

SI Appendix

Derivation of the model

The current-balance equation took the form

$$C \, dV/dt = -I_{\text{Leak}}(V) - I_{\text{Na}}(V, m, h) - I_{\text{K}}(V, n) - I_{\text{CAN}}(V, \text{Ca}) - I_{\text{syn}}(V, s_1 \dots s_N) - I_{\text{pump}}(\text{Na}) - I_{\text{noise}}$$

where

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

$$ds/dt = ((1 - s) s_{\infty}(V) - k_s s) / \tau_s$$

$$d\text{Ca}/dt = \varepsilon (s k_{\text{IP3}} - k_{\text{Ca}} (\text{Ca} - \text{Ca}_{\infty}))$$

$$d\text{Na}/dt = \alpha (-I_{\text{CAN}}(V, \text{Ca}) - I_{\text{pump}}(\text{Na}))$$

describe the evolution of the state variables, for each x in $\{m, h, n\}$.

Membrane currents were described with chord-conductance equations, in some cases modified for Ca^{2+} or Na^{+} gating (I_{CAN} and I_{pump}), with electrogenic pumps (I_{pump}) adapted from Li et al. (1):

$$I_{\text{Leak}}(V) = g_{\text{Leak}} (V - E_L)$$

$$I_{\text{Na}}(V, m, h) = g_{\text{Na}} m^3 h (V - E_{\text{Na}})$$

$$I_{\text{K}}(V, n) = g_{\text{K}} n^4 (V - E_{\text{K}})$$

$$I_{\text{CAN}}(V, \text{Ca}) = g_{\text{CAN}} (V - E_{\text{CAN}}) / (1 + \exp((\text{Ca} - k_{\text{CAN}})/\sigma_{\text{CAN}}))$$

$$I_{\text{syn}}(V, s_1 \dots s_N) = g_{\text{syn}} \sum_{i=1}^N s_i (V - E_{\text{syn}})$$

where N is the number of presynaptic neurons and $\{s_1 \dots s_N\}$ is the set of presynaptic s variables, and

$$I_{\text{pump}}(\text{Na}) = r_{\text{pump}} (\Phi(\text{Na}) - \Phi(\text{Na}_{\infty}))$$

$$I_{\text{noise}} = \xi(t)$$

where $\xi(t)$ is a Gaussian noise term with an average of zero and gamma (γ) as an adjustable parameter controlling the magnitude of the fluctuations, $\langle \xi(t) \rangle = 0$ pA, $\langle \xi(t) \xi(t') \rangle = \gamma \delta(t - t')$.

The remaining functions in the model, including those representing the voltage-dependence of channel kinetics, were

$$x_{\infty}(V) = 1 / (1 + \exp((V - \theta_x)/\sigma_x))$$

$$\tau_x(V) = \tau_{x-\max} / \cosh((V - \theta_x) / (2 \sigma_x))$$

$$\Phi(\text{Na}) = \text{Na}^3 / (\text{Na}^3 + k_{\text{Na}}^3).$$

Model parameters were set to the following values, unless otherwise specified: $C = 45$ pF, $g_{\text{leak}} = 3$ nS, $E_L = -60$ mV, $g_{\text{Na}} = 150$ nS, $E_{\text{Na}} = 85$ mV, $g_K = 30$ nS, $E_K = -75$ mV, $g_{\text{CAN}} = 4$ nS, $E_{\text{CAN}} = 0$ mV, $g_{\text{syn}} = 2.5$ nS, $E_{\text{syn}} = 0$ mV, $\theta_m = -36$ mV, $\sigma_m = -8.5$ mV, $\tau_{m-\max} = 1$ ms, $\theta_n = -30$ mV, $\sigma_n = 5$ mV, $\tau_{h-\max} = 15$ ms, $\theta_h = -30$ mV, $\sigma_h = -5$ mV, $\tau_{n-\max} = 30$ ms, $\theta_s = 15$ mV, $\sigma_s = -3$ mV, $\tau_s = 15$ ms, $k_{\text{Ca}} = 22.5$ ms⁻¹, $k_{\text{CAN}} = 0.9$, $\sigma_{\text{CAN}} = -0.05$ mV, $k_s = 1$, $k_{\text{ip3}} = 1200$ $\mu\text{M ms}^{-1}$, $r_{\text{pump}} = 200$ pA, $k_{\text{Na}} = 10$ mM, $\text{Ca}_{\infty} = 0.05$ μM , $\text{Na}_{\infty} = 5$ mM, $\varepsilon = 0.0007$, $\alpha = 6.6 \times 10^{-5}$ mM pA⁻¹ms⁻¹, $\gamma = 0$ pA². We scaled the synaptic parameters g_{syn} and k_{ip3} down by a factor of 5 for 200-neuron simulations.

Calcium currents and synaptic inputs. Voltage-dependent Ca²⁺ currents are present in the preBötC and contribute to burst generation (2, 3) but were omitted from the current-balance equation for the following reasons. Ca²⁺ currents are predominantly activated by AMPA receptor-mediated depolarization during inspiratory burst generation (4), so instead of deriving an explicit Ca²⁺ current that would respond specifically to synaptic depolarization, we coupled the synaptic variable s to the Ca²⁺ equation directly. The synaptic variable s therefore represents both ionotropic and metabotropic glutamatergic receptor (mGluR) activation: AMPA receptors cause Ca²⁺ changes via depolarization, whereas group I mGluRs elevate intracellular Ca²⁺ by evoking intracellular release from stores, which both contribute to Ca²⁺ increases that couple to and activate I_{CAN} during inspiratory burst generation (4). The variable s also appears in I_{syn} in the standard way, and the parameters k_{ip3} and g_{syn} scale its relative contributions to Ca²⁺ dynamics and to I_{syn} , respectively.

Parameter values. Synaptic coupling affects Ca^{2+} accumulation and I_{CAN} activation, which collectively underlie burst generation. Consequently, burst duration and the interburst interval depend on the parameter values for g_{CAN} , k_{IP3} , g_{syn} , Γ_{pump} , and k_{Ca} when I_{pump} is present. Rhythmic solutions in the model persisted over substantial variation in these parameters from nominal baseline values. Regulating these key parameters supported a wide range of interburst intervals and burst durations (Fig. S2). This robustness and capacity to generate bursts with varying characteristics support the relevance of this model to respiratory rhythmogenesis, where modulation and regulation of the basic rhythm is essential for the physiology of respiration (5-8).

Synaptic delay. In simulations of the self-coupled cell, we introduced a latency T_{delay} , with $s_{\infty} = 1 / (1 + \exp((V(t - T_{\text{delay}}) - \theta_s)/\sigma_s))$, to mimic the delay in synaptic coupling inherently present in the multi-cell simulations due to spike asynchrony (9). We found qualitatively similar dynamics for T_{delay} from 0 up to 19 ms. In general, we used $T_{\text{delay}} = 6$ ms to generate time courses in the self-coupled case (Figs. 3,4,S2,S3).

Activity-dependent outward currents. In some cases, I_{pump} was replaced by alternative net outward currents, as follows (see Figs. 4,S3,S4).

1) Slowly-activating M-like K^+ current (I_{M}):

$$I_{\text{M}}(V, n_{\text{M}}) = g_{\text{M}} n_{\text{M}} (V - E_{\text{K}})$$

$$dn_{\text{M}}/dt = (n_{\text{M}\infty}(V) - n_{\text{M}}) / \tau_{\text{nM}}(V)$$

$$\tau_{\text{nM}}(V) = \tau_{\text{nM-max}} / \cosh((V - \theta_{\text{n}}) / (2 \sigma_{\text{n}}))$$

where $E_{\text{L}} = -57.7$ mV, $\gamma = 500$ pA², $g_{\text{M}} = 2$ nS, $\tau_{\text{nM-max}} = 1000$ ms and $\theta_{\text{n}} = -30$ mV, $\sigma_{\text{n}} = -5$ mV, as above, was based on a delayed-rectifier current in a preBötC model by Butera and colleagues (10, 11) but with slower kinetics.

2) Ca^{2+} -dependent K^+ current ($I_{\text{K-Ca}}$):

$$I_{\text{K-Ca}}(V, z_{\text{K-Ca}}) = g_{\text{K-Ca}} z_{\text{K-Ca}}^2 (V - E_{\text{K}})$$

$$dz_{\text{K-Ca}}/dt = (z_{\infty}(\text{Ca}) - z_{\text{K-Ca}}) / \tau_{\text{K-Ca}}$$

$$z_{\infty}(\text{Ca}) = 1 / (1 + (k_{\text{K-Ca}}/\text{Ca})^4)$$

where $E_L = -60.15$ mV, $\gamma = 500$ pA², $g_{K-Ca} = 3$ nS, $k_{K-Ca} = 0.9$ μ M, $\tau_{K-Ca} = 100$ ms, was based on biophysical modeling and general properties of I_{K-Ca} (12, 13).

3) Persistent Na⁺ current (I_{Na-P}) with slow inactivation:

$$I_{Na-P}(V, h_{Na-P}) = g_{NaP} m_{NaP\infty}(V) h_{Na-P} (V - E_{Na})$$

$$dh_{Na-P}/dt = (h_{Na-P\infty}(V) - h_{Na-P}) / \tau_{Na-P}$$

$$m_{Na-P\infty}(V) = 1 / (1 + \exp((V - \theta_{m-Na-P})/\sigma_{m-Na-P}))$$

$$h_{Na-P\infty}(V) = 1 / (1 + \exp((V - \theta_{h-Na-P})/\sigma_{h-Na-P}))$$

where $E_L = -61.25$ mV, $\gamma = 500$ pA², $g_{Na-P} = 1$ nS, $\theta_{m-Na-P} = -40$ mV, $\sigma_{mNaP} = -6$ mV, $\theta_{hNaP} = -48$ mV, $\sigma_{h-Na-P} = 6$ mV, and $\tau_{Na-P} = 1000$ ms, was based on the preBötC model by Butera et al. (10, 11).

When any of these outward currents was included, rhythmic activity in the model was qualitatively similar to that generated with I_{pump} (Figs. 3,4,S3). In particular, the self-coupled single-cell model and the two-cell model produced repetitive activity patterns featuring spontaneous spiking prior to the burst, followed immediately by a higher-frequency spike rate during the burst, spike attenuation within each burst (i.e., depolarization block), and a transient quiescent period before low-rate pre-burst spiking resumed. In all cases except the model with I_{Na-P} , both Ca²⁺ and a slow activation variable associated with the additional outward current accumulated during the burst. In the case of I_{Na-P} , Ca²⁺ accumulation was accompanied by a decrease in the gating variable h_{NaP} , which caused I_{Na-P} inactivation and functioned analogously to activation of an outward current (Fig. 4C,S3D). No matter which current was used, the rhythmic activity depended crucially on synaptic interactions and I_{CAN} . The only significant difference observed among the currents considered was that with I_{pump} , bursting could occur even without including synaptic transmission latency, while the other currents required a small latency (T_{delay} above a small positive lower bound) to cause tonic spiking to ramp up the synaptic response, thereby promoting sufficient recurrent excitation to elicit bursting (Fig. S3B-D).

We also simulated 200-neuron networks (Fig. S4). For the I_{pump} simulations, E_L was drawn from a Gaussian (normal) distribution with a mean of -60.0 and standard deviation of 1 mV (i.e., -60.0 ± 1 mV). For the other net outward currents, the E_L distribution had the following means and standard deviations: -57.3 ± 1 mV (Fig. S4B), -60.1 ± 1 mV (Fig. S4C), and $-61. \pm 1$ mV (Fig. S4D). The differences in means were selected to compensate for the different tonic components of the outward currents (I_M , I_{K-Ca} , and I_{NaP} inactivation) that affected baseline membrane potentials in each case.

Caveats and limitations. Our preBötC-like model neuron lacks transient A-type K^+ current (I_A) and hyperpolarization-activated mixed cationic current (I_h). I_A influences the recruitment pattern of preBötC neurons during recurrent excitation in the 300-1000 ms prior to the inspiratory burst (14). I_h modulates respiratory frequency *in vitro* and influences the baseline membrane potential trajectory (15, 16). Our model predicts that the omitted currents may not be essential for burst generation, but rather influence inspiratory burst characteristics and regulate respiratory frequency (2-5).

The most problematic simplification may be that the model is limited to a single isopotential compartment in which synaptic integration and spike generation occur explicitly in the same location. Recent reports suggest that synaptic recruitment of I_{CAN} may occur principally in the dendrites (4, 17, 18), which is likely the primary site of Na/K pump activity, whereas most sodium influx via I_{Na} occurs in the axon hillock. These observations are reflected in our Na^+ equation, which includes dendritic Na^+ currents yet neglects I_{Na} , since diffusion of sodium from the axon hillock to the dendrites would be minimal; our model also generates qualitatively similar bursting when up to 10% of the Na^+ influx via I_{Na} is included in the dNa/dt equation. Despite this complication, maintaining a single-compartment model has a number of important advantages: i) the single-compartment construction facilitates bifurcation analyses; ii) because its morphology is not specific to preBötC neurons, the model provides a more general template to begin exploring group-pacemaker activity by modifying the model to match other neuronal

phenotypes found in CPGs in other parts of the brain stem and spinal cord; and iii) the simplicity of the present model elucidates the general principles underlying a novel group pacemaker mechanism for rhythmogenesis applicable to CPGs, yet retains its connection to inspiratory rhythm generation in the preBötC, which motivated our search for new rhythmogenic mechanisms.

References

1. Li YX, Bertram R, & Rinzel J (1996) Modeling N-methyl-D-aspartate-induced bursting in dopamine neurons. *Neuroscience* 71(2):397-410.
2. Elsen FP & Ramirez JM (1998) Calcium currents of rhythmic neurons recorded in the isolated respiratory network of neonatal mice. *J Neurosci* 18(24):10652-10662.
3. Onimaru H, Ballanyi K, & Homma I (2003) Contribution of Ca²⁺-dependent conductances to membrane potential fluctuations of medullary respiratory neurons of newborn rats in vitro. *J Physiol* 552(Pt 3):727-741.
4. Pace RW & Del Negro CA (2008) AMPA and metabotropic glutamate receptors cooperatively generate inspiratory-like depolarization in mouse respiratory neurons in vitro. *Eur J Neurosci* **In press**.
5. Feldman JL & Del Negro CA (2006) Looking for inspiration: new perspectives on respiratory rhythm. *Nat Rev Neurosci* 7(3):232-242.
6. Feldman JL, Mitchell GS, & Nattie EE (2003) Breathing: Rhythmicity, Plasticity, Chemosensitivity. *Annu Rev Neurosci* 26:239-266.
7. Richter DW & Spyer KM (2001) Studying rhythmogenesis of breathing: comparison of in vivo and in vitro models. *Trends Neurosci* 24(8):464-472.
8. Bianchi AL, Denavit-Saubie M, & Champagnat J (1995) Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. *Physiol Rev* 75(1):1-45.
9. Best J, Borisjuk A, Rubin J, Terman D, & Wechselberger M (2005) The dynamic range of bursting in a model respiratory pacemaker network. *SIAM J App. Dyn. Sys.* 4(4):1107-1139.
10. Butera RJ, Jr., Rinzel J, & Smith JC (1999) Models of respiratory rhythm generation in the pre-Bötzinger complex. I. Bursting pacemaker neurons. *J Neurophysiol* 82(1):382-397.
11. Butera RJ, Jr., Rinzel J, & Smith JC (1999) Models of respiratory rhythm generation in the pre-Bötzinger complex. II. Populations Of coupled pacemaker neurons. *J Neurophysiol* 82(1):398-415.
12. Engel J, Schultens HA, & Schild D (1999) Small conductance potassium channels cause an activity-dependent spike frequency adaptation and make the transfer function of neurons logarithmic. *Biophys J* 76(3):1310-1319.
13. Stocker M (2004) Ca²⁺-activated K⁺ channels: molecular determinants and function of the SK family. *Nat Rev Neurosci* 5(10):758-770.
14. Hayes JA, Mendenhall JL, Brush BR, & Del Negro CA (2008) 4-Aminopyridine-sensitive outward currents in preBötzinger complex neurons influence respiratory rhythm generation in neonatal mice. *J Physiol* 586(7):1921-1936.

15. Mironov SL, Langohr K, & Richter DW (2000) Hyperpolarization-activated current, I_h , in inspiratory brainstem neurons and its inhibition by hypoxia. *Eur J Neurosci* 12(2):520-526.
16. Thoby-Brisson M, Telgkamp P, & Ramirez JM (2000) The role of the hyperpolarization-activated current in modulating rhythmic activity in the isolated respiratory network of mice. *J Neurosci* 20(8):2994-3005.
17. Mironov SL (2008) Metabotropic glutamate receptors activate dendritic calcium waves and TRPM channels which drive rhythmic respiratory patterns in mice. *J Physiol* 586(9):2277-2291.
18. Morgado-Valle C, Beltran-Parrazal L, DiFranco M, Vergara JL, & Feldman JL (2008) Somatic Ca^{2+} transients do not contribute to inspiratory drive in preBötzinger Complex neurons. *J Physiol* 586(Pt 18):4531-4540.