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SPONTANEOUS EFFERENT ACTIVITY IN BRANCHES OF THE VAGUS NERVE CONTROLLING HEART RATE AND VENTILATION IN THE DOGFISH

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SUMMARY

Efferent activity was recorded from cranial nerves in the decerebrate dogfish (*Scyliorhinus canicula*) before and after injection of paralysing drugs. The recordings were made from the mandibular (Vth) and glossopharyngeal (IXth) nerves and the branchial (respiratory) and cardiac branches of the vagus (Xth) nerve. All the respiratory branches (Vth, IXth and Xth) and both cardiac branches fired rhythmic bursts of activity, synchronous with ventilation, which continued (at a higher rate) following paralysis, indicating that they originated in the CNS rather than arising reflexly from stimulation of pharyngeal mechanoreceptors. A burst of activity in the Vth nerve was followed by a burst in the IXth then, after a 30-ms delay, simultaneous bursts in the three respiratory branches of the Xth. The bursts in the branchial cardiac branches had a fixed phase relationship with activity in the respiratory branches, the onset of each burst preceding that in the immediately adjacent branch (branchial III), whereas the bursts in the visceral cardiac branches had a variable phase relationship with all other branches. The branchial cardiac branches alone contained units which fired sporadically between the bursts and increased their rate of firing during hypoxia. Both the bursting and non-bursting units responded to mechanical stimulation of the gill area. Separate oscillatory inputs driving the Vth, IXth and Xth respiratory motoneurones and an excitatory input to the bursting cardiac vagal motoneurones from expiratory motoneurones or the respiratory rhythm generator are implied by these relationships. The sporadically firing units in the branchial cardiac nerves clearly receive non-oscillatory inputs.

INTRODUCTION

The vagal innervation of the heart of the dogfish consists of two distinct pairs of nerves. The visceral cardiac branches arise close to the origin of the visceral branch in each side (Marshall & Hurst, 1905) whilst the branchial cardiac branches constitute the major part of the post-trematic limbs of the 4th branchial branches of the vagus nerve (Norris & Hughes, 1920). Parasympathetic control of the heart can be studied in the apparent absence of adrenergic sympathetic control (Young, 1933; Short,

Butler & Taylor, 1977). Previous studies (Short *et al.* 1977; Taylor, Short & Butler, 1977) have indicated that the branchial cardiac branches are more effective in cardio-inhibition than the visceral cardiac branches. Efferent activity has been recorded from the branchial cardiac vagus which included both sporadically active units that accelerated during hypoxia, possibly inducing the reflex bradycardia, and rhythmic bursts of action potentials that were synchronous with ventilatory movements (Taylor & Butler, 1982). It was speculated that the bursts of activity may arise reflexly from stimulation of pharyngeal mechanoreceptors by ventilatory movements. One of the aims of the present study was to determine whether the activity present in the branchial cardiac vagus is centrally or peripherally generated, by recording from the nerves of decerebrate fish following the injection of paralysing drugs, hence removing proprioceptive feedback from pharyngeal receptors.

A functional countercurrent exists over the gills of fish, with blood flowing across the gas exchange surfaces in the opposite direction to water flow. The flow of blood along the ventral aorta of the dogfish is markedly pulsatile (Taylor *et al.* 1977), and the flow of water over the gills, although continuous, is likely to be pulsatile on the basis of measurements of differential water pressures (Hughes & Shelton, 1962). It has frequently been suggested (Satchell, 1960, 1968; Shelton & Randall, 1962; Hughes, 1972) that the exchange of gases between water and blood may be rendered more effective by a degree of synchrony between the cardiac and respiratory pumps, causing periods of maximum water and blood flow to coincide during each cardiac cycle. Such synchrony would require nervous mechanisms to establish the appropriate phase relationship (Satchell, 1968). The present study includes an investigation of the temporal relationships between activity in the cardiac branches of the vagus nerve, the branchial branches of the vagus and the glossopharyngeal nerves, which together innervate the intrinsic respiratory muscles of the gill arches, and the mandibular branch of the Vth cranial nerve, which innervates muscles in the jaw involved in the mouth-closing phase of the ventilatory cycle (Hughes & Ballintijn, 1965).

METHODS

This investigation used 29 lesser spotted dogfish, *Scyliorhinus canicula* L., of either sex and body length between 53 and 72 cm. During the experiments performed in Birmingham (22) the experimental water temperature was $15 \pm 1\,°C$ and the fish were maintained at this temperature for a minimum of 2 weeks prior to experiments. The remainder of the experiments (7) were performed at the Laboratories of the Marine Biological Association, Plymouth at a water temperature of $18 \pm 1\,°C$. The fish were anaesthetized in 10 litres of sea water containing $0.7\,g$ tricaine (MS 222, Sandoz) until ventilatory movements became weak (approximately 20 min). The fish were then placed on an operating tray, packed in ice and left for a further 5 min (Williamson & Roberts, 1981). In some cases 0.05 ml Althesin (Glaxo Laboratories) was administered into the sub-orbital sinus to maintain anaesthesia. The forebrain was exposed to allow decerebration by extirpation of the prosencephalon and part of the diencephalon, taking care not to damage the cerebellum. The incision was closed and covered with a small patch of rubber membrane attached with cyanoacrylate

adhesive to prevent leakage. A polyethylene cannula containing heparinized dogfish saline (100 i.u. ml^{-1}, Evans Medicals) was inserted into the caudal artery and connected to a pressure transducer (Druck, PDCR, 75/2) to record dorsal aortic blood pressure. A cannula in the caudal vein was used for the injection of drugs. Two fine insulated copper wires, with approximately 2 mm of the tips exposed, were inserted close to the pericardial cavity and held in position by a patch of rubber membrane secured to the skin of the fish with cyanoacrylate adhesive. These wires were attached to a pre-amplifier (Isleworth, type A101) for the recording of ECG.

The vagus was exposed on the left side by a lateral incision starting from a point approximately 2 cm behind the spiracle and extending posteriorly just below and parallel with the lateral line, to a point dorsal to the fifth gill slit (Taylor & Butler, 1982). This incision opened into the dorsal wall of the anterior cardinal sinus, on the floor of which run the glossopharyngeal nerve and the origins of the major branches of the vagus nerve (Taylor *et al.* 1977). The required branch(es) of the vagus, or the glossopharyngeal nerve, was then carefully cleared of connective tissue, transected and replaced on the floor of the sinus. The incision was then tightly sutured. In four decerebrate fish the mandibular branch of the trigeminal nerve was exposed on the floor of the orbit. The nerve was cleared of connective tissue, transected and replaced on the floor of the orbit. Each fish was then transferred to the experimental tank filled with recirculated, aerated sea water at the experimental temperature, where it was clamped into a stereotaxic frame (Narishige Instruments) with a plate inserted into the mouth and clamped dorsally, and another lateral clamp holding the body of the fish posterior to the pectoral fins and dorsal to the midline to avoid constriction of the posterior cardinal sinus (Taylor & Butler, 1982).

The fish usually started to ventilate their gills immediately on placement in the experimental tank, unless Althesin had been administered during surgery, when the onset of ventilation was delayed by approximately 1 h. All fish were allowed 2 h for recovery from the effects of anaesthesia before the start of the experiment. The gills were force ventilated with aerated sea water *via* a tube inserted into the mouth below the clamp. Ventilatory movements were monitored by positioning a force transducer against the 1st gill slit on the right side of the fish. The movements recorded are simultaneous with the mouth closing (expiratory) phase of the ventilatory cycle (Hughes, 1960). Thirty minutes after placement in the experimental tank, or when ventilatory movements became strong, the incision into the cardinal sinus was re-opened and held open with lacrymal duct retractors (Macarthy's surgical). Local anaesthesia was obtained by injecting lignocaine hydrochloride (Xylocaine, Astra Chemicals) into the muscle and skin along the edge of the incision. By careful positioning of the retractors the incision was orientated so that sea water was excluded and blood continued to flow across the floor of the cardinal sinus and drain into the Cuverian duct, thus maintaining venous return to the heart. No problems were encountered with either blood loss or aspiration of air into the Cuverian duct. Short *et al.* (1977) established that blood flow along the ventral aorta was maintained after opening the cardinal sinus. The branch(es) of the vagus and/or glossopharyngeal and/or mandibular Vth nerves were then lifted clear of the blood on a pair of platinum hook electrodes attached to a manipulator (Narishige Instruments). Efferent activity in the nerve was relayed *via* a pre-amplifier (Isleworth, type A101) to an appropriate

set of filters and amplifiers (Neurolog system, Digitimer Ltd). The processed signal was displayed on a dual-beam storage oscilloscope (Tektronix R5031) and recorded together with ventilation and the ECG on magnetic tape (4-channel FM tape recorder, SE Labs. 8-40 or 4-channel Racal-Thermionic recorder, S113). The recordings were later transferred to paper using an ink-writing pen recorder with rectilinear coordinates (Gould 2200). Analysis of temporal phase relationships was performed using a planimeter (Summagraphics) programmed to measure the differences between the onset of bursts on pen-recorder traces of activity recorded simultaneously from selected pairs of nerves, and calibrated against the time-base on the recording. Some of the recordings were passed through an integrator (time constant 0·1 s) prior to being transferred to paper. Levels of tonic activity and changes in activity in the nerves were recorded before and during the application of stimuli known to cause a reflex bradycardia such as the hypoxia induced by stopping water flow to the gills or mechanical stimulation of a gill septum (Taylor & Butler, 1982).

After initial recordings had been obtained from the spontaneously ventilating fish, tubocurarine (Tubarine, Wellcome) was administered, at a dose of $7 \, \mathrm{mg \, kg^{-1}}$ which irreversibly abolished ventilatory movements, or at a lower dose of $2·5 \, \mathrm{mg \, kg^{-1}}$ from which the fish recovered after approximately 2 h (Williamson & Roberts, 1980).

Wherever possible measured variables are given as mean values ± S.E. with number of observations in parentheses.

<div align="center">RESULTS</div>

<div align="center">*Recordings from the branchial cardiac branch of the vagus*</div>

Activity recorded from the central cut end of the branchial cardiac branch of the vagus nerve in the spontaneously ventilating, decerebrate dogfish consisted of two types of units: rhythmically firing units synchronous with ventilatory movements, and smaller-amplitude units firing sporadically between the bursts. Efferent recordings from the 3rd branchial branch of the vagus, which innervates the intrinsic respiratory muscles of the 4th gill arch, also contained rhythmically bursting units which fired at a similar phase of the ventilatory cycle to the bursts in the branchial cardiac branch (Fig. 1A). In all experiments in which activity was recorded before and after injection of muscle relaxant drugs ($N = 15$), rhythmical activity with a similar phase relationship continued in the branchial cardiac branch and the 3rd branchial branch (Fig. 1B). Sporadic units were also identifiable in recordings from the branchial cardiac branch in the paralysed fish (Figs 1B, 2).

The rate of bursts of activity in the branches of the vagus after the injection of curare was variable in different fish. Usually, the injection of curare, and hence the removal of proprioceptive afferent feedback to the CNS from the gill arches, was accompanied by an increase in the bursting rate. In fish in which the majority of the respiratory nerves had been sectioned on one side, ventilation rate (f_R) and, therefore bursting rate, was $20 ± 2 \, \mathrm{bursts \, min^{-1}}$ (10) prior to the injection of curare, rising to $28 ± 4 \, \mathrm{bursts \, min^{-1}}$ (6) after curare was administered. In one fish with only the left branchial cardiac branch sectioned, f_R was similar to that previously reported for this species at 15 °C (e.g. Short, Taylor & Butler, 1979), at $43·5 \, \mathrm{bursts \, min^{-1}}$, rising to $52 \, \mathrm{bursts \, min^{-1}}$ after curare was injected. Curare was administered when the fish had
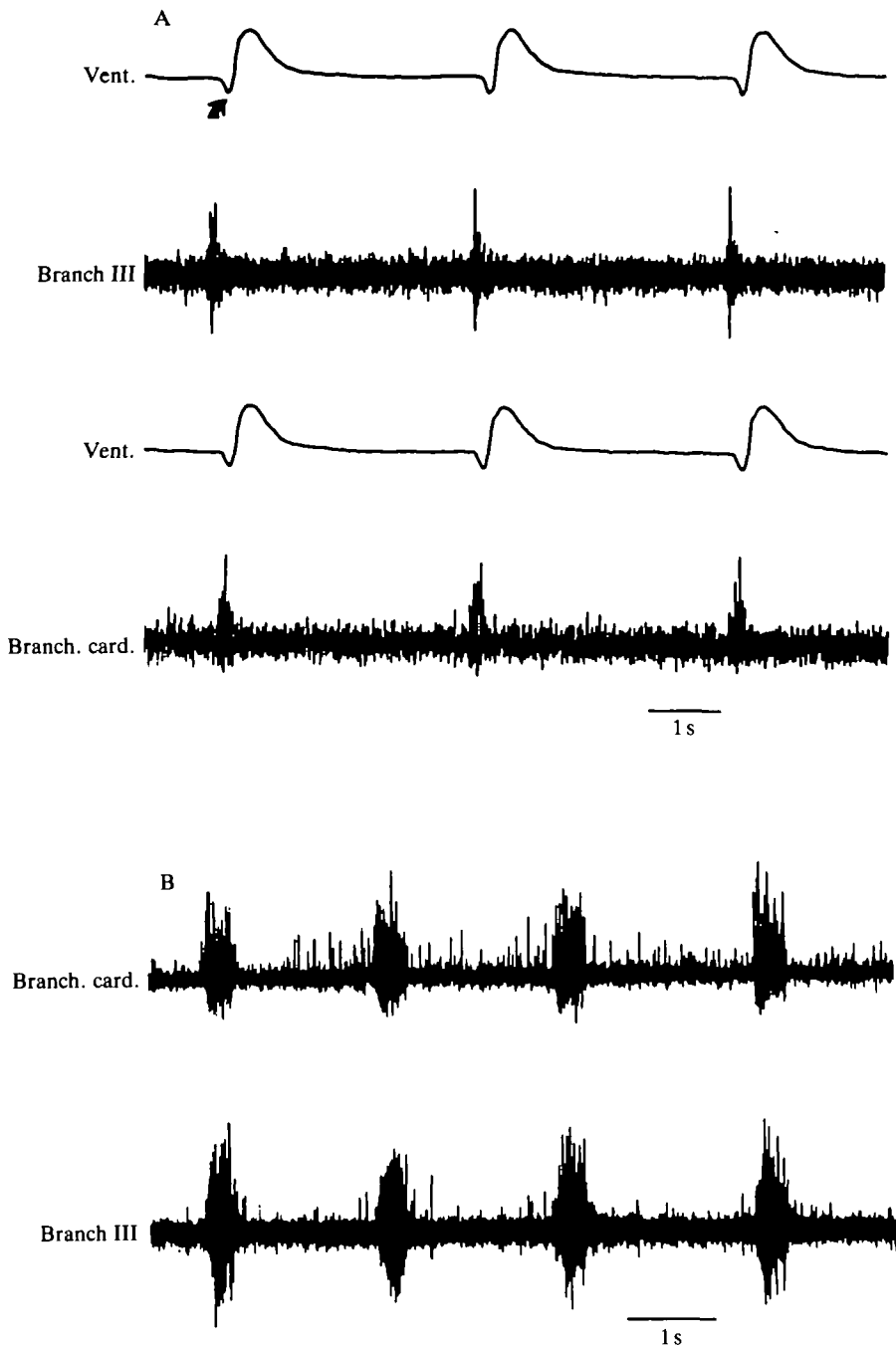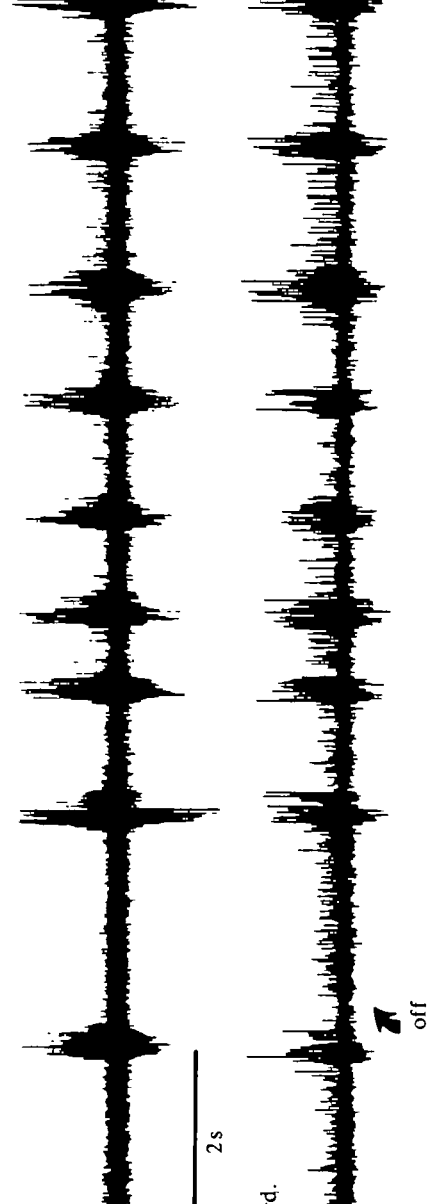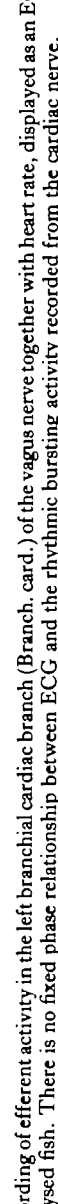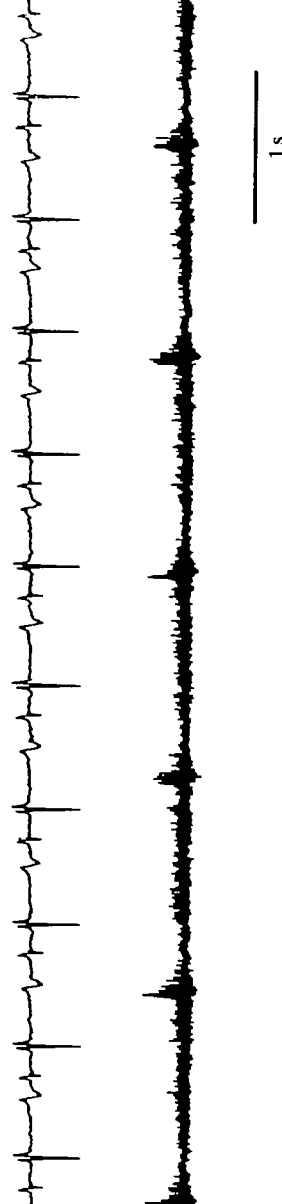
Fig. 1. (A) Rhythmic bursting of efferent activity synchronous with ventilatory movements (Vent.) recorded from the post-trematic 3rd branchial branch (Branch III) and from the branchial cardiac branch of the vagus nerve (Branch. card.) in the same, spontaneously ventilating fish. The arrow indicates the contraction of the first gill septum. (B) Simultaneous recordings from the same nerves in a paralysed fish. Note the relatively high rate of bursting in both nerves and the presence of sporadically firing units in the branchial cardiac nerve.

1 s

2 s

off

...rding of efferent activity in the left branchial cardiac branch (Branch. card.) of the vagus nerve together with heart rate, displayed as an E...
...ysed fish. There is no fixed phase relationship between ECG and the rhythmic bursting activity recorded from the cardiac nerve.

...rd.

...rdings from the post-trematic 3rd branchial branch (Branch III) and the branchial cardiac branch (Branch. card.) of the vagus nerve in...
...he water flow to the mouth was turned off (arrow) there was an initial increase in the rate of bursting activity in both nerves. After 7 s the...
...l higher than the rate prior to the flow being switched off. The increase in activity of the non-bursting units in the branchial cardiac...
...th bradycardia.

recovered from the vagolytic effect of MS 222 and could respond to stimuli, such as a 'prod' to the gill septa, known to elicit a transient bradycardia. The injection of curare caused no change in heart rate and curarized fish responded to the mechano-receptive stimulus of a prod to the gill septa with a marked bradycardia similar to that recorded prior to curarization.

Heart beats occurred during any phase of the bursting cycle in the branchial cardiac nerve branch and were occasionally observed to occur during a burst of activity in the nerve (Fig. 2). Each prod to the gill septa caused a huge increase in efferent activity in the branchial cardiac branch (cf. Taylor & Butler, 1982; Barrett & Taylor, 1985b) which was of long duration and contained many action potentials. This activity appeared to incorporate the bursting and non-bursting units and was mirrored by the activity recorded simultaneously from a branchial branch of the vagus.

The cessation of water flow to the mouth of the fish caused a reflex bradycardia accompanied by an increase in the rate of firing of the non-bursting units (Fig. 3), whilst the rate of bursting of the larger units varied in complex ways, sometimes accelerating (Fig. 3) at other times slowing (cf. Taylor & Butler, 1982). These variations in the rate of bursting in the branchial cardiac branch were exactly mirrored by the bursts recorded simultaneously from the branchial branch (Fig. 3).

Recordings from the visceral cardiac branch of the vagus

Prior to the injection of curare, efferent activity recorded from the visceral cardiac branch also contained rhythmic bursts of action potentials which continued, in the paralysed fish, at the same rate as bursts in the branchial cardiac and the branchial branches of the vagus (Fig. 4). They could be affected in a similar manner to those present in the branchial cardiac branch; for example, a prod to the gill septa elicited an immediate increase in activity in both cardiac branches. The phase relationship between the bursts of activity in the two pairs of cardiac branches was, however, highly variable. The bursts in the visceral cardiac branch were sometimes synchronous with bursts in the branchial cardiac branch (Fig. 4A), but in other animals preceded them and they were often out of phase with simultaneously recorded bursts in the branchial nerves (Fig. 4B), indicating that their phase relationship with the activity in the branchial and branchial cardiac branches was variable. There was no obvious relationship between heart rate and the variations in phase relationship between the bursts in the two pairs of cardiac nerves.

The sequence of the occurrence of bursts of action potentials in the ventilatory and cardiac nerves

Recordings of efferent activity from the nerves which innervate the intrinsic respiratory muscles of gill arches 1, 2, 3 and 4 (the glossopharyngeal and 1st, 2nd and 3rd branchial branches of the vagus respectively) and from the mandibular branch of the Vth cranial nerve, which can be taken to mark the beginning of the respiratory cycle by initiating jaw closure (Hughes & Ballintijn, 1965), contained rhythmic bursts of action potentials synchronous with ventilatory movements. The mean lag between the start of a burst in the mandibular Vth and one in the glossopharyngeal nerve was 152 ± 6 ms (10) (Fig. 5) the longest delay recorded in the sequence (Fig. 6). There was also a delay between the start of a burst in the glossopharyngeal and a burst

B

Visc. card.

Branch III

1 s

1 s
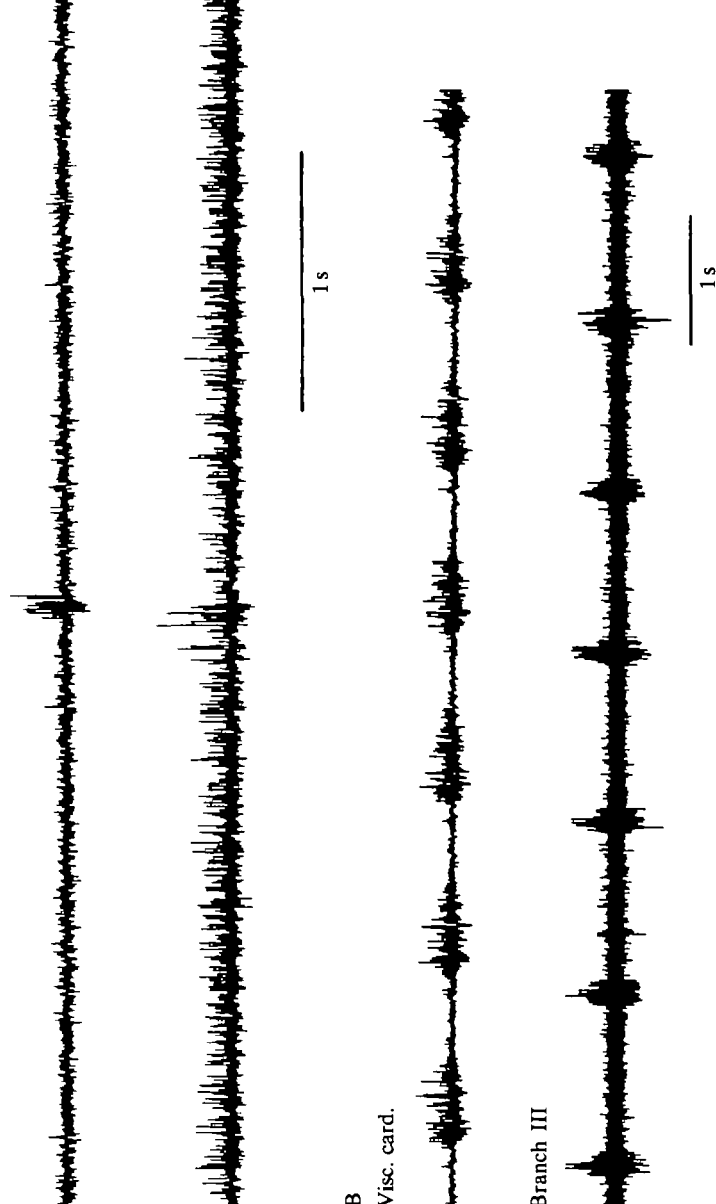
...ivity recorded simultaneously from the visceral cardiac (Visc. card.) and branchial cardiac (Branch. card.) branches of the vagus ne... ...The bursts of activity in the two cardiac branches are virtually synchronous. Note the presence of sporadically active units in the branchial ...re absent from the visceral cardiac branch. (B) Recordings from the visceral cardiac branch and the post-trematic 3rd branchial ... the vagus nerve in a paralysed fish. In this preparation the bursts of activity in the visceral cardiac branch occurred 215 ± 2 ms (10) a... ...ts in the 3rd branchial branch and were of longer duration [726 ± 10 ms (10) and 221 ± 8 ms (10) respectively]; whereas bursts in the b... ...(not shown) always preceded those in the 3rd branchial branch (cf. Figs 1 and 6).

Fig. 5. Regular bursting activity recorded from the post-trematic glossopharyngeal IXth (Gloss. IX) and the mandibular Vth (Mand. V) cranial nerves in a spontaneously ventilating fish. The start of a burst of activity in the mandibular Vth nerve preceded the start of a burst in the glossopharyngeal nerve by 152 ± 6 ms (10).

in the 1st branchial branch of the vagus [30 ± 14 ms (10)], whereas there was no detectable difference between the start of a burst in the 1st, 2nd and 3rd branchial branches of the vagus.

This implies that the contractions of gill arches 1 and 2 are temporally separated, but there is virtually no separation between the onset of contractions of gill arches 2, 3 and 4. It might be expected, due to the branchial cardiac branch's anatomical position, that the onset of a burst would be synchronous with those in the branchial branches of the vagus. In the nine fish investigated bursts of activity in the branchial cardiac branch always preceded those in the adjacent 3rd branchial branch by a mean interval of 60 ± 4 ms. This phase relationship was variable between fish but constant in any individual fish, whether the animal was paralysed or spontaneously ventilating, and whatever the length of the inter-burst interval. This was also found in the temporal relationships of other branches; for example, a doubling in the duration of the ventilatory cycle from 0.5 s^{-1} to 1.0 s^{-1} caused no change in the phase of the ventilatory cycle (one complete ventilatory cycle measured as unity) in which a burst in a branchial branch started, being 0.113 ± 0.01 (10) at the slower ventilation rate and 0.117 ± 0.01 (9) at the faster ventilation rate. The visceral cardiac branch was the only branch of the vagus in which the phase relationship between its burst and the bursts of activity in other branches did not remain constant in the same fish (Fig. 4A,B).

Fig. 6 also demonstrates the high level of inter-burst activity present in the branchial cardiac vagus in comparison with the recordings from the branchial branches of the vagus. This difference is also apparent between recordings from the branchial cardiac and visceral cardiac branches (Fig. 4A).

DISCUSSION

There has been much debate in recent years about the existence and possible functions of synchrony between heart beat and ventilation in fish (Satchell, 1960; Taylor & Butler, 1971; Hughes, 1972). A very precise phase relationship between the circulatory and respiratory pumps was reported in the dogfish *Squalus lebruni* (Vaillant) by Satchell (1960). He described the heart as exhibiting 'a regular rhythm locked to that of respiration' with the P-wave of the ECG occurring most often during the mouth-opening phase of the ventilatory cycle. As the heart beats at a lower frequency than the respiratory pump in fish under experimental conditions this synchrony resulted in the two pumps bearing a simple, whole number, ratio to one another (i.e. 1:2, 1:3, etc.) sometimes with alternation between two of these ratios. The discovery of rhythmic bursts of activity, synchronous with ventilatory movements, in the cardiac rami of the vagus nerve, which are solely responsible for the chronotropic responses of the heart in the dogfish (e.g. Young, 1933), could provide the mechanism underlying this apparent synchrony. The bursts would be expected to be inhibitory in nature and as the bursts in the branchial cardiac branches invariably occur during the mouth closing (expiratory) phase of the ventilatory cycle, their arrival at the cardiac pacemaker could result in the P-wave occurring most often during the mouth opening (inspiratory) phase of the cycle.

Taylor & Butler (1971) showed that a precise temporal relationship between heart rate and respiratory frequency did not occur in the dogfish *Scyliorhinus canicula*
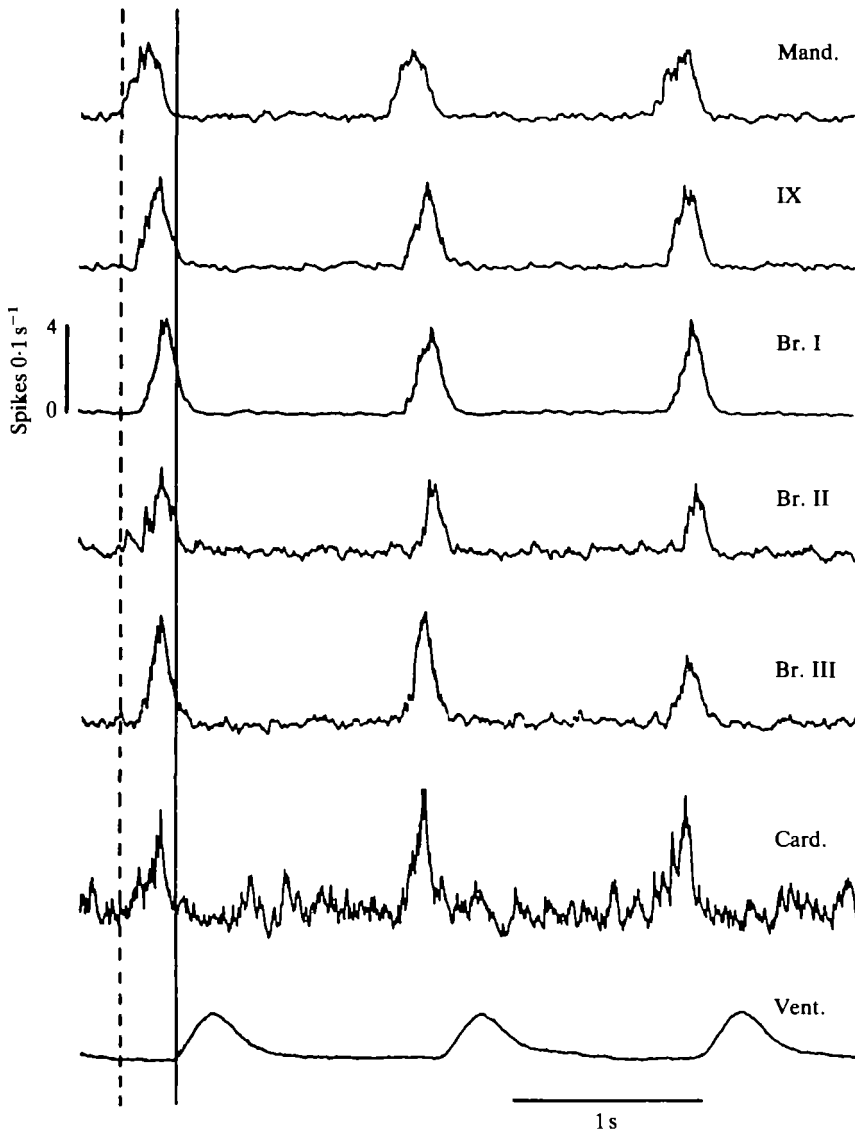
Fig. 6. An example of efferent activity recorded from the mandibular Vth (Mand.), glossopharyngeal (IX), the 1st, 2nd and 3rd branchial branches of the vagus nerve (Br. I, Br. II, Br. III) and the branchial cardiac branch of the vagus nerve (Card.), from the left side of the same fish, together with ventilatory movements (Vent.). The taped recordings (cf. Fig. 5) were passed through an integrator acting as a spike counter (time constant 0.1 s) and the approximate spiking rate is indicated by the vertical bar. The vertical solid line drawn through all the traces indicates the onset of a contraction of the 1st gill septum; the dashed line indicates the start of a burst of activity in the mandibular nerve which preceded that in all other nerves. The bursts in the glossopharyngeal preceded those in the 1st, 2nd and 3rd branchial branches of the vagus which occurred virtually simultaneously. The onset of the bursts in the branchial cardiac branch typically preceded those in the adjacent 3rd branchial branch and recordings from this nerve include sporadic activity between the bursts which is absent from the respiratory nerves.

exposed to a similar experimental regime to the present study but not decerebrated. It remains possible that synchrony between the cardiac and respiratory pumps is of functional significance in unrestrained dogfish, not subjected to operative procedures. Respiratory frequency is relatively low in unrestrained and unoperated dogfish, with a mean of 34 beats min^{-1} at 15 °C (Metcalfe & Butler, 1984) and in settled, unrestrained but cannulated fish, heart rate has been found to be elevated to a level which approximates to respiratory frequency (J. D. Metcalfe, personal communication). Exposure to moderately hyperoxic water, which reduces vagal tone on the heart, causes mean heart rate to rise to 34 ± 1 (10) beats min^{-1} in restrained dogfish at 15 °C, whilst respiratory frequency falls towards this value (Barrett & Taylor, 1984b). A 1:1 relationship exists between heart rate and respiratory rate in unrestrained and inactive dogfish allowed to settle in a large tank of sea water at 23 °C (E. W. Taylor & D. J. Barrett, in preparation) and coincident bursts of activity from cardiac and respiratory motoneurones could serve to couple the respiratory and cardiac pumps in these animals, increasing the probability of concurrence between the period of maximum water and blood flow (Taylor & Butler, 1982). The cardio-inhibitory action of the bursting units may exert a controlling influence over heart beats in conditions when the sporadically firing units, present in recordings from the branchial cardiac branches, are silent. These sporadic units accelerate during a reflex bradycardia induced by stimuli such as hypoxia (Fig. 3) and may be responsible for the observation that the branchial cardiac branches are more potent in cardio-inhibition than the visceral cardiac branches (Short et al. 1977; Taylor et al. 1977) which do not contain sporadically firing units (Fig. 4A).

The continuation of the bursts of activity in peripheral recordings from both the branchial cardiac and visceral cardiac branches in the paralysed fish has some interesting implications. When the bursts in the branchial cardiac branch were first described (Taylor & Butler, 1982) they were thought to arise most probably from an indirect reflex route, by movements of the gill arches stimulating pharyngeal mechanoreceptors present on the branchial processes lining the internal openings of the gill pouches (Satchell & Way, 1962). The present investigation has provided two pieces of evidence indicating that the bursts of activity in the cardiac vagi are not generated primarily via a reflex mechanoreceptive pathway: the continuation of the bursts in the paralysed fish and the occurrence of the bursts prior to the contraction of the gill septa (Fig. 5).

Although in the paralysed fish the bursts are generated in the absence of proprioceptive feedback, they are subject to modulation. Removal of afferent feedback appears to cause an increase in the rate of bursting in the branchial branches of the vagus which is mirrored by the rate of bursting in the cardiac nerves. The central generation of the bursts of action potentials could arise either from direct links with respiratory rhythm generator cells located diffusely in the medulla (Ballintijn, 1982), or from axon collaterals from respiratory motoneurones, terminating on the cardiac vagal motoneurones.

It has been suggested that neighbouring inspiratory motoneurones may innervate and inhibit cardiac vagal motoneurones in mammals (Spyer, 1982). The motoneurones nearest to the cardiac vagal neurones in the dogfish are the respiratory vagal motoneurones innervating the intrinsic muscles of the gill arches (Barrett, Roberts &

Taylor, 1984) which are active during expiration. In contrast to mammals, expiration is the active phase of ventilation in the dogfish (Hughes & Ballintijn, 1965). It is possible that these adjacent expiratory motoneurones could directly innervate and excite cardiac vagal motoneurones, the discharge of which could delay the cardiac cycle, providing the basis for the observation that the heart tends to beat immediately after the mouth opening (inspiratory) phase in *Squalus lebruni* (Satchell, 1960).

The close proximity of expiratory vagal motoneurones to the medial cardiac vagal motoneurones (Barrett *et al.* 1984; Barrett & Taylor, 1985*a*) and the demonstration that the medial cardiac motoneurones are responsible for the rhythmic bursts of activity present in peripheral recordings from the branchial cardiac branch (Barrett & Taylor, 1984*a*, 1985*b*), perhaps supports the idea that the projection of axon collaterals from respiratory motoneurones to cardiac motoneurones is the basis for the generation of the bursts synchronous with ventilatory movements. Such a pathway, however, would introduce a synaptic delay and whether excitatory or inhibitory, it would be expected that the bursts in the branchial branches of the vagus would precede those in the branchial cardiac branch. It has been demonstrated that this is not the case, but axon collaterals from respiratory motoneurones to the branchial cardiac motoneurones could still be implicated if the fibres in the branchial cardiac branch have a faster conduction velocity than those in the branchial branches. Calculations from the mean lag between the bursts in these branches and the distances from the brain, indicate that fibres in the branchial cardiac vagus would need conduction velocities at least $1 \mathrm{~m~s^{-1}}$ faster than the equivalent branchial fibres.

Peripheral recordings from the cardiac vagi which have an inhibitory effect on the heart of the teleost fish the tench, *Tinca tinca*, contain regular bursts of action potentials which are synchronous with ventilatory movements and similar to those recorded from the cardiac vagi of the dogfish (Randall, 1966). Heart beats never occur during the inhibitory impulses in the cardiac vagi of the tench. Randall (1966) concluded that the inhibitory impulses in the vagus prevent the heart beating during the mouth-opening phase (i.e. inspiration) of each breathing cycle and during respiratory coughs. These observations suggest similar central connections, i.e. inhibitory innervation of cardiac vagal motoneurones from inspiratory neurones, to those postulated in mammals.

A close relationship between the motor control of ventilation and blood flow is suggested by the anatomy of the branchial cardiac branch, which arises from the post-trematic 4th branchial branch of the vagus (Norris & Hughes, 1920). The occurrence of bursts of activity, having a fixed phase relationship with ventilatory movements, in peripheral recordings from this branch is predictable. The presence of regular bursts of activity synchronous with ventilatory movements in the visceral cardiac branch recordings is perhaps less predictable, since this branch arises close to the origin of the visceral branch of the vagus (Marshall & Hurst, 1905) which innervates the gastro-intestinal tract. Both pairs of cardiac vagi show rhythmic bursts of activity, but the relationship between the bursts in the visceral cardiac branch is not constant with respect to the bursts in the respiratory nerves or the branchial cardiac branch, which may indicate separate origins of, or inputs to, the visceral cardiac motoneurones in the CNS. Bertrand, Hugelin & Vibert (1973) have suggested that there are a large number of coupled local respiratory oscillators in the medulla which fluctuate in

dominance according to circumstances. The two cardiac branches may receive inputs from two different local oscillators, which normally are closely coupled but can be asymmetrically altered by extraneous circumstances (e.g. chemoreceptor feedback) and thus cause fluctuations in the phasing between the bursts in the two cardiac branches. It is also possible that the motoneurones responsible for the bursts of activity in one branch may receive input from adjacent respiratory neurones, whilst the other branch is affected by a local oscillator elsewhere in the medulla. Since the phase of the bursts in the branchial cardiac branch was fixed with respect to the activity in the branchial branches of the vagus, it can be assumed that the onset of bursts in the visceral cardiac branch is the variable component.

Respiration-related discharges are seen in both the vagal and sympathetic cardiac nerves of mammals. The activity in the vagus nerve occurs only in the absence of phrenic bursts which fire during the active inspiratory phase of ventilation (Richter, 1982), whilst sympathetic discharges increase during phrenic bursts (e.g. Kollai & Koizumi, 1979) and the sympathetic and parasympathetic nerves innervating the mammalian heart act reciprocally in response to most stimuli (Koizumi & Brooks, 1972). The circumstances in which bursts in the branchial cardiac and visceral cardiac branches occur either simultaneously or at different phases is not understood and deserves further investigation. An important difference between the two pairs of cardiac branches in dogfish, compared to mammals, is that both pairs have an inhibitory action on the heart. The dogfish may nevertheless possess the rudiments of a double, interactive innervation of the heart showing some of the characteristics of that present in higher vertebrates.

Recordings from the branchial cardiac branches are characterized by units firing sporadically between the regular bursts. Branchial cardiac preganglionic vagal motoneurones are found in two locations in the medulla around the level of obex. They are located medially and scattered laterally (Barrett, Roberts & Taylor, 1983; Barrett & Taylor 1985a). Inputs to the lateral branchial cardiac motoneurones, which are thought to be responsible for the non-respiratory related activity in peripheral recordings from the branchial cardiac branch (Barrett & Taylor, 1984a), are likely to differ from those to the medial branchial cardiac motoneurones since hypoxia induced by cessation of forced ventilation reduced the respiratory-related activity in the partially paralysed decerebrate dogfish whereas the sporadically firing units present in normoxia increased in rate during hypoxia (Taylor & Butler, 1982). There are also situations when both types of activity are enhanced, e.g. a prod to the gill septa. There is a possibility of interconnections between, or common inputs to, the two populations of branchial cardiac motoneurones.

The sequence of firing of bursts of action potentials in the nerves innervating the respiratory muscles of the jaw (the mandibular branch of the trigeminal) and the gill arches (the glossopharyngeal and branchial branches of the vagus) agrees well with data of B. L. Roberts & C. M. Ballintijn (personal communication). They found, using simultaneous electromyogram recordings from the adductor mandibulae and the constrictor muscles of the gill arches, that there was a lag between the constriction of the first and second gill arches and that the remaining gill arches contracted simultaneously. An identical pattern was found by the analysis of the bursts of activity in the glossopharyngeal, which innervates the 1st gill arch, and the branches of the vagus

which innervate the 2nd and succeeding arches (Fig. 6). This lag may arise from heterogeneous connections (possibly from different local oscillators in the medulla) to the pools of motoneurones supplying axons to these nerves, although the motoneurones are found adjacent to each other (Barrett *et al.* 1984). The existence of independent motor innervation of the 1st gill arch *via* the glossopharyngeal could be responsible for the observations made by Grigg (1970) on another species of elasmobranch, the Port Jackson shark *Heterodontus portusjacksoni*, a species similar to *Scyliorhinus* in general form. Under experimental hypoxic conditions these sharks relaxed the first gill flaps on either side, although they were still observed to beat weakly at the same rate as the other gill flaps. This caused them to flare open and water was shown to be drawn through them into the orobranchial cavity, soon to reappear from the remaining gill slits which were actively contracting. This use of the 1st gill slits for water intake was thought to allow respiration to continue during either the crushing of food or obstruction of the mouth while scavenging for food on the substratum. The need for independent operation of the first gill slit in certain circumstances may answer the question posed by Young (1950) as to why the IXth cranial nerve is not incorporated into the Xth as it leaves the brain.

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