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Diving into the mammalian swamp of respiratory rhythm generation with the bullfrog

Mufaddal I. Baghdadwala[a], Maryana Duchcherer[a], William M. Trask[a], Paul A. Gray[b], Richard J.A. Wilson[a,*]

[a] Hotchkiss Brain Institute and Alberta Children's Hospital Research Institute, Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[b] Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, USA

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ABSTRACT

All vertebrates produce some form of respiratory rhythm, whether to pump water over gills or ventilate lungs. Yet despite the critical importance of ventilation for survival, the architecture of the respiratory central pattern generator has not been resolved. In frogs and mammals, there is increasing evidence for multiple burst-generating regions in the ventral respiratory group. These regions work together to produce the respiratory rhythm. However, each region appears to be pivotally important to a different phase of the motor act. Regions also exhibit differing rhythmogenic capabilities when isolated and have different CO_2 sensitivity and pharmacological profiles. Interestingly, in both frogs and rats the regions with the most robust rhythmogenic capabilities when isolated are located in rhombomeres 7/8. In addition, rhombomeres 4/5 in both clades are critical for controlling phases of the motor pattern most strongly modulated by CO_2 (expiration in mammals, and recruitment of lung bursts in frogs). These key signatures may indicate that these cell clusters arose in a common ancestor at least 400 million years ago.

1. Introduction

Breathing is perhaps the most vital, robust and rhythmic behavior in vertebrates, but despite intense effort, the architecture of the neuronal controller is not resolved (Feldman and Del Negro, 2006; Gargaglioni and Milsom, 2007). In mammals, on which most studies have focused, there is good evidence that the ventral respiratory column in the brainstem plays a critical role in producing the respiratory motor pattern but the functional organization within this column is still debated (McCrimmon et al., 2000; Onimaru et al., 2004). At the heart of this debate is a key question: is the rhythm generator restricted to a single brainstem kernel (*i.e.*, a small anatomical cluster of cells), several kernels or distributed widely throughout the ventral respiratory column (Abdala et al., 2009; Lindsey et al., 2012; Smith et al., 2007)?

The neurobiology of the respiratory controller has only been investigated in a handful of non-mammalian species and thus insight from comparative studies, a mainstay of advance in other fields of neuroscience, is limited (Gargaglioni and Milsom, 2007). However, recent studies from select species of lampreys, frogs, turtles and birds are adding a new richness to our knowledge and may hold promise to understanding how the breathing circuit is organized and evolved (Bass and Baker, 1997; Cinelli et al., 2014; Johnson et al., 2007; Wilson et al., 2002). In this review, we will focus on respiratory rhythm generation in the frog, comparing discoveries in the frog with advances in mammal research.

2. Rhythm, phase and burst-shape

In all vertebrates, each breath consists of a complex coordinated act involving diverse muscle recruitment tuned to ever changing metabolic, biomechanical and behavioral needs. For the purpose of discussion, we will divide this act into 3 features (depicted in Fig. 1): (1) rhythm generation, the act of producing a sequence of breaths; (2) phase generation, the act of recruiting motor neurons at different phases during a single breath; and (3) burst-shape generation, the act of shaping the burst discharge to single muscles. Phase and burst shape generation can be described as pattern formation. Within the respiratory system, these functions operate over

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* Corresponding author at: Dept. of Physiology and Pharmacology, Hotchkiss Brain Institute Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada.
E-mail address: wilsonr@ucalgary.ca (R.J.A. Wilson).

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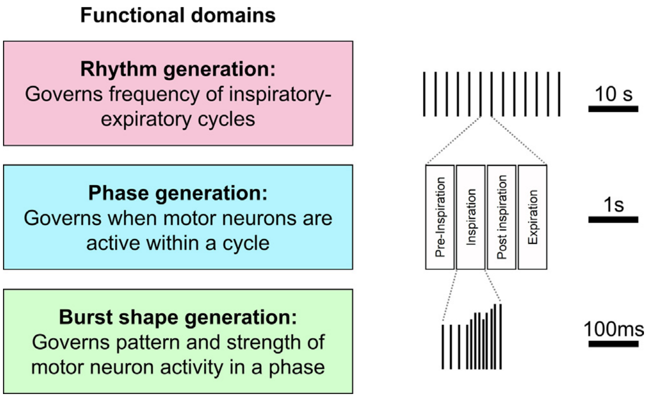

Fig. 1. Motor network involved in generating rhythmic behavior must coordinate 3 functional domains: rhythm (cycles time^{-1}), phase generation (onset and offset of motor neuron pools) and burst shape generation (temporal profile of action potential barrage within each pool). Phase generation and burst shape generation has traditionally been lumped together and referred to as "pattern generation". The extent to which these functional domains are anatomically separated within the CNS is a key question in respiratory neurobiology.

different time scales: rhythm generation occurs over seconds or more, phase generation occurs within a second and burst-shape generation occurs within hundreds of milliseconds. Having varying time domains does not necessitate these functions are produced by different circuit elements. Indeed, in many invertebrates—in which exploration of rhythm generating networks has been assisted by large, identifiable and robust cells—rhythm, phase and burst-shape features are usually produced by the same network of neurons (Bal et al., 1988; Cinelli et al., 2014; Guertin, 2009; Kristan et al., 2005). In these systems, all 3 of these features are commonly shaped by phasic sensory input, but the rudiments of most rhythmic motor patterns are produced by a single ensemble of neurons, the central pattern generator (defined formally as a circuit capable of producing phasic motor output without phasic sensory input). The concept of a central pattern generator is equally current for the spinal locomotor networks of mammals, with researchers reluctant to parcel out rhythm, phase and burst-shape generation to sub-networks of neurons, and despite intense effort and early indications to the contrary, no single rhythm generating kernel or cell type has been definitely identified; all three functions—rhythm, phase and burst-shape generation—appear in large part to be served by the circuit ensemble (Guertin, 2009; Hägglund et al., 2013). The locomotor ensemble may even include motor neurons, with increasing evidence that motor neurons are involved in rhythmicity, phasing and patterning of muscle recruitment (Manuel et al., 2012; Menelaou and McLean, 2012; Roberts et al., 1998).

Discordant to our understanding of most other systems, respiration rhythm and pattern generation have historically been considered as produced by a hierarchical network, wherein rhythm generation (the master layer assumed to reside in the rostral half of the ventral respiratory group) instructs the pattern-forming network (assumed to mainly reside in the caudal ventral respiratory group and/or motor neurons), with scant reciprocity (Feldman et al., 1990; Ramirez and Richter, 1996; Richter et al., 1992; Smith et al., 2007) (Fig. 2). This view gained steam in the 1980s after the momentous discovery of the preBötzinger complex (preBötC), a cluster of ~1000 neurons located on either side of the ventral lateral medulla, capable of independent rhythmogenesis and necessary for inspiratory activity (Gray et al., 2010; Smith et al., 1991). While the preBötC concept, *i.e.*, that of a single respiratory oscillator serving as the nodus vital of the respiratory rhythm, has survived after almost three decades of intense experimental challenge *in vitro* and *in vivo*, several lines of evidence suggest that rhythm generation may be more complex and more distributed:

1. In the superfused hindbrain of embryonic rodents, primordial respiratory bursts appear to originate rostral of the preBötC, in the pre-I area/para-facial respiratory group (pFRG) (Onimaru and Homma, 2003; Onimaru et al., 2004).
2. Imaging neuronal activity on the surface of the ventral brainstem of superfused neonatal opossum suggests respiratory bursts recorded in cranial and phrenic nerves originate randomly within the brainstem (Zou, 1994).
3. Opioids, which inhibit putative inspiratory rhythm generating neurons within the preBötC, cause failed inspiratory bursts. Yet the underlying tempo of the rhythm continues in the presence of opioids suggesting that opioid-dependent inspiratory bursting in the preBötC is not a pre-requisite for the breathing rhythm (Janczewski and Feldman, 2006; Mellen et al., 2003).
4. Ablating the preBötC in goats causes an initial apnea but if animals are maintained on a ventilator for 24 h, spontaneous breathing recovers, suggesting the role of the preBötC in inspiration can be supplanted by other regions through neuronal plasticity (Forster et al., 2014; Krause et al., 2009).

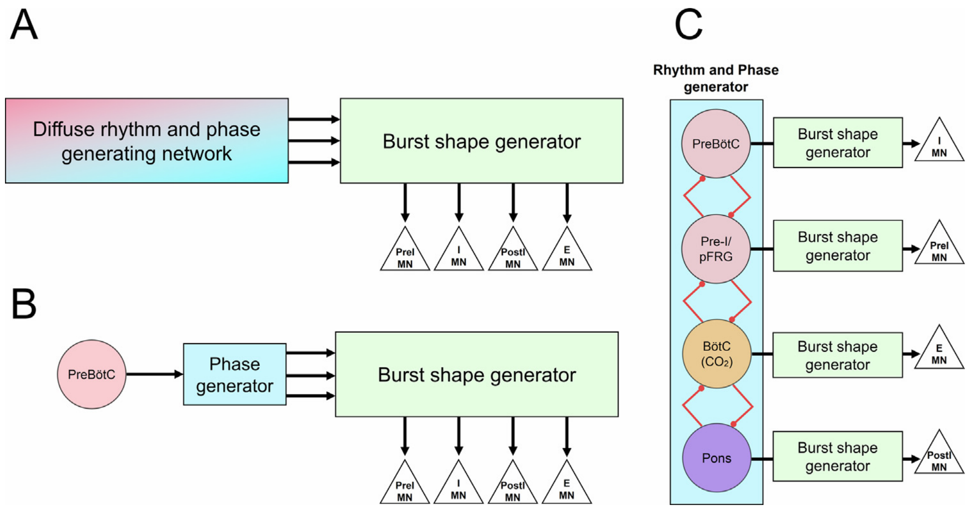

Fig. 2. Three different models for respiratory rhythm and pattern generation. (A) A diffuse rhythm and phase generating module that feeds into a diffuse burst shape generating module. (B) A distinct element in the circuit generates the rhythm, which is then passed to diffuse phase and burst shape modules. (C) Interconnected rhythm generating modules intrinsically produce phasic properties of pattern and project to phase-specific burst generating subunits.