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MOTOR CONTROL OF TAIL SPINE ROTATION OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

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INTRODUCTION

Rotation is a striking movement of the tail spine of the horseshoe crab, *Limulus polyphemus*. Overturned animals use this movement to right their large bodies. Richter (1964, 1966) studied the righting behaviour, which consists of the following actions. Upon coming to rest on the dorsum after being upset, the animal deflects its tail spine ventrally. For some time it holds the tail spine over the venter and flexes the abdomen upon the prosoma. The animal next extends the abdomen relative to the prosoma and deflects the tail spine dorsally, slowly at first and then with increasing speed, through the vertical plane. At the end of this movement the body is arched between an edge of the prosoma and tip of the tail spine. The animal then rotates the body about the tail spine to one side or the other. During rotation of the body the tip of the tail spine sometimes loses contact with the substrate. When this occurs the arch of the body collapses and the animal rotates the tail spine opposite to the direction in which the body was moving until the tip touches once again. It repeats the arching and rotating actions until, with contributions from grasping and pushing legs and beating gills, it pivots over upon a side or upon the anterior edge of the prosoma, thereby turning itself right side up.

Rotation of the tail spine is of neurophysiological interest because of its bearing on the question of how motor control systems promote behaviour. This question requires consideration of the motor pattern and the muscle response, because behaviour is expressed through integration of the two (Horridge, 1968). The importance of muscle response to motor control has been brought out most clearly by Harold Atwood. His studies on the functional and structural properties of crustacean muscles has indicated the extent to which integration can occur peripherally. He has shown in crustacean neuromuscular systems that a great variety of behavioural movements is possible through activation at different frequencies and of different combinations of fast, slow and inhibitory axons to fast, intermediate and slow muscle fibres (Atwood, 1967).

The importance of motor patterns to motor control in determination of particular classes of movement has been shown by studies of rhythmic and non-rhythmic systems, for example, insect flight (Wilson, 1961) and the crab claw opener (Wilson & Davis, 1965) respectively. Nerve recordings from some of these systems, namely the crayfish abdomen flexor (Gillary & Kennedy, 1969*a*, *b*), the cockroach coxal levator

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and depressor (Pearson & Iles, 1970) and the dragonfly larva expiratory system (Mill, 1970), show distinct motoneurones firing at different times in the firing patterns. The consequent movement of these systems is an effect of the sequence in which each motoneurone fired in the pattern. Analysis of motoneurone sequence during natural movements together with the knowledge of the neuromuscular effects of the motoneurones should provide a more full understanding of the significance of the firing pattern.

In at least one behavioural system both issues of motor control have been examined, and effective relationships have been shown among motor pattern, muscle properties and behaviour. Gillary & Kennedy (1969*a*, *b*) demonstrated that the burst-firing pattern of the largest motoneurone innervating the superficial or tonic flexors of the crayfish abdomen facilitated the response of these slow muscles (Kennedy & Takeda, 1965*b*). Facilitation did not occur when the motoneurone was driven in a non-burst mode. Behaviourally, the muscle response to the burst firing pattern gave rise to rapid flexion or to strong flexion against a barrier (Larimer & Eggleston, 1971).

In approaching the question of motor control of the *Limulus* tail spine in this work, the integrative aspects of motor pattern and muscle response were of concern. Much attention was directed to the motor pattern, and events in the firing pattern were correlated with movement of the tail spine, muscle tension, and activity of the muscles in order to show how the output pattern generated rotation. Attention to muscle response was given through an investigation of neuromuscular and histological properties in order to determine the mechanisms and structures through which the output was expressed. Movement and use of the tail spine during behaviour and the arrangement and action of the tail spine muscles were also examined, because these properties specified the behavioural and functional limits of the system. The result of these considerations provides an explanation of a motor control system which uses many neurones to recruit functionally and structurally similar muscle fibres and excites them efficiently and in inverse sequences to rotate the tail spine appendage in two directions, clockwise and counterclockwise.

MATERIALS AND METHODS

Limulus polyphemus (L.) was collected locally throughout the year on nights of the full moon at the spring tide, when animals ascended beaches to mate as described by Teale (1957). Selected animals were fed on fish and squid and kept in running sea water until shortly before use when they were taken to a filtered and aerated sea-water pond in the laboratory. Average-sized males – that is, 15–19 cm across the carapace – were used in experiments, because females were too large and powerful.

Results are given of some single experiments, which were conducted slightly differently from others in the same group of experiments. For example, in a set of experiments in which firing and tension were examined rotation was evoked in one animal and recorded, and this result is presented (Text-fig. 11). Furthermore, the analysis of motoneurones that is presented in Text-figs. 7–9 and in Text-fig. 12 are from single experiments, because it was impossible to identify the same motoneurones in different animals. Nevertheless, analysis of motoneurones in separate experiments did not differ qualitatively.

Electrical recording. The experiments required extracellular recording of muscle and nerve potentials and in some cases intracellular recording from muscle fibres. Extracellular muscle potentials were recorded by means of paired copper-wire electrodes approximately 95 μm in diameter. These were inserted through holes drilled in the carapace above the desired muscle. The wires were insulated to within 1 mm of the tip. These recordings were taken while animals moved without restraint in a sea-water aquarium. In some experiments involving restrained animals muscle potentials were recorded by means of suction electrodes attached to the sides of muscle fibres.

Nerve potentials were recorded by means of long flexible polyethylene suction electrodes. About 5 in. of P.E. 60 tubing, into which a silver wire was inserted, was sufficiently flexible to stay on the nerve during the vigorous movements of the contracting muscles. Electrode tips were placed on branches of nerves which ran just beneath the surface of muscles. Muscles were exposed by cutting windows in the dorsal or ventral wall of the carapace. In order to reduce bleeding and clotting about exposed tissue blood was withdrawn from the heart or pericardium by hypodermic syringe until no more came with ease. The volume withdrawn was ordinarily 50 ml. This withdrawal reduced both the blood pressure and the amount of blood circulating through the muscles but did not impair the behaviour of the animals during the course of experiments lasting 2–6 h. In order to eliminate prosoma–opisthosoma flexion movement, which would have dislodged electrodes and moved the tail spine in and out of the plane of view of the motion picture camera, a plate was bolted across the joint of these two sections of the body. Animals were clamped rigidly in a sea-water bath which was sufficiently deep to allow the tail spine to rotate without hindrance.

In both the muscle and nerve recording experiments movements of the tail spine were filmed with a Beaulieu 16 mm cine camera at 25 frames/sec. A Texas Instruments LS 400 phototransistor placed in the viewfinder produced pulses on the oscilloscope between frames when light was deflected away from the shutter and through the viewfinder. Films were taken from the rear of the animal and examined from this direction so that descriptions of rotations, namely clockwise and counterclockwise, refer to a viewing position behind the animal.

Intracellular potentials were recorded by means of 3 M-KCl-filled glass capillaries of 4–14 MΩ impedance. Only about 1 cm of a capillary was used and this was suspended from a flexible silver wire as a 'floating' microelectrode. Signals were amplified by means of a Bioelectric NF 1 neutralized input-capacity amplifier. Most recordings were made in tail-spine muscles of abdomens which had been separated from the prosoma and therefore deprived of circulation. Potentials were evoked in muscle fibres of these preparations by tactile stimulation, which triggered output to the dorsal muscles, or by directly stimulating nerves by means of suction electrodes. The remainder of the recordings were made in muscles of whole animals.

Experiments on motor pattern and on neuromuscular properties were performed on each of the muscles. The results suggested that motor pattern and neuromuscular properties were the same in each of the muscles. Most of the experiments, however, were conducted on the dorsal lateral muscles because these were the most approachable, provided at least one long nerve branch for recording and permitted work with the animal in a position from which tail spine rotation was readily elicited.

Mechanical recording. Tension was recorded in some experiments. Recordings were made with a Grass Ft. 03 force transducer tied with a nylon monofilament to a muscle tendon, which had been severed at its insertion. This method was applied to muscles in isolated abdomens and in intact animals. In the latter the tail spine was cut very close to the base so that when it rotated it would not interfere with the thread connecting the tendon to the transducer, and the tendon was not severed at its insertion. In order to record force developed by the tail spine during rotation the end of the tail spine was tied to the tip of a rod which was fixed to the transducer so that the tip was suspended only a few millimeters above the substrate. When the animal was turned over the tail spine pushed down upon the rod as if it were the substrate and the force measured was assumed to be the same as the force generated against a natural substrate. The animals were sometimes prevented from righting and the additional force generated was accepted as the reserve force it could generate.

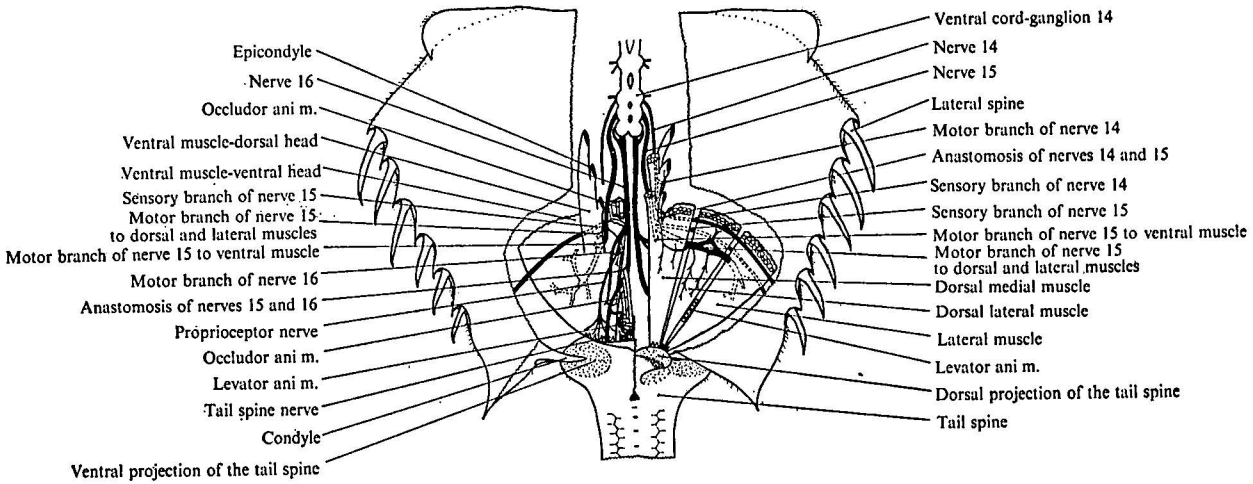
Histology. Nerve and muscle tissue from the dorsal lateral muscles was prepared for light microscopy after the method of Fourtner & Sherman (1972). Muscles were first relaxed in a 5·5 mM-$MgSO_4$ and 445 mM-NaCl solution. With the tail spine in its resting position tissue was then pre-fixed in glutaraldehyde, which was made 4% in 0·1 M sodium cacodylate buffer adjusted to pH 7·3 and osmotically adjusted with 2% NaCl and 0·2% $CaCl_2$. Tiny pieces of tissue were dissected out, and after washing in buffer post-fixed in 1% osmium tetroxide were washed, pre-stained in uranyl acetate, dehydrated in ethanol and transferred to propylene oxide before embedding in Araldite (6005). Thick sections were cut at 0·250 μm, stained with toluidene blue and examined with bright-field optics.

RESULTS

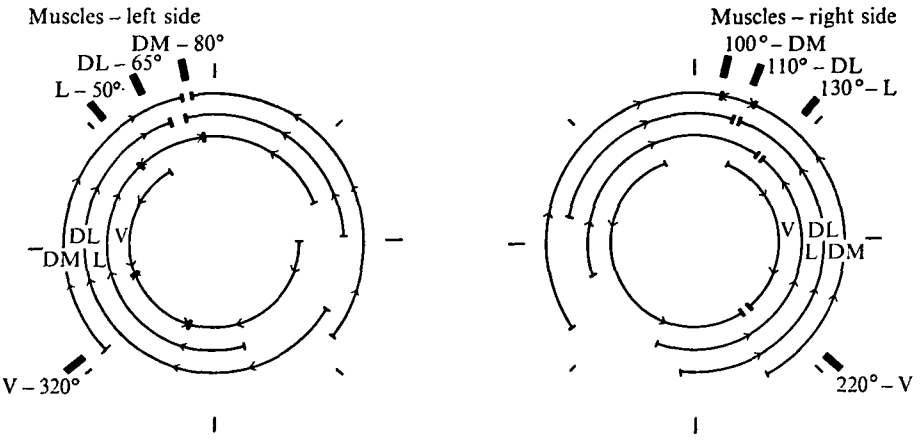
Functional morphology

Eight muscles move the tail spine of *Limulus*. These are housed in a chamber in the posterior of the abdomen and are symmetrically and adjacently arranged about the tail spine (Text-fig. 1 and inset diagram in Text-fig. 7). Patten & Redenbaugh (1899), who described the anatomy of *Limulus* in some detail, recognized only six muscles. Connective tissue, however, does separate the muscle mass into eight discrete muscles or bundles of muscle fibres. Occasionally, however, a few muscle fibres which originate with one muscle fuse or insert with an adjacent muscle. Patten and Redenbaugh designated the muscles as extensors and flexors but these terms do not accurately describe the action of the muscles. None of the muscles extends or straightens the tail spine relative to the longitudinal axis of the body yet all of them flex or deflect the tail spine relative to this axis. The action of each deflects the tail spine through some angle until the tail spine is perpendicular or nearly so to the normal resting position. The resting position is characterized by the tail spine pointing posteriorly and slightly ventrally and in a plane parallel to the vertical plane of the body. The angle through which any single muscle deflects the tail spine is given in Text-fig. 2.

The names dorsal medial, dorsal lateral, lateral and ventral have been given to the pairs of muscles relative to their position about the horizontal and vertical planes. The dorsal medial muscle, which corresponds to the telson extensor muscle *a* of Patten & Redenbaugh (1899), originates on the medial side of the last three abdominal epicondyles and on the underside of the dorsal wall of the muscle chamber. The dorsal



Text-fig. 1. Sketch of the nerves and muscles of the tail-spine apparatus from a dorsal aspect. The right side shows dorsal and lateral muscles beneath the wall of the carapace. The left side, a deeper view with dorsal and lateral muscles removed and dorsal projection of the tail spine cut away, shows a ventral muscle. Solid lines represent nerves running above or between muscles in the plane of view. Dashed lines represent nerves running beneath or within muscles. The eight tail-spine muscles are arranged symmetrically and contiguously (cf. upper inset in Text-fig. 7). The dorsal and lateral muscles insert on the dorsal projections of the tail spine, the ventral muscles on the ventral projections. The co-operative action of these muscles moves the tail spine about the condyles.



Text-fig. 2. Diagrams showing the angles to which individually contracted muscles deflect the tail spine and the arcs through which the major burst is active in each muscle during clockwise and counterclockwise rotation of the tail spine. The diagrams are drawn as if one were looking at the animal from behind. The isolated action of each muscle was elicited either by stimulation of the largest nerve innervating the muscle or by direct stimulation of the muscle. Rotations were produced centrally in whole animals in response to sensory input. The period of major burst activity in a given muscle was determined from several recordings of nerves innervating the muscle or of muscle potentials in the case of the left ventral muscle and was correlated with the arc through which the tail spine moved during this period. The most striking feature is that for each muscle the end-point of firing after rotation in either direction coincided closely with the angle to which the individual contraction of the same muscle deflected the tail spine. This point is indicated by the short, heavy bars at the end of arcs. During rotation there was no period of time when fewer than two muscles were active. DM, Dorsal medial; DL, dorsal lateral; L, lateral; and V, ventral muscles.

lateral and lateral muscles, which correspond to the telson extensor muscle *b* of Patten & Redenbaugh, originate on the underside of the dorsal wall of the muscle chamber and on its anterior wall. These three muscles insert on the dorsal projection of the tail spine (cf. right side of Text-fig. 1). The ventral muscle, which corresponds to the telson flexor muscle of Patten & Redenbaugh, originates by small heads on the lateral side of the last three abdominal epicondyles and on the lower portion of the anterior wall of the muscle chamber and inserts on the ventral projection of the tail spine (cf. left side of Text-fig. 1). Although the ventral muscle originates by dorsal heads, that is, by those on the epiconcyles (which are apodemes that project from the dorsal wall downward into the abdominal body cavity) as well as by a large ventral head, all of its force is directed upon the ventral projection.

Each of the eight muscles inserts on a tail-spine projection by more than one tendon. The dorsal lateral muscles, for example, insert by five or six tendons. Functionally, this arrangement allows the tail spine to be deflected to different angles by contraction of different portions of the muscle. Pulling on one and then the other of the most widely separated tendons of a dorsal lateral muscle deflects the tail spine to points separated by 5° of arc.

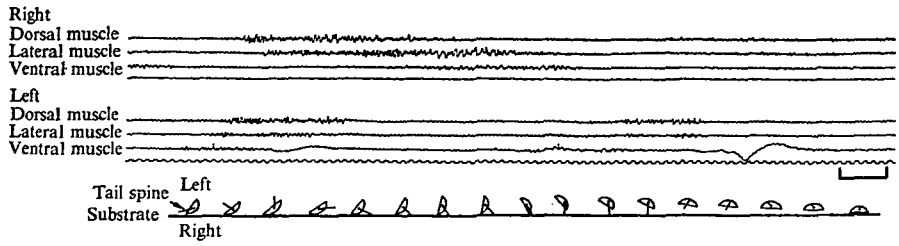
The tail spine articulates upon two condyles, which arise from the sides of the abdominal wall that forms the tail spine and anal foramen. The condyles project into spacious sockets, which exist on both sides of the head of the tail spine between dorsal

and ventral projections. Thin cuticle unites the condyles to the tail spine. The tail spine can swivel freely through 360° at its articulation. The tail spine can be maximally deflected to almost 90° in the vertical plane. However, as the tail spine is rotated toward the horizontal plane the angle of deflection is reduced to about 60° because the pointed, wing-like ends of the abdomen restrict lateral movement. This arrangement prevents the arc developed by the tip of a rotating, fully deflected tail spine from describing a perfect circle.

Three pairs of nerves innervate the tail-spine muscles. Nerve pairs arise from each of the last three ganglia of the ventral cord (Text-fig. 1). The nerves correspond to haemal nerves 14, 15 and 16 of Patten & Redenbaugh (1899) but are here called nerves 14, 15 and 16, because these are the only nerves from the last three ganglia involved with the tail-spine musculature. Nerve 14 innervates the dorsal medial muscle by a motor branch and the wall of the abdomen in the region of the last lateral spine by a sensory branch. Nerve 15 innervates all of the muscles by motor branches and also the wall of the abdomen posterior to the last lateral spine by a sensory branch. Nerve 16 innervates the medial portion of the ventral muscle by a motor branch. Nerve 16 also innervates a proprioceptor (Eagles, 1972, and personal observation) in the ventral muscles, muscles of the gut and anus and the tail spine. Anastomoses occur between motor branches of nerves 14 and 15 and nerves 15 and 16.

Rotation consists of a clockwise or counterclockwise turning of the tail spine about its point of articulation. Rotation starts with a deflexion of the tail spine through any plane perpendicular to the longitudinal axis at the point of articulation. The deflexion movement is usually turned into a rotational movement before the tail spine reaches the full extent of its deflexion. In nature, rotation occurs when the animal is turned over and starts the struggle which results in righting. Rotation also occurs in nature when the animal is tipped. In such cases the animal deflects its tail spine and swings it downward through the side of the body that is lower in order to bring this side up by the force of the moving tail spine. In the laboratory full rotations could be elicited when the animal was rigidly fixed, lifted above the substrate in order that the tail spine could revolve freely and stroked on the sides of the abdomen. Stroking on the right side evoked counterclockwise rotation and on the left side clockwise rotation. Persistent rotation required an intact central nervous system. However, following severance of the ventral cord as far posterior as ganglion 13, rotation could still be elicited by tactile stimulation of the abdomen but for only a brief period of time.

Righting requires the development of sufficient force to lift up the body and swivel it upon the tail spine. In some animals the force developed by the rotating tail spine approximated to the body weight in air, which was about 500 g for an average-sized male. The weight in water, however, as determined from weighings of several animals, was only 6·5 % of that in air. The average force developed by the rotating tail spine of four animals in the course of righting was 65 g, but more than twice this was generated when the tail spine was prevented from rotating. Thus, the animal is capable of developing sufficient force to lift itself upon its tail spine and to provide some additional force if it encounters resistance.



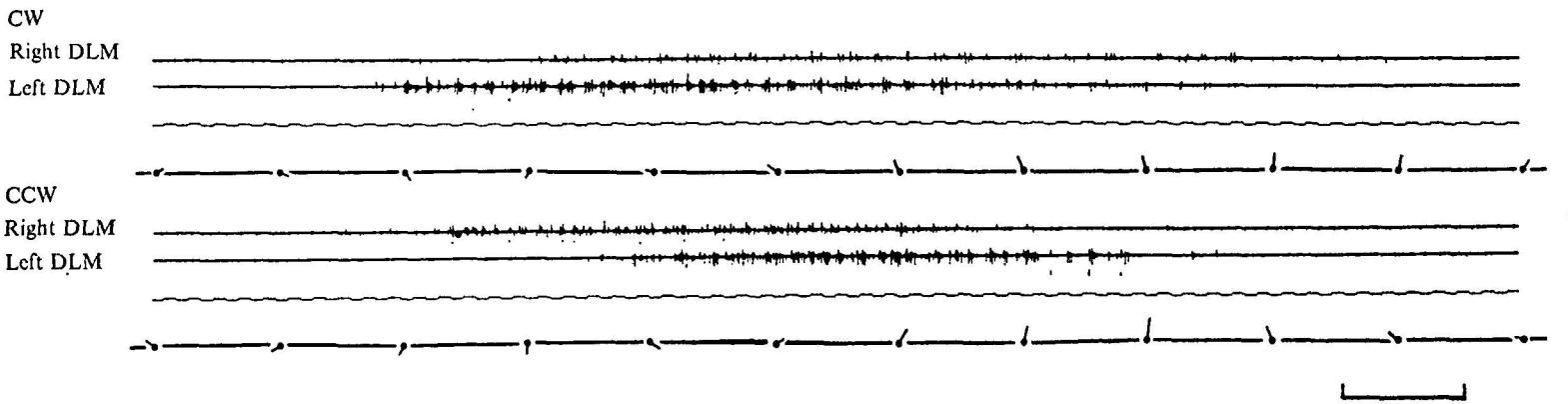
Text-fig. 3. Extracellular recording of muscle potentials from six muscles during righting of an unrestrained animal. The sketches below the records show movements of the animal as viewed from the rear and directions of movement are described from this viewpoint. The recordings are from two separated righting sequences but each consisted of approximately the same movements and took about the same time. The records have been placed together in order to show the sequential activation of each muscle during the production of rotation. The muscles fired in clockwise sequence, beginning with the left ventral muscle, and the tail spine was moved clockwise until it touched the substrate. At this point the body was moved counterclockwise until it left the substrate. From the point of leaving the substrate the tail spine was brought further around clockwise. The dorsal muscle is the dorsal medial muscle. The fourth and eighth traces monitored the frames filmed. Time: 200 msec.

The motor pattern

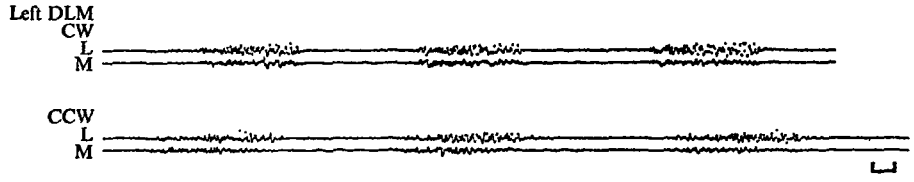
Rotation of the tail spine is produced when muscles contract sequentially. The records in Text-fig. 3 of potentials from three contralateral pairs of muscles during a righting manoeuvre indicate that this is the case. Just prior to the records shown the animal was turned ventral side up. At the start of the recording the animal rotated its tail spine clockwise until the spine touched and anchored. From that point on the body moved counterclockwise until the body left the substrate. The animal then settled and brought the tail spine further around clockwise. During this action muscles were recruited in a clockwise sequence. First the left ventral muscle fired, next the left lateral, left dorsal and then muscles on the right, dorsal, lateral, ventral and on around. Thus, sequential activation of the muscles in one direction leads to a rotation of the tail spine in the same direction or, when the tail spine becomes anchored, to rotation of the body in the opposite direction.

Sequential firing in the nerves produces the muscle activity, which is responsible for rotation. This is shown by recordings in Text-fig. 4 from a pair of contralateral nerves, one of which innervated the right dorsal lateral muscle, the other the left dorsal lateral muscle. In the upper record firing in the nerve to the left muscle preceded that in the nerve to the right muscle and the tail spine moved clockwise as indicated by the figures below the frame-counting trace. In the lower record firing to the right muscle preceded that to the left muscle and the tail spine was moved counterclockwise.

Activity also occurs in sequence within single muscles. Fibres in a muscle became active at different times during rotations as shown in Text-fig. 5. The order of firing to different fibres corresponded to the direction of rotation of the tail spine. The example in Text-fig. 5 is from the left dorsal lateral muscle and shows that lateral muscle fibres fired before medial fibres during clockwise rotation and vice versa during counterclockwise rotation. The changes in time between the beginning and the end of firing of one portion of the muscle relative to the beginning and end of firing of another portion of the same muscle during opposite rotations differed from 100 to 500 msec in



Text-fig. 4. Recordings from nerves to contralateral muscles during opposite rotations. The figures below the frame-counting trace indicate the position in rotation and relative degree of deflexion of the tail spine at 200 msec intervals. Firing to the left muscle preceded that to the right muscle for the generation of clockwise rotation; firing to the right preceded that to the left for counterclockwise rotation. This indicates that nervous activity occurs in opposite sequences to produce opposite rotations. Note that the angle of the tail spine at the end of firing to each muscle was approximately that to which the action of each muscle alone drew the tail spine as described in Text-fig. 2. This point at the end of firing of a nerve to a muscle also corresponded to the peak of tension developed by a muscle (Text-figs. 11, 12). Note also that the frequency and duration of firing were greater in a nerve when the muscle it innervated brought the tail spine upward on the side of the muscle than when the muscle brought the tail spine downward. For example, the left dorsal lateral muscle lifted the tail spine during clockwise rotation but carried it downward during counterclockwise rotation. The frequency and duration of firing were greater to this muscle during the former rotation than during the latter. This phenomenon suggests that the tail-spine mechanism is compensating for a load, and evidence for compensation is presented in greater detail in Table 2 for all rotations of the series from which these records were taken. Time: 200 msec. CW, clockwise; CCW, counterclockwise; DLM, dorsal lateral muscle.



Text-fig. 5. Muscle potentials recorded with a suction electrode from laterally and medially situated fibres in the same muscle, the left dorsal lateral muscle (DLM), during three clockwise and three counterclockwise rotations. During clockwise (CW) rotations the lateral fibres (L) fired slightly before the medial (M) but both stopped at about the same time. During counterclockwise (CCW) rotation the medial fibres started and stopped firing before the lateral fibres. Thus, fibres of a muscle are excited sequentially and effect rotation in the same direction as that in which they are excited. Time: 200 msec.

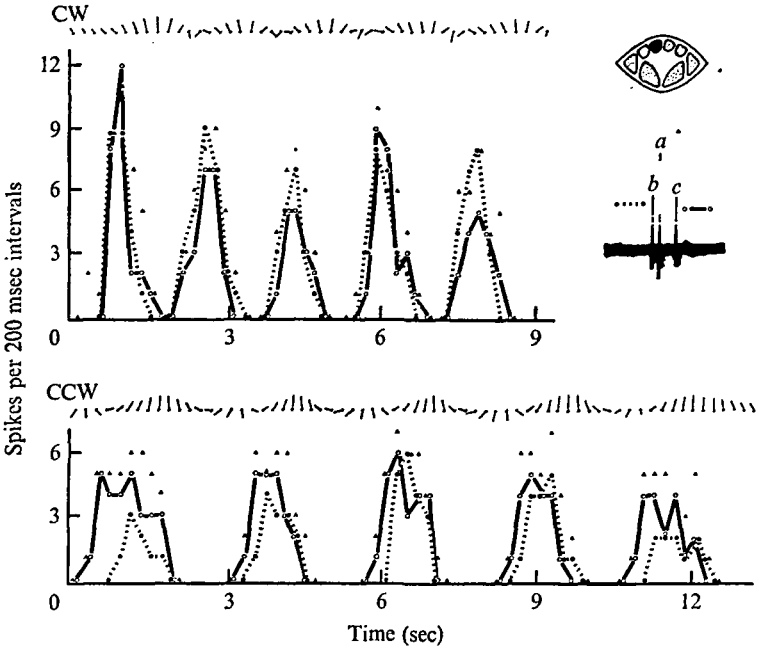
lead and lag. Since each muscle inserts by several tendons, sequential activation of fibres in a muscle means that the wave of contraction passing across a muscle pulls on the tendons consecutively. Such action would move the tail spine through the arc that can be described between the angles of deflexion created by pulling on the most distantly separated tendons of the muscle.

The motor pattern during rotation consists of a major burst of variously sized nerve spikes followed by a minor burst of small spikes. Each burst ranged from 800 to 1800 msec in duration. These features were seen in nerves to all of the muscles. A record from a nerve during a single rotation is shown in Text-fig. 6. The record demonstrates a major burst with three distinct large spikes, *a*, *b* and *c*, and some smaller ones followed by a minor burst of small spikes. In most nerve recordings several units appeared in the major burst. Nevertheless, one or more of the largest units in such a burst could usually be distinguished and followed throughout the record. In the minor burst only a few units fired, and presumably these were small neurones, because the recorded amplitude of the units in minor bursts was consistently small. These units did not fire during the major burst but began as the major burst ended. The units of the minor burst fired fastest at their onset and declined to a very low rate of firing or ceased altogether just before the next major burst began.

In major bursts motoneurones begin and finish firing consecutively and the sequence in which the units are recruited and drop out changes as the direction of rotation changes. Relationships among the three largest units in the major burst of Text-fig. 6 and accompanying bursts from the same nerve during five clockwise and five counterclockwise rotations are presented graphically in Text-fig. 7. Units *a*, *b* and *c* innervated some of the fibres of the left dorsal medial muscle and were distinguishable by their heights and waveforms (cf. lower inset in Text-fig. 7). Spikes of each unit were counted during 200 msec intervals and plotted against time. The most interesting feature shown by the graph is the relationship of spike *b* to spike *c* (Text-fig. 7; Table 1). During clockwise rotation *b* typically began to fire slightly before *c* and stopped sooner than *c* stopped. During counterclockwise rotation *b* did not begin firing until a few hundred milliseconds after *c* began and stopped slightly after *c* stopped. Thus, firing among motoneurones that innervate the same muscle occurs in sequences, which change during opposite rotations.

In major bursts units fire in clusters – that is, in groups of spikes. Each group usually includes one large spike and several smaller ones. Clusters are separated by silent

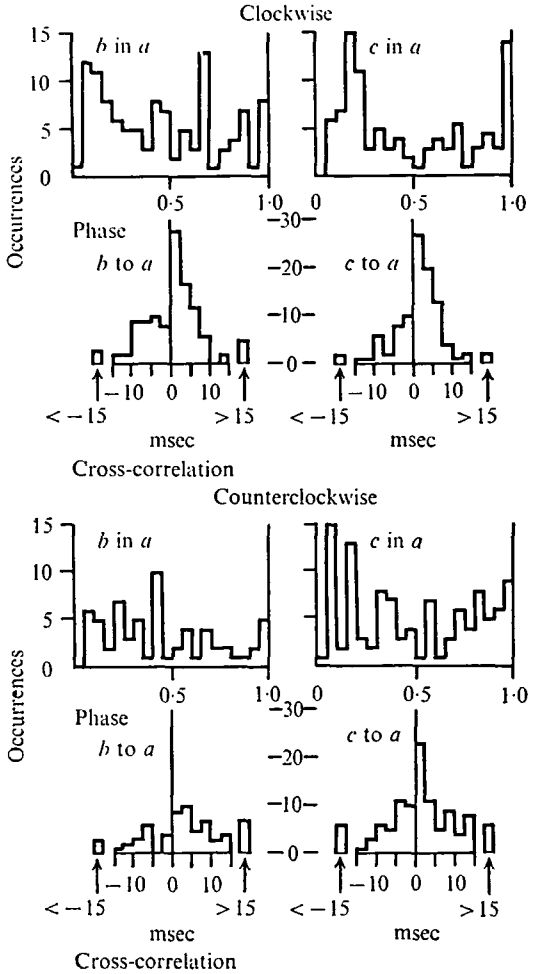
Text-fig. 6. Record showing details of firing in a nerve which innervated the left dorsal medial muscle during one complete rotation, the second in a series of five uninterrupted clockwise rotations. Firing consisted of a major burst with large, intermediate and small spikes and a minor burst of small spikes. The minor burst declined in frequency or ceased altogether before onset of the next major burst. In the major burst spikes fired in clusters; that is, in groups consisting of a few spikes each. The clusters are recognizable about the largest spike, spike *a*, which may serve as a unit of reference. The firing characteristics and relationships of neurones in this record and others in the same experiment are described in the next three figures. Time: 200 msec.



Text-fig. 7. Graph of the firing frequency of the three largest neurons shown in the record of Text-fig. 6 during an unbroken series of five clockwise and five counterclockwise rotations. The neurones innervated the left dorsal medial muscle as indicated by the blackened muscle in the upper inset which represents a posterior view into a cross-section of the abdomen and indicates the symmetrical and continuous arrangement of the muscles. The neurones could be distinguished by their height and waveforms as indicated by the lower inset, which is an enlargement from Text-fig. 6. Spikes of the three neurones were counted during successive 200 msec intervals and plotted against time. The figures above the graphs indicate the rotational position and relative deflexion of the tail spine at the end of each interval. The most interesting feature is the timing relation of spike *b* to spike *c*. *b* preceded *c* during clockwise rotation but followed *c* during counterclockwise rotation. Duration of lead and lag are presented in Table 1. This relationship means that firing sequences of motoneurons which innervate fibres in the same muscle change during opposite rotations and presumably in such a way as to cause sequential firing in a muscle (Text-fig. 5). Note also the higher firing frequency of the three units during clockwise rotation when the left muscles lifted the tail spine upward through the left side.

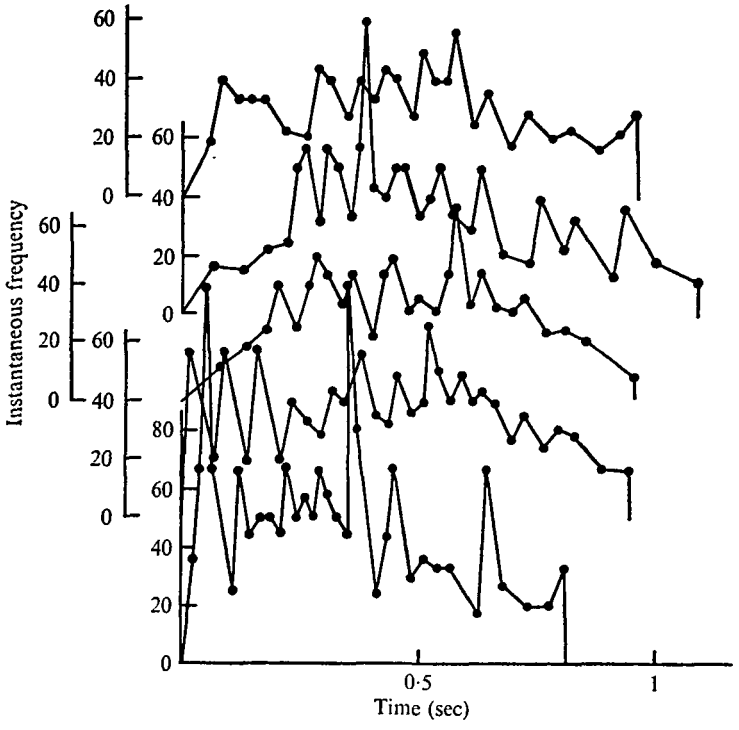
Table 1. *Time in milliseconds at the start and finish of firing of unit* b *relative to unit* c *of Text-figs.* 6 *and* 7

	Clockwise bursts				Counterclockwise bursts			
	Start		Finish		Start		Finish	
	b before *c*	*b* after *c*	*b* before *c*	*b* after *c*	*b* before *c*	*b* after *c*	*b* before *c*	*b* after *c*
	—	15·0	177·5	—	—	600·0	15·0	—
	2·5	—	—	135·0	—	222·5	—	7·5
	157·5	—	142·5	—	—	285·0	—	5·0
	22·5	—	160·0	—	—	180·0	—	327·5
	50·0	—	197·5	—	—	300·0	—	72·5
Mean	43·5		108·5			317·5		79·5



Text-fig. 8. Histograms of phase relationships and cross-correlations between spikes *a* and *b* and *a* and *c* from the experiment described in Text-figs. 6 and 7. *a* was chosen as the reference unit because it was the largest spike and fired most often. For cross-correlations spikes *b* and *c* were counted in 2·5 msec intervals preceding (negative values) and following (positive values) spike *a*. Spikes falling near the middle of an interval was assigned to the closer *a* spike. Phase histograms show at best a weak association between units. Cross-correlation histograms, however, indicate that more than two-thirds of the firings occurred within ± 10 msec of the reference unit and bear out the observation that motoneurones fire in clusters.

periods of several milliseconds, as can be seen in the record in Text-fig. 6 (see also Text-figs. 4 and 11). Analysis of the experiment described in Text-figs. 6 and 7 revealed the following relationships. The units were not tightly coupled as indicated in Text-fig. 8 by the absence of definite phase relationships among them. However, cross-correlation histograms (Text-fig. 8), which show the time of firing of one unit relative to another, indicated that more than two-thirds of the firings occurred within ± 10 msec of spike *a*, the reference unit and the largest spike in the record (cf. Text-figs. 6, 7). The average interspike interval of spike *a* was $32 \pm 15{\cdot}6$ (S.D., $N = 151$) msec for clockwise rotation and $40 \pm 14{\cdot}4$ (S.D., $N = 154$) msec for counterclockwise rotation.



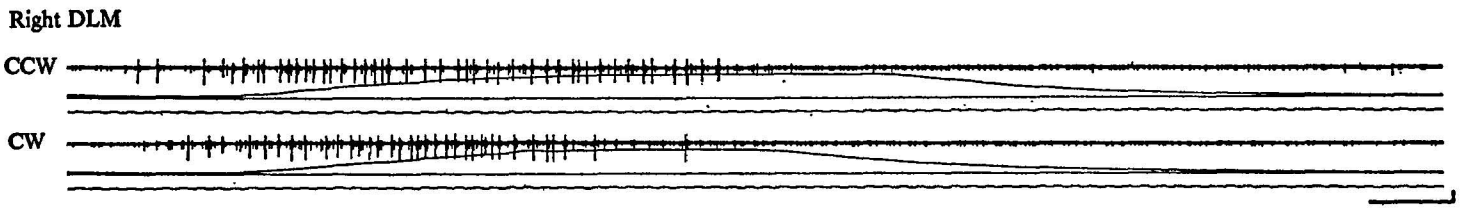
Text-fig. 9. Plots of instantaneous frequency of unit *a* from the experiment in Text-figs. 6 and 7 during the five consecutive clockwise rotations. Points were connected by lines in order to make it possible to follow the changes in frequency. Frequencies in successive rotations are plotted from bottom to top along the vertical axis. Instantaneous frequency was calculated as the reciprocal of the interspike interval, which was measured from the recordings and rounded to the nearest whole number. The main feature of interest is the great variability in the rate of firing, which increased at times to 130/sec although the mean frequency was 39/sec. Firing frequency increased rapidly, fluctuated and dropped to low values in the final few hundred milliseconds of the burst. Despite the variations, smooth tail-spine rotations were produced.

This means that if the first and last spike within a cluster fell 10 msec before and after spike *a* there was usually an interval of 10 msec or more between adjacent clusters. This analysis shows that although units in the major burst did not fire in near synchrony with other units, as definite phase relationships would have suggested, units nevertheless tended to be triggered closely to one another. In minor bursts the small units appeared to fire more randomly than units in major bursts (cf. Text-fig. 6, 11).

In major bursts the firing frequency of any given motoneurone is irregular. This is apparent from the plot of instantaneous frequency in Text-fig. 9 for unit *a* of the experiment described in Text-figs. 6 and 7 during clockwise rotation. Firing commenced at a rapid rate, fluctuated about the mean of 39 impulses/sec with occasional rapid discharges at 130 impulses/sec and declined gradually in the final few hundred milliseconds of the burst. The firing rate of the unit varied similarly during counterclockwise rotation but the mean frequency was lower, 28 impulses/sec. Thus, there is a good deal of variability in the interspike interval.

Both the major and the minor burst excite muscle fibres. The records of nerve and muscle potentials taken during the rotation in Text-fig. 10 show that the major burst

Text-fig. 10. Correlation of the firing of neurones in the major and minor bursts with muscle potentials during three clockwise rotations of the tail spine. The extracellular recordings were made with suction electrodes, one attached to a nerve branch (top trace) and the other to one or more muscle fibres (bottom trace) near the insertion of the right dorsal lateral muscle. During the major burst large muscle potentials occurred; during the minor burst small muscle potentials occurred. These potentials indicate the excitatory nature of the neurones in both bursts. The large muscle potentials obscure the activity of the major burst in the nerve trace. Time: 200 msec.



Text-fig. 11. Firing in the same nerve during opposite rotations while tension was recorded from the right dorsal lateral muscle on the second trace. The peak of tension occurred at the end of the major burst as indicated by the cessation in firing of the largest spike. Tension persisted for a time as the minor burst began. Tension then declined along with the minor burst. The rate of fall in tension was approximately the same as the rate of rise. Note also the greater duration of firing during counterclockwise rotation when the right dorsal lateral muscle lifted the tail spine upward on the right side. Note also the change in order of appearance of large-sized and intermediate-sized spikes at the beginning and end of the opposite rotations. This is another example of changed firing sequences among motoneurones to muscle fibres within a muscle as seen in Text-fig. 7 and Table 1. Third trace is a base line. Time: 200 msec. Tension: 100 g.

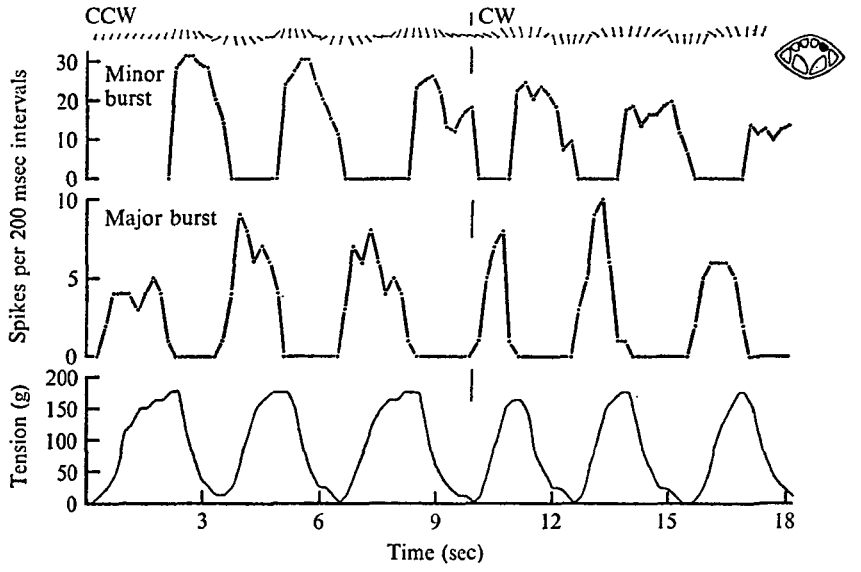
Table 2. *Burst duration and frequency of the largest spikes in each of the nerves innervating the contralateral muscles of the animal of Text-fig.* 4 *during three clockwise and three counterclockwise rotations*

	Clockwise			Counterclockwise		
	Duration (msec)	No. of firings	Mean frequency	Duration (msec)	No. of firings	Mean frequency
Right dorsal lateral muscle	560	14	—	760	23	—
	640	17	—	640	25	—
	880	14	—	760	30	—
Totals	2080	45	22/sec	2160	78	36/sec
Left dorsal lateral muscle	720	24	—	840	24	—
	880	32	—	640	24	—
	1120	36	—	840	24	—
Totals	2720	92	34/sec	2320	72	31/sec

produced large and numerous muscle potentials and that the minor burst produced small and fewer muscle potentials.

The major burst occurs during the development of tension and the minor burst during the decline of tension. This relationship of bursts to tension is shown by the records in Text-fig. 11 and the graph in Text-fig. 12. In the experiment nervous output to the right dorsal lateral muscle and tension from the muscle were recorded together while the animal rotated its tail spine. The major burst excited the muscle to rotate the tail spine to the same angle as that to which contraction of only that muscle would have brought the tail spine (Text-fig. 2). At the point of maximum tension the major burst ceased and the minor burst began. Tension persisted for about 200 msec after cessation of the major burst then declined as firing of the smaller units built up, peaked and then also declined. Tension decreased to zero and the minor burst ceased. This occurred at a point about 180° of tail-spine rotation from the point where maximum tension occurred and the minor burst began. The next major burst began at the end of the minor burst and tension rose once again. Thus, the major burst leads to development of tension and to rotation of the tail spine through a segment of the path of rotation. This burst, regardless of the direction of rotation, shuts off at the point to which the muscle it excites would draw the tail spine if the muscle contracted by itself (compare action of right DLM in Text-fig. 2 to (that in) Text-fig. 12). The minor burst acts during extension of the muscle and presumably impedes lengthening, because of the excitatory nature of the small units.

The nervous output to a given muscle varies in duration and frequency depending on the side of the body through which the tail spine moves. In general the duration (Text-figs. 11, 12), frequency (Text-fig. 7) or both (Text-fig. 4, Table 2) were greater when the muscle lifted the tail spine through its own side rather than when the muscle carried the tail spine down through the same side as the muscle. For example, Text-fig. 4 and Table 2 show that the output to the right dorsal lateral muscle was greater both in duration frequency during counterclockwise rotations when the tail spine was brought up on the right side of the body than during clockwise rotation when the tail spine was brought up on the left side and across the body. The duration and frequency of output were greater to the left dorsal lateral muscle during clockwise rotation than during counterclockwise rotation. In water the weight of the tail spine

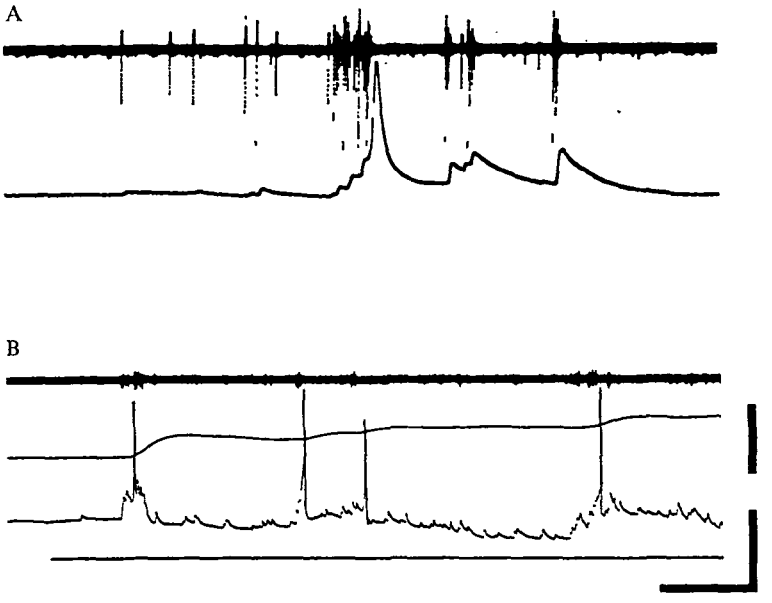


Text-fig. 12. Graph of three clockwise and three counterclockwise rotations from the experiment in Text-fig. 11. The largest spike in the major burst and all spikes in the minor burst were counted in 200 msec intervals. Tension was measured at the end of each interval. Both counts of spikes and measurements of tension were plotted against time. The main feature of interest is the correspondence of the minor burst with the period of relaxation when the muscle extended. This indicates that extension is actively resisted.

would appear to be negligible in itself because of the buoyancy of the body of *Limulus*. Yet when friction of the water is considered movement of the tail spine may develop a sufficient load to evoke the observed compensation. The reciprocal results between contralateral muscles (Text-fig. 4; Table 2), which were treated similarly, indicate that this phenomenon was not due to operative, recording and mechanical factors. The increased duration and frequency of firing to the muscle pulling the tail spine upward suggests that the tail-spine control system was compensating for load.

Neuromuscular properties

Responses of muscle fibres and patterns of innervation were studied by means of intracellular recordings in order to determine the effect of motor output upon the muscles. Membrane potentials averaged 50 ± 12 (S.D., $N = 233$) mV. Motoneurone activity, evoked centrally by tactile stimulation of the surface and spines of the abdomen, produced excitatory junctional potentials (e.j.p.s) no greater than 5 mV (Text-fig. 13). Junctional potentials summed and facilitated. During bursts of nervous activity, when the units innervating a muscle fibre fired together rapidly, spike-like potentials of 10–20 mV arose. Presumably these spikes initiated stronger contractions than junctional potentials because bursts in the nerve accompanied by spikes in the muscle fibre preceded large increments in tension (Text-fig. 13*b*). Tension increased in a graded manner in response to centrally initiated firing (Text-figs. 11, 13*b*). Ten-



Text-fig. 13. Intracellular recordings within muscle fibres.

(A) *En passant* recording in which the largest and next-to-largest spikes correlated with e.j.p.s in a fibre of the right dorsal lateral muscle in an intact animal. A burst of spikes produced a large summation and a spike-like potential of about 15 mV.

(B) *En passant* and intracellular recording coupled with tension measurement from the right dorsal lateral muscle. Bursts of clusters in the nerve led to summation and spiking in the muscle fibre and rapid but graded increments in tension. Continuing but slower nervous activity in the latter part of the record led to slight development or maintenance of tension. Spikes were about 10 mV and have been retouched. The bottom trace indicates the period of tactile stimulation to an isolated abdomen. The extent of facilitation in *en passant* recordings in which central activity initiated e.j.p.s was never clear, because spikes in bursts could not be clearly correlated with e.j.p.s. Presumably the combination of summation and facilitation led to spike-like potentials.

Voltage: (A) 10 mV, (B) 8 mV. Time: (A) 200 msec, (B) 1 sec. Tension: (B) 100 g.

sion began to develop at motor unit frequencies of about 5/sec as indicated by experiments in which nerves were stimulated for short periods at different frequencies. However, muscle fibres contracted in response to single nerve stimuli. Muscle fibres therefore showed functional characteristics intermediate between those of fast and slow muscle fibres of arthropods. They gave graded potentials, graded contractions and other responses which are characteristic of slow muscle fibres of arthropods, but they also generated spike-like potentials and gave twitch responses, which are characteristic of fast muscle fibres of arthropods (Atwood, 1963, 1965; Dorai Raj, 1964; Parnas & Atwood, 1966). These findings are the same as those of Eagles (1972) for the tail-spine muscles and are similar to those of Fourtner & Pax (1972) and Parnas *et al.* (1968) for leg muscles of *Limulus*.

Single muscle fibres are innervated by several excitatory motoneurones. Stimulation of nerves at increasing intensities, in order to progressively recruit axons, showed several e.j.p.s in the muscle fibre from which recordings were taken. Stimulation of two large nerves, namely motor branches of nerve 15 and nerve 16 to the ventral

Table 3. *Distribution of axon diameters in nerve branches of the size from which recordings were made*

Nerve*	Dimensions of nerve (μm)	No. of fibres > 3 μm	Diameters: Small (3–10 μm) No.	(%)	Intermediate (11–19 μm) No.	(%)	Large: 22–25 μm No.	(%)	27–32 μm No.	(%)	No. of very small fibres (< 3 μm)
A	157 × 85	48	28	(58)	18	(38)	2	(4)	0	(0)	> 35
B	157 × 110	50	31	(62)	18	(36)	1	(2)	0	(0)	*ca.* 50
C	242 × 121	52	25	(48)	22	(42)	3	(6)	2	(4)	*ca.* 15

* A and B are from one animal, C from another. Cross-section of nerve C is shown in Plate 1.

muscles, evoked e.j.p.s in the same muscle fibre. These results indicate that muscle fibres are innervated polyneuronally. In no instance was a hyperpolarizing junctional potential recorded. Neither reductions in the amplitude of an e.j.p. nor decreases in tension were recorded during recruitment of motoneurones by increasing the intensity of stimulation to nerves innervating a muscle. Thus, inhibitory fibres do not appear to innervate the muscles. Eagles (1972) also failed to demonstrate inhibition in the tail-spine muscles. This is also the case in the merocarpopodite flexor muscles of *Limulus* (Fourtner & Pax, 1972) but not in the closer muscles (Parnas *et al.* 1968), which have an inhibitor.

Two findings in the course of the innervation study revealed some additional characteristics of the neuromuscular arrangement. One was that activity from one neurone appeared in nerves entering adjacent muscles. Another was that e.j.p.s which correlated with the firing of an axon were not seen in all fibres of a muscle. These findings indicate a pattern of innervation in which motoneurones are not specific to a muscle because they overlap the separation between muscles, and in which motoneurones excite only some fibres within a muscle because their e.j.p.s could not be recorded in every portion of the muscle.

Histological properties

Nerves and muscles were studied histologically in order to confirm the physiological results, which suggested that nerves carried numerous fibres of many sizes and that muscles were of a type which showed features of both fast and slow muscles. Cross-sections of branches of the size from which recordings were made showed that these branches carried 75–100 fibres (Plate 1; Table 3). Some of these axons had quite large diameters of 22 to 32 μm. Most of the axons had intermediate (11–19 μm) and small (3–10 μm) diameters but the nerves also contained very small axons of diameter < 3 μm.

Muscle structure was studied in order to determine the degree of morphological variation among fibres and to correlate structure with function. Muscle fibres ranged in length from 2·5 to 5·0 cm. The diameters of fibres ranged between 10 and 60 μm and many were flattened. These variations can in great part be accounted for by the fact that muscle fibres fuse and taper along the distance from origin to insertion of the muscles. Fourtner & Sherman (1972) also indicated a similar variability between 10

and 60 μm in the range of diameters of leg-muscle fibres. The average sarcomere length was $6{\cdot}5 \pm 0{\cdot}8$ (S.D., $N = 63$) μm with a range in lengths between 4·9 and 8·4 μm. This indicates that the fibres are very similar. Similar uniform sarcomere lengths have been found in other *Limulus* muscles (Eastwood, 1971; Fourtner & Sherman, 1972). A mean sarcomere length of 6·5 μm indicates that *Limulus* muscle fibres are of an intermediate type, because arthropod fast muscles typically have short sarcomere lengths of < 6 μm with a range between 2 and 6 μm; arthropod slow muscles have sarcomere lengths of > 6 μm (Atwood, 1963, 1965; Doraj Raj, 1964; Kennedy & Takeda, 1965*a*; Parnas & Atwood, 1966; Gilai & Parnas, 1970; Jahromi & Atwood, 1969, 1971). In general, the histological properties of the tail-spine muscles allow them to be described as an intermediate type and provide a structural correlation to the observed intermediate neuromuscular properties.

DISCUSSION

The sequential firing pattern

Motor control of the tail spine in *Limulus polyphemus* produces rotation in opposite directions by exciting muscle fibres in opposite sequences. Muscle fibres or groups of fibres are activated one after the other in a clockwise or a counterclockwise sequence (Fig. 5), either of which can be driven continuously to generate several uninterrupted rotations. Presumably the changes observed in the order of recruitment of motoneurones in a nerve (Fig. 7; Table 1) are responsible for the changed firing order in fibres of a muscle. The firing sequences of the motoneurones during opposite rotations are not mirror images, because the time of firing of a motoneurone changes disproportionately relative to the start and end of firing of other motoneurones (Table 1).

Functionally, the sequential firing observed in the time of activation of muscle fibres within the same muscle is quite significant, because it means that fibres or groups of fibres within a muscle contract separately rather than the whole muscle at once. This further means that sequentially activated muscle fibres within a discrete muscle bundle sequentially exert tension through the several separate tendons by which the bundle inserts. This action would seem to contribute to a smoother rotation than if all the fibres in a muscle contracted at once.

Conceptually, the findings on sequential firing indicate that the functional contractile entity is not an entire muscle. Sequential firing shows that nerve and muscle fibres function independently of muscle boundaries. The findings that single motoneurones innervate portions of a muscle and not the whole muscle, that some nerve fibres innervate muscle fibres in adjacent muscles and that some muscle fibres originate with one muscle but insert with another show that the nerve and muscle fibres are arranged independently of muscle boundaries. Thus populations of fibres within a muscle appear to be the functional contractile entities. This finding extends the concept that a muscle – that is, a discrete bundle of muscle fibres – is not necessarily the functional entity. Davis (1968) pointed this out for the lobster swimmeret muscles, and Burrows' work (1967) on the eyecup muscles of the crab implies this but for a different reason than in the *Limulus* tail-spine system. In their systems two or more muscles have the same innervation and these muscles act as the functional contractile entity. In *Limulus*, the functional muscle appears to be different sections of the same

muscle or of adjacent muscle bundles. A functional muscle unit in *Limulus* may possibly be the fibres which insert by a single tendon. The tail spine in this sense is moved by a cone of muscle which differentiates movement through the separate contraction of its muscle units. Rotation is effected by muscle units pulling consecutively upon the more than a few dozen tendons.

These findings have certain comparative and functional implications. Other motor systems of arthropods show sequential firing patterns. Sequential firing occurs in antagonistic muscles in systems such as flight in insects (Wilson, 1961; Kammer, 1968), righting in lobsters (Davis, 1968), swimming in crayfish (Schrameck, 1970), ventilation in dragonfly larvae (Mill, 1970), gill-plate beating in *Limulus* (Fourtner, Drews & Pax, 1971) and walking in the cockroach (Pearson & Iles, 1970; Pearson, 1972). In these systems one muscle or one group of muscles fires alternately with another. In some of these systems a delay occurs between alternate bursts so that contractions in the sequence do not overlap. This delay is seen between the cockroach coxal depressor and elevator muscles during walking (Pearson & Iles, 1970). Non-overlapping of contraction is enhanced by peripheral inhibitors, which increase the rate of muscle relaxation (Iles & Pearson, 1971). Furthermore, in these systems movement typically does not occur in two directions as in the tail-spine system, although reverse movement occasionally takes place in walking systems (observations on crayfish and crabs in this laboratory; Davis & Ayers, 1972). Sequential firing is seen among the eleven muscles which sweep and pivot the scaphognathite or gill bailer in the branchial chamber of the crayfish in order to bring in water, move it across the gill and force it back out (Pasztor, 1968). The muscles fire one after the other to achieve movement, but this movement occurs in one direction and it is not continuous, because there is a short period of inactivity between beats of the bailer. Thus, there is a single motor program that generates sequential but not uninterrupted firing to the muscles of the bailer. Sequential firing occurs among muscles in the crab eyecup motor system, which promotes optokinetic nystagmus in two directions (Burrows & Horridge, 1968*a*). Not all the muscles, however, are active during any given fast or slow phase of a nystagmus, and opposite nystagmus movements require different muscles. Thus, only some muscles in a given nystagmus fire one after the other. This means that there are two central programs which drive different muscles in two quite dissimilar sequences. Sequential firing among several muscles and requiring two motor programs probably occurs in the crayfish uropod motor system, because the uropods move in opposite directions (Larimer & Kennedy, 1969*a*, *b*) and presumably do so through the co-operation of their several muscles. Thus, among the arthropods rhythmic movement is produced by sequential activation of two or more muscles or groups of muscles. Tail-spine rotation in *Limulus* is similarly produced by sequential firing but differs in the number of functional contractile units activated. Many functional contractile entities firing consecutively produce rotations in such a way that the periods of activity in adjacent entities overlap.

Function of the minor burst and the significance of extension

The small excitatory units of the minor burst appear to contribute to rotational movement of the tail spine by maintaining a degree of tension in muscles as they are passively extended. Activity in these units may affect both deflexion and rotation of

the tail spine. The effect of deflexion would be to keep the tail spine at a desired angle. Without some degree of tension in muscles other than those that are contracting the tail spine might not be rotated steadily nor be held firmly at angles between resting and maximum deflexion, because support is needed in all directions about the base of the tail spine. Tension maintained in muscles being extended would provide this stabilization, in much the same way as low-level firing in some antagonistic muscles of the leg of salamanders provides stabilization during walking (Székely, Czéh & Vörös, 1969). Tension maintained to provide stability is also seen in some crab eyecup muscles, which undergo no detectable change in impulse frequency and presumably contribute to eyecup movement by resisting lengthening while other muscles change the position of the eyecup (Burrows & Horridge, 1968*b*). This function of maintaining tension in lengthening muscles seems to be necessary in other systems. In walking cockroaches the coxal depressor muscle is inhibited during the contraction of its antagonist, the coxal elevator (Pearson & Iles, 1970; Iles & Pearson, 1971).

The effect of the small excitatory units on rotation is based on the following relationships. Tension reaches a maximum at the end of the major burst (Text-figs. 11, 12). At this point, firing of the small units begins and is maximal. Activity in the small units and tension decline simultaneously until the tail spine is rotated about 180° from the point of maximum tension (see sketches of the tail spine position and plots of tension and firing frequency in Text-fig. 12). Thus, during rotation a major burst causes a muscle to draw the tail spine toward the position attained during active contraction of the muscle and away from the position to which the previously contracting muscle brought it. The minor burst tends to keep the tail spine at the position attained during active contraction of a muscle. Without the minor burst and the tension it presumably maintains in extending muscles the tail spine might jerk as it moves in rotation and fail to move in its normal smooth pattern. Moreover, it might tend to fall toward the resting position rather than continue around the outer circumference of the circle which the tip of the rotating spine describes. The small units, therefore, possibly provide tension to extending muscles in order to maintain outward deflection and smooth rotation of the tail spine.

A similar role of excitatory activity during extension of muscles in another rotating system is seen in the vertebrate eye. In the vertebrate eye continuous but declining activity is seen in extending eye muscles. For example, slow nerve fibres to the lateral rectus muscle fire at decreasing rates but never cease completely during extension of the lateral rectus in the slow lateral-to-medial tracking phase of nystagmus (Yamanka & Bach-y-Rita, 1968). During naso-temporal movements of the eye the intensity of firing in the lateral and medial recti muscles fluctuates reciprocally. Firing increases in one muscle concomitantly with decreasing, but not terminating, firing in the antagonist (Björk & Kugelberg, 1963). Boeder (1962) has pointed out that active modulation of muscle extension is just as important from a mechanical point of view as contraction. Without the resistance contributed by active muscles during extension some component of abduction or adduction, elevation or depression of the eye would be lacking and the eye would deviate from its normal path of movement.

Expression of the firing pattern

The firing pattern causes the greatest effect in this motor system which lacks great neuromuscular and structural diversity by exciting small to large axons in clusters. Clusters evoke the strongest contractions in the muscles. Most spikes in the cluster fire within ± 10 msec of a reference unit and produce summed and facilitated e.j.p.s and frequently spike-like potentials in the muscle fibre (Text-fig. 13). A similar phenomenon of closely spaced firing affecting muscles more strongly than random or unspaced firing is seen in the motor patterns to some crustacean muscles. For example, the largest axon innervating the superficial flexors of the crayfish abdomen (Gillary & Kennedy, 1969*a*, *b*) fires in a bursting pattern. This neurone evokes small e.j.p.s that undergo facilitation when the neurone fires in its normal bursting mode of two to four occurrences per burst. Stronger contractions result from firing in bursts than when the axon is driven at a constant rate for a number of firings equal to the total in the bursts. Since this axon innervates more than 60% of the superficial flexors (Kennedy & Takeda, 1965*b*), strong contractions of these muscles are produced by the activity of this axon (Larimer & Eggleston, 1971). The effectiveness of closely spaced firing is also seen in the paired firing patterns of motoneurones that innervate the crayfish carpopodite extensor (Atwood & Wiersma, 1967), crayfish opener (Wilson & Davis, 1965) and some oculomotor muscles of the crab (Burrows & Horridge, 1968*b*). The interspike interval between the pairs ranges between 5 and 13 msec and this corresponds to the most effective interval for increasing tension in muscle (Ripley & Wiersma, 1953). This interval is of the same order of magnitude as the interval between some of the spikes in a cluster of the *Limulus* tail-spine system. Since some of the units in a cluster innervate the same muscle fibre (Text-fig. 13*a*), the effect could be similar to the paired or burst firing in crustacean axons. That is, closely spaced firing among the clustered units develops tension more effectively than if the units fired wholly at random.

In *Limulus* changes in the firing frequency of clusters and the duration of the firing period lead to variations in the rate and strength of movement of the tail spine. Such variations in frequency and duration of firing occurred (Text-figs. 2, 4, 5, 7, 11, 12; Table 2) when a muscle, in particular the dorsal lateral muscle, lifted the tail spine upward on the same side of the body rather than when the muscle brought the tail spine downward on the same side of the body. Changes in the firing frequency and duration of clusters suggest that these variations in the firing pattern are a motor mechanism that compensates for load.

The firing pattern appears to express itself without peripheral inhibition. This absence seems understandable if the excitatory activity during extension (Text-fig. 10) indicates that the main function of the system is to maintain tension in order to achieve desired tail-spine deflexions and to effect smoothness. Inhibition would release tension, which would interfere with stable movement of the tail spine and cause instability. Two other systems, which employ a sequential activation of muscles, namely those of the gill bailer (Pasztor, 1968) and the eye of the crab (Burrows & Horridge, 1968*a*) also lack peripheral inhibition. These systems require a high degree of co-ordination among muscles, which in the scaphognathite system fire in sequence during rhythmic beating. Inhibition appears in systems which tend to function with

greater reciprocity between muscles than do these systems, for example, in lobster swimmeret beating (Davis, 1968), cockroach walking (Iles & Pearson, 1971) and crayfish tail positioning (Page & Sokolove, 1972). However, this does not seem to be an absolute generalization, because tonic muscles in the crayfish uropods, which would appear to require cooperative interaction of muscles in order to perform the variety of movements of which they are capable, have peripheral inhibitors (Larimer & Kennedy, 1969*a*, *b*).

Central organization of the motoneurones

The results of the experiments on motor output give some insight into the central organization of the motoneurones. The findings that motoneurones tend to fire in clusters suggests a common input which triggers several neurones at about the same time. The finding that individual motoneurones fire in different sequences in opposite rotations suggests that each motoneurone also receives a specific input. Thus, each motoneurone may be driven by both a common excitor and a specific excitor. The interaction of the two leads to a suitable duration, sequence and frequency of units in the firing pattern for effecting rotations of the tail spine. Furthermore, since the motoneurones fire in inverse (although not mirror-image) patterns during opposite rotations, interactions appear to take place in the terminal fused ganglia 14–16 of the ventral cord, because the nerves that innervate the muscles arise from these ganglia and because rotation continues to occur, at least for a short time, following severance of the cord as far posterior as the 13th ganglion. Exploration of the central mechanisms within these ganglia in quest of understanding the organization of the motoneurones, the origin of the motor pattern, sequencing and the motor programs will be interesting.

SUMMARY

1. *Limulus polyphemus* (L.), the horseshoe crab, rotates its tail spine in order to right itself and to keep itself balanced.

2. Eight muscles, discrete bundles of muscle fibres, move the tail spine. Fibres of the muscles contract in sequence and thereby pull consecutively on the several tendons of each muscle in order to rotate the tail spine in either a clockwise or counter-clockwise direction.

3. Motoneurones in nerves to different muscles, fibres within a muscle and units in a nerve to a single muscle fire in different sequences during clockwise and counter-clockwise rotation of the tail spine.

4. The firing pattern consists of a major burst of small to large neurons which fire in clusters, and a minor burst of small neurones which appear to fire randomly. Motoneurones in both bursts are excitatory. The major burst develops tension, the minor burst acts during extension of the muscle and presumably impedes relaxation in order to produce stable deflexion and smooth rotation of the tail spine.

5. Muscle fibres respond to motor output with small excitatory junctional potentials of < 5 mV. E.j.p.s sum and show facilitation, and in some cases develop spike-like potentials of 10–20 mV. Both spiking and the greatest increase in tension occur during the clustered firings in major bursts.

6. Muscle fibres have sarcomere lengths of $6{\cdot}5 \pm 0{\cdot}8$ μm and diameters of 10–60 μm.

Nerve fibres range from less than 3 to 32 μm in diameter in large nerve branches which contain between 50 and 100 fibres.

7. These findings indicate that two different motor programs evoke contraction of muscle fibres in opposite sequences. Sequential contraction of fibres within a muscle means that muscle fibres which are activated together, rather than whole muscles, are the functional contractile entities.

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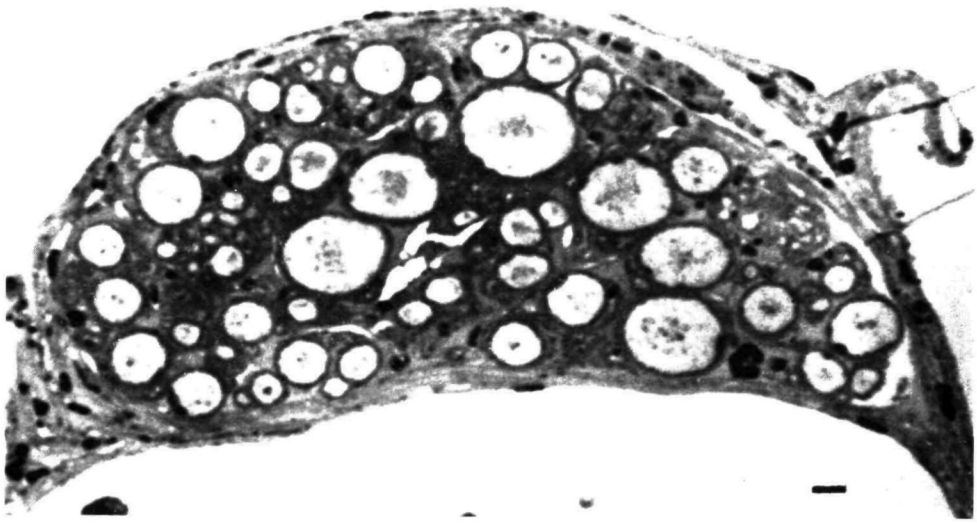

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EXPLANATION OF PLATE

Light micrograph of a cross-section of a nerve branch in the dorsal lateral muscle stained with toluidine blue. The section is from a branch of the size and in the position of a nerve commonly found and recorded from. Fibres with a variety of diameters are seen. Size distributions of the fibres are given in Table 3 under nerve C. Scale: 10 μm.