Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 147 (2007) 665–684
CBP
www.elsevier.com/locate/cbpa

Review

Control of breathing in anuran amphibians ☆

Luciane H. Gargaglioni [a,*], William K. Milsom [b]

[a] Department of Animal Morphology and Physiology, Sao Paulo State University-FCAV at Jaboticabal, SP, Brazil
[b] Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

Received 5 January 2006; received in revised form 21 June 2006; accepted 24 June 2006
Available online 28 June 2006

Abstract

The primary role of the respiratory system is to ensure adequate tissue oxygenation, eliminate carbon dioxide and help to regulate acid–base status. To maintain this homeostasis, amphibians possess an array of receptors located at peripheral and central chemoreceptive sites that sense respiration-related variables in both internal and external environments. As in mammals, input from these receptors is integrated at central rhythmogenic and pattern-forming elements in the medulla in a manner that meets the demands determined by the environment within the constraints of the behavior and breathing pattern of the animal. Also as in mammals, while outputs from areas in the midbrain may modulate respiration directly, they do not play a significant role in the production of the normal respiratory rhythm. However, despite these similarities, the breathing patterns of the two classes are different: mammals maintain homeostasis of arterial blood gases through rhythmic and continuous breathing, whereas amphibians display an intermittent pattern of aerial respiration. While the latter is also often rhythmic, it allows a degree of fluctuation in key respiratory variables that has led some to suggest that control is not as tight in these animals. In this review we will focus specifically on recent advances in studies of the control of ventilation in anuran amphibians. This is the group of amphibians that has attracted the most recent attention from respiratory physiologists.

Keywords: Respiration; Anuran; Hypoxia; Hypercarbia; Tadpole; Toad; Frog

Contents

☆ This paper is part of the 3rd special issue of CBP dedicated to The Face of Latin American Comparative Biochemistry and Physiology organized by Marcelo Hermes-Lima (Brazil) and co-edited by Carlos Navas (Brazil), Rene Beleboni (Brazil), Rodrigo Stabeli (Brazil), Tania Zenteno-Savín (Mexico) and the editors of CBP. This issue is dedicated to the memory of two exceptional men, Peter L. Lutz, one of the pioneers of comparative and integrative physiology, and Cicero Lima, journalist, science lover and Hermes-Lima's dad.

* Corresponding author. Rodovia Carlos Tonanni Km 05, 14870-000, Departamento de Morfologia e Fisiologia Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, SP, Brazil. Tel.: +55 16 32092656; fax: +55 16 32024275.
E-mail address: lucihel@fcav.unesp.br (L.H. Gargaglioni).

1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.06.040

1. Introduction

One of the defining characteristics of many amphibians is their free-living aquatic larval stage and semi-aquatic/terrestrial adult stage. This gave rise to their name that derives from the Greek for "double life" (amphibios). In many ways the larval stage reflects their ancestral origin from fish, while their adult stage resembles that of the more derived tetrapods. The three living orders (caecilians, salamanders and frogs/toads), however, include almost 4000 species that display a wide range of life histories and that are evolutionarily distant from their ancestral past. Thus, while amphibians stand as an intermediate stage in the evolution of the tetrapods, all modern amphibians are highly specialized and represent a significant departure in morphology, ecology and behaviour from the stem group that gave rise to the later tetrapods (Milner, 1988; Kardong, 2005).

Despite their high degree of specialization, and the tremendous differences that exist between the three major lineages, there are also many features that they share, including up to three respiratory surfaces (skin, gills and lungs). In most amphibians at least two of these are functional at any given time during development. Aquatic amphibians (including all larvae) primarily rely on gills for gas exchange while terrestrial amphibians primarily rely on lungs. In both, however, the skin may serve as a major surface for gas exchange. Indeed, some terrestrial species have reduced or lost their lungs (such as the plethodontid salamanders) and some aquatic forms have lost their gills (such as the fully aquatic cryptobranchid salamanders), both groups now relying solely on cutaneous gas exchange (Feder and Burggren, 1985). Given this, study of the control of breathing in amphibians may be regarded as a regulatory physiologists dream (or nightmare). These animals can 'ventilate' up to three different exchange surfaces (skin, gills and lungs) with different respiratory media (water and air) and can independently perfuse these surfaces in different proportions due to the existence of highly regulated intra- and extracardiac shunts (Wang et al., 1999b).

A review of all exchange processes occurring at all surfaces in all groups is beyond the scope of this article. The focus of this review will be solely on the control of gill and lung ventilation in anuran amphibians (primarily of the genera *Rana* and *Bufo*). These are the groups of amphibians that have attracted the most

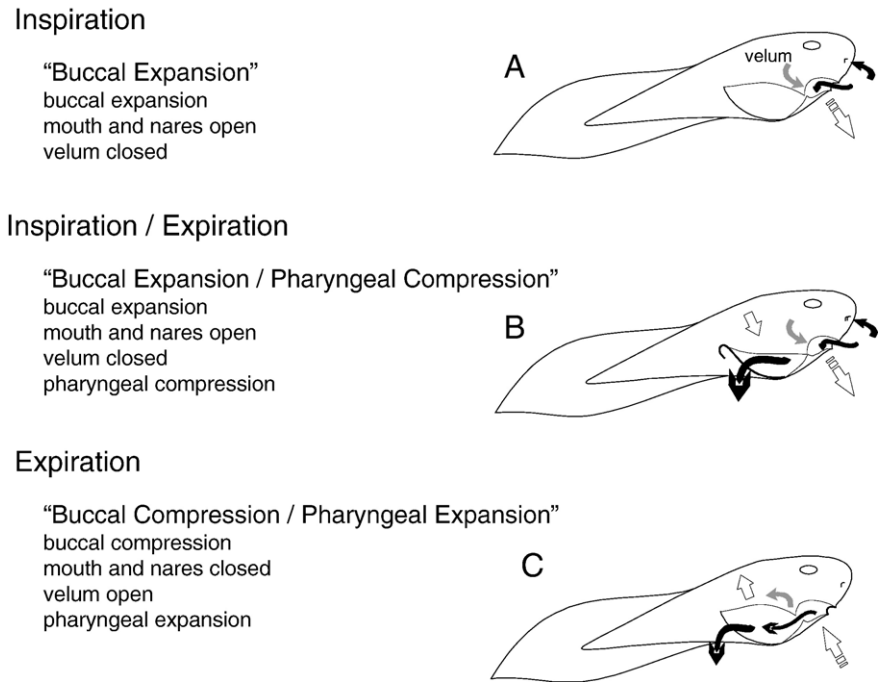

Fig. 1. Schematic diagram illustrating the ventilatory movements in an anuran tadpole. Open arrows indicate movement of the buccal and/or pharyngeal walls, gray arrow indicates movement of the velum, and black arrows indicate the path of water flow — see text for details.

attention from respiratory physiologists and ventilatory control is an area that has attracted much recent research and is ripe for review. Throughout this review the reader should bear in mind (1) that this group is not necessarily representative of other amphibians (such as caecilians and salamanders, or even other anurans such as *Xenopus*) and (2) that although the tendency is to assume that these animals have retained ancestral traits representative of the stem tetrapods, it is not yet clear to what extent the trends seen in respiratory control in anurans reflect unique specializations or shared-derived features found in all tetrapods.

2. The respiratory pump and respiratory patterns

Ventilation is dependent on the precise timing of neuromuscular outputs to the muscles of the respiratory pumps, the nares and the glottis. The interaction of these events in anuran amphibians result in a variety of breath types and ventilatory patterns, which in turn produce either water flow over the gills or air flow in and out of the lungs. In this section we will review the mechanical and neural events associated with both gill and lung ventilation in tadpoles and adult anurans respectively.

2.1. Gill ventilation in larvae

2.1.1. The respiratory pump

Anuran larvae use both buccal and pharyngeal force pumps to produce a unidirectional flow of water across the gills. The buccal and pharyngeal cavities are separated by a valvular velum. When the floor of the buccal cavity is depressed, the pressure within this chamber decreases drawing water in through the oral valve and the nares (Fig 1A). The negative pressure also pulls the velum shut preventing water from entering the pharyngeal cavity. Near the end of the buccal inhalation phase, pharyngeal constriction causes a rise in pressure within the pharyngeal cavity, which keeps the velum closed and expels water across the gills (Fig 1B). Next, the floor of the buccal cavity is elevated raising the pressure within this chamber and closing the oral valve and internal narial valve. This also pushes open the velum and forces water into the pharyngeal cavity (Fig. 1C). The pharyngeal cavity, which was just compressed, expands simultaneously and the two actions together (buccal compression and pharyngeal expansion) refill the pharyngeal cavity (Fig 1C). The net effect is not only to fill the pharyngeal chamber, but to displace water over the gills since buccal ejection exceeds the rate of pharyngeal expansion. As a consequence, as with water breathing fishes, there is a unidirectional flow of water over the gills during both phases of the ventilatory cycle.

With this pumping mechanism "inhalation" is accompanied by buccal expansion and pharyngeal compression, and "exhalation" is accompanied by buccal compression and pharyngeal expansion (Gradwell, 1972; Wassersug and Hoff, 1979). Both phases of the ventilatory cycle are active and are accompanied by motor output to appropriate levator and dilator muscles.

Gill ventilation in tadpoles is produced by high-frequency low-amplitude bursts of motor activity to the respiratory muscles (Gdovin et al., 1998, 1999; Torgerson et al., 1998; Liao et al.,

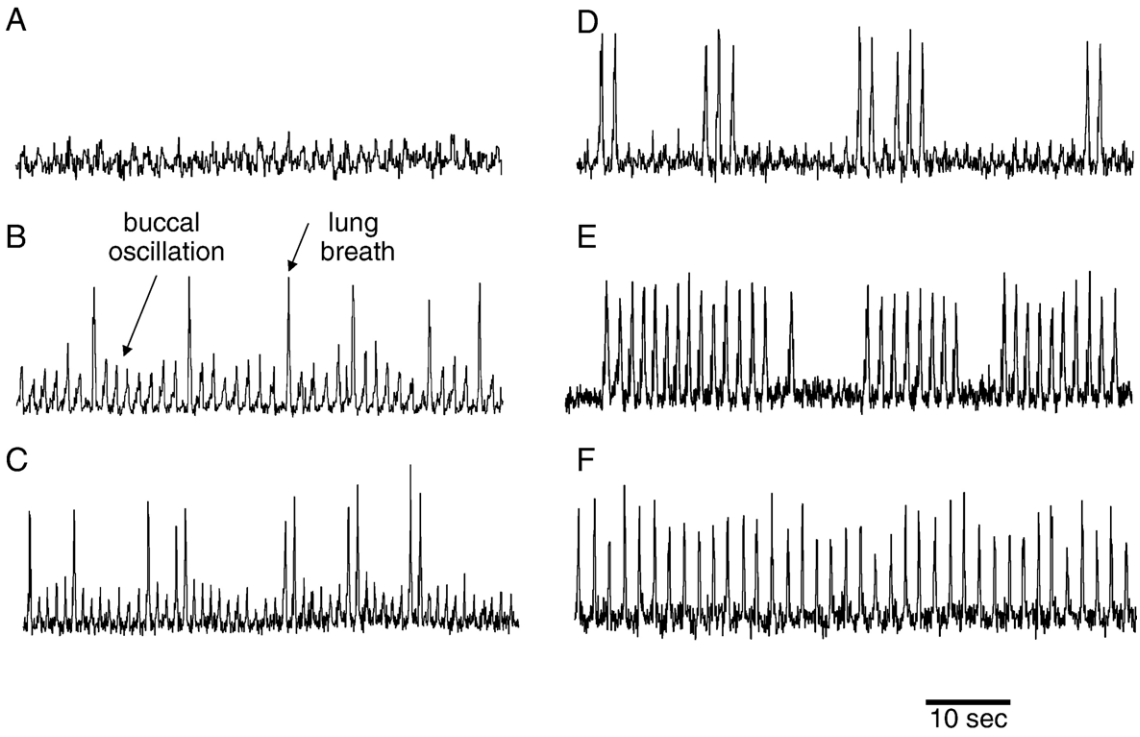

Fig. 2. Traces illustrating various parts of the anuran respiratory pattern continuum under progressively increasing tonic drive (due to slowly elevating CO_2) recorded in situ from the trigeminal nerve of a bullfrog. Small amplitude oscillations represent buccal ventilation while the larger amplitude oscillations in this figure represent lung breaths. Due to the time scale used for this figure, periods of prolonged apnea punctuated by isolated single breaths are not shown. (A) Buccal oscillations with no lung ventilation. (B) Evenly-spaced single lung breaths. (C) Single and doublet lung breaths. (D) Small episodes of variable length. (E) Large episodes of variable length. (F) Continuous lung ventilation (from Chatburn and Milsom, unpublished).

1996; Gradwell, 1971a,b). This activity is carried in the trigeminal, facial and vagus nerves (Vth, VIIth and Xth cranial nerves, respectively) innervating the oropharyngeal muscles (Gradwell, 1971a,b). It produces the continuous uni-directional flow of water from the mouth to the gill arches and over the gills described above. In tadpoles, there is a rostral to caudal burst sequence of gill motor output. Activity appears first in the Vth cranial nerve and is followed in sequence by bursts of equal time to peak in the VIIth and Xth cranial nerves (Gradwell, 1971a,b; Gdovin et al., 1998; Torgerson et al., 1998). In froglets, however, the burst cycles are not equal in each nerve, the time to peak of CN VII bursts is greater than the time to peak in CN V, X, and SN II bursts, and the time to peak of SN II burst activity is greater than that of CN V and X (Torgerson et al., 1998).

2.2. Air breathing in adults

2.2.1. Types of breaths

There are four basic types of breaths that make up the adult anuran breathing repertoire; buccal oscillations, balanced breaths, inflation breaths and deflation breaths. (1) Buccal oscillations ventilate the oropharynx alone. The nares remain open while the floor of the buccal cavity pumps air in and out of this cavity. No air enters or leaves the lungs. Balanced breaths, inflation breaths and deflation breaths ventilate the lungs. (2) Balanced breaths are ones in which pulmonary pressure and volume return to roughly similar levels at the end of each ventilation cycle. The same amount of air leaves and re-enters the lungs with each breath (De Jongh and Gans, 1969; Vitalis and Shelton, 1990). (3) Inflation breaths are ones in which the period of lung deflation is reduced or eliminated and thus more air enters the lungs than leaves. In certain species and/or under certain conditions, multiple breaths may occur in rapid succession without allowing time for lung emptying. This gives rise to a lung inflation cycle characterized by progressive increases in lung volume and pressure (West and Jones, 1975; Macintyre and Toews, 1976; Vitalis and Shelton, 1990; Sanders and Milsom, 2001). (4) Deflation breaths are ones in which more air leaves the lungs than is pumped back. Lung deflation cycles also occur and consist of a series of breaths in which pulmonary pressure and volume fall progressively over a series of breaths. More air is exhaled than is inspired with each successive breath (Vitalis and Shelton, 1990; Sanders and Milsom, 2001).

2.2.2. Breathing pattern

Anurans typically ventilate their buccopharyngeal cavity continuously (buccal oscillations) and the lungs intermittently (De Jongh and Gans, 1969; West and Jones, 1975; Vitalis and Shelton, 1990). Given the normally low metabolic rates of most adult anurans, it is not surprising that lung ventilation is rarely continuous. Various patterns of ventilation have now been described that form part of what can best be described as parts of a breathing pattern continuum that ranges from extensive periods of apnea with only occasional breaths to periods of continuous breathing.

In *Ranid* frogs, when respiratory drive is low, periods of apnea occur that can last hours and that are punctuated by isolated single breaths. As respiratory drive increases, the time between breaths decreases and breathing often begins to occur in episodes, usually of a few breaths each (Fig. 2C–D). As drive increases further, the number of breaths in each episode increases and the periods of apnea between them decrease (Fig. 2E). Eventually, if drive becomes high enough, breathing becomes continuous (Fig. 2F). The number of breaths per episode and the frequency of occurrence of episodes become controlled variables in these breathing patterns along with tidal volume and breathing frequency. And while it is true that most changes in respiratory drive that increase the number of breaths in each episode also tend to reduce the length of the apnea between episodes, these two variables can be controlled independently. Much of the time, however, the period of apnea is the major controlled variable in the breathing pattern (Boutilier and Toews, 1976; Milsom, 1991; Kinkead and Milsom, 1994; Kinkead, 1997).

Bufonid toads, on the other hand, have a more highly structured breathing sequence. Even under conditions of low respiratory drive they tend to breathe with episodes containing a large number of breaths (they are "biased" to the higher end of the breathing continuum). These episodes begin with a series of deflation breaths that progress into balanced breaths and end with an inflation sequence followed by a prolonged apnea (Fig. 3). These periods of apnea are generally much longer than those seen in ranids (Kruhøffer et al., 1987; Branco et al., 1992; Wang, 1994; Gamperl et al., 1999; Hou and Huang, 1999; Coelho and Smatresk, 2003).

The occurrence of inflation and deflation cycles (episodes of multiple inflation or deflation breaths in succession) varies from species to species as a function of the level of respiratory drive and behavioural state. Inflation cycles tend to be associated with defensive behaviours, preparation for vocalization, or increased

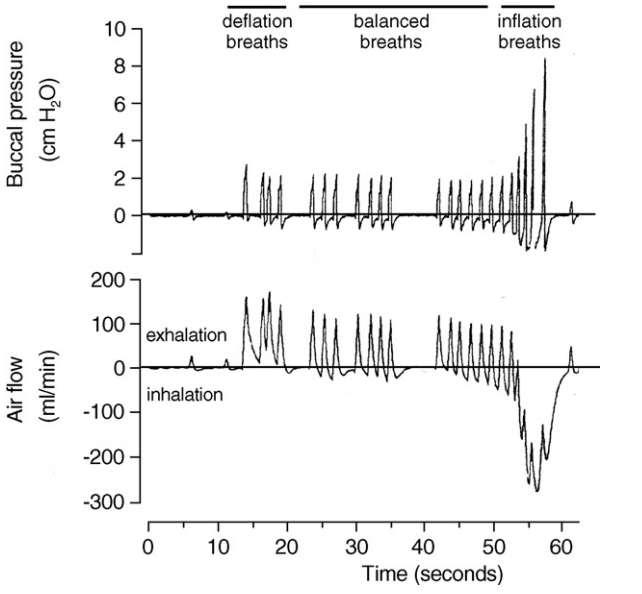

Fig. 3. Buccal pressures and nasal airflows in a hypoxic toad (*Bufo marinus*) during one breathing episode illustrating the series of deflation breaths followed by inflation breaths and culminating in a series of inflation breaths. (from Wang et al., 1994). Reproduced with permission from Comparative Biochemistry and Physiology A, Elsevier.

levels of respiratory drive (Macintyre and Toews, 1976; Pinder and Burggren, 1986; Vitalis and Shelton, 1990). They are more common in toads than frogs and this may reflect the more terrestrial life style of the toads (Hutchinson et al., 1968). Toads have decreased cutaneous gas exchange and to produce prolonged periods of apnea may require larger lung volumes for sufficient gas exchange. Frogs have a high cutaneous gas exchange capacity and their diving behaviour may necessitate smaller lung volumes (Hutchinson et al., 1968). Deflation cycles generally follow inflation cycles (usually after a prolonged period of apnea) (De Jongh and Gans, 1969; West and Jones, 1975; Vitalis and Shelton, 1990).

Given the size of the lungs relative to the size of the buccal cavity, it is not surprising that to fully inflate the lungs takes several buccal compressions in succession. What may be surprising is that anurans do not simply deflate their lungs by opening the glottis for a prolonged period. They do so by producing a series of purely expiratory breaths in succession. As a result, during these breaths, buccal expansion is reduced and buccal compression is associated only with emptying residual gas from the buccal cavity; it does not contribute to lung inflation. Thus, with inflation and deflation cycles, there can be breaths with no deflation and breaths with no inflation respectively, despite the fact that all breaths have an "inspiratory" and an "expiratory" phase with respect to buccal expansion/compression.

2.2.3. The respiratory pump

While the basic mechanism of amphibian gill ventilation is similar to that of fish, the mechanism of air breathing is very different from that of all other tetrapods. The buccal pump of the dual pump seen in larval anurans remains as the sole pump in most adult anurans (but see next section). The buccal pump may operate using either a two-stroke or a four-stroke mechanism. With the two-stroke buccal pump (used by most adult anurans) initial expansion (the first stroke) of the buccal cavity brings fresh air into the mouth (phase 1 in Fig. 4). This first phase of the lung breath (the buccal dilatory phase) is indistinguishable from the first phase of the buccal oscillation (De Jongh and Gans, 1969; Kinkead and Milsom, 1994; Kogo et al., 1994; Vasilakos et al., 2005). With a lung breath, the glottis then opens and gas from the lungs enters the buccal cavity where it mixes with the fresh air to varying degrees as it exits via the mouth and nares that remain open (phase 2 in Fig. 4). The nares and mouth then close and buccal compression (the second stroke) forces buccal gas into the lungs (phase 3 in Fig. 4). The glottis then closes and any excess gas left in the buccal cavity is expelled through the nares or mouth at the end of the buccal compression phase (phase 4 in Fig. 4). With the four-stroke buccal pump (found in *Xenopus* and a few other aquatic amphibians), the first buccal expansion (first stroke) draws gas from the lungs into the mouth. Buccal compression (the second stroke) then forces this

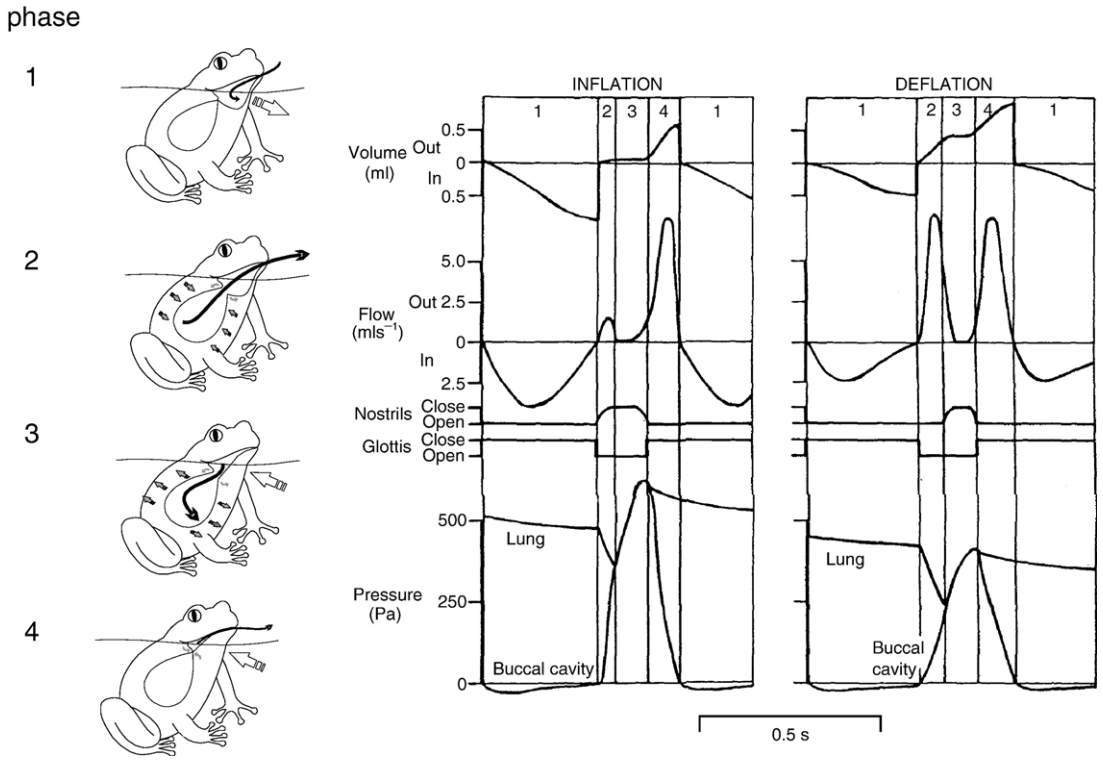

Fig. 4. Left hand panels: schematic diagram illustrating the ventilatory movements in an adult anuran. The ventilation cycle is divided into four phases as described in the text: (1) initial expansion (the first stroke) of the buccal cavity brings fresh air into the mouth; (2) the glottis opens and gas from the lungs enters the buccal cavity where it mixes with the fresh air to varying degrees as it exits via the mouth and nares that remain open; (3) the nares and mouth then close and buccal compression (the second stroke) forces buccal gas into the lungs; (4) the glottis then closes and any excess gas left in the buccal cavity is expelled through the nares or mouth at the end of the buccal compression phase. Right hand panels: summary diagrams illustrating the changes in lung volume, air flow at the nostrils, and pressures in the lungs and buccal cavity, along with the timing of nostril and glottis movement during an inflation and a deflation breath (from Vitalis and Shelton, 1990). Reproduced with permission from Journal Experimental Biology, The Company of Biologists Ltd.

gas out through the nares. Subsequent buccal expansion (the third stroke) draws in fresh air through the nares and buccal compression (the fourth stroke) then forces this air into the lungs.

The variety of breath types and ventilatory patterns exhibited by frogs and toads described above reflects the precise coordination of the neuromuscular events associated with the buccal force pump, the nares, and the glottis (Fig. 3) (West and Jones, 1975; Vitalis and Shelton, 1990). The buccal depressors are responsible for expansion of the buccal floor and are innervated by the sternohyoid branch of the hypoglossal nerve. The buccal levators facilitate compression of the buccal floor and are innervated by the mandibular branch of the trigeminal nerve and the main branch of the hypoglossal nerve. The muscles that facilitate movements of the glottis are the glottal dilators and constrictors, both innervated by the laryngeal branch of the vagus nerve (Sakakibara, 1984; Kogo et al., 1994).

Balanced breaths, lung inflation breaths, and lung deflation breaths are composed of four slightly different neuromuscular phases, the synchronization of which defines the type of breath. The right hand side of Fig. 4 illustrates the temporal sequence of neuromuscular and mechanical events and the resulting pressure and airflow changes associated with a deflation and an inflation lung breath in *Rana pipiens*. During both types of lung ventilation, in phase 1 (buccal inspiration) discharge from the sternohyoid branch of the hypoglossal nerve activates the buccal depressors causing the floor of the buccal cavity to lower and draws air in through the nares, while firing of the laryngeal branch of the vagus nerve activates the glottal constrictors sealing off the lungs. In phase 2 (lung emptying and expiration 1), while the nares remain open, discharge from the laryngeal branch of the vagus nerve causes glottal dilation allowing the air in the lungs to escape and the pressure between the lungs and the buccal cavity to equalize. In phase 3 (lung filling), while the glottis is still open and the nares are closed, discharge from the main branch of the hypoglossal nerve, and the mandibular branch of the trigeminal nerve causes buccal elevation, forcing the air from the buccal cavity into the lungs. In phase 4 (expiration 2), discharge from the laryngeal branch of the vagus nerve causes activation of the glottal constrictors, trapping the air and maintaining lung inflation while the remaining air in the buccal cavity passes out the nares. Ultimately, it is the volume of air that is expelled from, and subsequently pumped back into the lungs during the second and third neuromuscular phases of this cycle that determines whether the breath is inflation, deflation, or balanced in nature (Vitalis and Shelton, 1990). In other words, it is the timing of glottal opening/closing and narial opening/closing relative to buccal compression that determines the nature of the breath.

Neural discharge can be recorded from the nerves innervating the respiratory muscles and has been shown to closely coincide with the muscular and mechanical events responsible for buccal and lung ventilation in the intact animal (Sakakibara, 1984; Kogo et al., 1994). When this activity is recorded from the *in situ* paralyzed, decerebrate, unidirectionally ventilated (UDV) animal, or the *in vitro* isolated brainstem–spinal cord preparation, it is considered to represent the neural correlates of the ventilatory movements (De Jongh and Gans, 1969; Sakakibara, 1984). The neural discharge pattern associated with buccal oscillations occurs as high-frequency, low-amplitude, reciprocating bursts from the mandibular branch of the trigeminal nerve and the sternohyid branch of the hypoglossal nerve. The neural discharge associated with lung ventilation occurs in two phases. Remember that lung ventilation consists of 4 phases, where the first phase involves buccal dilation, and the third phase involves buccal compression. There are neural correlates to each of these phases. Lung ventilation begins with activity in the sternohyoid branch of the hypoglossal nerve which is often accompanied by a small amount of activity in Vm. This activity is associated with buccal dilation and is identical to that associated with a buccal oscillation (Vasilakos et al., 2005). This activity is followed by low-frequency, high-amplitude, simultaneous bursts from the mandibular branch of the trigeminal nerve, the main branch of the hypoglossal nerve and laryngeal branch of the vagus nerve (Kogo et al., 1994; Vasilakos et al., 2005). While the muscle groups recruited to produce buccal oscillations and lung breaths are essentially the same, the corresponding neural discharge patterns are considered to be distinct ventilatory rhythms (Fig. 2) (Wilson et al., 2002; see below).

The net result of these mechanical events is that a typical breath in an anuran consists of lung deflation followed by inflation and breath holding. The lung deflation occurs in the middle of the buccal cycle (between buccal expansion and compression) and is usually purely passive due to glottal opening (De Jongh and Gans, 1969). Glottal opening between buccal expansion and compression, however, does not always result in lung deflation, as during a lung inflation cycle (West and Jones, 1975; Vitalis and Shelton, 1990). And, while lung inflation is a result of buccal compression, buccal compression, even during a lung breath, does not always result in lung inflation, such as during a lung deflation cycle (Branco et al., 1992; Wang, 1994). Finally, air often exits the nares twice during the ventilation cycle, once (mostly from the lungs) at the end of the buccal expansion phase due to passive mechanisms, and again at the end of the buccal compression phase (mostly excess gas from the buccal cavity) (Vitalis and Shelton, 1990). As a result of these complexities, use of subjective terms such as inspiration/inhalation and expiration/exhalation can be problematic. Care must be taken to clearly define the nature and source of the power stroke(s) involved (buccal expansion/compression), the specific chamber being referred to (buccal, lung, etc.), and the path of air flow.

2.2.4. Active expiration and the origins of aspiration breathing

It has traditionally been believed that lung ventilation in amphibians, as just described, is similar to that in air-breathing fishes. Both use a buccal force pump employing expansion and compression of the mouth cavity with cranial and hypobranchial muscles. Reptiles, birds and mammals, on the other hand, use an aspiration pump that employs expansion of the thorax by axial muscles. Until recently there was no evidence of an intermediate mechanism. It has now been shown, however, that many amphibians (including *Xenopus*, an anuran) use axial muscles

for active expiration along with the buccal pump for active inspiration (see Brainerd, 1999 for review). This suggests that aspiration breathing evolved in two steps: (1) from buccal pumping alone to buccal pumping for inspiration and axial muscles for expiration and then (2) to aspiration breathing alone using axial muscles for both expiration and inspiration (Fig. 5). In the process, use of the buccal pump became reduced although it now appears that remnants of this behaviour remain in all tetrapods except, perhaps, the mammals (Brainerd, 1999). Importantly, this suggests that the ability to recruit axial musculature for exhalation is a shared-derived character found in all tetrapods. It further suggests that, despite our earlier warnings, modern amphibians may well serve as a model of the intermediate stages in the evolution of respiratory control.

3. Receptors and control of breathing

The regulation of breathing is dependent on the complex interaction of three components of the respiratory system: (1) the sensors, (2) the control centers and (3) the effector organs. In amphibians, receptors monitoring ventilatory function are located in the periphery and in the central nervous system (CNS). The former group includes the chemoreceptors and mechanoreceptors located in the vascular system, skin, airways and lungs while the latter group includes chemoreceptors located in the CNS. These receptors help to regulate the breathing pattern and also to assure appropriate matching of ventilation and perfusion at the interface between the respiratory medium and the blood. In this section we review studies on the involvement of receptors in the control of breathing in anuran amphibians.

3.1. O_2 receptors

Many anuran amphibians occupy environments that range from severely hypoxic (such as burrows at altitude), to hyperoxic (as can occur with daily changes in partial pressure of O_2 (PO_2) in some aquatic habitats) (Ultsch, 1973; Heisler et al., 1982). Thus, their respiratory demands can be diverse as can be the strategies they employ to match oxygen availability to tissue demand (Dejours, 1981).

In tadpoles oxygen may be acquired at multiple sites; gills, skin and/or lungs (Burggren and West, 1982; Burggren and Doyle, 1986; Burggren and Infantino, 1994). However, the interdependence between, and relative roles of the different exchange sites changes during ontogeny (Burggren and West, 1982; West and Burggren, 1984) (Fig. 6A). During the early stages of development, cutaneous uptake and gill ventilation are the principal pathways for oxygen acquisition. Most studies have shown that, as development proceeds, lung ventilation becomes more important until, at metamorphic climax (stages XVIII–XXIII), the gills degenerate and the lungs become the dominant site for O_2 exchange (Burggren and West, 1982; Burggren and Pinder, 1991). In one study, however, "surfacing events" did not increase across developmental stages, despite degeneration of the gills in late stage bullfrog tadpoles. The authors found that all stages breathed air, but air-breathing was not required for tadpoles to advance through their growth stages until stage XXII. They suggested that the animals breathed air, even when it was not required (1) to help lung development; (2) to avoid delayed metamorphosis and hypertrophy of the heart and to (3) to prevent lung collapse. Regardless, the data suggest that the ultimate increase in dominance of pulmonary gas exchange may not be tightly linked to loss of the gills alone (Crowder et al., 1998).

To determine the O_2 chemosensitive sites in tadpoles, Jia and Burggren (1997a,b) examined the ventilatory responses to hypoxia as well as to bolus injections of cyanide (NaCN) into the central circulation in larval bullfrogs before and after ablation of the first branchial arch. In non-ablated larvae, both internal and external stimuli increased gill ventilation. Ablation of the first gill arches eliminated the initial, rapid increase in gill ventilation upon changes in the PO_2 of inspired water and to the presence of cyanide, at all developmental stages (Fig 6B–D). Thus, these studies provided the first direct evidence that there are O_2 chemoreceptors in larval bullfrogs located on the first gill arch that are responsible for the initial rapid hypoxic ventilatory response. This was subsequently confirmed by Straus et al. (2001) who recorded directly from gill afferents exhibiting sensitivity to changes in P_{O_2}. A residual slow response remained, however, and was interpreted to arise from stimulation of a second population of receptors, possibly monitoring the cerebrospinal fluid. More recently, Winmill et al. (2005), using an isolated brainstem preparation, failed to find any evidence of a respiratory response to central hypoxia in premetamorphic tadpoles. Rather they found that metamorphic and adult preparations increased fictive lung burst episodes, at least initially, although the source of the stimulation was not explored. They also found that hypoxia then reversibly abolished respiratory-related activity. These investigators suggested that the reversible cessation of respiratory activity in the hypoxic metamorphic and adult bullfrog brain might be an adaptive, energy-saving response mediated by a brainstem oxygen sensor.

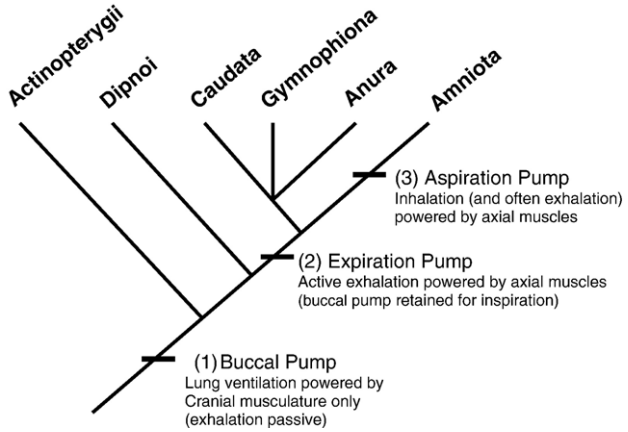

Fig. 5. Simplified cladogram of vertebrate relationships with the acquisition of aspiration breathing characters mapped onto it. Ray-finned fishes (Actinopterygii) and lungfishes (Dipnoi) utilize a buccal pump for lung ventilation. Both salamanders (Caudata) and amniotes use the transverse abdominis for exhalation (expiration pump). Amniotes inhale using an aspiration pump (from Brainerd, 1999). Reproduced with permission from Experimental Biology, Springer Science and Business Media.

It is now well established that the hyperventilation induced by hypoxia in adult anurans primarily results from activation of peripheral chemoreceptors (for review see Van Vliet and West, 1992). The first chemoreceptors to be described in amphibians were situated within the carotid labyrinth at the bifurcation of the internal and external carotid arteries (Adams, 1958). Information from the carotid labyrinth is carried along the glossopharyngeal nerve (the ninth cranial nerve) to the nucleus of the solitary tract (Stuesse et al., 1984). The output from this homologue of the mammalian carotid body (and the larval first gill arch) is thought to provide information that allows immediate regulation of breathing by the respiratory centre in the medulla. As with other tissues associated with O_2 chemoreception, the carotid labyrinth has a characteristic association of glomus cells (type I cells or chief cells), supporting cells (type II or sustencular cells), other cell types and nerve terminals (Fig. 7) (Rogers, 1963). The ultrastructural characteristics of the glomus cells change during metamorphosis in a manner that suggests that the glomus cells only begin to contribute to chemoreception immediately after metamorphosis (Kusakabe, 1992). In a recent study, Kusakabe (2002) reported that there are differences in the ultrastructure of the carotid labyrinth between anuran species suggesting that the carotid labyrinth of the genus *Xenopus* may be a more primitive form of this organ. These data are consistent with studies suggesting that *Xenopus* occupies a lower position on the evolutionary tree than other anurans (Cannatella and Trueb, 1988). Interestingly, Kusakabe et al. (1993) have also reported that when rats are exposed to long-term hypoxia (10–12 weeks) there is an enlargement of the carotid body, primarily due to vascular enlargement under peptidergic control. As a result, the hypoxic rat carotid body becomes very similar in appearance to the amphibian carotid labyrinth (Fig. 7). In a later study Kusakabe et al. (1998) observed that the immunohistochemical affinity for neuropeptides in the hypoxic rat carotid body was also extremely similar to that of the amphibian carotid labyrinth. Arterial PO_2 is low in amphibians by mammalian standards and these authors suggested that the similarities between the hypoxic mammalian carotid body and the normoxic amphibian carotid labyrinth might reflect general features of hypoxia adaptations that facilitate both oxygen uptake from blood and release of catecholamines into the blood by glomus tissue.

The suggestion that the carotid labyrinth has a chemoreceptor function was initially based on histological evidence (Boissezon, 1939; Chowdhary, 1951; Carman, 1955; Rogers, 1963, 1966; Banister et al., 1967) and on denervation studies (Smyth, 1939). By means of electrophysiological recordings, subsequent studies have confirmed that the carotid labyrinth can detect changes in arterial blood pressure, O_2 (Ishii et al., 1966) and CO_2/H^+ (Van Vliet and West, 1992). More recent studies have shown that the specific stimulus modality for the carotid labyrinth is arterial PO_2 (PaO_2) (Wang et al., 1994, 2004; Andersen et al., 2003). Toads breathing normoxic air did not increase ventilation when oxygen content was reduced by

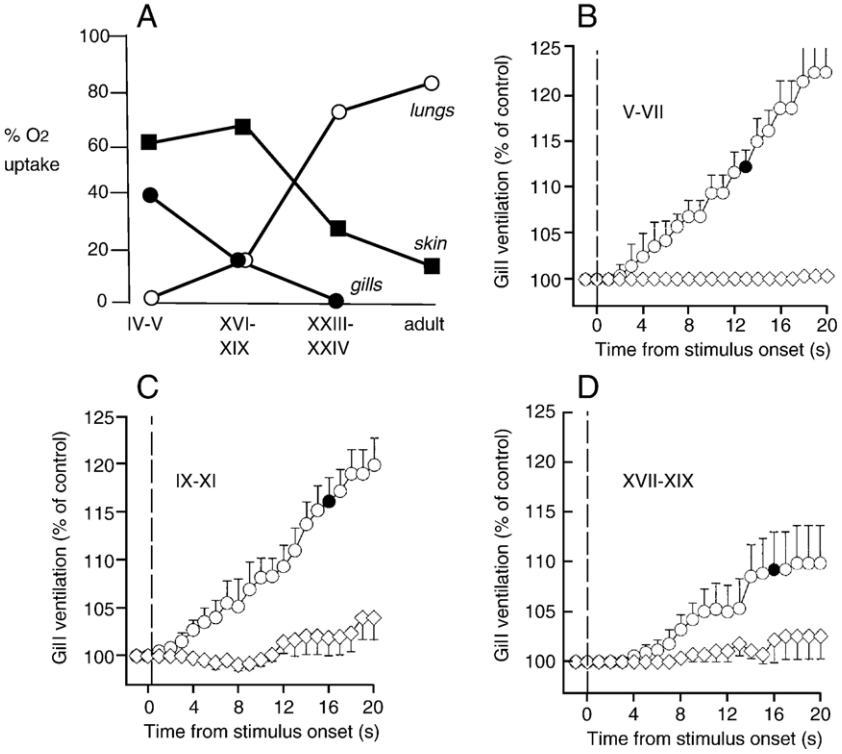

Fig. 6. (A) Change in the % of total O_2 taken up across the skin, gills and lungs during metamorphosis at 20 °C in the bullfrog. (B–D) Changes in gill ventilation frequency in tadpoles of various ages to a step-decrease in inspired water PO_2 (from normoxia to hypoxia at the broken vertical line in each panel) before (open circles) and after (open diamonds) denervation of the first gill arch. The onset of a significant change from time 0 is indicated for each data set by a single filled symbol (from Burggren and West, 1982; Jia and Burggren, 1997a,b). Reproduced with permission from Journal Experimental Biology, The Company of Biologists Ltd.

anaemia or carbon monoxide inhalation (Wang et al., 1994; Branco and Glass, 1995) although heart rate increased (Wang et al., 1994; Andersen et al., 2003). By independently infusing cyanide into both carotid and pulmonary circulations, Wang et al. (2004) then showed that systemic arterial receptors sensitive to PaO_2, were responsible for ventilatory control, while pulmonary arterial receptors, sensitive to oxygen content, were primarily responsible for cardiovascular responses to hypoxia.

Bilateral denervation of the carotid labyrinths decreases pulmonary ventilation but does not abolish the respiratory sensitivity to hypoxia in terrestrial or aquatic toads (Jones and Chu, 1988; West et al., 1987), suggesting that chemoreceptors within the carotid labyrinth may contribute to, but are not solely responsible for, the hypoxemic ventilatory drive in anurans. Special cells closely resembling the glomus cells in the carotid labyrinth have been found in the aortic wall of the toads *Bufo vulgaris* (Ishii et al., 1985) and *Bufo marinus* (Van Vliet and West, 1992). Chemoreceptors also appear to be present on the pulmocutaneous artery (Hoffmann and de Souza, 1982; Wang et al., 2004). Thus, injection of NaCN into the pulmocutaneous arches of conscious toads and anesthetized bullfrogs stimulated ventilation (Lillo, 1979; Hoffmann and de Souza, 1982; Van Vliet and West, 1987). However, the relative contribution of the aortic and pulmocutaneous chemoreceptive sites to ventilatory and cardiovascular control remains to be quantified.

In summary, it would appear that chemoreceptors on the first gill arch predominate in larval anurans while those in the carotid labyrinth predominate in the adults. Given that the carotid labyrinth is believed to be a homologue of the chemoreceptors from the first gill arch, it is intriguing to ponder what occurs in terms of chemoreceptor location and O_2 sensitivity at metamorphic climax (stages XVIII–XIX) when the gills are degenerating and the lungs are becoming the dominant site for O_2 exchange.

3.2. CO_2 receptors

Progressive larval development is associated with a transfer of the site of ventilatory exchange from the gills to the lungs, and is accompanied by the emergence of CO_2 as a source of the respiratory drive (Torgerson et al., 1997). There is a shift in the control system from one that is almost entirely oxygen-driven to one that is both oxygen and acid–base/CO_2-driven (Macintyre and Toews, 1976; Smatresk and Smiths, 1991; Burggren and Pinder, 1991; Branco et al., 1992, 1993; Kinkead and Milsom, 1997; Torgerson et al., 2001; Wang et al., 1999b, 2004).

There are conflicting reports in the literature regarding the onset of ventilatory responses to CO_2 in early stage tadpoles. While Infantino (1992) reported that hypercapnic challenge evoked no change in gill ventilation in early anuran larvae

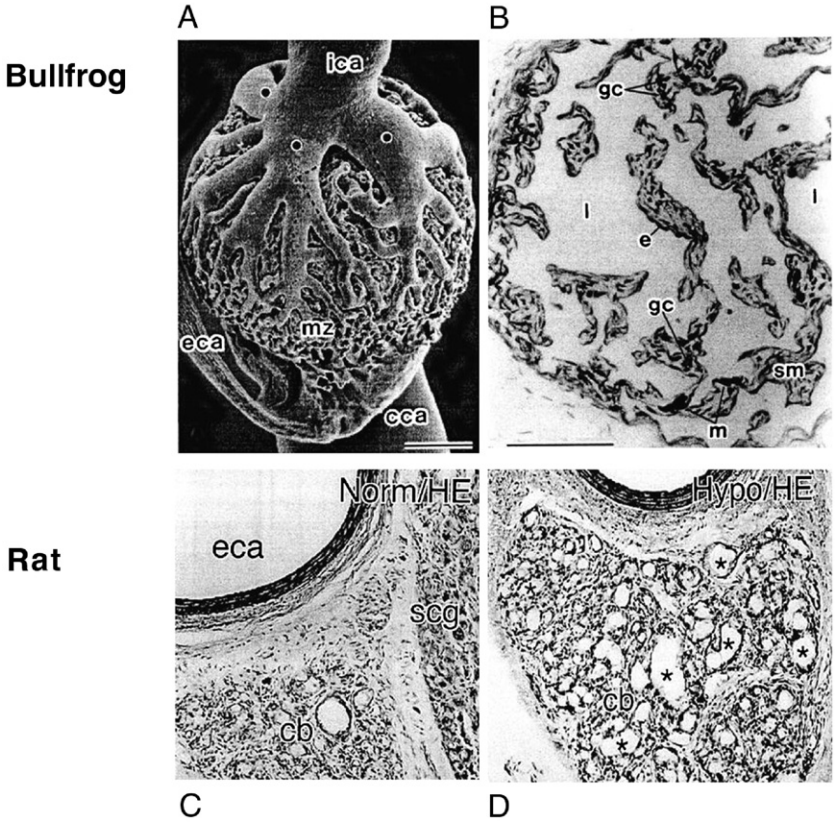

Fig. 7. (A) Scanning electron micrograph of a vascular resin cast of the left carotid labyrinth in *Rana catesbeiana*. Scale bar=200 μm. cca, common carotid artery; eca, external carotid artery; ica, internal carotid artery; mz, vascular maze (from Kusakabe, 1990). (B) Semithin section from the centre of the carotid labyrinth of *Rana catesbeiana* showing the maze like arrangement of blood vessels and the large sinusoidal spaces (e, endothelial cells; gc, glomus cells; l, lumen of sinusoid; m, melanophore; sm, smooth muscle. Scale bar=50 μm (from Kusakabe et al., 1995). (C–D) Comparison of semithin sections from the centre of a normoxic (C) and a hypoxic (D) rat carotid body. Note the increase in the volume of the hypoxic carotid body and the enlargement of the vasculature (*) (from Kusakabe et al., 1998).

(stages IX–XIV), Torgerson et al. (1997) reported that gill ventilation responded vigorously to central hypercapnic stimulation in premetamorphic larvae (stages X–XIX) but not in metamorphic animals, and Taylor et al. (2003a,b) found hypercapnic lung ventilatory response to be present from the earliest stages of bullfrog development. As for the onset of lung ventilatory responses to CO_2, both Torgerson et al. (1997) and Infantino (1992) reported that CO_2 only stimulated lung ventilation after metamorphosis when the lungs became the predominant site for gas exchange. The discrepancies between these various studies may reflect differences in the level of CO_2 used in each study or differences in the preparations used (intact versus isolated preparations). Whatever the cause, the overwhelming evidence suggests that central chemoreceptor reflexes shift from regulating gill ventilation to regulating lung ventilation just prior to metamorphosis from the aquatic larval stage into the air breathing adult form. Interestingly, the evidence also suggests that while central CO_2 chemosensitivity arises from both caudal and rostral sites in the medulla of premetamorphic tadpoles, it becomes concentrated at the rostral sites postmetamorphosis (Torgerson et al., 2001).

In adult anuran amphibians, central respiratory CO_2 chemoreceptors have been clearly documented and are thought to be distributed throughout the medulla, surrounding the fourth ventricle (Smatresk and Smiths, 1991; Branco et al., 1992). In intact anaesthetized toads, the central chemoreceptors contribute about 80% of the hypercapnic respiratory drive (Branco et al., 1992), suggesting a dominant role in the ventilatory acid–base regulation. Nevertheless, in *in vitro* brainstem preparations, varying the pH/CO_2 of the superfusate does not elicit the same magnitude of response (Kinkead et al., 1994; McLean et al., 1995a,b; Reid et al., 2000; Morales and Hedrick, 2002), suggesting that central chemoreception is modulated by peripheral feedback (see Section 3.4 below).

In mammals, these receptors were once thought to be located only on the surface of the ventral medulla but now it is thought that they are probably distributed more widely. Recently, sites have been identified in the ventrolateral medulla, nucleus of the solitary tract, ventral respiratory group, *locus coeruleus*, caudal medullary raphe, and fastigial nucleus of the cerebellum that are pH/CO_2 sensitive although it remains to be determined to what extent each is involved in the control of breathing (for a review, see Nattie, 2001; Richerson, 2004; Mulkey et al., 2004). It also remains unclear if the same is true for amphibians although a recent study by Noronha-de-Souza et al. (in press) provides evidence (via c-fos expression, catecholaminergic neuron lesioning and focal acidosis) that the area in the toad brainstem homologous to the locus coeruleus is chemosensitive suggesting that non-mammalian vertebrates may possess multiple sites of central chemosensitivity also.

In addition to central CO_2 chemoreceptors, several peripheral sites of CO_2/pH sensing have also been reported. Although there have been many studies about the CO_2-drive to breathe in amphibians, few have focused on the role of these peripheral receptors. As noted earlier, the carotid labyrinth can detect changes in CO_2/H^+ (Van Vliet and West, 1992) although the relative role of the labyrinth in eliciting ventilatory responses has not been specifically documented. Olfactory receptors sensitive to CO_2 powerfully inhibit breathing in unanesthetized bullfrogs (Sakakibara, 1978; Coates and Ballam, 1990). These receptors are relatively rare, and are stimulated by CO_2 concentrations ranging from 0.4 to 4% to produce a reflex inhibition of breathing (Coates, 2001). Sakakibara (1978) showed that transection of the olfactory nerves of the bullfrogs eliminates the ventilatory response to upper airway CO_2, whereas transection of the trigeminal nerve does not affect the response, suggesting that CO_2 receptors are located in the sensory olfactory epithelium innervated by the olfactory nerves. Additionally, Coates et al. (1998) reported that inhibition of carbonic anhydrase (CA) causes an immediate 65% reduction in the response of the electro-olfacto-gram to CO_2. These results, along with the histochemical localization of CA in some olfactory receptor neurons, indicate that CA plays a role in the detection of CO_2 in frog olfactory neurons and that only a small population of olfactory receptor neurons are CO_2 sensitive. It has been postulated that stimulation of olfactory receptors by changes in CO_2/H^+ might function to inhibit breathing when the ambient partial pressure of CO_2 is higher than the level in systemic blood, reducing CO_2 uptake while the animal seeks fresh air (Coates and Ballam, 1987; Milsom et al., 2004).

Finally, in adult anurans, pulmonary stretch receptors (PSR), for which the primary stimuli are changes in the lung volume, pressure or wall tension, are also CO_2 sensitive (Milsom and Jones, 1977; Fedde and Kuhlmann, 1978; Kuhlmann and Fedde, 1979). The information conveyed by PSR reports to the CNS via afferent fibers in the vagus nerve (Milsom and Jones, 1977; Fedde and Kuhlmann, 1978; Kuhlmann and Fedde, 1979). These receptors are mostly slowly adapting and decrease their firing rates as pulmonary concentrations of CO_2 increase (Milsom and Jones, 1977). The addition of 10% CO_2 to the lungs of *Rana pipiens* reduced by 50% the discharge frequency of 20 to 30% of the PSR (Milsom and Jones, 1977). On the other hand, in *Rana catesbeiana*, most receptors reduced discharge frequency when lungs were ventilated with only 4% CO_2 (Fedde and Kuhlmann, 1978; Kuhlmann and Fedde, 1979). According to these authors, a large group of receptors are refractory to CO_2 as a stimulus, but respond when intrapulmonary pressure is increased, suggesting that an interaction exists between the two sensory modalities (see Section 3.4 below). The combined chemosensitivity of PSR in frogs led Milsom and Jones (1977) to suggest that these receptors may represent the functional precursor of the highly CO_2 sensitive intrapulmonary chemoreceptors found in birds (Scheid and Piiper, 1986) and the relatively CO_2 insensitive lung mechanoreceptors found in mammals (Mitchell et al., 1980).

3.3. Mechanoreceptors

Pulmonary stretch receptors (PSR) in amphibians monitor the rate and degree of inflation and deflation of the lungs and project to the nucleus of the solitary tract through the vagus nerve (Milsom and Jones, 1977; Fedde and Kuhlmann, 1978; Kuhlmann and Fedde, 1979; Wang et al., 1999b). There are three different types of PSR in amphibians: (1) proportional

sensitive receptors that respond to the degree of tonic lung inflation, (2) rate sensitive receptors that respond to the rate of phasic changes in volume, and (3) receptors that respond to both stimuli (Milsom and Jones, 1977).

Although it is generally believed that amphibians possess a Hering–Breuer reflex as seen in other vertebrates (activation of stretch receptors suppresses inspiration and enhances expiration) (Tenney, 1979), there has been some controversy surrounding this issue. Thus, while there have been studies suggesting that lung inflation inhibits breathing and lung deflation stimulates breathing (De Marneffe-Foulon, 1962; Shelton and Boutilier, 1982; Wang et al., 1999a), there have also been studies suggesting the opposite (Kinkead et al., 1994; Kinkead and Milsom, 1996, 1997; Reid and Milsom, 1998), and a few studies that have obtained both results (Kogo and Remmers, 1994; Kogo et al., 1994; Reid et al., 2000). Many of these studies, however, have involved the analysis of anuran respiration in reduced preparations that monitor fictive breathing from eletroneurograms from the mandibular branch of the trigeminal nerve. This nerve innervates the buccal elevator and depressor muscles in the oropharyngeal region of anurans that are responsible for inducing contraction of the bucopharyngeal muscles that pump air into the lungs (Sakakibara, 1978). By analysis of activity in the trigeminal nerve alone, however, it is not possible to distinguish small lung breaths from buccal oscillations. To do this, it is essential to analyze the activity of the vagus nerve, which innervates the glottis, to confirm when lung ventilation occurs. When this is done (Sanders and Milsom, 2001), it appears that lung deflation produces infrequent, large-amplitude inflation breaths or cycles while progressive lung inflation changes the breathing pattern to one of high-frequency attempts to deflate the lungs that are largely passive, and accompanied by contractions of the buccal pump no larger than those associated with normal buccal oscillations (Fig. 8). These observations would suggest that anuran amphibians possess both Hering–Breuer inflation and deflation reflexes that take on unique characteristics associated with the deflation and inflation cycles seen in their breathing patterns, respectively.

These studies just described focused more on the role of tonic (prolonged) changes in lung volume on breathing pattern. In earlier studies, Kinkead and Milsom (1997) concluded that tonic and phasic PSR feedback had identical effects on breathing pattern suggesting that phasic input contributed little that was unique to ventilatory control. Recently, Reid and West (2004) analyzed the effects of the phasic feedback from PSR that occurs within a breath on breathing pattern. Their study showed that increasing phasic receptor feedback increased overall breathing frequency by increasing the number of breaths occurring in individual breathing episodes without altering the frequency of breathing within an episode (i.e. it prolonged the episode), and that this was influenced by the timing of that feedback with respect to the breath (Reid and West, 2004). The reasons for these discrepant results are not clear but may reflect differences in the procedures used in the two studies. In the study by Kinkead and Milsom (1997), phasic PSR feedback was provided by deflation of the lung from an inflated state while in the study of Reid and West (2004) it was provided by inflation of the lungs from a deflated state. This may suggest that the role of PSR feedback is reduced with increasing levels of tonic receptor feedback but this intriguing idea remains to be tested.

Finally, in anuran larvae, West and Burggren (1983) provided evidence that bullfrog tadpoles also possess pulmonary receptors that respond to changes in lung volume. Stimulation of these receptors with air, N_2 or O_2, however, reduced gill (not lung) ventilation frequency. The authors concluded that both lung inflation *per se* and the resulting

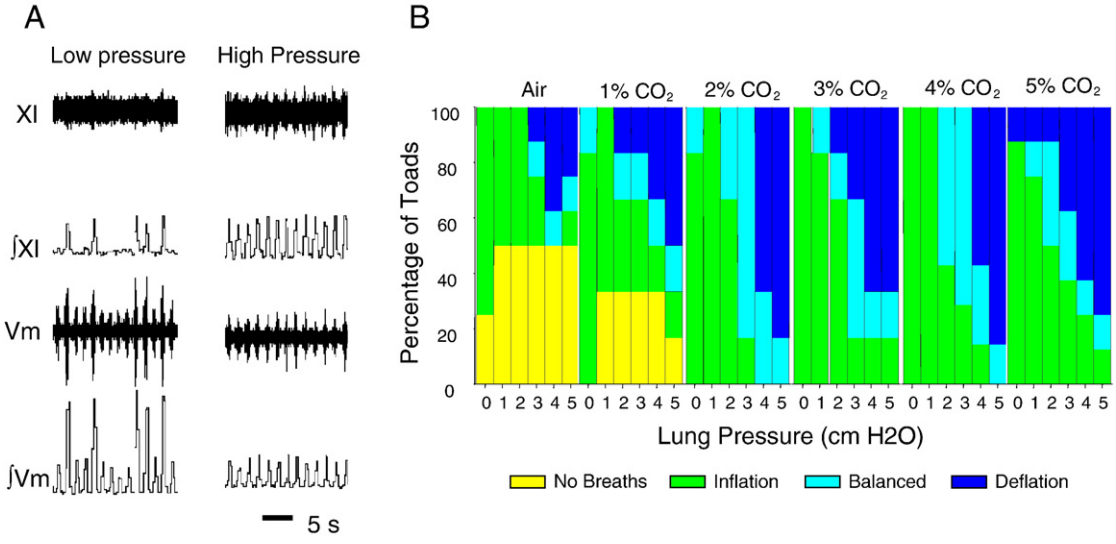

Fig. 8. (A) Recordings of raw and integrated (∫) electroneurograms from the laryngeal branch of the vagus (Xl) and the mandibular branch of the trigeminal nerve (Vm) in a bull frog with low (1 cm H_2O) and high (5 cm H_2O) inflation pressure. All oscillations in Xl were associated with lung breaths. Note that small oscillations in Vm could be associated with either buccal oscillations only (low pressure) or with lung ventilations (high pressure) (from Sanders and Milsom, 2001). (B) Proportion of toads (*Bufo marinus*) exhibiting inflation, balanced and deflation breaths under different degrees of lung inflation and respiratory drive) (from Sanders and Milsom, unpublished).

increase in PO_2, contribute to the suppression of gill ventilation following spontaneous air breathing, which serves to limit O_2 loss across the gills in aquatic hypoxia.

3.4. Interactions between mechanoreceptors and chemoreceptors

The respiratory system utilizes a host of different sensory receptor groups, including chemoreceptors and mechanoreceptors, to provide feedback to the CNS concerning the effectiveness of gas exchange. In general, ventilatory responses to altered receptor inputs involve an interaction between several receptor groups, central integrative mechanisms, and other modulatory inputs (Mitchell et al., 1990). In anuran amphibians there are few studies that have examined the interaction between chemo- and mechanoreceptors and how these modulate breathing pattern and ventilatory reflexes. West et al. (1987), investigating the role of the carotid labyrinth in intact and labyrinth-denervated toads, first suggested that stimulatory effects of hypoxia and hypercapnia on ventilation are interactive. More recently, Wang et al. (2004) reported that the fictive ventilatory responses to cyanide injections into both pulmonary and carotid circulations were increased by hypercapnia. According to these authors the interaction between O_2 and CO_2 chemoreceptor inputs may occur peripherally or centrally at integrative sites. However, the mechanism of this interaction is still unknown and deserves further study.

Additionally, there are interactions between PSR feedback and the respiratory drive provided by hypoxia and hypercapnia (as noted above). Several authors have shown that vagotomy in bullfrogs and cane toads blunts or abolishes the increase in breathing frequency displayed during hypercapnia (Kinkead and Milsom, 1996, 1997; Reid et al., 2000) and that increasing levels of CO_2 alter the ventilatory response to lung inflation/deflation (there is a positive interaction on breathing frequency between the level of CO_2 and the level of lung inflation, and an increasing tendency to produce deflation breaths) (Wang et al., 1999a, 2004; Sanders and Milsom, 2001) (see Sections 3.2 and 3.3; Fig. 8). Recently, it has been shown that PSR feedback also interacts with peripheral O_2 receptor input (Wang et al., 2004). This study demonstrated that lung inflation decreased the effect of cyanide injection and in several animals the cyanide injections failed to elicit any response when the lungs were fully inflated. Unfortunately, it is not possible to draw any firm conclusions concerning exactly where these interactions are mediated.

3.5. Nucleus isthmi and the hypoxic and hypercapnic-drive to breathe

Little is known about the role of CNS in the modulation of ventilatory responses to hypoxia and hypercapnia. To date, the only brain structure that has been investigated in this regard is the nucleus isthmi (NI). The role of this structure in modulating ventilatory responses was recently reviewed by Gargaglioni and Branco (2004).

The NI is a mesencephalic structure located in the amphibian brain between the roof of the midbrain and the cerebellum, which differentiates during metamorphosis, the period when pulmonary ventilation develops in bullfrogs (Senn, 1972). In an early study, lesion of this area with kainic acid (Kinkead et al., 1997) attenuated the increase in fictive breathing induced by hypercapnia. On the other hand, more recent studies demonstrated that both electrolytic and ibotenic acid lesions of the NI increased the ventilatory response to hypercapnia (3% inspired CO_2) and also to hypoxia (7% and 5% inspired O_2) suggesting that the NI inhibits both hypoxic and hypercapnic ventilatory response in toads (Gargaglioni and Branco, 2000; Gargaglioni et al., 2002). The differences between these studies may reside in the fact that kainic acid is an excito-toxin that stimulates cells (especially at glutamatergic synapses) for a prolonged period before it kills them (Watanabe et al., 1987). Since Kinkead et al. (1997) performed their experiments only 1.5 h after the administration of kainic acid, NI neurons may still have been activated, rather than chemically lesioned. The differences between studies may also arise from the fact that ibotenic acid produces more selective and limited lesions than kainic acid (Guldin and Markowitsch, 1982). The increased ventilation observed after hypoxia and hypercapnia in ibotenic lesioned toads resulted from an elevated tidal volume. Thus, the NI appears to provide an inhibitory input to respiratory sites, limiting breathing amplitude when the respiratory drive is high, possibly acting as a relay site for PSR information. There may be a number of putative mediators for these responses, but so far only glutamate and nitric oxide have been shown to be involved (Gargaglioni and Branco, 2001, 2003).

More recently, studies have examined the role of the amphibian *locus coeruleus* in the modulation of the hyperventilation induced by hypercapnia (Noronha-de-Souza et al., in press). The *locus coeruleus* (LC) is a noradrenergic pontine nucleus that has been proposed to act as a chemoreceptive site in mammals (for review see Nattie, 2001). In amphibians, the LC is located in the isthmus region at the rostral end of the hindbrain and is considered to be homologous to the LC of mammals (Marin et al., 1996). 6-hydroxydopamine (6-OHDA, a toxin that kills catecholaminergic neurons) lesions placed into the LC of the toad *Bufo paracnemis* reduced the hyperventilation induced by hypercapnia, suggesting that the LC modulates the hypercapnic ventilatory response in an excitatory fashion (Noronha-de-Souza et al., in press). Clearly, further investigation is required to elucidate the role of CNS sites in the modulation of ventilatory responses to hypoxia and hypercapnia in amphibians.

4. Central control of breathing

4.1. Central rhythm generation

The basis of central respiratory rhythm generation in mammals has been under consistent investigation since 1923 when Lumsden first demonstrated a progression from eupnea to apnoea following transections through the cat brainstem (Lumsden, 1923). A critical role for rhythm generating neurons in the medulla is now generally accepted in mammals and other vertebrates (Rekling and Feldman, 1998; Feldman et al., 2003)

and recent studies using neonatal rats suggest that two coupled rhythmogenic networks may be involved in the generation of the mammalian respiratory rhythm (Mellen et al., 2003; Onimaru and Homma, 2003; Janczewski and Feldman, 2006a,b; Feldman and Del Negro, 2006; Onimaru et al., 2006) Over the last decade, several studies have suggested that inspiratory (I) neurons (i.e., neurons that fire during inspiration) located within a small portion of the rostral medulla called the preBötzinger complex (preBötzC; Smith et al., 1991; Gray et al., 1999) are particularly important in mammalian respiratory rhythmogenesis. In addition to the I neurons within the preBötzC, pre-I neurons located within and rostral to the preBötzC, originally described and characterized by Onimaru et al. (1995), also appear to participate in rhythm generation. These neurons lie within the parafacial region, fire immediately before inspiration and are coupled to the neurons within the preBötzC (Onimaru and Homma, 2003). Recently, Janczewski and Feldman (2006a) demonstrated that these two rhythms are normally coupled but can function independently to spawn inspiration and active expiration in juvenile rats. Additionally, the authors point out that the preBötzC is essential for the respiratory rhythm generation, since breathing in mammals is dominated by inspiration. Another view hypothesizes that pre-I neurons in the parafacial respiratory group determines the respiratory activity in mammals (Onimaru et al., 2006). Further investigation will be necessary to elucidate the function and interaction between the two regions.

The concept of coupled respiratory rhythmogenic networks in mammals is consonant with results from nonmammalian vertebrates. Multiple neural networks which operate as a unit when the brain is intact have been described in lampreys, skates, teleost fish, anuran amphibians and chicks (Hyde, 1904; Rovainen, 1983; Thompson, 1985; Russel, 1986; Fortin et al., 1995, 1999; Champagnat and Fortin, 1997; Sundin et al., 2000; Wilson et al., 2002). In adult lampreys, central respiratory rhythm generation has been attributed to a medullary system comprised of two pairs of oscillators and the motoneurons with which they synapse (Thompson, 1985; Russel, 1986). The most rostral pair, which predominate in normal breathing, lie in the trigeminal region of the medulla, whereas a second pair, that generate low amplitude excitatory output termed “coughs”, “arousal breathing” and “weak breathing” lie in the vicinity of the facial, glossopharyngeal and vagal motor nuclei (Rovainen, 1983; Thompson, 1985). In fish (Chondrichthyes and Osteichthyes) there is also evidence for multiple respiratory rhythm generators in the medulla, as well as for a diffusely (rostral–caudal) distributed respiratory rhythm generating network (Shelton, 1961; Ballintijn, 1987). Finally, Fortin et al. (1995), using transverse slices of the hindbrain of the chick embryo have shown that there are multiple paired populations of neurons within the medulla in birds that are able to generate rhythmic respiratory activity in isolation. The chick data suggest that each pair of rhombomeric segments contains the necessary

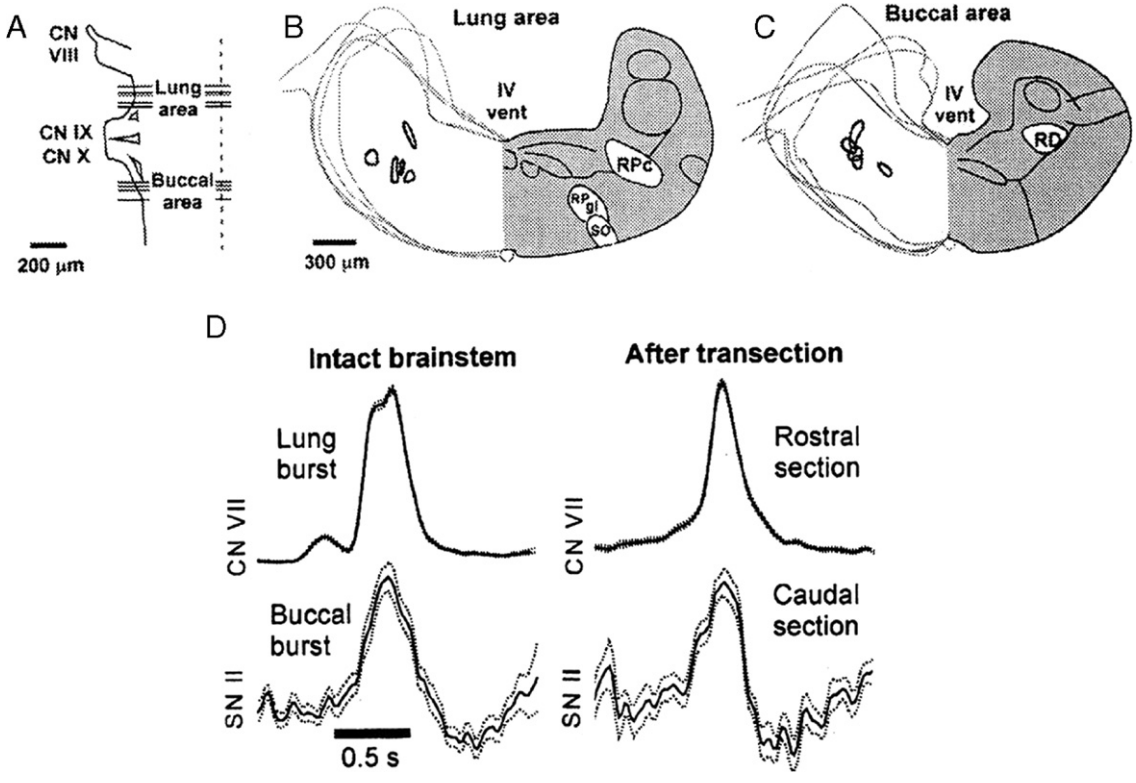

Fig. 9. Rhythmogenic sites in the bullfrog brainstem. (A) horizontal lines illustrate the level of the transverse slices shown to the right. (B) and (C) are outlines of 5 transverse slices from different brainstems containing the areas associated with lung and buccal oscillations respectively. Nearest anatomical landmarks are illustrated on the right (Vent, ventricle; RPc, reticularis parvocellularis; RPgl, reticularis paragigantocellularis lateralis, SO, superior olive, RD, reticularis dorsalis). (D) Comparison of discharge in the rostral (cranial nerve VII) and caudal (spinal nerve II) brainstem sections before and after transection to separate them. Each trace from SNII shows the average of 25 bursts (continuous line)±S.E.M. (dotted lines) (from Wilson et al., 2002). Reproduced with permission from Journal of Physiology, Blackwell Publishing.

circuitry to generate respiratory rhythm early in development (Fortin et al., 1995).

Multiple paired rhythm generating sites have now been described in anuran amphibians as well. Presently, there are numerous studies in amphibian tadpoles and adult frogs that suggest that the endogenous respiratory rhythm is generated by the medulla (Langendorff, 1887; McLean et al., 1995a; Reid and Milsom, 1998; Reid et al., 2000; Torgerson et al., 2001). Studies in which GABA (Gamma Amino Butyric Acid, the major inhibitory neurotransmitter in the brain) and AMPA [(±)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid, a potent excitatory amino acid that interacts selectively with certain glutamate receptors in the brain] (i.e. inhibitory and excitatory neurotransmitter/modulators respectively) have been microinjected into the medulla of bullfrogs have located two specific rhythmogenic sites within the ventral medullary reticular formation that appear to be involved in the generation of endogenous respiratory activity; one between the VIIth and IXth cranial nerves, and the other at the level of the vagus nerve root (McLean et al., 1995b; Wilson et al., 2002) (Fig. 9).

It should be noted that these paired rhythm generators are both involved in producing lung ventilation. While only one appears to be involved exclusively with ventilation of the buccal cavity, both appear to be involved with the production of lung breaths (Vasilakos et al., 2005). Given the mechanical events associated with these activities, the distinction between a buccal oscillation and a lung breath primarily involves differences in the force of muscle contraction and control of glottal versus narial opening and closing. In adults, buccal oscillations usually generate less force and are performed with the nares open and the glottis closed while lung ventilation may require more force (balanced or inflation breaths only) and involves complex timing of glottal opening and narial closing, depending on whether the breath results in net lung inflation or deflation (see Sections 2.2.1 and 2.2.3). In the premetamorphic tadpole the rhythm generating circuitry for lung ventilation appears to be placed in the more caudal region, just caudal to cranial nerve X (Torgerson et al., 2001). During metamorphosis to the adult form, however, as ventilatory function shifts from gills to lungs, the site of lung rhythmogenesis switches from the caudal to the rostral brain stem site (Torgerson et al., 2001).

While the mechanistic basis of rhythmogenesis at each of the two sites remains speculative, as does the nature of the interaction between them, there are data to suggest that the buccal oscillator depends on reciprocal postsynaptic inhibition between buccal interneurons (i.e. it depends on network interactions to run) (Galante et al., 1996) and runs continuously (Wilson et al., 2002). The lung oscillator, on the other hand, requires some form of activation and, once activated, drives the buccal oscillator. It depends largely on pacemaker-like properties of interneurons but during development, at least in *Rana catesbeiana*, becomes increasingly dependent upon fast Cl^- synaptic inhibition mediated by GABA and glycine receptors (Wilson et al., 2002; Broch et al., 2002). Neither oscillator is critical for the action of the other but interaction between the two areas is important in generating normal ventilatory motor patterns (Wilson et al., 2002).

More recently, Vasilakos et al. (2005) explored possible homologies between the coupled oscillators in frogs (the buccal and lung rhythm generators) and rats (the parafacial and preBötzC rhythm generators). Interestingly, the Pre-I and I neurons of the parafacial and preBötzC complexes respectively, differ in their sensitivity to opioids (Takeda et al., 2003); while Pre-I neurons are opiate-insensitive, activation of μ and κ receptors on I neurons causes respiratory depression. In frogs (intact and *in vitro*) (Vasilakos et al., 2005), opioids also significantly suppress the lung oscillator (suggesting it may be homologous to the preBötzC complex) but have little effect on the buccal oscillator (suggesting that it may be homologous to the parafacial respiratory group). In support of the homology between the mammalian preBötzC and amphibian lung oscillator, both utilize neurons with voltage dependent, pacemaker-like properties (Galante et al., 1996; Smith et al., 1991; Winmill and Hedrick, 2003a) that are dependent upon gap junctions (Winmill and Hedrick, 2003b) to generate the respiratory

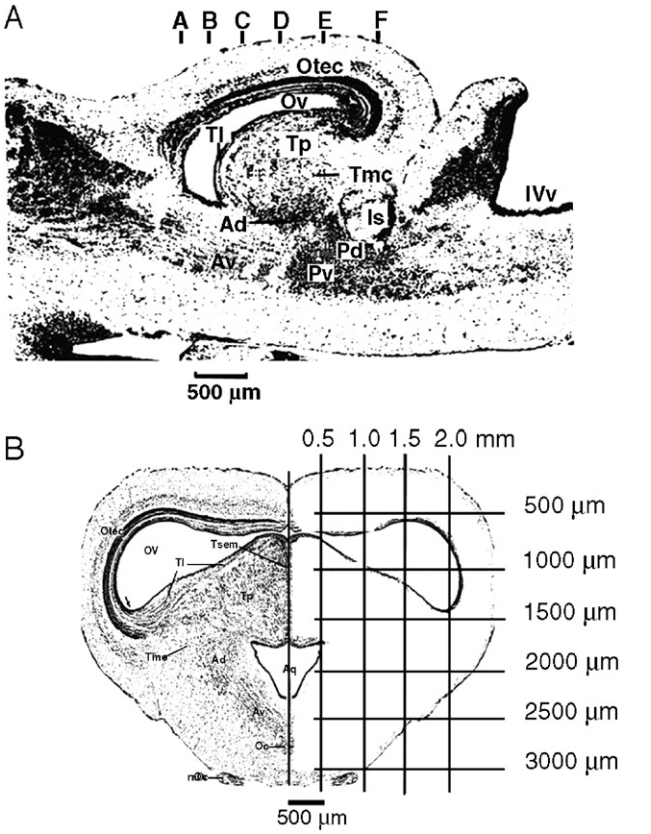

Fig. 10. (A) Parasagittal section of the bullfrog brain approximately 0.63 mm from the midline. Left is rostral and right is caudal. Electrical stimulations were administered at 3 or 4 medial to lateral sites at each of 6 rostral to caudal levels of the midbrain (A–F) while transections were made at two rostral to caudal levels of the midbrain (1 and 2). (B) Transverse section of the bull frog brain to illustrate the four medial to lateral sites used for electrode penetration and the six dorsal to ventral sites at which stimuli were applied. Abbreviations: Ad, anterodorsal tegmentum; Aq, Aqueduct of Sylvius; Av, anteroventral tegmentum; Is, nucleus isthmi; nOc, oculomotor nerve; Oc, nucleus of oculomotor nerve; Otec, optic tectum; Ov, optic ventricle; Pd, posterodorsal tegmentum; Pv, posteroventral tegmentum; Tl, laminar nucleus of torus semicircularis; Tmc, magnocellular nucleus of torus semicircularis; Tp, principal nucleus of torus semicircularis; Tsem, subpendymal midline nucleus.

rhythm. The pre I neurons of the parafacial region, however, also have pacemaker-like properties in rats, whereas neurons of the buccal oscillator in amphibians appear to depend exclusively on network synaptic inhibition (Galante et al., 1996; Broch et al., 2002). Based on this evidence, Vasilakos et al. (2005) suggested that the homology between the mammalian preBötzC and amphibian lung oscillator is strong, while that between the mammalian parafacial and the amphibian buccal

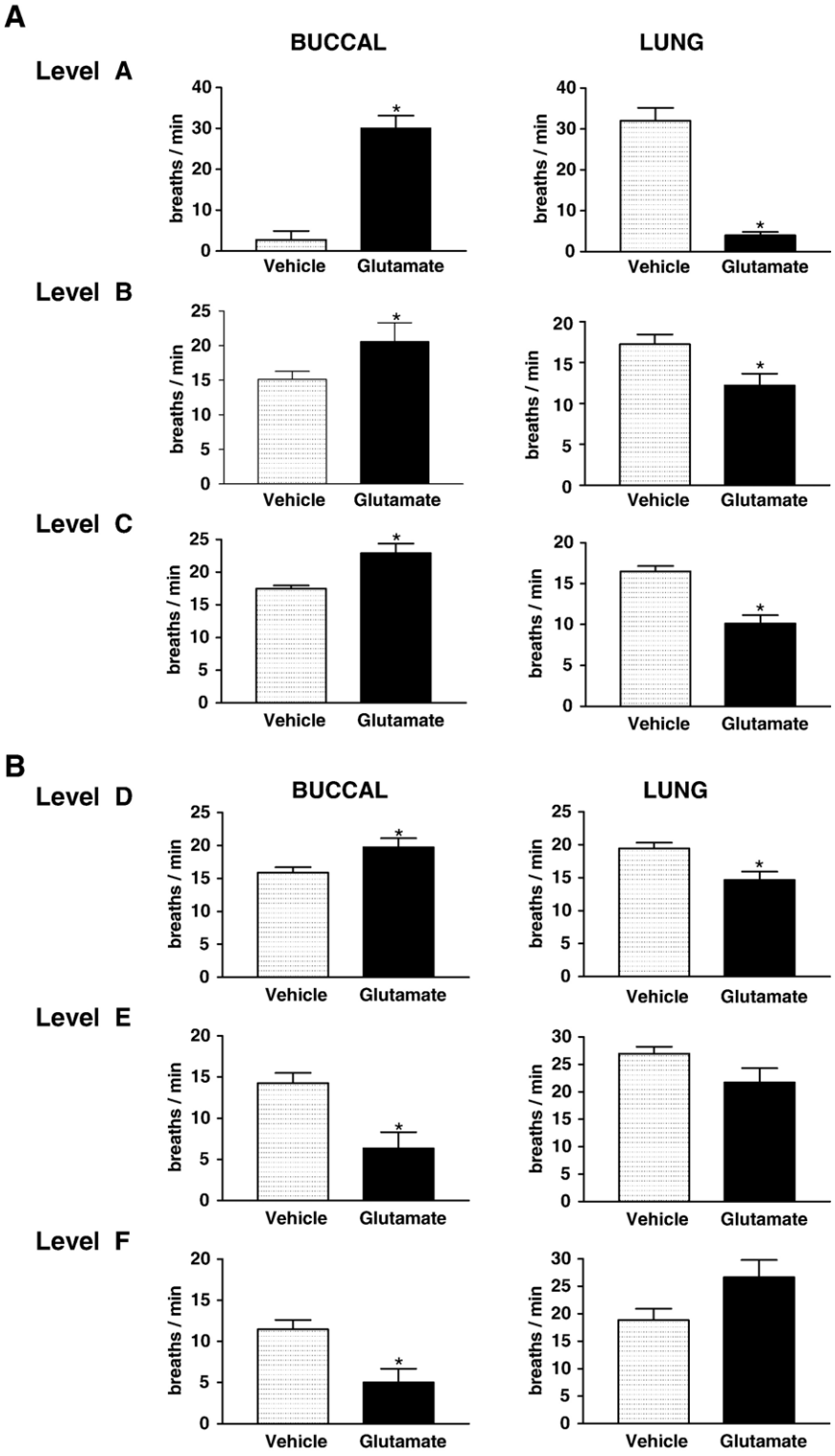

Fig. 11. (A) Mean effects of vehicle and glutamate stimulations in the frequency of buccal oscillations and lung ventilations at sites ranging from 0.5 to 2.0 mm lateral to the midline at depths ranging from 500 to 3000 μm below the brain surface at levels A, B and C in Fig. 10. (B) Mean effects of vehicle and glutamate stimulations in the frequency of buccal oscillations and lung ventilations at sites ranging from 0.5 to 2.0 mm lateral to the midline at depths ranging from 500 to 3000 μm below the brain surface at levels D, E and F in Fig. 10. * means statistic difference from vehicle values (*T*-test).

oscillator is weak. Even so, the authors considered the parafacial and buccal oscillators to be homologous and suggested that the paired coupled oscillators of the parafacial and preBötzC regions may have originated from the gill and lung oscillators, respectively, of the earliest air breathers.

Finally, as noted earlier (Section 2.2.2), adult amphibians often breathe intermittently and a variety of inputs act to promote or inhibit expression of the central rhythm (see following section). Of note, cumulative data imply that the neurogenesis of episodic breathing requires sites located in the caudal midbrain (Oka, 1958a,b; Reid et al., 2000; Gargaglioni et al., unpublished). Recent studies suggest that this caudal midbrain site may contain yet another paired oscillator that interacts with those located in the medulla to produce breathing episodes (Chatburn and Milsom, unpublished). There is also evidence that neurons in the dorsal mesencephalic tegmentum of fish, more specifically in a small area near the oculomotor nucleus in carp, have rhythm generating neurons that discharge before the first breath in each breathing episode (Jüch and Ballintijn, 1983).

Rhythm generation aside, multiple inputs to the respiratory control system, including those arising from tegmental and medullary sites, also play a role in shaping the burst pattern of the motor output associated with each breath. Slower rhythms of longer burst duration are generated by the more caudal hindbrain sites in isolation (Reid et al., 2000). Chemoreceptor, mechanoreceptor and other inputs act to modify the motor burst (see earlier sections).

4.2. Supra-medullary sites modulating the breathing pattern

It was previously thought that episodic breathing in amphibians arose as a result of a low metabolic rate coupled with inherent oscillations in blood gases (O_2 and CO_2/pH). In this model, lung ventilations were induced following apnea when a certain PaO_2 or $PaCO_2$ threshold was reached and breathing ceased when blood gas values had been brought back within a certain range (cf. Shelton et al., 1986). Within this context, many studies were undertaken to assess the role of the input from different groups of receptors (central and peripheral chemoreceptors: West et al., 1987; Smatresk and Smiths, 1991; Kinkead and Milsom, 1994; pulmonary stretch receptors: Kinkead and Milsom, 1996, 1997; olfactory receptors: Kinkead and Milsom, 1996) in the production of breathing episodes in anuran amphibians. These studies showed that while blood gas oscillations certainly modulate episodic breathing, they were not essential for the production of this breathing pattern. The data suggested that while some minimal level of chemoreceptor drive was required for respiratory rhythmogenesis (a biasing input to bring the overall system to threshold) (Kinkead and Milsom, 1994), oscillatory or phasic feedback was not required to turn on and off the breathing episodes (West et al., 1987; Smatresk and Smiths, 1991; Kinkead and Milsom, 1994, 1996, 1997; Kinkead et al., 1994). It appeared that the episodic pattern was an intrinsic property of the central respiratory control system that was further modulated by oscillations in blood gases and by phasic feedback from other peripheral sources (Kinkead, 1997). This conclusion was subsequently validated by the observation that the motor output produced by the isolated brainstem–spinal cord preparation from bullfrogs, in which the only afferent input is from central chemoreceptors and is tonic, was also episodic (Kogo and Remmers, 1994; Kinkead et al., 1994; Milsom et al., 1999).

The role of several putative neurotransmitters/neuromodulators in the production of lung episodes have now been investigated. Straus et al. (2000) demonstrated that low doses of baclofen converted episodic breathing into continuous ventilation under conditions of constant respiratory drive, suggesting that a GABAB dependent pathway may regulate the clustering of breaths into episodes. More recently, NO has also been implicated as a putative mediator of breathing episodes. Treatment with 7-NI (a neuronal NOS inhibitor) produced a dose-dependent reduction in the occurrence of lung bursts in the larval frog isolated brainstem (Harris et al., 2002). In addition, application of L-nitroarginine (a non specific NOS inhibitor) to post-metamorphic brainstems reversibly blocked the production of lung burst episodes (Hedrick et al., 2005). Both studies suggest that NO is important for the production of episodic breathing in the amphibian brainstem. However, the specific sites at which these neurotransmitters/neuromodulators act are still unknown.

In this isolated brainstem–spinal cord preparation, the clustering of the breaths into distinct episodes could only be completely eliminated by transection behind the optic lobes (i.e. behind the midbrain), just in front of the cerebellum (Oka, 1958a,b). This suggested that a more rostral site was essential for the production of breathing episodes and subsequent studies employing *in vitro* (Reid et al., 2000) and *in situ* preparations (Gargaglioni et al., unpublished) identified areas in caudal half of the midbrain of the bullfrog (*Rana catesbeiana*) that appear responsible for the production of episodic breathing patterns (Milsom et al., 1999; Reid et al., 2000; Gargaglioni et al., unpublished).

It was initially hypothesized that the nucleus isthmi (NI) located in the caudal half of the midbrain might be responsible for clustering breaths into episodes (Kinkead et al., 1997). Recent studies, however, show that ablation of the NI (by chemical or electrolytic lesion or drug microinjection) fails to eliminate the episodic pattern, suggesting that the NI is not directly responsible for turning breathing episodes on and off (Kinkead et al., 1997; Gargaglioni and Branco, 2000, 2001, 2003, 2004; Gargaglioni et al., 2002). More recently, the midbrain of anuran amphibians was systematically explored using an *in situ* preparation with chemical (glutamate) microinjections, to identify sites capable of exciting and inhibiting breathing (Fig. 10; Gargaglioni et al., unpublished). It was found that the principal sites in the midbrain capable of exciting or inhibiting the frequency of buccal oscillations and lung ventilations appear to be those primarily involved in visual and auditory integration and in the regulation of motor function and attentional state. Based on these results, it seems that there is a tendency for more rostral regions to facilitate buccal oscillations and inhibit lung ventilation (Fig. 11A) and for more caudal regions to inhibit buccal oscillations and excite lung ventilation

(Fig. 11B). Similar data exist for fish where evidence suggests that neurons near the oculomotor nucleus in the dorsal mesencephalic tegmentum of carp (a fish that also breathes episodically) discharge before the first breath in each breathing episode (Jüch and Ballintijn, 1983). Electrical stimulation at this site shortened the interbout interval and brought forward the onset of the next bout of breathing (i.e. it turned a breathing episode on). It did not, however, prolong the breathing episode suggesting that this site was not involved in terminating the breathing episode (Jüch and Ballintijn, 1983).

Finally, it has been shown recently that while the midbrain may be essential for the production of distinct breathing episodes, the medulla may also be able to generate episodic breathing patterns, of a sort, in isolation (Chatburn and Milsom; unpublished). It was found that following transection of the isthmus from the medulla in *in vivo* preparations of the brainstem and spinal cord, an episodic respiratory pattern would return, but only after a significant time. The spatio-temporal coordination of breaths within the breathing pattern, however, was less precise resulting in episodes that were less discrete and inconsistent in size and occurrence, and apneas between episodes that were dramatically prolonged. This raises new questions about the nature of the interactions between the dual paired oscillators in the medulla and the midbrain sites in producing and allowing the expression of the respiratory rhythm as it is manifest in the highly varied breathing patterns that we see in the anuran amphibians.

5. Conclusions

The control of breathing in amphibians is complex and intriguing. These animals can 'ventilate' up to three different exchange surfaces (skin, gills and lungs) with different respiratory media (water and air) and can independently perfuse these surfaces in different proportions due to the existence of highly regulated intra and extra-cardiac shunts. In adults, lung ventilation can be of four different types (buccal oscillations, balanced breaths, inflation breaths and deflation breaths). Finally, while anurans typically ventilate their buccopharyngeal cavity continuously (buccal oscillations) they ventilate the lungs intermittently. Various patterns of ventilation have now been described that form part of what can best be described as a breathing pattern continuum. With inflation and deflation cycles, there can be breaths with no deflation and breaths with no inflation respectively, despite the fact that all breaths have an "inspiratory" and an "expiratory" phase with respect to buccal expansion/compression. As a result of these complexities, comparisons of the anuran ventilation cycle to the aspiration cycle of other tetrapods can be problematic unless care is taken to clearly define the nature and source of the power stroke(s) involved (buccal expansion/compression), the specific chamber being referred to (buccal, lung, etc.), and the path of air (or water) flow.

Despite these complexities, anuran amphibians have attracted much attention from respiratory physiologists since they stand as an intermediate stage in the evolutionary transition from aquatic to aerial respiration. Because present day anurans are highly specialized and represent a significant departure in morphology, ecology and behaviour from the stem group that gave rise to the later tetrapods, it is not clear to what extent the trends seen in respiratory control in anurans reflect unique specializations or shared-derived features found in all tetrapods. The recent studies reviewed here provide exciting indications that they may well serve as a model of the intermediate stages in the evolution of respiratory control and suggest that further research in this area should be highly rewarding.

Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the NSERC of Canada.

References

Adams, W.E., 1958. The comparative morphology of the chemoreceptor cell in the amphibian carotid labyrinth. J. Anat. 104, 263–280.

Andersen, J.B., Hedrick, M.S., Wang, T., 2003. Cardiovascular responses to hypoxia and anaemia in the toad *Bufo marinus*. J. Exp. Biol. 206, 857–865.

Ballintijn, C.M., 1987. Evolution of central nervous control of ventilation in vertebrates. In: Taylor, E.W. (Ed.), The Neurobiology of the Cardio-respiratory System. Manchester University Press, Manchester, UK, pp. 3–27.

Banister, R.J., Portig, P.H., Vogt, M., 1967. The content and localization of catecholamines in the carotid labyrinths and aortic arches of *Rana temporaria*. J. Physiol. 192 (2), 529–535.

Boissezon, P., 1939. Le labyrinthe carotidien de la grenouille. rousse. addte. Bd. Soc. Hist. Nat. Toulouse 73, 145–152.

Boutilier, R.G., Toews, D.P., 1976. The effect of progressive hypoxia on respiration in the toad *Bufo marinus*. J. Exp. Biol. 68, 99–107.

Brainerd, E.L., 1999. New perspectives on the evolution of lung ventilation mechanisms in vertebrates. J. Exp. Biol. 4, 11–28.

Branco, L.G.S., Glass, M.L., 1995. Ventilatory responses to carboxyhaemoglobinaemia and hypoxic hypoxia in *Bufo paracnemis*. J. Exp. Biol. 198, 1417–1421.

Branco, L.G.S., Glass, M.L., Hoffmann, A., 1992. Central chemoreceptor drive to breathing in unanesthetized toads, *Bufo paracnemis*. Respir. Physiol. 87, 195–204.

Branco, L.G.S., Glass, M.L., Wang, T., Hoffmann, A., 1993. Temperature and central chemoreceptor drive to ventilation in toad (*Bufo paracnemis*). Respir. Physiol. 93, 337–346.

Broch, L., Sandoval, A.V., Morales, R.D., Hedrick, M.S., 2002. Regulation of the respiratory central pattern generator by chloride-dependent inhibition during development in the bullfrog (*Rana catesbeiana*). J. Exp. Biol. 205, 1161–1169.

Burggren, W.W., Doyle, M., 1986. Ontogeny of regulation of gill and lung ventilation in the bullfrog, *Rana catesbeiana*. Respir. Physiol. 66, 279–291.

Burggren, W.W., Infantino, R.L., 1994. The respiratory transition from water to air breathing during amphibian metamorphosis. Am. Zool. 34, 238–246.

Burggren, W.W., Pinder, A.W., 1991. Ontogeny of cardiovascular and respiratory physiology in lower vertebrates. Annu. Rev. Physiol. 53, 107–135.

Burggren, W.W., West, N., 1982. Changing respiratory importance of gills, lungs, and skin during metamorphosis in the bullfrog *Rana catesbeiana*. Respir. Physiol. 47, 151–164.

Cannatella, D.C., Trueb, L., 1988. Evolution of pipid frogs: intergeneric relationships of the aquatic frog family Pipidae (Anura). Zool. J. Linn. Soc. 94, 1–38.

Carman, J.B., 1955. The carotid labyrinth in *Hyla aurea*, with a note on that in *Leiopelma hochstetteri*. J. Anat. 89, 503–525.

Champagnat, J., Fortin, G., 1997. Primordial respiratory-like rhythm generation in the vertebrate embryo. Trends Neurosci. 20, 119–124.

Chowdhary, D.S., 1951. The carotid labyrinth of *Rana tigrina*. Nature 167 (4261), 1074.

Coates, E.L., 2001. Olfactory CO_2 chemoreceptors. Respir. Physiol. 129, 219–229.

Coates, E.L., Ballam, G.O., 1987. Upper airway CO_2 receptors in tegu lizards: localization and ventilatory sensitivity. J. Comp. Physiol. B 157, 483–489.

Coates, E.L., Ballam, G.O., 1990. Olfactory receptor response to CO_2 in bullfrogs. Am. J. Physiol. 258, R1207–R1212.

Coates, E.L., Wells, C.M., Smith, R.P., 1998. Identification of carbonic anhydrase activity in bullfrog olfactory receptor neurons: histochemical localization and role in CO_2 chemoreception. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 182, 163–174.

Coelho, F.C., Smatresk, N.J., 2003. Resting respiratory behaviour in minimally instrumented toads; effects of very long apneas on blood gases and pH. Braz. J. Biol. 63 (1), 35–45.

Crowder, W.C., Nie, M., Ultsch, G.R., 1998. Oxygen uptake in bullfrog tadpoles (*Rana catesbeiana*). J. Exp. Zool. 280, 121–134.

De Jongh, H.J., Gans, C., 1969. On the mechanism of respiration in the bullfrog, *Rana catesbeiana*: a reassessment. J. Morphol. 127, 259–290.

Dejours, P., 1981. Principle of Comparative Respiratory Physiology, 2nd edition. Elsevier/North-Holland Biomedical, New York.

De Marneffe-Foulon, C., 1962. Contribution á l'etude du mechanisme et du controle des mouvements respiratoires chez *Rana*. Ann. Soc. R. Zool. Belg. 92, 81–132.

Fedde, M.R., Kuhlmann, W.D., 1978. Intrapulmonary carbon dioxide sensitive receptors: amphibians to mammals. In: Piiper, J. (Ed.), Respiratory Function in Birds, Adult to Embryonic (Proceedings in Life Sciences). Springer-Verlag, Berlin, Heidelberg, Germany, pp. 34–50.

Feder, M.E., Burggren, W.W., 1985. Cutaneous gas exchange in vertebrates: design, patterns, control and implications. Biol. Rev. Camb. Philos. Soc. 60, 1–45.

Feldman, J.L., Del Negro, C.A., 2006. Looking for inspiration: new perspectives on respiratory rhythm. Nat. Rev., Neurosci. 7, 232–242.

Feldman, J.L., Mitchell, G.S., Nattie, E.E., 2003. Breathing: rhythmicity, plasticity, chemosensitivity. Annu. Rev. Neurosci. 26, 239–266.

Fortin, G., Kato, F., Lumsden, A., Champagnat, J., 1995. Rhythm generation in the segmented hindbrain of chick embryos. J. Physiol. 486, 735–744.

Fortin, G., Jungbltuh, F.S., Lumsden, A., Champagnat, J., 1999. Segmental specification of GABAergic during development of hindbrain neural networks. Nat. Neurosci. 2, 873–887.

Galante, R.J., Kubin, L., Fishman, A.P., Pack, A.I., 1996. Role of chloride-mediated inhibition in respiratory rhythmogenesis in an in vitro brainstem of tadpole, *Rana catesbeiana*. J. Physiol. 492, 545–558.

Gamperl, A.K., Milsom, W.K., Farrell, A.P., Wang, T., 1999. Cardiorespiratory responses of the toad (*Bufo marinus*) to hypoxia at two different temperatures. J. Exp. Biol. 202, 3647–3658.

Gargaglioni, L.H., Branco, L.G.S., 2000. Role of nucleus isthmi in the ventilatory response to hypoxia of *Bufo paracnemis*. Respir. Physiol. 119, 31–39.

Gargaglioni, L.H., Branco, L.G.S., 2001. Effect of nitric oxide in the nucleus isthmi on the hypoxic- and hypercarbic-drive to breathing of toads. Am. J. Physiol. 281, 338–345.

Gargaglioni, L.H., Branco, L.G.S., 2003. Role of glutamate in the nucleus isthmi on the hypoxia- and hypercarbia-induced hyperventilation of toads. Respir. Physiol. Neurobiol. 135, 47–58.

Gargaglioni, L.H., Branco, L.G.S., 2004. Nucleus isthmi and control of breathing in amphibians. Respir. Physiol. Neurobiol. 143, 177–186.

Gargaglioni, L.H., Coimbra, N.C., Branco, L.G.S., 2002. Chemical lesions of the nucleus isthmi increase the hypoxic and hypercarbic drive to breathing of toads. Respir. Physiol. Neurobiol. 132, 289–299.

Gdovin, M.J., Torgerson, C.S., Remmers, J.E., 1998. Neurorespiratory pattern of gill and lung ventilation in the decerebrate spontaneously breathing tadpole. Respir. Physiol. 113, 135–146.

Gdovin, M.J., Torgerson, C.S., Remmers, J.E., 1999. The fictively breathing tadpole brainstem preparation as a model for the development of respiratory pattern generation and central chemoreception. Comp. Biochem. Physiol. A. 124, 275–286.

Gradwell, N., 1971a. Gill irrigation in *Rana catesbeiana*. Part I. On the anatomical basis. Can. J. Zool. 50, 481–499.

Gradwell, N., 1971b. Gill irrigation in *Rana catesbeiana*. Part II. On the musculoskeletal mechanism. Can. J. Zool. 50, 501–521.

Gradwell, N., 1972. Gill irrigation in *Rana catesbeiana*. II. On the musculoskeletal mechanism. Can. J. Zool. 50 (5), 501–521.

Gray, P.A., Rekling, J.C., Bocchiaro, C.M., Feldman, J.L., 1999. Modulation of respiratory frequency by peptidergic input to rhythmogenic neurons in the preBotzinger complex. Science 286, 1566–1568.

Guldin, W.O., Markowitsch, H.J., 1982. No detectable remote lesions following massive intrastriatal injections of ibotenic acid. Brain Res. 225, 446–451.

Harris, M.B., Wilson, R.J., Vasilakos, K., Taylor, B.E., Remmers, J.E., 2002. Central respiratory activity of the tadpole in vitro brain stem is modulated diversely by nitric oxide. Am. J. Physiol. 283, 417–428.

Hedrick, M.S., Chen, A.K., Jessop, K.L., 2005. Nitric oxide changes its role as a modulator of respiratory motor activity during development in the bullfrog (*Rana catesbeiana*). Comp. Biochem. Physiol. A 142, 231–240.

Heisler, N., Forcht, G., Ultsch, G.R., Anderson, J.F., 1982. Acid–base regulation in response to environmental hypercapnia in two aquatic salamanders, *Siren lacertina* and *Amphiuma means*. Respir. Physiol. 49, 141–158.

Hoffmann, A., de Souza, M.B., 1982. Cardiovascular reflexes in conscious toads. J. Auton. Nerv. Syst. 5, 345–355.

Hou, P.C.L., Huang, S.P., 1999. Metabolic and ventilatory responses to hypoxia in two altitudinal populations of the toad *Bufo bankorensis*. Comp. Biochem. Physiol. 124, 413–421.

Hutchinson, V.H., Whitford, W.G., Kohl, M., 1968. Relationship of body size and surface area to gas exchange in anurans. Physiol. Soc. 41, 65–85.

Hyde, I.H., 1904. Localization of the respiratory centre in the skate. Am. J. Physiol. 10, 236–258.

Infantino, R.L., Jr. 1992. Ontogeny of Ventilatory Regulation in the Bullfrog *Rana catesbeiana* (PhD Dissertation). Amherst, MA: Univ. of Massachusetts.

Ishii, K., Honda, K., Ishii, K., 1966. The function of the carotid labyrinth in the toad. Tohoku J. Exp. Med. 88, 103–116.

Ishii, K., Ishii, K., Kusakabe, T., 1985. Chemo- and baroreceptor innervation of the aortic trunk of the toad *Bufo vulgaris*. Respir. Physiol. 60, 365–375.

Janczewski, W.A., Feldman, J.L., 2006a. Novel data supporting the two respiratory rhythm oscillator hypothesis. Focus on "respiration-related rhythmic activity in the rostral medulla of newborn rats". J. Neurophysiol. 96 (1), 1–2.

Janczewski, W.A., Feldman, J.L., 2006b. Distinct rhythm generators for inspiration and expiration in the juvenile rat. J. Physiol. 570, 407–420.

Jia, X., Burggren, W., 1997a. Developmental changes in chemoreceptive control of gill ventilation in larval bullfrogs (*Rana catesbeiana*). I. Reflex ventilatory responses to ambient hyperoxia, hypoxia and NaCN. J. Exp. Biol. 200, 2229–2236.

Jia, X., Burggren, W., 1997b. Developmental changes in chemoreceptive control of gill ventilation in larval bullfrogs (*Rana catesbeiana*). II. Sites of O_2-sensitive chemoreceptors. J. Exp. Biol. 200, 2237–2248.

Jones, D.R., Chu, C., 1988. Effect of denervation of carotid labyrinths on breathing in unrestrained *Xenopus* laevis. Respir. Physiol. 73, 243–255.

Jüch, P.J.W., Ballintijn, C.M., 1983. Tegmental neurons controlling medullary respiratory centre activity in the carp. Respir. Physiol. 51, 95–108.

Kardong, K.V., 2005. Vertebrates: Comparative Anatomy, Function, Evolution, 4th edition. McGraw Hill Publishers, New York, NY.

Kinkead, R., 1997. Episodic breathing in frogs: converging hypothesis on neural control of respiration in air breathing vertebrates. Am. Zool. 37, 31–40.

Kinkead, R., Milsom, W.K., 1994. Chemoreceptors and the control of episodic breathing in the bullfrog (*Rana catesbieana*). Respir. Physiol. 95, 81–98.

Kinkead, R., Milsom, W.K., 1996. CO_2-sensitive olfactory and pulmonary receptor modulation of episodic breathing in bullfrogs. Am. J. Physiol. 270, R134–R144.

Kinkead, R., Milsom, W.K., 1997. Role of pulmonary stretch receptor feedback in control of episodic breathing in the bullfrog. Am. J. Physiol. 272, 497–508.

Kinkead, R., Filmyer, W.G., Mitchell, G.S., Milsom, W.K., 1994. Vagal input enhances responsiveness of respiratory discharge to central changes in pH/pCO_2 in bullfrogs. J. Appl. Physiol. 77, 2048–2051.

Kinkead, R., Harris, M.B., Milsom, W.K., 1997. The role of the nucleus isthmi in respiratory pattern formation in bullfrogs. J. Exp. Biol. 200, 1781–1793.

Kogo, N., Remmers, J.E., 1994. Neural organization of the ventilatory activity in the frog, *Rana catesbeiana*. II. J. Neurobiol. 25, 1080–1094.

Kogo, N., Perry, S.F., Remmers, J.E., 1994. Neural organization of the ventilatory activity in the frog, *Rana catesbeiana.* I. J. Neurobiol. 25, 1067–1079.

Kruhøffer, M., Glass, M.L., Abe, A.S., Johansen, K., 1987. Control of breathing in an amphibian *Bufo paracnemis*: effects of temperature and hypoxia. Respir. Physiol. 69, 267–275.

Kuhlmann, W.D., Fedde, M.R., 1979. Intrapulmonary receptors in the bullfrog: sensitivity to CO_2. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 132, 69–75.

Kusakabe, T., 1990. Comparative studies on the vascular organization of carotid labyrinths of anurans and caudates. J Morphol. 204, 47–55.

Kusakabe, T., 1992. Ultrastructural characteristics of glomus cells in the external carotid artery during larval development and metamorphosis in bullfrogs, *Rana catesbeiana*. Anat. Rec. 233, 461–466.

Kusakabe, T., 2002. Carotid labyrinth of amphibians. Microsc. Res. Tech. 59 (3), 207–226.

Kusakabe, T., Powell, F.L., Ellisman, M.H., 1993. Ultrastructure of the glomus cells in the carotid body of chronically hypoxic rats: with special reference to the similarity of amphibian glomus cells. Anat. Rec. 237, 220–227.

Kusakabe, T., Hayashida, Y., Matsuda, H., Gono, Y., Powell, F.L., Ellisman, M.H., Kawakami, T., Takenaka, T., 1998. Hypoxic adaptation of the peptidergic innervation in the rat carotid body. Brain Res. 806, 165–174.

Langendorff, O., 1887. Die automatie des attemzentrums. Acch. Anat. Physiol. 285–295.

Liao, G.S., Kubin, L., Galante, R.J., Fishman, A.P., Pack, A., 1996. I. Respiratory activity in the facial nucleus in an in vitro brainstem of tadpole, *Rana catesbeiana*. J. Physiol. (Lond.) 492, 529–544.

Lillo, R.S., 1979. Autonomic cardiovascular control during submergence and emergence in bullfrogs. Am. J. Physiol. 237, 210–216.

Lumsden, T., 1923. Observations in the respiratory centre in the cat. J. Physiol. 57, 153–160.

Macintyre, D.H., Toews, D.P., 1976. The mechanics of lung ventilation and the effects of hypercapnia on respiration in *Bufo marinus*. Can. J. Zool. 54, 1364–1374.

Marin, O., Smeets, W.J., Gonzales, A., 1996. Do amphibians have a true locus coeruleus? Neuroreport. 7, 1447–1451.

McLean, H.A., Perry, S.F., Remmers, J.E., 1995a. Two regions in the isolated brainstem of the frog that modulate respiratory-related activity. J. Comp. Physiol. 177, 135–144.

McLean, H.A., Kimura, N., Kogo, N., Perry, S.F., Remmers, J.E., 1995b. Fictive respiratory rhythm in the isolated brainstem of frogs. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 176, 703–713.

Mellen, N.M., Janczewski, W.A., Bocchiaro, C.M., Feldman, J.L., 2003. Opioid-induced quantal slowing reveals dual networks for respiratory rhythm generation. Neuron 37, 821–826.

Milner, A.R., 1988. The relationships and origin of living amphibians. In: Benton, M.J. (Ed.), The Phylogeny and Classification of the Tetrapods. Amphibians, Reptiles Birds. Systematics Assoc. Special, vol. 35A. Clarendon Press, Oxford, pp. 59–102.

Milsom, W.K., 1991. Intermittent breathing in vertebrates. Annu. Rev. Physiol. 53, 87–105.

Milsom, W.K., Jones, D.R., 1977. Carbon dioxide sensitivity of pulmonary receptors in the frog. Experientia 33, 1167–1168.

Milsom, W.K., Reid, S.G., Meier, J.T., Kinkead, R., 1999. Central respiratory pattern generation in the bullfrog, *Rana catesbeiana*. Comp. Biochem. Physiol., A 124, 253–264.

Milsom, W.K., Chatburn, J., Zimmer, M.B., 2004. Pontine influences on respiratory control in ectothermic and heterothermic vertebrates. Respir. Physiol. Neurobiol. 143, 263–280.

Mitchell, G.S., Cross, B.A., Hiramoto, T., Scheid, P., 1980. Effects of intrapulmonary CO_2 and airway pressure on phrenic activity and pulmonary stretch receptor discharge in dogs. Respir. Physiol. 41, 29–48.

Mitchell, G.S., Douse, M.A., Foley, K.T., 1990. Receptor interactions in modulating ventilatory activity. Am. J. Physiol. 259, 911–920.

Morales, R.D., Hedrick, M.S., 2002. Temperature and pH/CO_2 modulate respiratory activity in the isolated brainstem of the bullfrog (*Rana catesbeiana*). Comp. Biochem. Physiol. A 132, 477–487.

Mulkey, D.K., Stornetta, R.L., Weston, M.C., Simmons, J.R., Parker, A., Bayliss, D.A., Guyenet, P.G., 2004. Respiratory control by ventral surface chemoreceptor neurons in rats. Nat. Neurosci. 7, 1360–1369.

Nattie, E.E., 2001. Central chemosensitivity, sleep, and wakefulness. Respir Physiol. 129, 257–268.

Noronha-de-Souza, C.R., Bicego, K.C., Michel, G., Glass, M.L., Branco, L.G., Gargaglioni, L.H., in press. Locus coeruleus is a central chemoreceptive site in toads. Am. J. Physiol.

Oka, K., 1958a. The influence of the transection of the brain upon the respiratory movement of the frog. J. Phys. Soc. Jpn. 20, 513–519.

Oka, K., 1958b. Further studies on the localization of the respiratory centers of the frog. J. Phys. Soc. Jpn. 20, 520–524.

Onimaru, H., Homma, I., 2003. A novel functional neuron group for respiratory rhythm generation in the ventral medulla. J. Neurosci. 23 (4), 1478–1486.

Onimaru, H., Arata, A., Homma, I., 1995. Intrinsic burst generation of preinspiratory neurons in the medulla of brainstem–spinal cord preparations isolated from newborn rats. Exp. Brain Res. 106, 57–68.

Onimaru, H., Kumagawa, Y., Homma, I., 2006. Respiration-related rhythmic activity in the rostral medulla of newborn rats. J. Neurophysiol. 96 (1), 55–61.

Pinder, A.W., Burggren, W.W., 1986. Ventilation and partitioning of oxygen uptake in the frog *Rana pipiens*: effects of hypoxia and activity. J. Exp. Biol. 126, 453–468.

Reid, S.G., Milsom, W.K., 1998. Respiratory pattern formation in the isolated bullfrog (*Rana catesbeiana*) brainstem–spinal cord. Respir. Physiol. 114, 239–255.

Reid, S.G., West, N.H., 2004. Modulation of breathing by phasic pulmonary stretch receptor feedback in an amphibian, *Bufo marinus*. Respir. Physiol. Neurobiol. 142, 165–183.

Reid, S.G., Milsom, W.K., Meier, J.T., Munns, S., West, N.H., 2000. Pulmonary vagal modulation of ventilation in toads (*Bufo marinus*). Respir. Physiol. 120, 213–230.

Rekling, J.C., Feldman, J.L., 1998. PreBotzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. Annu. Rev. Physiol. 60, 385–405.

Richerson, G.B., 2004. Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. Nat. Rev., Neurosci. 5, 449–461.

Rogers, D.C., 1963. Distinctive cell types in the amphibian carotid labyrinth. Nature 200, 492–493.

Rogers, D.C., 1966. A histological and histochemical study of the carotid labyrinth in the anuran amphibians, *Bufo marinus*, *Hyla aurea* and *Neobatrachus pictus*. Acta Anat. (Basel) 63, 249–280.

Rovainen, C.M., 1983. Generation of respiratory activity by the lamprey brain exposed to picrotoxin and strychnine, and weak synaptic inhibition in motoneurons. J. Neurosci. 10, 875–882.

Russel, D.F., 1986. Respiratory pattern generation in adult lampreys (*Lampetra fluviatilis*): interneurons and burst resetting. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 158, 91–102.

Sakakibara, Y., 1978. Localization of CO_2 sensor related to the inhibition of the bullfrog respiration. Jpn. J. Physiol. 28, 721–735.

Sakakibara, Y., 1984. The pattern of respiratory nerve activity in the bullfrog. Jpn. J. Physiol. 34 (2), 269–282.

Sanders, C.E., Milsom, W.K., 2001. The effects of tonic lung inflation on ventilation in the American bullfrog *Rana catesbeiana* Shaw. J. Exp. Biol. 204, 2647–2656.

Scheid, P., Piiper, P., 1986. Control of breathing in birds. In: Fishman, A.P., Cherniack, N.S., Widdicombe, J.G., Geiger, S.R. (Eds.), Handbook of Physiology, Section 3, the Respiratory System Control of Breathing, Part II. American Physiological Society, Bethesda, MD, pp. 815–832.

Senn, D.G., 1972. Development of tegmental and rhombencephalic structures in a frog (*Rana temporaria* L.). Acta Anat. (Basel) 82, 525–548.

Shelton, G., 1961. The respiratory centre in the tench (*Tinca tinca* L.) II. Respiratory neuronal activity in the medulla oblongata. J. Exp. Biol. 38, 79–92.

Shelton, G., Boutilier, R.G., 1982. Apnoea in amphibians and reptiles. J. Exp. Biol. 100, 245–273.

Shelton, G., Jones, D.R., Milsom, W.K., 1986. Control of breathing in ectothermic vertebrates. In: Fishman, A.P., Cherniack, N.S., Widdicombe, J.G., Geiger, S.R. (Eds.), Handbook of Physiology, Sect. 3, The Respiratory System, Vol. 2, Control of Breathing, Part 2. American Physiological Society, Bethesda, MD, pp. 857–909.

Smatresk, N.J., Smits, W., 1991. Effects of central and peripheral chemoreceptor stimulation on ventilation in the marine toad, *Bufo marinus*. Respir. Physiol. 83, 223–238.

Smith, J.C., Ellenberger, H.H., Ballanyi, K., Richter, D.W., Feldman, J.L., 1991. Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254, 726–729.

Smyth, D.H., 1939. The central and reflex control of respiration in the frog. J. Physiol. 95, 305–327.

Straus, C., Wilson, R.J., Remmers, J.E., 2000. Baclofen eliminates cluster lung breathing of the tadpole brainstem, in vitro. Neurosci. Lett. 292, 13–16.

Straus, C., Wilson, R.J., Remmers, J.E., 2001. Oxygen sensitive chemoreceptors in the first gill arch of the tadpole, *Rana catesbeiana*. Can. J. Physiol. Pharm. 79 (11), 959–962.

Stuesse, S.L., Cruce, W.L., Powell, K.S., 1984. Organization within the cranial IX–X complex in ranid frogs: a horseradish peroxidase transport study. J. Comp. Neurol. 222, 358–365.

Sundin, L., Reid, S., Rantin, T., Milsom, W.K., 2000. Branchial receptors and cardiorespiratory reflexes in a neotropical fish, the Tambaqui, *Colossoma macropomum*. J. Exp. Biol. 203, 1225–1239.

Takeda, S., Eriksson, L.I., Yamamoto, Y., Joensen, H., Onimaru, H., Lindahl, S.G., 2003. Opioid action on respiratory neuron activity of the isolated respiratory network in newborn rats. Anesthesiology 95, 740–749.

Taylor, B.E., Harris, M.B., Leiter, J.C., Gdovin, M.J., 2003a. Ontogeny of central CO_2 chemoreception: chemosensitivity in the ventral medulla of developing bullfrogs. Am. J. Physiol. 285 (6), 1461–1472.

Taylor, B.E., Harris, M.B., Coates, E.L., Gdovin, M.J., Leiter, J.C., 2003b. Central CO_2 chemoreception in developing bullfrogs: anomalous response to acetazolamide. J. Appl. Physiol. 94, 1204–1212.

Tenney, S.M., 1979. A synopsis of breathing mechanisms. In: Wood, S.C., Lenfant, C. (Eds.), Lung Biology in Health and Disease, Vol. 13. Evolution of Respiratory Processes: A Comparative Approach. Marcel Dekker Inc., New York, pp. 51–106.

Thompson, K.J., 1985. Organization of inputs to motoneurons during fictive respiration in the isolated lamprey brain. J. Comp. Physiol., A Sens. Neural Behav. Physiol. 157, 291–302.

Torgerson, C.S., Gdovin, M.J., Remmers, J.E., 1997. Ontogeny of central chemoreception during fictive gill and lung ventilation in an in vitro brainstem preparation of *Rana catesbeiana*. J. Exp. Biol. 200, 2063–2072.

Torgerson, C.S., Gdovin, M.J., Remmers, J.E., 1998. Fictive gill and lung ventilation in the pre- and post-metamorphic tadpole brainstem. J. Neurophysiol. 80, 2015–2022.

Torgerson, C.S., Gdovin, M.J., Brandt, R., Remmers, J.E., 2001. Location of central respiratory chemoreceptors in the developing tadpole. Am. J. Physiol. 280, 921–928.

Ultsch, G.R., 1973. A theoretical and experimental investigation of the relationships between metabolic rate, body size, and oxygen exchange capacity. Respir. Physiol. 18, 143–160.

Van Vliet, B.N., West, N.H., 1987. Response characteristics of pulmocutaneous arterial baroreceptors in the toad, *Bufo marinus*. J. Physiol. 388, 55–70.

Van Vliet, B.N., West, N.H., 1992. Functional characteristics of arterial chemoreceptors in the toad (*Bufo marinus*). Respir. Physiol. 88, 113–127.

Vasilakos, K., Wilson, R.J., Kimura, N., Remmers, J.E., 2005. Ancient gill and lung oscillators may generate the respiratory rhythm of frogs and rats. J. Neurobiol. 62, 369–385.

Vitalis, T.Z., Shelton, G., 1990. Breathing in *Rana pipiens*: the mechanism of ventilation. J. Exp. Biol. 154, 537–556.

Wang, T., 1994. Measurement of ventilatory responses in the toad *Bufo marinus*: a comparison of pneumotachography and buccal pressures. Comp. Biochem. Physiol. A 109, 793–798.

Wang, T., Branco, L.G.S., Glass, M.L., 1994. Ventilatory responses to hypoxia in the toad *Bufo paracnemis* before and after a decrease in haemoglobin oxygen-carrying capacity. J. Exp. Biol. 186, 1–8.

Wang, T., Taylor, E.W., Reid, S.G., Milsom, W.K., 1999a. Lung deflation stimulates fictive ventilation in decerebrate, paralyzed and unidirectionally ventilated toads (*Bufo marinus*). Respir. Physiol. 118, 181–191.

Wang, T., Hedrick, M.S., Ihmied, Y.M., Taylor, E.W., 1999b. Control and interaction of the cardiovascular and respiratory systems in anuran amphibians. Comp. Biochem. Physiol. A 124, 393–406.

Wang, T., Taylor, E.W., Reid, S.G., Milsom, W.K., 2004. Interactive effects of mechano- and chemo-receptor inputs on cardio-respiratory outputs in the toad. Respir. Physiol. Neurobiol. 140, 63–76.

Wassersug, R.J., Hoff, K., 1979. A comparative study of the buccal pumping mechanism of tadpoles. Biol. J. Linn. Soc. 12, 225–259.

Watanabe, T., Tanaka, T., Yonemasu, Y., 1987. Ibotenic acid-induced limbic seizures and neuronal degeneration. Brain Nerve 39, 505–508.

West, N.H., Burggren, W.W., 1983. Reflex interactions between aerial and aquatic gas exchange organs in larval bullfrogs. Am. J. Physiol. 244, R770–R777.

West, N.H., Burggren, W.W., 1984. Factors influencing pulmonary and cutaneous arterial blood flow in the toad, *Bufo marinus*. Am. J. Physiol. 247, 884–894.

West, N.H., Jones, D.R., 1975. Breathing movements in the frog *Rana pipiens.* I. The mechanical events associated with lung and buccal ventilation. Can. J. Zool. 53, 332–344.

West, N.H., Topor, Z.L., Van Vliet, B.N., 1987. Hypoxemic threshold for lung ventilation in the toad. Respir. Physiol. 70, 377–390.

Wilson, R.J., Vasilakos, K., Harris, M.B., Straus, C., Remmers, J.E., 2002. Evidence that ventilatory rhythmogenesis in the frog involves two distinct neuronal oscillators. J. Physiol. 540, 557–570.

Winmill, R.E., Hedrick, M.S., 2003a. Gap junction blockade with carbenoxolone differentially affects fictive breathing in larval and adult bullfrogs. Respir. Physiol. Neurobiol. 138, 239–251.

Winmill, R.E., Hedrick, M.S., 2003b. Developmental changes in the modulation of respiratory rhythm generation by extracellular K^+ in the isolated bullfrog brainstem. J. Neurobiol. 55, 278–287.

Winmill, R.E., Chen, A.K., Hedrick, M.S., 2005. Development of the respiratory response to hypoxia in the isolated brainstem of the bullfrog *Rana catesbeiana*. J. Exp. Biol. 208, 213–222.