

MODELLING RESPIRATORY RHYTHMOGENESIS: FOCUS ON PHASE SWITCHING MECHANISMS

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1. INTRODUCTION

It has been established that the normal respiratory pattern (“eupnea”) in mammals is generated in the lower brainstem^{1,2} and may involve several medullary and pontine regions. Although some researchers suggest that a smaller region within the medulla (e.g., the pre-Bötzinger Complex (pre-BötC)) may be sufficient for the respiratory rhythm generation³⁻⁵, the eupnoic respiratory rhythm (as well as apneustic breathing) has never been reproduced in reduced medullary preparations without the pons. At the same time, the specific ponto-medullary interactions related to genesis, shaping and control of the respiratory pattern have not been well characterized so far. Here we present a preliminary computational model of the ponto-medullary respiratory network that is considered a basis for the future interactive modeling-experimental studies. The model has been developed using a series of initial assumptions. Specifically, we have suggested that, under normal conditions *in vivo*, the eupnoic respiratory rhythm is generated by a ponto-medullary network. Hence, although the pre-BötC is a necessary part of this network, the intrinsic oscillations in this region are suppressed during eupnoea by the ponto-medullary interactions. These endogenous oscillations, however, may be released under some specific conditions, e.g., *in vitro*, because of the lack of the pons, or during hypoxia *in vivo*⁶. We have also assumed that the medullary part of the respiratory network contains special neural circuits performing the respiratory phase switching. Moreover, these circuits are also a target for pulmonary feedback and inputs from the pons and from major afferent nerves, which all use the same medullary switching circuits to regulate the timing of phase transitions and modulate the respiratory motor pattern⁷.

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2. MODEL OF THE PONTO-MEDULLARY RESPIRATORY NETWORK

The model (Figure 1) contains several interacting populations of respiratory neurons characterized in the rostroventrolateral medulla and pons *in vivo*, including the rostral ventral respiratory group, (rVRG); pre-BötC, and Bötzing Complex (BötC). The following neural populations were included in the model: ramp-I, and late-I (both in rVRG); early-I and pre-I (in pre-BötC); post-I and aug-E (in BötC); I-modulated, E-modulated and two distinct IE-modulated, IE₁ and IE₂, (in the rostral pons, rPons, that was supposed to include the dorsolateral (NPMB/KF) and ventrolateral pontine regions); tonic (in the caudal pons, cPons). Network interactions within the rVRG (i.e., between the ramp-I, early-I, and late-I populations) and between rVRG and BötC populations define the basic circuitry for the inspiratory off-switching (IOS) mechanism with the late-I population playing the key role in the inspiratory off-switching^{2,7-9}. Interactions among the rVRG populations, post-I population and pre-I population of pre-BötC define the basic circuitry for the expiratory off-switching (EOS) mechanism with the pre-I population performing inspiratory on-switching function⁸. Importantly, IOS and EOS mechanisms operate under control of both pontine input and pulmonary feedback which both are excitatory to the late-I, ramp-I and post-I population^{7,9}.

The model generates the stable “eupnoeic” respiratory rhythm with “augmenting” phrenic discharges and demonstrates realistic firing patterns and membrane potential trajectories of individual respiratory neurons (Figure 2A). In addition, the model reproduces a number of known physiological phenomena. For example, the disconnection of vagal feedback produces an increase in the amplitude and duration of phrenic discharges (Figure 2B), whereas a moderate vagal stimulation shortens inspiration and prolongs expiration. Both these effects are consistent with the Hering-Breuer (HB) reflex^{2,10}. A relatively strong stimulation of the vagus produces “postinspiratory apnea”^{11,12} (Figure 2C1). Short stimulation of the vagus delivered during inspiration can terminate the inspiratory phase, and the threshold for inspiratory termination reduces during inspiration^{2,10,13} (Figure 2C2). Stimuli delivered during post-inspiration prolong expiration^{10,12}, whereas the late part of expiration is insensitive to vagal stimulation^{10,14} (Figure 2C3). Short stimulation of pontine IE₁ population terminates inspiration^{1,2,15} (Figure 3B). The continuous pontine stimulation shortens inspiration and prolongs expiration¹⁶ (Figure 3A,C), which suggests the existence of ponto-medullary (PM) reflexes. The removal of rPons converts the eupnoeic pattern to apneusis^{1,2,6,15,17} (Figure 3D2). Short vagal stimulation may terminate the apneustic burst; (Figure 3E); continuous stimulation shortens the apneustic inspiratory discharges¹⁶ (Figure 3F). The complete removal of the pons releases a pacemaker-driven rhythm in the pre-BötC and converts the eupnoeic pattern to a gasping-like (*in vitro*-like) “decrementing” pattern^{1,2,6,18} (Figure 3D3). The latter is characterized by significant reduction of postinspiratory activity and shortening the delay between hypoglossal and phrenic discharges^{6,19} (compare Figure 3B3 with Figures 1 and 3B1).

3. CONCLUSIONS AND MODEL PREDICTIONS

Our modeling studies support the following suggestions: (1) The eupnoeic respiratory rhythm is generated by a ponto-medullary network mechanism; (2) The pontine inputs suppress the intrinsic, pacemaker-driven oscillations in the pre-BötC

(specifically via the activation of post-I neurons that intermediate pontine inhibition of the pre-I population and through a general excitatory drive to the medulla); (3) rPons provides inspiration-inhibitory and expiration-facilitatory PM reflexes that are independent on HB reflex and partly suppressed by pulmonary feedback; (4) Both HB and PM reflexes operate through the same medullary phase switching circuits.

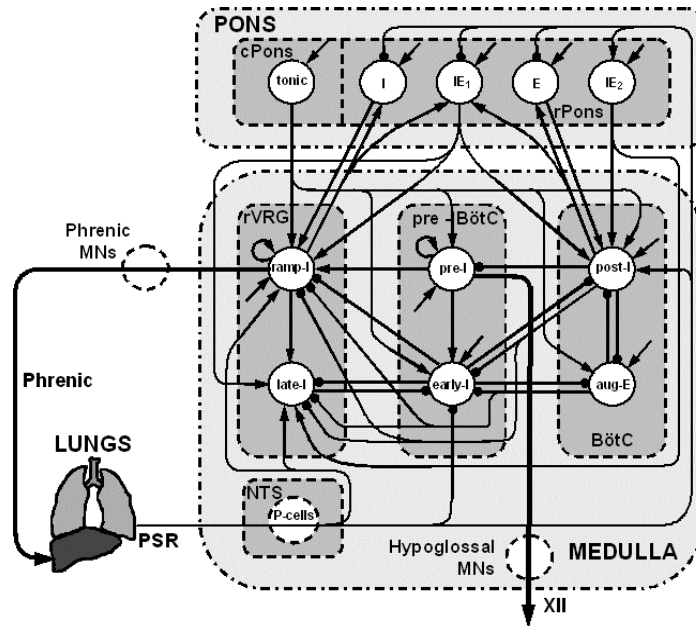


Figure 1. Schematic of the model of the ponto-medullary respiratory network. The white circles represent populations of different respiratory neurons (the populations of phrenic and hypoglossal motoneurons and NTS pump (P) cells are not present in the current model). Each population consisted of 50 neurons. All neurons were modeled in the Hodgkin-Huxley style and incorporated biophysical properties and channel kinetics characterized in respiratory neurons *in vitro*. Specifically, the fast sodium currents in all neurons and the persistent sodium currents in the pre-I population were described using recent experimental data on rat RVLM neurons²⁰; the high-threshold and low-threshold calcium currents were described using data of Elsen and Ramirez²¹; intracellular calcium dynamic was based on data of Frermann et al.²²; other cellular data were accepted from the previous models²³⁻²⁴. Neuronal parameters were randomly distributed within each neural population. All populations received tonic excitatory drive. The excitatory (arrows) and inhibitory (small black circles) connectivities among the neural populations within the medulla were assigned using the existing direct or indirect physiological data. Some additional connectivities were assigned based on the suggested IOS and EOS mechanisms. Reciprocal excitatory connections were assigned between the medullary ramp-I and the pontine I and IE₁ populations, and between the medullary post-I and the pontine IE₁ and E populations. These connections provided I, IE or E modulation of activity of the corresponding pontine populations. We also assumed that reticular neurons from caudal pons (tonic population) provided an additional excitatory tonic drive to the medullary respiratory populations. Simplified models of the lungs and slowly adapting pulmonary stretch receptors (PCR) were included in the model to provide pulmonary feedback to the respiratory network. The pulmonary feedback controlled activity of the key neural population involved in IOS and EOS circuits (see text for details) and hence provided regulation of the duration of the respiratory phases and HB reflex. In addition, this feedback inhibited the activity of the pontine neural populations that received excitation from the medullary populations (I, IE₁, E) and hence suppressed the pontine control of the respiratory pattern. At the same time one pontine population (IE₂) received excitation from the feedback and contributed to HB reflex. Integrated activity of ramp-I and pre-I population were considered as, respectively, phrenic and hypoglossal output of the model.

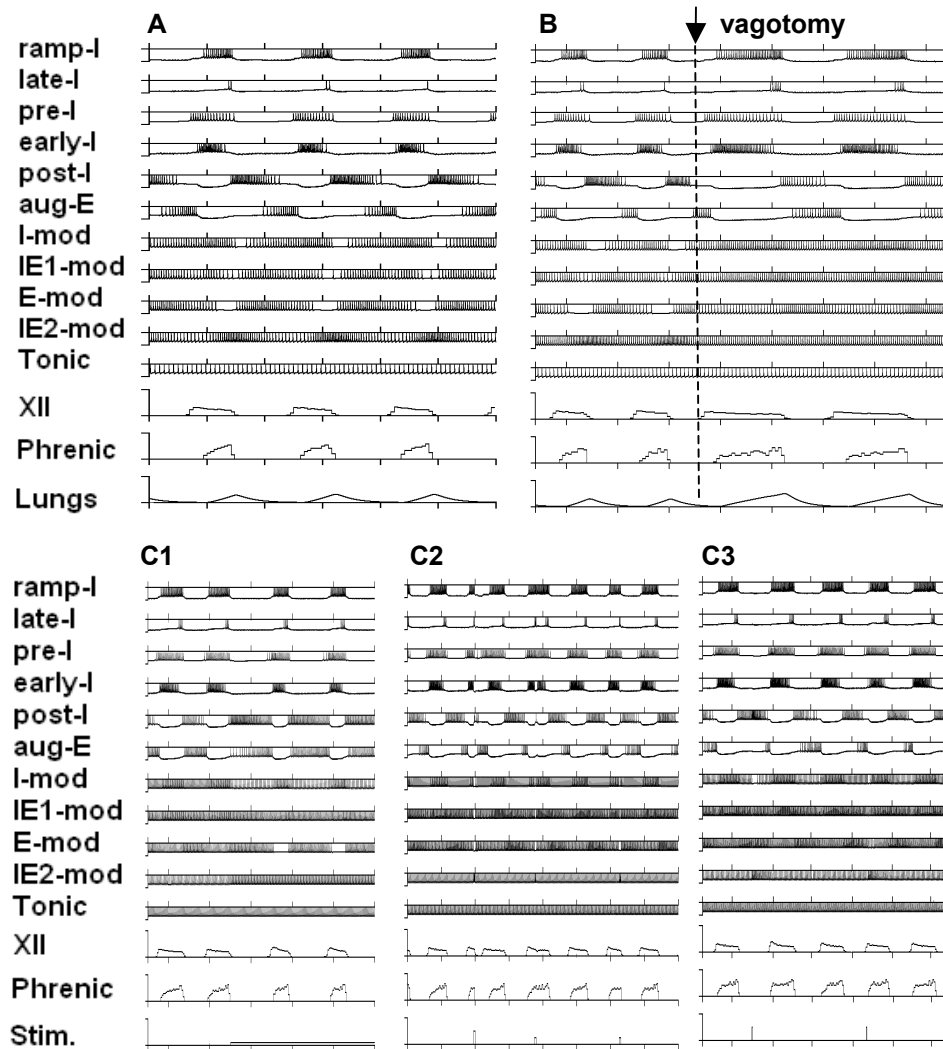


Figure 2. Model performance. Activity of each neural population is represented by the activity (trace of the membrane potential) of one representative neuron from the population. Tick marks on the time axes correspond to seconds. **A.** The eupnoeic respiratory pattern generated by the model under the normal conditions. **B.** The effect of "vagotomy" (disconnection of vagal feedback). Note an increase in the amplitude and duration of phrenic discharges. **C1-C3:** The effects of various vagus nerve stimulations. A relatively strong continuous stimulation of the vagus produces "postinspiratory apnea" (not shown). **C1.** A moderate continuous vagal stimulation shortens inspiration and prolongs expiration reflecting the Hering-Breuer reflex. **C2.** Application of short stimuli of the vagus during inspiration demonstrates a hyperbolic decrease of the threshold for inspiratory termination during the inspiratory phase. The first, high-amplitude stimulus applied at the beginning of inspiration terminated inspiration. The second stimulus with smaller amplitude was applied at the same phase, but could not terminate inspiration. The third stimulus had the same amplitude as the second one, but being delivered at a later phase of inspiration terminated current inspiration. **C3.** Short vagal stimulation delivered in the middle of expiration (first stimulus) prolongs expiration. The same stimulus delivered at the end of expiration has no effect on the duration of expiration (demonstrates an "insensitive period" at the end of expiration).

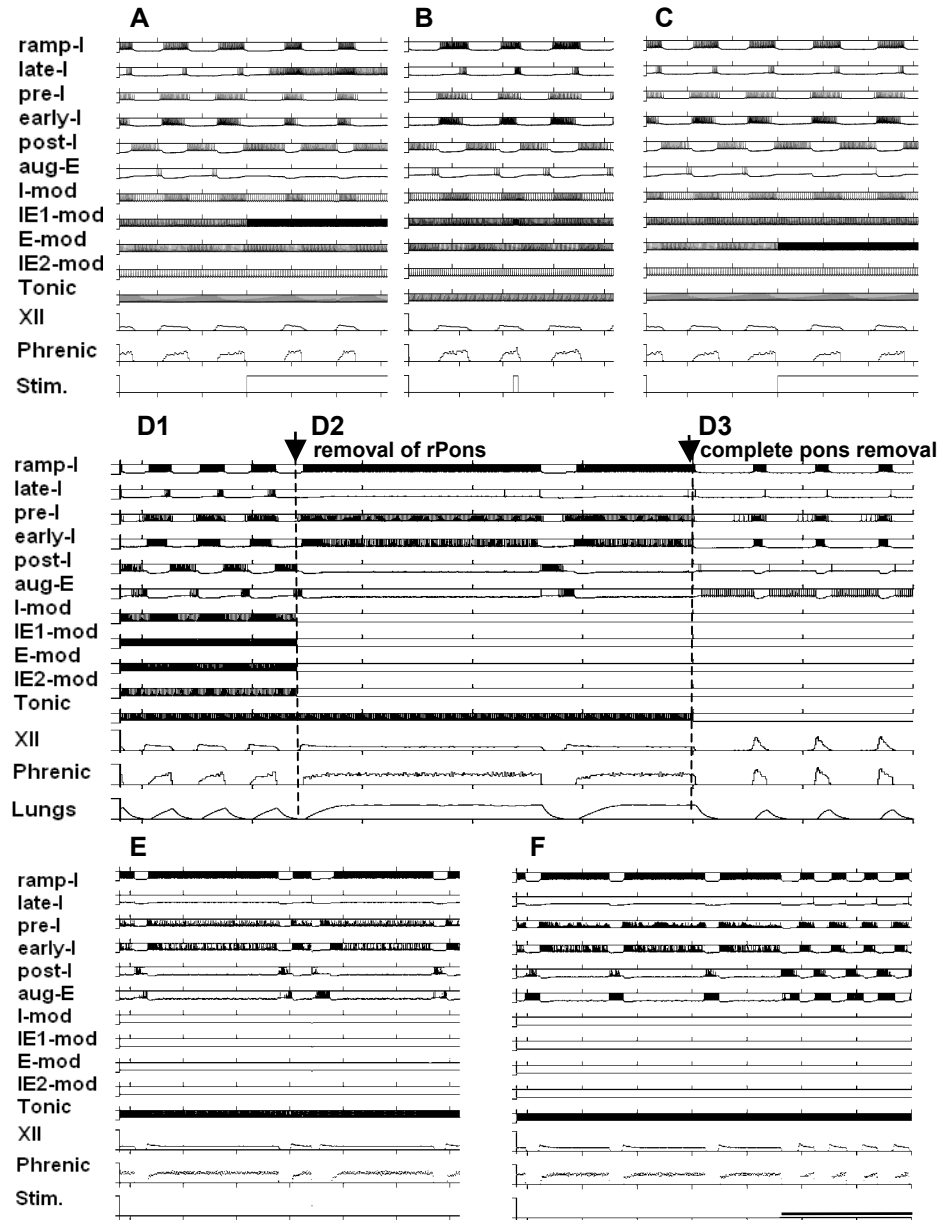


Figure 3. Model performance: the role of the pons (vagal feedback is off). **A.** Continuous stimulation of the pontine IE₁ population shortens inspiration and prolongs expiration. **B.** Short stimulation of IE₁ population terminates inspiration. **C.** Continuous stimulation of the pontine E(-mod) population prolongs expiration. **D1-D3:** Simulation of pontine lesions. **D1.** The eupnoeic respiratory pattern (the pons is intact). **D2.** Removal of rPons (see Figure 1) converts the eupnoeic pattern to apneusis. **D3.** The following removal of cPons releases an intrinsic pacemaker-driven rhythm in the pre-I population of pre-BötC and converts apneusis to a gasping-like (*in vitro*-like) “decrementing” discharges. **E and F:** Apneusis (rPons is off). **E.** Short vagal stimulation terminates apneustic inspiration. **F.** Continuous vagal stimulation shortens the apneustic inspiratory discharges.

4. ACKNOWLEDGEMENTS

This study was supported by NSF (0091942) and NIH (NS046062-02 and HL072415-01) grants to I. A. Rybak.

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