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INNERVATION PATTERNS OF FAST AND SLOW MUSCLE IN THE UROPODS OF CRAYFISH

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INTRODUCTION

In previous publications from this laboratory (Kennedy & Takeda, 1965*a*, *b*; Evoy & Kennedy, 1967; Kennedy, Evoy & Hanawalt, 1966; Evoy, Kennedy & Wilson, 1967) we have analysed the innervation of abdominal flexor and extensor muscles in the crayfish, and have specified the role of central interneurones in evoking co-ordinated, multisegmental postural movements. Movements involving the axial muscles of the abdomen occur in only a single plane, and are based upon relatively simple reciprocal relationships between excitatory and inhibitory motor outflows to the antagonistic slow flexor and extensor muscles. In the investigations described in this and the following paper we have extended this method of analysis to a more complex system controlling the appendages of the terminal abdominal segment. The uropods of crayfish perform a variety of movements concerned with righting responses, steering during locomotion and the like; and their motor control is more complex in several respects than that of the axial musculature. First, they are movable about three axes (rotation, extension/flexion and promotion/remotion) instead of just one. Secondly, their movements are often bilaterally asymmetric, as in steering reactions. Thirdly, phasic and tonic elements are more intermixed than in the abdominal musculature, and various types of movements can be produced by either category. Finally, some of the muscles operating the uropods show homologies primarily with the abdominal series, while others belong more clearly to the appendages themselves. The situation is thus intermediate between the neuromuscular systems of the abdomen and those of the well-studied thoracic limbs (for reviews see Kennedy, 1967; Atwood, 1967).

The first paper in this series describes the innervation and electrical characteristics of 12 of the more accessible uropod muscles, and provides some information on nearby muscles that flex or extend the telson. It will be shown that some muscles are purely phasic; these exhibit electrically excitable membrane responses and are innervated by phasic motoneurones lacking spontaneous activity. Others are tonic; they show only graded junctional potentials (j.p.s) generated by tonic, often 'spontaneous' motor activity. Still others have tonic and phasic portions, either with or without overlap of innervation from motoneurones of the two classes. We have gathered data on the number of motoneurones innervating the different muscles, the number innervating

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individual fibres, the properties of the j.p.s produced, the reflex sources of activation for various motoneurones and the presence or absence of peripheral inhibition.

In addition to expanding our knowledge of the variations in innervation pattern of crustacean muscles, the observations reported in the present paper provide a basis for experiments on the central nervous control of appendage movements. The second paper in this series will deal with postural movements evoked by the stimulation of single central 'command' interneurones.

METHODS

Crayfish (*Procambarus clarkii*, collected locally) were pinned ventral side up in a Lucite dish in Van Harreveld's (1936) solution after their blood had been replaced by perfusion with the physiological solution (Pabst & Kennedy, 1967). The soft cuticle overlying the uropod muscles was removed, along with appropriate portions of the protopodite and exopodite wall, so as to expose the muscles (see Fig. 1, Plate 1). In order to record from some of the deeper-lying muscles, the more superficial ones were removed as well. The nerves were left intact and were stimulated or recorded from through suction electrodes made from large-diameter micropipettes connected through polyethylene tubing to a micrometer syringe. Such electrodes were mounted flexibly on a micromanipulator, manoeuvred into the vicinity of the nerve and attached to it with light suction. This arrangement allowed *en passant* recordings to be made without altering impulse traffic in the nerves or distorting visualization through the use of oil. A silver wire sealed into the tubing led to one input grid of a standard capacity-coupled pre-amplifier; the indifferent lead was another piece of silver wire wrapped around the pipette. Similar electrodes were used to apply brief-pulse (*c.* 0·1 msec.) stimuli from electronic pulse generators to the nerves. Intracellular recording from muscle fibres was accomplished using 3 M-KCl-filled micropipettes; these had resistances in the range of 5–20 MΩ. They were mounted on flexible wires suspended from micromanipulators, in a 'floating' configuration which allowed stable penetrations to be made even in phasic muscles which twitched vigorously upon stimulation. Intracellularly recorded signals were led through neutralized-capacity pre-amplifiers (Bioelectric Instruments, Inc.). Conventional multichannel oscilloscope displays were photographed on film or positive paper to make permanent records.

The procedures used in these experiments were, for the most part, aimed at correlating the discharge of specific neurones with junctional events recorded intracellularly in one or more muscle fibres. The general method has been described in earlier papers (Kennedy & Takeda, 1965*a*, *b*; Fields, 1966). In the case of tonic muscles an *en passant* record of activity in the appropriate nerve was displayed simultaneously with one of junctional activity in a muscle fibre; the animal was subjected to a variety of natural stimuli, and attempts were made to correlate each size-class of impulses in the nerve with a particular type of junctional event in the muscle fibre. For phasic muscles the nerve was stimulated electrically with gradually increasing intensity, usually while the efferent volley was monitored with a second, more distal electrode. Increments in the amplitude of j.p.s evoked in the muscle fibre(s) could thus be associated with the recruitment of new motor axons in the efferent volley.

Measurements of sarcomere length were carried out on freshly teased fibres, using a phase-contrast microscope equipped with an ocular micrometer.

RESULTS

(1) *Anatomy and organization*

Schmidt (1915) has described the arrangement of uropod and telson muscles in *Astacus*. We have found that his anatomical conclusions apply to *Procambarus* as well, except for minor differences in the shape and position of certain muscles. Plate 1 is a version of one of Schmidt's figures that has been redrawn and corrected for our species. Schmidt's names for the muscles are often incorrect and misleading. They sometimes are based upon actions inferred from their origins and insertions (e.g. posterior telson flexor), sometimes are strictly anatomical (e.g. telson-uropodalis muscle) and sometimes mix anatomical and functional terms (e.g. dorsal rotator). We have used his terminology in the present account, but suggest that some of the names be changed as indicated in Table 1.

The muscles fall naturally into several groups, based upon their actions. We consider here only those muscles that lie entirely within the fifth segment or in the appendages themselves, and serve to move the uropods and telson with respect to the segment. Obviously, other muscles—such as the main abdominal oblique and deep extensor muscles—will move the tail fan indirectly as a result of their actions on segment 5. The main groups of uropod and telson muscles are indicated in Table 1. This listing classifies individual muscles into functional groups and gives data on their innervation. The following account of muscle groups and their actions is a useful preliminary to the analysis of innervation which forms the main body of this paper.

Movements in the plane of extension/flexion involve a large number of muscles, most of which appear to be serially homologous with axial abdominal muscles. Both groups of antagonists are innervated from the fifth as well as the sixth abdominal ganglion; each contains phasic as well as tonic muscles. The flexor muscles are more numerous and powerful than the extensors, as is the case with the abdominal muscle of more anterior segments. The rotator muscles form a special group. Though they accomplish some flexion, their most evident action is to twist the uropods so that their lateral margins move ventrally; the effect is to 'cup' the tail fan. The two rotator muscles contain tonic and phasic components, and have a rich innervation derived from different neurotomes. The final grouping comprises those muscles that promote and remote the uropods. These are presumably the homologues of coxopodite and basopodite muscles in the thorax (Pilgrim & Wiersma, 1963) and of corresponding muscles in the swimmerets (Davis, 1969). Here again, each group of synergists includes a tonic muscle. The individual muscles are all small, and the phasic ones have an innervation that is relatively simple compared to that of the larger flexors.

(2) *Phasic/tonic differentiation*

For the most part the muscles of the uropods and telson are either purely phasic or purely tonic. The phasic ones have short sarcomeres, often produce electrically excitable membrane responses, give brief twitches to single stimuli and are innervated by relatively large, normally silent motoneurones. The tonic muscles, on the other hand, have long sarcomeres, seldom show 'spikes' rising out of junctional potentials, respond with graded contractions to constant-frequency stimulation but fail to respond to single shocks, and are innervated by motoneurones that usually show spontaneous

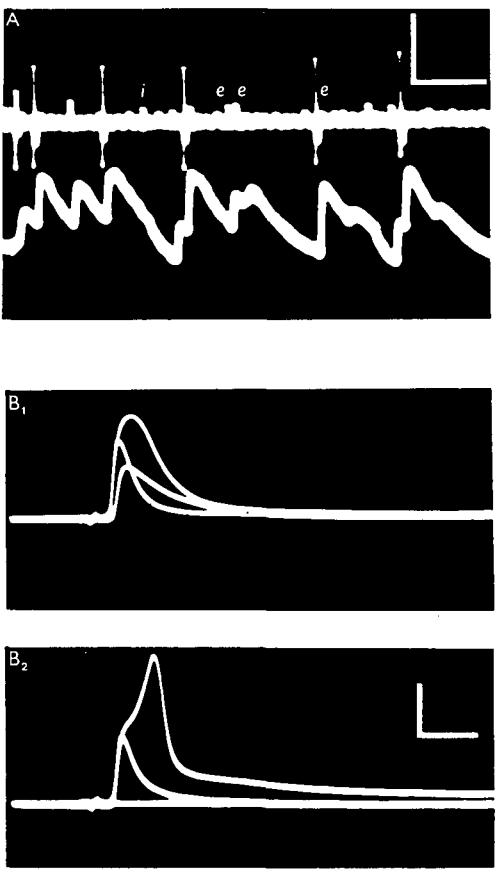
Table 1. *Actions and innervation of uropod muscles*

Muscle*	Action	Type	Innervation	Axons/muscle	Max. no. axons/fibre
Flexors					
Anal compressor (ventral telson flexor)	Telson flexion	Phasic	R6 G6	3E, 1I	3
Anal dilator (slow telson flexor)	Telson flexion, uropod abduction	Tonic	R6 G6	3+E, 1I	4
Posterior telson flexor	Telson flexion	Phasic	R6 G6	5E, 1I	5
Anterior telson flexor	Telson flexion	Phasic	R6 G6	1E	1
Telson uropodalis anterior	All flex, and close uropods on telson	Phasic	R2 G6	2+E	2
Telson uropodalis posterior		Phasic	R2 G6	2+E	2
Telson uropodalis lateral		Phasic	R2 G6	2+E	2
Rotators					
Ventral rotator	Rotation, some adduction and flexion	Mixed	R3 G5	2+tonic 3+phasic	–
Dorsal rotator	Rotation and flexion	Mixed	R1 G6	2E, 1I tonic 4+E phasic	3
Extensors					
Superficial extensor	Telson extension	Tonic	R2 G5	3+E, 1I	4
Lateral remotor	Remotion and extension of uropod	Phasic	R3 G6	2E	2
Medial remotor	Slight remotion and extension or uropod	Phasic	R3 G6	3E	2
Promotors					
Abductor expodite lateral (lateral promotor)	Promotion	Phasic	R3 G6	2E, 1I	3
Abductor exopodite dorsal (dorsal promotor)	Promotion	Phasic	R3 G6	2+E, 1I	3
Abductor exopodite ventral (slow promotor)	Promotion, some rotation	Tonic	R3 G6	2+E, 1I	3
Remotors					
Adductor exopodite (lateral remotor)	Remotion, closing exopodite upon endopodite	Phasic	R2 G6	1E	1
Productor exopodite (dorsal remotor)	Remotion, closing exopodite upon endopodite	Phasic	R2 G6	1E	1
Reductor exopodite (slow remotor)	Remotion, closing exopodite upon endopodite	Tonic	R2 G6	2E, 1I	3

* The names in the first column are those of Schmidt (1915). Where renaming is necessary, the proposed new names are given in parentheses.

activity. Tonic and phasic muscles are sometimes found in sets having roughly the same action but entirely separate innervation. The anal dilator and anal compressor muscles provide a typical example of this division. Sarcomeres in the former muscle average 10 μ in length, those of the latter 5 μ. Both originate near the base of the uropod, and insert on soft cuticle near the anus. The latter feature led Schmidt (1915) to misname both muscles; while they incidentally change the shape of the anal opening, their more important action is to flex the tail fan via their action upon the telson. The anal dilator may in addition produce some abduction of the uropod.

Both muscles are innervated via the sixth roots of the sixth abdominal ganglion



Text-fig. 1. Responses of tonic and phasic flexor muscles. A, anal dilator; lower trace, intracellular electrode in a single muscle fibre; upper trace, simultaneous *en passant* record from the sixth root, sixth ganglion. All activity is spontaneous. An impulse in the inhibitory axon is marked '*i*', impulses in three excitatory axons are marked '*e*'. Calibrations: 10 mV., 50 msec. B, anal compressor: both records are from microelectrodes in single fibres in the same muscle. The superimposed traces represent three intensities of stimulation of the sixth root, ganglion six. In B_1 two excitatory axons innervated the fibre; the smaller of the two long-duration junctional potentials is due to the lower-threshold axon, and the larger resulted from recruitment of the second. The inhibitor had the highest threshold; when it was recruited the shorter-duration, attenuated potential resulted. In B_2 only one of the excitatory axons innervated the muscle fibre. When the inhibitor was recruited (smaller potential) the excitatory junctional potential was reduced below the level required to initiate the electrically excitable membrane response. Calibrations: 20 mV., 10 msec.

(a diagram of the roots of the sixth ganglion is given by Barth, 1964). As Text-fig. 1 shows, however, they receive an entirely separate innervation. Fibres of the tonic anal dilator show continuous junctional activity, and individual j.p.s can be associated with the activity of identifiable motoneurones in an *en passant* recording from the sixth root. Text-fig. 1A shows that the muscle fibre in question receives input from the small and from one large excitatory axon, and from a peripheral inhibitory axon as well. The fibres of the anal compressor muscle never exhibit spontaneous junctional activity. They do, however, respond with large j.p.s to electrical stimulation of the sixth root. Text-fig. 1B shows such responses in two different fibres from the same preparation. That in Text-fig. $1B_1$ received two excitatory axons, that in Text-fig. $1B_2$ only one; each also was innervated by a peripheral inhibitor with a threshold higher than that of the exciters. The effect of the inhibitor, as in the abdominal muscles, was to reduce the amplitude and hasten the falling phase of excitatory j.p.s (cf. Kennedy & Takeda, 1965*a*; Parnas & Atwood, 1966). In Text-fig. $1B_2$ the excitatory j.p. produced an electrically excitable membrane response, which the inhibitor blocked by reducing the amplitude of the triggering junctional potential below the firing level.

(3) '*Mixed*' *muscles*

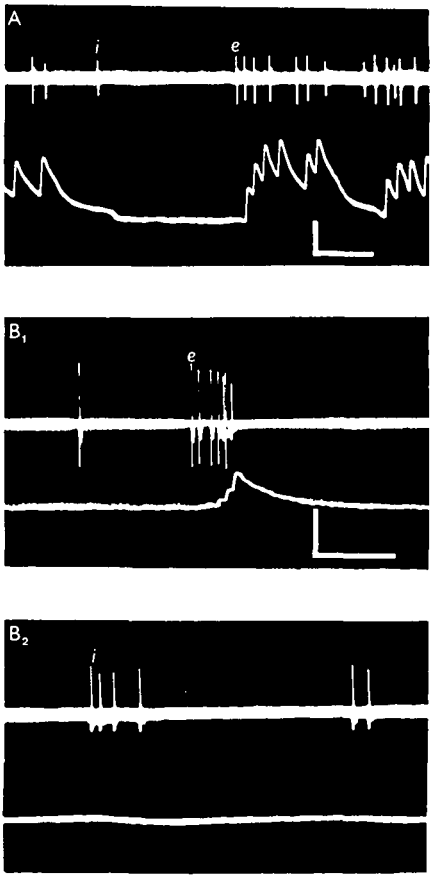
In several of the muscles investigated we found evidence for a separation into tonic and phasic regions. In no case were tonic fibres found intermingled with phasic ones, as in many limb muscles (Atwood, 1963, 1965); instead, one margin of the muscle mass was occupied by a tonic 'slip', which was discrete though not anatomically separated into a distinct head. The dorsal rotator muscle was chosen as an example of this arrangement for detailed study, since it is unusually accessible and is innervated by a single, purely motor nerve, the posterior branch of the first root from the sixth abdominal ganglion.

Tonic fibres of the dorsal rotator are located near the lateral margin of the muscle mass, and have sarcomeres averaging 8·5 μ in length. The phasic fibres that comprise most of the muscle have 4 μ sarcomeres. In no case were we able to observe spontaneous j.p.s by penetrating fibres away from the lateral slip. The tonic fibres, however, received excitatory and inhibitory innervation from spontaneously active axons. The record shown in Text-fig. 2A shows one excitatory and one inhibitory axon in an *en passant* recording, correlated with j.p.s of the appropriate sign from a microelectrode located in a tonic muscle fibre. On some occasions a second excitatory axon was seen, but it does not appear in the record shown.

The clear difference in size and hence in spike amplitude between the single exciter that was usually active and the peripheral inhibitor made it possible to analyse the reflex sources of activity for these two axons. As Text-fig. 2B and 2C show, stimulation of sensory hairs in the abdominal region and the uropods evoked discharge in the excitatory axon, while stroking the thorax normally produced inhibitory outflow. This reciprocity between exciter and inhibitor axons has been documented further by the results of stimulating central interneurones (Larimer & Kennedy, 1969).

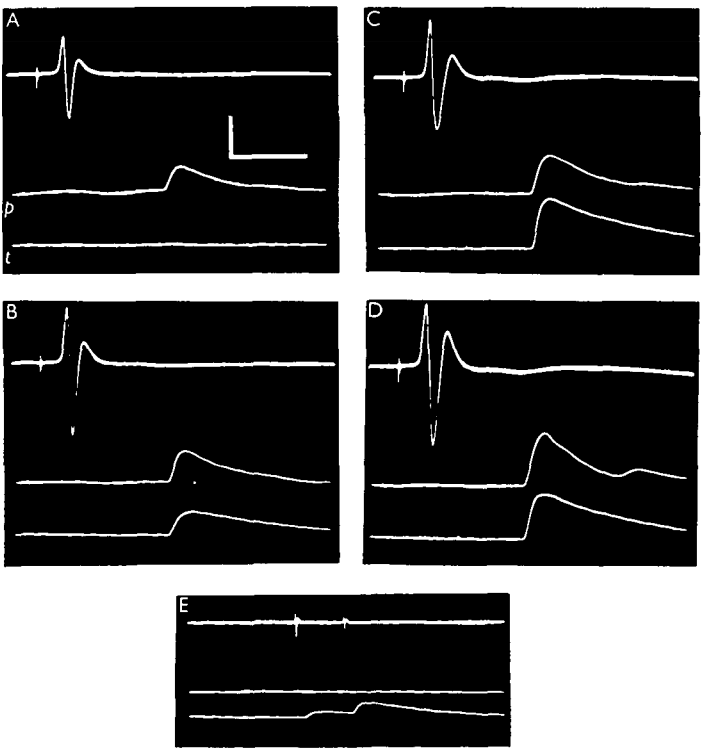
None of the phasic fibres in the dorsal rotator muscle showed spontaneous j.p.s, but the number of axons innervating a fibre could be revealed by increments in the response to shocks of increasing voltage delivered to the motor branch of the first root. Experiments of this kind showed that single fibres received as many as four excitatory axons; no inhibitory innervation was found for any phasic dorsal rotator fibres.

Simultaneous microelectrode penetrations were made of tonic and phasic fibres in the dorsal rotator to see whether the former received innervation from any axons supplying the phasic portion of the muscle. Text-fig. 3 shows the results of such an experiment. The tonic muscle fibre (bottom traces) showed j.p. increments that were associated with impulses in axons that also produced j.p.s in the phasic fibre (Text-fig. 3 B, C); the axons of lowest and highest threshold innervated the phasic muscle



Text-fig. 2. Inhibitory and excitatory innervation of tonic dorsal rotator muscle fibres. A, identification of inhibitory (i) and excitatory (e) impulses in the first root of ganglion six (upper trace), and their correlation with hyperpolarizing and depolarizing junctional potentials recorded intracellularly in a muscle fibre (lower trace). B, Reflex sources for activation of excitatory and inhibitory outflow; recording as in A. B_1, Stimulation of abdominal hairs; B_2, stimulation of thoracic hairs. Calibrations for A and B: 5 mV., 50 msec.

fibre exclusively. Neither of the axons recruited by electrical stimulation was the same as that showing spontaneous activity (Text-fig. 3 E); the j.p.s produced by the latter were smaller than either increment. Thus the tonic and phasic portions of the dorsal rotator muscle share some axons, while retaining separate innervation from others. Specifically, the tonic fibres alone receive the axon(s) showing spontaneous activity, while the phasic fibres are exclusively innervated by at least one and perhaps two low-threshold axons.



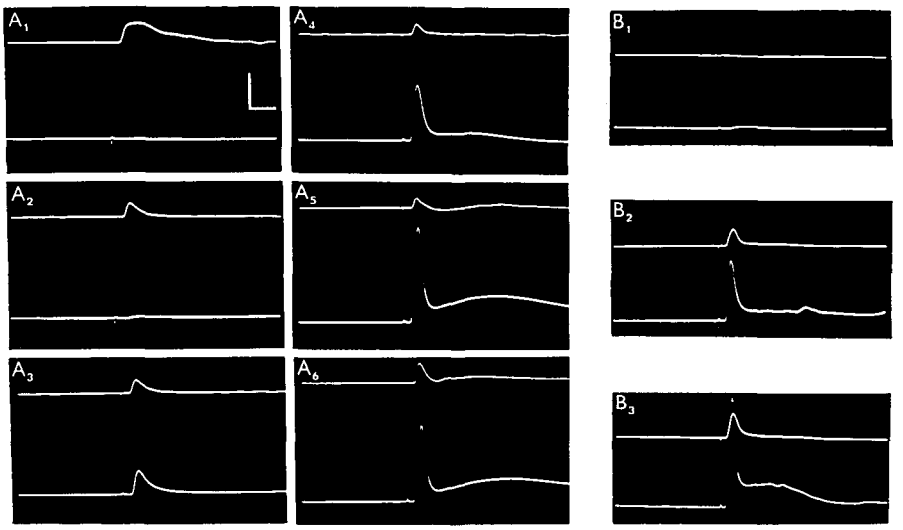
Text-fig. 3. Innervation of tonic and phasic fibres in the dorsal rotator muscle. First trace, *en passant* record from the first root, sixth ganglion; second trace, intracellular record from a phasic fibre (*p*); third trace, intracellular record from a tonic fibre (*t*). In A–D shocks of increasing intensity were delivered to the first root through a proximally located electrode; E shows impulses in a spontaneously active axon going only to the tonic fibre. Calibrations: 20 mV. for all records; 20 msec. for A–D, 50 msec. for E. The time scale for the upper trace in A–D should be magnified 10×. For further description, see text.

(4) *Phasic muscles*

The sharing of innervation between synergistic phasic muscles was also investigated. The anal compressor, discussed above, flexes the telson; so do two exclusively phasic muscles named the anterior and posterior telson flexor by Schmidt. All three are innervated by the sixth root of the sixth abdominal ganglion, though some axons may actually emerge from the fifth root since it is fused with the sixth along part of its length. The posterior telson flexor has a rich innervation; Text-fig. 4 shows a comparison between it and the anal compressor in this regard. In each case dual penetrations were made of fibres in different regions of the muscles, and responses to graded stimulation of the sixth root were recorded. The two fibres from the posterior telson flexor had a completely non-overlapping excitatory innervation, but shared a peripheral inhibitor (the threshold for this axon was exceeded in Text-fig. $4A_2$; though it produced a depolarizing inhibitory j.p. in the lower trace, it shortened and attenuated the excitatory j.p. in the top trace, and subsequent responses in the bottom trace were similarly short). Two increments of j.p. amplitude were seen in the top trace, and three in the bottom trace; thus a total of at least five excitatory axons innervate the posterior telson flexor, and they are distributed to it in a regionally differentiated

fashion. The inhibitor, however, is common to fibres in all regions. The axons innervating the anal compressor muscle are, in contrast, fewer in number and more ubiquitous. In Text-fig. 4B the inhibitory axon is seen to have the lowest threshold; it produced small j.p.s in Text-fig. $4B_1$, depolarizing in one case and hyperpolarizing in the other, and subsequent responses were brief in duration. The two excitatory axons found in this experiment each produced j.p. increments in both muscle fibres.

To determine whether any of the axons supplied by the sixth root to these two muscles were shared, experiments like that shown in Text-fig. 5 were performed.

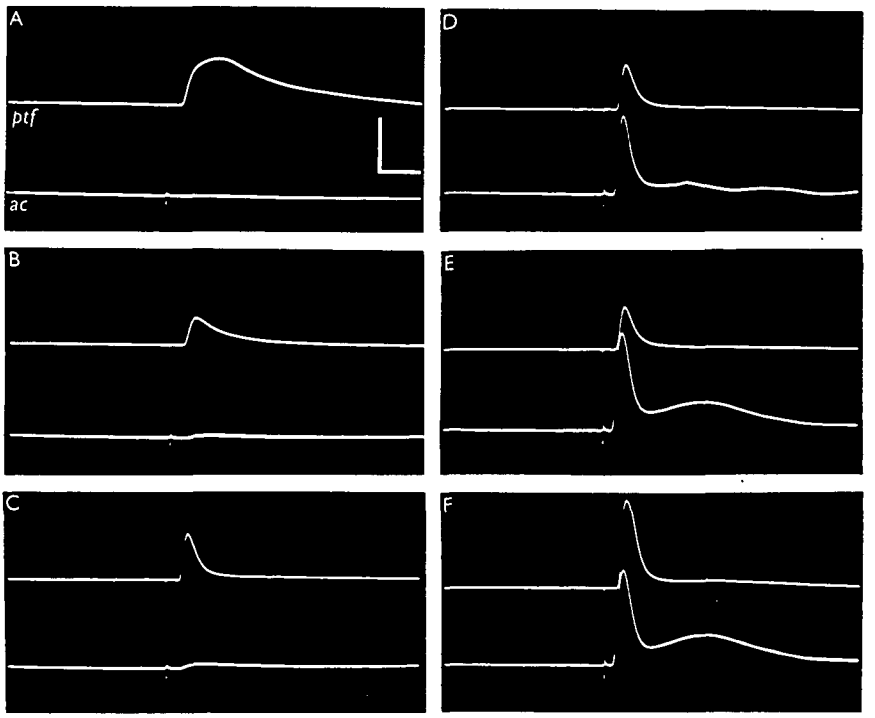


Text-fig. 4. Simultaneous intracellular recording from two muscle fibres in the posterior telson flexor (A) and the anal compressor (B). In each series, shocks of increasing intensity were delivered to the sixth root, ganglion six; all increments in the amplitude of the junctional potentials are illustrated. Calibrations: 25 mV., 10 msec. For further description, see text.

Simultaneous microelectrode recordings were made from fibres in each of the two muscles while a series of graded stimuli were delivered to the sixth root. The fibre in the anal compressor received two excitatory axons and that in the posterior telson flexor three; none of the motor neurones innervated *both* fibres. The inhibitor, however, was clearly common to the two muscles. Its appearance in Text-fig. 5B was signalled by a shortening and attenuation of the response in the posterior telson flexor, and by a slight depolarization in the anal compressor. Subsequent responses in the anal compressor muscle were short, as is characteristic for fibres receiving simultaneous excitatory and inhibitory input. The anterior telson flexor was shown to receive but a single excitatory axon, and no inhibitor. Its excitatory innervation is not shared with either of its phasic synergists.

Similar sharing experiments were conducted on two other synergistic groups of phasic muscles. The lateral and medial remotor muscles lie in a peripheral position just anterior to the uropods; they originate on the dorsal exoskeleton and insert on the basal segment of the uropod. Their action is thus to extend and remote the uropod, with the medial muscle accomplishing relatively more extension and less remotion than its lateral synergist. Each muscle receives two excitatory axons via the third root

of the sixth abdominal ganglion, and neither appears to be supplied with an inhibitor. One of the exciters to the lateral remotor is ubiquitous, while the other innervates only fibres on the medial aspect of the muscle. The axons supplying the medial remotor are distributed fairly widely in that muscle, but neither supplies the synergist as well.



Text-fig. 5. Simultaneous intracellular recording from muscle fibres in the posterior telson flexor (upper trace, *ptf*) and the anal compressor (lower trace, *ac*). In A–F shocks of increasing intensity were delivered to the sixth root, ganglion six; all increments in the amplitudes of junctional potentials are illustrated. Calibrations: 25 mV., 10 msec. For further description, see text.

The productor and adductor of the exopodite are thin phasic muscles located side by side in the protopodite; their origin is on the medial protopodite wall and their insertion on the exopodite. Both serve to remote and rotate the exopodite, closing it on the endopodite. Each receives but a single excitatory axon and is apparently without a peripheral inhibitor. As with the remotor and telson flexor series, the excitatory axons innervating the muscles are different.

(5) *Other muscle groups*

(*a*) *Muscles of the fifth segment*

Several muscles innervated from the fifth abdominal ganglion act primarily upon the terminal appendages; these include the superficial flexor muscle, the ventral rotator and the superficial extensors. These muscles are related to those of more anterior segments; since the latter are well known in terms of their innervation

(Kennedy & Takeda, 1965*a*, *b*; Fields & Kennedy, 1965), we have tried to compare the groups in the two regions.

In the fifth segment the third root—which innervates all flexor muscles—branches in a more complex way than in more anterior segments; a lateral component, proximally associated with the main third root, innervates the ventral rotator muscle. This lateral component contains large-diameter, phasic axons as well as tonic ones, which is to be expected since the ventral rotator is a mixed muscle. The superficial branch contains tonic axons alone, and innervates the superficial flexors. These muscles, not shown in Pl. 1, are located medially in the fifth segment. It is possible that their missing lateral portions are represented by the tonic part of the ventral rotator. Simultaneous recording from the roots indicated that the lateral division supplying the ventral rotator contains two or more axons that are *not* shared with the superficial branch. Some other axons appear in both branches; but in a few experiments we did not find evidence for sharing of innervation between individual pairs of superficial flexor and ventral rotator fibres. The tonic axons that supply the superficial flexor muscles are identical in number and function with those of more anterior segments, but their size relationships are considerably altered. The inhibitory axon is the fourth instead of the second largest, and the three largest excitatory axons from the third root of the fifth segment are much more alike in diameter than those of more anterior segments. Cutting the nerve cord between the third roots and the fifth ganglion eliminated *all* tonic motor outflow, indicating that—unlike anterior segments—the posterior ganglion does not contribute a small axon to the superficial flexor supply.

The extensor muscles lie directly under the dorsal exoskeleton of segment 5, and insert on the base of the telson. Records from the second root of the fifth ganglion demonstrated that at least three tonically active excitatory axons and one inhibitor innervate the extensors of each side. In addition, the root contains a phasic and a tonic muscle receptor organ (MRO), like those found in more anterior segments; but interestingly, no efferent axon could be found that inhibited the discharge of the slow MRO, even when that MRO was discharging at a frequency adequate to produce a strong 'feedback inhibition' reflex (Eckert, 1961; Fields, Evoy & Kennedy, 1967). We conclude that the accessory nerve is missing in the terminal segment.

(*b*) '*Horizontal positioners*'

Promotion and remotion are accomplished in part by small muscles located in the protopodite, which include various abductors and adductors of the two uropods. Schmidt's terminology for these muscles is perplexingly diverse; in fact, they can be classified in two antagonistic sets. The abductors comprise one group, and all produce promotion; they are innervated from the third root of the sixth ganglion. The remotors comprise the other which includes the adductor exopodite, productor exopodite and reductor exopodite. These muscles are all innervated from the second root of the sixth ganglion. One muscle in each set is tonic (abductor exopodite ventral and reductor exopodite); each of the tonic muscles is supplied by at least two excitatory axons and an inhibitor. The phasic remotor muscles each have only a single excitatory axon, which is not shared. The phasic promotors are more complexly innervated, each having two exciters and an inhibitor; the sharing relationships among these are described in section 4 above.

(c) Other flexors and rotators

Muscles of the anal compressor-telson flexor and rotator series have been described above. The small muscles of Schmidt's telson-uropodalis series also probably contribute to flexion as well as holding the telson and uropods together, so that the larger muscles may move the tail fan as a unit. The fibres in the telson-uropodalis muscles all seem to receive dual phasic excitatory innervation.

DISCUSSION

Phasic-tonic differentiation

The uropod muscles exhibit, for the most part, a degree of differentiation between fast and slow elements that is intermediate between the complete separation characteristic of abdominal muscles (Kennedy & Takeda, 1965*a*, *b*; Fields & Kennedy, 1965; Parnas & Atwood, 1966) and the more extensive mixing seen in limb muscles (e.g. Hoyle & Wiersma, 1958; Atwood, 1963). In the abdomen there are completely separate muscles with pronounced histological differences and an entirely separate innervation. In the limbs a variety of arrangements are found. The fibres of a muscle innervated by a single motor nerve may display a spectrum of structural and physiological 'speeds', from long-sarcomere fibres with passive membranes to short-sarcomere fibres with fully electrically excitable membranes, as in the accessory flexor of the meropodite (Cohen, 1963; Dorai Raj, 1964; Atwood & Dorai Raj, 1964). Some crustacean opener muscles display a similar range of muscle fibre properties, though in others, like that of the crayfish, it is the differentiation of nerve terminals rather than of muscle fibres that produces regional differences in frequency response (Bittner, 1968). Most of the limb muscles receive two or more excitatory axons; these often produce responses that show marked temporal differences, and are thus termed 'fast' and 'slow'. Within the muscles there may be fibres with a range of properties; presumably the more phasic fibres tend to receive the fast axon and more tonic ones the slow, but many are innervated by both, and muscle fibres with intermediate properties are common (Atwood, 1965). Even where muscle fibres are clearly either phasic or tonic, they may either be mixed or regionally segregated by type. Commonly, there is a high degree of mingling.

The majority of the uropod muscles are clearly in either a phasic or a tonic category, resembling the axial muscles of the abdomen much more than the limb muscles. In the only cases where muscles are mixed the tonic portion is an identifiable slip of the phasic one, with little intermingling of fibres. In these cases there is, however, an overlap in innervation: some motor axons serve both tonic and phasic portions of the muscle. At the extremes of the phasic-tonic spectrum there is still some private innervation: the largest axon exclusively serves fast muscle fibres and the smallest—the only one with spontaneous activity—exclusively serves tonic ones.

There is no evidence of sharing of excitatory innervation between any two muscles that are anatomically separate. This situation generally parallels that in the limbs, though in the limbs there is the well-known exception that the opener of the dactylopodite and the stretcher of the carpopodite share an axon (Wiersma & Ripley, 1952).

The frequency of shared inhibitory outflow in the uropod muscles parallels that in the limbs.

A striking feature of the uropod muscles is their tendency to receive a rich phasic innervation. Fibres with a quadruple excitatory innervation have been encountered a number of times in our experiments; several rather small phasic muscles receive three, four, or five excitatory axons. Some of these show a restricted regional distribution (e.g. one of the two supplying the lateral remotor muscle); others are ubiquitous. Little use has been found thus far for the motor unit concept for crustacean muscles, but the wealth of innervation supplying these twitch muscles suggests that recruitment might play a role in the gradation of power.

Finally, it is clear that the system of muscles and neurones required to run a relatively simple appendage is complex, even in a group of organisms renowned for the economical design of their motor systems. Eighteen muscles and at least 55 efferent neurones are invested in the task of moving the appendages of a single half-segment.

Homologies

The strict phasic/tonic dichotomy, and the location as well as the function of some of the muscles, suggests that they are serially allied with the axial muscles of more anterior segments. In all these respects the anal dilator and anal compressor muscles behave respectively like serial homologues of the superficial flexor and anterior oblique muscles in more anterior segments (Kennedy & Takeda, 1965*a*, *b*). The phasic muscles, i.e. the anal compressor and anterior oblique muscles, in both cases usually receive no more than two excitatory axons and one inhibitory axon in a given segment; the tonic muscles, the anal dilator and superficial flexors, in contrast, are innervated by several spontaneously active motoneurones. The sarcomere lengths agree fairly closely with those of the presumed anterior homologues (Kennedy & Takeda, 1965*b*) and the two muscles of the sixth segment are innervated by the sixth roots of the last abdominal ganglion, which may correspond to the third roots of more anterior ganglia. It is also possible that the anterior and posterior telson flexor muscles, both of which are innervated by the sixth root of the sixth abdominal ganglion, correspond to the posterior oblique and transverse muscles of more anterior segments. The superficial extensors and flexors innervated from segment 5, together with the ventral rotator, are apparently allied with the main axial muscles. Other muscles clearly have specific involvement with the tail appendages and cannot readily be homologized.

Some of the direct comparisons of neurones and muscles with more anterior homologues are worth special mention. The superficial flexor muscles of the fifth abdominal segment are more medial than those of rostral segments, and their innervation is altered in three detailed respects (cf. Kennedy & Takeda, 1965*b*). First, instead of being the second-largest axon, as it is in the third segment, the peripheral inhibitor is the fourth largest. Secondly, the largest exciter is more nearly equal in size to the others than it is in more rostral segments. Finally, one of the two smallest exciters does not come from the next caudal ganglion, as it does in the third segment (Evoy & Kennedy, 1967). These are minor differences, but they suggest substantial changes in the development and mode of connexion of neuronal units which have clear cellular homologues in adjacent ganglia.

An interesting difference between the innervation of the telson extensor muscles

and the slow extensors of more anterior ganglia involves the absence of the accessory nerve in the former case. This axon innervates the dendrites of the muscle receptor organs, and when active, it inhibits the discharge of the sensory neurone. It is involved in a reflex which Eckert (1961) first described as 'feedback inhibition' because activity in an MRO produced efferent discharge in the accessory nerve. More recently it has been shown that in fact this reflex is much more strongly directed to the accessory nerve of the next anterior segment than to that of the stimulated one; the effect is such that flexion in a given segment, which produces MRO discharge, inhibits the next anterior MRO (Fields *et al.* 1967). It has been postulated that this reflex relationship might function as an intersegmental co-ordinating mechanism for postural accommodation, by suppressing the resistance reflex mediated by each MRO on its own extensors and thus causing a rostral spread of 'flexor bias'. If the fifth segment had an accessory nerve, it could be useful only for self-inhibition, since there would be no more posterior MRO available to drive it intersegmentally. The fact that it is absent is consistent with the argument that the inhibition of MRO's by the accessory nerve is basically a phenomenon involved in intersegmental control.

SUMMARY

1. The innervation of 18 uropod and telson muscles in the crayfish has been studied in varying detail. Some muscles display purely phasic properties (short sarcomeres, electrically excitable membrane responses); others are strictly tonic (long sarcomeres, junctional potentials only, spontaneously active motor neurones); a few are mixed, but with anatomical segregation of fibre types. At least 55 efferent neurones are employed to operate these muscles.

2. The tonic muscles all receive peripheral inhibition, and a given inhibitory axon serves only a single tonic muscle. Inhibitory and excitatory axons serving a given muscle discharge reciprocally and often respond to different reflex inputs. The average number of excitatory axons innervating tonic muscles is 2–3.

3. The phasic muscles differ in the richness of excitatory innervation. Some receive as many as five axons per muscle, and as many as four per fibre; others are innervated by only a single motor axon. Many lack inhibitory innervation; where present, peripheral inhibitors may be shared between different phasic muscles. In no case were excitatory axons found to innervate anatomically separate muscles.

4. In 'mixed' muscles there is predominantly separate innervation of phasic and tonic fibres; but in at least one case axons were found to serve fibres of both types. The shared axon was phasic in character.

5. Some of the uropod and telson muscles are clearly homologous with axial muscles in more anterior abdominal segments. In a few of the former it was possible to make direct comparisons with the efferent neurones innervating their serial homologues. Differences were found in the size ratios of homologous axons, in their central location, and in their presence or absence.

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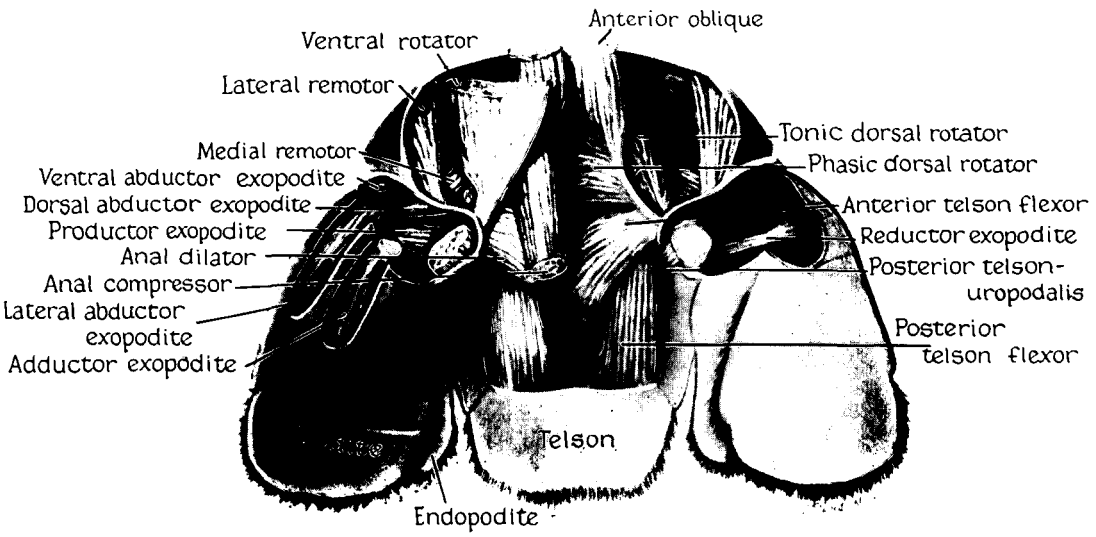
Anterior oblique
Ventral rotator
Lateral remotor
Medial remotor
Ventral abductor exopodite
Dorsal abductor exopodite
Productor exopodite
Anal dilator
Anal compressor
Lateral abductor exopodite
Adductor exopodite
Tonic dorsal rotator
Phasic dorsal rotator
Anterior telson flexor
Reductor exopodite
Posterior telson-uropodalis
Posterior telson flexor
Telson
Endopodite

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EXPLANATION OF PLATE

Ventral view of the telson and uropod muscles, corrected from the drawing of Schmidt (1915) for *Procambarus clarkii*. Suggested changes in Schmidt's terminology are given in parentheses (Table 1).