ENTRAINMENT OF THE BREATHING RHYTHM OF THE CARP BY IMPOSED OSCILLATION OF THE GILL ARCHES

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Summary

Artificial oscillation imposed onto the gill arches could modify the respiratory rhythm in the carp *Cyprinus carpio*. The degree of modification depended upon the frequency and amplitude of the applied movement. Oscillation at frequencies close to the spontaneous respiratory rhythm and at amplitudes in the range of 2–3 mm resulted in a cyclic pattern of lengthening and shortening of the breathing cycles. When the gill arches were maximally abducted artificially at the end of inspiration or early in expiration, the breathing cycle was shortened. A slight lengthening occurred when maximal gill arch abduction fell during the second half of expiration or during the first half of inspiration. An increase in oscillation amplitude led to entrainment of ventilation at a one-to-one ratio with the imposed movement. After oscillation stopped an aftereffect was seen. It is argued that gill arch proprioceptors contribute to the observed responses.

Introduction

Electrical stimulation of a single epibranchial vagal ganglion in the carp has been shown in previous studies to have several effects on the breathing movements of the gills (Ballintijn *et al.* 1983; De Graaf and Roberts, 1991). One of the most striking effects is seen when short trains of stimuli are given repeatedly at a frequency that is similar to, but not identical with, the existing ventilatory rhythm. The cycle period of the breathing rhythm in response to such a stimulus either shortens or lengthens so as to match that of the stimulus, and the rhythms of stimulation and respiration then become synchronized.

The gills move rhythmically during normal ventilation. They are actively abducted during inspiration, as a result of the contraction of the branchial arch levator muscles, and adducted in the following expiration as these muscles relax and as the hyoid arch moves forwards and upwards (Ballintijn and Punt, 1985). As

the gill arches move, the discharge rate of proprioceptors located in the gill arches changes in relation to the gill arch position (De Graaf and Ballintijn, 1987), thereby monitoring the degree of expansion of the branchial system. It was suggested in the previous paper (De Graaf and Roberts, 1991) that it was the stimulation of the sensory fibres that originate from these gill arch proprioceptors that resulted in the entrainment of the respiratory rhythm. However, other sense organs could be involved, because the vagus ganglion houses the cell bodies of other types of sensory fibres, all of which would be stimulated synchronously by ganglionic stimulation.

To examine the central impact of the gill arch proprioceptors, we stimulated them in the present study by imposing oscillatory movements upon the gill arches, so as to modify in as natural a way as possible the pattern of mechanoreceptive input transmitted to the central nervous system. The results support the view that activity of the gill arch proprioceptors can exert a powerful effect on the generating circuits and may play a significant role in the control of respiration.

Materials and methods

This study is based on five carp (*Cyprinus carpio* L.), of body lengths 21–27 cm, maintained under light anaesthesia ('Saffan', Glaxovet, 0.72–$1.44\,\mathrm{mg}\,100\,\mathrm{g}^{-1}$, injected into the dorsal body musculature) throughout the experiment. Each carp was held in an experimental tank by a clamp on the orbital ridges of the skull and by a plastic form to support the body. The spinal cord was transected to abolish body movement. All experiments were performed in a temperature-controlled room at 20°C.

Imposed gill arch movements

The operculum on one side of the head was removed and a stainless-steel clamp was placed around the second or third gill arch at the level of the epibranchial–ceratobranchial connection. These manipulations had no significant influence on the existing respiratory movements. But, although the clamp was applied only to one gill arch, the other arches also moved. The clamping surfaces (2.5 mm in height) were coated with Teflon to prevent tissue damage. The clamp was connected to a d.c. motor, provided with a sinusoidally varying voltage from a function generator so as to move the gill arches with amplitudes (0.5–4.5 mm) and frequencies (0.5 and 1.5 Hz) within the normal physiological range.

Recording

Electromyograms (EMGs) were recorded ipsilateral to the levator hyomandibulae (LH) and the A3 part of the adductor mandibulae (A3) using bipolar electrodes made from enamelled copper wire (100 μm diameter) with tin-coated tips. Respiratory movements of the preoperculum and movements of the arches during oscillation were monitored with mechanotransducers. The EMGs and the transducer signals were amplified and stored on magnetic tape and later written

out on paper, using an ink-jet recorder (Siemens Oscillomink-E) or *via* an A/D converter to a dot matrix printer.

Data analysis

All measurements were made from the paper recordings. The temporal relationship between respiratory activity and the imposed gill arch movements was expressed in terms of phase. This was calculated as the time of maximal gill arch abduction ABD (Fig. 1: *L*) from the start of the respiratory cycle, divided by the cycle period of the respiratory rhythm as measured from the EMG record (Fig. 1: *R*). The start of inspiratory muscle (LH) activity, which corresponds with the onset of active abduction of the respiratory system, was chosen as phase reference (phase=0). The patterns of activity of the various muscles in relation to the respiratory movements are illustrated in Fig. 1 of the previous paper (De Graaf and Roberts, 1991).

Results

Both the timing and the duration of respiratory muscle activity changed when the gill arches were oscillated; the effects depended on the amplitude and the frequency of the applied oscillation and on its temporal relationship to the existing respiratory cycle.

Phase dependency

The impact of an imposed oscillation depended upon its temporal relationship to the spontaneous breathing rhythm. In Fig. 1 the phase position of the maximal gill arch ABD shifts continuously in relation to the movement cycle, because the cycle periods of the applied oscillation and of the existing spontaneous respiration are not identical. Nevertheless, the stimulus affects the respiratory cycle period, which is modified in a phase-dependent manner. In general, we found that a breathing cycle would shorten when the gill arches were maximally abducted between phase positions 0.25 and 0.75, whereas it would lengthen when the gill arches were abducted earlier or later in the respiratory cycle (phase: 0–0.25 or 0.75–1.0). In the experiment illustrated in Fig. 2, maximal lengthening was about
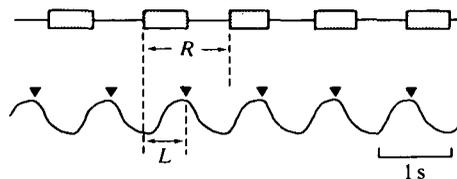

Fig. 1. The temporal relationship between respiratory muscle activity, indicated by the bars in the upper trace, and the oscillatory movement imposed onto the gill arch (lower trace, abduction upwards). *R*, respiratory cycle period; *L*, time between the moment of maximal gill arch abduction (triangles) and the start of the breathing cycle.

6 % and maximal shortening about 14 % when compared to cycles measured before oscillation (solid horizontal line). As a consequence of these modifications of the cycle period, the respiratory rhythm was regularly reset by the imposed oscillations of the gill arch. The cycle period was modified because the durations of activity of the muscles that contribute to the different phases of the respiratory cycle changed in such a way that the normal temporal relationship between the end of inspiratory muscle activity and the start of expiratory muscle activity, and *vice versa*, was unaltered. Consequently, the time of maximal ABD or adduction (ADD) resulting from activity of respiratory muscles coincided with the moment of imposed maximal ABD or ADD of the gill arches.

Entrainment of the respiratory rhythm

Artificial gill arch movements given at frequencies similar to those of the spontaneous respiratory rhythm could result in 1:1 entrainment of the respiratory muscle activity to the imposed oscillatory movement. An example is given in Fig. 3A, where the respiratory output has a cycle period of 1484 ms. The cycle period of the respiratory rhythm shortened when an oscillation with a cycle period of 1330 ms was applied to the gill arches. Imposed oscillations could also lengthen the cycle period.

The range of oscillation frequencies that resulted in 1:1 entrainment is limited. Beyond these frequencies, entrainment patterns with coupling ratios other than 1:1 could be obtained. For example, an applied oscillation given with a cycle
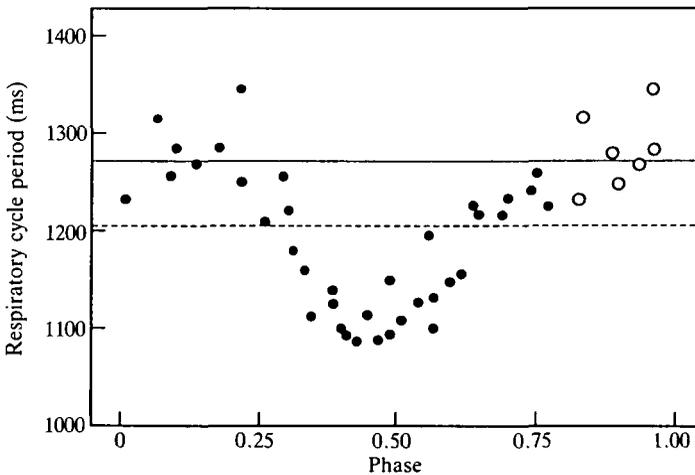

Fig. 2. Analysis of the phase-dependent change of respiratory cycle period during gill arch oscillation. The respiratory cycle period is plotted against the phase of maximal gill arch abduction (ABD) in the breathing cycle. If maximal ABD of gill arches occurred early in the breathing cycle (phase 0–0.2), a second imposed ABD occurred late in the same breathing cycle (open circles). The solid line indicates the mean cycle period before oscillation (1272 ms); the dashed line shows the cycle period after oscillation (1204 ms). The oscillation period was 1000 ms.

period of approximately twice the spontaneous breathing cycle could result in 1:2 entrainment.

Although all respiratory cycles were modified as soon as an oscillation was given, several cycles were needed before entrainment was achieved. This slow onset of entrainment is seen in Fig. 3B as an initial shifting of the phase position of ABD in relation to the motor output: maximal gill arch ABD comes progressively earlier in the breathing cycle until it 'locks in' around a phase position of 0.35. Thereafter, the constant phase relationship between the imposed movement and the motor output ensures entrainment.

After an imposed oscillation was stopped, the cycle period of the respiratory rhythm was different from that before the oscillation had been applied. Whether the period was longer or shorter than before the oscillation did not depend on the ratio of the imposed to the spontaneous cycle periods.

Applied oscillation and coughing

Shortly after the start of an applied oscillation, an EMG pattern was often observed that is characteristic of a cough, which is a normal breathing cycle in
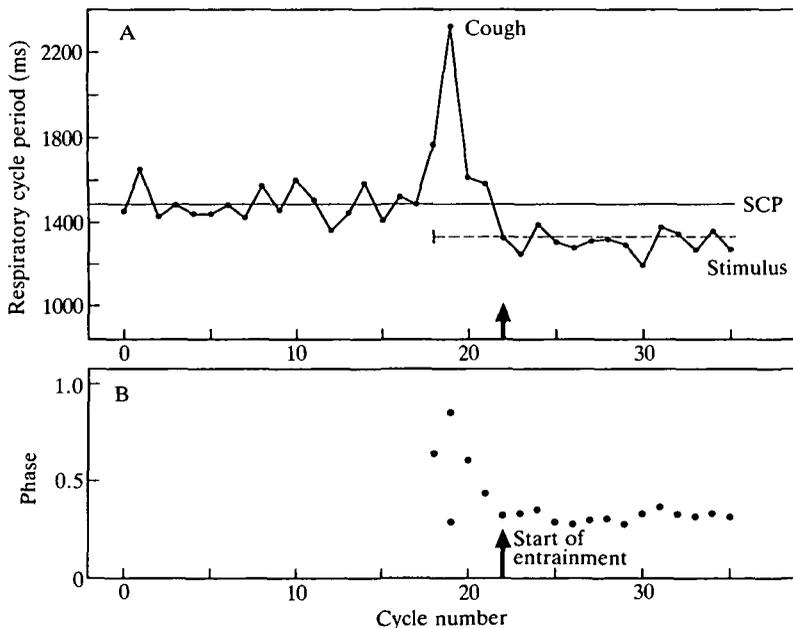

Fig. 3. Establishment of entrainment of the respiratory rhythm in response to oscillatory movements applied to the gill arches. In A the respiratory cycle period is plotted against cycle number. The solid line indicates the mean spontaneous cycle period (SCP) before oscillation (1484 ms). The dashed line indicates the duration and the period of oscillation (1330 ms). In B the phase positions of maximal abduction within the respiratory cycle period are plotted for the cycles illustrated in A. The graph shows the process of 'locking in' following the onset of gill arch oscillation. Entrainment is established as soon as the phase position is constant.
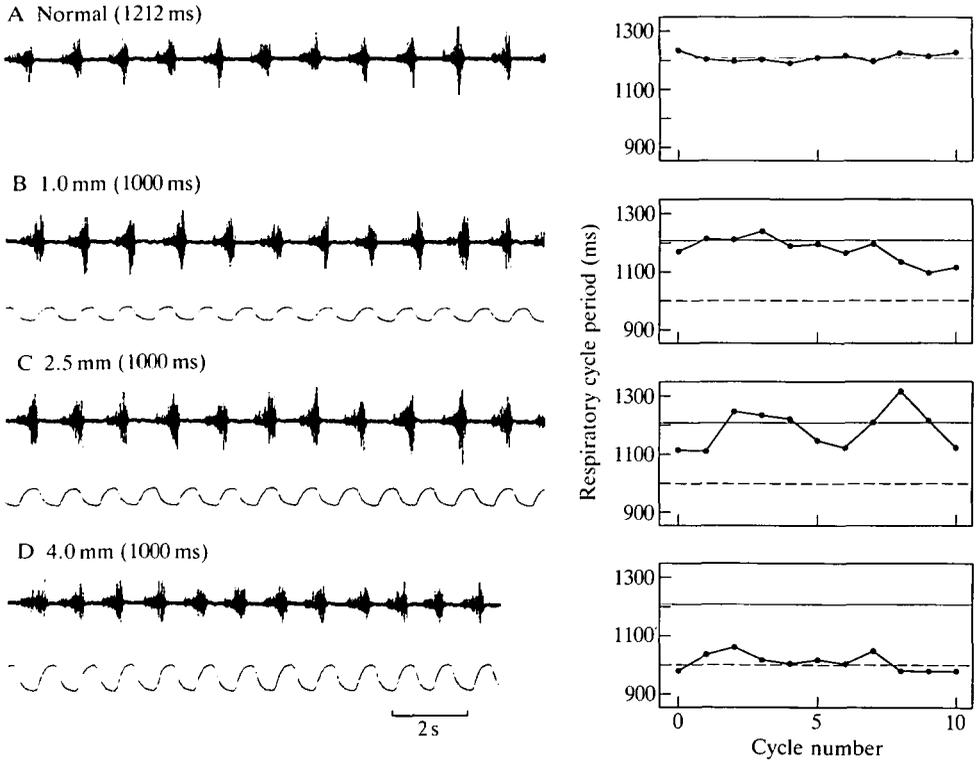
Fig. 4. The influence of imposed gill arch movements on respiration. On the left: respiratory muscle activity during normal breathing (A), and during imposed oscillation with a constant cycle period, but at increasing amplitude (B–D). Recordings represent steady-state situations. Upper trace, EMG of levator hyomandibulae; lower trace, imposed movement of gill clamp (abduction upwards). In the panels on the right, respiratory cycle period is plotted against cycle number for the four amplitudes of oscillation. The solid horizontal line indicates the mean cycle period before oscillation (1212 ms); the dashed line, the cycle period of the imposed oscillation (1000 ms). The points indicate the cycle period of ventilation during the oscillation. Respiration is clearly synchronized with the oscillation in D.

which ADD is interrupted by an intermediate ABD movement. In the case illustrated in Fig. 3, the second, relatively long breathing cycle after the onset of oscillation was a cough. This was possibly an effect caused by mechanical irritation by the clamp, because activation of gill filament mechanoreceptors is known to elicit coughing (De Graaf et al. 1987).

Effect of the amplitude of the imposed movement

The EMG recordings provided in Fig. 4 (left) illustrate the effect of the amplitude of the gill arch oscillation on the motor output. The diagrams, in which respiratory cycle period is plotted against cycle number (Fig. 4, right), demonstrate the influence of the oscillation on the respiratory rhythm. Oscillation at amplitudes up to about 1.5 mm has only a minor effect on the breathing rhythm

(Fig. 4B), but an increase of amplitude to 2–3 mm results in a clear modification of cycle period (Fig. 4C). Although nearly all cycles are modified, there is no synchronization between respiratory muscle activity and the applied gill arch movements, until a further increase in amplitude establishes complete entrainment (Fig. 4D).

Discussion

The imposed oscillatory movements roughly mimic the gill arch movements produced during normal breathing, which are known to modulate the activity of gill arch proprioceptors (De Graaf and Ballintijn, 1987). The afferent fibres from these proprioceptors pass through the vagal epibranchial ganglia, stimulation of which modifies the respiratory pattern (Ballintijn *et al.* 1983; De Graaf and Roberts, 1991). We believe that the entrainment that can be produced by oscillation of the gill arch and by electrical stimulation of the ganglia depends upon the activation of gill arch proprioceptor afferent fibres.

Comparison with electrical stimulation

The impacts of electrical stimulation and of gill arch oscillation on the respiratory rhythm are similar: both result in a phase-dependent modification of the respiratory cycle, an entrainment of the respiratory rhythm and a long-lasting aftereffect.

During oscillation entrainment, respiration locks to the imposed oscillation at phase values of 0.3–0.4. These values correspond in normal respiration to maximal gill arch ABD and maximum gill proprioceptor input (De Graaf and Ballintijn, 1987). In the entrainment that results from electrical stimulation of a vagal ganglion, however, locking occurs at a slightly later phase of 0.5–0.8, when ADD occurs in the respiratory cycle. The reason for this discrepancy is unclear, but possibly relates to the differences in the stimulation. Imposed oscillation modifies the pattern of sensory input that is sent from all the ipsilateral gill arches, whereas electrical stimuli only influence the input that is provided by a single ganglion. In addition, electrical stimulation simultaneously activates afferent fibres that arise from different types of receptor.

Circuitry for respiratory control

Sensory fibres from the branchial nerves terminate in different locations within the central nervous system (reviewed in De Graaf, 1989). Mechanoreceptive input from the gills can reach the motor nuclei that provide the efferent innervation to the respiratory pump and branchial muscles *via* several brainstem integrating nuclei. Short routes that do not involve pattern-generating neurones may be involved in local reflex movements. For example, it seems likely that the vagal fibres, which project to the reticular formation (RF) in the vicinity of vagal motoneurones (nMX) (Morita and Finger, 1985), connect either directly or indirectly with these motoneurones, which innervate the branchial musculature

and form a short loop. This pathway may be involved in the reflex contraction of the adductor muscles of the gill filaments in response to mechanical stimulation of gill filaments or gill rakers. Stronger stimulation results in coughing, which involves the respiratory pump muscles that also receive input from the reticular formation (RF). Peripheral vagal afferent fibres connect centrally with the descending trigeminal nucleus (nDV) and the medial funicular nucleus (nFM) (Morita et al. 1980; Morita and Finger, 1985). These are probable pathways for sensory input from gill arch proprioceptors involved in respiratory motor control. This view is supported by the finding that these nuclei, which are part of the trigeminal complex, also receive afferent input from proprioceptors in the respiratory pump muscles via the trigeminal nerve (Luiten, 1975). If, as we suggest, these receptors are involved in entrainment, they must be connecting fairly directly with the rhythm-generating neurones. The neuronal circuit involved in the generation of the rhythm of respiration in fishes has not yet been identified. However, the effects of local destruction of neurones in the rhombencephalon of the tench (*Tinca tinca*) suggest that an extended, diffusely organized neuronal network is involved (Shelton, 1961). Lesion and stimulation experiments performed on lampreys *Lampetra japonica* and other species, *Entosphenus japonica*, *Ichthyomyzon unicuspis* and *Petromyzon marinus*, have indicated that the rhythm generation may involve neurones located within the trigeminal region (Rovainen, 1983a,b) or more caudally, among the glossopharyngeal and vagal motoneurones (Kawasaki, 1981, 1984; Thompson, 1985).

Significance of the rhythmical sensory input

Although the central networks can generate a rhythm without peripheral information, the present experiments on carp are in line with those on other rhythmical systems in showing that sensory input has a significant impact on the action of the generating circuitry. In the carp, as in the dogfish *Scyliorhinus canicula* (Roberts and Ballintijn, 1988), the sensory input exerts three effects on the respiratory system, which depend on the type of sensory fibre that is activated, on the strength of the stimulus and on the 'state' of the existing respiratory rhythm. The first effect is seen in response to strong stimuli which evoke protective movements – either local contractions of the respiratory muscles ('direct twitch response', Ballintijn et al. 1983) that would protect a single gill, or a cough that protects the whole system.

The second effect of sensory stimulation depends on the time of presentation during the respiratory cycle and results in a change in the cycle period and a switching of the respiratory phases. Such phase-dependent modulation has now been reported to follow sensory stimulation in several rhythmical systems. For example, in mammalian respiration, the vagal input from the lung receptors during inspiration terminates this phase and reduces the duration of the next expiration (e.g. Cohen and Feldman, 1977). Phase switching has also been reported in mastication and in locomotion in several species (see Rossignol et al. 1988).

If the stimulus is repeated regularly, a third effect of sensory input is seen. Over a limited range of frequencies the ventilatory cycle becomes the same as that of the stimulus rhythm. Similar entrainment of the motor system has been seen in a variety of rhythmic motor systems, such as fish swimming (Roberts, 1969; Andersson *et al.* 1981; Grillner and Wallén, 1982; Williamson and Roberts, 1986; McClellan and Sigvardt, 1988) and locust flight (Wendler, 1974; Möhl, 1985; Reye and Pearson, 1988), and may well be a general feature of rhythmical sensorimotor interaction.

We have suggested previously (Roberts and Ballintijn, 1988) that the significance of entrainment is that it stabilises the system and prevents the central generating circuits from being disrupted by other types of stimuli. This stabilising action is demonstrated in the carp experiments (De Graaf and Roberts, 1991), when spontaneous coughing is seen to be eliminated during entrainment in response to electrical vagal ganglion stimulation. The entrainment 'capture range' is small, however, and is dependent upon the existing respiratory rhythm. The fact that the range is limited, but also variable, is important for the stabilising role of entrainment and means that the incoming sensory activity will hold the respiratory rhythm near its existing value and prevent its disruption by 'noisy' inputs to the generating circuitry. Important, powerful inputs to these circuits, however, will readily shift the respiratory rhythm onto a new level and away from the restraint of the old capture range. Once shifted to a new level, of course, entrainment will be re-established and will again provide stability.

References

ANDERSSON, O., FORSSBERG, H., GRILLNER, S. AND WALLÉN, P. (1981). Peripheral feedback mechanisms acting on the central pattern generators for locomotion in fish and cat. *Can. J. Physiol. Pharmac.* **59**, 713–726.

BALLINTIJN, C. M. AND PUNT, G. J. (1985). Gill arch movements and the function of the dorsal gill arch muscles in the carp. *Respir. Physiol.* **60**, 39–57.

BALLINTIJN, C. M., ROBERTS, B. L. AND LUITEN, P. G. M. (1983). Respiratory responses to stimulation of branchial vagus nerve ganglia of a teleost fish. *Respir. Physiol.* **51**, 241–257.

COHEN, M. I. AND FELDMAN, J. L. (1977). Models of respiratory phase switching. *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 2367–2374.

DE GRAAF, P. J. F. (1989). Control of respiration in the carp: mechanoreceptor input and respiratory rhythm. PhD thesis, University of Groningen.

DE GRAAF, P. J. F. AND BALLINTIJN, C. M. (1987). Mechanoreceptor activity in the gills of the carp. II. Gill arch proprioceptors. *Respir. Physiol.* **69**, 183–194.

DE GRAAF, P. J. F., BALLINTIJN, C. M. AND MAES, F. W. (1987). Mechanoreceptor activity in the gills of the carp. I. Gill filament and gill raker mechanoreceptors. *Respir. Physiol.* **69**, 173–182.

DE GRAAF, P. J. F. AND ROBERTS, B. L. (1991). Effects of vagal sensory input on the breathing rhythm of the carp. *J. exp. Biol.* **155**, 77–91.

Grillner, S. and Wallén, P. (1982). On peripheral control mechanisms acting on the central pattern generators for swimming in the dogfish. *J. exp. Biol.* **98**, 1–22.

Kawasaki, R. (1981). Artificial pacemaking of breathing movements by medullary stimulation in adult lampreys. *Jap. J. Physiol.* **31**, 571–583.

Kawasaki, R. (1984). Breathing rhythm-generation mechanism in the adult lamprey (*Lampetra japonica*). *Jap. J. Physiol.* **34**, 319–335.

Luiten, P. G. M. (1975). The central projections of the trigeminal, facial and anterior lateral line nerves in the carp (*Cyprinus carpio* L.). *J. comp. Neurol.* **160**, 399–418.

McClellan, A. D. and Sigvardt, K. A. (1988). Features of entrainment of spinal pattern generators for locomotor activity in the lamprey spinal cord. *J. Neurosci.* **8**, 133–145.

Möhl, B. (1985). The role of proprioception in locust flight control. III. The influence of afferent stimulation of the stretch receptor nerve. *J. comp. Physiol.* **156**, 281–291.

Morita, Y. and Finger, T. E. (1985). Topographic and laminar organization of the vagal gustatory system in the goldfish, *Carassius auratus*. *J. comp. Neurol.* **238**, 187–201.

Morita, Y., Ito, H. and Masai, H. (1980). Central gustatory paths in the crucian carp, *Carassius carassius*. *J. comp. Neurol.* **191**, 119–132.

Reye, D. N. and Pearson, K. G. (1988). Entrainment of the locust central flight oscillator by wing stretch receptor stimulation. *J. comp. Physiol.* **162**, 77–89.

Roberts, B. L. (1969). The coordination of the rhythmical fin movements of the dogfish. *J. mar. biol. Ass. U.K.* **49**, 357–378.

Roberts, B. L. and Ballintijn, C. M. (1988). Sensory interaction with central 'generators' during respiration in the dogfish. *J. comp. Physiol.* **162**, 695–704.

Rossignol, S., Lund, J. P. and Drew, T. (1988). The role of sensory inputs in regulating patterns of rhythmical movements in higher vertebrates. In *Neural Control of Rhythmic Movements in Vertebrates* (ed. A. H. Cohen, S. Rossignol and S. Grillner), pp. 201–284. New York: Wiley & Sons Inc.

Rovainen, C. M. (1983a). A trigeminal component of the central pattern generator for respiration in the adult lamprey. *Soc. Neurosci. Abstr.* **9**, 541.

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

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

Thompson, K. J. (1985). Organization of inputs to motoneurons during fictive respiration in the isolated lamprey brain. *J. comp. Physiol.* **157**, 291–302.

Wendler, G. (1974). The influence of proprioceptive feedback on locust flight coordination. *J. comp. Physiol.* **88**, 173–200.

Williamson, R. M. and Roberts, B. L. (1986). Sensory and motor interactions during movement in the spinal dogfish. *Proc. R. Soc.* **227**, 103–119.