PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Interneuronal Activity Patterns during Fictive Locomotion of Spinal Dogfish

W. Mos, B. L. Roberts and R. Williamson

Phil. Trans. R. Soc. Lond. B 1990 **330**, 341-349
doi: 10.1098/rstb.1990.0204

Interneuronal activity patterns during fictive locomotion of spinal dogfish

W. MOS[1], B. L. ROBERTS[1] AND R. WILLIAMSON[2]

[1] *Department of Experimental Zoology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands*
[2] *The Laboratory of the Marine Biological Association, PL1 2PB Plymouth, England*

SUMMARY

Interneuronal activity was recorded from the spinal cord of paralysed spinal dogfish (*Scyliorhinus canicula*) showing fictive swimming as indicated by rhythmic activity in the motor nerves.

The interneurons from which spike activity was recorded during unstimulated fictive swimming ($n = 282$) were divided into three groups, according to their firing patterns. Group I units (29%) discharged steadily (mean interspike interval 25–100 ms); their firing patterns were not or only weakly modulated in phase with the spinal cord motor output; when fish with different swimming rhythms were compared, no correlation was found between the average frequency of firing of these units and the mean cycle period of the motor rhythm; when spinal motor output stopped, these neurons remained active. Group II units (19%) discharged throughout the entire cycle of the motor rhythm although a few became phasically active at short cycles; their firing was clearly modulated in line with the motor rhythm and during shorter cycles increased in frequency; when motor output stopped, their spike activity was strongly reduced or absent. Group III units (52%) discharged bursts of action potentials in time with the motor rhythm, each unit firing during its own characteristic phase within the motor output cycle; their firing frequencies increased linearly with locomotor frequency; these units were silent when motor output stopped.

1. INTRODUCTION

The slow, regular swimming movements of fish result from the rhythmical activation of the lateral ('red') myotomal muscle fibres (Bone 1966; Bone *et al.* 1978; Johnston *et al.* 1977; van Leeuwen *et al.* 1990; Rome *et al.* 1984). Similar movements are carried out by spinal preparations of dogfish and recordings taken from these preparations from the red muscle or from motor nerves show rhythmical bursts of activity (Roberts 1969; Grillner *et al.* 1976; Williamson & Roberts 1980, 1986). By recording from spinal preparations paralysed with curare we have shown (Mos *et al.* 1990) that the rhythmic burst of impulses produced by the individual motoneuron has a pattern (firing frequency and duration) which is highly correlated with the cycle period of the motor rhythm. In addition, we showed that the mononeurons of each segment which are active during fictive swimming behave similarly, suggesting that they are driven by a coordinated common input that originates from interneuronal circuits.

In any neural circuit, the interneurons are usually the hardest to investigate. They are often small and are difficult to identify physiologically. They are heterogeneous, utilizing various transmitters and exerting different excitatory and inhibitory effects on diverse targets. They are particularly difficult to approach in vertebrates because they are so numerous and seem to have an effect only when they operate in concert, unlike certain identifiable invertebrate interneurons whose individual activation can initiate or modulate regular motor output (see, for example, Weeks & Kristan (1978); Getting (1983). Thus it is not surprising that we have incomplete information about the interneuronal components of most locomotor circuits, particularly for vertebrate preparations. Most studies in higher vertebrates have been focused on interneurons associated with reflex pathways (see, for example, Edgley & Jankowska (1987)) although some data are available for rhythmic fictive movements (Berkinblit *et al.* 1978; Deliagina *et al.* 1983; Shefchyk *et al.* 1990). Only for the lamprey (Grillner *et al.* 1986) and frog tadpole (Roberts *et al.* 1986) have the excitatory and inhibitory interneurons that are involved in rhythmic motor output production been well characterized.

In this paper we describe the firing behaviour of interneurons in the spinal cord of the paralysed spinal dogfish preparation when it is fictively swimming. The interneurons have been classified into three broad categories on the basis of their activity patterns and these have been compared with the behaviour of the motoneurons.

2. MATERIALS AND METHODS

The experiments were performed at the Laboratory of the Marine Biological Association, Plymouth, England, on 47 dogfish, *Scyliorhinus canicula* L., which had been kept in large tanks of circulating sea water. The fish were prepared as described in detail in the

Phil. Trans. R. Soc. Lond. B (1990) **330**, 341–349
Printed in Great Britain

previous publication (Mos *et al.* 1990). They were decerebrated under MS 222 anaesthesia and the spinal cord and nerves in the region of segment 35 were exposed. The motor and sensory parts of the nerves were separated from each other; the nerve branch used for recording the motor activity contained the axons of about 75% of the motoneuronal population of the ipsilateral spinal cord segment from which it emerged (Mos & Williamson 1986). The spinal cord was transected close to the obex and the muscles were paralysed by an injection of 7 mg kg^{-1} d-tubocurarine (Wellcome) into the suborbital sinus, before the microelectrode recording.

In all the fish used in this study, a rhythmical motor output was recorded from the motor nerves of the paralysed preparation (fictive swimming). The frequency of the motor rhythm differed from fish to fish and could vary during the course of the experiment. For example, in one fish the cycle period was steady at 14 s for the first hour of the experiment, but later shortened to 4 s. In another fish the cycle period was as short as 1.1 s. For most, the average cycle period was between 2 to 4 s. The cycle period would shorten at times spontaneously in brief episodes, for no apparent reason, but could also be experimentally altered by a variety of stimuli. Cutaneous stimulation, touch to the body, or a pinch to the tail, was very effective in doing this but the cycle period could also change in response to electrical stimulation of the spinal cord or sensory nerves.

(*a*) *Recording and stimulation*

The motor nerve was placed over bipolar platinum hook electrodes so as to monitor the motor output of the segment and to enable the axons of motoneurons to be stimulated antidromically for identification. The sensory nerve and spinal cord were also stimulated to aid in the identification of interneurons; for this, the sensory nerve bundle was placed over hook electrodes and a concentric needle electrode was placed into the spinal cord some 140–150 mm rostral to the segment used for recording. However, spinal cord and sensory nerve stimulation exerted long-lasting effects on the pattern of the motor output, and so these stimulation sites were activated only sparingly.

Unit recordings were made with 3 M KCl-filled microelectrodes (10–20 MΩ) from the ipsilateral half of the spinal cord segment giving rise to the spinal nerve used for stimulation and recording.

(*b*) *Analysis*

The unit and motor nerve recordings were amplified, stored on magnetic tape and subsequently written out on paper and analysed with a computer (PDP11/34 Digital Equipment Corp or Exorset 165, Motorola Corp). The spike data were analysed in relation to the rhythmical activity of the motor nerve, and in relation to stimuli applied to peripheral nerves or spinal cord. The nerve recordings (neurograms) were full-wave rectified and filtered with a leaky integrator (time constant 0.1 s) to provide a signal, the amplitude of which is a measure of the peak motoneuronal output of one segment (see Mos *et al.* 1990). The cycle period of the motor output was measured by passing the filtered neurogram through a level detector to provide a trigger for the computer at the start of each nerve burst, which is taken as the beginning of a new cycle. Spike data were stored as the time at which spike discharges occurred in each individual cycle and were displayed as rasters in real time. To compensate for the variation in cycle duration, the data were normalized by transforming the time of each spike occurrence in a cycle to a phase value; the result from 50 cycles was displayed as a phase histogram on a scale of 0 to 1 representing the length of a complete cycle. The histograms were corrected for any delays in triggering so that 0 showed the precise start of each burst recorded from the motor nerve. As in the preceding paper (Mos *et al.* 1990), locomotor frequency is defined as the reciprocal of cycle period.

3. RESULTS

Units that were antidromically activated by stimulation of the ipsilateral motor nerve of the same segment were classified as motoneurons. They were usually found at a depth below the surface of the spinal cord of around 1000 µm, which is the region of the ventral horn; their activity has been described in the accompanying paper (Mos *et al.* 1990).

All units which were not antidromically activated were classed as interneurons. Of course it is possible that we have recorded from a few motoneurons that do not project into the stimulated motor nerve or from visceromotoneurons, but their number would have been small and, therefore, they would not influence our analysis of interneuronal firing significantly. As far as we are able to judge from the shape of the potentials, and from the presence or absence of synaptic activity in intracellular recordings, unit activity was recorded both from neuronal somata and axons (the majority).

Of the 415 units analysed, 32% showed no spontaneous activity. These inactive units were located as they responded to high spinal cord (figure 1) or sensory nerve stimulation, or because they could be activated by intracellularly injected current. Many inactive units discharged if the skin was touched or the tail pinched.

The other, active units ($n = 282$) could be separated into three groups on the basis of their discharge patterns during unstimulated fictive swimming and

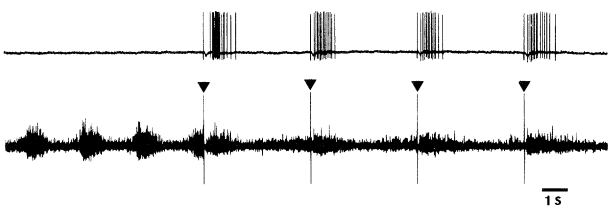

Figure 1. Recording from an inactive interneuron (top trace) that discharges bursts of spikes (spike size 25 mV) in response to stimuli given to the high spinal cord, as shown by arrows. In the nerve record (bottom trace) it is seen that this type of stimulation profoundly influences the output of the spinal cord.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

during periods of silenced motor output obtained after a strong tail pinch. These are: I (29 % of the active units), neurons that are continuously active at a more or less stable firing frequency which does not change markedly when fictive swimming ceases temporarily; II (19 %), continuously active cells which become silent or reduced in activity when the motor rhythm stops, and III (52 %), neurons discharging a burst of action potentials during each cycle of the motor rhythm. Neurons of all three groups could be recorded at positions from medial to lateral in the spinal cord and at all depths below the surface, but most units were found within or just dorsal or lateral to the ventral horn.

The group I interneurons discharge steadily at a relatively high frequency (mean interspike interval between 25 and 100 ms). When the motor rhythm stops for a short period of ten to several tens of seconds, a condition that occasionally occurs spontaneously but that always can be evoked by a strong tail pinch, group

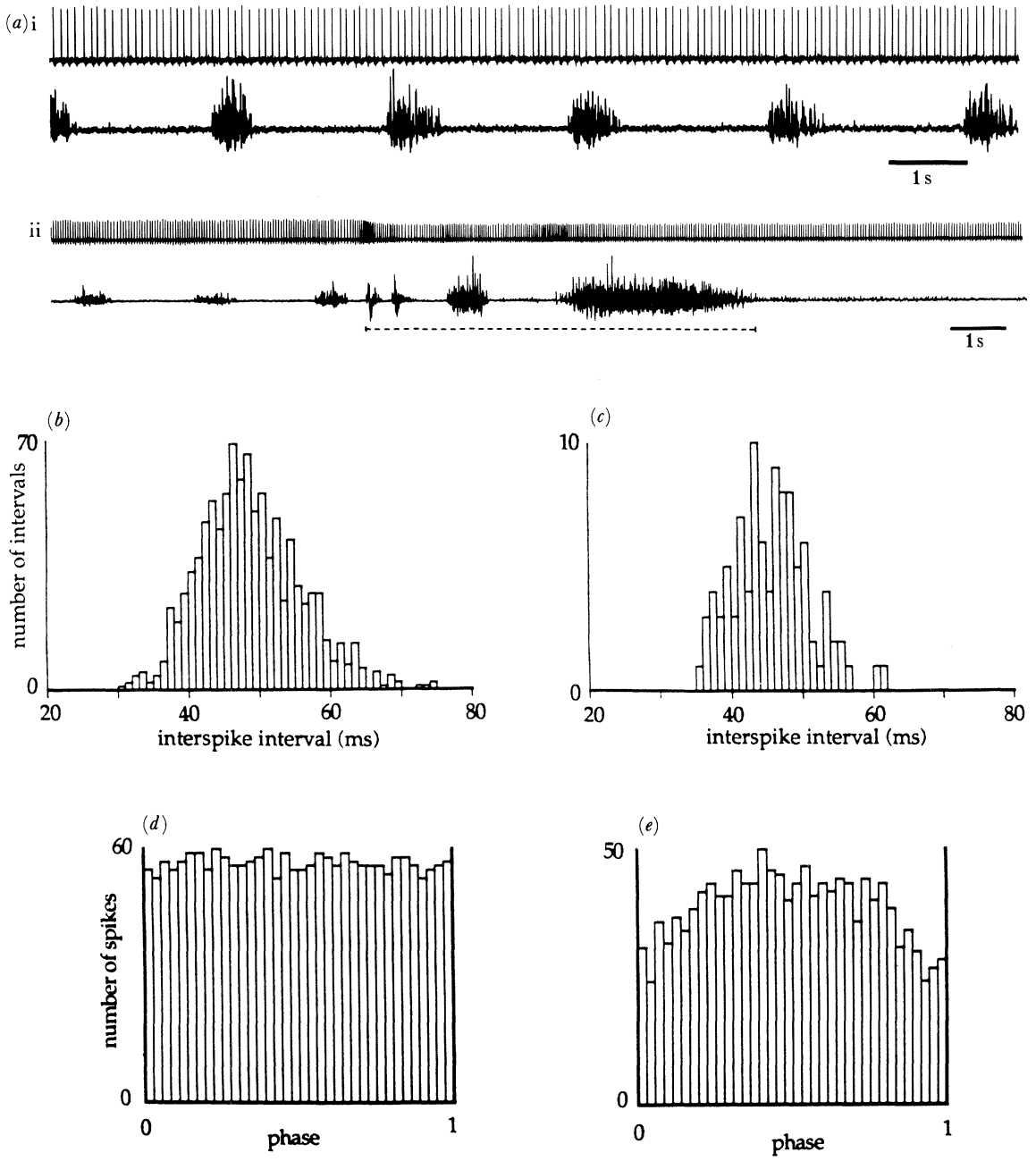

Figure 2. The activity of group I interneurons. (*a*) Extracellular recordings of interneurons (top traces) with a large (i) and a small (ii) mean interspike interval, and the simultaneously recorded motor nerve neurograms (bottom traces). (*a*) (i) Unstimulated fictive swimming; mean spike interval: 94 ms; spikes: 18 mV. (*a*) (ii) Unstimulated fictive swimming followed by a tail pinch, as shown by broken line, during which the motor activity is irregular. After the pinch, motor output stops for about 7 s before rhythmic activity resumes at the normal rate. The activity of the interneuron is slightly modulated in time with the motor output; spikes: 15 mV. (*b*, *c*) Interspike interval histograms of the neuron shown in (*a*) (ii) for a period of spontaneous rhythmic motor nerve activity (*b*) and for a period during which the motor rhythm ceases in response to a tail pinch (*c*). The mean intervals for the two periods are similar (48.2 and 45.3 ms, respectively). (*d*, *e*) Phase histograms of the activities of two neurons showing a poorer (*d*) and better (*e*) correlation with the motor rhythm than did the neuron in (*a*) (ii).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

I units continue to discharge at frequencies similar to those detected during fictive swimming, as is shown for one unit in figures 2*a–c*. Strong cutaneous stimulation was usually followed by a transient increase in firing rate (figure 2*a*).

The phase histograms for some of the units classed as group I show that the firing pattern is weakly modulated in phase with the motor nerve activity, whereas for others this modulation is absent (figures 2*d*, *e*). When the motor rhythm stops, no modulation of firing is observed. An analysis of the spike data from group I units recorded from fish with different locomotor frequencies showed that their firing levels are not clearly correlated to the rhythm of the motor output. No correlation could be detected with signal averaging between action potentials established in these neurons by intracellular current injection and the action potentials recorded from the motor nerve, suggesting that these neurons individually do not activate the motoneurons.

The firing patterns of units classified as group II are intermediate between those of groups I and III. Group II units fire throughout the cycle of the motor rhythm, as do group I units, but their activities are clearly

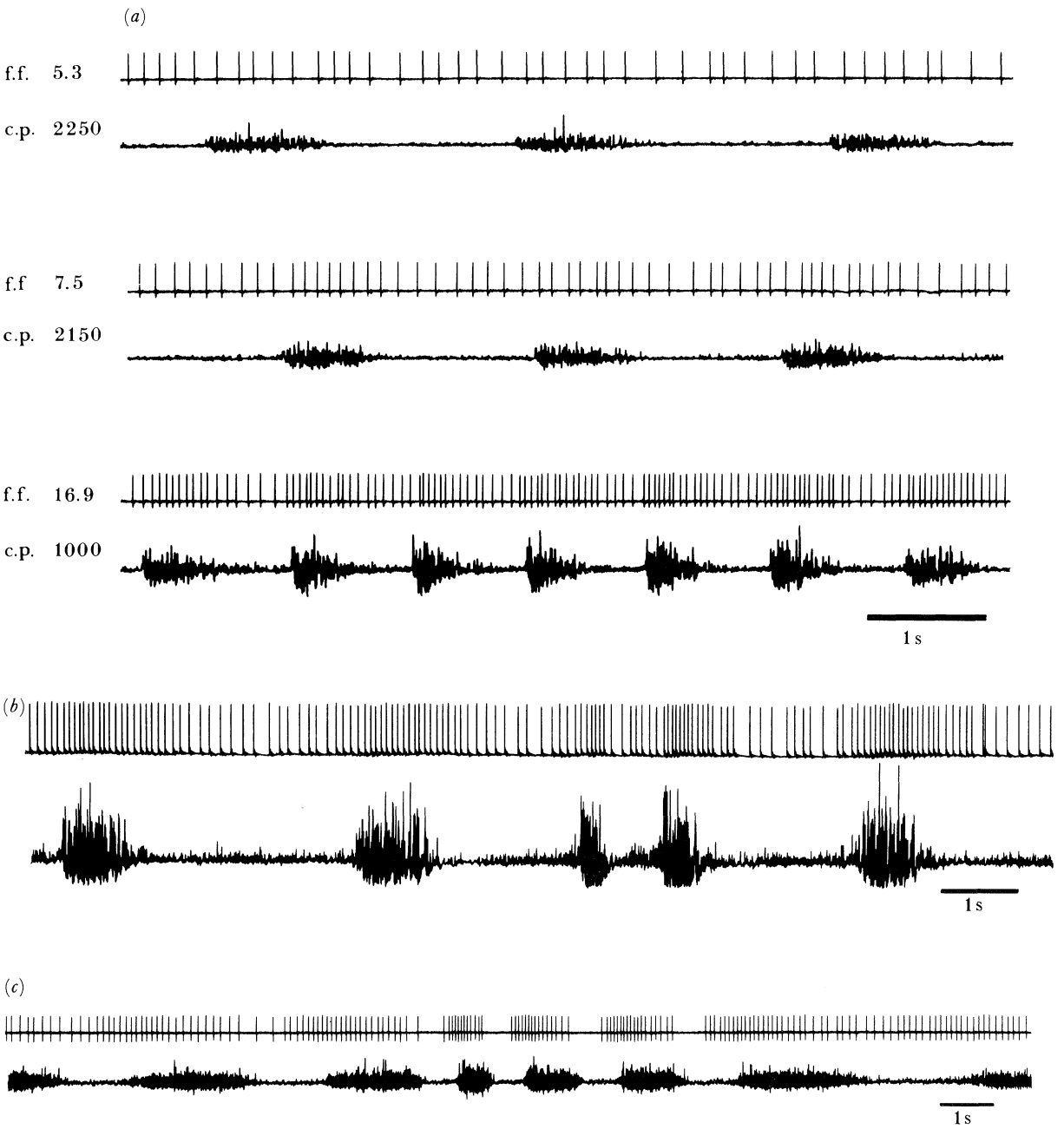

Figure 3. The activity of group II interneurons. (*a*) Three examples of the activity of the same neuron (top traces) during episodes of unstimulated steady fictive swimming at different cycle periods as demonstrated by the motor nerve neurogram recorded simultaneously (bottom traces). The mean firing frequency of the neuron (f.f. in impulses per second) and the mean cycle period of the motor output (c.p. in milliseconds) are indicated. Note that mean firing frequency increases as the cycle period shortens. (*b*) Activity of a neuron which fires much faster than the one shown in *a* (mean firing frequency at cycle periods of 3700 ms: 10.3 imp s^{-1}. (*c*) Activity of a group II neuron which changes its continuous activity to a bursting pattern when the cycle period of the motor rhythm shortens spontaneously. Spike sizes: 15–25 mV.

modulated in each cycle and their firing levels increase with decreasing cycle period (figure 3*a*), similar to group III units. They differ in their firing levels and in the phase relation with the motor rhythm (figure 3). From figure 3*a* it can be seen that the rhythmical modulation may become more obvious during shorter cycles. For some units the modulation increases dramatically with the discharge consisting of bursts of

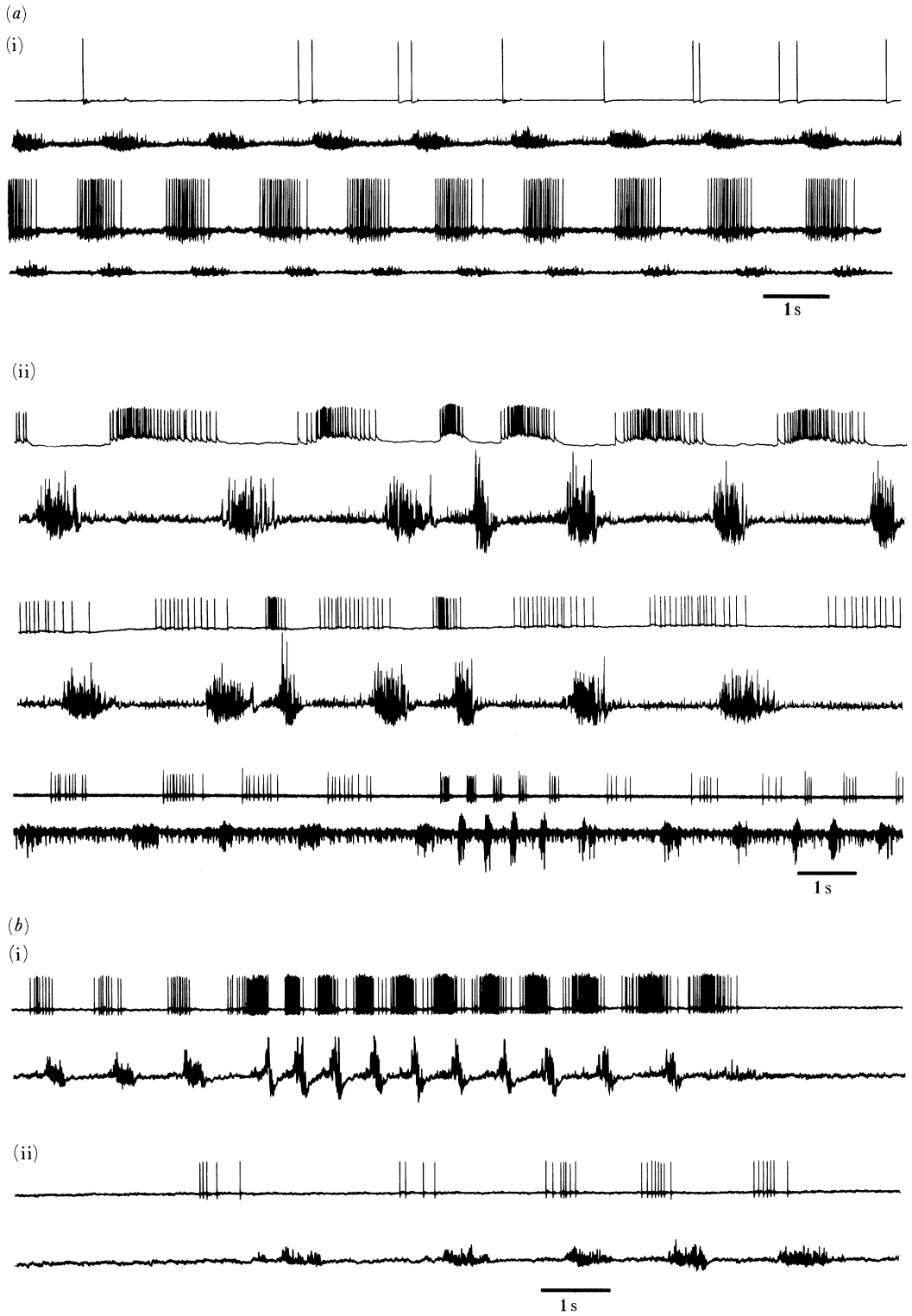

Figure 4. The activity of group III interneurons. Examples of interneuronal activity (top traces) in relation to motor nerve neurogram (bottom traces) during unstimulated fictive swimming. All neurons are extracellularly recorded (spikes between 10 and 25 mV), except the top neuron in (*a*) (ii) (spikes 30 mV). (*a*) (i) Two neurons firing few and many spikes, respectively, during steady fictive swimming at approximately the same cycle period. (*a*) (ii) Three neurons, recorded during changing cycle period, firing at different frequencies and phase positions. (*b*) (i,ii) Neuron that is silent when motor output stops for a short period after a spontaneous acceleration of the motor rhythm (*b*) (i). It resumes activity just before the recurrence of motor output (*b*) (ii) which after two cycles attains its normal frequency. This neuron is atypical in not shortening its burst length with cycle period.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

impulses separated by interburst intervals, either when short cycles occur spontaneously (figure 3*c*) or when they are evoked by sensory stimulation. Some units are inactive when the motor output stops but others maintain a low level of spontaneous activity.

Group III units discharge periodic bursts of action potentials in time with the rhythm of the motor output recorded from the motor nerve (figure 4*a*). Wide variations in the patterns of activity are observed between the units, even when they are compared for motor cycles of similar durations: the duration of the burst, the number of impulses per burst (1–40 impulses for cycle periods of around 2000 ms), firing frequency, and phase position within the output cycle, all differ from unit to unit. They are unlikely, therefore, to form a homogeneous group and probably they have different roles. Group III units stop discharging when the motor output ceases (figure 4*b* (i)) and usually fire one burst (occasionally two bursts) prior to the resumption of the motor activity (figure 4*b* (ii)).

As can be seen from the phase histograms of figures 5*a–c*, some group III interneurons begin to discharge at the start of motoneuronal activity, others discharge earlier, and some are only active during the interburst interval of the motor rhythm. Consequently, values for the phase position within the motor rhythm for the first spike of a burst range from 0 to 1 for different group III interneurons. However, most are maximally active just before the mononeurons discharge, as can be seen from the distribution of the modes of the phase histograms (figure 5*d*) derived from many units.

When the cycle period of the motor output is steady, the pattern of firing of group III neurons is also fairly constant. The pattern changes, however, if the motor rhythm alters. The burst duration of a group III interneuron usually varies linearly with the cycle period ($r > 0.9$ for most units) and its firing frequency increases linearly with locomotor frequency (figure 6*a*). Some units discharge as fast as 80 impulses per second (imp s^{-1}) at short cycle periods. When the mean impulse interval per burst is plotted against the cycle period, it is seen that for different neurons the slopes of the linear relationships that are obtained may differ considerably (figure 6*b*); some units change the impulse interval little as the cycle period shortens, which means that they fire fewer spikes in each cycle due to the reduction in burst duration, whereas others shorten the impulse interval markedly and consequently fire a relatively constant number of spikes in each burst. The average firing frequency of a group III neuron is linearly related to the maximum height of the integrated motor nerve neurogram (figure 6*c*) with neurogram activity linearly increasing with locomotor frequency (figure 6*d*). The phase position of its impulses within the motor rhythm usually remains unaltered when locomotor frequency and neurogram height change. Only a small minority of the group III units failed to be recruited at longer cycles with low amplitude of motor activity; nearly all units remained active over the whole range of locomotor frequencies that they were recorded.

Occasionally, we have recorded from a neuron whose activity did not fit any of the three categories described above. Such neurons were irregularly active and, according to their phase histograms, were not directly related to the motor output.

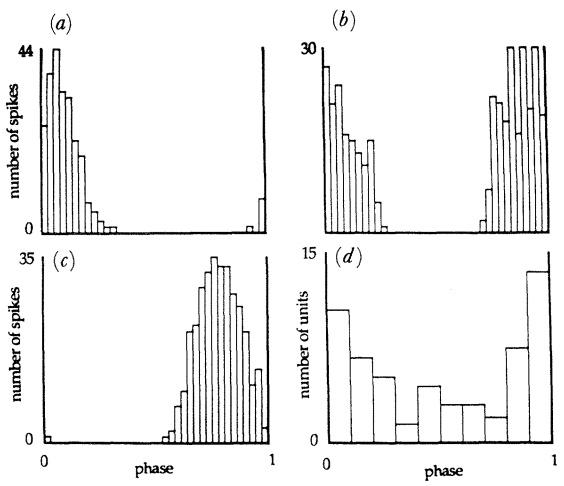

Figure 5. (*a–c*) Examples of phase histograms of three different group III interneurons recorded from the same fish at different times. The neurons begin to discharge at different moments during the motor nerve output. (*d*) The distribution of the modes of phase histograms of 77 group III interneurons, recorded from 21 different fish. Note that the majority of these modes is distributed about a phase value of 0, which is the beginning of the motor nerve discharge.

4. DISCUSSION

(*a*) *Interneuronal activity during fictive swimming*

We have separated the active interneurons recorded from the paralysed spinal dogfish preparation into three broad groups, on the basis of their firing patterns during fictive swimming. These divisions do not necessarily contain neurons that have distinctive morphological and physiological properties. Indeed, from what is known of other preparations, we would expect each of these groups to be heterogeneous. In the lamprey, for example, rhythmically active premotoneurons can be further subdivided into four classes (Buchanan 1986). Furthermore in the dogfish, some units (less than 5% of the cells we recorded) change their behaviour from one group to another as the frequency of the motor rhythm changes (e.g. figure 3*c*). Group I neurons, distinguished by their very regular firing pattern, maybe stand apart and perhaps constitute a distinctive identifiable neuronal class. These neurons, in contrast to most of the other cells that are active during unstimulated fictive swimming, remain active when motor output ceases and, in conformity with this finding, they have been observed in the spinal cord of the decerebrate dogfish (Paul & Roberts 1990) where spontaneous motor activity is absent. It seems unlikely, therefore, that these neurons contribute to motor pattern generation, although they may be important in sustaining the excitability of the spinal cord. Perhaps they provide tonic drive to the group II motoneurons (see Mos *et al.* 1990).

The activity of the other neuronal groups is highly correlated with the motor output. Most group III units become active prior to motoneuronal activity and so

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

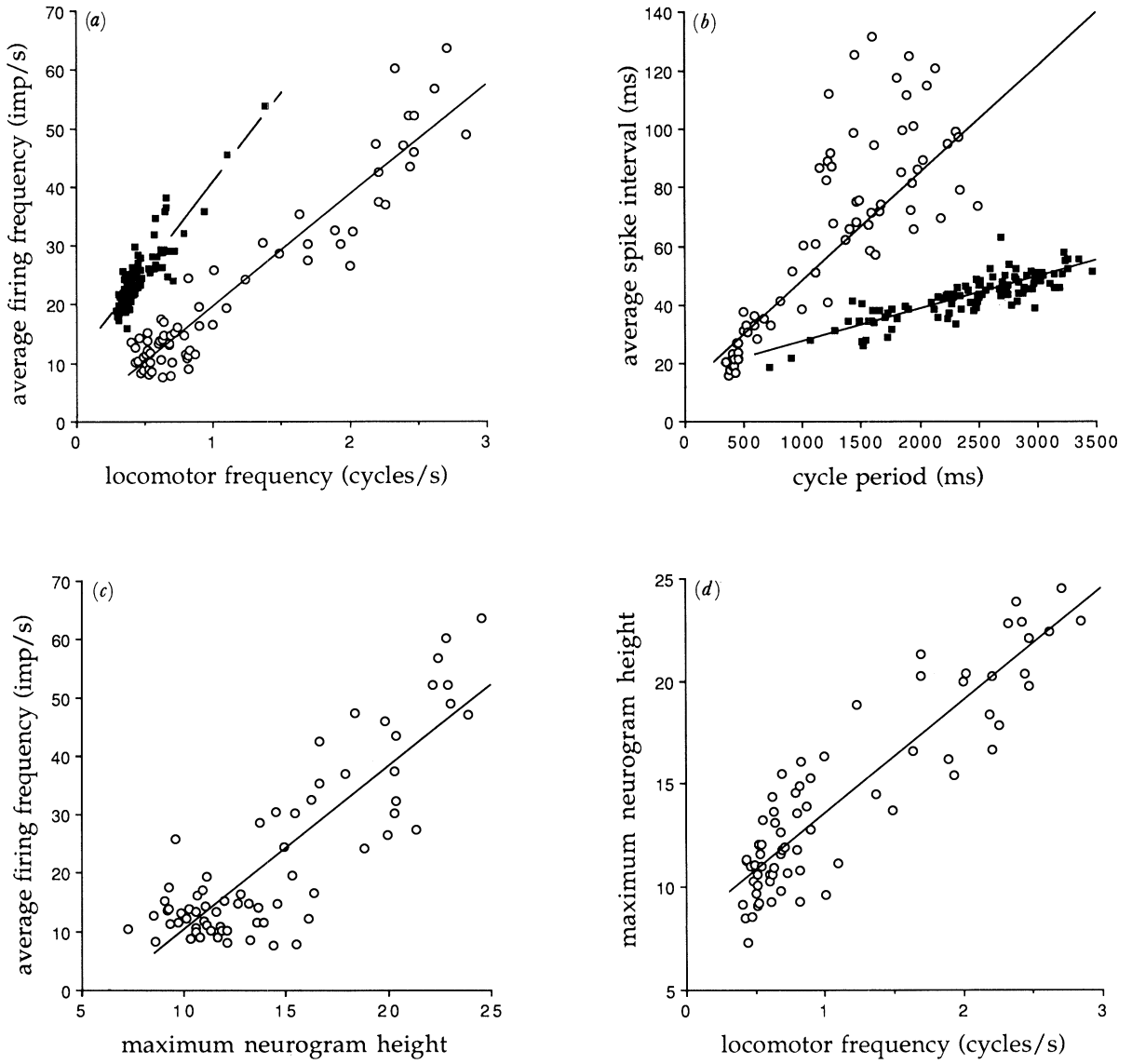

Figure 6. Changes in firing pattern of group III interneurons in relation to spontaneous changes in the locomotor frequency. (*a*) Average firing frequency during rhythmic bursts of two neurons plotted against locomotor frequency. Filled squares ($r = 0.89$) and open circles ($r = 0.95$) represent values for the top and bottom neuron, respectively, shown in figure 4*a*(ii). (*b*) Average impulse interval of the same two neurons as in (*a*) plotted against the instantaneous cycle period ($r = 0.85$ and 0.82, respectively). (*c*) Average firing frequency of the bottom neuron of figure 4*a*(ii) plotted against maximum height of the integrated neurogram (in arbitrary units) showing a linear correlation ($r = 0.85$). (*d*) Change of the maximum neurogram height with locomotor frequency for the same cycles as in (*c*) ($r = 0.90$).

this group may include premotor interneurons. During changing motor activity they show the same behaviour as the motoneurons they may be controlling. As their firing frequency increases, so does the amplitude of the motor nerve neurogram, and, thus, motoneuron firing frequency (see Mos *et al.* 1990). Further, just like motoneurons, they shorten the burst duration linearly with cycle period.

The linearities observed in the chain of events from motoneuron depolarization, through motoneuron firing to neurogram activity (see Mos *et al.* 1990), and the linear relation between interneuron firing and neurogram activity described in this paper, suggest a simple linear transfer function between premotor interneuronal and motoneuronal firing over a certain range. The fact that the linear increase of neurogram height with firing frequency was observed for all group III interneurons that we examined, even though they fired at a variety of phase positions in the cycle and, therefore, probably serve different functions, suggests further that: (i) between interneurons the transfer functions for firing are also highly linear, and (ii) the common input to the active motoneurons (see Mos *et al.* 1990) originates at an early point in the generator circuitry.

During periods of steady motor output, group II interneurons fire faster as the locomotor frequency is higher (figure 3). However, sudden changes in the locomotor frequency are not always mirrored by equivalent instantaneous changes in the firing frequency of these neurons, such as is very obvious in group III, but are rather reflected in a gradual shift in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

firing that takes place over a period of several cycles before the appropriate new firing level is reached. Such neurons not only might play a role in rhythm generation but also could establish a stabilizing influence on the motor output.

It is probable that most neurons in the cord of the paralysed spinal dogfish preparation are inactive in the absence of sensory and supraspinal input; what is their function? Some of these units are the descending axons of brainstem neurons because they discharge individual spikes to high spinal cord stimulation. They do not contribute to the fictive locomotion, although they are the route whereby the brain controls the cord. Others are interneurons resident within the cord that discharge multiple spikes in response to cord and sensory nerve stimulation. Their task might be to initiate activity in the motoneurons that innervate the white musculature, or to drive the associated interneurons. As described in the preceding paper (Mos *et al.* 1990), the motoneurons of this muscle system are probably quiescent in the unstimulated paralysed spinal preparation but may become active at times of 'noxious' stimulation, such as when the tail is pinched hard.

(*b*) *The interneuronal network underlying fictive swimming*

In this study we have recorded from many interneurons at positions throughout the entire ipsilateral half of the spinal cord segment that gave rise to the motor nerve from which fictive swimming was recorded, but it may well be that small categories of neurons that are important for generating, maintaining and modulating fictive swimming were missed. Nevertheless, the firing patterns of the three groups of interneurons that we have distinguished can give some idea about the operation of the spinal network. First, the increasingly strong correlation of interneuronal firing with motor behaviour from group I to group III units is suggestive of a hierarchical arrangement between the groups, and the neurons within a group, which leads to a gradual narrowing of the firing patterns towards that produced by the motoneurons. Secondly, in the unstimulated fish it was rare for an active neuron to stop firing when the rhythm of fictive swimming slowed down or for an inactive neuron to become active at short cycles. This could mean that virtually a constant population of interneurons is involved in unstimulated fictive swimming. Thirdly, when fictive swimming temporarily stops, a condition that we could always obtain by applying a strong tail pinch, all activity in group III and most of the activity in group II ceases, but returns immediately before the resumption of the motor activity. This observation also suggests that these neurons are organized in a network that behaves as an entity that is either active or quiescent. Fourthly, no interneuron was observed that increased its activity when the cycle period of the swimming rhythm lengthened. This suggests that a decrease in the level of motor activity is caused by a decrease in excitation rather than by an increase in inhibition within the generating interneuronal circuitry.

These are all general comments on the operation of the network. For a description of the network's structure and to understand in detail the differences between the neurons of each group in, for instance, the degree of modulation of the firing during fictive swimming and the phase position of the spike activity in the swimming cycle, it is necessary to combine electrophysiological with morphological identification of the neurons. Our preliminary results from intracellular horseradish peroxidase-labelling revealed that many neurons of all three groups are not resident in the spinal cord segment where the recordings were made, but possess a non-branching axon that runs unilaterally along two or more spinal cord segments. Such neurons belonging to group I would maintain a constant level of neuronal excitability over a longer stretch of spinal cord, whereas those of groups II and III might provide intersegmental coordination.

We are grateful to the Director and staff of the Laboratory of the Marine Biological Association, Plymouth, England, for their help during these experiments. We also thank A. J. A. Juta for the computer program for spike-train analysis and Dr W. van Raamsdonk for his helpful comments on the manuscript. R.W. was supported by a grant from the Wellcome Trust; W.M. was also supported by the Wellcome Trust, and by the British Council.

REFERENCES

Berkinblit, M. B., Deliagina, T. G., Feldman, A. G., Gelfand, I. M. & Orlovsky, G. N. 1978 Generation of scratching. I. Activity of spinal interneurons during scratching. *J. Neurophysiol.* **41**, 1040–1057.

Bone, Q. 1966 On the function of the two types of myotomal muscle fibre in elasmobranch fish. *J. mar. biol. Ass. U.K.* **46**, 321–349.

Bone, Q., Kiceniuk, J. & Jones, D. R. 1978 On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *Fish. Bull.* **76**, 691–699.

Buchanan, J. T. 1986 Premotor interneurons in the lamprey spinal cord: morphology, synaptic interactions and activities during fictive swimming. In *Neurobiology of vertebrate locomotion* (ed. S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg & R. M. Herman), pp. 321–333. London: Macmillan.

Deliagina, T. G., Orlovsky, G. N. & Pavlova, G. A. 1983 The capacity for generation of rhythmic oscillations is distributed in the lumbosacral spinal cord of the cat. *Expl Brain Res.* **53**, 81–90.

Edgley, S. A. & Jankowska, E. 1987 An interneuronal relay for group I and II muscle afferents in the midlumbar segments of the cat spinal cord. *J. Physiol.* **389**, 647–674.

Getting, P. 1983 Mechanisms of pattern generation underlying swimming in Tritionia. II. Network reconstruction. *J. Neurophysiol.* **49**, 1017–1035.

Grillner, S., Brodin, L., Sigvardt, K. & Dale, N. 1986 On the spinal network generating locomotion in lamprey: transmitters, membrane properties and circuitry. In *Neurobiology of vertebrate locomotion* (ed. S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg & R. M. Herman), pp. 335–352. London: Macmillan.

Grillner, S., Perret, C. & Zangger, P. 1976 Central generation of locomotion in the spinal dogfish. *Brain Res.* **109**, 255–269.

Johnston, I. A., Davison, W. & Goldspink, G. 1977 Energy metabolism of carp swimming muscles. *J. comp. Physiol.* **114**, 203–216.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Leeuwen, J. L. van, Lankheet, M. J. M., Akster, H. A. & Osse, J. W. M. 1990 Function of red axial muscles of carp (*Cyprinus carpio* L.): recruitment and normalized power output during swimming in different modes. *J. Zool. Lond.* **220**, 123–145.

Mos, W. & Williamson, R. 1986 A quantitative analysis of the spinal motor pool and its target muscle during growth in the dogfish, *Scyliorhinus canicula*. *J. comp. Neurol.* **248**, 431–440.

Mos, W., Roberts, B. L. & Williamson, R. 1990 Activity patterns of motoneurons in the spinal dogfish in relation to changing fictive locomotion. *Phil. Trans. R. Soc Lond.* B **330**, 329–339. (Previous paper.)

Paul, D. H. & Roberts, B. L. 1990 Spinal neuronal activity during the pectoral fin reflex of the dogfish: pathways for reflex generation and cerebellar control. *J. exp. Biol.* **148**, 403–414.

Roberts, A., Soffe, S. R. & Dale, N. 1986 Spinal interneurons and swimming in frog embryos. In *Neurobiology of vertebrate locomotion* (ed. S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg & R. M. Herman), pp. 279–306. London: Macmillan.

Roberts, B. L. 1969 The spinal nerves of the dogfish (*Scyliorhinus*). *J. mar. biol. Ass. UK.* **49**, 51–75.

Rome, L. C., Loughna, P. T. & Goldspink, G. 1984 Muscle fiber activity in carp as a function of swimming speed and muscle temperature. *Am. J. Physiol.* **247**, R272–R279.

Shefchyk, S., McCrea, D., Kriellaars, D., Fortier, P. & Jordan, L. 1990 Activity of interneurons within the L4 spinal segment of the cat during brainstem-evoked fictive locomotion. *Expl Brain Res.* **80**, 290–295.

Weeks, J. C. & Kristan, W. R. 1978 Initiation, maintenance and modulation of swimming in medicinal leeches by the activity of a single neurone. *J. exp. Biol.* **77**, 71–88.

Williamson, R. M. & Roberts, B. L. 1980 The timing of motoneuronal activity in the swimming spinal dogfish. *Proc. R. Soc. Lond.* B **211**, 119–133.

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

(*Received 12 April 1990; revised 13 August 1990; accepted 10 September*)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES