neurotransmission eliminates a coordinated rhythm at the level of the preBötC (Johnson et al., 1994; Ramirez et al., 1997; Koshiya and Smith, 1999). The frequency of the respiratory rhythm is modulated *via* release of excitatory neuromodulators by the Raphe nucleus (Gray et al., 1999; Doi and Ramirez, 2008).

The exact mechanism of the preBötC's rhythmicity is still debated. A number of mathematical models have been proposed to explain different aspects of preBötC rhythmic activity (Butera et al., 1999; Rybak et al., 2003; Purvis et al., 2007; Rubin et al., 2009; Cordovez et al., 2010), but none of these models explicitly addresses modulation of the respiratory rhythm by excitatory neuromodulators. However, a large fraction the excitatory neuromodulators endogenously released in the experimental brain slice preparation involve activation of multiple metabolic pathways (Doi and Ramirez, 2008) and lead to complex changes in neuronal dynamics. Thus, realistic models of neuromodulatory pathways are necessary to explain the frequency range seen in preBötC neurons.

Our recently published two-compartment model simulates the direct effect of neuromodulators on G_q -coupled receptors ($5HT_{2A}$ or NK_1) in preBötC inspiratory neurons (Toporikova and Butera, 2011). In this paper, we used our model to investigate the conditions for frequency modulation by excitatory neuromodulators in the preBötC, by considering its interaction with the Raphe. Since the exact connectivity and distribution of ionic properties of preBötC neurons still remain unresolved, we constrained our network by incorporating two additional important features of preBötC neurons: population activity during the respiratory cycle and the distribution of intrinsic bursting properties. The goal of this work is to build a network that (i) has a realistic distribution of intrinsic bursting properties, (ii) produces output similar to recordings from brainstem slices, with a high firing frequency during the inspiratory phase and a very low firing rate for the rest of the phase, and (iii) displays realistic frequency modulation by neuromodulators. To satisfy all of these conditions, we explored two possible connectivity schemes between the preBötC and Raphe. The first scheme hypothesizes that the Raphe nucleus exerts a constant neuromodulatory tone to the preBötC. The second scheme includes positive feedback from the preBötC to the Raphe. Our simulation results show that the positive neuromodulatory feedback scheme provides both a stable network rhythm and realistic frequency modulation by excitatory neuromodulators. To our knowledge, this is the first attempt to consider the dynamics of endogenous neuromodulator release in the preBötC rhythm generation.

2. Materials and methods

2.1. Network model

The network consisted of 50 model cells connected all-to-all through excitatory synapses. Each cell in the network simulation is a minimal two-compartment model of an inspiratory preBötC neuron (Toporikova and Butera, 2011). 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). The somatic compartment contains the essential spiking currents: fast inward Na^+ (I_{Na}), outward delayed rectifying K^+ (I_K), persistent Na^+ (I_{NaP}) and passive leak (I_L). 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:

$$C_{ms} = \frac{dVs}{dt} = -I_{Na} - I_{NaP} - I_K - \frac{gc}{1-k}(V_s - V_D) - I_L - I_{syn} \quad C_{md} = \frac{dV_D}{dt} = -I_{CaN} - \frac{gc}{k}(V_D - V_s)$$

where the currents are given by:

$$\begin{aligned} I_{Na} &= g_{Na} m^3 (1-n)(V_s - V_{Na}) \\ I_{NaP} &= g_{NaP} p_{\infty} h (V_s - V_{Na}) \\ I_K &= g_K n^4 (V_s - V_K) \\ I_L &= g_{LNa} (V_s - V_{Na}) + g_{LK} (V_s - V_K) \\ I_{syn} &= g_{syn} \sum s_j (V_s - V_{syn}) \\ I_{CaN} &= g_{CaN} f([Ca]_i)(V_D - V_{Na}) \end{aligned}$$

Here, the parameter g represents the maximal conductance of the appropriate current (Na , NaP , K , $Leak$, $synaptic$ or CaN), while n and h are activation and inactivation variables, respectively.

The synaptic activation variable s can be defined as:

$$\frac{ds}{dt} = \frac{(1-s)H(V) - k_{syn}s}{\tau_s(V)},$$

n and h are 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 curves and synaptic activation function $H(V)$ are modeled as a sigmoid, with x representing m , n , h or p :

$$x_{\infty}(V) = \frac{1}{1 + \exp((V_s - V_x)/s_x)}$$

while the time constant is modeled as follows

$$\tau_x(V) = \frac{\overline{\tau_x}}{\cosh((V_s - V_x)/2s_x)}$$

V_x , s_x , $\overline{\tau_x}$ 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, $s_h = 5$ mV, $V_{ss} = -10$ mV, $s_s = -5$ mV, $\overline{\tau_n} = 10$ ms, $\overline{\tau_h} = 10,000$ ms, $\overline{\tau_s} = 5$ ms, $V_K = -85$ mV, $V_{Na} = 50$ mV, $V_{syn} = 0$ mV, $V_{gNa} = 28$ nS, $g_K = 11.2$ nS, $g_{LNa} = 0.5$ nS, $g_{LK} = 1.9$ nS, $g_{CaN} = 1$ nS, $g_{syn} = 0.2$ nS, $k_{syn} = 1$.

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

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

where f_i is a constant reflecting the fraction of bound to free Ca^{2+} concentration in the cytosol (Wagner and Keizer, 1994), $f_i = 0.0001$; A is a scaling constant, $A = 0.005$; and K_d is the dissociation constant for IP_3 receptor inactivation by Ca^{2+} , $K_d = 0.4$.

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

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

where P_{IP_3R} is the maximum total permeability of IP_3 channels, $P_{IP_3R} = 31,000$ 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 $K_a = 0.4$ 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 that the changes in intracellular Ca^{2+} are small relative to the changes that occur at the cell membrane, which lead to conservation of the total amount of Ca^{2+} within the dendritic compartment. With this assumption, the ER Ca^{2+} concentration can be calculated as:

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

where 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).

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

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

Other parameter values include: the ratio of somatic to total area, $k = 0.3$; the membrane capacitances, $C_{mS} = 21$ μ F, $C_{mD} = 5$ μ F; and the conductance $g_c = 1$ nS.

2.2. Choice of parameters for g_{NaP} and $[Ca]_{tot}$

To reproduce heterogeneity in neuronal activity (Purvis et al., 2007), we randomly chose each parameter set (g_{NaP} and $[Ca]_{tot}$) from a uniform distribution. The boundary of the distribution was chosen such that the number of intrinsic bursting cells matched experimental recordings in a network with $K_{tonic} = 1$ (Del Negro et al., 2005). To match these data, we chose a 2-dimensional uniform distribution for parameters g_{NaP} and $[Ca]_{tot}$ with 5% of the distribution in the somatic bursting regime (I_{NaP} bursting mechanism) and 5% of the distribution in the dendritic and somato-dendritic (I_{CaN}) bursting regime (Fig. 1C). For each set of numerical experiments, we ran 10 simulations with different randomly chosen parameter sets (g_{NaP} and $[Ca]_{tot}$).

2.3. Dynamics of neuromodulator release

The mean concentration of neuromodulator in the preBötC is represented by the variable $[NT]$. Since we only consider excitatory neuromodulators that activate the G_q protein pathway (such as substance P or serotonin), the concentration of neuromodulator in the model is proportional to the concentration of IP_3

$$[IP_3] = \alpha [NT]$$

where α is the proportionality constant, $\alpha = 1$.

In a network where synaptic neurotransmission is pharmacologically blocked, $[NT]$ represents the concentration of either bath-applied neuromodulators or agonists/antagonists of G_q -coupled receptors. In an experiment on an intact network, neuromodulators can be both bath applied and endogenously released. Release of neuromodulators depends on the firing rate of secreting neurons located in the Raphe nucleus. It has been shown experimentally that there are two subpopulations of such neurons in the Raphe: neurons with a constant firing rate and neurons with a firing rate reflecting the activity of preBötC neurons (Ptak et al., 2009). Therefore, we chose a differential equation for $[NT]$, simulating both tonic and phasic release of excitatory neuromodulators.

Thus, dynamics of neuromodulator release were calculated as follow:

$$\frac{d[NT]}{dt} = \frac{K_{tonic} + K_{syn} g_{syn} \sum s_j - K_{deg} [NT]}{\tau_{NT}},$$

where K_{tonic} is the tonic rate of neuromodulator release, $K_{tonic} = 1 \mu M$; K_{syn} is the maximum rate of neuromodulator release in response to change of activity of population; $K_{syn} = 5 M/mS$; K_{deg} is the degradation rate, ($K_{deg} = 0.95$); τ_{NT} is time constant, $\tau_{NT} = 100 mS$.

2.4. Data analysis

The model equations were integrated with the 4th order Runge–Kutta method with a time step of 0.1 ms using the C++ programming language. Spike times were collected and analyzed using custom Python scripts. Simulations were run for at least 160 s of simulation time. The data from the first 60 s of all simulations were discarded to eliminate initial transient network activity. Spike times in all neurons in the model network were binned by 20 ms intervals and the number of spikes in each interval (f_{bin}) was calculated.

To quantify how well binned time series resembled traces of respiratory activity, we calculated network bursting index (NBI).

$$\text{NBI}(t) = \text{Standard Deviation}[f_{\text{bin}}(t)] \times (\text{Mean}[f_{\text{bin}}(t)] - \text{Median}[f_{\text{bin}}(t)])$$

This metric was not intended to be a rigorous definition of network-level synchrony, but rather to easily identify rhythmic patterns similar to mean field recordings of preBötC activity. This index reflects several crucial features of experimental preBötC recordings: (1) the background activity is low between the bursts; (2) there is a sudden rise in firing rate as bursting starts; (3) the population activity is high during the burst. The NBI value reflects the fact that time series with prominent peaks have large differences between mean and median. In addition, to encourage a sharp increase in spike frequency during the inspiratory phase, we multiply the difference between mean and median by standard deviation. The recordings with prominent sharp peaks in firing rate in our simulation have an $\text{NBI} > 100$. Smaller values of NBI indicate either absence of peaks or high level of background activity. Typical recordings were in the range of NBI between 200 and 700. Burst frequency was calculated using a threshold of 1.5 spikes per ms.

In addition to NBI, we have developed a *modulation metric*, which quantitatively evaluates the frequency modulation in the simulated network. We have noticed that in some configurations, burst frequency decreases with increase in neuromodulatory tone. Such a drop in burst frequency has not been reported in animals older than P2 (Shvarev et al., 2003; Doi and Ramirez, 2010). Therefore, we have designed *modulation metric* to identify network configuration in which application of excitatory neuromodulators create wide range of burst frequencies and frequency only increases with neuromodulatory tone.

$$\text{Modulation metrics} = \text{frequency range} \times (1 - \text{frequency decrease})$$

$$\begin{aligned} \text{frequency range} &= \frac{\text{Max}(BF) - \text{Min}(BF)}{BF(K_{\text{tonic}}=0)} \\ \text{frequency decrease} &= \frac{BF(K_{\text{tonic}}=0) - \text{Min}(BF)}{BF(K_{\text{tonic}}=0)} \end{aligned}$$

where $\text{Max}(BF)$ is a maximum mean burst frequency of ten random simulations over all values of neuromodulatory tone (K_{tonic}), $\text{Min}(BF)$ is a minimum mean burst frequency of ten random simulations over all values of neuromodulatory tone, and $BF(K_{\text{tonic}}=0)$ is a mean burst frequency for zero neuromodulatory tone.

3. Results

3.1. Electrical activity in synaptically isolated neurons

In our previous work, we developed a two-compartment model that accounted for two types of endogenous bursting in the preBötC. Ca^{2+} -dependent bursting follows rhythmic Ca^{2+} release from the endoplasmic reticulum (ER) of the dendritic compartment and depends on the dynamics of IP3 receptors. The Ca^{2+} -independent bursting relies on slow inactivation of the persistent sodium current in the soma. In the two-compartment model, endogenous bursting depends on the total amount of Ca^{2+} in the ER of the dendritic compartment ($[Ca]_{\text{tot}}$) and the conductance of the persistent sodium current (g_{NaP}). If both g_{NaP} and $[Ca]_{\text{tot}}$ are small, the cell is silent. If $[Ca]_{\text{tot}}$ is small, but g_{NaP} is in the bursting range, the model produces persistent sodium (*somatic*) bursting, and when $[Ca]_{\text{tot}}$ is sufficiently large, bursting activity follows Ca^{2+} oscillations (*dendritic* bursting).

Calcium oscillations in the two-compartment model also depend on the concentration of bath-applied excitatory neuromodulators, which activate the G_q protein pathway and induce IP3 release. The variable $[NT]$ in the model represents the concentration of such neuromodulators, presumably substance P (SP) or serotonin (5-HT). We further investigate electrical activity in the two-compartment model to show that the presence of Ca^{2+} oscillations, which indicate dendritic bursting, is non-linearly dependent on the values of $[NT]$ and $[Ca]_{tot}$ (Fig. 1A). For example, Ca^{2+} oscillations in the model can be generated at low levels of $[Ca]_{tot}$ if $[NT]$ is sufficiently large. It has been found that application of 5-HT can induce intrinsic bursting activity in non-bursting inspiratory neurons (Pena and Ramirez, 2004; Ptak et al., 2009). Our model is consistent with these data and confirms that the concentration of excitatory neuromodulators can change the dynamical properties of neurons and induce bursting.

To evaluate the changes in the fraction of bursting cells with changes in neuromodulatory tone, we have run simulations for a 50 cell neuronal network model, which was intended to represent the population of synaptically isolated preBötC neurons. We chose the distribution of endogenous bursting properties based on experimental results (Del Negro et al., 2005), where only approximately 10% of the preBötC neurons are intrinsic bursters (5% Ca^{2+} -dependent and 5% Ca^{2+} -independent). In control ($[NT] = 1$) simulation, the 10% of the area, from which parameters are randomly picked, is located in the bursting regime (5% in dendritic and somato-dendritic and 5% in somatic regime). For example, panels B, C, and D in Fig. 1 show 50 cells with randomly chosen parameters g_{NaP} and $[Ca]_{tot}$ and at three different concentration of excitatory neuromodulator. For the control case ($[NT] = 1$), only 5% of the chosen region is in the dendritic bursting regime (Fig. 1C). For $[NT] = 1.1$, the dendritic bursting regime extends toward lower values of $[Ca]_{tot}$ and includes a larger proportion of cells (Fig. 1B). The increase in the fraction of dendritic bursters is a result of the lower threshold for Ca oscillations. For larger values of NT (Fig. 1A), Ca oscillations can occur in cells with smaller total amount of Ca in the ER (Catot). As a result, for higher neuromodulatory tone, a larger fraction of cells exhibit dendritic bursting (Fig. 1D). For low neuromodulatory tone ($[NT] = 0.9$), the dendritic regime moves toward higher values of $[Ca]_{tot}$ and none of the cells are dendritic bursters (Fig. 1B). Thus, our model predicts that, for a fixed distribution of $[Ca]_{tot}$, the number of dendritic bursters increases with an increase in neuromodulatory tone (Fig. 1E). This prediction can be verified experimentally by monitoring electrical activity or changes in intracellular Ca^{2+} in populations of synaptically isolated preBötC cells at different levels of bath-applied agonists/antagonists of G_q -coupled receptors. For low neuromodulatory tone, induced by application of G_q agonist, very few cells sustain intrinsic Ca^{2+} oscillations (Fig. 1D), which leads to a constant low level of intracellular Ca^{2+} . In contrast, at high neuromodulatory tone, induced by application of G_q agonist, a large fraction of cells produce intrinsic Ca^{2+} oscillations, leading to a periodic increase in intracellular Ca^{2+} .

Since our model assumes that Ca^{2+} oscillations originate in the dendritic compartment, such changes would be hard to detect experimentally. One way to isolate dendritic Ca^{2+} oscillations is to record activity in synaptically isolated cells, where Ca^{2+} oscillations are induced by the depolarizing potential from the dendrites. Dendritic bursting activity in this preparation can be measured indirectly, by measuring somatic activity either directly through a microelectrode array or indirectly by Ca^{2+} imaging.

3.2. Dynamics of neuromodulator release in network of synaptically connected cells

The exact role of intrinsic bursters in the respiratory network is unresolved. There are a range of theories spanning the spectrum from a dominant role of intrinsic pacemakers to others suggesting bursting is an emergent property. Today's perspectives are more nuanced between these two extremes. We studied the role of intrinsic bursting activity in the network

by monitoring frequency in a synaptically coupled 50-cell model while simulating bath application of excitatory neuromodulators. It has been shown that an increase in the concentration of excitatory neuromodulators (SP or 5-HT), as well as agonists for G_q -protein coupled receptors, increases the frequency of the respiratory network (Doi and Ramirez, 2010).

Neuromodulatory tone in a preBötC slice preparation can be controlled by bath application of neuromodulators during the experiment. However, endogenous release of neuromodulators by the nearby Raphe nucleus also affects neuromodulatory tone in the preBötC. 5-HT and SP neurons project from the Raphe to the preBötC (Ptak et al., 2009), where they release these neuromodulators and activate G_q -coupled receptors ($5HT_{2A}$, $5HT_{2B}$, and NK_1). We considered the role of excitatory neuromodulators in preBötC rhythmic output by introducing two possible schemes of their endogenous release from the Raphe nucleus: tonic (Fig. 2A, referred as *Model A*) and a combination of tonic and phasic (Fig. 2B, referred as *Model B*).

When simulating *Model A*, we consider a constant rate of 5-HT or SP release, which would lead to a constant rate of receptor binding and a constant level of IP_3 synthesis. To simplify the model, we simulate the activity of the Raphe neurons as a change in concentration of excitatory neuromodulator ($[NT]$), which is proportional to IP_3 concentration (see Section 2). Thus, we model NT as a differential equation with a linear positive term (K_{tonic}), which represents neuromodulator synthesis rate and linear negative term (K_{deg}) which represents the rate of degradation. Since K_{tonic} does not change during the simulation, we will refer to it as a *neuromodulatory tone* to stress its difference from the phasic neuromodulator release in *Model B*.

3.3. Tonic increase of excitatory neuromodulator does not result in frequency modulation in the model of a preBötC network

Our goal is to identify network configuration, which produces stable respiratory rhythm whose frequency increases in response to larger neuromodulatory tone. To test if the increase in neuromodulatory tone increases the frequency of neuronal output in *Model A*, we connect 50 model cells through excitatory synaptic connections (see Section 2) and apply different levels of neuromodulatory tone by varying parameter K_{tonic} . The resulting network produced a stable respiratory rhythm for lower values of K_{tonic} (Fig. 3A). The frequency of network rhythm increases with an increase in tonic neuromodulatory tone (compare panel A and B in Fig. 3). However, there is a sudden drop in network frequency for K_{tonic} between 1 and 1.5. Such a drop of frequency is not representative of preBötC recordings for preparation older than P2, and is reflected in a low modulation metric (Table 1, first row). Since our goal is to find a model with frequency modulation similar to experimental preBötC recordings, we must either reject the *Model A* or try to find parameters regime where this model can produce realistic results.

It has been hypothesized that excitatory neurotransmitters increase synaptic drive in the respiratory network (Rybak et al., 2003). To test this hypothesis, we varied the strength of synaptic coupling (g_{syn}) in addition to neuromodulatory tone (Fig. 3D). Each curve in Fig. 3D represents the frequency of rhythmic activity in the network where K_{tonic} was progressively increased from 0 to 2. The change in K_{tonic} can be interpreted as either a progressive increase in concentration of bath-applied excitatory neuromodulator or electrical stimulation of the Raphe nucleus (Ptak et al., 2009; Doi and Ramirez, 2010). In agreement with experimental data, there is no network activity for a low level of synaptic coupling ($g_{syn} = 0$ or $g_{syn} = 0.1$, not shown), which represents application of synaptic blockers (not shown). Increase in synaptic connection to $g_{syn} = 0.2$ (Fig. 3D, red trace) results in network bursts with a constant frequency for low levels of neuromodulatory tone (up to $K_{tonic} = 0.8$).

For larger values of K_{tonic} , the response is biphasic with a transient frequency decrease which is followed by an increase. However, the frequency varied significantly between different runs of the simulations (see larger error bars for red curve for K_{tonic} between 1 and 2). We measured the Network Burst Index (NBI) to quantify how well our simulations incorporate features of respiratory rhythms (see Section 2). The NBI remains low for K_{tonic} between 0 and 1.5 (Fig. 3E, red trace), but increases for larger values of K_{tonic} . However, even for larger neuromodulatory tone, NBI remains lower than 100, which indicates low network activity for $g_{syn} = 0.2$ nS.

For a larger value of synaptic coupling ($g_{syn} = 0.3$ nS), there is a modest increase in NBI, with significant increase in NBI for larger neuromodulatory tone (Fig. 3D, E, blue trace). However, the range of burst frequencies are significantly lower than for $g_{syn} = 0.2$, which is indicated as a decrease in modulatory metric (Table 1, compare first and second row). A further increase in synaptic coupling ($g_{syn} = 0.4$ nS) increases NBI but shortens the range of network rhythm (Table 1, third row), with the output becoming arrhythmic for $K_{tonic} > 1.8$ (Fig. 3D, E, black traces).

These results show that *Model A* is unable to produce a network with the desired properties. For low synaptic coupling ($g_{syn} = 0.2$ nS), an increase in neuromodulatory tone induces significant frequency modulation, but the network activity does not resemble preBötC activity, which is indicated as a low NBI. For larger values of synaptic coupling, the NBI is high, but there is only small change in network frequency with neuromodulatory tone. We therefore conclude that tonic increases in neuromodulatory tone alone cannot account for realistic burst frequency modulation in preBötC.

The very weak rhythmic behavior in model A can be explained by the dendritic bursters' weak response to synaptic current. Since intrinsic Ca^{2+} oscillations are not affected by current injection in the soma (Toporikova and Butera, 2011), dendritic bursters are not easily synchronized with the rest of the network. Therefore, an increase in neuromodulatory tone in model A does not induce rhythmic activity in spite of a significant increase in the number of bursting cells.

3.4. Positive feedback produces realistic frequency modulation in respiratory network

In addition to tonically firing neurons, the Raphe nucleus contains a subpopulation of neurons whose firing rate reflects changes in preBötC neuronal activity (Ptak et al., 2009). We modeled this interaction between the Raphe and preBötC as positive feedback from firing activity of the network to release excitatory neuromodulator. We added a second positive term to the differential equation for $[NT]$ that is proportional to the synaptic activity in the preBötC network. Thus, for *Model B*, global concentration of excitatory neuromodulator increases with an increase in network firing activity.

Example raster plots for the *Model B* simulation are shown in Fig. 4A–C. The network shows stable rhythmic behavior with an increase in frequency for larger values of K_{tonic} . Compared to a model without feedback, there is much less firing activity between the bursts, even for larger values of K_{tonic} (notice the difference between Fig. 3C and Fig. 4C). For the rest of this work we show the simulations with $g_{syn} = 0.2$ nS. The results are similar for larger values of g_{syn} , but burst durations are longer than observed in experimental recordings of preBötC.

The burst frequency of the network depends on the strength of the positive feedback between the Raphe and the preBötC, which is represented by the parameter K_{phasic} in our model. Without feedback *Model B* is the same as *Model A*, with a biphasic response to an increase in neuromodulatory tone. An increase in coupling strength ($K_{phasic} = 5$) results in a

linear relationship between neuromodulatory tone and network frequency. Larger values of feedback strength produce a non-linear response, where the burst frequency saturates at higher values. The increase in strength of feedback also improves network rhythm, which is indicated by an increase in NBI (compare green, black, and blue curves in Fig. 4H). In addition, the range of frequency increases with an increase in strength of positive feedback, indicated by an increase in modulation metric (Table 2). Since our goal was to achieve a wide range of frequency modulation and stable rhythmic activity, we chose $K_{phasic} = 15$ for our model.

To further examine how rhythmicity in the *Model B* changes with feedback strength, we ran simulations for low ($K_{tonic} = 0.4$), medium ($K_{tonic} = 1$) and high ($K_{tonic} = 1.6$) neuromodulatory tone and analyze the burst frequency, burst duration, and NBI.

Burst frequency changes dramatically with changes in neuromodulatory tone. At low neuromodulatory tone ($K_{tonic} = 0.4$), frequency is low for all feedback strengths (Fig. 5A, red). At high neuromodulatory tone ($K_{tonic} = 1.6$), frequency modulation is also minimal but burst frequency remains high (Fig. 5A, blue). At the intermediate level ($K_{tonic} = 1$), burst frequency linearly depends on the strength of feedback (Fig. 5A, black).

Burst duration also varies with neuromodulatory tone: it increases with increasing feedback strength for all values of neuromodulatory tone, but the difference is less dramatic than for the frequency. For low neuromodulatory tone, the burst duration rises in a sigmoid fashion with an increase in feedback strength (Fig. 5B, red). With intermediate tone, the burst duration rises steeply with feedback strength (Fig. 5B, black) and further increases in neuromodulatory tone decrease burst duration (Fig. 5B, blue).

The NBI increases with increases in feedback strength for all values of neuromodulatory tone. However, for low neuromodulatory tone this increase is very slow (Fig. 5C, red). The slope increases with increased neuromodulatory tone (Fig. 5C, black), although further increases in neuromodulatory tone do not improve the NBI (Fig. 5C, blue).

The predictions of change in frequency, burst duration and NBI can be tested experimentally by measuring preBötC output while varying the activity of the Raphe nucleus (pharmacologically, electrophysiologically or optogenetically), in three different concentrations of bath-applied agonists/antagonists of G_q coupled receptors. Our model predicts that (i) manipulation of Raphe activity will cause significant changes in burst frequency and (ii) higher activity of Raphe produces preBötC bursts with a higher frequency. This predictions generalizes the findings of Ptak et al. (2009) from preBötC neurons. We further predict that the application of a G_q antagonist will eliminate this frequency modulation by Raphe and keep the network frequency constant and low. In contrast, application of a G_q agonist will result in a high frequency for all levels of Raphe stimulation.

3.5. Role of non-specific Ca current

The precise molecular identity of I_{CaN} currents in preBötC neurons is still debated. There is conflicting evidence for a role for either TRPM (Crowder et al., 2007; Mironov, 2008) or TRPC (Ben-Mabrouk and Tryba, 2010) types of currents. Experiments show that I_{CaN} blockers significantly affect the rhythmic properties of preBötC neurons (Pena et al., 2004; Pace et al., 2007). It has been shown that blocking I_{CaN} with flufenamic acid (FFA) decreases burst duration (Crowder et al., 2007; Ben-Mabrouk and Tryba, 2010). However, when this experiment was repeated in combination with bath-applied substance P, burst frequency and duration decreased significantly (Ben-Mabrouk and Tryba, 2010).

We simulated this experiment by progressively increasing g_{CaN} in our model and running simulations for different levels of neuromodulatory tone (Fig. 6). Similar to experimental data, the frequency and duration of the resulting network output depends on neuromodulatory tone. For low values of K_{tonic} , the burst frequency does not change but the burst duration increases with increasing g_{CaN} (Fig. 6A, C, D, black triangles). In contrast, for high values of K_{tonic} , both burst frequency and duration increase with increasing g_{CaN} (Fig. 6B C, D., red circles). In addition to reproducing trends in activity consistent with experimental data (Ben-Mabrouk and Tryba, 2010), our model predicts that the burst duration will also increase with increasing g_{CaN} (Fig. 6D).

Thus, in the context of our model, conflicting experimental data on I_{CaN} (Pace et al., 2007; Ben-Mabrouk and Tryba, 2010) may be due to differences in Ca^{2+} dynamics at high and low levels of neuromodulatory tone. At low neuromodulatory tone, very few neurons have Ca^{2+} oscillations, so an increase in g_{CaN} is identical to an increase in depolarizing bias current, which only increases burst duration. In contrast, at high neuromodulatory tone, large fraction of cells has intrinsic Ca^{2+} oscillations, which translated into depolarizing potential through action of I_{Can} . The advantage of such simultaneous Ca^{2+} oscillations is that they provide the mechanism for burst termination in a network. The IP_3 receptor is slowly inactivated by Ca^{2+} , which leads to a reduction of depolarizing potential and prevents the soma from spiking. Since neuromodulator release depends on preBötC firing, a reduction in somatic spiking reduces neuromodulator release from Raphe.

Because I_{CaN} translates Ca^{2+} oscillations to a depolarizing potential, the value of this current indirectly modulates the level of positive feedback, with larger values of g_{CaN} inducing faster and longer somatic spiking, which translated to a larger release of neuromodulators by the raphe. Thus for smaller values of g_{CaN} , Ca^{2+} oscillations create a small depolarizing potential and make the burst slightly longer, but do not induce significant changes in burst frequency (Fig. 6C, D, black). In contrast, larger values of g_{CaN} not only induce significant changes in the depolarizing potential (increase burst duration) but also induces significant increase in release of neuromodulators from the Raphe, thus increasing burst frequency (Fig. 6C, D, red). This change in frequency and amplitude of network rhythm is similar to the findings of (Ben-Mabrouk and Tryba, 2010).

3.6. High neuromodulatory tone and positive feedback help to maintain rhythm in low $[K^+]$

In brainstem slice preparations, rhythmic neuronal activity can only be achieved at concentrations of extracellular K^+ greater than 8 mM, which is significantly higher than the physiological K^+ level (~3 mM). If the K^+ concentration falls below 6 mM, the rhythm in the slice usually ceases. The absence of rhythmic activity at physiological $[K^+]$ raises an important question of how well this preparation represents *in vivo* conditions.

Our model results suggest that high $[K^+]$ may serve as a compensatory mechanism for low neuromodulatory tone. It has been shown experimentally that application of high concentrations of substance P can restore the rhythm in low K^+ (Pena and Ramirez, 2004). Our model reproduces these experimental results (compare panels A–C in Fig. 7 with Figure 7). We have carried out network simulations in control and low $[K^+]$ for different levels of neuromodulatory tone (Fig. 7). A representative example of simulation in high $[K^+]$ ($V_k = -85$ mV) is shown in Fig. 7A. When $[K^+]$ is lowered ($V_k = -87$ mV), the network rhythm abruptly stops (Fig. 7B). If in the same simulation, neuromodulatory tone is increased ($K_{tonic} = 2$), the rhythm returns with a frequency close to the original one (Fig. 7C). This rescue of the rhythm is critically dependent on the feedback from the raphe, since removal ($K_{phasic} = 0$) results in network rhythm cessation (Fig. 7D).

Thus, our model suggests that experimental preparations which require high $[K^+]$ for initiation of rhythmic activity have reduced endogenous neuromodulatory tone. There are number of reasons for low neuromodulatory tone in the slice preparation, including severed neuronal connections, reduced thickness of slices, and rundown of neuromodulators. *In vivo* sources of neuromodulatory tone to the preBötC are not limited to the Raphe, but also includes other brain nuclei such as the locus ceruleus, nucleus ambiguus, and hypothalamus (Doi and Ramirez, 2008), which are typically eliminated during slice preparation. It is also possible that Raphe basal activity is lower in the slice preparation, since other brain nuclei, such as the hypothalamus and locus ceruleus (LC), modulate the activity of Raphe neurons (Bernard et al., 2003; Tao et al., 2006). This prediction can be tested experimentally by measuring the concentrations of excitatory neuromodulators in the “punches” preBötC of tissues from slices which are active or silent in physiological $[K^+]$.

4. Discussion

In this work, we introduced a computational model which utilizes a positive feedback loop from the preBötC to the Raphe to achieve realistic frequency modulation, as observed in experimental recordings. Our approach was to reverse-engineer the network, constraining its parameters to those that produced an output consistent with identified dynamical properties observed in experiments.

The first property we choose to constrain our network was a realistic distribution of endogenous bursting properties. It has been shown that intrinsic bursters represent only a small fraction of cells in the population. Our model proposed that the small number of intrinsic Ca^{2+} bursting properties is a direct result of low neuromodulatory tone which is characteristic of the slice preparation. A prediction of our model is that number of neurons with intrinsic Ca^{2+} bursting properties will increase with excitatory neuromodulatory tone. Such predictions can be tested by recording activity in synaptically isolated cells at different levels of bath-applied excitatory neuromodulators.

The second property we chose is the general shape of the output of the respiratory network. Recordings of the mean field potential in the preBötC show a very fast firing rate during the activity of respiratory muscles in the diaphragm and a very low firing rate for the rest of the respiratory cycle. We therefore considered peaks in firing rate as our criteria for realistic network output and chose parameters in our model to minimize electrical activity during the inter-burst interval. We used the NBI metric to identify peaks in firing rate during the network simulations. If the mean in network firing rate exceeded the median, we classified such a network as bursty. The absence of clear peaks in firing activity was the primary reason for the rejection of a network without feedback. A large portion of networks we tested in these conditions failed to produce reliable peaks in the range of K_{tonic} between 1.1 and 1.5 (Fig. 3C).

The third criteria we identify to constrain the network parameters was a wide range of frequency modulation. Since the respiratory network has to adjust the respiratory frequency to metabolic demands of the body, the network frequency should be easily tunable. In the slice preparation, the population rhythmic output varies between preparations, but application of SP reliably induces 2–3 fold increases in frequency. In addition, simultaneous blocking NK1 and 5-HT2 receptors decrease the frequency of respiratory network output (Doi and Ramirez, 2010). This range of frequencies led to selection of a strength of positive feedback value ($K_{phasic} = 15$) to maximize the frequency range of the network.

We focused on the neuromodulators that are released from the raphe nuclei and activate G_q protein coupled receptors (presumably NK1 and 5-HT2). However, it must be emphasized

that respiratory rhythm generation also depends on numerous other neuromodulators affecting the same pathway. For example, endogenously released NE from the A5 region and LC bind α_1 receptors on preBötC neurons and contribute to respiratory rhythm generation (Hilaire et al., 2004). In addition, within the medullary slice there are several subpopulations of NE neurons, whose activity affect cardiorespiratory regulation (Guyenet, 1991). Their roles in respiratory rhythmogenesis remain unknown but can be essential for neuromodulatory feedback to preBötC neurons. Other sources for activation of G_q -coupled receptors include acetylcholine, which is released from the LC and binds to the M3 receptor (Shao and Feldman, 2005) and histamine, which is released from hypothalamus and binds to the H1 receptor (Dutschmann et al., 2003).

Our model predicts that the connectivity between the Raphe and preBötC is crucial for frequency modulation by excitatory neuromodulators. This prediction can be tested in the preBötC “island” (Johnson et al., 2001; Tryba et al., 2008; Vandam et al., 2008). It has been shown that the preBötC “island” can generate the rhythm with a smaller amplitude and higher cycle-to-cycle variability. Our model predicts that in the preBötC island preparation, application of excitatory neuromodulators will not induce significant frequency modulation. That is, the results will be similar to our simulations of *Model A* (Fig. 3), with either low NBI and only small changes in frequency, or high NBI and no significant change in frequency.

In addition to activation of G_q protein coupled receptors, the same neuromodulators can act on different receptor subtypes and therefore lead to activation of different second messenger pathways. For example, 5HT activates 5-HT₄ and 5-HT₇ receptors known to exert G_s protein-mediated excitatory effects on the respiratory rhythm (Doi and Ramirez, 2008). Thus, it is likely that these and many other yet unidentified modulatory actions will contribute to respiratory rhythm generation. In our future work we will introduce the interaction of different NK and 5-HT receptors subtypes and simulate their combined effect.

The choice of parameters g_{NaP} and $[Ca]_{tot}$ in our model is dictated by the distribution of firing properties of the preBötC neurons. We used data on the fraction of intrinsic bursting cells of both Ca and NaP types, and chose parameters to match fraction of bursting cells in the population. In addition, we considered simulations with a large fraction of bursting cells (up to 25%) and have not noticed any changes in the frequency modulation of the network (results not shown). However, networks with a larger fraction of NaP cells display higher frequency at low neuromodulatory tone, which can explain the higher excitability provided by these cell type. The rest of the cells in the population were considered quiescent. Experimental recordings of preBötC neurons report that in addition to silence and bursting, some cells produce regular firing activity, however the fraction of such tonically spiking cells remain unknown. The tonic behavior in our model can be achieved by adding a distribution of V_k . To make sure that our results are valid in the presence of tonically spiking cells, we ran our simulations with randomly selected values of V_k within an interval $[-82, -85]$, which resulted on average to ~20% of tonically spiking cells (results are not shown). The results presented in this work were still valid in the presence of tonically spiking cells. However, in order to keep presentation and analysis focused on the key findings, we did not include these simulations.

The single cell model we used in our simulation utilizes two independent bursting mechanisms. As neuromodulatory tone increases, a larger proportion of cells exhibit Ca^{2+} -oscillations. In a network with only tonic neuromodulatory tone (*Model A*), an increase in excitatory neuromodulator leads to a sharp transition from synaptic connection-induced bursting to Ca^{2+} -driven bursting. As a result, rhythmic output stops or significantly slows down at some values of neuromodulatory tone (Fig. 3D, red curve). However, if positive

feedback is included in the model (Model B), such unstable dynamics are eliminated. Thus, our model predicts that loss of the positive feedback from preBötC to Raphe will result in decreased frequency or even loss of rhythmicity for some degrees of neuromodulatory tone. It has been shown that in the neonatal rat brainstem-spinal cord preparation, the effects of SP can be biphasic at the very early age (P0–P1), with an initial drop or even cessation of the rhythm and prolonged period of increased frequency (Shvarev and Lagercrantz, 2006). This finding is identical to results of our model for the weak feedback strengths. For more mature preparations (P2–P3) SP did not induce a decrease in respiratory frequency, which is similar to our modeling results for larger values of positive feedback. Therefore, one possible interpretation of Shvarev and Lagercrantz (2006) experiments for early post-natal experiment is an increase in positive feedback between the Raphe and preBötC during the first few days of postnatal life. It has been reported that victims of sudden infant death syndrome (SIDS) have multiple defects in the serotonergic system (Duncan et al., 2010; Paterson et al., 2009). Experiments in animal models show that newborn animals with selective loss of 5-HT neurons lose their ability to resuscitate during anoxia or hypoxia (Cummings et al., 2011; Erickson and Sposato, 2009). It is possible that in such cases diminished or lost activity of serotonergic neurons in the Raphe leads to an inability to sustain stable respiratory rhythm during sudden changes in neuromodulatory tone imposed by hypoxia or anoxia.

In conclusion, we propose a novel model of connectivity between the raphe and preBötC nuclei, where neuronal activity in preBötC induces release of excitatory neuromodulators from the raphe. Such positive feedback between these nuclei produces a stable respiratory rhythm whose frequency can be easily modulated to adapt to metabolic demand for oxygen. To our knowledge, this is the first model which explicitly simulates the action of excitatory neuromodulators on the inspiratory network in the preBötC.

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References

- Ben-Mabrouk F, Tryba AK. Substance P modulation of TRPC3/7 channels improves respiratory rhythm regularity and ICAN-dependent pacemaker activity. *European Journal of Neuroscience*. 2010; 31:1219–1232. [PubMed: 20345918]
- Bernard R, Lydic R, Baghdoyan HA. Hypocretin-1 causes G protein activation and increases ACh release in rat pons. *European Journal of Neuroscience*. 2003; 18:1775–1785. [PubMed: 14622212]
- Butera RJ Jr, Rinzel J, Smith JC. Models of respiratory rhythm generation in the pre-Botzinger complex. II. Populations of coupled pacemaker neurons. *Journal of Neurophysiology*. 1999; 82:398–415. [PubMed: 10400967]
- Cho H, Kim MS, Shim WS, Yang YD, Koo J, Oh U. Calcium-activated cationic channel in rat sensory neurons. *European Journal of Neuroscience*. 2003; 17:2630–2638. [PubMed: 12823470]
- Crowder EA, Saha MS, Pace RW, Zhang H, Prestwich GD, Del Negro CA. Phosphatidylinositol 4,5-bisphosphate regulates inspiratory burst activity in the neonatal mouse preBotzinger complex. *Journal of Physiology*. 2007; 582:1047–1058. [PubMed: 17599963]
- Cummings KJ, Commons KG, Hewitt JC, Daubenspeck JA, Li A, Kinney HC, Nattie EE. Failed heart rate recovery at a critical age in 5-HT-deficient mice exposed to episodic anoxia: implications for SIDS. *Journal of Applied Physiology*. 2011; 111:825–833. [PubMed: 21680874]
- Del Negro CA, Morgado-Valle C, Hayes JA, Mackay DD, Pace RW, Crowder EA, Feldman JL. Sodium and calcium current-mediated pacemaker neurons and respiratory rhythm generation. *Journal of Neuroscience*. 2005; 25:446–453. [PubMed: 15647488]

- Doi A, Ramirez JM. Neuromodulation and the orchestration of the respiratory rhythm. *Respiratory Physiology & Neurobiology*. 2008; 164:96–104. [PubMed: 18602029]
- Doi A, Ramirez JM. State-dependent interactions between excitatory neuromodulators in the neuronal control of breathing. *Journal of Neuroscience*. 2010; 30:8251–8262. [PubMed: 20554877]
- Duncan JR, Paterson DS, Hoffman JM, Mokler DJ, Borenstein NS, Belliveau RA, Krous HF, Haas EA, Stanley C, Nattie EE, Trachtenberg FL, Kinney HC. Brainstem serotonergic deficiency in sudden infant death syndrome. *Journal of the American Medical Association*. 2010; 303:430–437. [PubMed: 20124538]
- Dutschmann M, Bischoff AM, Busselberg D, Richter DW. Histaminergic modulation of the intact respiratory network of adult mice. *Pflügers Archiv*. 2003; 445:570–576. [PubMed: 12634928]
- Erickson JT, Sposato BC. Autoresuscitation responses to hypoxia-induced apnea are delayed in newborn 5-HT-deficient Pet-1 homozygous mice. *Journal of Applied Physiology*. 2009; 106:1785–1792. [PubMed: 19213929]
- Gray PA, Reikling JC, Bocchiaro CM, Feldman JL. Modulation of respiratory frequency by peptidergic input to rhythmogenic neurons in the preBotzinger complex. *Science*. 1999; 286:1566–1568. [PubMed: 10567264]
- Cordovez JM, Wilson CG, Solomon IC. Geometrical analysis of bursting pacemaker neurons generated by computational models: comparison to in vitro pre-Bötzinger complex bursting neurons. *Advances in Experimental Medicine and Biology*. 2010; 669:45–48. [PubMed: 20217318]
- Guyenet PG. Central noradrenergic neurons: the autonomic connection. *Progress in Brain Research*. 1991; 88:365–380. [PubMed: 1813926]
- Hilaire G, Viemari JC, Coulon P, Simonneau M, Bevengut M. Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. *Respiratory Physiology & Neurobiology*. 2004; 143:187–197. [PubMed: 15519555]
- Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology*. 1952; 117:500–544. [PubMed: 12991237]
- Johnson SM, Koshiya N, Smith JC. Isolation of the kernel for respiratory rhythm generation in a novel preparation: the pre-Botzinger complex “island”. *Journal of Neurophysiology*. 2001; 85:1772–1776. [PubMed: 11287498]
- Johnson SM, Smith JC, Funk GD, Feldman JL. Pacemaker behavior of respiratory neurons in medullary slices from neonatal rat. *Journal of Neurophysiology*. 1994; 72:2598–2608. [PubMed: 7897477]
- Koshiya N, Smith JC. Neuronal pacemaker for breathing visualized in vitro. *Nature*. 1999; 400:360–363. [PubMed: 10432113]
- Li YX, Rinzel J. Equations for InsP3 receptor-mediated $[Ca^{2+}]_i$ oscillations derived from a detailed kinetic model: a Hodgkin-Huxley like formalism. *Journal of Theoretical Biology*. 1994; 166:461–473. [PubMed: 8176949]
- Mironov SL. Metabotropic glutamate receptors activate dendritic calcium waves and TRPM channels which drive rhythmic respiratory patterns in mice. *Journal of Physiology*. 2008; 586:2277–2291. [PubMed: 18308826]
- Pace RW, Mackay DD, Feldman JL, Del Negro CA. Inspiratory bursts in the preBotzinger complex depend on a calcium-activated non-specific cation current linked to glutamate receptors in neonatal mice. *Journal of Physiology*. 2007; 582:113–125. [PubMed: 17446214]
- Paterson DS, Hilaire G, Weese-Mayer DE. Medullary serotonin defects and respiratory dysfunction in sudden infant death syndrome. *Respiratory Physiology & Neurobiology*. 2009; 168:133–143. [PubMed: 19481178]
- Pena F, Ramirez JM. Substance P-mediated modulation of pacemaker properties in the mammalian respiratory network. *Journal of Neuroscience*. 2004; 24:7549–7556. [PubMed: 15329402]
- Pena F, Parkis MA, Tryba AK, Ramirez JM. Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia. *Neuron*. 2004; 43:105–117. [PubMed: 15233921]

- Ptak K, Yamanishi T, Aungst J, Milescu LS, Zhang R, Richerson GB, Smith JC. Raphe neurons stimulate respiratory circuit activity by multiple mechanisms via endogenously released serotonin and substance P. *Journal of Neuroscience*. 2009; 29:3720–3737. [PubMed: 19321769]
- Purvis LK, Smith JC, Koizumi H, Butera RJ. Intrinsic bursters increase the robustness of rhythm generation in an excitatory network. *Journal of Neurophysiology*. 2007; 97:1515–1526. [PubMed: 17167061]
- Ramirez JM, Telgkamp P, Elsen FP, Quellmalz UJ, Richter DW. Respiratory rhythm generation in mammals: synaptic and membrane properties. *Respiration Physiology*. 1997; 110:71–85. [PubMed: 9407602]
- Rubin JE, Hayes JA, Mendenhall JL, Del Negro CA. Calcium-activated nonspecific cation current and synaptic depression promote network-dependent burst oscillations. *Proceedings of the National Academy of Sciences USA*. 2009; 106:2939–2944.
- Rybak IA, Shevtsova NA, St-John WM, Paton JF, Pierrefiche O. Endogenous rhythm generation in the pre-Botzinger complex and ionic currents: modelling and in vitro studies. *European Journal of Neuroscience*. 2003; 18:239–257. [PubMed: 12887406]
- Schwarzacher SW, Smith JC, Richter DW. Pre-Botzinger complex in the cat. *Journal of Neurophysiology*. 1995; 73:1452–1461. [PubMed: 7643160]
- Shao XM, Feldman JL. Cholinergic neurotransmission in the preBotzinger complex modulates excitability of inspiratory neurons and regulates respiratory rhythm. *Neuroscience*. 2005; 130:1069–1081. [PubMed: 15653001]
- Shvarev YN, Lagercrantz H. Early postnatal changes in respiratory activity in rat in vitro and modulatory effects of substance P. *European Journal of Neuroscience*. 2006; 24:2253–2263. [PubMed: 17042798]
- Shvarev YN, Lagercrantz H, Yamamoto Y. Two types of rhythm in the respiratory network output in the isolated ventrolateral medulla in the neonatal rats. *Neuroscience Letters*. 2003; 347:53–56. [PubMed: 12865140]
- Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL. Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science*. 1991; 254:726–729. [PubMed: 1683005]
- Tao R, Ma Z, McKenna JT, Thakkar MM, Winston S, Strecker RE, McCarley RW. Differential effect of orexins (hypocretins) on serotonin release in the dorsal and median raphe nuclei of freely behaving rats. *Neuroscience*. 2006; 141:1101–1105. [PubMed: 16820265]
- Toporikova N, Butera RJ. Two types of independent bursting mechanisms in inspiratory neurons: an integrative model. *Journal of Computational Neuroscience*. 2011; 30:515–528. [PubMed: 20838868]
- Tryba AK, Pena F, Lieske SP, Viemari JC, Thoby-Brisson M, Ramirez JM. Differential modulation of neural network and pacemaker activity underlying eupnea and sigh-breathing activities. *Journal of Neurophysiology*. 2008; 99:2114–2125. [PubMed: 18287547]
- Vandam RJ, Shields EJ, Kelty JD. Rhythm generation by the pre-Botzinger complex in medullary slice and island preparations: effects of adenosine A(1) receptor activation. *BMC Neuroscience*. 2008; 9:95. [PubMed: 18826652]
- Wagner J, Keizer J. Effects of rapid buffers on Ca^{2+} diffusion and Ca^{2+} oscillations. *Biophysical Journal*. 1994; 67:447–456. [PubMed: 7919018]
- Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah TR, Davis S, Forster HV. Large lesions in the pre-Botzinger complex area eliminate eupneic respiratory rhythm in awake goats. *Journal of Applied Physiology*. 2004; 97:1629–1636. [PubMed: 15247161]

Fig. 1.

Bursting in the two-compartment model depends on the choice of parameters. (A) Dendritic bursting regime depends on neuromodulatory tone ([NT]) and total Ca²⁺ concentration in the ER ([Ca]_{tot}). (B–D) The level of neuromodulatory tone [NT] determines the fraction of dendritic bursters in a population of 50 cells. (B) For high neuromodulatory tone ([NT] = 1.1 μM), a large proportion of cells are in the dendritic bursting regime. (C) For a medium (control) level of neuromodulatory tone ([NT] = 1 μM), only 5% of the cells are in dendritic bursting regime. (D) For a low level of neuromodulatory tone ([NT] = 0.9 μM), none of the cells are in the dendritic bursting regime. (E) Fraction of dendritic bursters increases with increase in neuromodulatory tone.

Fig. 2.

Two diagrams representing possible connections between the Raphe nucleus and the preBötC. (A) In *Model A*, the Raphe releases excitatory neuromodulator with constant rate (K_{tonic}). (B) In *Model B*, in addition to tonic release of neuromodulator, there is positive feedback between the activity of neurons in preBötC and Raphe. The synaptic activity in the preBötC modulates population activity in the Raphe and thus affects the rate of neuromodulator release (K_{phasic}).

Fig. 3.

Response of *Model A* to increase in constant neuromodulatory tone. (A–C) Raster plot of representative simulation with $g_{syn} = 0.2$ nS for three levels of neuromodulatory tone. (A) For low value of neuromodulatory tone ($K_{tonic} = 0.4$) network frequency is low and burst duration is short, which results in a small NBI (NBI = 7.86). (B) Increase in rate of neuromodulator release ($K_{tonic} = 1$) increases network frequency, however the activity between the bursts also increases which results in small network bursting index (NBI = 6.21). (C) For higher values of neuromodulatory tone ($K_{tonic} = 1.6$), network frequency became even faster, but there is a lot of activity between the bursts and NBI index remain low (NBI = 21.61). (D) Burst frequency of network model as a function of neuromodulatory tone for different levels of synaptic strength (red – $g_{syn} = 0.2$ nS, blue – $g_{syn} = 0.3$ nS, black – $g_{syn} = 0.4$ nS). Bars represent standard error in network burst frequency. Only networks with rhythmic output were used in error calculation. (E) Network burst index (NBI) increases with increase in synaptic strength. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 4.

Frequency modulation in a network with positive feedback (*Model B*). (A–C) A raster plot of a representative network simulation with positive feedback ($K_{phasic} = 15$). Network frequency increases with an increase in neuromodulatory tone. (A) In low ($K_{tonic} = 0.4$) neuromodulator tone, the frequency is low but the NBI is larger than in *Model A* (NBI = 125). (B) In medium ($K_{tonic} = 1$) neuromodulatory tone, the frequency and the bursting index increase (NBI = 434). In high neuromodulatory tone ($K_{tonic} = 1.6$), the burst frequency increases further and the burst shape remains representative of preBötC neurons, indicated by high value of burst index (NBI=335). (D–F) Concentration of excitatory neuromodulator ($[NT]$) in the same simulations as A–C. The rise in $[NT]$ coincides with increases in spiking activity of the network. (G) Burst frequency responses to neuromodulatory tone for four different strengths of positive feedback (K_{phasic}). (H) Network burst frequency index (NBI) increases with strength of positive feedback.

Fig. 5.

Response to changes in positive feedback strength (K_{phasic}) for three different levels of neuromodulatory tone. (A) Burst frequency remains low for all feedback strengths in low neuromodulatory tone ($K_{tonic} = 0.4$, red), non-linearly increases with increase in feedback strengths for intermediate neuromodulatory tone ($K_{tonic} = 1$, black), and stays high for high neuromodulatory tone ($K_{tonic} = 1.6$, blue). (B) Burst duration increases with increase in feedback strength for all values of neuromodulatory tone. For low neuromodulatory tone this rise is sigmoid (red), in intermediate tone it rises more steeply at lower values of K_{phasic} (black) and a further increase in neuromodulatory tone saturates (blue). (C) Network burst index (NBI) increases with increase in strength of positive feedback. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 6.

Response to changes in g_{CaN} depends on neuromodulatory tone. (A and B) A raster plot of a representative simulation. (A) For low neuromodulatory tone ($K_{tonic} = 0.75$) an increase in g_{CaN} increases burst duration but does not affect frequency. (B) For high neuromodulatory tone ($K_{tonic} = 1.25$), an increase in neuromodulatory tone increases both frequency and burst duration. (C) For lower neuromodulatory tone, burst frequency does not change with increase of g_{CaN} tone, but for high K_{tonic} burst frequency increases with g_{CaN} . (D) Burst duration increases with an increase in g_{CaN} for all values of K_{tonic} . (E) NBI increases with an increase in g_{CaN} , with stronger effect for larger values of K_{tonic} .

Fig. 7.

Positive feedback is required to rescue the respiratory rhythm in low $[K^+]$ by high neuromodulatory tone. (A) In high $[K^+]$ ($V_k = -85$ mV), the network is rhythmic at low neuromodulatory tone ($K_{tonic} = 1$). (B) Reduction in $[K^+]$ ($V_k = -87$ mV) abolishes the rhythm. (C) Increase in neuromodulatory tone ($K_{tonic} = 2$) returns network to a rhythmic state. (D) Removal of the positive feedback ($K_{phasic} = 0$) stops network rhythm.

Summary of network activity for different values of synaptic coupling strength (g_{syn}). Results are mean often simulations for values of K_{ionic} from 0 to 2, shown in Fig. 3D, E. NBI increases with an increase in synaptic coupling, which indicates improvement in burst shape as synaptic coupling is increased. However, the range of respiratory frequencies decreases, which is indicated by a small modulation metric for larger values g_{syn} .

Table 1

g_{syn}	Frequency range	Frequency decrease	Modulation metrics	Mean NBI
0.2	2.30	0.45	1.26	20
0.3	0.80	0	0.80	179
0.4	0.61	0	0.61	445

Table 2

Summary of network activity for different values positive feedback strength (K_{phasic}). Results are mean often simulations for values of K_{tonic} from 0 to 2, shown in Fig. 4G, H. NBI increases with increase in positive feedback, which indicate improvement in burst shape as positive feedback strength is increased. In addition, the range of respiratory frequencies increases, which is indicated by the increase in modulation metric.

K_{phasic}	Frequency range	Frequency decrease	Modulation metrics	Mean NBI
0	2.30	0.45	1.27	20.10
5	3.43	0.27	2.50	135.39
10	4.48	0.09	4.08	203.59
15	8.30	0	8.30	271.72