

**REFLEX AND CENTRAL CONTROL
OF CARDIORESPIRATORY INTERACTIONS
IN ELASMOBRANCH FISHES: A REVIEW**

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Peripheral receptors

Oxygen sensitive chemoreceptors exert dominant control over cardiorespiratory reflexes in fish. The typical response to ambient hypoxia is a reflex bradycardia and increased ventilatory effort (Taylor, 1992). Many studies support the existence of peripheral oxygen receptors on or near the gills of elasmobranch fish. The gill arches in fishes are innervated by cranial nerves IX and X and it is these nerves which innervate the carotid and aortic bodies of mammals. Bilateral section of IX and X abolished the hypoxic bradycardia in the trout (Smith and Jones, 1978) but did not in elasmobranchs (Satchell, 1959). Butler *et al*, (1977) found it necessary to bilaterally section cranial nerves V, VII, IX and X to abolish the hypoxic bradycardia in the dogfish and concluded that the O₂ receptors are distributed diffusely in the orobranchial and parabranchial cavities.

Mechanical stimulation of the gill arches is known to elicit the 'cough' reflex in fish (e.g.Satchell, 1960), and a reflex bradycardia (Taylor, 1985). These mechanoreceptors will be stimulated by the ventilatory movements of the gill arches and filaments, but there is no direct evidence that they contribute to respiratory control on a breath-by-breath basis (Burlison *et al*, 1992). The branchial branches of cranial nerves IX and X provide the afferent arm for the reflex changes in ventilation and heart rate following stimulation of the gill arches or increases in arterial pressure. Central stimulation of branchial nerves in the dogfish caused a bradycardia (Taylor, 1992, Young *et al*, 1993). However this could have stimulated mechanoreceptor and/or chemoreceptor afferents (see below).

Efferent control of the heart

The heart in all fish, except cyclostomes, and in all tetrapods is supplied with inhibitory parasympathetic innervation via the vagus nerve. The inhibitory effect is mediated via muscarinic cholinoreceptors associated with the pacemaker and atrial myocardium (Holmgren, 1977). The heart in vertebrates typically operates under a degree of inhibitory vagal tone that varies with physiological state and environmental conditions. The elasmobranchs are phylogenetically the earliest group of vertebrates in which a well developed autonomic nervous system with clearly differentiated parasympathetic and sympathetic components has been described (Nichol, 1952). They are also the earliest group known to have an inhibitory vagal innervation of the heart. Heart rate in the dogfish varies directly with PO_2 ; hypoxia induces a reflex bradycardia, a normoxic vagal tone is released by exposure to moderate hyperoxia, and extreme hyperoxia induces a secondary reflex bradycardia, possibly resulting from stimulation of venous receptors (Barrett and Taylor, 1984). All of these effects can be abolished by injection of the muscarinic cholinergic blocker atropine (Taylor, 1992). In addition, cholinergic vagal tone, assessed as the proportional change in heart rate following atropinization or cardiac vagotomy, increases with increasing temperature of acclimation (Butler and Taylor, 1975, Taylor, 1992, Taylor *et al*, 1977). These data indicate that variations in the degree of cholinergic vagal tonus on the heart serve as the predominant mode of nervous cardioregulation in elasmobranchs and that the level of vagal tone on the heart varies with temperature and oxygen partial pressure.

Cardio-respiratory synchrony

The matching of the flow rates of water and blood over the counter-current at the gills of fish, according to their relative capacities for oxygen, may be essential for effective respiratory gas exchange. The pumping action of the heart generates a pulsatile flow of blood, which in fish is delivered directly down the ventral aorta to the afferent branchial vessels. To optimise respiratory gas exchange this pulsatile blood flow should probably be synchronised with the respiratory cycle, which typically consists of a double pumping action; with a buccal pressure pump alternating with an opercular or septal suction pump to maintain a constant but highly pulsatile water flow throughout the respiratory cycle. The flow is maximal early in the respiratory cycle and declines during the last two thirds of a cycle (Hughes, 1960, Hughes and Shelton, 1962). Recordings of differential blood pressure and gill opacity in the dogfish revealed a brief period of rapid blood flow

through the lamellae early in each cardiac cycle (Satchell, 1960), and as the ECG tended to occur at or near the mouth-opening phase of the ventilatory cycle this could result in coincidence of the periods of maximum flow rate of blood and water during each cardiac cycle (Shelton and Randall, 1970).

However, this relationship is not fully established. In the restrained dogfish, ventilation rate was approximately twice heart rate and these showed a drifting relationship (Taylor, 1985, Taylor and Butler, 1971). Experimentally restrained dogfish show no hypoxic ventilatory response (Butler and Taylor, 1971) and no evidence of maintained cardiorespiratory synchrony (Hughes, 1972, Taylor and Butler, 1971); while unrestrained fish show reduced normoxic ventilation rates, synchronous with heart beat, and also exhibit a ventilatory response to hypoxia (Metcalf and Butler, 1984). The absence of synchrony, or even consistent close coupling, as opposed to a drifting phase relationship, was most often attributable to changes in heart rate, which was more variable than ventilation rate in prepared dogfish (Hughes, 1972; Taylor, 1985; Taylor and Butler, 1971). As they lack sympathetic innervation to the heart, this may be reliably interpreted as variations in cardiac vagal tone. A decrease in vagal tone on the heart, such as that recorded during exposure to moderately hyperoxic water, caused heart rate to rise towards ventilation rate, suggesting that when vagal tone was relatively low a 1:1 synchrony could occur. When cannulated dogfish were allowed to settle in large tanks of running, aerated seawater at 23°C they showed 1:1 synchrony between heartbeat and ventilation for long periods. This relationship was abolished by atropine, confirming the role of the vagus in the maintenance of synchrony. Whenever the fish was spontaneously active or disturbed the relationship broke down due to a reflex bradycardia and acceleration of ventilation, so that the 2:1 relationship between ventilation and heart rate characteristic of the experimentally restrained animal was re-established (Taylor, 1985). Thus, it is possible that the elusiveness of data supporting the proposed existence of cardiorespiratory synchrony in dogfish has been due to experimental procedures that increase vagal tone on the heart.

The neural basis of synchrony

The heart in the dogfish operates under a variable degree of vagal tone (see above). This implies that the cardiac vagi will show continuous efferent activity. 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, which could be attributed to two types of unit (Barrett and Taylor, 1985a, Barrett and Taylor, 1985b, Taylor and Butler, 1982). 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 playing a role in the determination of the overall level of vagal tone on the heart, which as stated previously seems to vary according to oxygen supply. Other, typically larger units fired in rhythmical bursts which were synchronous with ventilatory movements (Taylor, 1992). We also hypothesised that these units, showing respiration related activity which was unaffected by hypoxia, may serve to synchronise heart beat with ventilation (Taylor and Butler, 1982). 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. 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). Neurons located ventro-laterally outside the DVN were either spontaneously active, firing regularly or sporadically but never rhythmically, or were silent. Thus the two types of efferent activity recorded from the cardiac nerve arise from the separate groups of CVPN, as identified by neuratomical studies (Taylor, 1992).

Activity recorded from the central cut end of the cardiac vagus, or centrally from CVPN, in the decerebrate, paralysed dogfish is likely to be centrally generated. In the intact fish, stimulation of peripheral receptors will affect patterns of activity. All of the spontaneously active CVPN from both divisions and some of the silent CVPN fired in response to mechanical stimulation of a gill arch, which implies that they could be entrained to ventilatory movements in the spontaneously breathing fish (Taylor, 1992). Support for this idea was provided by phasic electrical stimulation of the central cut end of a branchial branch of the vagus in the decerebrate dogfish (M. J. Young, E. W. Taylor and P. J. Butler - unpublished observations). Central stimulation of branchial branches of the vagus in the dogfish with bursts of electrical pulses entrained the efferent activity in neighbouring branchial and cardiac branches. This entrained the efferent bursting units recorded from the central cut end of the ipsilateral branchial cardiac branch, presumably due to stimulation of mechanoreceptor afferents (Taylor, 1992). The firing rates of the non-bursting units recorded from the branchial cardiac were also increased, suggesting that chemoreceptor afferents were being stimulated as well. The

entrained activity in the cardiac vagus drove the heart at rates either slower or faster than its intrinsic rate (see below).

Satchell (1960) described a cyclical pattern of cardiac inhibition in the dogfish which related to dilatation of the pharynx at each inspiration. This led to phasic increases in vagal tone, superimposed on a tonic background of vagal activity, which he suggested may relate to blood pressure, though there is no evidence of baroreceptor inputs in elasmobranch fish. Consequently, normal breathing movements in the intact fish may indirectly influence cardiac vagal outflow, and subsequently heart rate, by stimulating branchial mechanoreceptors. Thus, the typical reflex bradycardia in response to hypoxia may arise both directly, following stimulation of peripheral chemoreceptors and indirectly, via increased stimulation of ventilatory effort, 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). These data support a previous conclusion that synchrony in the dogfish was under reflex control, with mechanoreceptors on the gill arches constituting the afferent limb and the cardiac vagus the efferent limb of a reflex arc (Satchell, 1960). However, the spontaneous, respiration-related bursts recorded from the branchial cardiac nerve continued in decerebrate dogfish, after treatment with curare which stopped ventilatory movements, suggesting that they originated in the brainstem. 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. As the bursts are synchronous, the innervation of CVPN is likely to be excitatory rather than inhibitory as described for the mammal and it is equally possible that a direct drive from a central pattern generator operates both on the RVM and the CVPN (Taylor, 1992).

These data from elasmobranchs suggest that cardiorespiratory synchrony, when present, is due primarily to central interactions generating respiration-related activity in CVPN located in the DVN, which are then effective in determining synchronous heart beating when overall cardiac vagal tone, attributable primarily to activity in CVPN located outside the DVN, is relatively low in normoxic or hyperoxic fish. Synchrony will be reinforced in the spontaneously breathing fish by rhythmical stimulation of branchial mechanoreceptors. Confirmation that the heart may beat at a rate determined by bursts of efferent activity in the cardiac vagi was obtained by peripheral electrical stimulation of these nerves in the prepared dogfish. Although continuous vagal stimulation normally slows the heart, it proved possible to drive the denervated heart at a rate either lower or somewhat higher than

its intrinsic rate with brief bursts of stimuli, delivered down one branchial cardiac vagal branch. At a rate several beats higher than its intrinsic one, the heart responded to alternate bursts of electrical pulses so that it began beating at half the rate of the bursts (M. Young, E.W.Taylor and P. J. Butler, unpublished observations).

Work on teleosts has stressed the importance of inputs from peripheral receptors in the genesis of cardiorespiratory synchrony. Efferent nervous activity recorded from the cardiac branch of the vagus in the tench was synchronized with the mouth-opening phase of the breathing cycle (Randall, 1966). It was suggested that this activity maintains synchrony between heart beat and breathing movements and that both a hypoxic bradycardia and synchrony were mediated by reflex pathways. Randall and Smith (1967) described the development of an exact synchrony between breathing and heart beat in the trout during progressive hypoxia. In normoxia heart rate was faster than ventilation; hypoxia caused an increase in ventilation rate and a reflex bradycardia which converged to produce a 1:1 synchronisation of the two rhythms. Both the bradycardia and synchrony were abolished by atropine. In addition, they were able to demonstrate 1:1 synchronization of hypoxic heart rate with pulsatile forced ventilation, which was clearly generated by reflex pathways, presumably arising from mechanoreceptors on the gills, because the spontaneous breathing efforts of the intubated fish were out of phase with imposed changes in water velocity and were without effect on heart beat (Randall and Smith, 1967). It is interesting in this regard that heart rate was observed to rise immediately upon the onset of ram ventilation in the trout, implying a reduction in vagal tone (Taylor, 1992). As this can be attributed to the effect of cessation of activity both in the central respiratory pattern generator and in the respiratory apparatus it implies that respiratory activity to some extent generates cardiac vagal tone.

Thus, we are left with an apparent conflict of evidence on the mode of generation of cardiorespiratory synchrony. In elasmobranchs it may be centrally generated in inactive, normoxic, or hyperoxic fish when cardiac vagal tone is low; while in teleosts it appears during hypoxia and is generated reflexly by increased vagal tone. The differences between these two groups of fish may be real and it is of interest that branchial denervation increases fictive ventilation rate in elasmobranchs but decreases it in teleosts. However, it is as likely that further experimentation will establish that both central and peripheral mechanisms are important in each group. When cod were cannulated and released into large holding tanks of normoxic seawater they showed periods of 1:1 synchrony (Jones *et al*, 1974, Taylor, 1992). The importance of these observations is that they measured dorsal aortic blood

flow, which was markedly pulsatile in phase with variation in buccal pressure, confirming a role for cardiorespiratory synchrony in the generation of concurrent flow patterns of ventilation and perfusion over the gills. Thus, both unrestrained dogfish and cod can show synchrony, and as our understanding of the underlying mechanisms increases, it seems likely that elasmobranchs and teleosts will share common characteristics with respect to the generation and potential physiological advantages of cardiorespiratory synchrony. What emerges from our present understanding is that a potent mechanism for the generation of cardiorespiratory synchrony in fish exists in the form of entrainment of the heart by the bursting units present in recordings of efferent activity in the cardiac vagi, whether these are generated by central interactions, reflexly by stimulation of branchial mechanoreceptors or most likely by a combination of central and peripheral mechanisms. Entrainment of the heart with the bursts of efferent, respiration related activity in the cardiac vagi could explain the 1:1 synchrony observed in 'settled' normoxic dogfish and cod and in hypoxic trout. As discussed above, cardio-respiratory synchrony may serve to optimise the effectiveness and/or efficiency of respiratory gas exchange and transport in fish. To place this account in the context of a more substantial review of central control of cardiorespiratory interactions in vertebrates, see Taylor *et al.* (1999).

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