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Variability in respiratory rhythm generation: In vitro and in silico models

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ABSTRACT

The variability inherent in physiological rhythms is disruptive *in extremis* (too great or too little) but may also serve a functional and important role in homeostatic systems. Here we focus on the neural control of respiration which is critical for survival in many animals. The overall respiratory control system is comprised of multiple nuclei, each of which may have different contributions to rhythm variability. We focused on the pre-Bötzinger complex (preBötC) which is unique in that it can be studied *in vitro* as an isolated nucleus with autorhythmic behavior. The *in vitro* results show a bounded range of variability in which the upper and lower limits are functions of the respiratory rate. In addition, the correlation between variability and respiratory rate changes during development. We observed a weaker correlation in younger animals (0–3 days old) as compared to older animals (4–5 days old). Based on experimental observations, we developed a computational model that can be embedded in more comprehensive models of respiratory and cardiovascular autonomic control. Our simulation results successfully reproduce the variability we observed experimentally. The *in silico* model suggests that age-dependent variability may be due to a developmental increase in mean synaptic conductance between preBötC neurons. We also used simulations to explore the effects of stochastic spiking in sensory relay neurons. Our results suggest that stochastic spiking may actually stabilize modulation of both respiratory rate and its variability when the rate changes due to physiological demand.

1. Introduction

Variabilityis inherent in physiological rhythms and may arise from a variety of sources, both stochastic and deterministic. However, an open question remains: is variability a byproduct of rhythmogenesis or can it play a functional, homeostatic role in generation of a physiologic rhythm? Excessive variability may indicate instability in a rhythmic oscillator while insufficient variability could result from an inability to adapt to environmental influences. Heart rate variability is widely accepted as an indicator of cardiac health [1], and respiratory variability is gaining attention as a potential biomarker in respiratory conditions

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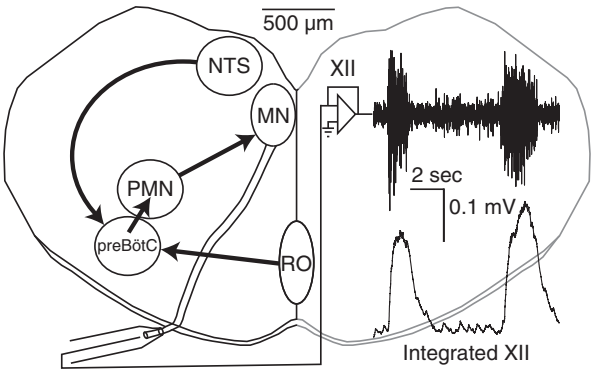

Fig. 1. *In vitro* slice (outline) shown with superimposed trace (on right) of two inspiratory bursts recorded unilaterally from cranial nerve XII using a suction electrode (bottom left). The trace includes the raw recording (top right) and the integrated signal (bottom right) used to identify burst onset times. The left hemisection includes a diagram of connectivity between the following anatomical regions: Tonically spiking neurons in NTS and RO modulate the preBötC which drives the PMN and, indirectly, the MN. Integrated XII is used to identify burst onset by threshold-crossing. preBötC = pre-Bötzinger complex, PMN = premotoneurons, MN = hypoglossal motoneurons, XII = hypoglossal nerve, NTS = nucleus tractus solitarii, RO = nucleus raphe obscurus.

[2]. Intuition might suggest that minimizing variability is optimal in the cardiorespiratory system. However, there is an association between higher levels of cardiorespiratory variability and better health, including respiratory health [3] and neurological conditions such as schizophrenia [4] and autism spectrum disorders [5]. A commonly accepted viewpoint is that regulatory systems are "designed" to seek a homeostatic set point [1]. However a complex system intended to interact with a highly variable environment might be better described as "homeodynamic" [3]. Variability may be more a reflection of dynamic plasticity in the underlying system, rather than a purposeful function unto itself.

The autonomic control circuitry in the brainstem is highly complex [6]. Coupling exists between the cardiac and respiratory systems [7], and mutual influence may change according to the particular dynamical regime in which the individual systems operate and interact [8,9]. We focus on the neural generation of the respiratory rhythm by the pre-Bötzinger Complex (preBötC), an important respiratory pattern generator located in the brainstem [10]. In particular, we look at the *in vitro* interburst interval (IBI) of the output of cranial nerve XII which serves as a proxy for the drive to respiratory muscles. The electrical pattern of cranial nerve XII has been shown to have an exact one-to-one relationship to the respiratory pattern in phrenic nerves (C4–C6) and thoracic nerves (T2, T5, T6, and T8) [11,12]. The IBI is analogous to the commonly studied heart beat-to-beat interval, or RR interval, which has become the primary feature of interest in cardiac variability [13].

During involuntary breathing, the respiratory system functions as a closed-loop system for the purpose of maintaining proper levels of oxygen (O_2) and carbon dioxide (CO_2) in the body. The nervous system rhythmically activates the diaphragm and intercostal muscles in order to expand and contract the lungs. The neural drive is in turn modulated by three types of sensory feedback: (1) chemosensation of O_2 and CO_2, (2) mechanosensation in pulmonary stretch receptors, and (3) barosensation of blood pressure. Neural generation of the resting respiratory rhythm occurs in the medulla and is critically dependent on a region called the pre-Bötzinger Complex (preBötC) [10,14]. The preBötC is unique in that it can be studied *in vitro* as an isolated nucleus with autorhythmic behavior [15]. Here we take the initial step of a "bottom up" strategy by looking only at the preBötC in order to understand key behaviors and establish a verifiable model of variability in the primary respiratory pattern.

Different mechanisms have been proposed to explain the generation of the rhythm in the preBötC. These include intrinsically bursting neurons, conditional bursting neurons, and emergent network bursting [6,10,16]. A possible source of variability is the stochastic spiking of sensory feedback neurons. The preBötC receives sensory feedback from various sources including chemo-, mechano- and barosensation through the *nucleus tractus solitarii* [17,18], as well as chemosensation through the *nucleus raphe obscurus* [19]. As shown in Fig. 1, the *in vitro* slice preparation retains portions of these sensory feedback nucleii and is ideally suited to evaluate rhythm variability in the preBötC, an important component in the generation of the respiratory rhythm.

Prior research on respiratory rhythm variability has included both experiments and modeling. Some *in vivo* studies have used chemical stimulation [20] and vagotomy [21] and analyzed the complexity of the recorded waveforms. Another study has looked at changes to variability in the output of the *in vitro* preBötC due to the effects of hypoxia and norepinephrine [22]. Models of the closed-loop system have been developed for extreme forms of rhythm variability such as coughing [23], gasping [24], and apnea [25–28]. One previous model of the *in vitro* preBötC has specifically looked at how bursting neurons stochastically contribute to the network rhythm [29].

In contrast, the present study analyzes variability in a normal (eupneic) pattern within the *in vitro* preBötC slice preparation (Fig. 1) across a wide range of respiratory rates. In addition, it introduces a model of stochastic synaptic drive to the preBötC (Fig. 2). In particular, we investigate the relationship between variability and the respiratory rate. Based on experimental observations, we develop an *in silico* model that replicates the variability characteristics seen *in vitro* to provide insight into the possible physiological sources of variability. The results are important for understanding the generation of variability in the overall respiratory control system, particularly the contribution of the preBötC. In addition, the present study provides insight into the influence of the stochastic nature of sensory feedback on central pattern generators in the nervous system.