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Control of respiration in fish, amphibians and reptiles

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Abstract

Fish and amphibians utilise a suction/force pump to ventilate gills or lungs, with the respiratory muscles innervated by cranial nerves, while reptiles have a thoracic, aspiratory pump innervated by spinal nerves. However, fish can recruit a hypobranchial pump for active jaw occlusion during hypoxia, using feeding muscles innervated by anterior spinal nerves. This same pump is used to ventilate the air-breathing organ in air-breathing fishes. Some reptiles retain a buccal force pump for use during hypoxia or exercise. All vertebrates have respiratory rhythm generators (RRG) located in the brainstem. In cyclostomes and possibly jawed fishes, this may comprise elements of the trigeminal nucleus, though in the latter group RRG neurons have been located in the reticular formation. In air-breathing fishes and amphibians, there may be separate RRG for gill and lung ventilation. There is some evidence for multiple RRG in reptiles. Both amphibians and reptiles show episodic breathing patterns that may be centrally generated, though they do respond to changes in oxygen supply. Fish and larval amphibians have chemoreceptors sensitive to oxygen partial pressure located on the gills. Hypoxia induces increased ventilation and a reflex bradycardia and may trigger aquatic surface respiration or air-breathing, though these latter activities also respond to behavioural cues. Adult amphibians and reptiles have peripheral chemoreceptors located on the carotid arteries and central chemoreceptors sensitive to blood carbon dioxide levels. Lung perfusion may be regulated by cardiac shunting and lung ventilation stimulates lung stretch receptors.

Key words: Vertebrates; Control of respiration; Respiratory rhythm generation; Water and air-breathing; Chemoreceptors; Mechanoreceptors

Introduction

There are important differences in the construction of the respiratory systems in ectothermic vertebrates, related to their modes of respiration. Fish typically propel water unidirectionally over the gills, using ventilatory muscles, which operate around the jaws and skeletal elements in the gill arches lining the pharynx. Adult amphibians retain the buccal force pump for tidal lung ventilation; their larvae are aquatic gill-breathers. Thus, in fish and amphibians the major respiratory muscles are cranial muscles, innervated by motor neurons with their cell bodies in the brainstem, located close to the presumed site of the central respiratory rhythm generator (RRG). Reptiles retain an elaborate buccal, hyoidean force pump, but ventilate the lungs primarily with a thoracic aspiratory pump, although they typically lack the diaphragm, characteristic of mammals. The RRG in the brainstem generates respiratory activity in descending fibres that drive respiratory activity in spinal motor neurons innervating intercostal muscles (Table 1) (1).

Mammals characteristically display continuous, rhythmic, aspiratory breathing to maintain their relatively high rates of oxygen uptake and carbon dioxide excretion. Exceptions are the foetus and neonate, which often show intermittent cycles of breathing related to sleep states (2), and diving or hibernating mammals, which suspend or markedly reduce breathing and heart rates for varying periods but otherwise show typical cardiorespiratory control mechanisms. Patterns of ventilatory mechanics are defined solely in terms of the time spent in inspiration and expiration and the rate of air flow. Combinations of these variables produce the familiar components of breathing, namely, frequency, tidal volume and minute ventilation. Based on neurophysiological data, the mammalian ventilatory cycle has been divided into three distinct neural phases, defined as inspiration, post-inspiration (passive expiration) and ex-

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Table 1. Respiratory pumps and their innervation.

Group	Pump	Primary muscle group	Primary innervation
Lampreys	Suction/force	Branchial (tidal ventilation)	V, VII
Elasmobranchs	Suction/force	Buccal/parabranchial	V, VII, IX, X
Active/hypoxic	*Suction/force*	*Hypobranchial*	*XII*
Teleosts	Suction/force	Buccal/opercular	V, VII, IX, X
Active/hypoxic	*Suction/force*	*Hypobranchial*	*XII*
Air-breathing fish	Suction/force	Buccal/parabranchial	V, VII, XII
Amphibians			
Larval	Suction/force	Buccal/parabranchial	V, VII
Adult	Suction/force	Buccal/pharyngeal/glottis/nares	V, VII, IX, X
Salamander	*Expiration*	*Hypaxial*	*Spinal*
Reptiles			
Squamates	Aspiration	Intercostal/abdominals	Spinal
Active/hypoxic lizards	*Suction/force*	*Gular pump/hypobranchial*	*XII*
Chelonians	Aspiration	Pectoral/glottis	Spinal, X
Crocodilians	Aspiration	Diaphragmaticus/intercostals	Spinal

Primary innervation refers to cranial (Vth to XIIth) or spinal nerves that supply the main sources of efferent nervous supply to the respiratory muscles. Information in italics is an exception to the general rule for that group of animals.

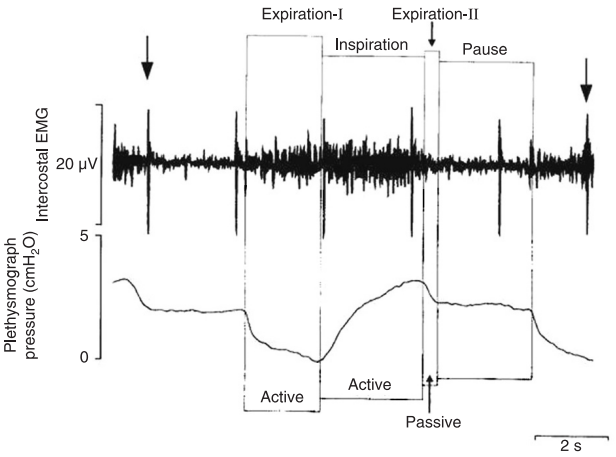

Figure 1. Recording of electromyographic (EMG) activity from intercostal muscles of *Uromastyx aegyptius microlepis*, together with the pressure generated within a plethysmograph by an aspiratory lung-inflation cycle. The inflation cycle is triphasic, with active expiration-I leading to active inspiration then a brief, passive, partial expiration-II, followed by a ventilatory pause with the lungs inflated, terminated by the next cycle. The recording from intercostal muscles include the ECG (large arrows) (Reproduced from Ref. 59, with permission).

piration (active expiration) (3). The pattern is more complex in arrhythmic or episodic breathers, such as the amphibians and reptiles, where the components of breathing frequency also include an apnoeic or non-ventilatory period of variable duration and number of breaths per episode. Recordings from respiratory neurons in bullfrogs demonstrated that lower vertebrates also have a three-phase respiratory cycle. However, the first phase is expiration, and it occurs when the glottis opens. This is then followed by inspiration, which is produced by the brisk activation of the buccal elevators to push air back into the lungs. The last phase corresponds to the post-inspiratory phase in mammals and is a period of breath holding, during which neurons other than those involved in the production of the two other phases were shown to be active. The lungfish, which also have a buccal force pump, have a post-inspiratory phase (1). Figure 1 shows the respiratory cycle in a lizard related to activity in intercostal muscles.

The cardiovascular system is not divided in a typical fish, with the heart delivering blood directly into the branchial vasculature and then on into the systemic circulation. In contrast, mammals and birds have a completely divided circulatory system, with separate pulmonary and systemic circuits. Air-breathing fish, amphibians and most reptiles have more or less incompletely divided circulatory systems, allowing differential perfusion of the pulmonary circuit. This ability may be an essential component of their intermittent patterns of ventilation, often associated with periods of submersion. Amphibians may, in addition, utilise bimodal respiration. Larval amphibians possess gills, often in combination with developing lungs, while adult amphibians can switch between cutaneous and lung breathing (e.g., during graded hypoxia or submersion), so that the distributing effects of vascular mechanisms are of paramount importance and may operate to regulate oxygen uptake in the absence

of changes in ventilation.

Despite these major differences in the construction and mode of operation of their respiratory and cardiovascular systems, evidence is accumulating that the vertebrates share some important similarities in the mechanisms of central generation of the respiratory rhythm, control of the cardiovascular system and in the central nervous and reflex generation of cardiorespiratory interactions. Knowledge of this complex area is dominated by the results of medically oriented research on mammals. Accordingly, this comparative review will consider control of the respiratory system, and its coordination with the cardiovascular system, in fish, amphibians and reptiles, in relation to our more thorough understanding of mammalian patterns. Reference will be made in the relevant sections to recent extensive reviews.

Generation of the respiratory rhythm

Despite years of detailed investigation, the nature of respiratory rhythm generation in all vertebrates, including mammals, is still in dispute with a role for pacemaker neurons showing spontaneous rhythmic oscillations contrasted with neural networks relying on synaptic interactions. What is not in dispute is the location of the RRG, which in all vertebrates is concentrated in the brainstem, and specifically the medulla oblongata. The mechanisms underlying respiratory rhythmogenesis in mammals are only now being resolved (1,4). Groups of respiratory pre-motor neurons and neurons innervating upper airway muscles are found in the caudal medulla near the nucleus ambiguous, and the Bötzinger complexes. In addition, at least one site of respiratory rhythmogenesis has been identified in neonatal mammals, in the 'pre-Bötzinger' complex, which is situated in the reticular formation (RF) of the rostral medulla, at the level of the hypoglossal nuclei (1). These outflows probably derive, in an evolutionary sense, from the branchial motoneurons of more primitive, gill-breathing vertebrates, which retain their primary roles in respiratory rhythm generation in present-day fish and larval amphibians. Accordingly, the RF is thought to be the site both of the primary respiratory rhythm generator in fish and amphibians and of the respiratory and suckling rhythms in neonatal mammals.

Cyclostomes

Our understanding of the nature and topography of the RRG in fish can be traced back, in evolutionary terms, as far as the jawless cyclostomes. This group of vertebrates is composed of the jawless fishes, the myxinoids (e.g., *Myxine*, the hagfish) and the petromyzontes (e.g., *Lampetra*, the lamprey). The lamprey (*Petromyzon* sp) has a larval form, the ammocoete, which has a muscular velum that pumps water and uses the pharyngeal clefts to filter feed. The feeding current is driven by muscular pumps associated with the velum and anterior branchial pouches that act against the elastic recoil of the branchial basket. The jawless adult has an oral sucker with which it attaches to fish such as the salmon. Consequently its mouth is closed off and the animal breathes via seven pairs of branchial pouches that are ventilated bidirectionally (i.e., it is a tidal breather). Ventilation is again by contraction of the muscles around the branchial basket forcing water out through the pouches, with elastic recoil drawing water back into the pouches. Generation of its respiratory rhythm has provided a model for what may be the ancestral form of the vertebrate RRG (4). Spontaneous bursts of respiration-related activity have been recorded from the isolated brainstem of the lamprey. Periodic bursts of activity recorded from motor nuclei supplying the Vth cranial nerve, located in the rostral half of the medulla, precede those recorded from respiratory motor nuclei in the caudal half of the medulla, innervating the VIIth, IXth and Xth cranial nerves (1,4). A brain-gill-velum preparation of the ammocoete larva showed that velar pumping was driven by an RRG in the trigeminal region with descending pathways driving the branchial motoneurons and this hierarchy is retained in the adult even though the trigeminal motoneurons are no longer active as the velum ceases to act as a pump. Activity continues in the trigeminal nucleus when it is isolated from the rest of the brain, suggesting that rhythm generation involves neurons located within this region. Recent evidence suggests that rhythm generation in these rostral neurons fits a "group pacemaker" model as their activity continues in an isolated brainstem, bathed with chloride-free saline (4). Electrical stimulation of this area excites the other respiratory motoneurons directly and could entrain the respiratory rhythm. These observations suggest that the motor pattern for respiration is at least partly generated and co-ordinated in the rostral half of the medulla in the lamprey, possibly in the trigeminal nucleus, and is transmitted to respiratory motoneurons through descending pathways (5). However, the caudal region of the brainstem, including the motor nuclei of the VIIth, IXth and Xth cranial nerves, is capable of generating rhythmic activity following transaction at the level of the Vth nucleus, suggesting a separate rhythm generator possibly responsible for strong contraction of the muscles of the gill pouches or "coughs" used to clear the branchial basket of obstruction. Midline transaction of the rostral medulla disrupts the normal respiratory rhythm while leaving the coughs unaffected (5). Although the true nature of the RRG remains unresolved, the mechanisms uncovered in the cyclostomes seem to be somewhat similar to those retained in jawed fishes, implying that they utilise a mechanism that may be an ancestral link back to velar ventilation of a feeding apparatus.

Jawed fishes

Because water contains less oxygen per unit volume than air and yet is considerably more dense and viscous, fish normally exhibit continuous rhythmical breathing movements of the buccal and septal or opercular pumps.

Rhythmic ventilatory movements continue in fish following brain transection to isolate the medulla oblongata, though changes in pattern indicate that there are influences from higher centres. Central recording and marking techniques have identified a longitudinal strip of neurons with spontaneous respiration-related bursting activity. These neurons make up elements of the trigeminal Vth, facial VIIth, glossopharyngeal IXth, and vagal Xth motor nuclei, which drive the respiratory muscles, together with the descending trigeminal nucleus and the reticular formation. Areas in the midbrain have efferent and afferent connections with the reticular formation (1). The respiratory rhythm apparently originates in a diffuse respiratory pattern generator in the reticular formation, though maintenance of a respiratory rhythm in intact animals generally relies on an element of respiratory drive from peripheral receptors. Vagal afferents from the gill arches that innervate a range of tonically and physically active mechanoreceptors and chemoreceptors project to the motor nuclei via the intermediate facial nucleus (1).

Simultaneous recordings of efferent activity from the central cut ends of the nerves innervating the respiratory muscles of the dogfish, and the pacu, revealed that the Vth cranial nerve fires in advance of the VIIth, IXth, and Xth cranial nerves (6,7). The resultant co-ordinated contractions of the appropriate respiratory muscles may relate to their original segmental arrangement before the evolution of the brain, head and jaws with their cranial musculature, an arrangement that is retained in the hindbrain of the dogfish in the sequential topographical arrangement of the motor nuclei, including the subdivisions of the vagal motor nucleus (6). This traditional view of the origin of the jaws and visceral arches and their innervation has recently been questioned on the basis of developmental studies of the role of neural crest cells. These suggest a separate origin for the jaws as feeding structures, independent of the visceral arches, which combined ventilation with filter-feeding, a view supported by study of marker genes. As suggested above, a possible evolutionary antecedent of the jaws may be the velum of filter feeding protochordates or larval cyclostomes. Fish often show markedly reduced ventilation rates when inactive in normoxic or hyperoxic waters and may interrupt their normal regular rhythm of gill ventilation and exhibit episodic breathing patterns. Carp were shown to possess a group of neurons with phase switching properties situated in the midbrain, that appear to play a key role in the control of episodic breathing. Stimulation of this area of the brainstem during a ventilatory pause brought forward the onset of the next breathing bout (1).

Both elasmobranchs and teleosts can recruit an additional group of hypobranchial muscles into the respiratory cycle to provide active jaw occlusion. These are a complex ventral sheet of muscle, inserted between the pectoral girdle, the lower jaw and the ventral processes of the hyoid and branchial skeleton, associated primarily with suction feeding and ingestion in water-breathing fishes, recruited into the respiratory cycle during periods of vigourous, forced ventilation such as may occur following exercise or deep hypoxia (Table 1) (8). These muscles are innervated by the hypobranchial nerve, which contains elements of the occipital nerves and the anterior spinal nerves. Injection of adrenaline into the dogfish stimulates active ventilation and induces activity in the hypobranchial nerve of the dogfish (8). The hypobranchial nerve in fish is the morphological equivalent of the hypoglossal nerve, which innervates the muscles of the tongue in reptiles, birds and mammals. These muscles are utilised in suckling by infant mammals, an activity likely to require its own central oscillator, which is thought to reside in the RF. The RRG in fish is thought to reside in the RF, suggesting an evolutionary link from fish to mammals (1).

Air-breathing fishes

Air-breathing fishes have evolved a variety of air-breathing organs (ABO) for obtaining oxygen from above the water surface but retain gills, ventilated by cranial muscles, for the uptake of a variable proportion of their oxygen requirements, dependent on species and conditions, and for excretion of most of their carbon dioxide. Many facultative air-breathers, such as the tarpon, rely on gill ventilation and restrict blood flow to the ABO in normoxic water but increase rates of air-breathing and perfusion of the ABO with hypoxic exposure and particularly during exercise (9). During these periods opercular beating becomes imperceptible, indicating cessation of effective gill breathing or a switch to ram ventilation whilst swimming. Access to air reduces the lactic acid load during burst swimming and prolongs aerobic exercise in tarpon but they are able to repay an accumulated oxygen debt during recovery by increased rates of gill ventilation (10).

In all air-breathing fish, gulping of air at the water surface is achieved through the action of the same muscles as used for feeding or for forced ventilation in water-breathing fishes. These are elements of the jaw musculature, innervated by cranial nerve V, together with the hypobranchial musculature, innervated by occipital and anterior spinal nerves (Table 1). They function together in a co-ordinated action either for feeding or gulping air, actions that are independent of the visceral arches and may derive from their separate evolutionary origins as feeding muscles. In the primitive ray-finned (actinopterygian) fish, the bowfin (*Amia calva*), that utilises a well-vascularized swimbladder as an ABO, there appear to be two types of airbreath, one that involves exhalation followed by inhalation (designated “type I” airbreaths) and one that simply involves inhalation (“type II” airbreaths). It is suggested that type I breaths are respiratory in nature whereas type II breaths have a buoyancy-regulating function. Spectral analysis indicates that there is an inherent rhythmicity to “type I” (i.e., respiratory-related) air-breathing, both in normoxia and hypoxia (11). This periodicity may be

driven by changes in blood O_2 status that occur during the interbreath interval, rather than by an RRG for air-breathing. Some authors have suggested that air-breathing is critically dependent on afferent feedback and, as stated above, is simply a reorganisation of coughing and suction feeding movements requiring relatively little neural reorganisation (4,12). Control of the switch between ventilation of the gills and the ABO is likely to relate to stimulation of chemoreceptors by reduced oxygen levels at the gills or in the ABO but the central sites responsible for control of air-breathing reflexes in fish are still unknown.

Reorganisation of the central nervous system (CNS) associated with the evolution of air-breathing has been poorly studied in fish. As regards actinopterygian, air-breathing fishes, there is probably an RRG for gill ventilation located in the reticular formation of the hindbrain, similar to that of water-breathing fish. In the bowfin, catecholamine infusion stimulates gill ventilation, apparently via a central mechanism, but has no effect on air-breathing in normoxia or hypoxia, indicating that central sites controlling gill ventilation and air-breathing are pharmacologically and possibly spatially different (13). However, the sequence of events associated with air-breathing in the bowfin suggests that the action of air-breathing would require little change in the pattern of neural control required for suction feeding and/or coughing, with the exception of control over opening of the glottis at the entrance to the ABO (12). Neural tracers revealed that the ABO in the bowfin is innervated by vagal motor neurons that may supply the glottis or smooth muscle in the walls of the ABO. An isolated brainstem preparation from the long-nosed gar, *Lepisosteus osseus*, showed two distinct motor patterns in fictive respiratory activity recorded from the root of the Vth cranial nerve, a high frequency, low amplitude pattern associated with gill breathing and a low frequency, high amplitude pattern associated with ventilation of the lung during air-breathing (14). This latter activity was associated with phasic activation of the Xth cranial nerve that innervates the glottis. The differing motor patterns and recruitment of vagal motor neurons suggest the presence of two separate oscillators.

There may be separate origins for air-pumping mechanisms in actinopterygian fishes, derived from the suction feeding/coughing pumps and the lobe-finned (sarcopterygian) lung fish and their evolutionary cousins the amphibians. However, both utilise the same sets of muscles to pump air into the lung, and consequently may possess the same central oscillators. It has been suggested that the African lungfish (*Protopterus aethiopicus*) possesses two separate central rhythm generators, one for gill ventilation and the other for air-breathing (1). The sequence of air flow in the breathing cycles of lungfish and amphibians such as bullfrogs is essentially similar. In amphibians, there is evidence that evolution of air-breathing rhythms may have required a new motor pattern in the CNS rather than one evolved from progressive modification of the branchial rhythm generator. Episodic breathing rhythms were recorded from an isolated brainstem of a bullfrog, implying that they are generated centrally in the absence of patterned inflow from receptors. Microinjection of glutamate into rostral areas of the bullfrog-isolated brainstem in an area of the reticular formation that corresponds to that identified as responsible for respiratory rhythmogenesis in foetal mammals caused brief breathing episodes (see below). The RF has been identified as the site for the RRG in fish (15). It seems that the neural networks associated with respiratory rhythmogenesis have been well conserved during vertebrate evolution.

Amphibians

Amphibian tadpole larvae have gills ventilated by activity in cranial muscles, with branchial performance comparable to teleost fish, but carry out a large proportion (60%) of respiratory gas exchange over their permeable skin. As development proceeds the lungs assume increasing importance in oxygen uptake, though the skin remains the major exchange surface until metamorphosis is nearly complete. In adult amphibians, most oxygen is taken up from the lungs, ventilated by the buccal cavity, but the skin retains a predominant role in the excretion of carbon dioxide (1,15).

The sequence of air flow in the breathing cycle of lungfish and amphibians such as bullfrogs is similar. However, unlike air-breathing fish, which must open their mouth to aspirate ambient air into their buccal cavity at the onset of the breathing cycle, frogs aspirate air via their nostrils. Lung ventilation usually occurs episodically in bullfrogs. The breathing cycle has recently been described by Gargaglioni and Milsom (15). Typically, after a bout of lung breathing, a series of elevations and depressions of the floor of the buccal cavity follows, called buccal oscillations. It has been suggested that they may be remnants of the mechanisms of gill ventilation used by the premetamorphic tadpole stages, and homologous to gill ventilations in fish, and that their rhythm may reflect vestiges of the central rhythm generator for gill ventilation (16). Buccal oscillations and lung ventilations are produced by the same muscles. The primary difference between these two events is the force of the contraction and the positions of the glottis and nares (Table 1). In resting animals, buccal oscillations occur more or less continuously and are interrupted by periodic lung ventilations, which normally occur at a time when another buccal oscillation would have been initiated.

There is accumulating evidence for multiple paired rhythm generators in amphibians, with expression of the lung rhythm being conditional upon a higher level of central and/or peripheral receptor input. However, the fact that lung ventilation always occurs at a time when a buccal oscillation would otherwise have occurred suggests that if there are separate rhythm generators, they are entrained to a large degree. There are some circumstantial evidence for the existence of two central respiratory rhythm generators

in the bullfrog (4). Hypercapnia had no effect on the frequency of lung inflations but reduced both the occurrence of buccal oscillations and their instantaneous frequency when they did occur. This might suggest that there are separate rhythms for lung inflation and buccal oscillation, which can be uncoupled. Microinjection of inhibitory and excitatory neurotransmitters/modulators into the medulla of bullfrogs has located two specific rhythmogenic sites within the ventral medullary RF (4).

A number of investigators have used *in vitro* preparations of the larval or adult anuran brainstem to examine the mechanisms of respiratory rhythmogenesis (15). Recordings of fictive breathing in isolated brainstem preparations revealed spontaneous neural output from the roots of cranial nerves V, VII, X, and XII. However, these bursts were synchronous, implying that the spatiotemporal relationships between bursts of activity in these nerves in the intact animal rely on feedback from peripheral receptors. Microinjections of glutamate into rostral areas of the bullfrog brainstem, near the VIIth motor nucleus, caused a brief excitation of fictive breathing. Interestingly, this area corresponds to the pre-Bötzinger area of the reticular formation in the mammalian brainstem, considered to be a primary site for respiratory rhythmogenesis in the neonate (5,15).

Extracellular recording from *in vitro* brainstem, spinal cord preparations of *Rana catesbeiana* tadpoles, and adults revealed that it is possible to manipulate the two types of neural activity associated with buccal or lung breathing independently, using pharmacological agents (4). Superfusion of an *in vitro* brainstem-spinal cord preparation from the bullfrog tadpole with chloride-free saline eliminated the rhythmic bursts associated with gill ventilation while augmenting lung bursts, indicating that the former arise from a GABAergic, network-type rhythm generator whereas lung ventilatory rhythms arise from pacemaker cells (16). However, there is some evidence for maturation of respiratory rhythmogenesis from a pacemaker-driven process in the tadpole to a network-driven process in the adult frog and for relocation of the site of the RRG during development. This apparent discrimination is of interest in comparison to the situation described in fish, where gill ventilation may depend on pacemaker cells located in the RF, and in adult mammals, where lung ventilation may be dependent on activity in neural networks (4,15).

The evolution/development of air-breathing rhythms may have required a new motor pattern in the CNS rather than one that evolved from progressive modification of the branchial rhythm generator (4,17). This may have evolved from the generator for the feeding rhythm, which can be recruited by the respiratory RRG during forced ventilation in fish, or when air-breathing fish gulp air at the water surface, as described above. For instance, the activity of the hypaxial muscles during exhalation in salamanders possibly represents a primitive condition, intermediate between the buccal force pump of fish and the thoracic/abdominal aspiration pump of reptiles, birds and mammals (see Table 1) (1,4). Although similar data are not available for anuran amphibians, which may have lost this function, these data raise important considerations regarding the evolution of the control of ventilation in amphibians, which imply that descending fibres from the brainstem, innervating spinal motoneurons, can have important roles in some species, anticipating their roles in the supposedly more advanced tetrapods.

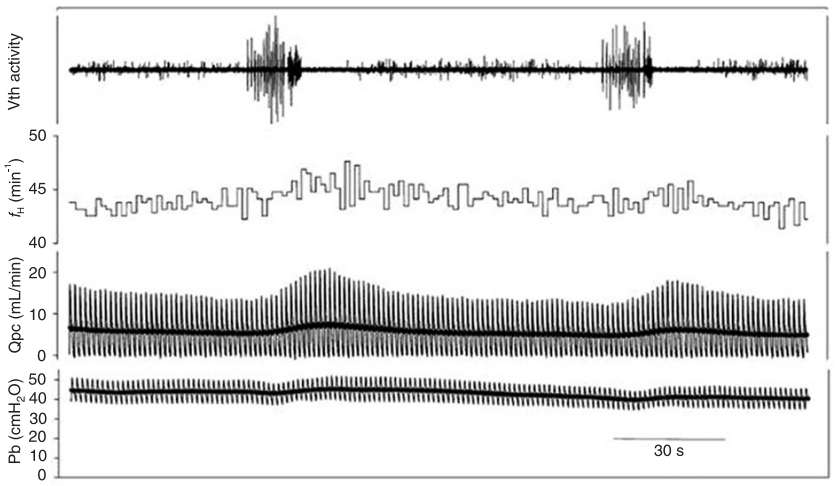

Figure 2. Cardiovascular changes associated with discontinuous ventilatory activity in a decerebrated and paralysed toad (*Bufo marinus*). Ventilatory activity was measured as nervous activity in the fifth cranial nerve, which innervates the respiratory muscles in the buccal cavity. As the toad was unidirectionally ventilated and paralysed, there were no changes in afferent input during bouts of fictive ventilation, and the rise in pulmocutaneous blood flow (Qpc) during ventilation is likely to be caused by central feed-forward mechanisms. There were no obvious changes in systemic blood pressure (Pb) or heart rate (f_H) (Modified from Ref. 53, with permission).

Amphibians often breathe intermittently, with bouts of ventilation interrupted by quiescent periods or, in aquatic species or stages, submersion. Intermittent breathing patterns are common in lower vertebrates, such as amphibians and reptiles, and contrast with the continuous breathing patterns of non-diving birds and mammals in their apparent lack of constancy and intrinsic rhythm (Figure 2). Many researchers have ascribed the genesis of breathing episodes in amphibians and reptiles to the inherent oscillations of blood O_2 and/or CO_2/pH levels associated with intermittent breathing, rather than to

the action of a “mammalian-type” central control mechanism. In this model, lung ventilations are induced when a certain PaO_2 or $PaCO_2$ threshold is reached and breathing ceases when the blood gas values have been brought back within a certain range (18). The observation that breathing is completely suppressed when convective requirements are met by unidirectional ventilation (17) indicates that lung ventilation is conditional upon a minimal stimulatory input. However, unidirectionally ventilated toads can still display episodic breathing or fictive ventilation (19), although this experimental procedure has been assumed to maintain blood gases constant and, in paralysed animals, lung distension constant, and thus produce only tonic chemoreceptor and mechanoreceptor input. These data imply that the mechanisms underlying episodic breathing may be an intrinsic property of the central respiratory control system, a view which seems to be confirmed by the observation that the motor output from a brainstem-spinal cord preparation of the bullfrog was episodic, in the absence of any possible feedback from the periphery (17). The central generation of these episodic breathing patterns has been localised to the nucleus isthmi in the brainstem of the bullfrog (17). This mesencephalic structure is the neuroanatomical equivalent of the pons in mammals, which contributes to the control of breathing pattern.

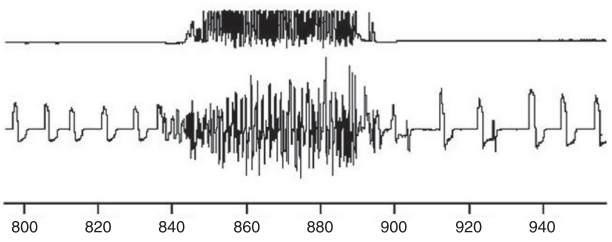

Figure 3. Ventilatory airflow rates in *Varanus exanthematicus* running on a treadmill. The upper trace is a recording of lateral bending of the trunk whilst running for 30 s; the lower trace is a pneumotachograph recording of ventilatory airflow at the nares, with expiration recorded as deviations above the baseline. The active lizard shows a marked disruption of normal airflow (Modified from Ref. 58, with permision).

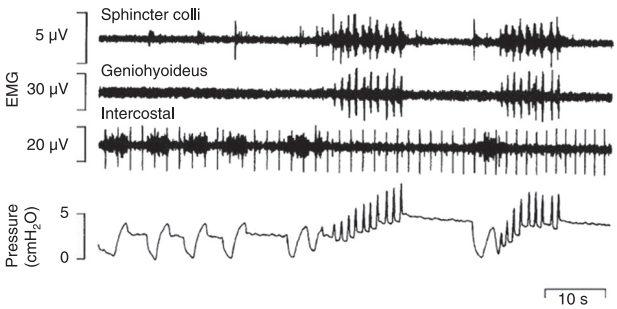

Figure 4. A series of thoracic plus buccal ventilatory cycles recorded from an anaesthetised *Uromastyx aegyptius microlepis* using whole body plethysmography. At 30°C, a cycle of thoracic ventilatory cycles terminated in the addition of buccal pumping. This started in the respiratory pause immediately after passive expiration and resulted in additional inflation of the lung signified by an increase in baseline plethysmograph pressure, which is held until the subsequent active expiration. Note the short interval between one respiratory pumping cycle and the next. EMG, electromyographic activity in sphincter colli and geniohyoideus muscles inserted on the pectoral girdle and from intercostal muscles, with this recording including the ECG (Modified from Ref. 59, with permission).

Reptiles

Reptiles are typically committed air-breathers, having dry scaly skin and well-developed lungs. They are an ancient and highly polyphyletic class of vertebrates. Extant members show highly diverse respiratory and cardiovascular mechanisms, including some they share with the amphibians, such as an incompletely divided circulatory system and periodic ventilation, often combined with periods of submersion. Lizards, in common with all other reptiles (except some crocodilians) lack a diaphragm. However, unlike modern amphibians they do have ribs, and lung ventilation has long been considered to be generated by intercostal muscles acting on the rib cage. As lizards run in a serpentine manner, employing segmental muscles from the body wall, it was asserted by some investigators that they are unable to breathe while running (Figure 3). However, some lizards have been shown to utilise an alternative mode of ventilation involving a gular pump, which alternates with the costal pump (see Table 1). Following a short passive expiration, a bout of buccal pumping caused a progressive increase in lung volume, followed by breath-hold (Figure 4). X-ray imaging of the varanid lizard, *Varanus exanthematicus*, revealed that it used an accessory gular pump when walking, thus overcoming the supposed mechanical constraint on active lung ventilation during exercise (reviewed by Ref. 1). As well as the intercostal muscles, crocodilians have a unique mechanism for lung ventilation in the form of the diaphragmatic muscle that moves the liver and viscera back and forth to aspirate the lung (see Table 1). This muscle is inserted on the pelvic girdle, which is also rotated to change abdominal volume. Chelonians have their ribs fused to the carapace and lung ventilation is achieved by movements of the forelimb and shoulder girdle, together with the glottis (Table 1).

The existence of anatomically and functionally separate thoracic and gular respiratory pumps in lizards would seem to require separate sites of central respiratory rhythm generation. However, this interesting possibility remains unexplored. Putative sites of respiratory pattern generation, having similarities in neural organisation and activation to those extensively documented for mammals, have been described for turtles. Recent evidence from study on the

isolated brainstem indicates that the RRG in turtles requires calcium-activated cation channels and resembles the group-pacemaker model described for mammals, though during synaptic inhibition blockade the rhythm generator appeared to be transformed into a pacemaker-driven network (20). However, the direct contribution of these populations of neurons and their potential integration of sensory information, in determining the generation of respiratory movements, remains unclear (21). As in amphibians, it has been suggested that the initiation of bouts of discontinuous breathing in reptiles may relate to thresholds for stimulation of central and peripheral chemoreceptors rather than to patterns dictated by a central rhythm generator (21). This may enable the flexibility of response essential for an ectothermic vertebrate, as the thresholds for stimulation will vary with temperature, in accord with the animal's oxygen demand. However, unidirectionally ventilated alligators display episodic breathing so that centrally generated rhythmicity may have a role in its initiation (1).

In turtles, the basic output of the RRG is episodic, even under experimental conditions when all sensory feedback appears to be tonic (22). Experiments performed on reptiles demonstrated that mild anaesthesia and brainstem section at the level of the rostral rhombencephalon (metencephalon) abolish these breathing episodes, i.e., the animals now breathe in an uninterrupted fashion. Vagotomy also affects the breathing pattern by reducing the number of breaths per episode in crocodilians. It is interesting to note, however, that vagotomy had no effect on the breathing pattern when it was performed after episodic breathing had been abolished by a caudal midbrain transection (reviewed in Ref. 1).

Afferent innervation of the respiratory and circulatory systems

Fish

Chemoreceptors. Fish typically respond to ambient hypoxia with a reflex bradycardia and increased ventilatory effort. These changes were interpreted as adaptive responses that improved respiratory gas exchange but the bradycardia is now considered to be a protective response for the highly aerobic cardiac muscle (1). The chemoreceptors inducing these responses vary among the studied species of fish in their location, distribution, innervation and also the reflex triggered by each receptor population. Many sites have been suggested as the reflexogenic origin of the O_2 chemosensory responses. The gill arches, innervated by cranial nerves IX and X, are the ubiquitous site of O_2 receptors in all fishes studied (23). Other sites can contribute to that response. Among them are the walls of the orobranchial cavity, innervated by cranial nerves V and VII (24) and the spiracle or pseudobranch, innervated by nerves VII and IX (23) and maybe the brain (25). The first gill arch presents putative O_2 chemosensory cells with ultrastructure similar to that of the mammals' carotid body (26,27). Activity recorded peripherally from branchial respiratory branches of the trout showed an exponential increase in afferent activity with a progressive decrease in O_2 supply. These responses resemble the recorded responses of the mammalian carotid body, similarly innervated by the IXth cranial nerve. They were shown to have similar embryonic origins (26), innervation and even chemoreceptive mechanisms, suggesting that they may be the evolutionary antecedents of the mammalian carotid body (26).

The O_2 receptors in fish occur in distinct populations, which characteristically generate very specific reflex responses. The populations of receptors associated with hypoxic bradycardia are restricted to the first gill arch in some species like *Salmo gairdneri*, *Gadus morrhua* and *Hoplias malabaricus*, but can be found throughout the other gill arches in other species, like *Ictalurus punctatus*, the pacu, *Piaractus mesopotamicus* and the tambaqui, *Colossoma macropomum*. The hypoxic ventilatory response, on the other hand, only arises from receptors confined to the gill arches in a few species like *Ictalurus punctatus*. In many others, such as *Hemipterus americanus*, *Hoplias malabaricus*, *Colossoma macropomum*, and *Piaractus mesopotamicus*, total gill denervation fails to eliminate the hypoxic ventilatory response and the remaining receptors appear to occur at extrabranchial sites that include the orobranchial cavity (28-31).

These populations of O_2 chemoreceptors can also be directed to monitor either the internal or the external environment. The chemoreceptors that engender a hypoxic bradycardia appear to sense the O_2 levels in the water passing over the gills. Changes in ventilation are most often triggered in response to changes in O_2 tension in the blood passing through the gills (29,31). Many teleosts have receptors that sense changes in both water and blood (28,29,32).

Immunofluorescence techniques (against serotonin) have made it possible to directly identify putative O_2 receptors on the gills by visualising the presence of 5-HT in dense-cored vesicles in neuroepithelial cells (NEC). Jonz and Nurse (27) reported that innervation of NEC in the gill filaments may account for the development of a functional O_2-sensing pathway and the hyperventilatory response to hypoxia in zebrafish, *Danio rerio*, larvae. Furthermore, these NECs are innervated by the same nerves and located at the gill sites that would provide internal and external O_2 monitoring as inferred by the reflexogenic experiments reported above. That was also observed by Coolidge et al. (31) studying the distribution of NEC in the gills of four species of fish: trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), trairão (*Hoplias lacerdae*), and traíra (*Hoplias malabaricus*). The authors described innervated NEC at the filament tips in all species in a prime location to sense PwO_2, in agreement with physiological data. It also appeared that there were NEC monitoring PaO_2 surrounding the efferent filament artery. The presence of these internal

chemoreceptors varied between the species and correlated with their ability to respond to internal arterial hypoxia. Furthermore, the authors also described a group of non-innervated NEC in the lamella that might have a paracrine role, acting directly on the pillar cells to enhance respiratory surface area when exposed to aquatic hypoxia.

To date, we are not able to link species-specific patterns of O_2 receptor distribution to particular phylogenetic or life history traits, and it may prove rewarding to continue to try to map locations and distribution as a function of phylogeny, habitat and life history. Short-term plasticity, due to a previous history of exposure to hypoxia, may also lead to changes in responsiveness and even functionality of receptors (29).

Mechanoreceptors. The respiratory muscles in fish contain length and tension receptors, in common with other vertebrate muscles, and the gill arches bear a number of mechanoreceptors with various functional characteristics. Mechanical stimulation of the gill arches is known to elicit the 'cough' reflex in fish and a reflex bradycardia and stimulation of branchial mechanoreceptors by increasing rates of water flow may be the trigger for the cessation of active ventilatory movements during 'ram ventilation' in fish (1,6). These mechanoreceptors will also be stimulated by the ventilatory movements of the gill arches and filaments and may be important in stabilising the respiratory rhythm. When gill arches of a lightly anaesthetised fish were artificially moved the respiratory rhythm was regularly reset by the imposed movements in a manner related to the phase of the respiratory cycle at which the movement was imposed, with 1:1 entrainment achieved when the imposed rhythm was close to the natural rhythm (33). These experiments suggest that phasic mechanoreceptor activity serves to stabilise the generation of the respiratory rhythm, preventing the central generating circuits from being disrupted by other inputs. Central stimulation (towards the brain) of nerves innervating respiratory muscles in the carp with short trains of electrical stimuli also entrained the respiratory rhythm to the imposed stimuli (33).

The branchial branches of the IXth and Xth cranial nerves innervate a range of tonically and phasically active mechanoreceptors as well as chemoreceptors on the gill arches of fish and project directly to a dorsal sensory nucleus lying above the equivalent motor nuclei in the medulla (23). The sensory area in turn projects centrally to the respiratory motor nuclei. However, sensory fibres from branchial receptors may terminate in different locations within the brainstem and consequently have different effects on integration.

Some vagal afferent fibres seem to project to vagal motoneurons innervating branchial muscles by short loops, either directly or via the RF and may be involved in the reflex contraction of adductor muscles on the gill filaments in response to mechanical stimulation of the gill filaments or gill rakers. Stronger stimulation may induce the coughing reflex with simultaneous contraction of respiratory pump muscles that receive inputs from the RF. Vagal afferent fibres also connect with the trigeminal complex that receives inputs from proprioceptors in the respiratory pump muscles innervated by the trigeminal Vth cranial nerve. As proprioceptive reflexes are involved in entrainment and stabilisation of the respiratory rhythm then their inputs must be connected fairly directly with the rhythm-generating neurons (1). Chemoreceptor stimulation transmitted in the vagus nerve, that affects ventilation, may be relayed via the medulla. However, microinjection of glutamic acid into identified areas of the vagal sensory projection in sculpin, identified by injection of a fluorescent tracer, elicited specific, highly localised responses, including changes in ventilation frequency and amplitude (34). Glutamate has been identified as the neurotransmitter for afferents into the nucleus tractus solitarii (NTS) in mammals.

Evidence for the involvement of baroreceptors in vasomotor control in fish was long contentious and it has been proposed that the evolution of barostatic control of the heart may be associated with the evolution of air-breathing because the gills of fish are supported by their neutral buoyancy in water. Ventilation of the gills generates hydrostatic pressures, which fluctuate around, but predominantly above ambient levels. Arterial blood pressures in the branchial circulation of fish and the pressure difference across the gill epithelia are relatively low, despite the fact that the highest systolic pressures are generated in the ventral aorta, which leaves the heart to supply the afferent branchial arteries. Consequently, the need for functional baroreceptors in fish is not clear. However, in teleosts, injection of adrenaline, which raised arterial pressure, caused a bradycardia, abolished by atropine, while low frequency oscillations in blood pressure, similar to the Mayer waves in mammals, were abolished by injection of the α-adrenoreceptor antagonist yohimbine. These data imply active regulation of vasomotor tone and the balance of evidence indicates that functional arterial baroreceptors may exist in the branchial circulation of teleost fishes (1).

Aquatic surface respiration and air-breathing in fish

It has generally been considered that hypoxia, consequent upon stagnation of tropical freshwater habitats, was the environmental spur for the evolution, in the Devonian era, of air-breathing in many bony fishes, which use a variety of different ABO, from modified swimbladders to diverticula of the branchial chambers (35). There are also a large number of highly derived marine teleosts that occupy the intertidal zone and which evolved air-breathing abilities and an amphibious lifestyle independently of the freshwater air-breathers (36). The selection pressures may have been an ability to tolerate emersion during low tide and to escape extremes of salinity and hypoxia in tidepools. These species typically use the skin, gills, and branchial chambers as ABO. However, it has been argued on the basis of fossil evidence

and the ionic composition of blood plasma that terrestrial vertebrates have evolved from freshwater ancestors, rather than from amphibious marine ancestors (35).

Chemoreceptor control of aquatic surface respiration. It has been suggested that true air-breathing evolved from a behaviour known as aquatic surface respiration (ASR) (37). As the name implies, ASR involves rising to the surface and ventilating the layer of water in contact with the atmosphere, which is richer in dissolved oxygen than the underlying bulk water. Many teleost species have evolved this behavioural response in both temperate and tropical environments, freshwater and marine. Many species also hold an air bubble (or bubbles) in their mouth when they perform ASR, which may have a dual role of increasing oxygen levels in the bucco-opercular cavity, and maintaining the fish buoyant at the water surface (38), and may have been the behavioural antecedent to true air-breathing. Indeed, although ASR is a behavioural response to hypoxia, it is in fact a reflex that is driven by oxygen-sensitive chemoreceptors (24,32). Shingles et al. (32) demonstrated that the ASR response, plus a typical teleost gill hyperventilation and bradycardia, were elicited by chemoreceptors sensitive to oxygen levels in the ventilatory water stream and the blood stream of flathead grey mullet (*Mugil cephalus*). Florindo et al. (24) demonstrated that the chemoreceptors that stimulate ASR in the tambaqui are diffusely distributed in the gills and orobranchial cavity, innervated by cranial nerves VII, IX, and X. These chemoreceptor sensory modalities and innervations would appear to be homologous, therefore, to those that drive reflex gill hyperventilation in all fish groups studied to date (1,23). Thus, ASR may use the pre-existing sensory arm of such hypoxic ventilatory reflexes, integrating a new motor output that involves rising to the water surface to ventilate the surface layer. Presumably, cessation of this behaviour is also driven by information from the same chemoreceptors (32).

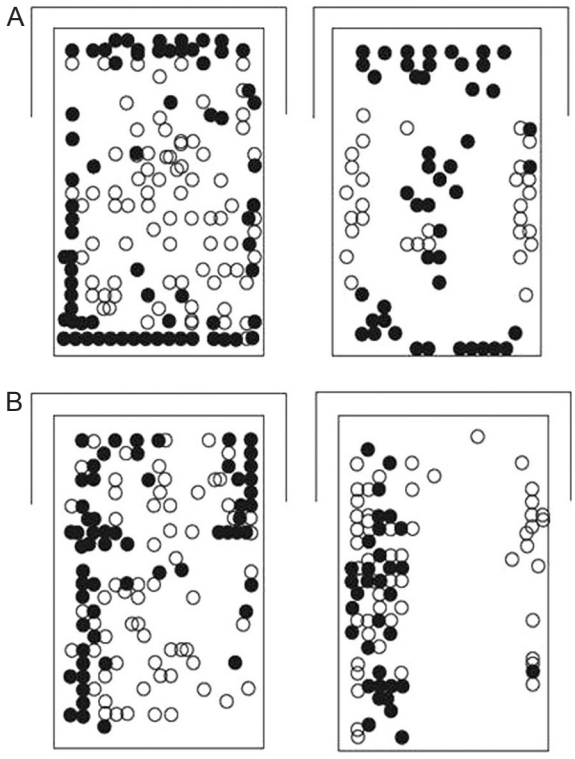

Figure 5. Aerial view of locations of aquatic surface respiration (ASR) events performed by flathead grey mullet *Mugil cephalus* in response to an external application of 300 mg NaCN into the ventilatory stream via a buccal cannula (A) or when exposed to aquatic hypoxia (B) in clear water (left panel) or in turbid water containing 300 NTU Polsperse 10 kaolin (right panel) at a temperature of 25°C. Open circles represent ASR events in the absence of a model avian predator; solid circles represent events in the presence of the model. The outer lines at the top of the diagrams represent a sheltered area in the aquarium (Reproduced from Ref. 32, with permission from the University of Chicago Press).

Clearly, ASR is a much more complex chemoreflex than changes in gill ventilation, with a very large behavioural component, which must involve significant inputs from higher brain centres (32). Teleost fish exhibit behavioural modulation of gill ventilation patterns, and such higher-order inputs to the respiratory medulla must, presumably, have been a prerequisite for the evolution of the complex motor responses of ASR and true air-breathing in fishes. One major ecological cost to reflexes such as ASR and air-breathing is that they place fish at significantly greater risk from aerial predation by birds. Shingles et al. (32) found that if grey mullet perceive a risk of predation they can modulate the behavioural component of the ASR chemoreflex. Exposure of the grey mullet to a model avian predator delayed the onset of ASR in hypoxia or in response to direct chemoreceptor stimulation with cyanide. Furthermore, the fish surfaced preferentially under a sheltered area in their experimental chamber or close to the walls (Figure 5A). In turbid water, the fish could not see the model predator and it had no effect on the onset of ASR but, in turbidity, all the mullet preferentially surfaced around the walls of their chamber (Figure 5B). Thus, the behavioural component of the ASR reflex is plastic; it can be modulated by inputs from higher centres, in particular as a function of perceived risk of predation (39).

A number of species have morphological features, such as upturned mouths and flattened heads, which appear to improve the efficiency of ASR. In some species, the morphological adaptations are very pronounced such as the dermal lip protuberances in various tropical teleosts. For example, in the Neotropical tambaqui, *C. macropomum*, hypoxia causes the lower lip to swell extensively, to form a funnel that skims the surface layer of water into the mouth (28). Florindo et al. (24) reported that this was not an oxygen chemoreflex but, rather, a direct effect of low oxygen on the lip tissue.

Chemoreceptor control of air-breathing. Stimuli for air-breathing in fish include hypoxia and hypercapnia, both modulated by increased temperature and exercise, which increase oxygen demand and CO_2 production (4,37). In the bowfin, air-breathing was only stimulated by changes in water or blood O_2 status, but not by changes in plasma acid-base status (13), and further evidence suggests that bowfin do not possess any central chemosensitivity controlling gill ventilation or air-breathing. Indeed, air-breathing is known to be a chemoreflex driven by oxygen-sensitive receptors (40). The surfacing and gulping response in freshwater air-breathing species can be stimulated by chemoreceptors that sense oxygen levels in either the ventilatory water or the blood stream (13,25).

The sites for O_2-chemoreception in these fishes regulate not only air-breathing, but also changes in heart and gill ventilation. Only a small number of species have been studied to date and there is a lack of information about the site for O_2 receptors, their innervation, the specific reflex triggered by each receptor population and also the interaction among them for controlling the switch between the water and air-breathing. Application of the O_2 chemoreceptor stimulant sodium cyanide (NaCN) into the ventilatory water stream of longnose gar (*Lepisosteus osseus*) inhibited gill ventilation and elicited air-breathing, whereas NaCN given into the bloodstream (dorsal aorta) stimulated both gill ventilation and air-breathing (25). In the bowfin, only externally applied NaCN elicited air-breathing, whereas both external and internal NaCN stimulate gill ventilation (13). In the obligate air breather the African lungfish, there were air-breathing responses to both internally and externally applied NaCN, whereas in the facultative air breather *Ancistrus*, which possesses suprabranchial chambers, hypoxia stimulated air-breathing but increased temperature and exercise did not.

O_2-sensitive chemoreceptors are found diffusely distributed in the gills and pseudobranch of the gar and bowfin, innervated by cranial nerves VII, IX and X. Gill denervation (with pseudobranch ablation in the latter case) almost completely abolished air-breathing in normoxia and abolished responses to hypoxia and NaCN (13,25). Air-breathing can also be stimulated by stretch receptors in the swim-bladder, which in the spotted gar (*Lepisosteus oculatus*) exhibit sensitivity to CO_2. Functional reflex studies in traíra (35), pacu (29) and jeju (Lopes JM, Leite CAC and Rantin FT, unpublished results) have shown a population on O_2 chemoreceptors, innervated by the IXth cranial nerve, that seems to work on decreasing the gill ventilation response to hypoxia. It is possible that those receptors would work in the air-breathing fish by stopping gill ventilation while the ABO organ provided oxygen to the blood in hypoxic waters.

As was the case for fish performing ASR, there is evidence that air-breathing reflexes are significantly influenced by higher central processing, in particular a perceived risk of predation. Smith and Kramer (41) reported that exposure of the Florida gar *Lepisosteus platyrhincus* to a model avian predator resulted in a decrease in air-breathing frequency and an increase in gill ventilation effort. Herbert and Wells (42) found that fear of predation reduced air-breathing frequency by the blue gourami, *Trichogaster trichopterus*, an obligate air breather, which compensated by reducing activity, presumably to conserve the O_2 stored in the ABO.

Very little is known about the reflex control of breathing in amphibious marine species. The giant mudskipper, *Periopthalmodon schlosseri*, lives on tidal mudflats, where it builds a deep burrow, from which it emerges to forage between shallow puddles and the exposed mudflats. It is an obligate air breather, which stores air in a highly vascularized orobranchial cavity, containing much reduced gills. The gills are only ventilated with water when the animal expires the air from its orobranchial cavity, at which point it will submerge the mouth and perform a few cycles of gill ventilation (McKenzie DJ, unpublished personal observations). Aquatic hypoxia has no effect on patterns of gill ventilation or air-breathing in the mudskipper (43). In normoxia at 25°C, mudskippers breathe air about once every 9 min, with each air breath causing a characteristic variation in heart rate that is observed in all air-breathing fishes, namely an expiration bradycardia followed by an inspiration tachycardia (35). A representative trace of this is shown in Figure 6A. Figure 6B shows the effects of aerial hypoxia (20%) on air-breathing, ventral aortic blood pressure and heart rate in the same mudskipper, with a pronounced hypoxic bradycardia and a large increase in air-breathing frequency. Figure 6C shows the effects of cyanide gas, given as a bolus into the buccal cavity, on heart rate, blood pressure and air-breathing in a giant mudskipper. There is a typical teleost bradycardia in response to cyanide, followed by vigourous air-breathing responses. Cyanide given as a bolus into the bloodstream, via an indwelling catheter in the ventral aorta, was without effect on air-breathing or heart rate. Exactly the same pattern of responses to hypoxia and cyanide was observed in a second fish (McKenzie DJ, Taylor EW, Ip YK, unpublished observations). Thus, this meagre data set demonstrates that the giant mudskipper possesses chemoreceptors, which elicit bradycardia and air-breathing, but that these apparently monitor oxygen levels in the air held in the mouth, with no sensitivity to blood oxygen levels.

Baroreceptors in air-breathing fish. There is no experimental evidence for baroreceptor responses in air-breathing fish. Most air-breathing fish supply their various air-breathing organs from the systemic circulation. Lungfish and all tetrapods have distinct pulmonary arteries and veins in association with true lungs, having highly permeable surfaces; the lungfish, *Protopterus*, has a diffusion distance of 0.5 mm over the ABO, which is similar to the mammalian lung (44). However, possibly because they retain gills, lungfish have similar, relatively low blood pressures in the respiratory and systemic circuits and, as a consequence, may not have a functional requirement

for baroreceptor responses to protect their lungs against oedema resulting from hypertension. It could of course be argued that control of blood pressure in a relatively low pressure system requires sensitive pressoreception. This remains to be demonstrated.

Amphibians

Chemoreceptors. A detailed review by West and Van Vliet (45) considered the roles of peripheral chemoreceptors and baroreceptors in cardiorespiratory control in amphibians, while the factors influencing the progressive transition from water to air-breathing during amphibian metamorphosis were reviewed by Burggren and Infantino (46). Responses to hypoxia in larval bullfrogs were eliminated by ablation of the first gill arches, suggesting that they are the site of the O_2-sensitive chemoreceptors. A residual slow response was interpreted as stimulation of a second population of receptors, possibly monitoring the cerebrospinal fluid (CSF). The rapid responses to hypoxia are blunted in later stage bullfrog larvae, in which the lungs are developing and the gills degenerating. In an earlier study of the bimodally breathing bullfrog tadpole, mild aquatic hypoxia was found to increase gill ventilation but more severe hypoxia promoted high frequencies of lung ventilation and a suppression of gill ventilation (1,47), which was in response both to lung inflation *per se* and to the resulting increase in PO_2 (47). In the neotenous, gill-bearing axolotl, *Ambystoma mexicanum*, both gill ventilation and air-breathing were stimulated by cyanide, infused either into the ventilatory water stream or into the blood stream (1). Cardiac responses were complex with an initial bradycardia, presumably in response to stimulation of peripheral chemoreceptors, followed by a tachycardia at the first air breath, possibly in response to stimulation of lung stretch receptors, a situation comparable to the mammalian response to hypoxia (1). Heart rate in the bullfrog tadpole did not change during aquatic hypoxia, with access to air (1,47).

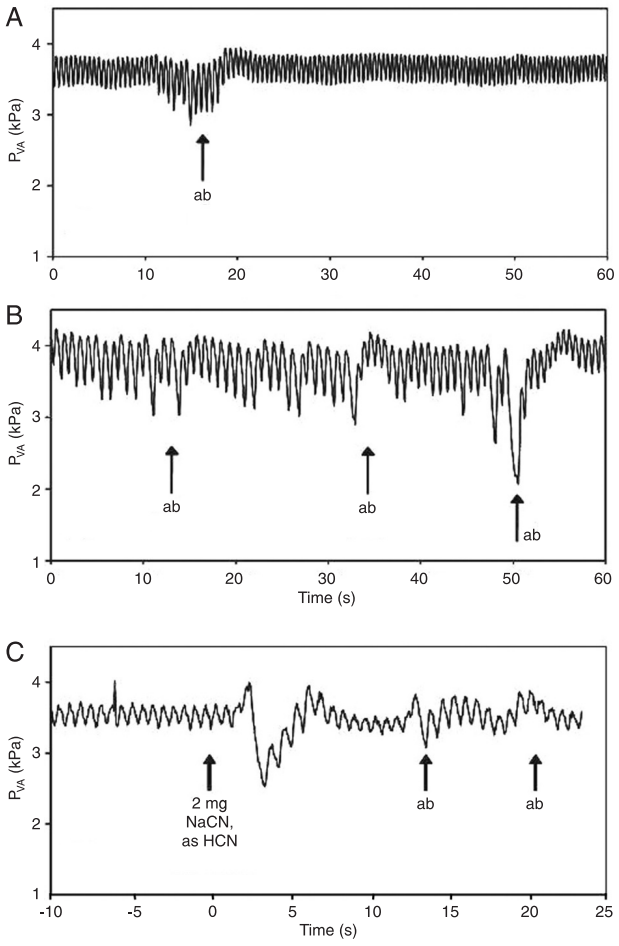

Figure 6. Representative traces of ventral aortic blood pressure (P_{VA}) in a giant mudskipper (*Periopthalmodon schlosseri*, body mass 142 g) in normoxia (A), aerial hypoxia at 5% of normal atmospheric O_2 saturation (B), and in response to a bolus injection of 2 mg NaCN (acidified to form gaseous HCN) given as a bolus into the orobranchial cavity during an air-breath holding period (C), see text for details. ab, air-breath, confirmed visually, with the typical variations in heart rate associated with exhalation and inhalation. In this animal, air-breathing frequency was 7/h in normoxia and rose to 85/h in aerial hypoxia.

While their larvae may retain functional oxygen receptors on the gill arches, the carotid labyrinths are the main putative sites for oxygen receptors in adult amphibians. They are situated at the bifurcation of the internal and external carotid arteries and innervated by branches of the glossopharyngeal nerve, which projects its afferent fibres to the NTS in the brainstem (48). These receptors are functionally similar to the mammalian carotid bodies, as they also respond to hypercapnia and their discharge can be modulated by sympathetic stimulation (1). More recent studies have also shown that the receptors are sensitive to oxygen partial pressure, rather than content (45); a finding consistent with the results of whole animal study of the stimulus modality of the hypoxic ventilatory response in toads (1). Elevated arterial levels of CO_2/H^+ increase discharge rate from the carotid labyrinth of toads (45).

Blood gas levels in adult amphibians are not determined solely by rates of lung ventilation; instead the degree of shunting of blood through the pulmonary circuit and/or the cutaneous vessels may have a major role in determining oxygen and CO_2 levels. The degree of shunting is likely to be referred to input from peripheral chemoreceptors. In the adult bullfrog, more blood is directed towards the lungs during aquatic hypoxia, while aerial hypoxia elicits an increase in cutaneous perfusion. The return of blood to the right side of the heart from the cutaneous circulation may specifically serve to improve oxygen supply to the myocardium, which in amphibians is devoid of a coronary circulation (1).

Perfusion of the brain in anaesthetised toads with artificial CSF having low pH/high CO_2 significantly increased

ventilation in normoxia (49). A similar response was recorded from the *in vitro* preparation of the bullfrog brainstem (1,4). It appears that, as in most vertebrates (other than some fish) investigated thus far, toads have central chemoreceptors, probably located on the ventral surface of the medulla, which respond to acidic/hypercapnic challenge. Repeating these experiments in unanesthetised animals indicated that the contribution of peripheral receptors to respiratory drive was secondary to the role of central chemoreceptors, which contributed about 80% of the total hypercapnic respiratory drive in the toad, a similar proportion to that observed in mammals (1,4,47).

This dominant role for central chemoreceptors in the generation of respiratory drive in amphibians appears at metamorphosis. An *in vitro* preparation of the isolated brainstem from the bullfrog tadpole displayed co-ordinated, rhythmic bursting activity in cranial nerves V, VII and X, which could be characterised as representing fictive gill or lung ventilation. In early stage larvae, variations in pH of the superfusate were without effect on gill or lung burst frequency. Later stage larvae showed an increasing predominance of neural lung burst activity, which markedly increased in acid pH (50). The onset of episodic breathing patterns during metamorphosis was coincident with developmental changes in the nucleus isthmi in the bullfrog, and it seems possible that this region of the brainstem is involved in integration of central chemoreceptor information (17).

Mechanoreceptors. Pulmonary stretch receptors (PSR) constitute another important source of feedback, contributing to the control of breathing in amphibians. There are three different types of PSR in amphibians responding to 1) the degree of lung inflation, 2) the rate at which lung volume changes or 3) both stimuli (51,52). These receptors are innervated by afferent fibres in the pulmonary vagi (51,52), which project to the solitary tract in the brainstem (48). The receptors are mostly slowly adapting and their firing rates decrease when the intrapulmonary CO_2 concentration is increased (47,52,53). There is evidence for interactions between mechanoreceptor and chemoreceptor reflexes. In the toad lung, inflation decreased the effect of cyanide injection, while these same effects were increased by hypercapnia (54).

Pulmonary afferent fibres play a key role in the termination of lung inflation in the adult and inhibition of buccal oscillation in the pre-metamorphic tadpoles. The evidence is that pulmonary deafferentation by vagotomy in *Xenopus* results in an increase in the number of inspirations in a ventilatory period, and overinflation of the lungs (1). Sectioning the pulmonary branch of the vagus nerve leads to an increase in the amplitude and frequency of resting ventilation in the bullfrog (1,55), indicating that PSR feedback modulates the breathing pattern; however, these data also suggest that PSR feedback is not responsible for the onset/termination of the breathing episodes. This matter remains unresolved as decerebrate paralysed anurans showed that lung inflation inhibited fictive breathing, as would be predicted from work on mammals, while another study, of a similar preparation, indicated that lung inflation stimulated breathing (1,55).

Amphibians which breathe discontinuously, often in association with periods of submersion, typically display large increases in heart rate and pulmonary blood flow at the onset of bouts of lung ventilation. However, the contribution of lung stretch receptors to this response is not resolved (45). Whereas artificial lung inflation increased heart rate in anaesthetised toads, in conscious *Xenopus laevis*, PSR denervation did not abolish the increase in heart rate associated with lung inflation and in lightly anaesthetised animals artificial lung inflation did not affect heart rate, though pulmo-cutaneous blood flow increased (1). A similar response was demonstrated in *Bufo marinus* (56).

Reptiles

Chemoreceptors. Scattered groups of glomus cells have been identified in the connective tissue surrounding the main and collateral branches of the carotid arteries in lizards. This area is profusely innervated by the superior laryngeal branch of the vagus nerve and possibly the glossopharyngeal nerve (1). All primary afferent fibres of the glossopharyngeal and the majority of vagal afferent fibres, enter the NTS in the monitor lizard. Although activity in these putative receptors has not been recorded, denervation of this area abolished the increase in ventilation shown by lizards when hypoxic or hypercapnic blood was injected into the carotid arch (1,4,53).

In turtles, O_2 chemoreceptors are not present at the anatomically defined carotid bifurcation because it is formed by division of the internal carotids, the external carotids having atrophied during development (51). Milsom (26) suggests that due to this process the site homologous to the carotid bifurcation in amphibians and mammals is part of the aortic arch in turtles. The largest aggregations of chemoreceptive tissue are found in this central cardiovascular area and are innervated by the superior and inferior branches of the vagus nerve arising from the nodose ganglion. The inferior branch also innervates chemoreceptors located on the pulmonary artery of the turtle (1).

These receptor groups have been shown to respond to changes in oxygen level but their roles in establishing resting ventilatory drive or in reflex responses to hypoxia are unknown (57). Peripheral oxygen receptors in reptiles seem to respond to a reduction in oxygen content (i.e., hypoxemia) or to the rate of delivery of oxygen to the receptor, which includes blood flow, rather than to systemic hypoxia (i.e., a reduction in PO_2). Similar characteristics have been attributed to arterial chemoreceptors in fish and in birds and mammals (57).

Reptiles, in common with some air-breathing fish and amphibians, have pulmonary and systemic circulations that are incompletely separated, so that some systemic venous

blood can bypass the lungs to re-enter the systemic circulation, while some arterialized blood can re-enter the pulmonary circulation. Consequently, arterial blood gas composition is affected by the degree of admixture of oxygenated arterialized blood and oxygen-depleted venous blood, rather than lung gas composition alone as it is in mammals. This presents the intriguing possibility that regulation of these central vascular shunts, with reference to peripheral chemoreceptors, may play an important role in control of arterial blood gas composition in reptiles, independent of ventilatory control (1,19).

The hypercapnic ventilatory response is well developed in reptiles, and changes in Pa, CO_2 rather than Pa, O_2 provide the dominant drive to breathe (21,49). Central chemical control of ventilation in an ectothermic, air-breathing vertebrate was first demonstrated in the unanaesthetized turtle, *Pseudemys scripta elegans* (53). Perfusion of the lateral and 4th cerebral ventricles with artificial CSF caused an increase in ventilation to 4 times control, following a calculated pH change of only 0.02 units. Inhalation of gas mixtures enriched with CO_2 stimulates ventilation in crocodilians. It causes ventilation volume to rise, decreases periods of breath hold and increases the number of breaths in each breathing episode. Snakes and lizards may respond to environmental hypercarbia, which stimulates lung receptors, with decreased ventilation but show a marked increase in response to venous CO_2 loading (49).

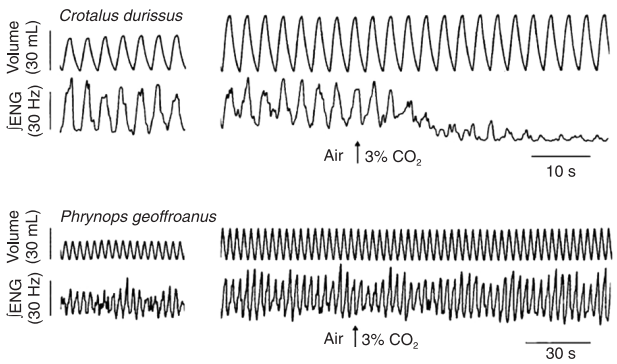

Figure 7. The effects of mechanical inflation of the lungs (left traces) and inhalation of 3% CO_2 (right traces) on integrated afferent activity recorded from the whole vagus nerve of the rattlesnake, *Crotalus durissus*, and the side-necked turtle, *Phrynops geoffroanus.* The upper trace records are changes in lung volume and the lower trace records are changes in nerve discharge rates (Modified from Ref. 60, with permission).

Mechanoreceptors. Pulmonary stretch receptors are present in testudines, lizards, snakes, and alligators. Recordings from the central cut end of the vagus in a range of reptilian species during artificial lung inflation have shown clear evidence for the presence of functional lung stretch receptors (Figure 6) (34). These receptors are sensitive to lung volume and thus provide feedback regarding lung filling and emptying, and with that, of tidal volume. In general, activation of stretch receptors suppresses inspiration and enhances expiration (the Breuer-Hering reflex). Snakes and alligators have both rapidly and slowly adapting stretch receptors (1). Consistent with mammals, the sensitivity of the pulmonary stretch receptors in reptiles is depressed and, in some instances, even silenced by CO_2. This was true in snakes but not in testudines (Figure 7).

In addition to the stretch receptors, most reptiles possess intrapulmonary chemoreceptors sensitive to CO_2 (1,53). Similar receptors are present in birds but have not been demonstrated in amphibians or mammals. The discharge frequency of the intrapulmonary chemoreceptors (IPC) is inversely proportional to PCO_2 and the depressing effect of hypercapnia on minute ventilation in both amphibians and reptiles has been attributed to an inhibitory effect of CO_2 on IPC (49). However, when lung CO_2 changes in a physiologically realistic fashion IPC may in fact stimulate, rather than depress, ventilation so that IPC may play an important role in regulating ventilation whenever metabolic rate is increased (57).

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