Control of the Heart and of Cardiorespiratory Interactions in Ectothermic Vertebrates

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Abstract The functional anatomy of the respiratory and cardiovascular systems and the neuro-anatomy and neuro-physiology of the systems that are implicated in the co-ordination of cardiac output with ventilation are reviewed in fish, including air-breathing fish, amphibians and reptiles. Recent data is reviewed in the light of previous studies on mammals. This account focuses on the roles of the autonomic nervous system in both feed-forward and feedback control of the respiration-related, beat-to-beat changes in heart rate that accompany continuous, rhythmical breathing as well as the marked changes in heart rate associated with bouts of discontinuous breathing. The control of cardiac shunting in species with incompletely separated systemic and pulmonary blood flow is also described.

1 Introduction

Vertebrates supply oxygen to the tissues and remove metabolically produced CO_2 through a concerted action of the respiratory and cardiovascular systems. An appropriate coordination of these systems is, therefore, important for effective gas exchange, and it is essential that both systems can respond in a coordinated manner when metabolic demands change or when gas composition in the environment is altered. It is not surprising, therefore, that the cardiovascular and respiratory systems of all vertebrates are functionally linked. For example, changes in heart rate with ventilation occur in all vertebrate groups and, in spite of the large anatomical differences, it is believed that the essential components of the cardiorespiratory control systems are similar among the different vertebrate taxa (e.g. Taylor et al. 1999). For example, in mammals, heart rate rises during inspiration, a phenomenon termed

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respiratory sinus arrhythmia (RSA), while fish may show periods of synchrony between heart beat and ventilation (CRS).

As a common feature of vertebrates, the respiratory rhythm is generated in the brainstem, while the heart beat is initiated by pacemaker cells at the sinoatrial node, or more diffusively within the vena cava. Cardiorespiratory interactions, therefore, arise from coordination of activities in the central respiratory rhythm and the cardiac pacemaker. Ventilatory and cardiovascular responses are initiated by feedback from peripheral and central chemo- and/or mechanoreceptors that sense, for example, changes in blood gas composition or lung stretch. Central integration of the afferent feedback from these receptors ultimately modifies motor output to the respiratory muscles and the autonomic regulation of the heart and blood vessels. In addition to reflexes that are initiated or mediated by afferent receptor input, cardiorespiratory interactions can be generated within the central nervous system (CNS) and, thus, serve as a feed-forward mechanism. These interactions occur in mammals in the nucleus ambiguus (NA), an area of the brainstem situated ventrolaterally from the dorsal motor nucleus of the vagus (DVN), where the bulk of cardiac vagal preganglionic neurons (CVPN) are situated (Jordan and Spyer 1987). Clear evidence has been obtained for two functional populations of CVPN in dogfish (Barrett and Taylor 1985c) and then in mammals (Daly and Kirkman 1989) and exploration of similar distributions and roles for CVPN in other non-mammalian vertebrates is a central theme of this review, briefly considered by Taylor (1993). Roles for reflex and central mechanisms are, of course, not mutually exclusive and it is entirely possible that both occur simultaneously in all groups of vertebrates. Although many ectothermic vertebrates exhibit changes in heart rate and blood flow during ventilation that are much larger than commonly observed in mammals, the underlying mechanisms are better understood in mammals than in other groups of vertebrates. Thus, in a series of studies on cross-perfused dogs, where the effects of lung stretch receptor feedback could be dissociated from central irradiation of ventilatory activity to the cardiovascular center, Anrep et al. (1936a, b) firmly established that both mechanisms contribute significantly to RSA in mammals. Now it is widely recognized that the following factors contribute to RSA in mammals:

(1) Central communication between the respiratory centres and the cardiac vagal motoneuron pools in the medulla, acting as feed-forward control
(2) Activation of pulmonary stretch receptor stimulation during ventilation acting as a feedback signal
(3) Modulation from CO_2- and O_2-sensitive chemoreceptors
(4) Mechanical effects on the heart and associated blood vessels due to ventilatory movements of the thoracic cavity

Here we will review the interactions between ventilation, heart rate and central vascular blood flows in the major groups of ectothermic vertebrates (i.e. excluding birds and mammals). A general model of these interactions is given in a schematic diagram in Fig. 1. Apart from giving a general introduction to the range of mechanisms underlying the control of cardiorespiratory interactions in each group, we have

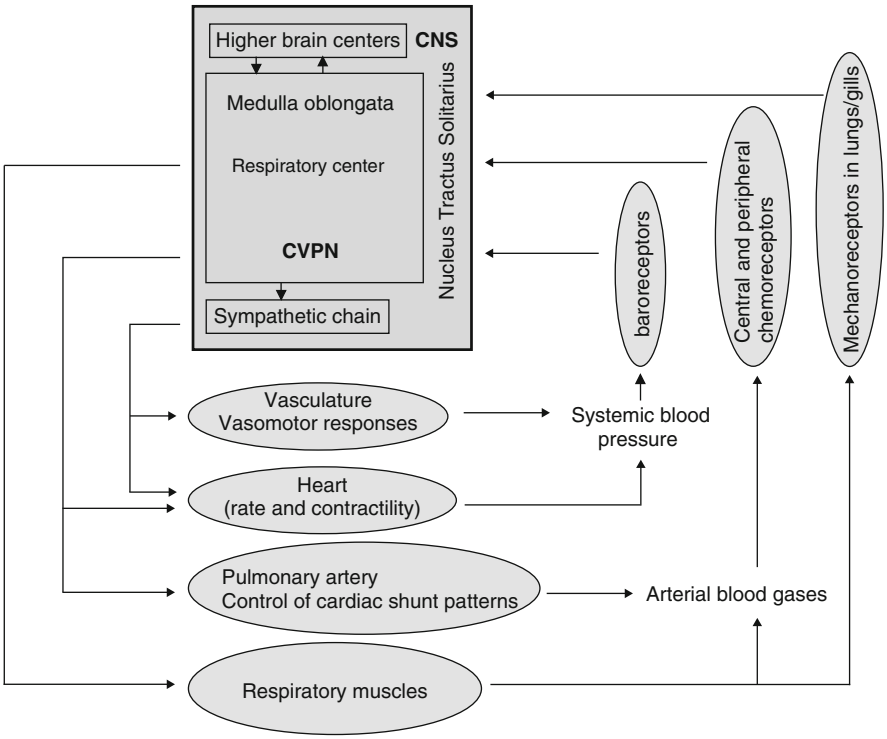


Fig. 1 Diagram of the major components mediating cardiorespiratory interactions in vertebrates. The respiratory rhythm is generated within the medulla oblongata, and leads to subsequent activation of respiratory muscles. Ventilation stimulates mechanoreceptors in the respiratory apparatus (gills or lungs), which, together with chemoreceptors sensing ambient and blood gas levels, provide feedback to the respiratory oscillator. Cardiovascular changes accompanying ventilation may arise from central interactions between the respiratory and cardiovascular centers within the central nervous system (CNS), as well as from feedback from central and/or peripheral chemoreceptors. In amphibians and reptiles, control of blood gases is also influenced by changes in pulmonary blood flow and cardiac shunt patterns. Variations in arterial blood pressure, resulting from altered cardiac output and changes in vasomotor tone, are sensed by baroreceptors. All of these inputs are integrated in the nucleus tractus solitarius (NTS). The heart and the pulmonary artery are innervated by the vagus, which exerts an inhibitory action on heart rate and causes a constriction of the pulmonary artery that increases pulmonary vascular resistance and induces right-to-left cardiac shunts. The sympathetic innervation normally increases both rate and contractility of the heart, and may lead to a minor relaxation of the sphincter surrounding the pulmonary artery

chosen a few examples to illustrate these mechanisms. Tonic and rapid phasic control of the heart that is likely to generate cardiorespiratory interactions is predominantly parasympathetic (Bootsma et al. 1994; Campbell et al. 2004, 2006). Accordingly, this synoptic review will concentrate on cardiac control from the parasympathetic nervous system, via the Xth cranial nerve, the vagus, and its interactions with activity in the nerves supplying the cranial respiratory muscles in fish and amphibians and the thoracic respiratory apparatus in reptiles. Some information

derived from mammalian studies is included to clarify our current understanding of certain mechanisms. For more detailed accounts of this topic the reader is directed to recent reviews (Taylor et al. 1999, 2001; Wang et al. 1999, 2001b).

2 Efferent Innervation of the Vertebrate Heart

The innervation of the heart has undergone marked evolutionary changes within vertebrates and may be viewed as a gradual transition from an aneural heart in hagfish, which are the most primitive extant craniates, to the dual innervation that is characteristic of all higher vertebrates (Fig. 2). Thus, while the heart of hagfish (myxinoids) does not appear to be innervated (Augustinsson et al. 1956; Jensen 1965), and seems insensitive to the classic neurotransmitters, the lamprey heart receives vagal innervation. In contrast to all vertebrates, however, this vagal innervation is excitatory, and is mediated by acetylcholine acting on nicotinic cholinoceptors (Taylor et al. 1999).

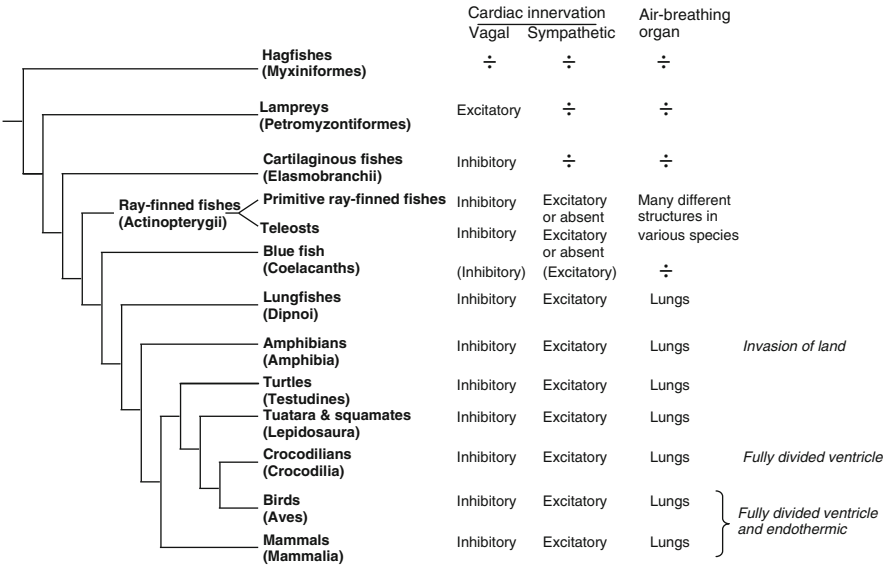


Fig. 2 Phylogeny of the cardiac innervation and air-breathing organs in the major groups of living craniates. The heart of hagfishes is aneural, while the heart of lampreys receives an excitatory vagal innervation. An inhibitory vagal innervation that relies on activation of muscarinic receptors on the heart appeared in cartilaginous fishes and was retained in all other vertebrates. The excitatory sympathetic innervation is present in most but not all ray-finned fishes, and all other higher vertebrates. Air-breathing organs evolved independently in several groups of ray-finned fishes, while true lungs first appeared within lungfishes and are found in all tetrapods. Endothermy seems to have evolved independently in birds and mammals

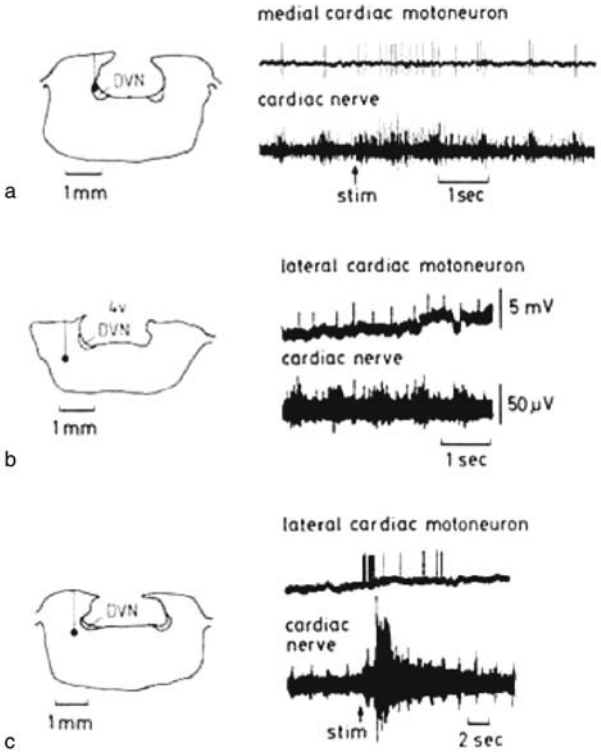
Elasmobranchs are the earliest group known to have an inhibitory vagal innervation of the heart, but there is no direct sympathetic innervation. Circulating catecholamines, however, are important in cardiac control (Randall and Taylor 1991). A dual innervation of the heart appeared in ray-finned fishes (Actinopterygii), and while sympathetic innervation may have been subsequently lost in some cases such as sturgeons, the combination of vagal inhibition and sympathetic stimulation is characteristic of all higher vertebrates. The inhibitory parasympathetic input from the tenth cranial nerve (the vagus) uses acetylcholine (ACh) as neurotransmitter that acts on muscarinic receptors and can be blocked by the muscarinic receptor antagonist atropine. This innervation predominantly affects heart rate. The excitatory sympathetic input from spinal nerves, on the other hand, stimulates both rate and force of contraction, and uses adrenaline or noradrenaline as neurotransmitter. These catecholamines act on β-adrenergic receptors that can be blocked by antagonists such as propranolol or sotalol.

There are plenty of studies showing that infusion of ACh or catecholamines mimic the effects of vagal or sympathetic stimulation respectively, but it is also well-established that the autonomic nervous system of many lower vertebrates uses a number of other agents in addition to the classic neurotransmitters. There is evidence for purinergic control of the heart in dogfish, while neuropeptide Y modulates its cardiac responses to noradrenaline (Xiang et al. 1994). In amphibians, there is a particular prevalence of non-adrenergic-non-cholinergic (NANC) factors (see Morris and Nilsson 1994), and in snakes a NANC factor seems to accelerate heart rate following a meal (Wang et al. 2001a). Within the context of cardiorespiratory interactions, the existence of these transmitter substances implies that the control exerted via the autonomic nervous system is subject to modulation by endocrine and endogenous factors.

2.1 Elasmobranchs

The well-developed autonomic nervous system of sharks and rays is clearly differentiated into parasympathetic and sympathetic components (Taylor 1992), but the sympathetic nervous system does not extend into the “head” (Young 1950). As a result, there is no direct sympathetic innervation of the heart or the gills, and nervous control of the heart and cardiorespiratory interactions is restricted to the inhibitory vagal innervation. Although variations in cholinergic vagal tonus on the heart are the sole source of nervous cardioregulation in elasmobranchs (Butler and Taylor 1971; Taylor et al. 1977), circulating catecholamines have been shown to modulate the sensitivity of the heart to parasympathetic control (Agnisola et al. 2003).

Injection of neural tracers to identify vagal preganglionic neurons (VPN) in the brainstem of the dogfish, *Scyliorhinus canicula* revealed that about 90% were located in the dorsal motor nucleus of the vagus (DVN). A clearly distinguishable group of cells with scattered distributions outside of the DVN that comprised 8% of the total population of VPN were identified as cardiac VPN (CVPN), innervating the heart via the branchial cardiac nerve (Fig. 3). They constitute about 45% of CVPN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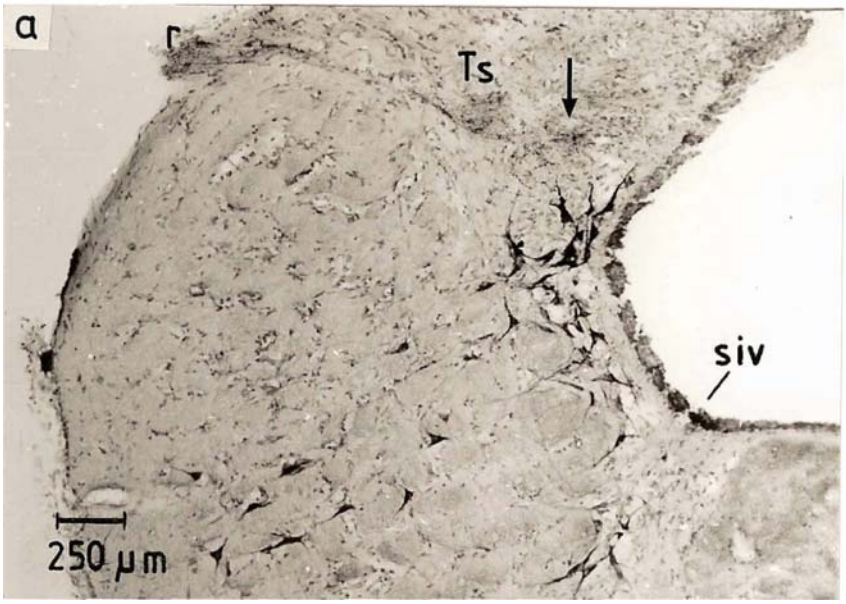
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with the rest located in the DVN, where they have an overlapping rostro-caudal distribution with neurones supplying respiratory muscles in the gill arches (Barrett and Taylor 1985b; Taylor 1992; Taylor et al. 2009c) This dual location of CVPN has been shown to have important functional implications (see below).

2.2 Teleosts

The sympathetic chains extend into the head in teleosts, where they contact cranial nerves, and form vagosympathetic trunk with the vagal fibers that exert cardioinhibitory control (e.g. Gannon and Burnstock 1969). Thus, teleosts may be considered the earliest group of vertebrates with both sympathetic and parasympathetic control of the heart (Taylor 1992 and see Fig. 2). However, the influence of the sympathetic innervation on the fish heart varies between species and is often virtually absent, so that parasympathetic influences predominate, particularly in the generation of cardiorespiratory interactions (Fig. 2).

A similar distribution of VPN and CVPN to that described above for the dogfish has been described in the cod, *Gadus morhua* and trout, *Oncorhynchus mykiss* (Withington-Wray et al. 1987). About 12% of VPN were located outside the DVN and, although most of these were CVPN, some of them innervated the gills, possibly supplying vasomotor input to branchial blood vessels (Taylor 1992). A current study of pacu, *Piaractus mesopotamicus* (E.W. Taylor and C.A.C. Leite, unpublished observations) revealed CVPN distributed in separate nuclei within the DVN, a ventral group containing about 60% of cell bodies, and a dorsal group containing about 40% of cell bodies. In addition, there were a small number of cell bodies scattered laterally outside of the DVN, constituting only about 2% of the total number of CVPN. So the neuranatomical basis for control of the heart and CRI is not consistent within the teleost fishes. In pacu, the CVPN had overlapping rostro-caudal distributions with motoneurons having their axons in the VIIth, IXth and Xth cranial nerves, supplying respiratory muscles, but not with those supplying the mandibular branch of the Vth cranial nerve (Fig. 4 and see Taylor et al. 2009b).

Fig. 3 (Continued) **a** A transverse section taken through the brainstem of the dogfish, *Scyliorhinus canicula*, to show cardiac vagal preganglionic neuron cell bodies (CVPN) stained black with horseradish peroxide, after its application to a cardiac nerve. They are located in the DVN close to the 4th ventricle (grouped directly below the *vertical arrow*) and in scattered locations ventrolateral to the DVN (*r*, rootlet of vagus nerve; *siv*, sulcus intermedius ventralis in the wall of the fourth ventricle; *Ts*, sensory projection from the cardiac vagus). **a–c** Extracellular recordings from CVPN in the brainstem of the dogfish, identified by antidromic stimulation of the cardiac nerve followed by dye injection (the course of the electrode and the recording position are shown by the *line* and *filled circle*); together with simultaneous recordings of activity in the ipselateral cardiac nerve. A unit located in the DVN (**a**) fired in bursts that contributed to those recorded from the cardiac nerve and responded to mechanical stimulation of a contra-lateral gill septum (stim). Units recorded from lateral locations, outside the DVN, fired regularly (**b**), sporadically, or were silent (**c**), except when responding to mechanical stimulation of a gill septum (stim). (Taken from Taylor 1992)

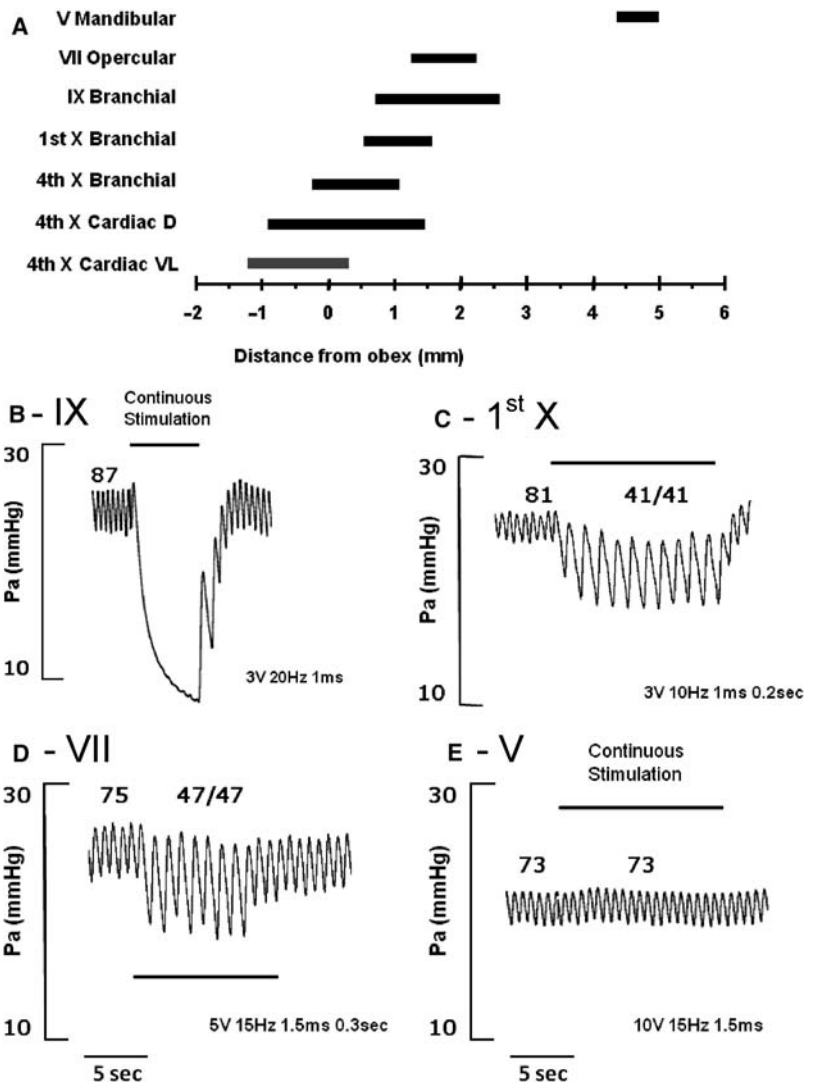

Fig. 4 a The rostro-caudal distribution in the brainstem of the pacu, *Piaractus mesopotamicus,* of cell bodies of respiratory visceral motor neurones (RVMN) supplying axons to the mandibular Vth, opercular VII, branchial IX, first and fourth branchial X, together with the CVPN in the DVN (Cardiac D) and ventro-lateral group (Cardiac VL). Data are taken from fish with the best fills (largest number of labelled cell bodies). Effects on heart rate, measured as blood pressure, of electrical stimulation of the central cut ends of: *B*, the glossopharyngeal IXth; *C*, the branchial Xth; *D*, the facial VIIth; and *E*, the mandibular Vth cranial nerves. The period of stimulation is indicated by the *horizontal bar above* or *beneath* each trace and the stimulation parameters are given *below* each trace. The initial, intrinsic heart rate (bpm), followed by the bursting rate of the electrical stimuli and the consequent heart rate are given *above* each trace. Continuous stimulation of the IXth caused cardiac arrest, but stimulation of the Vth was without effect. Phasic stimulation of the VIIth, XIIth or Xth recruited heart rate to the applied bursting frequency. (Taken from Taylor et al. 2009c)

2.3 Air-breathing Fish

Application of a neural tracer to branchial branches of the vagus nerve in the bowfin, *Amia calva*, labelled cell bodies in the dorsal motor nucleus of the vagus (DVN) and in lateral locations outside the DVN, as described in teleosts (e.g. cod) and all tetrapod vertebrates (Taylor et al. 1999). The nerve supplying the glottis and ABO had cell bodies in a ventrolateral location in the brainstem and the ventral horn of the anterior spinal cord. From their location it was possible to identify them as cell bodies which typically supply axons to the hypobranchial nerve that provides nerves to elements of musculature normally associated with feeding movements. In water-breathing fish these are used to gulp air at the surface. In addition, the ABO was innervated by a group of cell bodies in the DVN that may provide efferent axons to smooth muscle in the swimbladder wall, comparable with the vagal efferents controlling reflex broncho-constriction in the mammal (Taylor et al. 1996).

2.4 Amphibians

In the aquatic amphibian *Xenopus laevis*, the vagal motonucleus is located in the medulla oblongata over a rostro-caudal distance of about 4 mm, either side of obex, with over 90% of neuron cell bodies rostral of obex. A sensory projection of the vagus was revealed, by anterograde transport of HRP along afferent axons, with diffuse labelling of the dorsal visceral sensory nucleus over a rostro-caudal distance of about 2 mm, rostral of obex in an area identified as the nucleus of the solitary tract (NTS) (Wang et al. 1999). All branches of the vagus in *Xenopus* are supplied with efferent axons by neurons with their cell bodies, either in a medial nucleus, within the central grey, equivalent to the mammalian dorsal vagal motonucleus (DVN), or in a ventro-lateral nucleus, outside the central grey, which may be the amphibian equivalent of the nucleus ambiguus (NA). The cardiac vagus had 26% of its CVPM in the NA. Cardiac and pulmonary vagal motoneurons showed a largely overlapping distribution. In the axolotl *Ambystoma mexicanum*, the neotenous adult had no VPN outside of the DVN. Following metamorphosis, induced by injection of thyroxine, the number of VPN increased, and 15% were now located outside of the central grey matter. This ventro-lateral migration was accompanied by migration onto land and a switch to lung breathing (Taylor et al. 1999, 2001).

The neuranatomical evidence that cardiac and respiratory VPM are located in close proximity in the anuran brainstem implies that some cardiorespiratory interactions in anurans may be generated by synaptic interactions between central neurons generating efferent outflow to the heart and respiratory apparatus. This may be of particular importance in amphibians as their major respiratory muscles, as well as the airways, are innervated by cranial nerves, including the vagus, which have their cell bodies in the brainstem, in close proximity to CVPM. However, at present, there is no physiological evidence for such a synaptic interaction between

the populations of vagal preganglionic motoneurons controlling cardiovascular and respiratory function in amphibians.

As in mammals, the amphibian heart is innervated by spinal sympathetic stimulatory and cranial (vagal) parasympathetic inhibitory nerves. The sympathetic nerves join the vagus nerve before reaching the heart to form a vago-sympathetic trunk that innervates the atria, the ventricle and the sinus venosus. The vagosympathetic trunk also innervates a sphincter immediately distal to the point where the pulmocutaneous artery divides into the pulmonary artery proper and the cutaneous vessel (de Saint-Aubain and Wingstrand 1979; Morris and Nilsson 1994). This sphincter consists of concentrically arranged layer of smooth muscle cells, and provides a means to control pulmonary blood flow by redirecting cardiac output into the systemic circulation (Wang et al. 1999). Consistent with this pattern of innervation, injection of the muscarinic blocker, atropine, increases heart rate and pulmonary blood flow, while adrenergic blockade causes an immediate decrease in heart rate (Taylor and Ihmied 1995; Gamperl et al. 1999). Vagal tone on the heart dominates over-sympathetic tone in *Xenopus* (Taylor and Ihmied 1995) and the adrenergic regulation of the pulmonary circulation seems less important than the vagal tone. However, the extent to which withdrawal of vagal tone versus increased sympathetic tone accounts for the increased heart rate and pulmonary blood flows associated with ventilation in amphibians has not been determined. Numerous studies demonstrate the presence of other neurotransmitters than ACh and catecholamines in the autonomic nervous innervation of the cardiovascular system in amphibians, and atropine, for example, cannot fully block the decrease in heart rate as a result of vagal stimulation in *Bufo* (e.g. Preston and Courtice 1995). The existence of these NANC factors implies that it is technically difficult to study cardiovascular regulation in amphibians because traditional pharmacological approaches may not suffice.

2.5 Reptiles

The vagus nerve in reptiles runs to the heart, trachea, lungs, pulmonary and coronary vasculature, thymus, thyroid and gut, supplying preganglionic fibres. The vagus has an inhibitory effect on heart rate (Fig. 2), while effects on contractility appear to be rather small. The vagus also innervates smooth muscle surrounding the pulmonary artery, where increased vagal tone causes constriction and associated narrowing of the pulmonary artery. Thus, conditions of high vagal tone, characteristic of resting reptiles with low metabolic demands, are associated with low heart rates and low pulmonary blood flows, leading to right-to-left cardiac shunts where blood is recirculated into the systemic circulation. The vagal effects on both the heart and the pulmonary artery can normally be abolished with atropine, and while there is plenty of immunohistochemical evidence for the presence of NANC factors in the reptilian circulatory system, it seems that most of the efferent vagal control is mediated by ACh acting on the classic muscarinic receptors. The reptilian heart

also receives sympathetic innervation that exerts positive chronotropic and inotropic effects on both the atria and the ventricle. These effects can normally be blocked by β-adrenergic antagonists. In general, the vagus exerts larger effects on the pulmonary circulation than sympathetic innervation (Overgaard et al. 2002; Galli et al. 2007; Taylor et al. 2009), but this may depend on species, and may vary with metabolic state and certain types of behaviour.

The somatotopic representation of the vagus in reptiles is little-known and seems to vary between species. Early studies on reptiles described two divisions (medial and ventrolateral) of the vagal motor column in a variety of species, which were provisionally designated as the DVN and NA. The pattern of labelling is on the whole similar to that observed after applying neural tracers to the vagus nerve in other vertebrates with differences in the degree of representation of VPN in a lateral division. A "nucleus ambiguus" (NA) was identified adjacent to the DVN in the tortoise (Cruce and Niewenhuys 1974) with between 36 and 50% of VPN located in the NA of the terrapin (reported by Taylor et al. 1999). An initial HRP study of the vagal motor column in the agamid lizard *Uromastyx microlepis* revealed that the majority of VPN are in the DVN, with a small proportion (6%) ventrolaterally located in the NA. A putative NA was also described in a lizard and an alligator but was apparently absent from a snake (Taylor et al. 1999, 2001). However, in the rattlesnake *Crotalus durissus*, use of fluorescent markers revealed that the majority of VPN are in the DVN, with a small proportion (4%) ventrolaterally located in a putative NA. Despite the paucity of cells in the NA these reptiles showed clear respiratory modulation of heart rate (see below). The somatotropic representation of the cardiac and pulmonary innervation is virtually unknown in reptiles and further study is warranted.

The basis of the apparent variation in location of VPN is likely to be that the reptiles are not a homogeneous group, having wide evolutionary divisions separating the present-day reptiles (Taylor et al. 1999). The chelonians (turtles and tortoises) are anapsids, a group regarded as primitive, having arisen from close to the ancestral reptilian stock (evolved from primitive amphibians). The snakes and lizards are diapsids, from the same reptilian stock that produced the archosaurs. These in turn evolved into the ruling reptiles ("dinosaurs") represented today by the crocodiles and alligators and, on another evolutionary line, the birds. Birds have less than 5% of VPN outside of the DVN but 30% of CVPN are in a ventrolateral nucleus, and birds have been shown to exhibit RSA (Taylor et al. 2001). Mammals are recognised as having evolved from a separate, primitive reptilian stock, the synapsids. These were remote in evolutionary terms from the lines leading to the present-day reptiles and the birds, but may have been closer to their amphibian ancestors and to the primitive chelonians. In mammals the bulk of CVPN are located in the NA, where inhibitory influences from neighbouring inspiratory neurones are recognised as the major source of RSA (Taylor et al. 1999). Thus, the disposition of VPN may have phylogenetic as well as functional correlates, but there seems to be clear evidence that a dual location for CVPN is a factor in generating cardiorespiratory interactions in vertebrates.

3 Cardiorespiratory Interactions

3.1 Fish

In fish water and blood are delivered directly to either side of the respiratory gas exchange surfaces on the gills, and there is a close matching of respiratory water flow and cardiac output, according to their relative capacities for oxygen (the ventilation/perfusion ratio), which is thought to optimise respiratory gas exchange over the functional counter-current at the gills (Hughes and Shelton 1962; Piiper and Scheid 1977; Taylor 1992). As both water and blood flows over and within the gills are markedly pulsatile (e.g. Jones et al. 1974), close beat-to-beat temporal relationships between heart beat and ventilation or cardiorespiratory synchrony (CRS) have long been hypothesised as being important for the optimisation of respiratory gas exchange (Satchell 1960). More recent work has established direct evidence in some fishes for fine control of heart rate, including its beat-to-beat modulation by the respiratory cycle that generates cardiorespiratory interactions (CRI), culminating in CRS (Taylor 1992). In addition, more subtle modulation of heart rate by respiratory activity, termed cardiorespiratory coupling (CRC), has been demonstrated by power spectral analysis of cardiac intervals (Campbell et al. 2004; Taylor et al. 2006). Abolition of CRC by cardiac vagotomy was shown to affect oxygen uptake (Campbell and Egginton 2007).

In most fish it seems that variations in the inhibitory vagal tone, imposed by activity in cardiac vagal preganglionic neurones (CVPN) within the medulla oblongata, are the predominant factors generating cardiorespiratory interactions (Taylor et al. 1999, 2009–c). Below, we review the location of CVPN and the putative roles of feed-forward control from within the CNS and feed-back control from peripheral chemoreceptors and mechanoreceptors in determining their activity. Recordings of spontaneous efferent activity in cardiac vagi contain bursts of respiration-related activity. We have investigated the origins of this activity and its possible effects on the heart. Bursts of electrical stimuli delivered peripherally to the cardiac vagus or centrally to respiratory branches of cranial nerves VII, IX and X can recruit the heart at a range of frequencies (Taylor et al. 2006, 2009b). In elasmobranchs, phasic efferent activity in cardiac vagi that are the basis of cardiorespiratory interactions seems to originate primarily from central interactions between respiratory neurones and CVPN, and is characterised by relatively low levels of vagal tone on the heart (Taylor 1992). In teleosts the bursts seem to be driven reflexly by stimulation of peripheral chemoreceptors and mechanoreceptors when respiratory drive and cardiac vagal tone are high (Taylor et al. 1999, 2009b). These differences seem fundamental. However, reflex control from peripheral receptors is important in determining activity in CVPN, and consequently heart rate, in all fish including elasmobranchs (see Fig. 3a and c) as well as teleosts (see Fig. 4c and d), and evidence from current investigations suggests that there are elements of central feed-forward control of cardiorespiratory interactions in some teleosts as well as elasmobranchs. Consequently, it seems probable that variable combinations (relating to conditions

and possibly to species differences) of feed-forward control via central interactions, plus feed-back control from peripheral receptors, determine activity in CVPN in fish. This in turn can recruit the heart to the respiratory rhythm, though it may subserve different roles in different groups of fish.

3.2 Air-Breathing Fish

Many of the primitive ray-finned fishes are air-breathers and air-breathing also seems to have evolved numerous times within teoleosts (Graham 1997). The anatomy and physiology of the air-breathing organs vary enormously among species, but it is characteristic that heart rate increases during air-breathing. This tachycardia probably contributes to a temporal matching of perfusion and ventilation of the air-breathing organ, although the significance of the putative function of this matching remains to be studied experimentally. Thus, it is noteworthy that complete pharmacological abolition of the heart rate changes in the air-breathing jeju does not affect oxygen uptake (McKenzie et al. 2007), and it is clearly of interest to investigate the functional correlates of cardiorespiratory interactions in air-breathing fishes.

In lungfish (dipnoi), a vagal innervation of the heart is well established, whilst the sympathetic chain seems poorly developed (Axelsson et al. 1989). In the African lungfish *Protopterus sp*, there were very small changes in heart rate during the intermittent ventilation of the lungs, while heart rate increased both in the South American and in the Australian lungfishes, *Lepidosiren paradoxa* and *Neoceratodus forsteri* (Johansen and Hanson 1968; Axelsson et al. 1989; Fritsche et al. 1993; Sanchez et al. 2001). The rather small influence of lung ventilation may, at least in part, reflect the surgical difficulties associated with physiological instrumentation of these animals, and studies on minimally instrumented lungfish would be of great interest. In any event, although heart rate does not change much with breathing, pulmonary ventilation is associated with a marked rise in pulmonary blood flow in all species of lungfish (Johansen et al. 1968a; Fishman et al. 1985; Burggren and Johansen 1986; Axelsson et al. 1989; Fritsche et al. 1993). This rise in pulmonary blood flow is caused by a combination of β-adrenergic and cholinergic mechanism (Johansen and Reite 1968) but the afferent signals leading to the vascomotor responses remains to be studied. Lungfish are endowed with pulmonary stretch receptors as well as both central and peripheral chemoreceptors making it likely that feedback from these receptors is involved (Delaney et al. 1983).

Changes in heart rate during ventilation are also evident in *Arapaima gigas*, an obligatory air-breathing, osteoglossid fish that relies on the heavily vascularised swimbladder for gas exchange (Farrell 1978). In the jeju (*Hoplerythrinus unitaeniatus*), a teleost fish that also uses a modified swimbladder as air-breathing organ, heart rate decreases during expiration, but increases drastically during the subsequent inhalation that inflates the swim bladder (Farrell 1978; McKenzie et al. 2007). These heart rate changes were primarily of cholinergic origin and an in-depth analysis of the heart rate variability in this species indicated that the heart rate

changes were qualitatively similar to RSA in higher vertebrates (McKenzie et al. 2007). Air-breathing also causes heart rate to increase in the climbing perch where the air-breathing organ is located in a suprabranchial cavity (Singh and Hughes 1973), and similar responses have been reported for the electric eel and two related species of synbranchid eels, all of which use the buccopharyngeal cavity for gas exchange (Johansen et al. 1968b; Graham et al. 1995; Skals et al. 2006). An example of the rapid heart rate change upon an air-breath in *Synbranchus marmoratus* is shown in Fig. 5 (Skals et al. 2006). As seen in this example, expansion of the buccopharyngeal cavity leads to a marked rise in heart rate, which is attended by a rise in central venous pressure, which is likely to preserve cardiac filling, keeping stroke volume constant in spite of the decreased filling time (Skals et al. 2006).

While changes in cholinergic tone seem to dominate the afferent regulation of the heart rate changes associated with air-breathing in both primitive and derived fishes, the efferent mechanisms remain to be studied in more detail. However, in the electrical eel as well as the synbranchid eels, manual inflation of the buccopharyngeal cavity elicits heart-rate changes that are similar to those occurring during spontaneous air-breathing (e.g. Graham et al. 1995). This unequivocal evidence for stretch receptor feedback being involved in the regulation of the cardiorespiratory interactions is interesting, given the large variation in respiratory structures among the various air-breathing fish, and it would be interesting to identify the central projections in an array of different air-breathing fishes. The apparent central role of stretch receptor feedback obviously does not exclude the possibility of centrally generated interactions leading to feed-forward control. Also, it is quite likely that inputs from chemoreceptors are involved. Thus, as shown in the example of Synbranchus (Fig. 4), which is typical for other species of air-breathing fish as well, heart rate decreases progressively during the breath hold. During this time, PO_2 of the gas within the air-breathing organ decreases while CO_2 accumulates to a lesser extent, given that it may be excreted to the water over the gills or accumulates in body fluids.

3.3 Amphibians

The larvae of all amphibians are aquatic, but the gills are lost in virtually all species during development, but the adult forms maintain bimodal gas exchange where the structurally simple lungs are supplemented with cutaneous gas exchange. The lungs are ventilated by muscles inserted around the bucco-pharangeal cavity, innervated by efferent motor output from cranial nerves, which generate a positive pressure driving air into the lungs. The respiratory rhythm is generated within the central nervous system (CNS) and is modulated by peripheral chemo- and mechanoreceptors as well as central chemoreceptors in the medulla. The cardiovascular system of amphibians, as well as reptiles, is complex because the ventricle is undivided, allowing for mixture of divided ventricle with blood flow to the lungs, skin and body controlled independently. Thus, cardiorespiratory responses

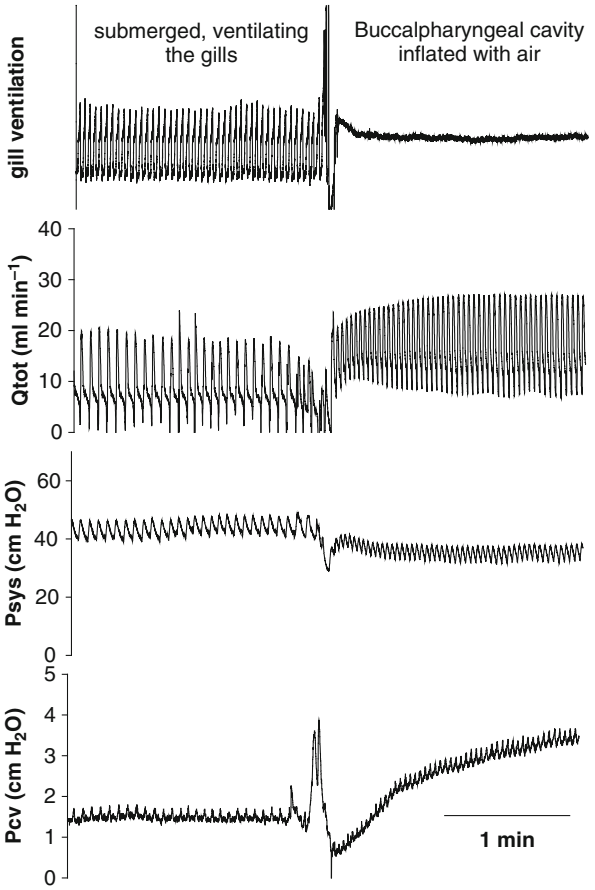

Fig. 5 The changes in heart rate and blood flow in the ventral aorta associated with the transition from aquatic gill ventilation to inflation of the buccopharyngeal cavity with air in the air-breathing fish *Synbranchus marmoratus*. Both heart rate and cardiac output (Qtot) increase rapidly in association with the air-breath because stroke volume is maintained by an increase in central venous pressure (*Pcv*) that probably is caused by α-adrenergic venous constriction (*Psys* is the pressure in the dorsal aorta). (Taken from Skals et al. 2006)

to increased metabolism or hypoxia can consist of increased ventilation, increased heart rate and/or redistributions of blood flows (see Wood 1984; Wang and Hicks 1996b).

In addition to the control of heart rate, the vagus (cranial nerve X) also regulates vascular resistance in the pulmonary artery which, in turn, affect cardiac shunts within the undivided anuran ventricle. Thus, increased vagal tone increases resistance to pulmonary blood flow and induces large right-to-left shunts and leads to lower oxygen concentrations in the arterial blood. Thus, in combination with the lower heart rate, high vagal tone is associated with a reduction in systemic oxygen delivery, and high vagal tone and the predominance of right-to-left cardiac shunts

are indeed most prevalent in resting undisturbed animals where metabolism is low (e.g. Gamperl et al. 1999; Wang et al. 2001b). In active animals, lung ventilation is associated with a relative left-to-right shunt, causing blood flow to be redirected to the lungs (Shelton 1970). However, little is known about the control of central vagal motor outflow to the heart and pulmocutaneous artery. Anatomical evidence indicates a close proximity of the centers responsible for respiratory rhythmogenesis and the vagal motoneurons involved in cardiovascular regulation. Furthermore, anurans in which phasic feedback from chemo- and mechanoreceptors is prevented by artificial ventilation exhibit cardiorespiratory interactions that appear similar to those of conscious animals. These observations indicate interactions between respiratory and cardiovascular centres within the CNS (Wang et al. 1999). Thus, similarly to mammals and other air-breathing vertebrates, the cardiorespiratory interactions in anurans result from both feed-back and feed-forward mechanisms. Because amphibians are positioned centrally in the phylogeny of air-breathing vertebrates and they metamorphose from gill-breathing larvae to lung-breathing adults, an understanding of the mechanisms controlling their cardiorespiratory systems may reveal the fundamental properties that were associated with the evolutionary emergence of air-breathing.

3.4 Reptiles

As discussed previously, reptiles represent an ancient polyphyletic group and generalisations regarding the topography and control of their cardiorespiratory systems must be avoided. Thus, while all extant members of reptiles are ectothermic and committed air-breathers, their mechanisms for pulmonary ventilation, lung structure and morphology of the heart vary considerably among taxa. Cutaneous gas exchange contributes significantly only under special situation such as hibernation at low temperature (Feder and Burggren 1985). Turtles and tortoises (the anapsids) are considered the most primitive extant group of reptiles, and most species of this clade have poorly divided ventricles and a ventilatory mechanism that is highly specialized to take account of their shell (Landberg et al. 2003). In common with the turtles, lizards and snakes (squamates) lack a diaphragm, and lung ventilation is normally generated by intercostal muscles acting on the rib cage. A primitive buccopharyngeal or gular pump, like that described in amphibians, was considered to be used primarily for generating airflows within the oral cavity, presumably for olfaction. 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 utilise thoracic, aspirational breathing while running. However, an alternative mode of ventilation, involving a gular pump which alternated with the costal pump, has been described in an agamid lizard (Al-Ghamdi et al. 2001) and during exercise in a varanid (Brainerd and Owerkowicz 1996; Owerkowicz et al. 1999).

Most reptiles breathe intermittently, and the breathing pattern, particularly of aquatic species, is often characterized by long-lasting breath-holds. In many of the

aquatic species, periods of pulmonary ventilation consist of several breaths taken continuously, while many terrestrial species tend to ventilate the lungs with single breaths separated by shorter intervals of apnea. Regardless of habitat, the breathing cycle is terminated by an inspiration, and the lungs therefore remain inflated during apnoea. As in amphibians, it has been suggested that the initiation of discontinuous bouts of breathing is initiated by a complex interplay of stimulation of central and peripheral chemoreceptors as PO_2 in the blood and lungs decreases and CO_2 increases, rather than by centrally generated ventilatory rhythms (Douse and Mitchell 1990; Milsom 1990). 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. However, unidirectionally ventilated alligators display episodic breathing (Douse and Mitchell 1991), so that centrally generated rhythmicity may have a role in its initiation.

The cardiac ventricle of all squamates is incompletely divided, but varanid lizards and pythons have evolved the capacity for ventricular pressure separation allowing for high systemic blood pressures, while keeping the pressure in the pulmonary circulation low. In crocodilians, which are in fact more closely related to birds than the rest of the reptiles, the ventricle is anatomically divided, and pulmonary ventilation is driven by muscles of the body wall moving the liver, which is attached to a transverse connective tissue sheet resembling a mammalian diaphragm (Farmer and Carrier 2000).

Given the incomplete anatomical separation of cardiac ventricle in reptiles, there is a possibility of admixture between the oxygenated blood that returns to heart from the lungs with the oxygen-poor systemic venous blood. The direction and magnitude of these cardiac shunts vary consistently with ventilation, and all experimental studies agree that periods of apneoea are associated with low pulmonary blood flows and a net right-to-left shunt, while ventilation is associated with a reduction in the right-to-left shunt, or even a development of left-to-right shunts where blood is recirculated with the pulmonary circulation (e.g. Lillywhite and Donald 1989; Wang and Hicks 1996b). It is also clear that large right-to left shunts are characteristic of undisturbed and resting animals, whilst exercise or handling stress caused pulmonary blood flow to increase (Wang et al. 1997, 2001a). The tight correlation between heart rate and the net shunt pattern makes it very likely that the smooth muscle surrounding the pulmonary artery is innervated by the same vagal motoneurons as those innervating the heart, securing a functional coupling.

The efferent control of pulmonary blood flow and the rise in heart rate during ventilation is not well understood. Many studies have noted that heart and pulmonary blood flow tend to increase before lung ventilation is initiated, which would indicate a centrally generated release of vagal tone on the heart and the pulmonary artery. Also, artificial inflation and deflation of the lungs in anaesthetized and fully recovered turtles did not elicit cardiovascular responses in one study on turtles (Herman et al. 1997), while similar manipulation of lung volume in the same species elicited marked changes in heart rate and pulmonary blood flow in an earlier study (Johansen et al. 1977). The reason for such disparate findings is not clear, but points to a complex interaction between stretch receptor feedback from the lungs

and the efferent cardiovascular control. The role of chemoreceptor stimulation is also uncertain and needs to account for the changes in ventilatory patterns, which in itself changes pulmonary blood flow and the direct effects of hypoxia on the pulmonary vasculature (Wang et al. 1997; Skovgaard and Wang 2006)

The functional significance of the tight interaction between heart rate and cardiac shunt patterns remains enigmatic (e.g. Hicks and Wang 1996). This is even the case in turtles, which probably exhibit very pronounced heart rate and blood flow changes. Thus, when the rise in pulmonary ventilation during ventilation was prevented by mechanical occlusion of the pulmonary artery, there was no effect on oxygen uptake or CO_2 excretion in fully recovered turtles (Wang and Hicks 2008).

4 The Neural Basis of Cardiorespiratory Interactions

In mammals, heart rate varies with the respiratory cycle, accelerating during inspiration. It is known to be driven by respiration-related fluctuations in the efferent, inhibitory supply to the heart via the cardiac vagus. This is generated centrally by an inhibitory input to cardiac-vagal preganglionic neurones (CVPN) in the ventro-lateral nucleus ambiguus (NA) from inspiratory neurones in the neighbouring ventral respiratory group. The consequent gating of vagal outflow causes heart rate to rise during inspiration (Jordan and Spyer 1987). Consequently, instantaneous heart rate varies on a beat-to-beat basis with respiration and these variations in the heart rate variability signal (HRV) are termed respiratory sinus arrthymia (RSA).Its functional role in improving pulmonary oxygen uptake was recently discussed (Hayano and Yasuma 2003). Analysis of these beat-by-beat changes, using frequency domain analysis, has been recognized as an important tool for examining the underlying autonomic effectors controlling cardiac output (Campbell et al. 2004). In conscious dogs, Akselrod et al. (1981) demonstrated that random process analysis of the HRV signal provided a sensitive, quantitative measure of rapidly reacting cardiovascular control mechanisms, revealing three distinct components. These were: (1) the high-frequency component (0.3–0.4 Hz) associated with central respiratory drive and solely vagally mediated, (2) a mid-(0.1–0.3 Hz), and (3) a low (0.07–0.1 Hz)-frequency component associated with blood pressure control systems and thermal vasomotor activity respectively. Both of these latter components had a mixture of sympathetic and vagal contributions (Bootsma et al. 1994). The “polyvagal theory” has suggested that the beat-to-beat modulation of heart rate that generates RSA is restricted to mammals, which have evolved myelinated vagal pathways that originate in the NA (Porges 1995). This assertion has been contested by Grossman and Taylor (2006) who pointed out that the beat-to-beat control of cardiac interval has been reported in both resting dogfish (Taylor 1992), and hypoxic trout (Randall and Smith 1967), and that settled rattlesnakes show respiratory modulation of heart rate resembling mammalian RSA (Campbell et al. 2006). These species have CVPN located both in the DVN and in a ventro-lateral

location outside the DVN that may constitute a primitive NA (Taylor 1992). So a dual location for CVPN seems to be a common feature of the vertebrate brainstem with putative links to control of heart rate variability and cardiorespiratory interactions.

5 Fish

Cardiorespiratory interactions (CRI) have been reported in both resting dogfish, *Scyliorhinus canicula* (Taylor 1992) and hypoxic trout, *Oncorhynchus mykiss* (Randall 1966). Cardiac vagotomy or injection of atropine abolished CRS in the dogfish (Taylor 1992), while in the sculpin, *Myoxocephalus scorpius*, injection of atropine raised mean heart rate in normoxia and abolished a hypoxic bradycardia, while cardiac vagotomy abolished heart rate variability (Campbell et al. 2004). These observations confirm the dependence of beat-to-beat variability of heart rate on tonic vagal control. However, the neurological basis for CRI in fishes in still largely unresolved.

6 Elasmobranchs

Recordings from the central cut end of a branchial cardiac branch of the vagus in decerebrate, paralysed dogfish revealed high levels of spontaneous efferent activity that could be attributed to two types of unit (Taylor and Butler 1982; Barrett and Taylor 1985a,c). Some units fired sporadically and increased their firing rate during hypoxia. Injection of capsaicin into the ventilatory stream of the dogfish, which was accompanied by a marked bradycardia, powerfully stimulated activity in these non-bursting units recorded from the central cut end of the cardiac vagus (Jones et al. 1993). Consequently, we suggested they may initiate reflex changes in heart rate, as well as play a role in the determination of the overall level of vagal tone on the heart. Other, typically larger, units fired in rhythmical bursts which were synchronous with ventilatory movements (Taylor and Butler 1982; Barrett and Taylor 1985a). We hypothesised that these units, showing respiration-related activity which was unaffected by hypoxia, may serve to synchronise heart beat with ventilation (Taylor 1992).

The separation of efferent cardiac vagal activity into respiration-related and non-respiration-related units was discovered to have a basis in the distribution of their neuron cell bodies in the brainstem, described above. Direct connections between bursting CVPN and RVM are possible in the dogfish hindbrain, as both are located in the DVN with an overlapping rostro-caudal distribution (Taylor 1992; Taylor et al. 1999). Extracellular recordings from CVPN identified in the hindbrain of decerebrate, paralysed dogfish by antidromic stimulation of a branchial cardiac branch revealed that neurons located in the DVN were spontaneously active, firing in

rhythmical bursts which contributed to the respiration-related bursts recorded from the intact nerve (Barrett and Taylor 1985c and see Fig. 3). Neurons located ventrolaterally outside of the DVN were either spontaneously active, firing regularly or sporadically but never rhythmically, or were silent (Fig. 3). Thus, the two types of efferent activity recorded from the cardiac nerve arise from the separate groups of CVPN, as identified by neuranatomical studies (Taylor 1992). There is clear experimental evidence for central, feed-forward control of CRI in dogfish, as well as reflexcontrol originating from mechanoreceptors on the gills (Barrett and Taylor 1985c; Taylor 1992 and see Fig. 3). Thus, in the intact fish, normal breathing movements that stimulate peripheral mechanoreceptors on the gills may, by a reflex pathway, generate activity in CVPN and consequently in the cardiac vagi, affecting heart rate. This implies that the typical reflex bradycardia in response to hypoxia may arise both directly, following stimulation of peripheral chemoreceptors, and indirectly, via increased ventilation, which by stimulating branchial mechanoreceptors may increase vagal outflow to the heart. This is reminiscent of, but opposite in kind to, the hypoxic response in the mammal, where stimulation of lung stretch receptors causes an increase in heart rate (Daly and Scott 1962). As well as slowing the heart, respiration-related efferent activity in the cardiac vagi may entrain the heart (Taylor et al. 2006, 2009c).

7 Teleosts

Most studies on teleosts have stressed the importance of inputs from peripheral receptors in the genesis of cardiorespiratory synchrony (Randall 1966; Randall and Smith 1967). In pacu, *Piaractus mesopotamicus*, respiration-related, bursting activity was only recorded from the cardiac vagus in normoxic fish when they were hyperventilating or coughing, implying that the bursts arise reflexly, following stimulation of branchial mechanoreceptors. In fish rendered moderately hypoxic by reduction of the flow of water irrigating the gills, a period of spontaneously increased ventilatory amplitude was accompanied by respiration-related bursts of activity in the cardiac vagus, which were not apparent in the inactive, normoxic fish, and appeared to recruit the heart. In both pacu and dogfish, phasic peripheral stimulation of the cardiac vagus or central stimulation of respiratory branches of cranial nerves VII, IX and X entrained the heart over a wide range of frequencies (Fig. 4). However, central stimulation of the mandibular branch of the Vth cranial nerve, innervating the jaw was without effect on heart rate, possibly reflecting the fact that the distribution of motoneurones supplying this nerve does not overlap with CVPN. Nevertheless, activity in the cardiac vagus was synchronous with activity in the Vth cranial nerve, and both anticipated activity in other cranial nerves supplying respiratory muscles, implying that central feed-forward control was determining this relationship (Taylor et al. 2009c).

8 Amphibians

The intermittent ventilatory patterns of anurans are often associated with concomitant cardiovascular changes. Thus, a number of studies have shown that heart rate increases upon inflation of the lungs, and it is also clear that the pulmonary blood flow increases (reviewed by Wang et al. 1999). These cardiovascular responses are primarily caused by release of vagal tone on the heart and pulmonary artery. Thus, vagotomy or atropine injection reduces or abolishes cardiorespiratory coupling; however, the underlying mechanisms are not known. Cardiorespiratory coupling in amphibians may originate from direct influences of activity in the centres responsible for respiratory rhythm generation on the cardiac and pulmonary arterial vagal motoneurons. These interactions occur within the CNS and are independent of afferent feedback. Thus, in decerebrated and paralyzed toads (*Bufo marinus*), where the lungs were unidirectionally ventilated to maintain constant degree of lung inflation, pulmonary blood flow increased during periods of ventilatory activity in the respiratory muscles innervating the buccal cavity (Wang et al. 2004). An example of such a response is shown Fig. 6.

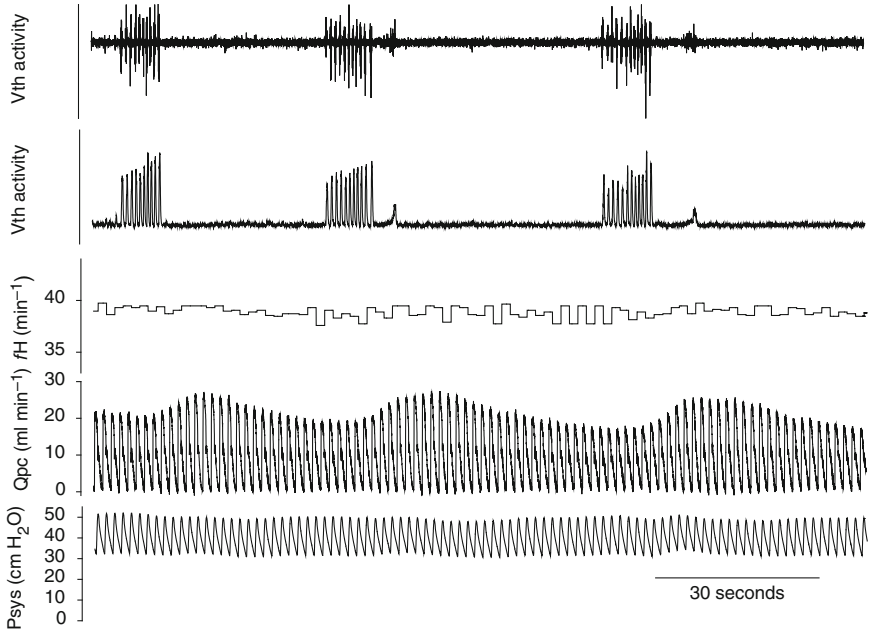

Fig. 6 Cardiovascular changes associated with ventilatory activity in a decerebrated and paralysed toad (*Bufo marinus*). Ventilatory activity was measured as nervous activity in the fifth cranial nerve (*two upper traces*), which innervates the respiratory muscles in the buccal cavity. Because the toad 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) and the attendant changes in systemic blood flow during ventilation seems to be caused by central feed-forward mechanisms. There were no obvious changes in systemic blood pressure (*Psys*) or heart rate (*fH*). (Taken from Wang et al. 2004)

Stretch receptor feedback from the lungs, however, is also important for the cardiovascular responses associated with lung ventilation, and inflation of the lungs of anurans often elicits a rise in pulmonary blood flow and heart rate (e.g. West and Burggren 1984; West and Van Vliet 1992) During breathing, changes in lung volume stimulate pulmonary stretch receptors and it is possible that their afferent activity serves as a feedback mechanism to the cardiovascular centres that, in turn, release cardiac vagal tone on the heart and pulmonary artery. In some experiments on anaesthetized *Bufo, Rana* and *Xenopus*, artificial lung inflation elicited cardiovascular responses that were similar to those observed during spontaneous breathing, and these responses were abolished following atropine injection and during deep anesthesia (West and Burggren 1984; Wang et al. 1999). Changes in thoracic pressure and volume during pulmonary ventilation may also alter the venous return to the heart and change cardiac output through the classical Frank-Starling mechanism (e.g. Segura et al. 1981). Apart from nervous control, resistance to pulmonary blood flow can be altered within the pulmonary circulation per se, where lung gas composition may exert a direct influence on vascular tone (West and Burggren 1984).

9 Reptiles

Reptiles are typically periodic breathers, and during bouts of breathing the degree of shunting of blood flow to the lung increases. Vasomotor control is important in diverting blood between the pulmonary and systemic systems. In turtles and lizards, the net direction and magnitude of shunt flow are affected by resistance in the pulmonary circuit, relative to the systemic circuit, by active vagal, cholinergic regulation of pulmonary arterial resistance. Control of pulmonary blood flow in reptiles is achieved by vagal cholinergic constriction of the pulmonary artery. Peripheral electrical stimulation of the vagus or intravenous injection of acetylcholine each result in bradycardia and an increase in pulmonary vascular resistance, which reduces pulmonary blood flow (Taylor et al. 1999). These cardiovascular changes are abolished by administration of atropine. The rattlesnake, *Crotalus durissus*, has a single lung and functional pulmonary arch, designated as the right arch, though it is innervated by the left vagus. Peripheral electrical stimulation of the left vagus slowed the heart and stopped blood flow to the pulmonary arch (Taylor et al. 2009). Blood flow is also under adrenergic control. In *Boa constrictor* vagal, cholinergic tone predominated over sympathetic, adrenergic tone in inactive animals. The increase in heart rate during enforced activity was due largely to complete withdrawal of inhibitory vagal tone, while the increase following a meal was mediated by small changes in overall autonomic tone with evidence of involvement of non-adrenergic-non-cholinergic (NANC) factors (Wang et al. 2001a).

Reptiles probably exhibit the most pronounced cardiovascular changes associated with ventilation of all vertebrate groups. Thus, a tachycardia during ventilation is characteristic of most species belonging to all major groups of reptiles (e.g.

Johansen 1959; Andersen 1961; Pough 1969; Huggins et al. 1970; Jacob and McDonald 1976; Heatwole 1977; Jacob 1980). The heart rate changes seem most pronounced in aquatic species, and an early description of the tachycardia during ventilation in turtles even suggested that bradycardia during breath-hold represent the normal state (Belkin 1964). In the free-diving turtle *Trachemys scripta*, pulmonary blood flow increased more than threefold at the onset of breathing, during recovery from breath-holds lasting longer than 5 min (Wang and Hicks 1996a). Systemic blood flow also increased during ventilation. These increases were accomplished entirely through changes in heart rate during ventilation, with stroke volume unchanged. Systemic blood flow always exceeded pulmonary flow, so that a net right-to-left cardiac shunt prevailed, regardless of ventilatory state. Nevertheless, because pulmonary flow increased markedly during ventilation, the ratio of pulmonary to systemic flow increased from 0.3 to 0.8. In both the turtle, *Pseudemys scripta*, and the tortoise, *Testudo graeca*, the onset of lung ventilation was closely accompanied by a tachycardia (Burggren 1975). As stimulation of pulmonary stretch receptors, arterial chemoreceptors, and baroreceptors or water receptors was without effect on heart rate, it was concluded that this ventilation tachycardia resulted from central interactions between respiratory and cardiac neurons in the medulla. However, lung stretch receptor afferents have been recorded from the vagus nerve of reptiles (Sundin et al. 2001). All changes in heart rate were mediated by alterations in vagal tone.

The heart rate changes with ventilation disappear upon blockade of the autonomic nervous innervation of the heart and are clearly regulated. Wang et al. (2001a, b) showed that there are slight changes in fH related to lung ventilation in snakes, but it is uncertain whether these components formed distinct oscillations in fH at the frequency of fV, and could therefore be categorised as RSA. On the basis of power spectral analysis of heart rate, Gonzalez and De Vera (1988) argued that there was no spectral component of the heart rate signal with ventilation in a small lizard *Galloti galloti*. However, the use of fH dataloggers for long-term monitoring in undisturbed rattlesnakes (Campbell et al. 2006) enabled us to determine HRV in settled, recovered animals when sympathetic tonus was low and vagal tonus high. These animals showed oscillatory components in the HRV signal at the frequency of ventilation that were abolished by injection of atropine. Results from this study agreed in part with Gonzalez and De Vera (1988), in that two peaks were detected in the fH spectra of the rattlesnake. However, the frequency and amplitude of these peaks were relative to fH, with high fH favouring the lower frequency peaks and low fH the high frequency peaks. The removal of these peaks, independently, by pharmacological blockade was accompanied by the associated changes in fH, suggesting that these peaks represented sympathetic and parasympathetic drive on the heart. Furthermore, the high peaks, that were removed by the cholinergic blocker atropine, indicated an oscillatory component in HRV that occurred at the frequency of the respiratory cycle. The respiratory cycle of rattlesnakes consists of a prolonged inspiration followed by a relatively short expiration. Heart rate slowed upon expiration and increased during inspiration, which is similar to the changes in heart rate observed in conscious unrestrained mammals and characterized as RSA (Hayano

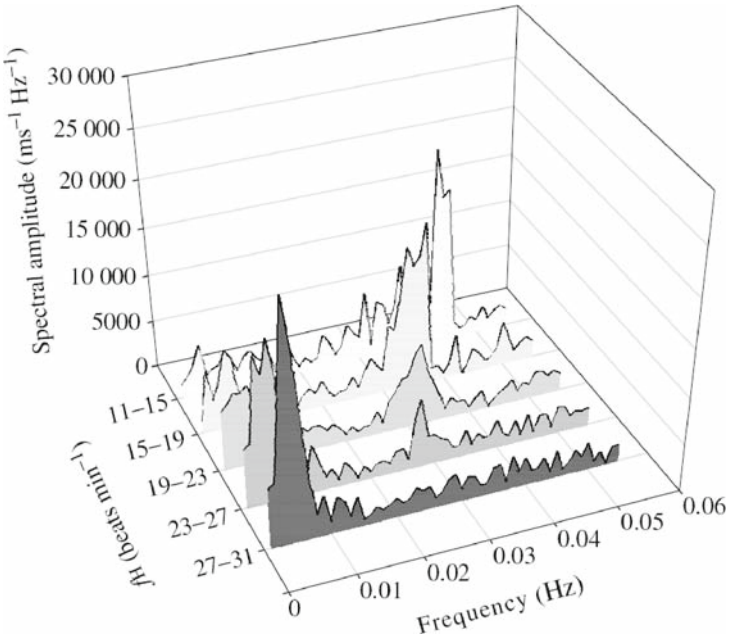

Fig. 7 Power spectra generated from heart beat interval data recorded for 110 h from four rattlesnakes, *Crotalus durissus*, fitted with external miniature dataloggers. Heart rate varied with recovery from operative procedures and over a diurnal cycle. For power spectral analysis, ten individual data sets, each consisting of 512 consecutive R-R intervals, were chosen from each animal, within each of 5 fH categories. The resultant power spectra within 0.001 Hz frequency bins were pooled to produce the plots. (Taken from Campbell et al. 2006)

and Yasuma 2003). These peaks showed up clearly following power spectral analysis of heart rate variability (Fig. 7). Thus, this study contrasts with that of Gonzalez and De Vera (1988), as we were able to present clear evidence for respiratory modulation of heart rate which closely resembled that recorded from mammals, and accordingly may be classed as RSA. These data refute the proposition that centrally controlled cardiorespiratory coupling is restricted to mammals, as propounded by the polyvagal theory of Porges (1995).

Changes in heart rate during bouts of ventilation that consist of many consecutive breaths are less easy to characterise. Thus, as shown in Fig. 8, which depicts an example of heart rate changes during intermittent ventilation of a turtle, it is clear that heart rate often remains high and invariable during the entire breathing episode. Thus, while heart rate clearly increases in connection with pulmonary ventilation, there is no variation associated with inspiration and expiration during the individual breaths. It may therefore be appropriate to label these interactions as cardiorespiratory coupling.

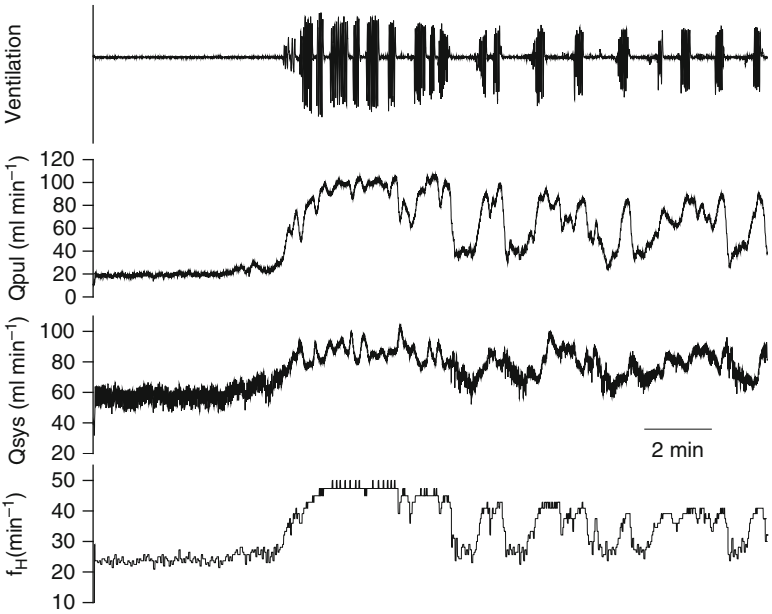

Fig. 8 Heart and central vascular blood flow in an aquatic turtle, *Trachemys scripta*, during spontaneous apnoea and ventilation. The ventilatory bouts, which consist of several consecutive breaths, are accompanied by a large rise in heart rate (fH) and pulmonary blood flow (*Qpul*), while systemic blood flow (*Qsys*) remains unchanged. As a consequence of a strong vagal tone on the smooth muscle that surrounds the pulmonary, large right-to-left cardiac shunts prevail during apnoea, while relaxation of this sphincter during ventilation reverses the cardiac hunt pattern. (Taken from Wang and Hicks 1996a)

10 Mechanisms of Phasic Vagal Control of the Heart

There are at present no definitive studies of the mechanisms by which respiration-related efferent activity in the cardiac vagus recruits the heart in fish, amphibians or reptiles. In the dogfish, injection of the muscarinic cholinoceptor antagonist, atropine, abolished both the cardiac arrest due to tonic efferent electrical stimulation of the cardiac vagus and the recruitment of the heart by phasic stimulation, so that both are due to stimulation of muscarinic receptors by acetylcholine (Taylor et al. 2006). In dogfish, these separate effects may relate to the topographical and physiological separation between CVPN in the DVN and putative NA (Fig. 3). The denervated heart in dogfish was entrained by bursts of activity delivered down the peripheral cut end of the cardiac vagus at frequencies lower and somewhat higher than its intrinsic rate. However, when these phasic stimuli were combined with lower amplitude continuous stimulation that simulated the activity recorded from the central cut end of the cardiac vagus, the heart was only entrained at frequencies lower than intrinsic heart rate (Taylor et al. 2006). A similar pattern of entrainment was observed when respiratory nerves were stimulated centrally in pacu (see Fig. 4).

Thus, as stated above, the non-bursting units recorded from the cardiac vagus may be responsible for slowing the heart while the respiration-related activity modulates heart rate on a beat-to-beat basis when overall vagal tone on the heart is low, possibly signifying that the non-bursting units are silent. Whether these separate neural units innervate different parts of the heart is unknown.

Finally, we are left with a leading question: How can activity in the cardiac vagus which is normally inhibitory, when delivered in pulses, recruit the heart at rates above its intrinsic rate? By its nature, this is likely to be a direct induction of the heart beat rather than an effect on cardiac intervals. When bursts of electrical stimuli were delivered peripherally down the cardiac vagus of the dogfish at 43 bursts min^{-1} the heart slowed, but it was observed to beat at exactly half the bursting rate, implying that it was induced to beat by alternate bursts (Taylor et al. 2006). This question has long been of interest to mammalian physiologists. In mammals the cardiac vagi show bursting activity of variable frequencies (Jewett 1964; Katona et al. 1970; Kunze 1972; Taylor et al. 1999), and the heart can be entrained by bursts of electrical activity delivered down the cardiac vagi over a range of stimulation frequencies both lower and higher than the intrinsic heart rate (e.g. Levy et al. 1969, 1972, 1981; Pokrovskii 1984, 2003). In a classic study of the chronotropic effects of brief cardiac vagal stimulation in cats, Brown and Eccles (1934) identified a relationship between the phase of the cardiac cycle during which the efferent stimulus was delivered and its chronotropic effect, with two inhibitory phases and a brief phase of relative or actual cardioacceleration. Martin (1977) noted that atrioventricular conduction time in the heart of the dog was shorter in the presence of vagal stimulation. Similar complex relationships were reviewed by Levy et al. (1981). Pace et al. (1984) showed that maximum R–R intervals were triggered when a brief efferent stimulus was delivered to the cardiac vagus during the phase of slow depolarization of the cardiac pacemaker cells. The minimum R–R intervals occurred when the stimulus was delivered before this phase. These observations imply that the effect of each burst depends on the phase of the cardiac cycle at which it is applied. Thus, the vagal effect on the heart cannot be measured merely in terms of the amount of acetylcholine delivered per unit time. Similar studies are long overdue on amphibians and reptiles.

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References

Agnisola, C., Randall, D.J. and Taylor, E.W. (2003) The modulatory effects of noradrenaline on vagal control of the heart in the dogfish, *Squalus acanthius*. Physiol. Biochem. Zool. 76: 310–320

Akselrod, S., Gordon, D., Ubel, F.A., Shannon, D.C., Barger, A.C. and Cohen, R.J. (1981) Power spectrum analysis of heart-rate fluctuation — a quantitative probe of beat-to-beat cardiovascular control. Science 213: 220–222

Al-Ghamdi, M.A.D., Jones, J.F.X. and Taylor, E.W. (2001) Evidence of a functional role in lung inflation for the buccal pump in the agamid lizard, *Uromastyx aegyptius microlepis*. J. Exp. Biol. 204: 521–531

Andersen, H.T. (1961) Physiological adjustments to prolonged diving in the American alligator *Alligator mississippiensis*. Acta Physiol. Scand. 53: 23–45

Anrep, G.V., Pascual, W. and Rossler, R. (1936a) Respiratory variations of the heart rate. I. The reflex mechanism of the respiratory arrhythmia. Proc. R. Soc. B 119: 191–217

Anrep, G.V., Pascual, W. and Rossler, R. (1936b) Respiratory variations of the heart rate. II. The central mechanism of the respiratory arrhythmia and the interactions between the central and the reflex mechanisms. Proc. R. Soc. B 119: 218–230

Augustinsson, A., Fange, R., Johnels, A. and Ostlund, E. (1956) Histological, physiological and biochemical studies on the heart of two cyclostomes, hagfish (*Myxine*) and lamprey (*Lampetra*). J. Physiol. Lond. 13: 257–276

Axelsson, M., Abe, A.S., Bicudo, J.E.P.W. and Nilsson, S. (1989) On cardiac control in the South American lungfish, *Lepidosiren paradoxa*. Comp. Biochem. Physiol. 93A: 561–565

Barrett, D.J. and Taylor, E.W. (1985a) Spontaneous efferent activity in branches of the vagus nerve controlling heart rate and ventilation in the dogfish. J. Exp. Biol. 117: 433–448

Barrett, D.J. and Taylor, E.W. (1985b) The location of cardiac vagal preganglionic neurones in the brainstem of the dogfish. J. Exp. Biol. 117: 449–458

Barrett, D.J. and Taylor, E.W. (1985c) The characteristics of cardiac vagal preganglionic motoneurones in the dogfish. J. Exp. Biol. 117: 459–470

Belkin, D.A. (1964) Variations in heart rate during voluntary diving in the turtle *Pseudemys concinna*. Copeia 1964: 321–330

Bootsma, M., Swenne, C.A., Vanbolhuis, H.H., Chang, P.C., Cats, V.M. and Bruschke, A.V.G. (1994) Heart-rate and heart-rate variability as indexes of sympathovagal balance. Am. J. Physiol. 266: H1565–H1571

Brainerd, E.L. and Owerkowicz, T. (1996) Role of the gular pump in lung ventilation during recovery from exercise in varanid lizards. Am. Zool. 36: 88A

Brown, G.L. and Eccles, J.C. (1934) The action of a single vagal volley on the rhythm of the heart beat. J. Physiol. 82: 211–241

Burggren, W. (1975) A quantitative analysis of ventilation tachycardia and its control in two chelonians, *Chrysemys scripta* and *Testudo graeca*. J. Exp. Biol. 63: 367–380

Burggren, W.W. and K. Johansen (1986) Circulation and respiration in lungfishes (Dipnoi). J. Morph. Suppl. 1: 217–236

Butler, P.J. and Taylor, E.W. (1971) Response of the dogfish (*Scyliorhinus canicula* L.) to slowly induced and rapidly induced hypoxia. Comp. Biochem. Physiol. 39A: 307–323

Campbell, H.A. and Egginton, S. (2007) The vagus nerve mediates cardiorespiratory coupling that changes with metabolic demand in a temperate nototheniod fish. J. Exp. Biol. 210: 2472–2480

Campbell, H.A., Taylor, E.W. and Egginton, S. (2004) The use of power spectral analysis to determine cardiorespiratory control in the short-horned sculpin *Myoxocephalus scorpius*. J. Exp. Biol. 207: 1969–1976

Campbell, H.A., Leite, C.A.C., Wang, T., Skals, M., Abe, A.S., Egginton, S., Rantin, F.T., Bishop, C.M. and Taylor, E.W. (2006) Evidence for a respiratory component, similar to mammalian respiratory sinus arrhythmia, in the heart rate variability signal from the rattlesnake, *Crotalus durissus terrificus*. J. Exp. Biol. 209: 2628–2636

Cruce, W.L.R. and Niewenhuys, R. (1974) The cell masses in the brain stem of the turtle (*Testudo hermanni*): a topographical and topological analysis. J. Comp. Neurol. 156: 277–306

Daly, M., de, B. and Scott, M.J. (1962) An analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors in the dog. J. Physiol. Lond. 162: 555–573

Daly, M. de B. and Kirkman, E. (1989) Differential modulation by pulmonary stretch afferents of some reflex cardioinhibitory responses in the cat. J. Physiol. Lond. 417: 323–341.

de Saint-Aubain, M.L. and Wingstrand, K.G. (1979) A sphincter in the pulmonary artery of the frog *Rana temporaria* and its influence on blood flow in skin and lungs. Acta Zool. 60: 163–172

Delaney, R.G., Laurent, P., Galante, R., Pack, A.I., Fishman, A.P. (1983) Pulmonary mechanoreceptors in the dipnoi lungfish *Protopterus* and *Lepidosiren*. Am. J. Physiol. 13: R418–R428

Douse, M.A. and Mitchell, G.S (1990) Episodic respiratory-related discharge in turtle cranial motorneurons: *in vivo* and *in vitro* studies. Brain Res. 536: 297–300

Douse, M.A. and Mitchell, G.S (1991) Time courses of temperature effects on arterial acid-base status in *Alligator mississippiensis*. Respir Physiol 83: 87–102

Farmer, C.G. and Carrier, D.R. (2000) Pelvic aspiration in the American alligator (*Alligator mississippiensis*). J. Exp. Biol. 203: 1679–1687

Farrell, A.P. (1978) Cardiovascular events associated with airbreathing in two teleosts, *Hoplerythrinus unitaeniatus* and *Arapaima gigas*. Can. J. Zool. 56: 953–958

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

Fishman, A.P., Delaney, R.G. and Laurent, P. (1985) Circulatory adaptation to bimodal respiration in the dipnoan lungfish. J. Appl. Physiol. 59: 285–294

Fritsche, R., Axelsson, M., Franklin, C.E., Grigg, G.G., Holmgren, S. and Nilsson, S. (1993) Respiratory and cardiovascular responses to hypoxia in the Australian lungfish. Respir. Physiol. 94: 173–187

Galli, G.L.J., Skovgaard, N., Abe, A.S., Taylor, E.W. and Wang, T. (2007) The adrenergic regulation of the cardiovascular system in the South American rattlesnake *Crotalus durissus*. Comp. Biochem. Physiol. 148: 510–520

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

Gannon, B.J. and Burnstock, G. (1969) Excitatory adrenergic innervation of the fish heart. Comp. Biochem. Physiol. 29: 765–773

Gonzalez, J. and De Vera L.D. (1988) Spectral analysis of heart-rate variability of lizard, *Gallotia galloti*. Am. J. Physiol. 254: R242–R248

Graham, J.B. (1997) Air-Breathing Fishes: Evolution, Diversity, and Adaptation. Academic Press, San Diego

Graham, J.B., Chiller, L.D. and Roberts, J.L. (1995) The transition to air breathing in fishes. V. Comparative aspects of cardiorespiratory regulation in *Synbranchus marmoratus* and *Monopterus albus* (Synbranchidae). J. Exp. Biol. 198: 1455–1467

Grossman, P. and Taylor, E.W. (2006) Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. Biol. Psychol. 74: 263–285

Hayano, J. and Yasuma, F. (2003) Hypothesis: respiratory sinus arrhythmia is an intrinsic resting function of cardiopulmonary system. Cardiovasc. Res. 58: 1–9

Heatwole, H. (1977) Heart rate during breathing and apnea in marine snakes (Reptilia, Serpentes). J. Herp. 11: 67–76

Herman, J., Wang, T., Smits, A.W. and Hicks, J.W. (1997) The effects of artificial lung inflation on pulmonary blood flow and heart rate in the turtle. J. Exp. Biol. 200: 2539–2549

Hicks, J.W. and Wang, T. (1996) Functional role of cardiac shunts in reptiles. J. Exp. Zool. 275: 204–216

Hughes, G.M. and Shelton, G. (1962) Respiratory mechanisms and their nervous control in fish. Adv. Comp. Physiol. Biochem. 1: 275–364

Huggins, S.E., Hoff, H.E. and Peña, R.V. (1970) The respiratory heart-rate response in crocodilian reptiles. Physiol. Zool. 43: 10–18

Jacob, J.S. (1980) Heart rate-ventilatory response of seven terrestrial species of North American snakes. Herpetologica 36(4): 326–335

Jacob, J.S. and McDonald, H.S. (1976) Diving bradycardia in four species of North American aquatic snakes. Comp. Biochem. Physiol. 53A: 69–72

Jensen, D. (1965) The aneural heart of the hagfish. Ann. N.Y. Acad. Sci. 127: 443–458

Jewett, D.L. (1964) Activity of single efferent fibres in the cervical vagus nerve of the dog, with special reference to possible cardio-inhibitory fibres. J. Physiol. 175: 321–357

Johansen, K. (1959) Heart activity during experimental diving of snakes. Am. J. Physiol. 197: 604–606

Johansen, K. and Hanson, D. (1968) Functional anatomy of the hearts of lungfishes and amphibians. Am. Zool. 8: 191–210

Johansen, K. and Reite, O.B. (1968) Influence of acetylcholine and biogenic amines on branchial, pulmonary and systemic vascular resistance in the African lungfish, Protopterus aethiopicus. Acta Physiol. Scand. 74: 465–471

Johansen, K., Lenfant, C. and Hanson, D. (1968a) Cardiovascular dynamics in the lungfishes. Z. Vergl. Physiol. 59: 157–186

Johansen, K., Lenfant, C., Schmidt-Nielsen, K. and Petersen, J. (1968b) Gas exchange and control of breathing in the electric eel, *Electrophorus electricus*. Z. Vergl. Physiol. 61: 137–163

Jones, D.R., Langille, B.L., Randall, D.J., and Shelton, G. (1974) Blood flow in dorsal and ventral aortas of the cod, *Gadus morhua*. Am. J. Physiol. 226: 90–95

Jones, J.F.X., Young, M., Jordan, D. and Taylor, E.W. (1993) Effect of capsaicin on heart rate and fictive ventilation in the decerebrate dogfish (*Scyliorhinus canicula*). J. Physiol. Lond. 473: 236P

Johansen, K., Burggren, W.W., and Glass, M.L. (1977) Pulmonary stretch receptors regulate heart rate and pulmonary blood flow in the turtle, *Pseudemys scripta*. Comp. Biochem. Physiol. 58A: 185–191

Jordan, D., Spyer, K.M. (1987) Central mechanisms mediating respiratory-cardiovascular interactions. In: Taylor EW (ed) Neurobiology of the Cardiorespiratory System. Manchester University Press, Manchester, pp 322–341

Katona, P.G., Poitras, J.W., Barnett, G.O. and Terry, B.S. (1970) Cardiac vagal efferent activity and heart period in the carotid sinus reflex. Am. J. Physiol. 218: 1030–1037

Kunze, D.L. (1972) Reflex discharge patterns of cardiac vagal efferent fibres. J. Physiol. 222: 1–15

Landberg, T., Mailhot, J.D., and Brainerd, E.L. (2003) Lung ventilation during treadmill locomotion in a terrestrial turtle, *Terrapene carolina*. J. Exp. Biol. 206: 3391–3404

Levy, M.N., Martin, P.J., Lano, T. and Zieske, H. (1969) Paradoxical effect of vagus nerve stimulation on heart rate in dogs. Circ. Res. 25: 303–314

Levy, M.N., Iano, T. and Zieske, H. (1972) Effects of repetitive bursts of vagal activity on heart rate. Circ. Res. 30: 186–195

Levy, M.N., Martin, P.J. and Stuesse, S.L. (1981) Neural regulation of the heart beat. Ann. Rev. Physiol. 43: 443–453

Lillywhite, H.B. and Donald, J.A. (1989) Pulmonary blood flow regulation in an aquatic snake. Science 245: 293–295

Martin, P.J. (1977) Paradoxical dynamic interactions of heart period and vagal activity on atrioventricular conduction in the dog. Circ. Res. 40: 81–89

McKenzie, D.J., Campbell, H.A., Taylor, E.W., Micheli, M., Rantin, F.T. and Abe, A.S. (2007) The autonomic control and functional significance of the changes in heart rate associated with air breathing in the jeju, *Hoplerythrinus unitaeniatus*. J. Exp. Biol. 210: 4224–4232

Milsom, W.K. (1990) Mechanoreceptor modulation of endogenous respiratory rhythms in vertebrates. Am. J. Physiol. 259: R898–R910

Morris JL, Nilsson S (1994) The circulatory system. In: Nilsson S, Holmgren S (eds) Comparative Physiology and Evolution of the Autonomic Nervous System. Harwood Academic Publishers, Chur Switzerland, pp 193–246

Overgaard, J., Stecyk, J., Farrell, A.P. and Wang, T. (2002) Adrenergic control of the cardiovascular system in the turtle *Trachemys scripta*. J. Exp. Biol. 205: 3335–3345

Owerkowicz, T. et al. (1999) Contribution of gular pumping to lung ventilation in monitor lizards. Science 284: 1661–1663

Pace, D.G., Masuda, Y., Eisenstein, I. and Levy, M.N. (1984) Effects of digoxin on the chronotropic responses to repetitive vagal stimulus bursts in the dog. Canad. J. Physiol. Pharmacol. 62: 1411–1415

Piiper, J., Scheid, P. (1977) Comparative physiology of respiration: functional analysis of gas exchange organs in vertebrates. In: Widdicombe JG (ed) International Review of Physiology, Respiratory Physiology, II, vol. 14. Baltimore: University Park Press, Baltimore, pp 219–253

Pokrovskii, M.V. (1984) Control of cardiac rhythm by stimulation of the vagus nerve in bursts in the rat. Byulleten Éksparimental noi Biologii i Meditsiny 97: 389–390

Pokrovskii, M.V. (2003) Alternative view on the mechanism of cardiac rhythmogenesis. Heart Lung Circulation 12: 18–24

Porges, S.W. (1995) Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A polyvagal theory. Psychology 32: 301–318

Pough, F.H. (1969) Physiological aspects of the burrowing of sand lizards (*Uma iguanidae*) and other lizards. Comp. Biochem. Physiol. 31, 869–884

Preston, E. and Courtice, G.P. (1995) Physiological correlates of vagal nerve innervation in lower vertebrates. Am. J. Physiol. 37: R1249–R1256

Randall D.J. (1966) The nervous control of cardiac activity in the tench (*Tinca tinca*) and the goldfish (*Carassius auratus*). Physiol. Zool. 39: 185–192

Randall D.J. and Smith J.C. (1967) The regulation of cardiac activity in fish in a hypoxia environment. Physiol. Zool. 40: 104–113

Randall D.J. and Taylor E.W. (1991) Evidence of a role for catecholamines in the control of breathing in fish. Rev. Fish Biol. Fisheries 1: 139–158

Sanchez, A., Soncini, R., Wang, T., Koldkjær, P., Taylor, E.W. and Glass, M.L. (2001) The differential cardiorespiratory responses to ambient hypoxia and systemic hypoxaemia in the South American lungfish, *Lepidosiren paradoxa*. Comp. Biochem. Physiol. 130: 677–687

Satchell, G.H. (1960) The reflex co-ordination of the heart beat with respiration in dogfish. J. Exp. Biol. 37: 719–731

Segura, E.T., Bronstein, A. and Schmajuk, N.A. (1981) Effect of breathing upon blood pressure and heart rate in the toad, *Bufo arenarum* Hensel. J. Comp. Physiol. 143: 223–227

Shelton, G. (1970) The effect of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. Respir. Physiol. 9: 183–196

Singh, B.N. and Hughes, G.M. (1973) Cardiac and respiratory responses in the climbing perch Anabas testudineus. J. Comp. Physiol. 84: 205–226

Skals, M., Skovgaard N., Taylor, E.W., Leite, C.A.C., Abe, A.S. and Wang, T. (2006) Cardiovascular changes under normoxic and hypoxic conditions in the air-breathing teleost *Synbranchus marmoratus*: importance of the venous system. J. Exp. Biol. 209: 4167–4173

Skovgaard, N. and Wang, T. (2006) Local control of pulmonary blood flow and lung structure in reptiles: Implications for ventilation perfusion matching. Resp. Physiol. Neurobiol. 154: 107–117

Sundin, L., Burleson, M., Wang, T., Reid, S., Salgado, H., Abe, A., Glass, M. and Milsom, W. (2001) Pulmonary receptors in reptiles: Discharge patterns of receptor populations in snakes versus turtles. J. Comp. Physiol. B 171: 103–111

Taylor EW (1992) Nervous control of the heart and cardiorespiratory interactions. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish Physiology, vol. 12B. Academic Press, New York, pp 343–387

Taylor, E.W. (1993) The neuranatomy of central cardiorespiratory control in vertebrates. Functionsanalyse Biologischer Systeme 23: 149–158

Taylor, E.W. and Butler, P.J. (1982) Nervous control of heart rate: activity in the cardiac vagus of the dogfish. J. Appl. Physiol. 53(6): 1330–1335

Taylor, E.W. and Ihmied, Y.M. (1995) Vagal and adrenergic tone on the heart of *Xenopus laevis* at different temperatures. J. Therm. Biol. 20: 55–59

Taylor, E.W., Short, S. and Butler, P.J. (1977) The role of the cardiac vagus in the response of the dogfish, *Scyliorhinus canicula* to hypoxia. J. Exp. Biol. 70: 57–75

Taylor, E.W., McKenzie, D.J., Levings, J.J., Randall, D.J. (1996) Control of ventilation in air-breathing fish. In: Val AL, Almeida-Val VMF, Randall DJ (eds) Physiology and Biochemistry of the Fishes of the Amazon. INPA, Alameda Cosme Ferreira, pp 155–167

Taylor E.W., Jordan D., Coote J.H. (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol. Rev. 79: 855–916

Taylor, E.W., Al-Ghamdi, M.A.D., Ihmied, Y.I., Wang, T. and Abe, A.S. (2001) The neuranatomical basis of central control of cardiorespiratory interactions in vertebrates. Exp. Phys. 86: 781–786

Taylor E.W., Campbell H.A., Levings J.J., Young M.J., Butler P.J., Egginton S. (2006) Coupling of the respiratory rhythm in fish with activity in hypobranchial nerves and with heart beat. Physiol. Biochem. Zool. 79: 1000–1009

Taylor, E.W., Andrade, D.V., Abe, A.S. and Wang, T. (2009a). The unequal influences of the left and right vagi on the control of the heart and pulmonary artery in the rattlesnake, *Crotalus durissus*. J. Exp. Biol. 212: 145–151

Taylor, E.W., Leite, C.A.C., Florindo, L.H., Beläo, T. and Rantin, F.T. (2009b). The basis of vagal efferent control of heart rate in a Neotropical fish, the pacu, *Piaractus mesopotamicus*. J. Exp. Biol. 212: 906–913

Taylor, E.W., Leite, C. and Levings, J.J. (2009c). Central control of cardiorespiratory interactions in fish. Acta Histochemica (in press)

Wang, T. and Hicks, J.W. (1996a) Cardiorespiratory synchrony in turtles. J. Exp. Biol. 199: 1791–1800

Wang, T. and Hicks, J.W. (1996b) The interaction of pulmonary ventilation and the right-left shunt on arterial oxygen levels. J. Exp. Biol. 199: 2121–2129

Wang, T. and Hicks, J.W. (2008) Changes in pulmonary blood flow do not affect gas exchange during intermittent ventilation in resting turtles. J. Exp. Biol. 211: 3759–3763

Wang, T. E. Krosniunas and Hicks, J.W. (1997) The role of cardiac shunts in the regulation of arterial blood gases. Am. Zool. 37: 12–22

Wang, T., Hedrick, M.S., Ihmied, Y.M., and Taylor, E.W. (1999) Control and interaction of the cardiovascular and respiratory systems in anuran amphibians. Comp. Biochem. Physiol. 124: 395–408

Wang, T., Taylor, E.W., Andrade, D. and Abe, A.S. (2001a) Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*. J. Exp. Biol. 204: 3553–3560

Wang, T., Warburton, S., Abe, A.S. and Taylor, E.W. (2001b) Vagal control of heart rate and cardiac shunts in reptiles: relation to metabolic state. Exp. Phys. 86: 777–784

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

West N.H. and Burggren W.W. (1984) Factors influencing pulmonary and cutaneous arterial blood flow in the toad, *Bufo marinus*. Am. J. Physiol. 247: R884–R894

West N.H., Van Vliet, B.N. (1992) Sensory mechanisms regulating cardiovascular and respiratory systems. In: Feder ME, Burggren WW (eds) Environmental Physiology of the Amphibians. University of Chicago Press, Chicago, pp 151–182

Withington-Wray DJ, Taylor EW, Metcalfe JD (1987) The location and distribution of vagal preganglionic neurones in the hindbrain of lower vertebrates, Chap. 16. In: Taylor EW (ed) Neurobiology of the Cardiorespiratory System. Manchester University Press, Manchester, pp 304–321

Wood, S.C. (1984) Cardiovascular shunts and oxygen transport in lower vertebrates. Am. J. Physiol. 247: R3–R14

Xiang, H., Taylor, E.W., Whiteley, N.M. and Randall, D.J. (1994) Modulation of noradrenergic action by neuropeptide Y in dogfish. Physiol. Zool. 67: 204–215

Young, J.Z. (1950) The Life of Vertebrates. Clarendon Press, Oxford