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## Evolution of Vertebrate Respiratory Control

**R J A Wilson**, University of Calgary, Calgary, AB, Canada

**B E Taylor and M B Harris**, University of Alaska, Fairbanks, AK, USA

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### Introduction

Neil Armstrong's first step on the moon is more than matched by our vertebrate ancestors who transitioned to land. Like the first astronauts, the first vertebrates to invade land were well prepared. Key aspects of the respiratory controller appear to have predated tetrapods and to have been present in fish some 400 Ma.

Our understanding of central respiratory control is rudimentary. There have been relatively few modern studies in fish, frogs, and reptiles; the vast majority of data come from studies in mammals. Evolutionary theories should be grounded by data derived from diverse species; in this case, unfortunately, they are not. In effect, investigators are putting together a jigsaw puzzle of which most of the pieces are currently missing. Consequently, the evolutionary framework on which this article is based is speculative, and the homology hypothesis is very much a work in progress. It is presented here in the hope of provoking more work in this exciting area.

### Evolution of Rhythm Generation

#### Evolution of Gill Ventilation: From Tunicate to Fish

The primordial motor patterns required for pumping water over gills can be traced back to the earliest chordates: aquatic animals that lived in water and were filter feeders (Figure 1). Living examples of primitive chordates include tunicates and amphioxus. The adult forms of these animals use rhythmic muscle contractions to pull water through their mouths, into pharyngeal-like structures, and out through secondary orifices.

In amphioxus, the passage of water through the pharynx and across the gills contributes little to gas exchange, suggesting that irrigation of the pharyngeal cavity in primordial vertebrates may have originated from the need to feed, not breathe. Nonetheless, recent molecular studies suggest that these rudimentary feeding behaviors may be the predecessor of vertebrate breathing. The motor neurons responsible for pulling water into the pharynx of adult tunicates,

located in the cerebral ganglion which until recently was thought to be a highly derived structure, express transcription factors homologous to *Phox2a* and *Phox2b* (Figure 2). In fish and tetrapods, expression of *Phox2a* and *Phox2b* within the central nervous system is restricted to the brain stem, occurring in brain stem cranial motor neurons, used extensively (but not exclusively) for ventilation. In larval tunicates, when the caudate body plan is most obvious, transcription factors homologous to *Phox2a* and *Phox2b* are similarly restricted to the neck region of the animal.

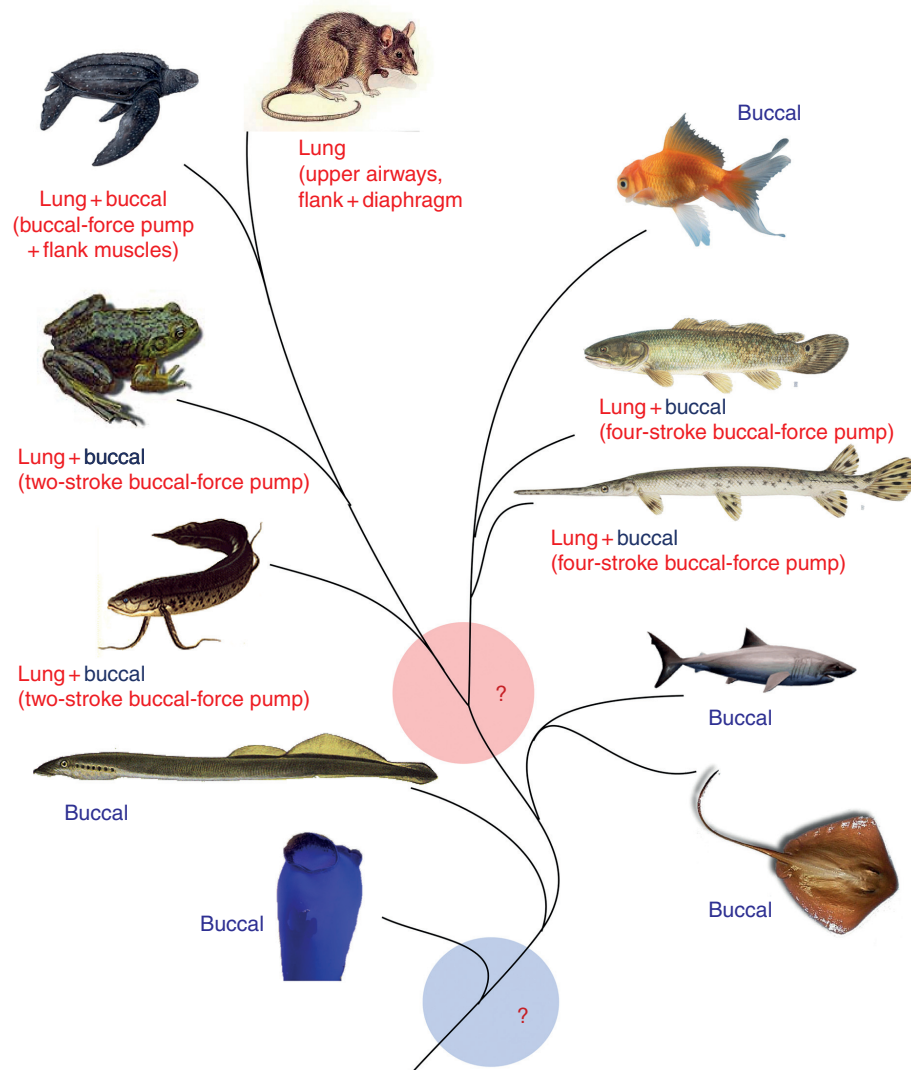
By the time the jawless fishes (agnatha) evolved, the walls of the gills had likely thinned and become highly vascularized, becoming the primary site for gas exchange, as exemplified in extant agnatha such as the lamprey and hag fish.

Most fish ventilate their gills with water more or less continuously, and therefore the neuronal mechanism responsible likely involves a central rhythm-generating circuit. The presence of a central rhythm generator is supported by the fact that fish brain stems continue to exhibit rhythmic cranial nerve discharges when isolated and superfused.

Where the rhythm generator resides, however, is poorly understood. Several early studies in lamprey and modern ray-finned fishes have addressed the location and organization of the respiratory rhythm generator for gill ventilation. These studies uniformly identify the brain stem as the site of rhythmogenesis, but the rostrocaudal location of the rhythm generator within the brain stem is less clear.

In lamprey and dogfish, a rostrocaudal wave of muscle contraction ensures movement of water across the gills. The rostrocaudal wave of somatic muscle activation involved in swimming is produced by a chain of coupled neuronal oscillators within the spinal cord. While some earlier studies suggest that multiple brain stem oscillators combine to produce the rostrocaudal wave of muscle contraction involved in ventilating the gills, more-recent work suggests that the rostrocaudal progression is produced by differences in conduction delay as a result of rostral and caudal motor neurons having axons of different lengths.

In dogfish, respiratory neurons are distributed along the length of the brain stem, leading some researchers to suggest that the rhythm generator is distributed within either the motor column or the reticular system that runs the length of the brain stem. In addition, local ablation experiments in dogfish have so far failed to identify a discrete location



**Figure 1** Phylogeny of breathing in vertebrates. Lower vertebrates use buccal and pharynx muscles to move water in and out of their buccal cavities (blue text). In the most primitive vertebrates (e.g., tunicates), this behavior may have been more important in feeding than in breathing. In bimodal ventilators that breathe air and water (e.g., gar, lungfish, and metamorphic tadpoles), the buccal musculature is also used as a 'buccal-force pump' to drive air into primitive lungs (red text). The motor pattern used to pump water appears to be retained in frogs and reptiles, co-opted into ventilating the buccal cavity with air. In reptiles, flank muscles assist the buccal-force pump to inflate the lungs. In mammals, lung inflation occurs through aspiration, the power stroke for which is provided almost entirely by the flank muscles and diaphragm. Nonetheless, even in mammals, phasic contractions of the buccal musculature retain an important respiratory role of maintaining patency of the upper airways. Blue circle, hypothesized origin of primordial neuronal circuit generating buccal contractions used to breathe water. Pink circle, hypothesized origin of air-breathing organ and primordial neuronal circuit, which inflates it. Top right, clockwise: Modern ray-finned fishes (teleosts), primitive ray-finned fishes (*Amia* and gar), elasmobranchs (sharks and rays), tunicates, agnatha (lamprey), lobe-finned fishes (lungfish), amphibians (bullfrog), reptiles (leatherback turtle), and mammals (rat).

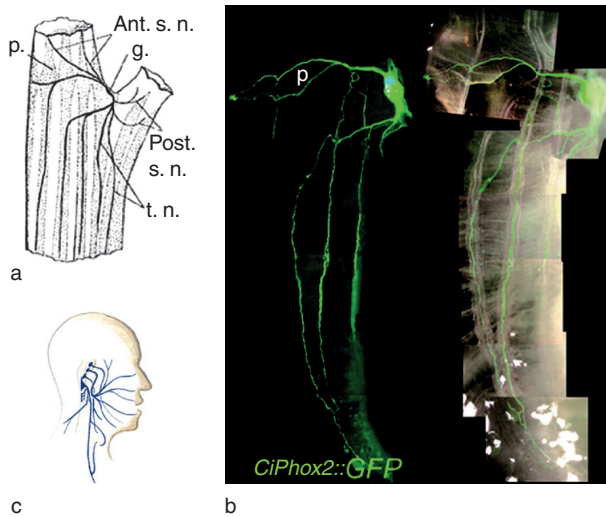
that stops rhythmic activity in motor nerves. However, the former evidence is correlative, and local ablation experiments may have missed a critical site. Thus, whether the rhythm generator for gill ventilation in dogfish is distributed remains an open question.

Brain stem transection studies in lamprey suggest a discrete rostral region of the brain stem, located at the level of the trigeminal nucleus, is responsible for gill ventilation. Studies of metamorphic amphibian tadpoles and juvenile frogs also suggest that a discrete

region of the brain stem may be uniquely responsible for gill ventilatory rhythmogenesis, albeit at a more caudal location.

#### Gill Ventilation in the Amphibian Tadpole

Work to resolve the central neuronal mechanisms involved in breathing in lower vertebrates has focused mainly on the bullfrog, *Rana catesbeiana*. Based on parsimony, the paucity of data from fish to refute claims of homology, and the low likelihood that a

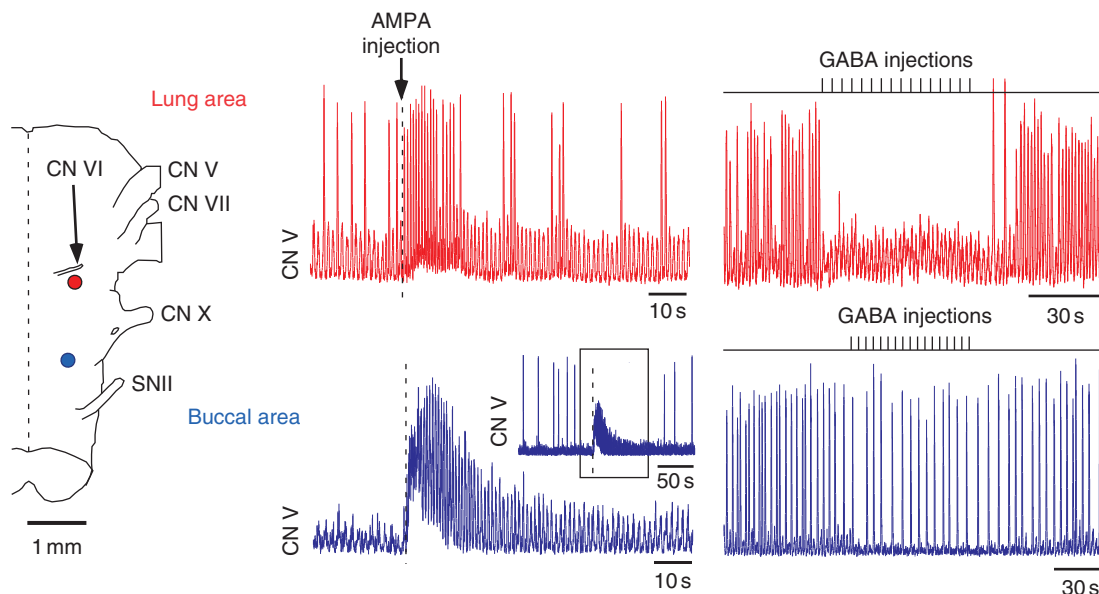


**Figure 2** Motor neurons expressing Phox2 innervate the buccal cavity of both tunicates and higher vertebrates. Contractions caused by activity of Phox2 motor neurons in tunicates (a) and (b) and water-breathing vertebrates move water through the 'buccal' cavity. In higher vertebrates, including rats and humans (c), the expression pattern likely includes  $\text{CO}_2/\text{H}^+$ -chemosensitive neurons and cranial motor neurons that play an important role in air breathing. GFP, green fluorescent protein. From Dufour HD, Chettouh Z, Deyts C, et al. (2006) Precranial origin of cranial motoneurons. *Proceedings of the National Academy of Sciences of the United States of America* 103(23): 8727–8732.

vital behavior such as gill ventilation would have been lost and re-evolved during the transition from fish to frogs, an assumption central to the field is that the neuronal mechanisms responsible for gill ventilation in fishes and bullfrog tadpoles are similar.

In tadpoles, which have both gills and lungs, a 500  $\mu\text{m}$  isolated transverse brain stem slice at the level of the vagus is capable of generating a gill-like motor pattern. During metamorphosis, tadpoles lose their gills and depend more heavily on their lungs for gas exchange. However, despite the loss of gills, a facsimile to the gill ventilatory motor pattern is retained for flushing air in and out of the buccal cavity (i.e., buccal ventilation). This behavior likely serves to prime the buccal cavity for lung inflation and may also be important for olfaction.

Transection studies and drug microinjection studies have been used to locate the rhythmogenic site within the isolated superfused brain stems responsible for buccal ventilation. A small, bilaterally iterated site has been identified proximal to the vagus nerve that appears to be important for generating the buccal motor pattern (Figure 3). Injections of the non-N-methyl-D-aspartate glutamate receptor agonist  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid into isolated superfused brain stems at this



**Figure 3** Two oscillators in frogs. Sufficiency (left traces) and necessity (right traces) tests suggest two areas important for generating ventilatory motor patterns, buccal (blue) and lung (red). Under control conditions the isolated juvenile frog brain stem produces buccal and lung ventilatory motor patterns in cranial nerves (CNs). These motor patterns are easily distinguished by burst amplitude (buccal small, lung large). Injecting the non-N-methyl-D-aspartate receptor agonist  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) into the lung area (for location see diagram on left) to produce local excitation causes an increase in lung burst frequency whereas injecting  $\gamma$ -aminobutyric acid (GABA) to produce local inhibition abolishes lung bursts but has no effect on buccal bursts. On the other hand, injecting AMPA into the buccal area causes an increase in buccal bursts, but abolishes lung bursts (inlay), whereas injecting GABA into the buccal area abolishes buccal bursts but has no effect on lung bursts. These data, in combination with the results of transection studies and the tight coordination of buccal and lung bursts suggest that the motor pattern is mediated by two coupled oscillators. From Wilson RJA, Vasilakos K, Harris MB, Straus C, and Remmers JE (2002) Evidence that ventilatory rhythmogenesis in the frog involves two distinct neuronal oscillators. *Journal of Physiology* 540: 557–570.

site caused an increase in the frequency of buccal bursts (sufficiency test), whereas an injection of  $\gamma$ -aminobutyric acid (GABA) into the same site caused cessation of buccal bursts (necessity test). These data demonstrate that the region of the brain stem at the level of the vagus is both necessary and sufficient for buccal ventilation.

The rhythmogenic mechanism responsible for generating buccal ventilation remains largely unexplored, although experiments in a number of lower vertebrates suggest that it requires postsynaptic chloride-mediated inhibition and can be abolished with strychnine and bicuculline, potent agonists of GABA and glycine receptors, respectively.

### Origins of Air Breathing

Ray-finned and lobe-finned fishes diverged at least 408 Ma. The ray-finned fish gave rise to modern teleosts, and the lobe-finned fish gave rise to tetrapods.

Extant but primitive ray-finned and lobe-finned fishes have saclike structures that can be opened to the atmosphere, serve a respiratory function, and have an embryonic origin similar to that of the tetrapod lung, suggesting that fish evolved lungs long before amphibians invaded land.

There are ~375 extant species of air-breathing fishes, including modern perciform teleosts such as the Siamese fighting fish, *Betta splendens*. Only a small portion of these so-called modern species use lunglike organs for gas exchange; most have secondarily derived structures that serve this purpose. Thus, while the first ray-finned fishes were capable of breathing air using a structure homologous to the tetrapod lung, air breathing was lost during the evolution of more-modern fishes, only to be rederived, in some cases, from structures unrelated to the proto-lung.

Despite the presence of lunglike organs in extant but primitive ray-finned fishes, such as the gar and amia, it has long been assumed that air breathing in this clade evolved independently from air breathing of lobe-finned fish. The following three lines of evidence, based on assumed differences in the neuronal mechanisms controlling air breathing, have been used to support this argument:

1. Compared with lobe-finned fishes and tetrapods, air breathing in ray-finned fishes is infrequent and rarely rhythmic but responds vigorously to stimulation of peripheral chemoreceptors. Therefore, some have argued that air breathing in fish is more likely to be reflex-mediated than produced by a central respiratory rhythm generator.

2. The mechanics of air breathing in ray- and lobe-finned fishes differ even though base members

of both clades use buccal musculature to push air into their lungs. Specifically, ray-finned fishes use a two-cycle mechanism (one cycle of buccal dilation and constriction to expel stale air from lungs to the atmosphere, another to acquire fresh air into the buccal cavity and pump it into the lungs) whereas lobe-finned fishes and most amphibians use a one-cycle mechanism (expulsion of stale air from the lung to the atmosphere and acquisition of fresh air into the buccal cavity occur simultaneously during a single phase of buccal dilation, and the subsequent buccal constriction pumps the freshly acquired air in the buccal cavity into the lungs).

3. Air breathing in ray-finned fishes differs from that in lobe-finned fishes and tetrapods by showing little sensitivity to arterial  $\text{CO}_2$ . Therefore, unlike air breathing in tetrapods, air breathing in ray-finned fishes is not modulated by central  $\text{CO}_2$  chemoreceptors.

Based on a study using the isolated gar brain stem, it has been proposed that the first air-breather may have predated the division of ray- and lobe-finned fish, the clades that gave rise to modern teleosts and tetrapods, respectively. Specifically, it has been shown that the isolated gar brain stem produces bursts of activity, similar to those associated with air breathing, that respond to changes in superfusate  $\text{PCO}_2$ . These controversial data suggest gar have a central  $\text{CO}_2$ -sensitive rhythm generator for air breathing, similar to lobe-finned fishes and their tetrapod descendants (Figure 4). If this is indeed the case, then the air-breathing ancestor of modern tetrapods likely evolved before the divergence of ray- and lobe-finned fishes some 400 Ma.

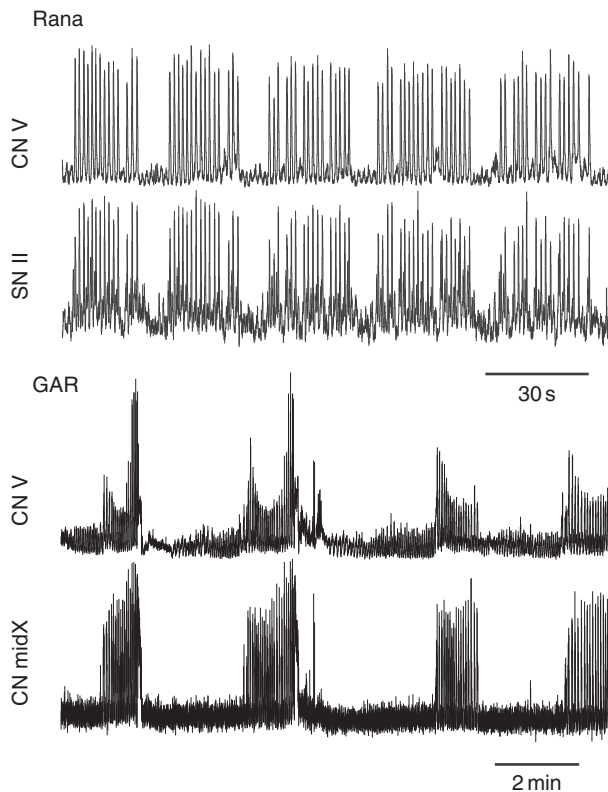
Not all evidence supports the presence of a  $\text{CO}_2$ -sensitive brain stem rhythm generator in primitive ray-finned fishes. Amia, for example, fail to increase their rate of air breathing when arterial  $\text{PCO}_2$  is increased by systemic injection of azetazolamide. However, brain stem isolation and superfusion experiments, which are most likely to demonstrate the presence of a central rhythm generator, have yet to be performed.

### The Air-Breathing Rhythm Generator in Amphibians

The presence of a central rhythm generator for air breathing in lungfish, while not investigated in any detail, is equivocal. Until data suggest otherwise, it seems reasonable to assume that the neuronal architecture is similar to that in frogs.

In frogs, a number of studies suggest that the central rhythm generator for air breathing comprises a buccal oscillator accompanied by another brain stem oscillator that is active only during lung inflation. The buccal and lung oscillators appear to be spatially distinct. Necessity, sufficiency, and transection





**Figure 4** Comparison of motor patterns produced by frog and gar brain stems. Few studies have investigated the neuronal control of breathing in primitive air-breathing ray-finned fishes, and therefore uncertainty remains about the neuronal mechanisms that mediate breathing in this clade. However, data from a study in which the brain stem was isolated and superfused suggest that the gar brain stem is capable of producing both buccal and lung motor patterns in the absence of phasic input; that is, it contains a central respiratory rhythm generator. Furthermore, the putative lung bursts are  $\text{CO}_2/\text{H}^+$ -sensitive. It is remarkable that the motor output produced by the brain stem of the gar is similar to that produced by the juvenile frog, suggesting that brain stems of primitive air-breathing ray-finned fish may also contain two respiratory oscillators. From Wilson RJA, Harris MB, Remmers JE, and Perry SF (2000) Evolution of air-breathing and central  $\text{CO}_2/\text{H}^+$  respiratory chemosensitivity: New insights from an old fish? *Journal of Experimental Biology* 203: 3505–3512.

studies suggest that the lung oscillator is located rostral to the buccal oscillator at a level just caudal to the facial nucleus (Figure 3). In contrast to the buccal oscillator, bursting of the lung oscillator does not depend on postsynaptic chloride-mediated inhibition, is inhibited by opioids, but is strongly stimulated by increased  $\text{CO}_2$ .

While spatially distinct, the buccal and lung oscillators interact functionally. In the intact brain stem, the buccal oscillator appears to play an important role in the timing of lung ventilation and may participate directly in lung inflation by priming the buccal cavity before the contraction phase (power stroke) mediated by the lung oscillator.

Given the strong temporal coordination between buccal and lung bursts, a two coupled oscillator model has been proposed. While the possibility of additional oscillators is not excluded, simulations suggest that two coupled oscillators are capable of recapitulating the salient characteristics of the motor pattern produced by the isolated brain stem.

### The Air-Breathing Rhythm Generator in Mammals

Unlike frogs and fish, which inflate their lungs using positive pressure generated by the buccal musculature, mammals inflate their lungs using negative pressure (aspiration) generated by the diaphragm. Despite the dichotomous biomechanics of the power stroke, in both frogs and mammals the power stroke is preceded by a phase of buccal expansion. In mammals this priming phase protects the upper airways from collapse during the ensuing negative pressure. In frogs, the buccal expansion draws fresh air into the mouth prior to the power stroke.

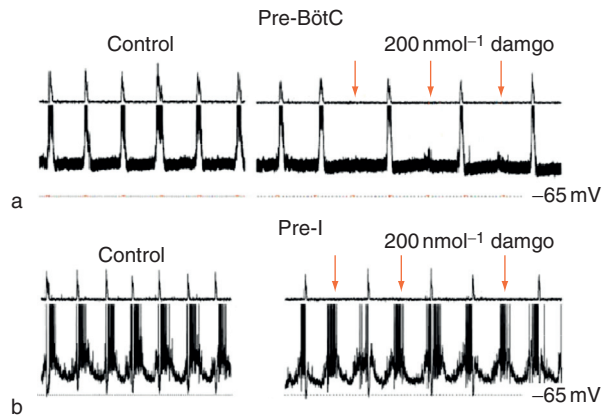
Recent data suggest that a neuronal architecture consisting of two coupled oscillators, the pre-Bötzinger complex (pre-BötC) and the preinspiratory (pre-I) oscillator, may participate in mammalian respiratory rhythmogenesis (Figure 5). The contribution of these oscillators when embedded within the intact respiratory network is currently under debate. The simplest viewpoint is that the pre-BötC generates inspiratory, power stroke activity, with the pre-I oscillator generating expiratory, pre-I, priming activity. However, on the other extreme, some have argued that within the context of an intact, possibly distributed rhythmogenic network, the contribution of each oscillator is minimal at best. Nonetheless, the presence of two putative oscillators in mammals raises the possibility that they evolved from the same ancestral air-breathing circuit that gave rise to the frog oscillators.

Figure 6 compares the properties of the priming and power stroke oscillators of frogs and mammals. Apparent from this comparison, the power stroke oscillators share a large number of similarities. The priming oscillators also share some similarity, but the resemblance is weaker. Therefore, the likelihood that the power stroke oscillators are homologous is good; the case for homology between priming oscillators is more tenuous.

## Evolution of Chemoreceptors

### Why Chemoreceptors?

The primary role of chemoreceptors is to maintain homeostasis. These receptors detect changes in blood and/or environmental levels of  $\text{O}_2$ ,  $\text{CO}_2$ , and pH and initiate cardiorespiratory adjustments to keep gas



**Figure 5** Recent data suggest two oscillators underlie respiratory rhythm generation in mammals. Until recently, the respiratory rhythm generator was widely considered to be composed of a single rhythmogenic kernel called the pre-Bötzinger complex (pre-BötC) embedded in a larger pattern-generating network. Substantive evidence supports the role of the pre-BötC in rhythm generation. However, recent experiments using the  $\mu$ -opioid agonist DAMGO suggest that a second oscillator is also involved. The rat neonatal isolated brain stem preparation normally produces rhythmic bursts in cranial nerves and cervical nerves including the phrenic. DAMGO produces ‘dropped beats’ whereby occasional bursts are lost (arrows), but the underlying rhythm (illustrated with vertical bars) is not affected. Intracellular recordings from neurons within the pre-BötC demonstrate that activity of the pre-BötC is abolished during a dropped beat (a), suggesting that pre-BötC does not pace the underlying rhythm. Instead, it appears that the rhythm originates rostrally, within the parafacial respiratory group. This region is rich in preinspiratory (pre-I) neurons that fire immediately before phrenic activity and has been implicated in respiratory rhythm generation previously. It is important to note that, unlike pre-BötC neurons, rhythmic activity of neurons within this region persists during dropped beats (b). Top trace in each panel: integrated phrenic neurogram. Lower trace in (a) and (b), respectively: intracellular recording from a pre-BötC and a pre-I neuron. From Mellen NM, Janczewski WA, Bocchiaro CM, and Feldman JL (2003) Opioid-induced quantal slowing reveals dual networks for respiratory rhythm generation. *Neuron* 37(5): 821–826.

tensions and pH within set limits despite changing metabolic demands.

Water-breathing vertebrates obtain  $O_2$  from water. The low capacity of water for  $O_2$  necessitates a high flow across gas-exchange surfaces. In contrast, the relatively high solubility of  $CO_2$  in water generally results in rapid  $CO_2$  excretion. In essence, water-breathing vertebrates ventilate in order to acquire sufficient  $O_2$  to meet metabolic demands and, in doing so, satisfy the need to excrete metabolically produced  $CO_2$ . In contrast to aquatic vertebrates, terrestrial vertebrates breathe a medium rich in  $O_2$  but face greater limitations in the ability to excrete metabolically produced  $CO_2$ . Consequently, at rest, air-breathing terrestrial vertebrates generally ventilate to satisfy needs for  $CO_2$  excretion and, in doing so, satisfy metabolic requirements for  $O_2$  uptake.

The evolution from water to air thus necessitated a transition from a respiratory system dominated by the need to satisfy  $O_2$  demand to one that is primarily preoccupied with excreting  $CO_2$ . These changing demands were met in part by the changing importance of two populations of chemoreceptors, peripheral and central, that have different sensitivities to  $O_2$  and  $CO_2$ .

### Peripheral Respiratory Chemoreceptors in Fish

The primary sites of respiratory chemoreceptors that modulate aquatic ventilation in fish are the gills. The anterior gill arches and, to a lesser extent, regions within the orobranchial pharynx contain populations of  $O_2$ -sensitive receptors oriented toward both the blood and the environment. This receptor distribution suggests that fish modulate ventilation in response to aquatic  $O_2$  levels, the effectiveness of  $O_2$  uptake, or the degree of metabolic  $O_2$  consumption as indicated by blood  $PO_2$  at the level of the gills. These locations also contain  $CO_2/H^+$ -sensitive receptors, which in most fish appear to be oriented toward the environment and, thus, likely monitor aquatic  $CO_2$  tension.

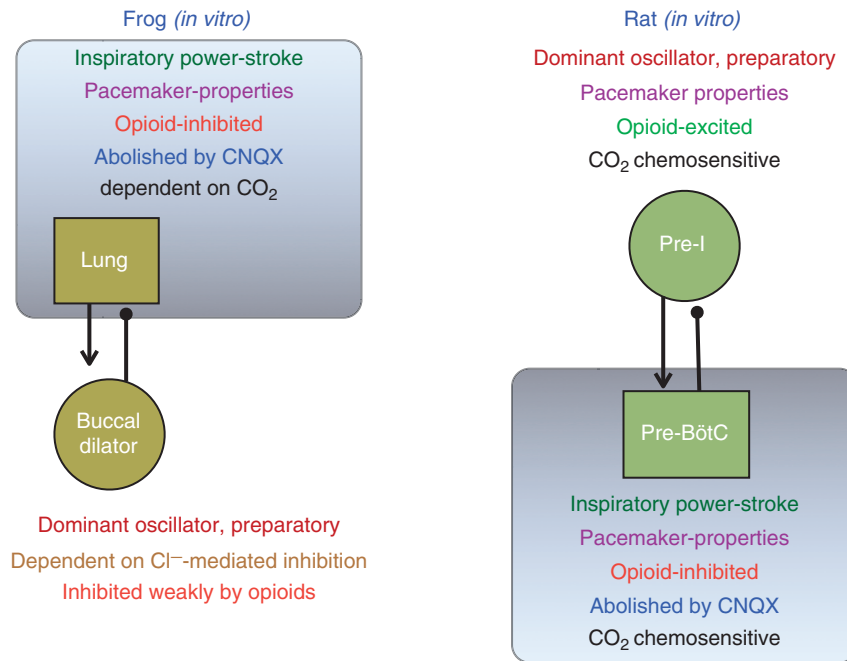
While branches of cranial nerves (CNs) VII (facial), IX (glossopharyngeal), and X (vagus) innervate the gills, sensory information from the chemoreceptors to the brain stem is carried predominantly in CNs IX and X.

In both air- and water-breathing fishes, the peripheral chemosensory information carried by these nerves has been considered almost absolute in stimulating ventilation; in air-breathing fishes, the input was so strong that air breathing was considered an on-demand behavior, controlled entirely by a peripheral respiratory chemoreceptor-mediated reflex arch. More recent data suggest the involvement of a central rhythm generator and modulation by other sensory modalities.

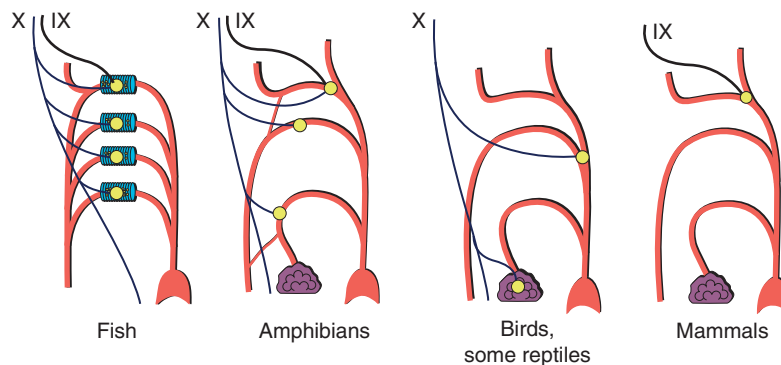
### Peripheral Respiratory Chemoreceptors in Tetrapods

The carotid and aortic arches house the primary peripheral respiratory chemoreceptors in tetrapods. These structures share a common embryonic origin with the gill arches of fish and, like the peripheral respiratory chemoreceptors in fish, are innervated by CNs IX and X (Figure 7). These similarities have led to the widely held view that the carotid and aortic arch chemoreceptors of tetrapods evolved from fish gill arch chemoreceptors.

In mammals, the carotid bodies dominate peripheral chemosensitivity: the ventilatory response to hypoxia decreases only slightly (<10%) when the aortic arch chemoreceptors are denervated. The carotid bodies are most strongly activated when stimulated concurrently by low levels of oxygen and high levels



**Figure 6** Functional similarities between frog and rat oscillators. There are a number of similarities between the putative pairs of oscillators in frogs and rats, as illustrated. The similarities between the frog lung oscillator and the rat pre-Bötzinger complex (pre-BötC) (squares) are strongest, and therefore these are most likely to be homologous. Arguments for homology of buccal and pre-I oscillators (circles) rely on their functional similarity: both are active immediately before inspiration, priming the upper airways for the ensuing power stroke, and appear to have a primary role in setting the underlying rhythm. Diagram illustrates the anatomical arrangement of the oscillators in frogs and rats. Note that anatomy does not support dual homology.



**Figure 7** Comparative anatomy of peripheral chemoreceptors. This diagram depicts the distribution of peripheral arterial and pulmonary CO<sub>2</sub>/H<sup>+</sup>-sensitive chemoreceptors (yellow circles) in various vertebrate groups. IX and X designate the ninth (glossopharyngeal) and tenth (vagus) cranial nerves (CNs), respectively. It is important that chemoreceptors associated with the first gill arch in fish, innervated by CN IX, are homologous to the primary peripheral chemoreceptors in all vertebrates. Modified from Milsom WK (2002) Phylogeny of CO<sub>2</sub>/H<sup>+</sup> chemoreception in vertebrates. *Respiratory Physiology & Neurobiology* 131(1–2): 29–41.

of CO<sub>2</sub>. This condition arises during suffocation, leading some to describe the peripheral chemoreceptors as the primary asphyxia sensor. Under normal conditions (i.e., when blood gases are normoxic and normocapnic), the contribution of the peripheral chemoreceptors to the overall ‘drive’ to breathe is still debated, with the consensus around 40% and estimates ranging from 0% to 70%. The debate is complicated by the state-dependent integration of

peripheral respiratory chemoreceptor afferent information, which is subject to complex modulation depending on, among other factors, the degree of lung inflation and brain stem PCO<sub>2</sub>.

### Central CO<sub>2</sub> Chemoreceptors in Air-Breathing Vertebrates

Although peripheral chemoreceptors contribute in important ways to ventilatory responses to transient

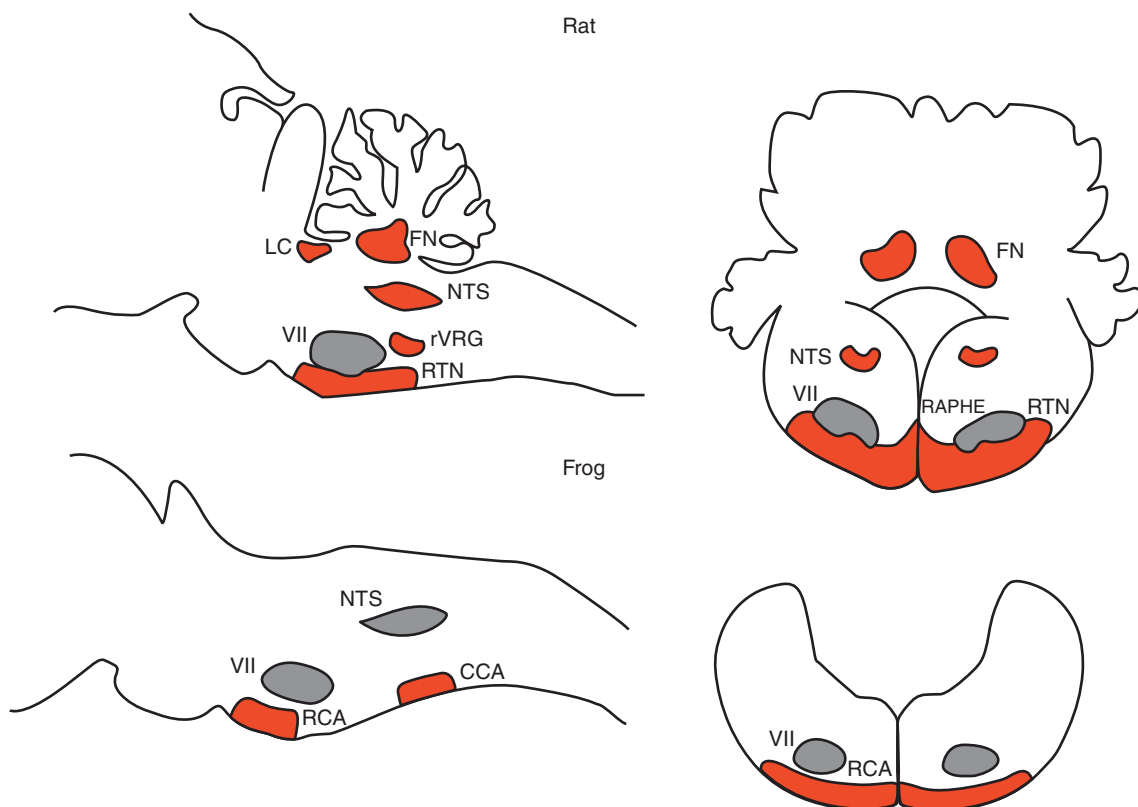


changes in  $O_2$  or  $CO_2/H^+$  in tetrapods, responses persist despite denervation of peripheral chemoreceptors. The dominant source of chemosensory drive under steady-state conditions is central chemoreceptors:  $CO_2/H^+$ -sensitive receptors located within the central nervous system. There is some evidence (discussed later) to suggest the presence of these receptors in primitive fishes, but their general presence in fish has yet to be substantiated.

Despite considerable research effort in mammals, the nature of central chemoreceptors has made their investigation difficult. In rodents and goats, activity of multiple populations of chemosensitive cells contributes to ventilatory responses invoked by central changes in  $CO_2/H^+$ . These populations are widely distributed throughout the brain stem in important respiratory areas such as the raphe, nucleus tractus solitarius, locus coeruleus, hypothalamus, and the subpopulations of the ventral lateral medulla, including the pre-BötC and retrotrapezoid nucleus. Chemostimulation of any one of these populations can elicit substantial ventilatory responses, suggesting

considerable redundancy. Moreover, these different populations may utilize a diverse array of chemosensory transduction mechanisms.

The origin of these chemoreceptor populations relative to the evolution of tetrapods and the water-to-air transition is enigmatic. There is some evidence of chemosensitive cells in the brain stem of some water-breathing fishes, including one of the most primitive extant groups, the lamprey. However, the preponderance of evidence indicates that water-breathing fishes lack central ventilatory chemosensitivity. As previously noted, air breathing is a gas exchange strategy that has evolved independently in a number of fish species. Within one of these air-breathing lineages, the tetrapod lung has its origin in now-extinct, primitive air-breathing fish. An extant species of air-breathing fish (the gar, *Lepisosteus osseus*) closely related to this lineage that gave rise to tetrapods has provided a key element in the understanding of the origin of central ventilatory chemosensitivity. Brains isolated from these fish produce neural correlates to gill ventilation and air breathing.



**Figure 8** Central chemoreceptors in frogs and mammals. This diagram depicts areas within the central nervous system that have been shown to contain  $CO_2/H^+$ -sensitive cells. Longitudinal (left) and coronal sections (right) of a typical mammal (top) and amphibian (below) are shown. Areas with documented chemosensitivity are red, and their locations are presented relative to the facial nucleus (VII; shaded). Chemosensitive areas include the following: locus coeruleus (LC); fastigial nucleus (FN); rostral ventral respiratory group (rVRG); retrotrapezoid nucleus (RTN); nucleus tractus solitarius (NTS); and the amphibian rostral chemosensitive area (RCA) and caudal chemosensitive area (CCA).

Exposing isolated brains to elevated  $\text{CO}_2$  prompts an increase in putative air breathing, indicating central ventilatory sensitivity to  $\text{CO}_2$ . Thus, like the tetrapod lung,  $\text{CO}_2/\text{H}^+$ -sensitive ventilatory chemoreceptors may also have their origin in a primitive air-breathing fish.

The amphibian tadpole metamorphoses from a fishlike, free-swimming larval stage that exchanges gas with water across its skin and gills into a mature semiterrestrial form that exchanges gas across its skin and lungs. Brains isolated from any of these stages produce neural correlates to gill ventilation and air breathing, which represent gill and lung ventilation in the intact animal. Mechanisms of ventilatory control are remarkably conserved among vertebrates, and these preparations have provided great insight into these mechanisms. Previous characterizations suggested that central  $\text{CO}_2/\text{H}^+$  chemosensitivity for air breathing was absent in early metamorphic stages despite the presence of a lung and that this sensitivity developed only in the late stages, associated with loss of the gills, the adoption of a semiterrestrial lifestyle, and use of the lung ventilation as a primary gas exchange strategy. Subsequent investigations, however, have established that exposing brains isolated from tadpole of any stage to elevated  $\text{CO}_2$  results in increases in putative air breathing. Thus, central  $\text{CO}_2/\text{H}^+$  chemosensitivity for air breathing is present throughout tadpole development, including fishlike stages with fully functional gills.

#### **Other Chemoreceptors: Upper Airways and Lung Epithelial Bodies**

Many vertebrate species have olfactory and perhaps gustatory  $\text{CO}_2/\text{H}^+$ -sensitive receptors that can influence ventilation and are believed to be involved in general defense responses to environmental conditions. These include  $\text{CO}_2/\text{H}^+$ -sensitive receptors associated with pulmonary arteries and lung tissues present in amphibians, reptiles, birds, and mammals. The significance of these receptors is unclear, and their evolutionary origin has not been investigated.

#### **Other Reflexes**

Ventilation is modulated by a number of reflexes other than those initiated by chemoreceptors. Pulmonary stretch has a profound influence on breathing, termed the Hering-Breuer reflexes. Receptors located in the lung and/or pleural tissues and stimulated by the pulmonary stretch associated with lung inflation terminate inspiration in a reflex that prevents overinflation of the lung. Similarly, pulmonary stretch lengthens the period between subsequent breaths, and pulmonary deflation hastens the next inspiration.

Such reflexes are present in amphibians as well as mammals. Air-breathing fishes such as gar and lungfish show similar alterations in ventilatory volume and frequency with pulmonary inflation and deflation. The origin of these reflexes may relate to either protection of the fragile lung or buoyancy control, but their presence in primitive air-breathing fishes suggests that Hering-Breuer pulmonary stretch reflexes are as old as the lung itself.

Another important reflex has been termed the mammalian diving reflex. This comprises a host of respiratory and cardiovascular adjustments associated with the presence of water in the pharynx or on the face. This reflex is more pronounced in neonates than in adults of most species, notably mammals, and in mammals it is exaggerated by low temperature. The ventilatory component of this reflex is airway closure and inhibition of breathing, measures that prevent water from entering the lungs. Some aspects of this reflex are observed in almost every air-breathing species, including fishes, suggesting that reflexes protecting the lung from water arose in conjunction with air breathing in aquatic species.

#### **Summary and Conclusions**

From a review of key aspects of respiratory control in vertebrates, it appears that many of the components characteristic of the neuronal controller for air breathing in mammals may have predated tetrapods. These include the oscillators that generate respiratory rhythm and the chemoreceptors that regulate it.

*See also:* Autonomic Nervous System: Respiratory Control; Autonomic Nervous System: Central Respiratory Control; Brainstem Respiratory Circuits; Respiration.

#### **Further Reading**

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