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THE MOTOR INNERVATION AND MUSCULATURE OF THE ANTENNULE OF THE HERMIT CRAB, *PAGURUS ALASKENSIS* (BENEDICT)

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INTRODUCTION

Although the motor innervation and musculature of the thoracic and abdominal appendages of Crustacea have been studied exhaustively (Wiersma & Ripley, 1952; Davis, 1968; Pasztor, 1968; Larimer & Kennedy, 1969; Paul, 1971; Sherman & Atwood, 1971), those appendages which are innervated by the brain have attracted little attention (Hoyle, 1968; Burrows & Horridge, 1968; Mendelson, 1969). This bias has probably resulted from the recent emphasis on the role of central programmes in determining the patterning of motoneuronal activity; many thoracic and abdominal appendages are involved in rhythmical activities during which the component movements show little cycle-to-cycle variability. Such systems have proved to be excellent for studying the involvement of command, oscillator and sensory neurones in determining motor output (DeLong, 1971; Davis & Kennedy, 1972*a*, *b*, *c*).

The antennules of the hermit crab, *Pagurus alaskensis*, show four types of activity: flicking, rotation, wiping and withdrawal. In life the antennules are flicked almost continuously, yet this activity cannot be regarded as rhythmical and there is no clear coordination between left and right antennules. Some components of flicking are fairly stereotyped from flick to flick, although other components of flicking and other antennular activities are more variable (Snow, 1973). These observations suggest that a study of the factors influencing activity in antennular motoneurones might provide an interesting comparison with similar studies in systems showing rhythmic activity.

The objective of this paper is to describe the motor innervation and contractile properties of the muscles of the medial and distal antennular segments and thus to provide a basis for studying the patterning of activity within and between specific motoneurones during various antennular activities. The major finding is that the antennular motor system is extremely simple and comprises a phasic, a phaso-tonic and a tonic component. The properties of the motor units are such that it is possible to make strong inferences regarding which motoneurones are involved in each antennular activity (see Snow, 1973).

MATERIALS AND METHODS

Specimens of *P. alaskensis* (Benedict) were dredged off Waldron Island, San Juan Archipelago, Washington, and maintained in running sea water at the Friday Harbor Laboratories where this work was carried out between July and September 1971. Physiological data presented here were collected from 128 preparations.

(1) *Saline*

The antennular muscles were found to be extremely sensitive to osmotic pressure. The following saline was developed on the basis of the osmolarity of sea-water samples taken from the holding tanks (av. 885 mOsm) and of fresh blood samples taken from six animals (av. 870 mOsm). In accordance with the ionic analyses of hermit-crab blood (Robertson, 1953), the saline was made 30 mM with respect to sulphate ions. The following number of millimoles of components were dissolved in one litre of distilled water in this order: 463 NaCl; 8 KCl; 10 $MgSO_4$; 20 $CaSO_4$; 10 Tris buffer. The pH was then adjusted to 7·4 with conc. HCl and NaOH. This saline averaged 887 mOsm and in it muscles frequently gave good responses for up to 4 h. The data reported are restricted to those collected within the first 1·5 h of any experiment.

(2) *Dissection*

Both left and right antennules of large (body length $\simeq$ 8 cm) hermit crabs were used. Antennules were excised at the base of their proximal segment and placed in a small Petri dish of saline. This was maintained between 12 and 14 °C by circulating sea water around the Petri dish. The bottom of the dish was covered with Sylgard 184 Encapsulating Resin (Dow Corning) which allowed rigid pinning and trans-illumination of the preparation.

In life the antennules are rotated frequently but in narcotized animals the aesthetasc hairs point ventrally. The side which bears the aesthetasc hairs will thus be referred to as the ventral side of the antennule. Joint movements which result in ventral displacement of the more distal limbs will be referred to as flexions (see Snow, 1973).

Using a sliver of razor blade the exoskeleton of the proximal segment was sliced longitudinally along the dorsal and ventral sides. The proximal segment was then pulled apart and the half containing the statocyst was removed by cutting its membranous attachment with the medial segment. The remaining half contains the nerves supplying the medial and distal antennular segments.

Cochran (1935) described and gave numbers to three muscles of the medial and distal segments of a brachyuran antennule: muscle 30 (musculus $productor_3$ I antennae); muscle 31 (musculus $reductor_3$ I antennae); muscle 32 (musculus $reductor_4$ I antennae). Dissection showed that in the antennule of the hermit crab three muscle groups of similar function to Cochran's muscles 30, 31 and 32 may be recognized, but two of these are subdivided into two separate muscles giving a total of five muscles in three muscle groups 30, 31 and 32. Muscle group 30 raises the distal segment while 31 depresses the distal segment and 32 depresses the outer flagellum.

Muscle groups 31 and 32 were exposed by longitudinally shaving the exoskeleton from the medial side of the medial and distal segments, respectively. Muscle group 30 was exposed by similarly removing the exoskeleton from the exterior side of the medial segment. Such cuts were made from the arthrodial membrane at the distal end of a segment to about one-third to one-half the length of the segment.

Each muscle group is connected to the basal exoskeleton of the more distal limb by a single tendon (Text-fig. 1). A small wedge of this exoskeleton attached to the tendon of a muscle group was cut and the rest of the more distal limb was excised. The

Antennule was stapled to the Sylgard with insect pins so that the muscle group was exposed dorsally and the wedge of exoskeleton was attached to a tension transducer. The remaining half of the proximal segment was pinned inner side dorsally and the muscles overlying the nerves in the proximal segment were removed by cutting their distal and proximal attachments and carefully severing associated nerve branches.

(3) *Stimulation and recording*

Initially the nerves present were stimulated with fine silver hooks while recording intracellularly in muscle groups 30, 31 and 32. It soon became apparent that the proximal portion of one nerve contained all the motor axons, and a fine suction electrode was used in most succeeding preparations. Motor axons were differentiated by exploiting their differences in threshold to electrical stimulation. Frequently intracellular junction potentials were simultaneously recorded in two fibres of one muscle group using an Adak Electronics FET follower and a WPI M4a electrometer. Whole-muscle tension was monitored using an RCA 5734 tension transducer. Recordings were displayed on a Tektronix 565 oscilloscope and photographed directly using a Nihon Kohden PC-2A continuous recording camera.

(4) *Histology*

The microanatomy of the antennules was determined by dissection of fresh material, thin sectioning of Epon-embedded nerves and serial sectioning of wax-embedded antennules. For measurements of axon diameters the nerves of the basal segment were fixed in a 2·5% phosphate-buffered gluteraldehyde and 1% phosphate-buffered osmium. These were embedded in Epon and 1 μm sections were stained in Richardson's stain. For serial sectioning the entire contents were withdrawn from the old exoskeleton of pre-moult antennules. These were secured to strips of Sylgard and fixed in Bouin's for 24 h. They were then dehydrated, cleared in toluene and embedded in wax. Serial sections were taken at 15 μm and stained in Masson's trichrome (Pantin, 1948).

(5) *Sarcomere measurements*

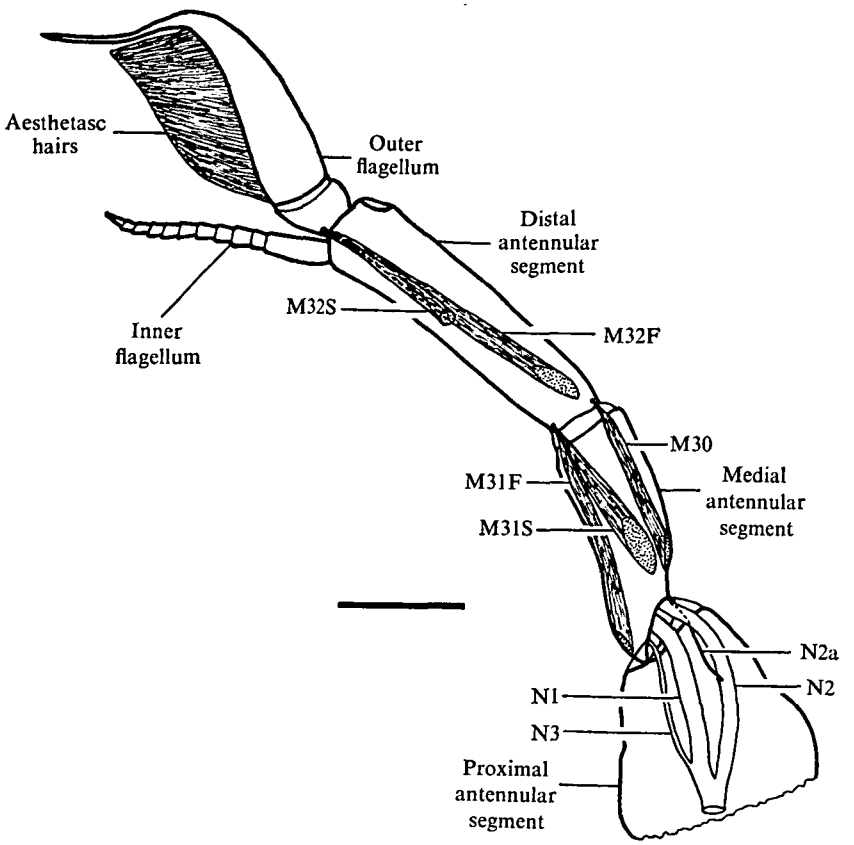
Sarcomere lengths were measured by excising the antennules of narcotized animals at the base of the proximal segment. All three muscle groups were then exposed as described above and the antennules were secured to Sylgard strips in the position which they adopt in narcotized animals. Antennules were fixed in 2·5% phosphate-buffered gluteraldehyde for 1 h and washed in sea water. The muscles were dissected out onto glass slides, teased apart and the mean sarcomere lengths were calculated in each muscle from direct measurements using the light microscope.

RESULTS

(1) *Anatomy*

A. *Muscles*

The distal and medial segments of the antennule contain a total of five muscles which are divided into three muscle groups. Muscle group 30 consists of a single muscle, 30 (M30). Proximally the fibres of M30 are attached to the dorsal exoskeleton at the base of the medial segment. Distally they are attached to a tendon which is



Text-fig. 1. Medial view of the right antennule. Showing the muscles of the distal and medial segments. The medial half of the proximal segment exoskeleton (see text) has been rotated 180° to expose the antennular nerves. Scale – 2 mm.

attached to the dorsal exoskeleton at the base of the distal segment (Text-fig. 1). Tension in M30 causes extension at the medial segment–distal segment joint. Cutting muscle groups 30 and 31 shows that elasticity in this joint opposes displacement of the distal segment from its resting position shown in Text-fig. 1. This elasticity is weak in recently moulted animals.

Muscle group 31 consists of two muscles. Sarcomere measurements, excitatory junction potential (EJP) shapes and whole-muscle tension responses have suggested that these muscles probably contain only slow (muscle 31S) and fast (muscle 31F) fibres, respectively. Distally the fibres of both muscles share a common tendon which attaches to the ventral exoskeleton at the base of the distal segment. The fibres of muscle 31S (M31S) lie along a diagonal to the medial segment and are attached proximally to the dorso-medial exoskeleton near the base of this segment. The fibres of muscle 31F (M31F) lie along the ventral exoskeleton of the medial segment to which they are attached in the proximal region (Text-fig. 1). Although tension in either M31S or M31F causes flexion of the medial segment–distal segment joint, the movement caused by M31F is small compared to that caused by M31S.

Muscle group 32 consists of two muscles. Sarcomere measurements, EJP shapes,

Table 1. *Mean sarcomere lengths (μm) of antennular muscles*

Antennule no.	Muscle 30	Muscle 31F	Muscle 31S	Muscle 32F	Muscle 32S
1	6·1	3·7	5·6	2·4	6·6
2	4·8	3·0	5·7	2·2	6·1
3	6·4	2·5	5·6	2·6	5·6
4	5·8	3·9	5·2	2·4	5·2
5	5·4	3·2	5·5	2·1	3·8
6	5·8	3·6	5·7	2·9	5·4
7	6·1	2·4	5·3	2·6	5·5
8	3·8	2·3	4·9	2·5	5·0

Means are based on five to ten measurements of ten sarcomeres in each muscle.

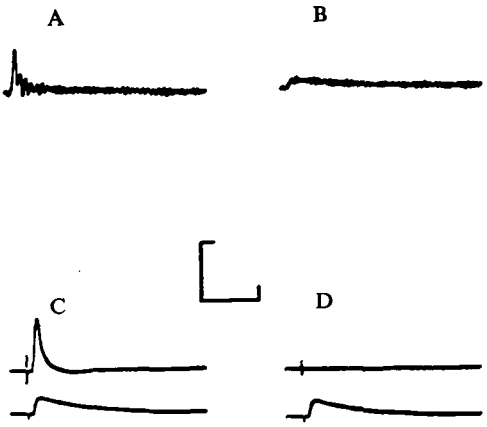
and whole-muscle tension responses have suggested that these muscles probably contain only slow (muscle 32S) and fast (muscle 32F) fibres, respectively. Distally the fibres of both muscles share a common tendon which attaches to the ventral exoskeleton at the base of the outer flagellum. The fibres of both muscles lie along a diagonal to the distal segment. Proximally, the fibres of muscle 32S (M32S) are attached to the medial exoskeleton about one-half to two-thirds of the way from the base of the distal segment, while those of muscle 32F (M32F) are attached to the dorso-medial exoskeleton near the base of the distal segment (Text-fig. 1). Although tension in either M32S or M32F causes flexion at the distal segment–outer flagellum joint, M32S consists of only about six fibres and it can thus develop very little tension in comparison with M32F. When muscle group 32 is removed the outer flagellum assumes a raised position to which it returns after manual depression suggesting that elasticity within its joint with the distal segment is important in raising the outer flagellum during normal activity.

The five muscles described above may be grouped on the basis of their coloration and sarcomere lengths. Muscles 31F and 32F have an orange coloration while muscles 30, 31S and 32S are relatively colourless. Data from eight antennules showed that muscles 31F and 32F had short sarcomeres (2·1–3·9 μm), while the sarcomeres of muscles 30, 31S and 32S were about twice as long (usually 4·8–6·6 μm) (Table 1).

B. *Nerves*

All nerves to the outer and inner flagella and the distal and medial segments branch from a single nerve which enters the base of the proximal segment. Within the base of this segment three major branches arise which will be referred to in order of decreasing diameter as nerves 1–3 (Text-fig. 1). Nerve 1 (N1) is about 280 μm in diameter and contains mainly the fine axons of the sensory neurones of the outer flagellum. Nerve 3 (N3) is of unknown function and varies considerably in diameter between animals. No contractions or junction potentials were observed in any of the above muscles on stimulation of N1 or N3.

Nerve 2 (N2) is about 170 μm in diameter. It is a mixed nerve containing the motor axons to all five muscles described above and sensory axons most of which arise from receptors on the inner flagellum. Within the proximal segment a fine nerve (approx. diameter 33 μm) branches off N2 and coils around its extero-lateral side before



Text-fig. 2. Tension responses and intracellular recordings in muscle 30 in response to a single high-intensity and low-intensity stimulus to nerve 2. High-intensity stimulation elicits a twitch of muscle 30 (A) and an EJP in the ventral fibres (C, upper trace) and fibres elsewhere in muscle 30 (C, lower trace). Low-intensity stimulation elicits a small slow contraction of muscle 30 (B) and an EJP in fibres elsewhere in muscle 30 (D, lower trace), but no EJP in the ventral fibres (D, upper trace). Scale – A and B: 54 mg, 150 msec; C and D: 60 mV, 30 msec.

entering the medial segment. This branch will be referred to as nerve 2a (N2a) (Text-fig. 1).

Thin Epon sections show that N2a contains only two axons of about 13–14 μm in diameter and that N2 contains two giant axons of about 40–42 μm in diameter (Pl. 1). Serial sections of antennules show that one giant axon leaves N2 in the basal half of the medial segment and can be seen running between M31S and M31F. The other giant axon enters the distal segment where it becomes associated with muscle group 32.

(2) *Physiology*

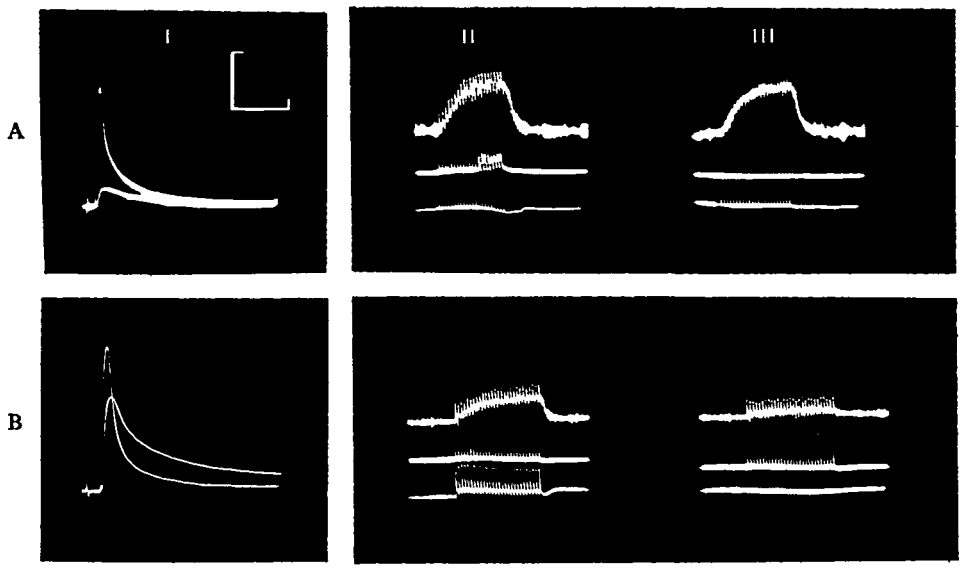
If the branch N2a is cut, stimulation of N2 evokes tension and EJPs in muscle groups 31 and 32 but not in muscle group 30. Stimulation of branch N2a gives rise to tension and EJP in muscle group 30. In most preparations N2 was stimulated proximally to branch N2a.

No inhibitory junction potentials were recorded in any of the muscles investigated on stimulation of any of the antennular nerves.

A. *Motor innervation and tension responses of muscle group* 30

Nerve 2a consists of two axons, and attempts were therefore made in each preparation to separate these axons by altering the intensity or duration of the stimulus. Two parameters were used to monitor the responses to stimulation: (1) the tension developed by M30 and (2) the occurrence of intracellularly recorded EJPs within different cells of M30. Successful separation was achieved in only 50% of these preparations, and examination of these records suggested that it was impossible to predict which axon would have the lower threshold. This would be expected from the similarity in diameter of the two axons (Pl. 1).

The following data were collected from preparations where the axons of N2a had different thresholds. When single stimuli of different intensities were applied to N2,



Text-fig. 3. AI and BI: junction potentials in the ventral fibres (large EJPs) and fibres elsewhere (smaller EJPs) in muscle 30 in response to a single high-intensity stimulus to nerve 2. AII and III and BII and III: intracellular recordings (lower two traces) and whole-muscle tension (top traces) in response to repetitive stimulation of nerve 2 at high (II) and low (III) intensity. Records A and B are from different preparations in which the axon to the ventral fibres (middle traces) had the highest and lowest threshold, respectively. Note the tonic tension development in the absence of a depolarization plateau (BII) (AIII). Scale – AI: 30 mV, 15 msec; BI: 15 mV, 15 msec; AII and III: 1500 msec, top traces 22 mg, middle traces 150 mV, lower traces 60 mV; BII and III: 1500 msec, top traces 54 mg, middle and lower traces 60 mV.

two tension responses were elicited in muscle 30: (1) a rapid, usually larger twitch response (Text-fig. 2A); and (2) a slower, usually smaller contraction (Text-fig. 2B). The larger and smaller responses were characterized by faster and slower rates of tension development and relaxation, respectively. The relative magnitude of the larger and smaller responses varied between preparations. Even when both axons were excited M30 seldom developed more than 14 mg tension.

Intracellular recording in different cells of M30 while stimulating N2 at different intensities revealed that each cell was innervated by only one axon. Two microelectrodes were employed to differentiate between the EJPs evoked by each axon of N2a. Although resting potentials among the fibres of M30 were usually between 50 and 60 mV, EJPs varied in size from 2 to 62 mV. It was noted that larger EJPs (39–62 mV) occurred only within a few fibres along the ventral surface of M30 and that elsewhere in the muscle EJPs were smaller (2–25 mV). The larger EJPs followed a faster time course than the smaller EJPs (Text-figs. 2C, 3 AI and BI). Simultaneous recording in the ventral fibres and fibres elsewhere in M30 showed that the EJPs in each group of fibres had different thresholds to stimulation of N2 (Text-figs. 2C, D). It was therefore concluded that each axon of N2a innervated a different population of fibres within M30. These axons have been named 30F and 30S in accordance with the fast and slow time course of the tension responses they elicit

in M30. Thus the axon innervating the ventral fibres will be referred to as 30F while the axon innervating the fibres elsewhere in the muscle will be referred to as 30S.

Repetitive stimulation of axon 30F (A30F) at frequencies of 5, 10, 20 and 40/sec did not result in facilitation of EJPs in the ventral fibres, but stimulation of axon 30S (A30S) at frequencies as low as 5/sec resulted in facilitation of EJPs in fibres elsewhere in M30. The degree of facilitation in the latter group of fibres was inversely related to the initial size of the EJP. Junction potentials of around 5 mV increased to three or four times their original height after four stimuli applied at 10/sec (Text-fig. 3 AIII), yet EJPs of around 25 mV increased by only a fraction of their original size (Text-fig. 3 BII). Junction potentials of A30F showed slight disfacilitation at frequencies of 20/sec.

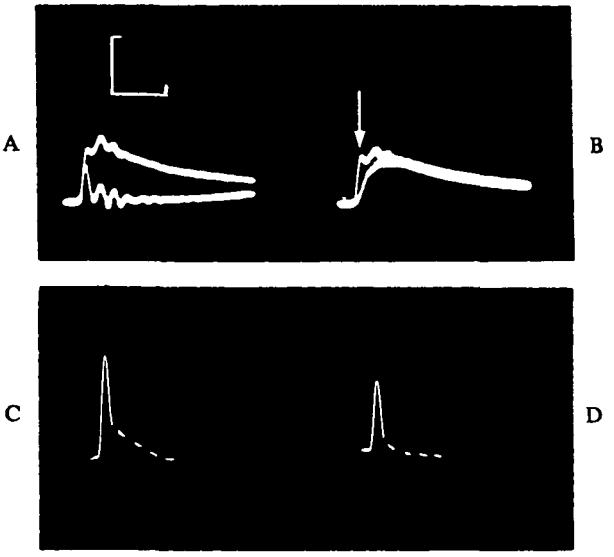
Monitoring of tension developed by M30 while simultaneously recording within fibres innervated by A30F and A30S, respectively, enabled the contractile properties of M30 to be related to its innervation. Preparations in which A30S had the lowest threshold were used to investigate the tension responses attributable to the excitation of this axon, while other preparations were used to investigate responses to excitation of A30F. The stimulation of A30S at frequencies as low as 5/sec resulted in a tonic contraction of M30 on which small contractions were superimposed. Each small contraction correlated with an EJP in those muscle fibres innervated by A30S (Text-fig. 3 AIII). Increasing the stimulus intensity resulted in an EJP in fibres innervated by A30F, and larger twitches became superimposed on the tonic contraction induced by A30S. At frequencies up to 10/sec stimulation of both axons did not result in increases in the magnitude of the tonic contraction (cf. Text-fig. 3 AII with AIII). In preparations where A30F had the lowest threshold its stimulation at frequencies up to 10/sec resulted in a series of large twitches with no tonic contraction (Text-fig. 3 BIII). Tonic contraction, however, resulted when the stimulus intensity was above threshold for A30S (Text-fig. 3 BII).

It should be noted that the tonic contractions observed in these experiments arose from summation of the slow 'twitches' evoked by stimulation of A30S and not by the build-up of a depolarization plateau from summation of the EJPs of this axon. The twitch response evoked by stimulation of A30F lasts for less than 50 msec whereas the decay of tonic contractions of the same magnitude may take up to 500 msec (cf. Text-fig. 2 AIII with 3 BII).

In conclusion, it appears that axons 30F and 30S innervate different populations of muscle fibres. The fibres innervated by A30F are fast in character while those innervated by A30S are slow. This distinction between populations, however, is not clearly apparent from sarcomere measurements of the fibres in M30.

B. *Motor innervation and tension responses of muscle group* 31

The application of a high-intensity single stimulus to N2 evoked a large contraction (150–560 mg) in muscle group 31. This tension developed rapidly and decayed slowly. Lowering the stimulus intensity resulted in a smaller contraction (90–270 mg) which developed at the same rate but which decayed rapidly (Text-fig. 4A). Visual inspection during this twitch suggested that only M31F was contracting. After cutting M31F a single high-intensity stimulus resulted in a contraction in which tension developed at a slower rate than in the intact preparation (Text-fig. 4B). Maximum

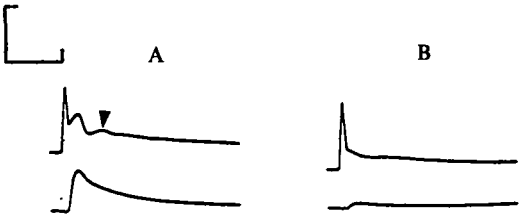


Text-fig. 4. Tension responses of muscle group 31 in response to a single stimulus to nerve 2. A: the tension responses to a high-intensity (larger response) and a low-intensity (smaller, faster decaying response) stimulus. B: the tension responses in the same preparation to a high-intensity stimulus, before (arrow) and after cutting muscle 31F. Note the slower rate of tension development when only muscle 31S is contributing to the response. C and D: the tension responses to a high-intensity (C) and low-intensity (D) stimulus in a preparation where muscle 31S was not contracting. Note the difference in magnitude of the two responses. Owing to vibration following the twitch, records C and D have been touched by dotting through the mean points of the vibrations. Scale – 280 mg, 60 msec.

tension development and the rate of decay of tension were the same as in the intact preparation.

Examination of Text-fig. 4A shows that at high intensities of stimulation tension developed rapidly up to 310 mg, while at low intensities tension developed rapidly up to 190 mg. Upon cutting M31F no rapid tension development occurred. These data suggested that M31F was responsible for rapid tension development and that it gave different responses to high-intensity and low-intensity stimulation of N2. Attempts to record these by cutting M31S were unsuccessful, probably due to damage of the motor nerve branches to M31F. In a few intact preparations, however, visual examination showed that M31S failed to respond to stimulation of N2 and the tension responses of M31F to high-intensity and low-intensity stimulation could thus be recorded. Both high-intensity (Text-fig. 4C) and low-intensity (Text-fig. 4D) stimuli evoked a twitch response. The former ranged from 200 to 490 mg in different preparations while the latter was smaller, ranging from 90 to 200 mg. These results suggest that M31F is innervated by at least two axons and that stimulation of one or both of these results in small or large rapid tension development in intact preparations.

Resting potentials in cells of M31F and M31S were from 55 to 65 mV and from 40 to 70 mV, respectively. Simultaneous intracellular recordings in M31F and M31S during high-intensity stimulation of N2 resulted in a double-peaked response in most of the fibres of M31F and a single-peaked response in all fibres of M31S (Text-fig. 5A). The response in fibres of M31F consisted of a large (40–60 mV),



Text-fig. 5. Intracellular recordings in muscles 31F (top traces) and 31S (bottom traces) in response to a high-intensity (A) and low-intensity (B) stimulus to nerve 2. Note the two peaks in the top trace of A (arrow marks mechanical artifact) the second of which occurs synchronously with the peak in the lower trace. Record B shows only the EJPs of axons 31F (top trace) and 31S (bottom trace). Scale – 60 mV, 15 msec.

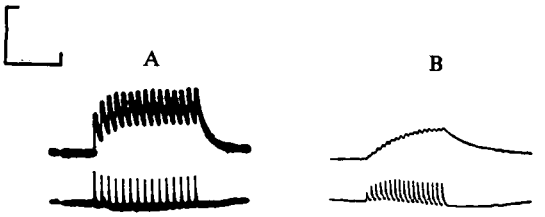
sharp, initial peak which decayed rapidly but incompletely before being followed by a smaller (20–40 mV) blunter peak. The second peak initially decayed at about the same rate as the first but this phase was usually interrupted by movement artifacts (Text-fig. 5A). The first and second peaks were absent in about 5 and 20% of M31F fibres, respectively.

The electrical response in fibres of M31S consisted of a blunt peak (20–40 mV) which had about the same magnitude and latency, in any preparation, as the second peak in fibres of M31F. Subsequent decay of the response in M31S was slow in comparison with both peaks in M31F. In all preparations the second peak in M31F had the same threshold as the response in M31S and it was thus concluded that they were EJPs of a single axon which innervates both muscles. As this axon may elicit slow and fast tension responses in muscle group 31 it will be referred to as axon 31F–S (A31F–S).

Stimulation of N2 at an intensity just below the threshold of A31F–S resulted in a small EJP (1–5 mV) in fibres of M31S (Text-fig. 5B). In a few preparations no small EJPs were seen but this was probably because the axon responsible for the small EJP had a higher threshold than A31F–S in these instances. In the few fibres of M31F which did not respond to stimulation of A31F no EJP was observed on stimulation subthreshold to A31F–S. In these preparations small EJPs could be simultaneously recorded in M31S and it was therefore concluded that these resulted from excitation of a single axon which innervates only M31S. Because of the low rate of decay of the EJPs of this axon and the slow tension responses and long sarcomeres of M31S, this axon will be referred to as axon 31S (A31S).

Stimulation at intensities subthreshold to A31F–S and A31S or just A31F–S evoked only the first peak in most fibres of M31F (Text-fig. 5B). This showed an all-or-none behaviour to further decrements of the stimulus intensity.

Simultaneous recording of tension of muscle group 31 and EJPs within fibres of M31F and M31S showed that when only A31F was stimulated a brief (20–30 msec) twitch contraction, similar to that shown in Text-fig. 4D, was elicited. Visual inspection showed that only M31F was involved in this response. At intensities just above-threshold for A31S, M31S gave a small contraction which did not appear to alter the recorded tension. When M31F was cut this small contraction of M31S could be measured as ranging from 4 to 23 mg in different preparations. Simultaneous



Text-fig. 6. Tension responses and intracellular recordings in muscle 31S during repetitive stimulation of nerve 2 at high (A) and low (B) intensity. Muscle 31F has been cut. A: high-intensity stimulation at 5/sec results in tonic tension development (top traces) due to summation of the single contractions of muscle 31S. B: low-intensity stimulation at 10/sec still results in tonic tension development (top trace) due to summation of single small contractions of muscle 31S. Scale – A: 104 mg, 60 mV, 1500 msec; B: 166 mg, 48 mV, 2400 msec.

stimulation of A31S, A31F–S and A31F in intact preparations resulted in a rapidly developing, slowly decaying, large contraction of muscle group 31 (Text-fig. 4A).

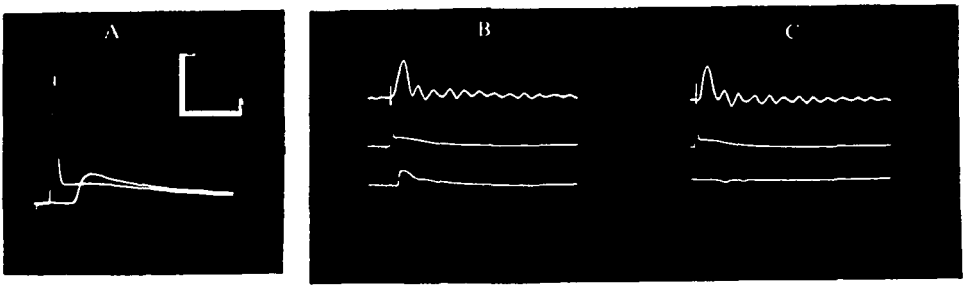
Repetitive stimulation up to 40/sec of A31F did not result in facilitation of EJPs recorded in M31F or any tonic tension development. Similarly, although stimulation of A31F–S at up to 40/sec did not result in facilitation of EJPs recorded in M31F or M31S, tonic tension development occurred at frequencies as low as 5/sec. This tonic tension was observed in preparations in which M31F had been severed and is due to the summation of the slowly decaying contractions of M31S (Text-fig. 6A). Repetitive stimulation of A31S at about 20/sec in such preparations resulted in threefold facilitation of EJPs and tonic tension development. This tonic tension was due to the summation of the small, slowly decaying contractions of M31S elicited by stimulation of A31S and not to the build-up of a depolarization plateau from summation of the EJPs of this axon (Text-fig. 6B).

In conclusion, it appears that there are three axons, 31F, 31S and 31F–S, innervating muscle group 31. Muscles 31F and 31S are composed of fibres which are fast and slow in character, respectively. The fibres of M31S are innervated by A31S. Axon 31F innervates 95% of the fibres of M31F. Its low threshold suggests that A31F is the giant axon of N2 which associates with muscle group 31. Axon 31F–S innervates all the fibres of M31S and 80% of the fibres of M31F.

C. *Motor innervation and tension responses of muscle group* 32

The application of single stimuli to N2 elicited twitch contractions (60–220 mg) of muscle group 32. Careful observation of the exposed muscles showed that at a lower stimulus intensity only M32F contracted. Repetitive stimulation at frequencies up to 40/sec at this intensity resulted in a series of twitches without any tonic tension development. Increasing the stimulus intensity resulted in tonic tension development at frequencies of only 10/sec. The threshold for tonic tension was identical to that for contractions in M32S and, furthermore, the rate of decay of the tonic tension was far less than that of the twitch contraction (Text-fig. 8A).

Simultaneous intracellular recording in M32F and M32S suggested that each muscle was innervated by a single axon. The EJPs in fibres of M32F were large (40–72 mV) and followed a very short time course (duration: 4 msec) while those in fibres of M32S were smaller (12–18 mV) and followed a longer time course (duration:



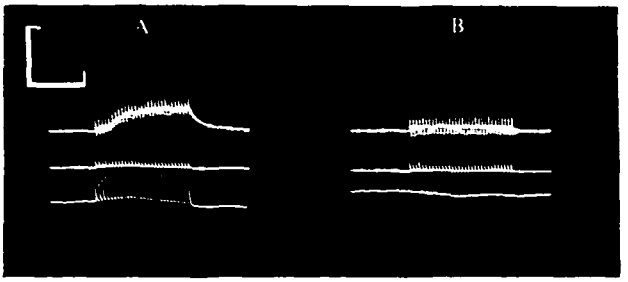
Text-fig. 7. A: junction potentials in muscle 32F (large EJP) and 32S (smaller EJP) in response to a single high-intensity stimulus to nerve 2. B and C: tension responses in muscle group 32 (top traces) and intracellular recordings in muscles 32F (middle traces) and 32S (lower traces) in response to a high-intensity (B) and low-intensity (C) stimulus to nerve 2. Scale – A: 30 mV, 15 msec; B and C: 100 mg, 63 mV, 63 msec.

50 msec) (Text-fig. 7A). The fibres of M32F and M32S had similar resting potentials of 58–75 and 60–70 mV, respectively.

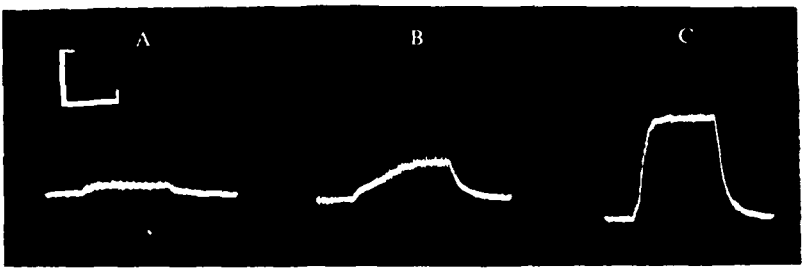
The axons innervating M32F and M32S will be referred to as A32F and A32S, respectively, in accordance with the time courses of the tension responses they elicit in muscle group 32. In all preparations, A32F had a very low threshold and is therefore considered to be the giant axon in N2 which associates with muscle group 32. The marked difference in the latencies of the EJP in M32F and in M32S further suggests that the motor axon responsible for the former is of relatively large diameter (Text-fig. 7A).

Simultaneous recording of tension developed by muscle group 32 and the EJPs within fibres of M32F and M32S made possible further analysis of the contractile properties of muscle group 32 in relation to its innervation. High-intensity stimulation of N2 elicited twitch contractions of muscle group 32 yet stimulating subthreshold to A32S did not elicit a detectably different contractile response (cf. Text-fig. 7B with 7C). Repetitive stimulation of A32F at 5–40/sec did not result in facilitation or summation of EJPs in M32F. Disfacilitation was only very slight at frequencies from 20 to 40/sec. No tonic tension was developed, and at frequencies of 10–40/sec the twitch magnitude decreased by 10–30% over the first few twitches (Text-fig. 8B). Repetitive stimulation of A32S at 5–10/sec elicited two- to threefold facilitation of EJPs in M32S (Text-fig. 8A). At frequencies of 10/sec this was coupled with tonic tension development by muscle group 32, but no depolarization plateau developed in the fibres of M32S until A32S was stimulated at 20/sec. Tonic tension below this frequency can only be explained by the summation of small contractions in M32S each of which arose from a single stimulus to the motor nerve.

In order to record the contractile responses elicited by A32S alone, M32F was cut and A32F was selectively stimulated at 100/sec until the remnants of M32F ceased to respond to stimulation (90 sec). A single stimulus to N2 at just above-threshold intensity to A32S elicited a contraction in M32S. Such contractions were too small in magnitude (approx. 1 mg) to be accurately monitored on the recording system and therefore did not register at all during previous recordings of the twitches of M32F. Using high amplification and repetitive stimulation at frequencies of 5–20/sec.



Text-fig. 8. Tension responses and intracellular recordings in muscles 32F (middle traces) and 32S (lower traces) during repetitive (10/sec) stimulation of nerve 2 at high (A) and low (B) intensity. The tonic tension development is threshold-linked to the appearance of EJPs in muscle 32S but not to the presence of a depolarization plateau. Scale – A and B: 1600 msec, top traces 110 mg, middle traces 160 mV, bottom traces 64 mV.



Text-fig. 9. Tension responses in muscle 32S during stimulation of nerve 2 at 5/sec (A), 10/sec (B) and 20/sec (C). Muscle 32F has been cut (see text). The tonic tension development results from summation of the single contractions of muscle 32S which can be distinguished during low-frequency stimulation (A). Scale – 54 mg, 1500 msec.

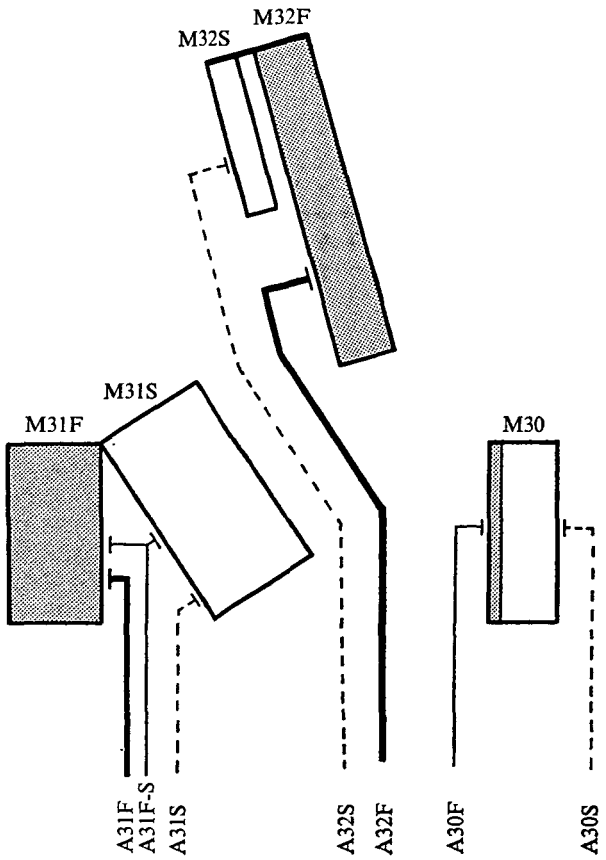
a tonic tension development could be recorded (Text-fig. 9A–C). At frequencies of 5/sec the responses to each stimulus pulse can be seen superimposed on the tonic response (Text-fig. 9A).

In summary, the muscle fibres of M32F and M32S are fast and slow in character, respectively. All the fibres of M32F are innervated by the giant axon 32F while all those of M32S are innervated by axon 32S.

DISCUSSION

(1) *Muscular organization*

No overshooting EJPs were observed in any muscles studied. The fibres of these muscles could, however, be divided into two groups on the basis of the rates of decay of their EJPs, their sarcomere length and their rates of contraction and relaxation. Atwood (1965) has shown that the EJPs in the rapidly relaxing, short-sarcomered, thick fibres of the opener muscle of *Chionectes* have a shorter time constant than those in the slowly relaxing, long-sarcomered, thin fibres. Although recording of tension from single muscle fibres of the antennular muscles was not practicable, the present experiments show that EJPs with slow rates of decay occurred in muscle fibres with long sarcomeres and were always threshold-linked with slow rates of contraction and relaxation. In addition, EJPs with fast rates of decay were recorded



Text-fig. 10. Schematic diagram of the motor innervation and musculature of the distal and medial antennular segments. Stippled areas of the blocks represent fast muscle cells while unmarked areas represent slow muscle cells. Continuous lines represent motor axons whose EJPs do not facilitate. The thicker lines represent the giant axons 31F and 32F. Broken lines represent motor axons whose EJPs show facilitation.

in muscles which had short sarcomeres and were threshold-linked with fast rates of contraction and relaxation.

A possible exception to this scheme are those fibres of muscle 30 which are innervated by axon 30F. The EJPs of axon 30F decayed rapidly and could be threshold-linked with a brief twitch in muscle 30, yet fibres with short sarcomeres were only rarely observed in this muscle. Frequently fibres innervated by axon 30F were difficult to find, suggesting that they represent only a small percentage of the fibre population of muscle 30. During the measurement of sarcomeres a few fibres with sarcomere lengths of about 3·5 μm were found in three preparations of muscle 30. Failure to discover such fibres in other preparations may only reflect their scarcity.

(2) *Comparison with other systems*

The neuromuscular organization of the antennular motor system is summarized in Text-fig. 10. With the exception of axon 31F–S there is no sharing of motoneurones between anatomically separate muscles. This situation parallels that of the

crayfish uropods (Larimer & Kennedy, 1969) and, with the exception of the common excitor to the stretcher and opener muscles, that of the crustacean limb (Wiersma & Ripley, 1952; Sherman & Atwood, 1971). The anatomical distribution of axon 31F–S differs from that of the common excitor of the crustacean limb in that it innervates synergistic muscles (M31F and M31S) and in this respect it is more analogous to axon D_s which innervates four of the six cockroach mesothoracic and metathoracic coxal depressor muscles (Pearson & Iles, 1971). Separate synergistic bundles with shared motoneurones have been identified in the lobster swimmeret system, but Davis (1968) has chosen to consider these as parts of a single functional muscle whose division simply represents an optimal mechanical arrangement of the muscle and its attachments within an irregular enclosure.

A second interesting feature of the antennular system is that, with the exception of axon 31F–S, all motoneurones innervate either fast or slow muscle fibres (Text-fig. 10). A rigid separation between the innervation of fast or slow muscle fibres has been documented in the crayfish abdominal musculature (Kennedy & Takeda, 1965*a*, *b*; Parnas & Atwood, 1966). In contrast, however, the motoneurones of the crustacean leg innervate a mixed population of muscle fibres (see Hoyle & Wiersma, 1958; Atwood, 1963). The crayfish abdomen and the crustacean leg represent extremes, and more intermediate situations are found in the crayfish uropods and the cockroach leg. Most motoneurones to the uropod muscles innervate only fast or slow fibres but in at least one case Larimer & Kennedy (1969) observed a motoneurone innervating both fast and slow fibres of a mixed muscle. Similarly, although the four fast coxal depressor muscles of the cockroach leg are innervated by a single axon D_f, the two slow muscles and two of the fast muscles are innervated by a single axon D_s (Pearson & Iles, 1971; Iles & Pearson, 1971). In both these systems the axon innervating both fast and slow muscle fibres may be compared with axon 31F–S in the antennular motor system.

(3) *Role of the motor system components in antennular movements*

Although the antennular motor system contains a purely phasic and a purely tonic component, the dual innervation of muscles 31F and 31S by axon 31F–S gives this system a phasic-tonic component. The various activities of the antennule have been described in the preceding paper (Snow, 1973) and it is useful to consider how the phasic, phasic-tonic and tonic components may be involved in these activities.

Most easily accounted for is the phasic system which is probably functional only during antennular flicking. This system consists of axons 32F, 31F and 30F and muscles 32F, 31F and the fast portion of muscle 30. During flicking a short, high-frequency train of spikes in the giant motor axons 31F and 32F could elicit almost synchronous twitches in muscles 31F and 32F which would result in a phasic flexion at the medial segment–distal segment and distal segment–outer flagellum joints, respectively. Maynard (1965) shows an electromyogram recorded in muscle group 32 during a flick of the lobster antennule. This record consists of a burst of seven spikes which occur at a frequency of about 170/sec. Similar records from muscles 31F and 32F of *P. ochotensis* during flicking show that activity in these muscles occurs almost synchronously and consists of two to three spikes per flick with an intraburst frequency of 200/sec (Snow & Field, unpublished). Extension at the medial segment–

distal segment joint could similarly be caused by activity in axon 30F eliciting rapid twitch in the fast component of muscle 30. This movement would be facilitated by the slight elasticity in this joint. Extension at the distal segment–outer flagellum joint is completely dependent on joint elasticity.

The phasic-tonic system consists of axon 31F–S and muscles 31F and 31S. This system controls movements of the medial segment–distal segment joint only and is probably active during the fast flexion-withdrawal reflex. During this activity the medial segment–distal segment joint is rapidly flexed through 50–80° (Snow, 1973). The water resistance to such a movement would be considerable, requiring a large amount of tension from the underlying musculature. The proximal attachment of muscle 31S is such that any tension developed would be most effective following partial flexion while tension in muscle 31F would become less effective as flexion continued (Text-fig. 1). An action potential in axon 31F–S would elicit rapid tension development in muscle 31F which would be immediately followed by the slower longer-lasting contraction of muscle 31S. A second action potential in axon 31F–S within 100 msec would elicit a similar contraction which would summate with the first contraction of muscle 31S (see Text-fig. 4A). A relatively low-frequency burst in axon 31F–S could thus elicit the powerful and rapid contraction of muscle group 31 necessary for the fast flexion reflex.

The tonic system consists of axon 30S to the slow component of muscle 30 and axons 31S and 32S to muscles 31S and 32S, respectively. Part or all of this system is probably employed to control the slower antennular movements such as wiping, slow flexion-withdrawal reflexes, extension-withdrawal reflexes, any other smooth movements about the medial segment–distal segment and distal segment–outer flagellum joints and posture during tonic flexion withdrawal. Tonic flexion of the medial segment–distal segment joint during antennular flicking is probably also mediated via the tonic system (Snow, 1973). An interesting feature of this system is the very small, slow tension responses which may be elicited by a single stimulus to the motor nerve. Such a response facilitates the development of extremely small tonic tensions upon low-frequency (5–10/sec) excitation of the motor axons involved (Text-figs. 3 AIII, 6B, 9A, B). If a similar frequency–tension relationship exists *in vivo* then this system could be interpreted as extending the low-frequency response range of slow muscle to motoneuronal activity. Functionally this would be extremely useful for the fine postural control of appendages with little joint resistance and whose weight is largely balanced by the density of the environmental medium.

(4) *Absence of peripheral inhibition*

From the present experiments there was no evidence of postsynaptic peripheral inhibition in the antennular motor system, but no attempt was made to test for presynaptic inhibition (Dudel & Kuffler, 1961). In the lobster the observation that activity in muscle group 32 decreased in frequency in response to some passive movements of the outer or inner flagellum (Maynard, 1965) suggests that central and not peripheral inhibition influences antennular muscle contractions.

Further inferences for the absence of postsynaptic inhibition may be drawn from a consideration of the role of peripheral inhibition in relation to the movements required during the antennular activities (Snow, 1973). The role of peripheral inhibition in

crustacean muscles other than those of the claws and legs is unclear (see Bush, 1962*a*, *b*; Evoy, Kennedy & Wilson, 1967; Iles & Pearson, 1971). In insects, Iles & Pearson (1971) have proposed that postsynaptic inhibition is important in eliciting rapid relaxation of slow coxal depressor muscles during walking in the cockroach. Unlike cockroach walking, however, antennular activities are never rhythmical (Snow, 1973) and thus the phasing of contractions of the antennular muscles need not conform to any centrally imposed cycle time (Pearson & Iles, 1970). In addition, a study of the antennular activities has suggested that only the phasic, phaso-tonic or tonic component of the motor system is utilized during any one type of activity.

It could be argued that postsynaptic peripheral inhibition could effect rapid relaxation of slow antennular muscles upon initiation of antennular activities which involve contractions of fast antennular muscles. Without measurements of the overlap of activity between motor units of the phasic or phasic-tonic component and antagonistic motor units of the tonic component of the motor system, little can be said about this possibillity.

SUMMARY

1. The motor innervation and musculature of the medial and distal segments of the hermit-crab antennule have been described anatomically.

2. Intracellular recordings within these muscles and simultaneous monitoring of whole-muscle tension have been used to define the motoneurones and contractile properties of the muscle fibres they innervate.

3. The motor system consists of two fast, two slow and one mixed muscle which are innervated by seven motoneurones.

4. The motor innervation is such that this system may be divided into three components: phasic, phasic-tonic and tonic. The possible involvement of these components in the antennular activities is discussed.

5. The tonic component is adapted to produce fine tonic tension in response to relatively low-frequency (5–10/sec) motoneurone discharge. It is suggested that this may be important for the postural control of appendages which, owing to the density of the environmental medium, are relatively weightless.

6. No evidence of postsynaptic inhibition was found, and this is discussed in relation to the movements of the antennule.

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REFERENCES

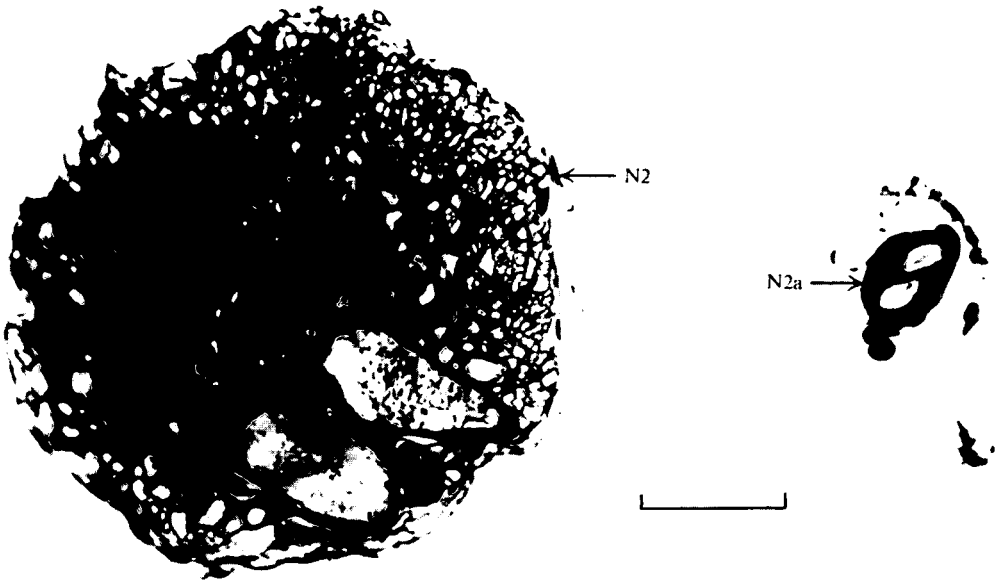
ATWOOD, H. L. (1963). Differences in muscle fibre properties as a factor in 'fast' and 'slow' contraction in *Carcinus*. *Comp. Biochem. Physiol.* **10**, 17–32.

ATWOOD, H. L. (1965). Excitation and inhibition in crab muscle fibres. *Comp. Biochem. Physiol.* **16**, 409–26.

BURROWS, M. & HORRIDGE, G. A. (1968). The action of the eyecup muscles of the crab *Carcinus* during optokinetic movements. *J. exp. Biol.* **49**, 223–50.
BUSH, B. M. H. (1962*a*). Peripheral reflex inhibition in the claw of the crab, *Carcinus maenas* (L.). *J. exp. Biol.* **39**, 71–88.
BUSH, B. M. H. (1962*b*). Proprioceptive reflexes in the legs of *Carcinus maenas* (L.). *J. exp. Biol.* **39**, 89–105.
COCHRAN, D. M. (1935). The skeletal musculature of the blue crab, *Callinectes sapidus*, Rathbun. *Smithsonian misc. Collns.* **92** (9), 1–76.
DAVIS, W. J. (1968). The neuromuscular basis of lobster swimmeret beating. *J. exp. Zool.* **168**, 363–78.
DAVIS, W. J. & KENNEDY, D. (1972*a*). Command interneurons controlling swimmeret movements in the lobster. I. Types of effects on motoneurons. *J. Neurophysiol.* **35**, 1–12.
DAVIS, W. J. & KENNEDY, D. (1972*b*). Command interneurons controlling swimmeret movements in the lobster. II. Interaction of effects on motoneurons. *J. Neurophysiol.* **35**, 13–19.
DAVIS, W. J. & KENNEDY, D. (1972*c*). Command interneurons controlling swimmeret movements in the lobster. III. Temporal relationships among bursts in different motoneurons. *J. Neurophysiol.* **35**, 20–9.
DELONG, M. (1971). Central patterning of movement. *Neurosciences Res. Prog. Bull.* **9**, 10–30.
DUDEL, J. & KUFFLER, S. W. (1961). Presynaptic inhibition at the crayfish neuromuscular junction. *J. Physiol.* **155**, 543–62.
EVOY, W. H., KENNEDY, D. & WILSON, D. M. (1967). Discharge patterns of neurons supplying tonic abdominal flexor muscles in the crayfish. *J. exp. Biol.* **46**, 393–412.
HOYLE, G. (1968). Correlated physiological and ultrastructural studies on specialized muscles. 1*a*. Neuromuscular physiology of the levator of the eyestalk of *Podophthalamus vigil* (Weber). *J. exp. Zool.* **167**, 471–86.
HOYLE, G. & WIERSMA, C. A. G. (1958). Excitation at neuromuscular junctions in crustacea. *J. Physiol.* **143**, 441–53.
ILES, J. F. & PEARSON, K. G. (1971). Coxal depressor muscles of the cockroach and the role of peripheral inhibition. *J. exp. Biol.* **55**, 151–64.
KENNEDY, D. & TAKEDA, K. (1965*a*). Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. *J. exp. Biol.* **43**, 211–27.
KENNEDY, D. & TAKEDA, K. (1965*b*). Reflex control of abdominal flexor muscles in the crayfish. II. The tonic system. *J. exp. Biol.* **43**, 229–46.
LARIMER, J. L. & KENNEDY, D. (1969). The innervation patterns of fast and slow muscles in the uropods of crayfish. *J. exp. Biol.* **51**, 119–33.
MAYNARD, D. M. (1965). The occurrence and functional characteristics of heteromorph antennules in an experimental population of spiny lobsters, *Panulirus argus*. *J. exp. Biol.* **43**, 79–106.
MENDELSON, M. (1969). Electrical and mechanical characteristics of a very fast lobster muscle. *J. Cell Biol.* **42**, 548–63.
PANTIN, C. F. A. (1948). *Notes on Microscopical Technique for Zoologists.* Cambridge University Press.
PARNAS, I. & ATWOOD, H. L. (1966). Phasic and tonic neuromuscular systems in the abdominal extensor muscles of the crayfish and rock lobster. *Comp. Biochem. Physiol.* **18**, 701–23.
PASZTOR, V. M. (1968). The neurophysiology of respiration in decapod crustacea. I. The motor system. *Can. J. Zool.* **46**, 585–96.
PAUL, D. H. (1971). Swimming behaviour of the sand crab, *Emerita analoga* (Crustacea, Anomura). II. Morphology and physiology of the uropod neuromuscular system. *Z. vergl. Physiol.* **75**, 259–85.
PEARSON, K. G. & ILES, J. F. (1970). Discharge patterns of coxal levator and depressor motoneurons of the cockroach, *Periplaneta americana*. *J. exp. Biol.* **52**, 139–65.
PEARSON, K. G. & ILES, J. F. (1971). Innervation of coxal depressor muscles in the cockroach, *Periplaneta americana*. *J. exp. Biol.* **54**, 215–32.
ROBERTSON, J. D. (1953). Further studies of ionic regulation in marine invertebrates. *J. exp. Biol.* **30**, 277–96.
SHERMAN, R. G. & ATWOOD, H. L. (1971). Structure and neuromuscular physiology of a newly discovered muscle in the walking legs of the lobster *Homarus americanus*. *J. exp. Zool.* **176**, 461–74.
SNOW, P. J. (1973). The antennular activities of the hermit crab, *Pagurus alaskensis* (Benedict). *J. exp. Biol.* (preceding paper).
WIERSMA, C. A. G. & RIPLEY, S. H. (1952). Innervation patterns of crustacean limbs. *Physiologia comp. Oecol.* **2**, 391–405.

EXPLANATION OF PLATE

A light micrograph of a cross-section of nerves 2 and 2a. Note the two axons 30F and 30S in nerve 2a and the giant axons 31F and 32F of nerve 2. Scale – 50 μm.


N2
N2a