Point:Counterpoint: Medullary pacemaker neurons are essential for both eupnea and gasping in mammals vs. medullary pacemaker neurons are essential for gasping, but not eupnea, in mammals

**Point:** MEDULLARY PACEMAKER NEURONS ARE ESSENTIAL FOR BOTH EUPNEA AND GASPING IN MAMMALS

Despite intense research it remains unresolved whether pacemakers are essential for eupnea and/or gasping. Recordings of neural activity in vivo and in vitro and attempts to eliminate pacemaker activity through pharmacological lesions have led to controversial interpretations. Yet it must be emphasized that major progress has been achieved over the past decade.

**Medullary network located within the pre-Bötzinger complex is essential for eupnea.** Sophisticated pharmacological lesions demonstrate that the pre-Bötzinger complex is critical for generating eupnea in vivo (3, 5, 11, 21). There is no evidence that the pre-Bötzinger complex is suppressed during eupnea, nor that medullary neuronal activities are not critical for the neurogenesis of eupnea, as hypothesized by St. John in 1996 (13). Moreover, a detailed cellular analysis by Lieske et al. (6) led to the identification of pacemakers and the demonstration that the isolated medullary respiratory network can generate more than one respiratory pattern. Core properties of eupnea and the ability to reconfigure into gasping remain functional in the isolated respiratory network. This allowed an in-depth comparison of pacemaker activity during different states of the network.

**Core properties of network reconfiguration are preserved in isolated medullary network.** Core properties of eupnea include inspiratory rise time, burst duration, activity pattern, and discharge phases of respiratory neurons. In eupnea, the rise time of inspiratory activity is significantly slower than during severe hypoxia in vitro (6) and in vivo (15, 20). Moreover, the pre-Bötzinger complex contains postinspiratory and expiratory neurons in vivo and in vitro (6, 12). Gasping is typically characterized by an absence of expiratory activity and a reconfiguration of postinspiratory activity in vivo (14). Likewise, postinspiratory neurons shift to an inspiratory discharge pattern or become quiescent during anoxia in vitro (6). Consistent with the reconfiguration from eupnea to gasping are also changes in burst duration, amplitude, and frequency, which behave similar in vitro and in vivo (6). The concept that eupnea and gasping are generated by network reconfiguration rather than a switch between two distinct rhythm generators or centers (“pneumatactic vs. gasping” centers) can also explain the gradual transition from eupnea into gasping in vivo (4, 20), and in vitro (6).

**The reconfiguration from eupnea to gasping is associated with the reconfiguration in the role of pacemaker neurons.** The concurrent reconfiguration of network and pacemaker activity was first identified in vitro by Thoby-Brisson and Ramirez (16) and Peña et al. (10).

**In normoxia,** two types of pacemakers are active: Cd-sensitive pacemakers that burst presumably by activating the calcium-activated, non-specific cation current (ICAN) and Cd-insensitive pacemakers that depend on the persistent sodium current (INaP). Blockade of both burst mechanisms eliminates fictive eupneic activity (3, 10), while fictive eupnea persists upon blockade of INaP alone (2, 10). Paton et al. (7) reproduced a very similar finding in vivo and in situ, as blockade of INaP does not block eupnea. Unfortunately these authors did not attempt a combined blockade of ICAN and INaP, which we expect would block eupnea in vivo. Because pacemaker activity depends not only on INaP, the conclusion that “pacemakers are not essential for eupnea” can not be drawn from blocking INaP alone.

**In hypoxia**, Cd-sensitive pacemakers and many nonpacemakers cease, whereas Cd-insensitive pacemakers continue to burst. In this reconfigured network, blockade of INaP alone is sufficient to eliminate fictive gasping (10). This finding was later confirmed in vivo and in situ (7). We concluded that pacemakers are essential for gasping based on lesions of INaP and the demonstration that the majority of neurons (Cd-sensitive pacemakers and nonpacemakers) shut down, whereas Cd-insensitive pacemakers persist in gasping (10).

We proposed that rhythm generation is more complex during normoxia, involving a heterogeneous pacemaker population, as well as expiratory and inspiratory nonpacemakers that are likely to contribute to respiratory rhythm generation (10). Peña et al. (10) further proposed that the transition from eupnea to gasping is accompanied by a reduction in pacemaker diversity, such that during anoxia gasping is driven only by Cd-insensitive pacemakers. This conclusion was also confirmed by Paton et al. (7) stating: “Here we demonstrated transformation within the central respiratory oscillator from a rhythm that is probably generated by a complex combination of cellular properties and synaptic interactions to one based critically on intrinsic membrane properties.” This is essentially the same as our conclusion. The important message is that network and cellular properties play a critical role in eupnea. None of the existing experiments can demonstrate that pacemaker neurons are not essential for eupnea.

**Pacemaker activity is highly modulated in eupnea.** To address the question whether pacemakers are essential for eupnea, we have to consider that the contribution of pacemakers to the overall network activity is not hard wired (1). Synaptic inhibition, which is very strong in normoxia, tends to inhibit bursting properties in many, but not all, pacemakers (2, 17). A variety of neuromodulators (8, 9, 18, 19) enhance and induce bursting properties. These inhibitory and excitatory influences are not static but continuously regulated. We thus propose that the number and degree of bursting depends on dynamic factors such as state and neuromodulatory milieu. Consequently, the question whether pacemakers are essential for eupnea can not have a simple answer: there will be conditions when pacemakers are more or less important.
Pacemaker activity imbues the respiratory network with the plasticity essential for eupnea. The extreme view, i.e., that pacemakers play no role in eupnea, would require that all bursting properties are suppressed in normoxia. For this notion, there is as little evidence as for the proposal that the pre-Bötzinger complex is suppressed during eupnea. Endogenous neuromodulators are essential for bursting during well-oxygenated conditions (i.e., normoxia). Blockade of endogenously activated serotonin receptors (5-HT2A) abolishes bursting in Cd-insensitive pacemakers, but retains action potential generation. At the network level, 5-HT2A blockade dramatically affects regularity and frequency, which coincides with the blockade of bursting in Cd-insensitive pacemakers (8). Moreover, the remaining network becomes dependent on ICAN. Thus the persistence of network activity does not indicate that rhythm-generating mechanisms are unaffected (18). The same is true following blockade of INaP. While this manipulation does not abolish respiratory rhythm generation in normoxia (2, 7, 10), rhythm generation is altered and destabilized in presence of norepinephrine (19).

If eupnea characterizes breathing with all its plasticity and ability to adapt to changes in environmental and metabolic conditions, then these experiments are consistent with the notion that pacemaker properties are essential for generating eupnea and gasping. Neuromodulators and synaptic mechanisms are capable of continuously regulating the relative contribution of different types of bursting mechanisms, which stabilizes network activity and imubes the network with the enormous plasticity that characterizes eupnea. By reducing some of these properties, the respiratory rhythm may persist, but the persisting rhythm is limited in its adaptive capability, which is one of the hallmarks of eupnea.

REFERENCES


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COUNTERPOINT: MEDULLARY PACEMAKER NEURONS ARE ESSENTIAL FOR GASPING, BUT NOT EUPNEA, IN MAMMALS

For more than 80 years, the brain stem mechanisms that might underlie the neurogenesis of automatic ventilatory activity have been debated. Mechanisms proposed have included the discharge of pacemaker neurons, inhibitory synaptic interactions within a neuronal circuit, or a combination of these processes. Inherent to these discussions has been the question as to whether there are state-dependent changes in the mechanisms for respiratory rhythm generation such as occur during the transition from eupnea to gasping.

Eupnea and gasping differ in multiple aspects, with a primary difference being the rate of rise of inspiratory motor activity. In eupnea, phrenic activity increases gradually. In gasping, phrenic discharge reaches a peak almost immediately after onset and has a decrementing pattern (Fig. 1). During gasping, the temporal dispersion of cranial (X and XIIth nerves) vs. spinal motor outflows is lost as they synchronize, and laryngeal adductor activity (post-inspiratory discharge) is abolished (12, 19–21, 29; Fig 1). Therefore, we propose that multiple, simultaneously recorded motor outflows are necessary to assign behavioral terms to respiratory motor patterns.

Rhythms in vitro. Although an in vitro en bloc preparation was introduced more than 20 years ago (26) and slice preparations soon thereafter (17), the rhythms generated by these