SI Appendix

Derivation of the model

The current-balance equation took the form

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

where

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

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

$$dCa/dt = \varepsilon\,(s\, k_{IP3} - k_{Ca}\,(Ca - Ca_\infty))$$

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

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):

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

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

$$I_K(V,n) = g_K\, n^4\,(V - E_K)$$

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

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

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

$$I_{pump}(Na) = r_{pump}\,(\Phi(Na) - \Phi(Na_\infty))$$

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

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, $\langle\xi(t)\,\xi(t')\rangle = \gamma\cdot\delta(t - t')$.

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

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

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

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

Model parameters were set to the following values, unless otherwise specified: C = 45 pF, g_{leak} = 3 nS, E_L = –60 mV, g_{Na} = 150 nS, E_{Na} = 85 mV, g_K = 30 nS, E_K = -75 mV, g_{CAN} = 4 nS, E_{CAN} = 0 mV, g_{syn} = 2.5 nS, E_{syn} = 0 mV, θ_m = -36 mV, σ_m = -8.5 mV, $\tau_{m\text{-}max}$ = 1 ms, θ_h = -30 mV, σ_h = 5 mV, $\tau_{h\text{-}max}$ = 15 ms, θ_n = -30 mV, σ_n = -5 mV, $\tau_{n\text{-}max}$ = 30 ms, θ_s = 15 mV, σ_s = -3 mV, τ_s = 15 ms, k_{Ca} = 22.5 ms^{-1}, k_{CAN} = 0.9, σ_{CAN} = -0.05 mV, k_s = 1, k_{ip3} = 1200 μM ms^{-1}, r_{pump} = 200 pA, k_{Na} = 10 mM, Ca_∞ = 0.05 μM, Na_∞ = 5 mM, ε = 0.0007, α = 6.6x10^{-5} mM $pA^{-1}ms^{-1}$, γ = 0 pA^2. 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 Ca^{2+} 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. Ca^{2+} currents are predominantly activated by AMPA receptor-mediated depolarization during inspiratory burst generation (4), so instead of deriving an explicit Ca^{2+} current that would respond specifically to synaptic depolarization, we coupled the synaptic variable *s* to the Ca^{2+} equation directly. The synaptic variable *s* therefore represents both ionotropic and metabotropic glutamatergic receptor (mGluR) activation: AMPA receptors cause Ca^{2+} changes via depolarization, whereas group I mGluRs elevate intracellular Ca^{2+} by evoking intracellular release from stores, which both contribute to Ca^{2+} 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 Ca^{2+} 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} , r_{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 $s_\infty = 1 / (1 + \exp((V(t - T_{delay}) - \theta_s)/\sigma_s))$, 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_{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):

$$I_M(V, n_M) = g_M n_M (V - E_K)$$

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

$$\tau_{nM}(V) = \tau_{nM\text{-}max} / \cosh((V - \theta_n) / (2 \sigma_n))$$

where E_L = –57.7 mV, γ = ~~500~~ pA^2, g_M = 2 nS, $\tau_{nM\text{-}max}$ = 1000 ms and θ_n = -30 mV, σ_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 ($I_{K\text{-}Ca}$):

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

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

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

where E_L = –60.15 mV, γ = 500 pA^2, $g_{K\text{-}Ca}$ = 3 nS, $k_{K\text{-}Ca}$ = 0.9 μM, $\tau_{K\text{-}Ca}$ = 100 ms, was based on biophysical modeling and general properties of $I_{K\text{-}Ca}$ (12, 13).

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

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

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

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

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

where E_L = –61.25 mV, γ = 500 pA^2, $g_{Na\text{-}P}$ = 1 nS, $\theta_{m\text{-}Na\text{-}P}$ = –40 mV, σ_{mNaP} = –6 mV, θ_{hNaP} = –48 mV, $\sigma_{h\text{-}Na\text{-}P}$ = 6 mV, and $\tau_{Na\text{-}P}$ = 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 $I_{Na\text{-}P}$, both Ca^{2+} and a slow activation variable associated with the additional outward current accumulated during the burst. In the case of $I_{Na\text{-}P}$, Ca^{2+} accumulation was accompanied by a decrease in the gating variable h_{NaP}, which caused $I_{Na\text{-}P}$ 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.±1 mV (Fig. S4D). The differences in means were selected to compensate for the different tonic components of the outward currents (I_M, $I_{K\text{-}Ca}$, 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.

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