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Phil. Trans. R. Soc. Lond. B 1990 **330**, 329-339
doi: 10.1098/rstb.1990.0203

Activity patterns of motoneurons in the spinal dogfish in relation to changing fictive locomotion

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SUMMARY

Rhythmic motoneuronal activity was recorded from segmental motor nerves of moving (spinal swimming) and paralysed (fictive swimming) spinal dogfish (*Scyliorhinus canicula*), and, in the paralysed preparation, microelectrode recordings were made from spinal cord motoneurons.

The motoneurons could be divided into two groups, according to their activity patterns. Group I ($n = 31$) were inactive during fictive swimming and did not respond to gentle tactile stimulation; when recorded from intracellularly they showed stable to weakly oscillating (< 1 mV) membrane potentials. Group II ($n = 15$) fired bursts of action potentials in phase with the motor nerve activity, which were superimposed upon larger (up to 17 mV) depolarizations, and responded to gentle tactile stimulation. Two of these cells discharged also in the interburst interval of the nerve activity.

Decreases in cycle period of the fictive swimming (i.e. increases in locomotor frequency) were instantaneously accompanied by increases in the amplitude of the rectified and integrated motor nerve signal, which represents peak activity of group II motoneurons, and decreases in the duration of the motor burst. Similar instantaneous changes were seen in the firing frequency and burst duration of individual group II motoneurons. The conformity between unit and population behaviour with changing speed of fictive swimming, and the close correspondence observed between the form of the excitatory postsynaptic potentials recorded from individual motoneurons and the form of the integrated neurogram, suggest that the group II motoneurons receive a common excitatory drive. Re- and decruitment of motoneurons were virtually absent during these changes of speed.

During unstimulated spinal swimming, regular left–right alternating EMG activity is recorded from the red but not from the white part of the myotome. The ratio of group I to group II motoneurons (31:15) recorded in this study agrees with the previously reported proportion of axons in the spinal motor nerve that project to the white and red muscle fibres, respectively. We suggest, therefore, that group II motoneurons innervate the red and superficial muscle fibres and group I the white fibres. The different activity patterns of the two motoneuronal groups in the spinal fish probably reflect the different ways the red and white muscle systems are used during locomotion.

1. INTRODUCTION

The swimming movements of fish result from the contraction of the myotomal musculature that is composed of several muscle sets that can be distinguished by their location, colour, morphology, enzymatic profile and function (Bone 1966, 1978; Bone *et al.* 1986). The two major divisions, which are easily recognized because of their red and white colours, have properties that are suitable for different types of locomotion: the red musculature for regular sustained swimming and the white for abrupt short-lasting movement. The muscle divisions are innervated by populations of motoneurons that, on average, differ in location, size and enzymatic content (Fetcho 1986; Mos & Williamson 1986; van Raamsdonk *et al.* 1983, 1987), but whether these motoneuronal sets have different firing behaviours and are differently controlled is not well known. To study these questions we have examined the motoneuronal activity in paralysed and moving spinal dogfish. The dogfish was chosen because, as a spinal preparation, its spinal cord is sufficiently active to generate a regular motor output spontaneously (see Healey (1957) and Roberts & Williamson (1983) for literature).

In the subsequent paper (Mos *et al.* 1990) we expand this study to the firing patterns of spinal interneurons during fictive swimming. The general outcome of the two papers is that the motor pattern for regular swimming generated by the dogfish spinal cord, when it is isolated from descending input from the brain, is based upon the activity of a circumscribed set of interneurons and motoneurons. We will argue in this paper that these motoneurons innervate the red and superficial muscle fibres, whereas those that are inactive during fictive swimming innervate the white part of the myotome.

Phil. Trans. R. Soc. Lond. B (1990) **330**, 329–339
Printed in Great Britain

2. MATERIALS AND METHODS

(*a*) *Animal preparation*

The experiments were done at the Laboratory of the Marine Biological Association, Plymouth, England, on 31 dogfish, *Scyliorhinus canicula* L., of lengths 55–75 cm. Each fish was anaesthetized in a seawater solution of 0.2 g l^{-1} MS222 (Sandoz) and then surrounded by ice (Williamson & Roberts 1981). The fish was decerebrated and the hindbrain and rostral spinal cord were exposed. The spinal cord, in the region of segment 35, was revealed by removing the overlying dorsal vertebral cartilage, great care being taken not to damage the blood vessels enveloping the spinal cord. These blood vessels were usually distributed as a dense network that limited the cord surface available for microelectrode penetration. The spinal nerves of the exposed segments were dissected free of adjacent tissue and then split into the motor and sensory parts (Roberts 1969*a*) and cut peripherally. The nerve branch used for recording contained the majority of the motor axons that supply the red, and the lateral and ventral white muscle fibres, but not the dorsal white fibres; the axons of the red myotomal division constitute approximately one third of the total axonal population in that branch (Mos & Williamson 1986).

The fish was then placed in a tank with its head firmly held in a holder and aerated seawater was passed over the gills (figure 1*a*). The temperature of the seawater was normally 17 °C but could be varied over a 10° range by recirculating it through an exchange system. When the fish had recovered from the anaesthetic, the spinal cord was completely severed by cautery just caudal to the brain. The resulting spinal preparation then performed continuous undulating body movements (spinal swimming), similar to those of the intact fish.

For microelectrode studies, movement of the spinal preparation was prevented by injecting intravenously 7 mg kg^{-1} d-tubocurarine (Wellcome) to paralyse the musculature. This dose of curare has no observable influence on the spontaneous activity of the neurons (Williamson & Roberts 1980). The vertebral column in the region of the exposed spinal cord was then rigidly fixed to a supporting frame so that the body was lifted from the bottom of the tank. In this preparation, rhythmic activity was recorded from the spinal cord and nerves (fictive swimming).

(*b*) *Recordings*

In the swimming animal the activity of the red muscle was recorded with unipolar enamelled copper wires (0.2 mm diameter). Motoneuronal activity was monitored from the central stump of the nerve that was drawn into a suction electrode made from flexible polyvinyl tubing (Williamson & Roberts 1986).

In the paralysed animal (figure 1*a*), the motor nerve was placed over bipolar platinum wire electrodes so as to monitor the motor output of the segment. Intra- and extracellular recordings from motoneurons were made with glass microelectrodes filled with 3 M KCl (resistances 10–20 MΩ). Examples of records from motor nerves and motoneurons are given in figures 1*b*, *c*. Ipsilateral motoneurons were identified by their antidromic (constant latency) response to stimulation of the motor nerve. In addition to this, active motoneurons were shown to project into this nerve by averaging the nerve signal, by using the orthodromic spikes recorded with the microelectrode as triggers. The maximum distance between the stimulating electrodes and the entry of the nerve into the vertebral column was about 9 mm. Signals were amplified and stored on magnetic tape for later analysis. Motoneurons that were spontaneously active in the undisturbed fish will be indicated in this paper as MN followed by a number.

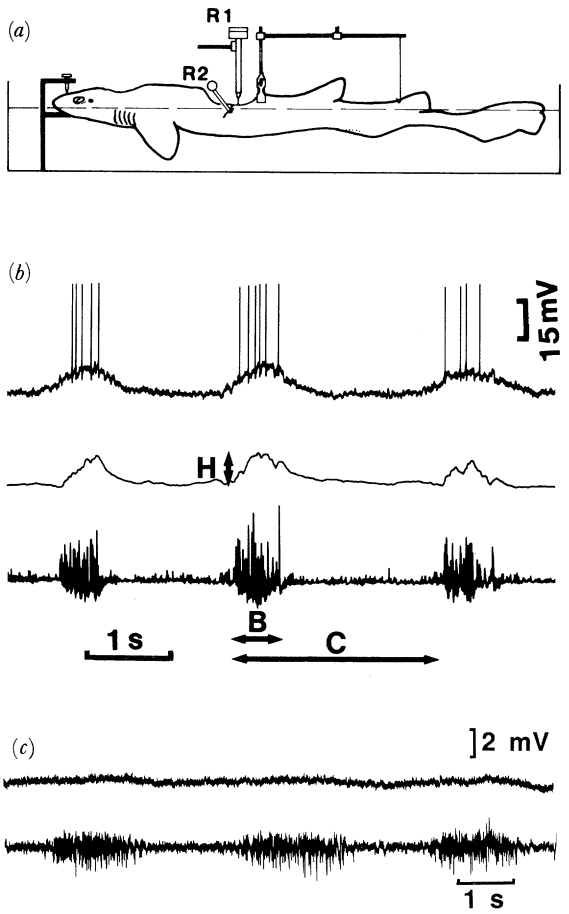

Figure 1. (*a*) Diagram of the decerebrate, spinal preparation, paralysed with curare, held in a head holder in a tank of sea-water. The vertebral column just caudal to the exposed spinal cord segment is clamped rigidly. R1, recording microelectrode inserted into spinal cord; R2, motor nerve of the spinal cord segment of R1 placed over hook electrodes for stimulation of and recording from motoneuron axons. (*b*) Example of the spontaneous activity of a motoneuron and the motor nerve of the same spinal cord segment. Top trace, intracellular microelectrode recording of motoneuron MN4; bottom trace, recording from the motor nerve; middle trace, the rectified and integrated nerve signal. How cycle period (C), burst duration (B) and maximum height of the rectified and integrated neurogram (H) are measured is shown. (*c*) Example of an intracellular recording from a silent motoneuron (upper trace) showing weak membrane potential oscillations in phase with the motor nerve activity (bottom trace).

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(*c*) *Analysis*

Samples of the nerve recordings were written out on paper and the cycle period, defined as the time elapsed between the onsets of two consecutive nerve discharges, and the duration of a nerve discharge were determined. Mean values were calculated from at least 200 cycles. Nerve signals were rectified and smoothed with a leaky integrator (time constant 0.1 s) to provide a measure of motor activity. The amplitude of the output signal of the integrator was linear with the size of the input signal as determined by applying different pre-amplifications to the same raw nerve signal and by systematically varying the size of electronically generated input signals. In the case of the transformed nerve signal the height will depend on how many units are simultaneously active and on their frequencies of firing. Of course, it will also depend on the recording conditions, which are different for different nerves and probably also for the same nerve during the course of an experiment. Therefore, comparisons were made only between integrated neurograms derived from a limited number of consecutive cycles. The height measurements made from these signals are given in arbitrary units which actually represent mm on the pen recordings of the signals.

In the example given in figure 1*b* the cycle period, burst duration and maximum height of the integrated neurogram are shown; the reciprocal of the cycle period measured in seconds is the locomotor frequency. The correlations between pairs of these parameters of the activity of the total motoneuronal population were determined, as well as their correlations with the following parameters for individual motoneurons: peak and average frequency of firing during a burst, duration of the discharge, number of spikes in a burst, and duration, amplitude and rate of rise of the membrane depolarization. For spike-train analysis, the microelectrode signal was passed through a window discriminator, which sent a 1 ms standard pulse to the computer (Motorola: Exorset 165) at each spike occurrence. The time resolution for spike intervals was 0.1 ms. Peak and average firing frequencies of motoneurons (i.e. the reciprocals of the shortest and the mean interspike intervals, respectively) always showed strong positive correlations with each other. Average firing frequency showed slightly higher correlations with the other parameters than did peak frequency and was therefore chosen to describe the firing levels of the motoneurons during changing conditions. Firing patterns were similar whether the data were from intracellular or extracellular recordings.

3. RESULTS

(*a*) *Motor output in swimming fish*

The spinal dogfishes used in these experiments performed continuous undulatory body movements of relatively constant form and frequency (usually 0.5–0.65 Hz, cycle periods 1500–2000 ms). In time with these movements, rhythmical bursts of activity were recorded from the motor nerves and, in conformity with the previous report by Bone (1966), from the lateral red, but not from the white musculature.

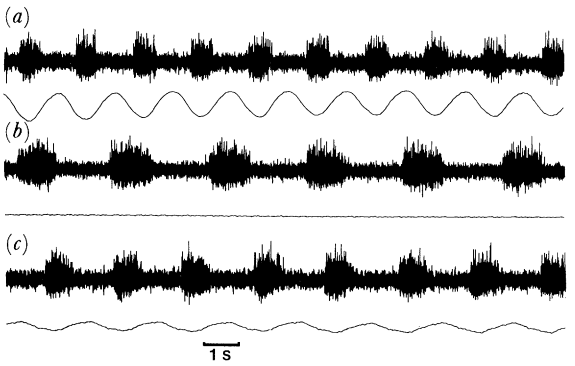

Figure 2. Motor activity of a spinal fish when it is not paralysed (*a*) (spinal swimming), and during complete (*b*) (fictive swimming), and partial (*c*) paralysis. Upper traces, motor nerve record; bottom traces, output from mechano-transducer showing the body movements of the fish. Paralysis slows the motor rhythm down but the duration of the nerve discharge remains the same proportion of the cycle and the intensity of the discharge is little changed.

Occasionally the frequency of these movements changed for a few cycles, sometimes spontaneously, but also in response to certain types of gentle stimulation (e.g. body stroke), giving cycle periods of longer or shorter duration. From these cycles it was seen that the bursts of activity recorded in the myogram and neurogram varied in amplitude approximately linearly with the locomotor frequency and that the burst duration was linearly related to the cycle period.

These relations between amplitude and duration of the motor activity, and the frequency and cycle period of the locomotory rhythm, were further investigated in paralysed animals. To be confident that the same population of motoneurons is active during spinal and fictive swimming, we analysed the spinal cord motor output at different levels of paralysis. Figure 2 shows an example of the motor activity of a fish before, and during complete and incomplete paralysis. Although the paralysis is seen to reduce the locomotor frequency with a small amount (from about 0.60 to 0.36 cycles per second), the amplitude of the neurogram does not change markedly and the duration of the motor burst remains the same proportion of the cycle period. This neurogram pattern is similar to that recorded from unparalysed fish when the spinal swimming slows down spontaneously. We, therefore, think that the same motoneurons are active in spinal and fictive swimming.

(*b*) *Motor output in paralysed fish*

All paralysed fish showed a rhythmical motor output from the spinal cord which appeared as burst discharges in the motor nerves. In most fish a low-level tonic activity was recorded between the rhythmic bursts (see the neurograms of figures 1*b*, 3*a* and 7). An example of a neurogram, together with the simultaneous extracellular recording of a motoneuron (MN8) from the same segment, is shown in figure 3*a*. Various parameters were determined from such recordings, as described in §2, and some examples are

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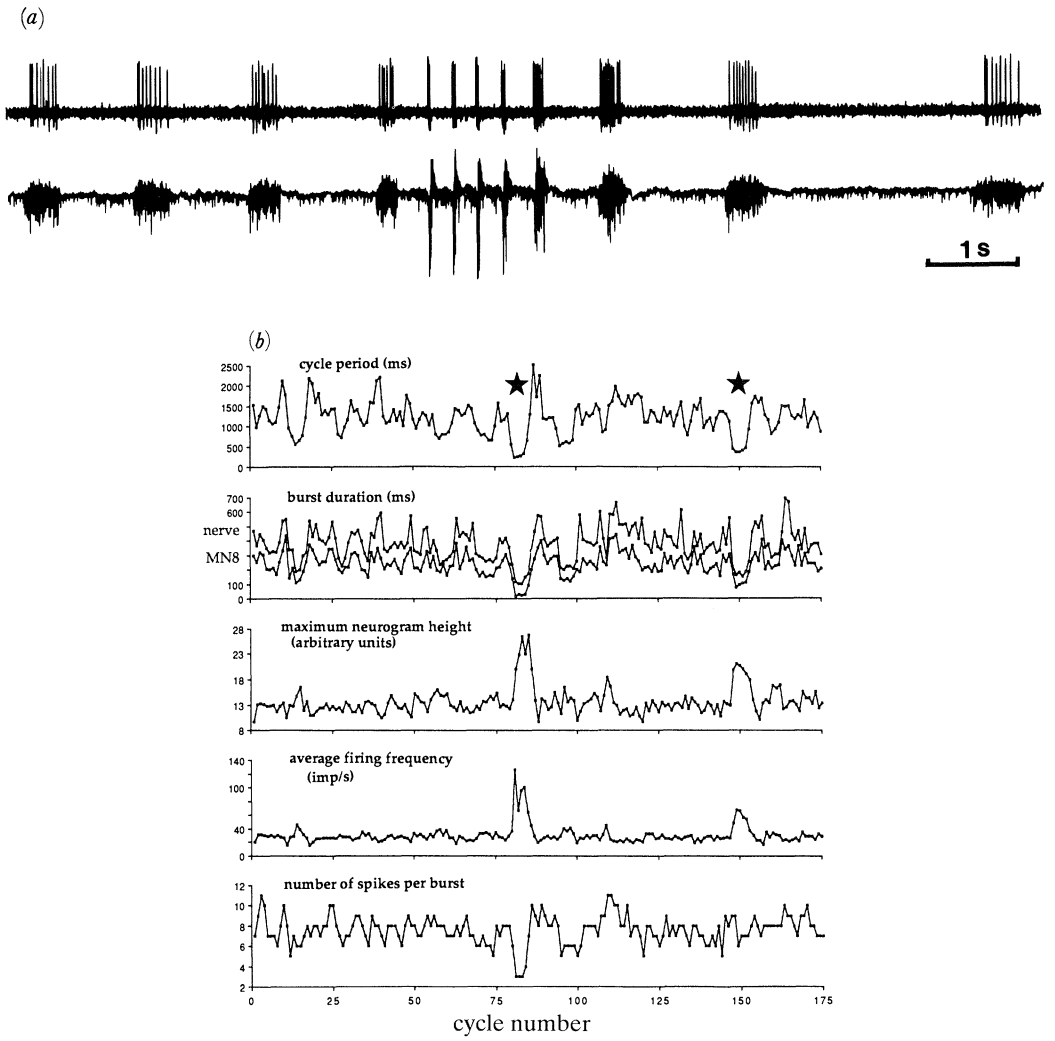

Figure 3. Activity of a group II motoneuron (MN8) during fictive swimming. (*a*) Top trace, extracellular microelectrode recording (spike size 15 mV); bottom trace, recording from the motor nerve of the same spinal cord segment. This episode was chosen from a longer period of recording, which is represented in (*b*), to show that during a rather steady motor pattern spontaneous accelerations of bursts may occur. (*b*) The cycle period, duration of the bursts of the nerve and MN8, maximum integrated neurogram height (in arbitrary units), average firing frequency, and number of spikes plotted against the number of the cycle for 175 consecutive cycles of unstimulated fictive swimming. Stars show bouts of fast cycles. Note the absence of systematic changes with cycle number, indicating stable recording conditions.

provided for MN8 in figure 3*b*, where cycle period, duration of the burst discharge and maximum height of the rectified and integrated neurogram, as well as average firing frequency and number of spikes for MN8, are plotted for 175 consecutive cycles.

The mean cycle period of fictive swimming, under undisturbed conditions, ranged from 1100–5100 ms for different fish. In many fish the motor rhythm was very constant, with a coefficient of variation of 3–5 % for the cycle period, but in others, larger variations in cycle period were observed (coefficients of variation of 10–40 %). The high values found in some of these fish relate to the presence of bouts of 3–7 much shorter cycles which recurred spontaneously every 50–100 cycles (figure 3*a* and * in figure 3*b*). During these brief episodes, the average cycle period could be as short as 250 ms.

Changes in the motor rhythm were accompanied by instantaneous changes in peak motoneuronal activity, as measured by the maximum height of the rectified and integrated neurogram (figure 3*b*), and in the duration of the motor burst. As reported previously for *Scyliorhinus* (Williamson & Roberts 1980), the burst duration was linearly related to the cycle period (correlation coefficients ranging from 0.58–0.88 in different fish). For each fish the average duration of the nerve burst was between 20 and 45 % of the average cycle period. The maximum neurogram height was linearly related to the locomotor frequency as shown in figure 4*a*. This figure shows a considerable scatter of the data points that are derived from individual cycles in a fish with a highly variable motor rhythm. The scatter was less when the locomotor frequency was manipulated experimentally in fish that showed otherwise very stable rhythms of fictive swimming (e.g. figure 4*b*). To obtain a range of locomotor frequencies in these fish, the temperature of the surrounding seawater was changed gradually (0.1 °C min^{-1}) be-

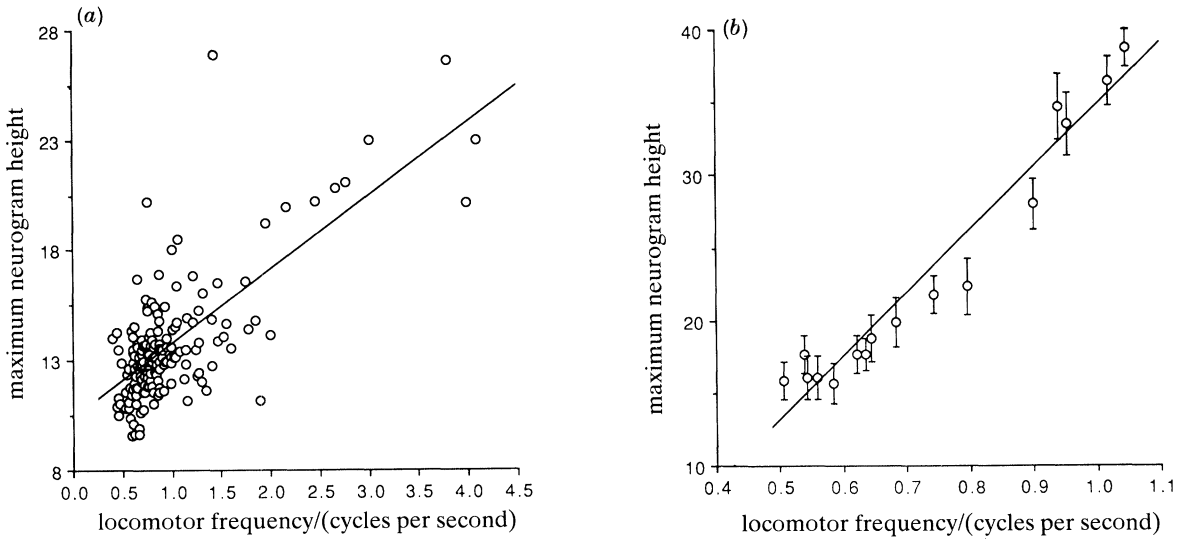

Figure 4. Relation between the level of nerve activity and the instantaneous frequency of fictive swimming in two different spinal fish. (*a*) Maximum integrated neurogram height (in arbitrary units) plotted against locomotor frequency, showing a linear correlation ($r = 0.72$). The data are from the series of cycles shown in figure 3*b*. (*b*) Change in maximum integrated neurogram height in a fish that showed fictive swimming at rather constant frequency that was gradually altered by slowly changing the temperature of the surrounding water. The values for neurogram activity are means ± s.d. of 50 cycles ($r = 0.97$). The different scales of the abscissa in (*a*) and (*b*) reflect the difference in the range of locomotor frequencies in the two fish.

tween 10 and 20 °C, the frequency diminishing as the temperature decreased.

(*c*) *Firing behaviour of individual motoneurons during fictive swimming*

The spinal motoneurons could be divided into two groups, designated I and II, on the basis of their activity in the unstimulated spinal, paralysed preparation. Recordings were made from 46 motoneurons, 31 of which were classed as group I because they did not show spike activity during fictive swimming. The other 15 motoneurons (group II) produced periodical bursts of action potentials in phase with the burst activity recorded from the motor nerve. Except for MN1 (see figure 7), all group II motoneurons were recruited in each cycle. Thus, no decruitment of a motoneuron was observed when the motor rhythm slowed down and no silent motoneuron, classified as belonging to group I, was recruited as the rhythm spontaneously or in response to gentle tactile stimulation accelerated. Thirteen group II motoneurons were inactive during the interburst intervals; two also discharged at a low frequency between the nerve bursts and may therefore belong to a separate motoneuronal class. These two were recorded extracellularly and so their interburst firing is not an artefact resulting from microelectrode penetration.

Antidromic latencies of the group II motoneurons varied between 0.2 and 1.5 ms as did those of group I. One group II motoneuron was intracellularly labelled with horseradish peroxidase (HRP) (microelectrode filled with 8% HRP). Its soma was found to be located at the ventrolateral edge of the ventral horn and its axon emerged from the medial dendrite and ran caudally over some 800 μm before entering the ventral rootlet where it left the spinal cord; its diameter at this point was about 2 μm.

The duration of the discharge of a group II motoneuron was linearly related to the duration of the discharge recorded from the motor nerve, except for the shortest nerve bursts where the discharges of some units were disproportionately brief. This extra reduction was associated with a decrease in the relatively constant number of spikes in a burst (see figure 3*b*).

The shortest interspike intervals for each group II motoneuron were usually found at the start of the burst or nearer to the middle, while the frequency of firing declined towards the end. The average firing frequency during a burst showed an approximately linear relation with the locomotor frequency (correlation coefficients from 0.72 to 0.91 for the 15 group II motoneurons). Figure 5*a* shows this relation for three motoneurons recorded from the same fish. These three motoneurons differed with respect to their firing levels. For instance, the average firing frequency at cycle periods of around 1000 ms ranged from 15 to 23 impulses per second (imp s^{-1}) for MN6; from 23 to 37 imp s^{-1} for MN8 and from 40 to 53 imp s^{-1} for MN7. One motoneuron, recorded from a different fish, fired at lower frequencies than did MN6 and one fired faster than MN7. The other motoneurons, all from different fish, had firing frequencies intermediate between those of MN6 and MN7. The highest frequency recorded from any group II motoneuron was 135 imp s^{-1} in a spontaneously occurring burst and 141 imp s^{-1} in a burst evoked by forceful tactile stimulation.

The average firing frequency of each group II motoneuron correlated positively with maximum integrated neurogram height (correlation coefficients 0.64–0.92 for the 15 group II motoneurons; figure 5*b*) as expected from the linear relations of firing frequency

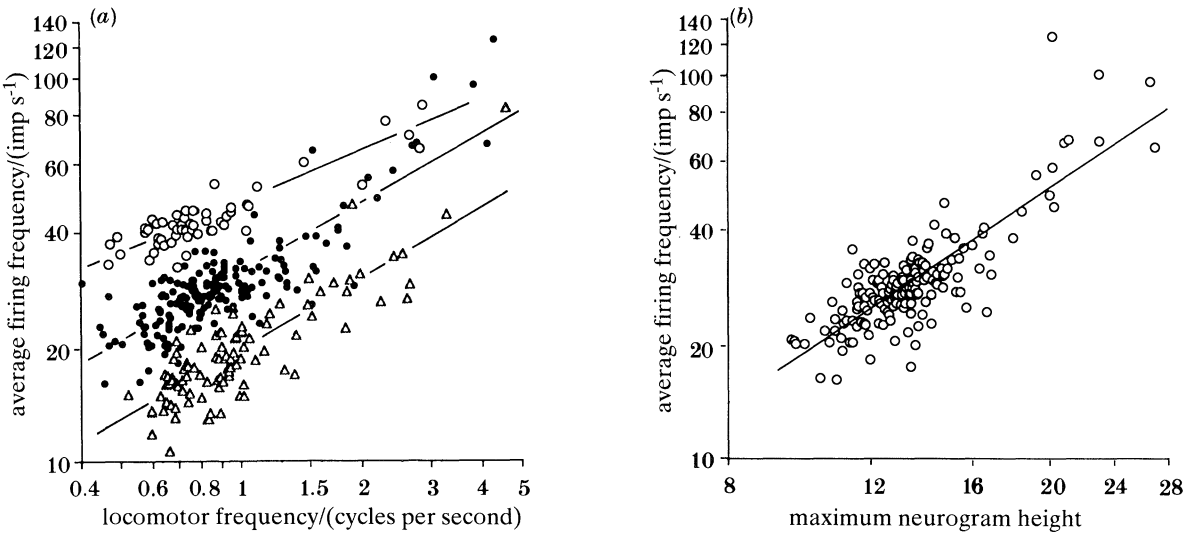

Figure 5. Average firing frequencies of three group II motoneurons, recorded from the same fish, plotted against locomotor frequency. The motoneurons increase their firing frequency linearly with locomotor frequency (MN6, $r = 0.88$; MN7, $r = 0.91$; MN8, $r = 0.89$) with approximately similar slopes but at any given locomotor frequency their rates of firing are different. (△, MN6; ○, MN7; ● MN8.) (*b*) Average firing frequency of MN8 in relation to maximum height of the rectified and integrated neurogram. The data are obtained from the 175 cycles of figure 3*b* and show a positive correlation ($r = 0.81$). Note that logarithmic scales are used in (*a*) and (*b*) for a better separation of the individual data points.

and neurogram height with locomotor frequency (figures 4 and 5*a*). A similar positive correlation was found when the data were selected from cycles of a narrow range of durations, so as to eliminate any influence of locomotor frequency upon this relation.

A marked difference between the motoneuronal groups existed in their responses to tactile stimulation. Group I units did not discharge in response to gentle cutaneous stimulation with a glass rod and only rarely to a strong tail pinch applied with forceps, whereas group II units readily changed their firing behaviour to such stimuli (figure 6*a*, *b*).

The same type of stimulus evoked similar motor responses in different fish. Thus, a tail pinch always evoked a strong motor burst in the nerve (sometimes several strong bursts alternating between the left and right body half) and in each group II motoneuron that was tested, followed by a period during which the motor rhythm ceased; after the fish were released from the pinch a variable amount of time (1 to tens of seconds) elapsed before the motor rhythm returned, simultaneously in the group II motoneuron and the nerve. Similarly, gently stroking the skin on different midline and ipsi- and contralateral positions along the body evoked characteristic responses that could be distinguished from the fictive swimming in the undisturbed fish by one or more of the following: the maximum height of the integrated neurogram was smaller or larger than according to the range found during unstimulated fictive swimming although the locomotor frequency stayed within the 'normal' range; the burst duration was shorter or longer than would be proportionate to the cycle period; and cessation of left–right alternating burst activity, with one side of the spinal segment showing a sustained motor output.

The changes in motor output seen during gentle stimulation did not alter the relation between the intensity of the neurogram activity and the firing frequency of the individual group II motoneuron, as is shown for one position of cutaneous stimulation in figure 6*c*. This suggests that the changes in the motor output are fully carried by the group II motoneurons and that no recruitment of motoneurons occurs during this type of stimulation, which is in line with the finding that group I units remain silent. The firing frequency of the individual group II motoneuron during a motor burst obtained by a tail pinch was at the low end of the frequency range that matches the size of the neurogram or might even be slightly below it (figure 6*c*), suggesting that some additional motoneurons are recruited. This fits well with our observation that some of the group I motoneurons fired in response to a tail pinch.

(*d*) *Membrane potentials of motoneurons during fictive swimming*

Intracellular recordings from group I motoneurons ($n = 18$) showed membrane potentials at −70 to −75 mV which were either stable or weakly oscillating (less than 1 mV) in phase with the motor rhythm (figure 1*c*). In contrast, the membrane potentials recorded from group II motoneurons ($n = 6$) were not below −60 to −65 mV and showed larger depolarizations, in phase with the discharges in the motor nerve, on which were superimposed bursts of action potentials (figure 7). The general shape of each phasic depolarization was very similar to that of the integrated neurogram of the concomitant nerve activity (figure 7). Thus, strong positive correlations were found between the rate of rise, the maximum height (figure 8*a*) and the duration of the phasic depolarization, and

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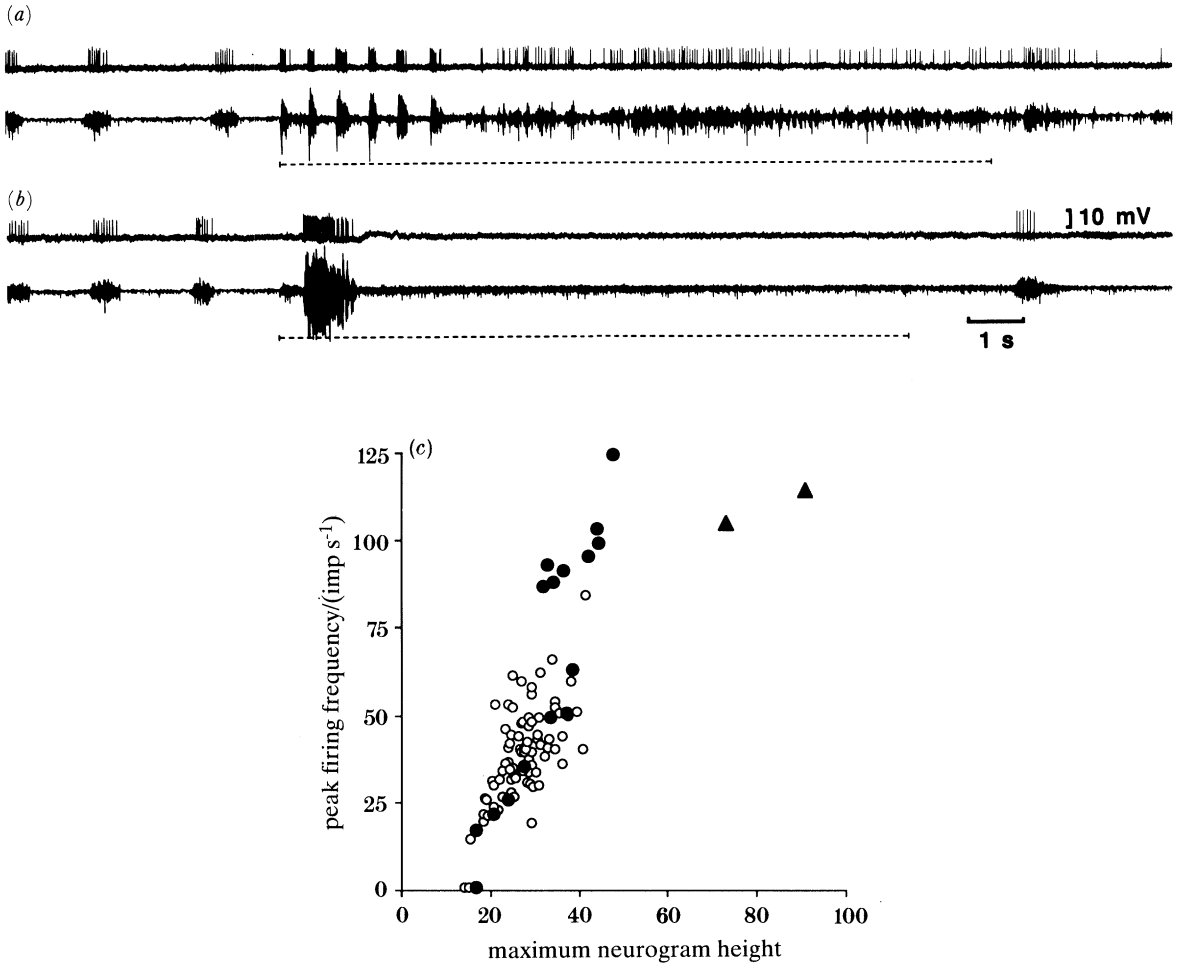

Figure 6. Changes in the activity of a group II motoneuron (MN8) (upper traces) and the motor nerve of the same segment (bottom traces) in response to gentle and strong tactile stimulation. (*a*) Gently stroking the skin at the level of the pectoral fin, ipsilateral and rostral to the site of recording, elicits a characteristic response which consists of a number of short swimming cycles followed by continuous activity, and inactivity in the contralateral nerve of this segment (not shown), as stimulation is continued. (*b*) A strong tail pinch evokes a massive discharge whereafter all regularly alternating motor activity has vanished. The motor rhythm returns with some delay when the tail pinch is ended, simultaneously in the motoneuron and the motor nerve. Broken lines in (*a*) and (*b*) show the stimulus period. (*c*) Peak firing frequency of MN8 plotted against maximum integrated neurogram height (in arbitrary units) during unstimulated fictive swimming (○) and during the two stimulations shown in (*a*) (●) and (*b*) (▲), each of which was repeated to increase the number of data points. The data of unstimulated swimming are from just before any stimulation was applied and from periods between stimulations during which the motor rhythm had returned to 'normal'. Peak rather than average frequency of firing was chosen for this plot to relate frequency to neurogram activity at a series of instants during the prolonged activity shown in (*a*). The firing frequency was arbitrarily set at 1 for cycles during which MN8 fired only once. The considerable increase in neurogram activity obtained after a tail pinch compared to the relatively moderate increase in firing frequency of MN8 suggests the possibility of recruitment of some group I motoneurons by this type of stimulation. Increases in neurogram activity in response to gentle stimulation can be fully explained by the increase in firing of group II motoneurons.

the corresponding features of the integrated neurogram. Moreover, it was striking that complex nerve discharges, with more than one peak of activity, could be associated with similar, multi-peaked depolarizations recorded from the motoneuron (arrows in figure 7*b*). The amplitude of phasic depolarization was strongly correlated to the maximum rate of depolarization ($r = 0.83$ for the cell shown in figure 7).

A linear relation was found between the amplitude of the phasic depolarization of a motoneuron and its average and peak firing frequency in a burst (figure 8*b*). Only one motoneuron was recorded intracellularly during a spontaneous bout of very fast cycles (i.e. locomotor frequency > 2 cycles per second). This neuron did not show a marked increase in the size of the depolarizations during this bout, although the firing frequencies were much higher than during normal cycles. This might suggest that the observed linear relation does not extend into the range of firing levels found at high locomotor frequencies but becomes steeper, a conclusion that is also arrived at when the depolarization-firing frequency relation is extrapolated to higher values. For instance, the relation of figure 8*b* extrapolated to 90 imp s^{-1} leads to a synaptic depolarization of around 40 mV, which seems quite unreal. The steepening of the relation at high firing levels

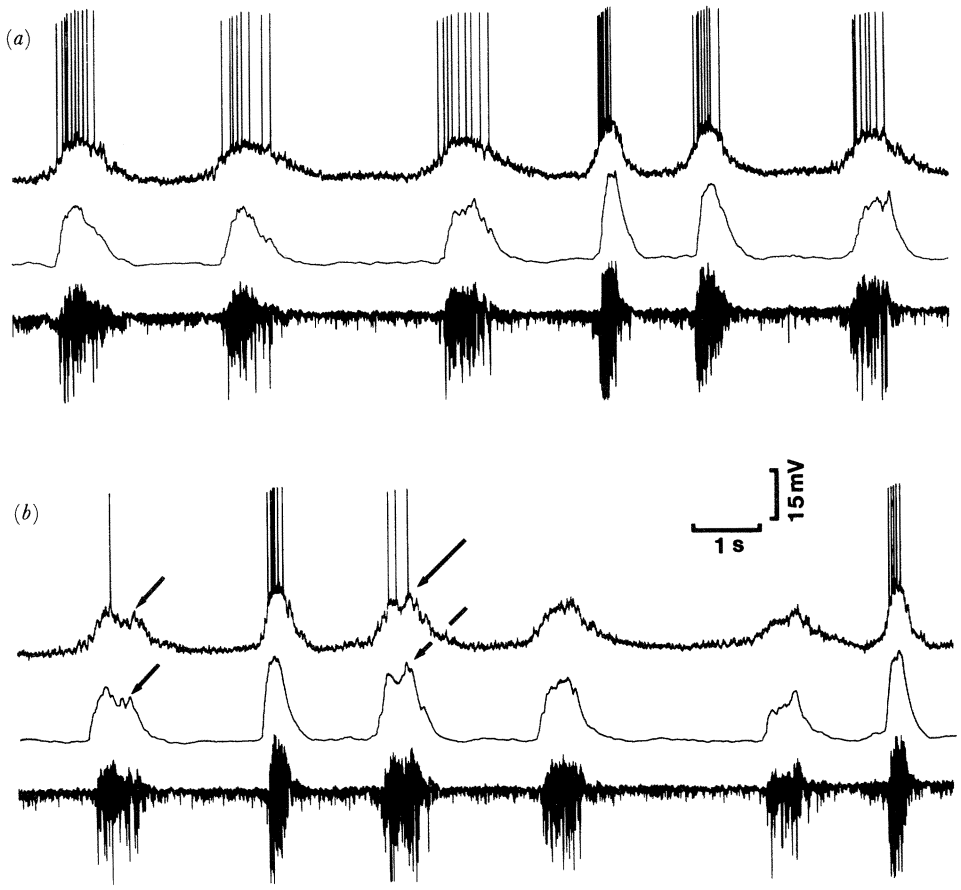

Figure 7. (*a*, *b*) Two examples from the intracellular recording from MN1 (top traces) and the motor nerve (bottom traces) during fictive swimming. The middle traces show the rectified and integrated nerve signals. In (*b*) two cycles are seen during which the excitation of MN1 is subthreshold for firing, a condition not met in the other group II motoneurons. During bursts with higher firing frequencies, spike generation is inactivated at membrane potential levels that are well above the recruitment threshold, possibly by inactivation of sodium channels. The shape of the depolarization corresponds with that of the burst discharge of the nerve. Arrows show where multiple peaks in the integrated nerve record resemble similar peaks in the membrane potential of MN1. Timescale applies to all traces; amplitude calibration applies to top traces.

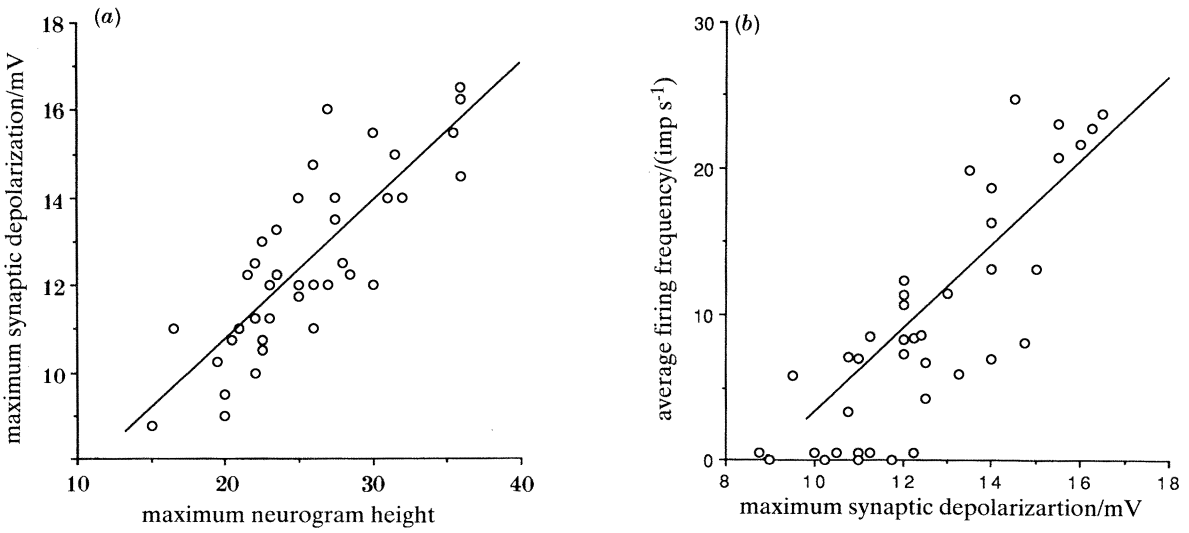

Figure 8. (*a*) Maximum synaptic depolarization of MN1 plotted against maximum height of the rectified and integrated neurogram, showing a linear relation ($r = 0.82$). (*b*) Average firing frequency of MN1 plotted against maximum synaptic depolarization. The average firing frequency was arbitrarily set at 0.5 or 0 for cycles during which MN1 fired only one spike or did not fire at all. The linear regression line applies to cycles during which at least two spikes were fired ($r = 0.79$).

could compare to the phenomenon of the 'secondary range of firing' of mammalian motoneurons described by Kernell (1965).

4. DISCUSSION

There are four main findings from this study that are significant for our understanding of motor coordination. The first is that the motoneuronal population supplying the myotome does not behave homogeneously, but can be divided into a set that is inactive during fictive swimming (group I motoneurons) and one that is active (group II). The second finding is that the firing frequencies and burst durations of the active motoneurons vary predictably in relation to the motor rhythm. Thirdly, there is a conformity between unit and set behaviour that suggests that the group II motoneurons receive a common excitatory drive. Finally, there is little sign of recruitment of motoneurons at higher speeds of fictive swimming and decruitment when the speed slows down, which means that the gradation of force exerted by the myotomal part that is innervated by the group II motoneurons takes place by alteration of the motoneuronal firing frequencies, rather than by a change of the number of 'motor units' active.

(*a*) *Division of the motoneuronal population*

We believe that the group II motoneurons innervate red muscle fibres, because electromyographic recordings have shown that during unstimulated spinal swimming the red muscle fibres are regularly active whereas the white fibres are not (Bone 1966). The lateral position and small axonal diameter of the group II motoneuron that was intracellularly labelled also suggest that it innervates red muscle, as application of HRP in the red muscle retrogradely labels lateral motoneurons with thin axons (Mos & Williamson 1986). However, such morphological data are not conclusive as the red and white muscle motoneurons overlap both in position and size. Finally, approximately one third of the axons in the motor nerve used in this study to monitor the spinal cord output projects to the red musculature (Mos & Williamson 1986), a proportion that agrees with the percentage of the motoneurons from which spontaneous activity was recorded. This would mean, assuming that our recordings are randomly taken from the motoneuronal population, that the silent motoneurons of our study innervate white muscle fibres.

The two motoneurons that fired continuously might be a group II subset and they would seem appropriate to innervate the superficial muscle fibres, which constitute a single fibre layer between the red fibres and the skin and possibly serve a postural role (Bone *et al.* 1986). Although the white muscle motoneurons are, on average, larger than red muscle motoneurons and have larger axons (Mos & Williamson 1986), we found no clear separation between the axonal conduction velocities of active and inactive motoneurons (range: 10–20 m s^{-1}). This may reflect, however, the difficulty of obtaining precise measurements of the short conduction distance.

The two groups of motoneuron have distinctive properties. First, the membrane potentials of group II motoneurons were less negative than those recorded from group I, perhaps suggesting that they were tonically depolarized. In *Xenopus* tadpoles, a tonic excitation has been observed in spinal motoneurons that discharge in fictive swimming (Soffe & Roberts 1982). This tonic excitation is lost when the tadpole stops swimming and, therefore, seems to be essential for the generation of a rhythmical motor output (Dale & Roberts 1984). Secondly, phasic membrane depolarizations larger than 1 mV were only recorded from group II motoneurons, but not from group I. Thirdly, group II motoneurons always responded to a gentle touch of the body or tail with a change in firing pattern, whereas inactive motoneurons remained silent. Indeed very few of the group I motoneurons could be made to discharge to tactile stimulation, and for these a prolonged forceful stimulus was needed. This contrasts with the situation in the non-paralysed swimming spinal dogfish where the white musculature is readily brought into action by strong cutaneous stimulation (*Scyliorhinus*: Bone 1966; *Squalus*: Grillner 1974), resulting in vigorous movements of large amplitude which, on occasion, may be more or less rhythmical (Bone 1966). In swimming fish, movement related sensory signals perhaps enhance the excitability of or gate the input to the white muscle motoneurons.

The finding that tactile stimulation mainly influences the activity of group II motoneurons conforms to the results obtained by Teräväinen & Rovainen (1971) in the lamprey. They used a non-paralysed spinal preparation in which movements were impaired mechanically. Stimulation of the skin of the body evoked activity only in the slow muscle fibres, but not in the fast. A strong stimulus applied to the tail also activated the slow fibres and, in addition, a few fast fibres.

Another activity related division of the lamprey spinal motor population was observed by Wallén *et al.* (1985). The lamprey spinal cord when separated from the brain does not show a rhythmic motor output but such output can be obtained by tonic excitation, for instance by bath-applied excitatory agents (Poon 1980). By using an excitatory amino acid to elicit fictive locomotion in the *in vitro* spinal cord, Wallén *et al.* (1985) found that the motoneurons supplying the ventral third of the myotome received a different drive from the interneuronal circuitry compared to those supplying the dorsal third of the myotome. Within each group, however, the inputs to simultaneously recorded motoneurons were very similar. Although these results cannot be directly compared with our findings in the dogfish, both studies show that the spinal motor population consists of subsets that differ with respect to their connections with intrinsic spinal cord interneuronal circuitry.

(*b*) *Motoneurons and muscle fibres*

The red muscle fibres of the dogfish, as of other

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fishes, are multiply innervated and do not propagate action potentials, but instead show local graded junctional potentials (Roberts 1969*b*; Stanfield 1972). When stimulated with pulse trains, fish red muscle fibres develop half maximum tetanic tension at activation frequencies between 20 and 50 Hz and attain 75% of maximum tetanic tension at about 80–125 Hz (*Scyliorhinus*, *Gadus*, *Tilapia*: Johnston 1982; *Cyprinus*: Granzier *et al*. 1983). These values correspond to the range of firing frequencies observed in our study. Individual group II motoneurons do not discharge at constant frequency, but reach maximum near to the start or at the middle of the burst, a pattern which matches that seen in the amplitude of junctional potentials recorded from red muscle fibres (Roberts 1969*b*).

The similar way in which the group II motoneurons behave when the rate of fictive swimming changes suggests that they are driven by the same set of interneurons. This conclusion is confirmed by the good correspondence observed between the form and height of the integrated neurogram, which we take to represent the population output, and membrane events and firing frequencies of individual group II motoneurons. Fluctuations in the activity of the motor nerve thus seem to reflect rather exactly the changes of firing of individual motoneurons and of their premotor interneurons. In their study of cat hindlimb motoneurons, Hoffer *et al*. (1987) concluded similarly that the fluctuations observed in the integrated myogram reflected ongoing changes in a central driving that is common to the motoneuron pool and so the whole-muscle electromyogram can provide a good measure of individual motoneuronal input.

(*c*) *Neuronal activity in fictive, spinal and natural swimming*

An important consideration in the present study is whether the sharp distinctions in activity and excitability observed between the two motoneuronal sets represent the situation in the intact swimming animal, or are a product of the experimental procedure. In mammals, for example, motoneuronal activity patterns do seem to differ in different experimental preparations. Hoffer *et al*. (1987) showed that the output pattern in the walking cat differs in essential respects from that observed in decerebrated cats during electrical stimulation of the brain stem, both when the cats are walking on a moving belt (Zajac & Young 1980) and when paralysed (Jordan 1983).

Comparisons between the motor outputs of paralysed and swimming spinal *Scyliorhinus* (Williamson & Roberts 1980, 1986) have shown that many features of the pattern of the motor program remain in the paralysed preparation. Similar conclusions have been obtained for the lamprey (Wallén & Williams 1984) and the stingray (Droge & Leonard 1983). However, motoneurons in the free-swimming fish are undoubtedly influenced by supraspinal and sensory systems and this input may modify motoneuronal behaviour. Thus white muscle motoneurons in *Scyliorhinus* might under these conditions contribute to regular locomotion. We do not know whether this is so because, unfortunately, all data about muscle activity of the dogfish have been derived from spinal preparations and no electromyographic recordings have been made from free-swimming animals. However, in the herring, which has a similar pattern of muscle innervation to the dogfish, that is multiple innervation of red and focal innervation of white muscle fibres, such recordings have shown a sharp distinction between the red musculature, used for slow swimming, and the white musculature, which is recruited only for rapid movements (Bone *et al*. 1978). Such a sharp division in the operation of the muscle systems of the intact dogfish could mean that the findings of this study, indicating a task group organization according to a 'red' and a 'white' motor system which are differently controlled, are not just an artefact produced by the spinalization of the fish.

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.

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