

REFLEX CONTROL OF ABDOMINAL FLEXOR MUSCLES IN THE CRAYFISH

I. THE TWITCH SYSTEM

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The polyneuronal innervation of crustacean muscles has been well explored in decapod limbs, both comparatively at the level of entire muscles (Wiersma & Ripley, 1952) and, by means of single motor axon stimulation and microelectrode recording, at the level of single muscle fibres (Fatt & Katz, 1953*a, b*; Furshpan, 1955; Hoyle & Wiersma, 1958). Experiments of the latter sort have shown that motor axons produce excitatory junctional potentials (e.j.p.s) that may or may not give rise to active electrogenic events (spikes). The e.j.p.s evoked by different motor axons may differ in amplitude, rate of rise or fall, and tendency to facilitate upon repetitive stimulation; axons that evoke more sharply rising e.j.p.s exhibiting little facilitation have been termed 'fast', and others 'slow.'

While the fast/slow dichotomy between motor nerves was once thought to be the only agent capable of producing different kinds of contraction in the same muscle, recent evidence indicates that muscle fibres themselves may be differentiated as to the electrical constants of their membranes (Atwood, 1963; Dorai Raj & Cohen, 1964; Dorai Raj, 1964; Atwood & Dorai Raj, 1964), and that this in turn may be a reflexion of more basic structural differences (Jasper & Pezard, 1934; Cohen, 1963). The physiological evidence provided by these authors shows clearly that some muscle fibres produce twitches because their membranes can support active electrogenic responses, while others contract only tonically because they lack this ability.

Despite this wealth of information about the actions of motor nerves upon crustacean muscle fibres, and despite the presence of a well-studied system of peripheral inhibitory axons, few attempts have been made to correlate these neuromuscular actions with the reflex connexions and discharge patterns of the motoneurons themselves. The crayfish abdomen presents several features which have encouraged us to attempt this sort of analysis with it. First, the relevant regions of central nervous system and of musculature are readily exposed in otherwise intact preparations; secondly, recent experiments in this laboratory (Takeda & Kennedy, 1964), following up the earlier analysis by Wiersma (1947), have defined in some detail the location and sources of central activation for large flexor motoneurons. Thirdly, there is a clear physical separation between the flexor systems producing fast twitches and those producing slow, tonic contractions. A superficial ventral sheet of fibres, which always exhibits smoothly graded shortening, is supplied by a bundle of six small motor axons. These

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superficial muscles are sharply differentiated histologically as well as functionally from the massive underlying twitch muscles. The latter are responsible for the extremely rapid flexion that occurs during the swimming reflex, do not produce visible tonic contractions, and are supplied by ten axons of large diameter.

The emphasis in the present study is upon the modes of regulation which the central nervous system imposes upon the flexor muscles. In the second paper (Kennedy & Takeda, 1965) the tonic system will be considered; it will be shown that a complex bombardment of spontaneous excitation and inhibition reaches the slow muscle fibres, and that this efferent discharge in the six small motor axons is under the delicate control of a variety of sensory inputs. In contrast, the large axons innervating the twitch muscles normally show no spontaneous activity, and one of the effective central routes for activating them is the central giant fibres. It was this fact, indeed, which first prompted this investigation. Since the discovery that the largest flexor motoneurone of the third root, the motor giant, is activated by an electrotonic junction of short delay (Furshpan & Potter, 1959), there seemed little reason to postulate the need for additional fast flexor innervation. The fact that other motoneurones appearing to serve the same muscles have identical inputs from the central giants (cf. Wiersma & Schallek, 1947), albeit a longer delay in activation, thus constitutes something of a paradox. It will be shown that giant and non-giant motoneurones do in fact innervate the same muscle fibres (along with an inhibitory axon); an impulse in either motor axon produces an extraordinarily large junctional potential with superimposed spike, leading to a fast twitch. The differentiation between them involves their recovery cycles rather than the form of individual post-junctional events.

METHODS

All preparations were of intact crayfish (*Procambarus clarkii* Girard), pinned ventral-side up in a paraffined Perspex dissecting dish. The superficial exoskeleton was carefully removed from abdominal segments 1 to 5 inclusive, exposing the flexor muscles and the ventral nerve cord with all of its branches intact. Depending upon specific requirements of the experiment ligatures were then made around the cord in various segments with fine silk thread; these served to isolate particular regions of the central nervous system and also allowed the cord to be pulled and rolled over toward one side to expose both the underlying muscles and a greater length of the main portion of the third root. For stimulation, pairs of fine platinum wires mounted on low-power micromanipulators were placed on the third root, on the connective of that segment, or on both. In addition, a fine metal electrode (25–50 μ in tip diameter, insulated to the tip) could be manoeuvred directly on to the third root and positioned so that it selectively stimulated particular axons. The gross electrodes normally activated the largest axon (the motor giant) at lowest threshold, but this could sometimes be altered by changing the strength/duration relation of the stimulus. The junctions between central giant fibres and the motor giant axon are notoriously labile (Furshpan & Potter, 1959), so that the responses from central giant fibre stimulation often involved only the non-giant motoneurones. By combining these various stimuli with stimuli delivered from the roving microelectrode it was thus possible to study the combined effects of specific pairs of motor axons without isolating them and

disturbing their central connexions. Stimuli were square pulses of 0.1 msec. or less fed to the electrodes through RF isolation units.

Electrical events in muscle fibres were recorded using 'floating' glass capillary microelectrodes. The micropipettes, filled with 3M-KCl, had resistances (measured in physiological solution) of 10–30 M Ω : they were suspended from a vertical microdrive with a thin piece of silver wire which allowed fairly free lateral movement but still provided the requisite vertical stiffness for penetration. Often, muscle fibres could be held by the microelectrode even during the lateral movements of 1–2 mm. that accompanied violent contractions of the main flexors. In conjunction with microelectrode recording, neutralized capacity preamplifiers (Bioelectric Instruments, Inc.) were used, with conventional dual-beam oscilloscopic display and recording. For tension measurements small bundles of fibres were gripped in a pair of forceps attached to the moving anode of an RCA 5734 transducer.

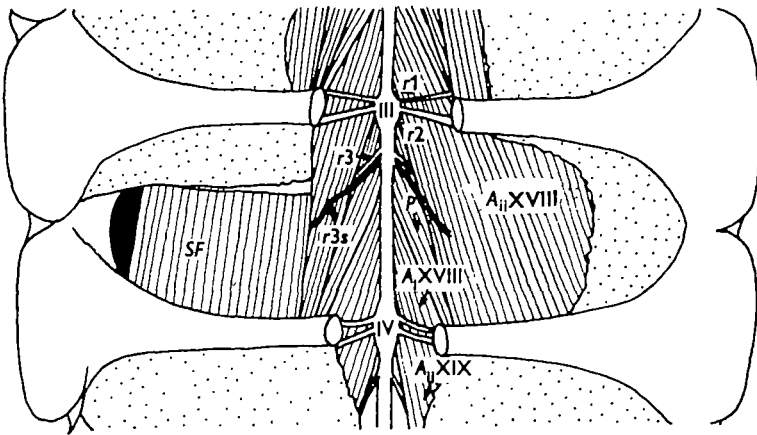
Nerves and muscles were fixed for histological examination in Bouin's or Gilson's fluid, paraffin-embedded after dehydration, and sectioned at 5 μ . Sections were stained with Masson's Trichrome or the silver procedure of Rowell (1963). Preliminary tracing of the nerve fibres, as well as analysis of the branching patterns of axons within the muscle, were accomplished with the aid of methylene blue staining of fresh preparations.

ANATOMY

The general features of the supply of motor axons to the main third root of the third abdominal ganglion have been described earlier (Takeda & Kennedy, 1964). The root contains ten efferent axons, of which one is the motor giant; two of the remaining nine originate in a posterior ganglion and not in the third. The cell bodies of non-giant motoneurons in the third ganglion may have either a homolateral or heterolateral location. The synapses responsible for activating these non-giant motoneurons are in the neuropile of the third ganglion (Takeda & Kennedy, 1964), but the motor giant fibre is activated near the root exit, across a set of *en passant* junctions which link it to the central giant fibres (Wiersma, 1947; Furshpan & Potter, 1959).

Within 2 mm. of its exit from the connective the main third root branches several times to supply the main flexor muscles of its segment. The direct flexor muscles are complex, spiralling bands in which a single unit, extending over three segments, actually consists of three separate segmental muscles abutting end-to-end (Rayner & Wiersma, 1965). A particular root in a mid-abdominal segment will thus innervate the component muscles of three oblique series; conversely, a given oblique series is innervated from three consecutive third roots along its length. Rayner & Wiersma (1965) have developed a nomenclature for these muscles which replaces that of Schmidt (1915). They term the three 'series' A_I , A_{II} and A_{III} (proceeding from medial to lateral) in any given segment and indicate the segment by following such a designation with Roman numerals. Thus A_I , XVIII (see Text-fig. 1) refers to the most medial 'series' in segment 18 (4th abdominal). In the next posterior segment, this muscle series becomes A_{II} , XIX. Methylene blue staining of the third root of the third abdominal ganglion showed that the largest (motor giant) axon branches at both the first two bifurcations of the root, but that several of the medium-sized axons do not. This observation was confirmed by examining serial sections. A more complete

analysis of the innervation of flexor muscles, as well as of their anatomy and function, has been carried out by Rayner & Wiersma. The anatomical studies (Rayner & Wiersma, 1964) show that in addition to the direct action of the oblique flexor muscles in the 'tail flip', other muscles participate in an indirect manner; these latter were not studied in our experiments. Their analysis of innervation (Rayner & Wiersma, personal communication) is in general agreement with ours in showing that oblique muscle fibres receive the motor giant axon, one special (non-giant) motor axon, and a common inhibitor axon.



Text-fig. 1. Diagrammatic representation of ventral flexor muscles of one segment and their innervation from the third root (r_3 and r_{3s}) of the *third* ganglion (III). The superficial flexors (*SF*) have been removed on the animal's left side. A_1 and A_2 are the direct anterior 'oblique' flexor muscles of segment XVIII; in the next posterior segment, A_1 , XVIII becomes A_2 , XIX. A_3 is too lateral to be seen. *P*, posterior oblique muscle.

The diameters of the ten main third-root axons before they branch for the first time range between 20 and 50μ in a specimen 8 cm. long. By comparison, the six axons that form the bundle innervating the ventral superficial flexor muscles ('membrane muscles' of Pilgrim & Wiersma, 1963) have diameters of less than 10μ (see Plate I). None of these six axons is a branch of any of those in the main third root; instead they form a separate bundle of small fibres which may be followed rostrally in the connective all the way into the neuropile of the third ganglion.

The superficial muscles and the main flexors differ both in arrangement and in internal organization. Their general topography, and that of the nerves innervating them, is shown in Text-fig. 1. The superficial flexors form a sheet which is essentially a single-fibre layer near the medial edge, 1 mm. or so lateral to the nerve cord. This sheet thickens laterally, but is never more than a few fibres thick; there is a division in angle and in the details of insertion between a medial and a lateral 'head' within the sheet (Pilgrim & Wiersma, 1963), but their innervation is common. By contrast, the underlying oblique muscles are thick, massive bundles in which many fibres have a wholly internal location. Cross-sections of the fibres reveal some differences: there is a suggestion that the myofibrils of the oblique muscles are regularly packed, and that those of the superficiais are more loosely clumped. The difference may not be sufficiently dramatic to suggest the application of the vertebrate categories *Fibrillenstruktur*

and *Felderstruktur*, which appear to correspond nicely with the differences found in another crustacean muscle (Cohen, 1963); but they are of the same sort. In other muscles, the arrangement of the myofibrils has been correlated with a sharp difference in sarcomere length—the twitch (*Fibrillenstruktur*) muscles having fine striations (sarcomere length 2–3 μ) and the slow (*Felderstruktur*) muscles having much coarser striations (sarcomere length 10–12 μ) (Cohen, 1963). A similar differentiation was noted earlier for closer and opener muscles in crab claws (Jasper & Pezard, 1934), and for the fast and slow dorsal stretch-receptor muscles (Alexandrowicz, 1951). With respect to this property the difference between superficial and oblique flexors is profound (Plate I). Like other slow muscles the superficial fibres have sarcomere lengths of around 10 μ ; the obliques, in contrast, have sarcomere lengths of about 2–3 μ .

RESULTS

Functions of the muscles

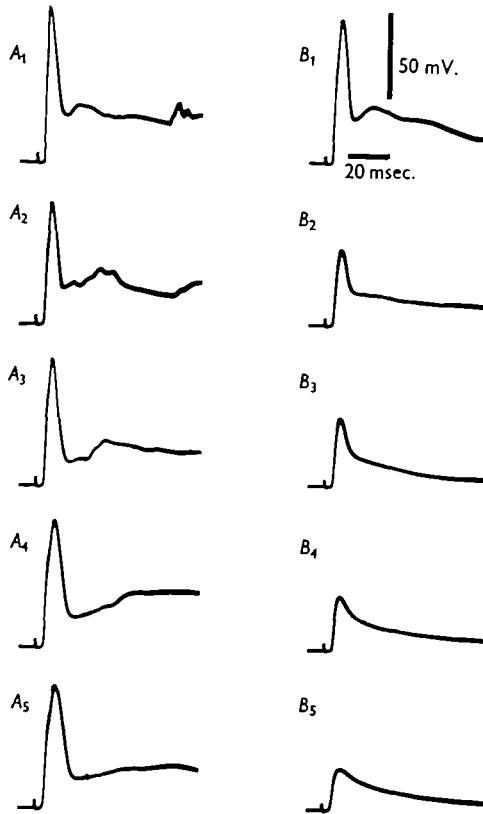
The oblique muscles undergo violent twitches upon stimulation of the central giant fibre, and it is clear that they are responsible for producing the flexor thrust during backward swimming. The function of the much less prominent superficial muscles is more difficult to ascertain. Pilgrim & Wiersma (1963) decided that they withdraw the intersegmental membrane during contraction of the main flexors. The present experiments show that although they may have such a function they perform the much more significant task of generating all slow postural flexion of the abdomen.

Crayfish pinned ventral-side up often show strong, maintained abdominal flexion. When the nerve cord was exposed in such animals and the posterior branches of the third roots were cut in segments 1–5, no flexor tone whatever remained in the abdomen notwithstanding that the innervation to the oblique muscles through the main third root branches was still intact. Even various reflex manoeuvres which normally evoke slow flexion failed to produce any in such preparations, though the flexion of the telson which normally accompanies that of the abdomen was present. The reciprocal experiment was also performed. After the main third root branches were cut the animal could still fully flex the abdomen, even against gravity; stimulation of the central giant fibre became ineffective, but repetitive stimulation of the ventral nerve cord produced tonic, *frequency-dependent* contractions in the superficial muscles which achieved full flexion. It is therefore justified to conclude that the superficial muscles are necessary and sufficient for all 'tonic' abdominal flexion, and that the oblique muscles function only in fast, twitch responses usually associated with swimming.

Electrical responses of twitch fibres

In a fresh preparation which had not been stimulated previously, a low-intensity shock to the third root produced a response like that shown in Text-fig. 2, *B*₁. Such sharply rising, inflected responses often overshoot the zero potential and produced vigorous twitches; they consisted of large junctional potentials with active secondary responses superimposed. The surprising feature of these potentials, which were evoked by the lowest-threshold axon in the third root, was their rapid diminution upon repetitive stimulation. Text-fig. 2, column *B*, shows the first, second, fifth, tenth and fifteenth responses from a series in which a well-rested preparation was stimulated

once every 2 seconds. The junctional potential quickly (in this case after a single stimulus) fell below the level necessary to trigger a full secondary response. Thereafter it declined to a low maintained value that depended upon the rate of stimulation. Following repetitive stimulation at higher frequencies, recovery was slow but complete. Text-fig. 3 is a plot of the amplitudes of responses to stimulation of the motor giant following a tetanus at 5/sec. delivered for 40 sec. There is some indication that the testing stimuli themselves have a small retarding effect, but it is clear that the recovery

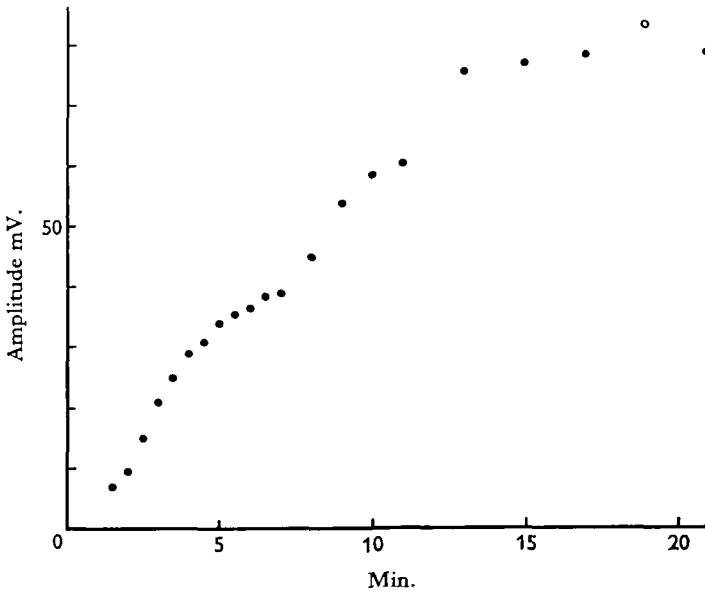


Text-fig. 2. Intracellular records from the same muscle fibre (in *A*₁₁, XIX) in response to stimulation of the intact third root of the fourth ganglion. In column *A* the third root was stimulated at higher intensity than in column *B*, so that the responses in *A* are to impulses in both non-giant and motor giant axons and those in *B* are to motor giant impulses alone. Stimulus frequency, 0.5/sec.; first, second, fifth, tenth and fifteenth responses are shown for each column.

time is substantially longer than 10 min. The response to the motor giant displayed this lability to a varying extent in different preparations, but always showed a marked contrast to the behaviour of non-giant motor axons.

In Text-fig. 2, column *A*, the experiment already described for the motor giant (column *B*) was performed at a higher stimulus intensity, so that the motor giant was activated together with one of the non-giant axons. Some diminution of the second response (*A*₂) occurred, but thereafter there was little change; the amplitudes of the fifth, tenth and fifteenth responses (*A*₃₋₅) were approximately equal. It is evident that

the potential evoked by the non-giant motoneurons does not show the same rapid decrement as that generated by the motor giants. In fact, stimulation of the non-giant motoneurone alone (after the motor giant potential had diminished to a very low value following repetitive stimulation) was often adequate to produce an active secondary response (Text-figs. 5 *C*, 7 *D*). In other cases (Text-figs. 4 *C*, 5 *A*₂) only large junctional potentials resulted from non-giant impulses, though these sometimes led to secondary responses through facilitation (Text-fig. 4 *D*).

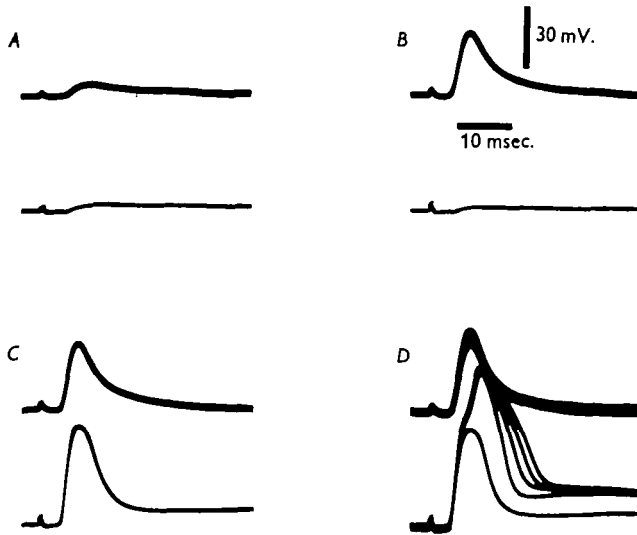


Text-fig. 3. Recovery of amplitude of the excitatory junctional potential produced by the motor giant axon; same unit as in Fig. 2 *B*. The measurements were made at intervals following a tetanus at 5/sec. lasting 40 sec.; note that the duration of the intervals between measurements was increased during recovery, first from 30 sec. to 1 min. and later to 2 min. The point (open circle) at 19 min. represents a response to a higher intensity stimulus, which activated both motor giant and non-giant motor axons.

The innervation pattern typical of these oblique flexor fibres is illustrated by the experiment shown in Text-fig. 4. The third root of ganglion 3 was stimulated; the upper trace is the intracellularly recorded response of a muscle fibre in anterior oblique muscle *A*₁₁, XVIII (terminology of Rayner & Wiersma, 1965). The lower trace is from a microelectrode in the more medially located anterior oblique muscle (*A*₁, XVIII) belonging to the next consecutive oblique 'series'. The intensity of the stimulus was increased, and the responses shown are all barely suprathreshold; the root had been stimulated repetitively prior to the series of records. In *A*, the junctional potential evoked by the motor giant axon appears at the same threshold in both muscle fibres; since it was in the early stages of recovery from the preceding tetanus, its amplitude was low. At a higher value of stimulus intensity (*B*) a non-giant motoneurone innervating the lateral muscle was recruited, and the analogous response in the medial muscle was added upon a further intensity increase (*C*). Upon repetitive stimulation, the junctional potentials evoked by non-giant motoneurons showed facilitation. At the frequency used the increase in the e.j.p. of the medial muscle fibre

was sufficient to evoke a secondary response; that in the lateral muscle fibre was not, and resulted only in an augmented junctional potential (*D*). This variation in the ability of non-giant motor impulses to evoke spikes was typical, though in general a higher proportion of spiking was seen in fresh preparations.

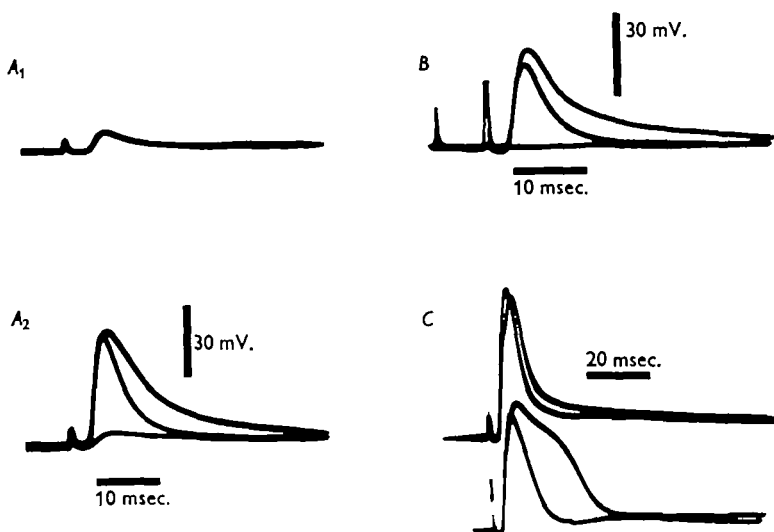
Since the oblique muscle series are of multisegmental origin, it is to be expected that at a particular point along one series the segmental source of the innervation will change. Text-fig. 5A illustrates the location of this point. The third roots of ganglia 3 and 4 were exposed for stimulation, and an intracellular microelectrode was used for



Text-fig. 4. Responses of muscle fibres innervated by the third root of ganglion 3. Upper trace, fibre in A_{11} , XVIII; lower trace, A_1 , XVIII. The stimulus (to the 3rd root of ganglion 3) was gradually increased in intensity from *A* to *D* inclusive; in *D*, the stimulus was repeated at 1/sec. for 5 superimposed sweeps, and both responses increased in amplitude progressively.

longitudinal exploration of the boundary between A_1 , XVIII and A_{11} , XIX. The recording site in A_1 was no more than 300μ rostral to that in A_2 , and the stimuli in both cases were delivered to the root of the fourth ganglion. In A_2 the three responses shown were obtained at successively higher stimulus intensities; the low-amplitude junctional potential showed rapid decrement upon repeated stimulation, and is therefore the response to the motor giant axon. The longer of the large responses resulted from recruitment of a non-giant motoneurone, and the briefer response was due to the additional recruitment of an inhibitory fibre having a still higher threshold (see below). The response in the more rostral location (A_1) was evoked at precisely the same stimulus voltage as that required to produce the large, non-giant junctional potential in A_2 , so the same motor axon was responsible for these two potentials. Though the responses are not shown, stimulation of the *rostral* third root evoked junctional potentials of reciprocal amplitudes at the two locations. Other experiments confirmed that there is a very sharp line of demarcation between regions of the muscle innervated from the two segments. The line runs across the muscle, beginning at the rostral angle of the fourth ganglion and proceeding anterolaterally, perpendicular to the long axis of the muscle fibres. The results suggest that this region of discontinuity is one in which one

set of muscle fibres abuts upon another, rather than one in which the innervation changes along one set of fibres. The small potential seen on the rostral side of the boundary upon stimulation of the caudal root could result either from relatively low-resistance connexions where the fibres abut upon one another, or from a weak 'accidental' overlap of innervation. The general pattern of triple innervation from a common motor giant axon, a specific non-giant motor fibre and a common inhibitor is in agreement with the findings of Rayner & Wiersma (personal communication).

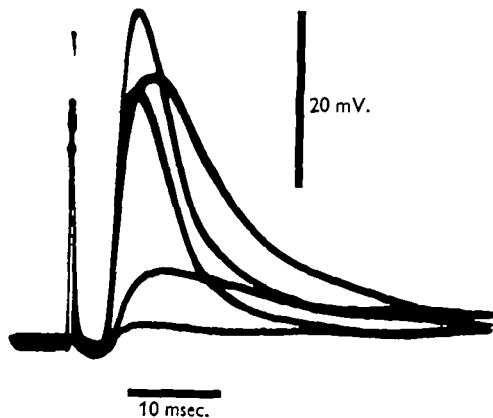


Text-fig. 5. (A) Responses from fibres at different locations near the boundary of A_1 , XVIII and A_2 , XIX. A_1 , more rostral location; stimulus to 4th ganglion, third root. A_2 , about 300μ caudal to A_1 ; same stimulus, superimposed sweeps at three different intensity levels. (B) Action of the inhibitory axon; recording in A_{II} , XVIII. A stimulus to the third root of ganglion 3 was delivered alone on one sweep; on the second, it was preceded by 8 msec. by a stimulus (via a microelectrode) that selectively activated the inhibitory fibre. The third sweep shows effect of the inhibitor axon alone. (C) Effect of inhibitor on responses of different muscle fibres to stimulation of the third root of ganglion 3. Upper trace, A_{II} , XVIII; lower, A_1 , XVIII. Two successive sweeps, first at lower intensity, then at higher. Both responses are shortened by recruitment of the inhibitor; the slight increase in rise rate may be due to additional recruitment of an excitatory axon. 30 mV. calibration in A_2 applies also to A_1 and C; 10 msec. calibration in A_1 applies also to A_2 .

Text-fig. 6 shows a relatively rare case of apparent quintuple innervation. The smallest potential was evoked at lowest threshold and showed rapid decrement. In addition to this response from the motor giant, four further stages were found: the second was another small e.j.p., the third a very large one, the fourth a sudden reduction in duration and amplitude due to recruitment of the inhibitor axon, and the fifth an additional, comparatively small increment in e.j.p. amplitude. Such responses were found only in a very few cases; triple innervation, as in Text-fig. 5 A_2 , was the rule for fibres of the oblique muscles.

That the abrupt decrease in duration of the junctional potential such as in Text-fig. 5 A_2 is due to the recruitment of a peripheral inhibitor axon was shown in the experiment of Text-fig. 5B, on a different preparation. A stimulating microelectrode was moved across the third root near its exit zone until a point was found at which it could

selectively activate the axon producing the reduction. When this stimulus preceded activation of a non-giant motoneurone by a few milliseconds the response was attenuated in amplitude and duration. Text-fig. 5*B* shows three superimposed sweeps: inhibitory stimulus alone, excitatory stimulus alone, and both together with the inhibition preceding by about 7 msec. This was approximately the optimal interval for inhibition, though the time course of the inhibitory conductance change—as judged by acceleration of the rate of fall of the e.j.p.—was nearly 100 msec. The inhibitory axon was also effective against e.j.p.s produced by the motor giant fibre, but a quantitative comparison could not be made because of the rapid antifacilitation characteristic of motor giant e.j.p.s. As Text-fig. 5*C* shows, the effect of the inhibitory conductance change was most dramatic in those muscle fibres in which the



Text-fig. 6. Responses of a fibre in *A*_{II}, XIX to graded stimulation of the third root of the fourth ganglion, illustrating five distinct stages of potential. For further explanation, see text.

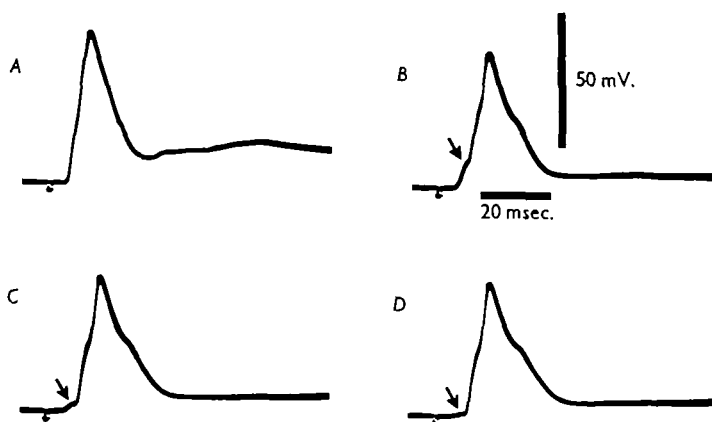
duration of excitatory response was long. In most fibres stimulation of the inhibitory nerve had only a small effect on the amplitude of the excitatory response, especially if spikes were present. Thus the dramatic instances of inhibitory effect upon potential were in those fibres whose responses resembled those shown in the bottom trace in Text-fig. 5*C*. Visual observation indicated that the extent of contraction was not noticeably different when an inhibitor discharge was included in an efferent third-root volley than when it was absent. It is therefore difficult to believe that peripheral inhibition has a substantial effect in attenuating the peak tension developed by the flexors during a twitch. It may, on the other hand, function significantly in limiting the time course of single twitches through its action in accelerating repolarization.

Function of dual excitatory innervation

An attempt was made to gain some insight into the odd situation that the vast majority of flexor muscle fibres receive a dual excitatory innervation in which the motor axons not only have similar action upon the muscle but are both activated by the central giant fibres.

Text-fig. 7 shows a series of responses to stimulation of the central giant fibres, the normal route of activation for twitch flexors during vigorous backward swimming. Since the motor giant and non-giant axons are being excited synaptically, the differ-

ence in synaptic delay and in central conduction time is added to that due to peripheral conduction time; one would thus predict that the motor giant impulses would arrive earlier relative to the non-giant ones than when the root is stimulated. The results show that this is the case. Stimuli at just above the intensity necessary to evoke central giant fibre activity were delivered to the nerve cord once every 2 sec.; *A*, *B*, *C* and *D* are the first, fourth, seventh and tenth responses respectively. The junctional potential due to the motor giant axon (indicated by the arrow in *B–D*) decreased in amplitude at the usual rapid rate. Concomitantly, the response became somewhat more prolonged. We attribute the latter phenomenon to the fact that secondary, active responses are often graded in character, and that the weakened electrogenic activity should be less effective in repolarizing the residual junctional potential from

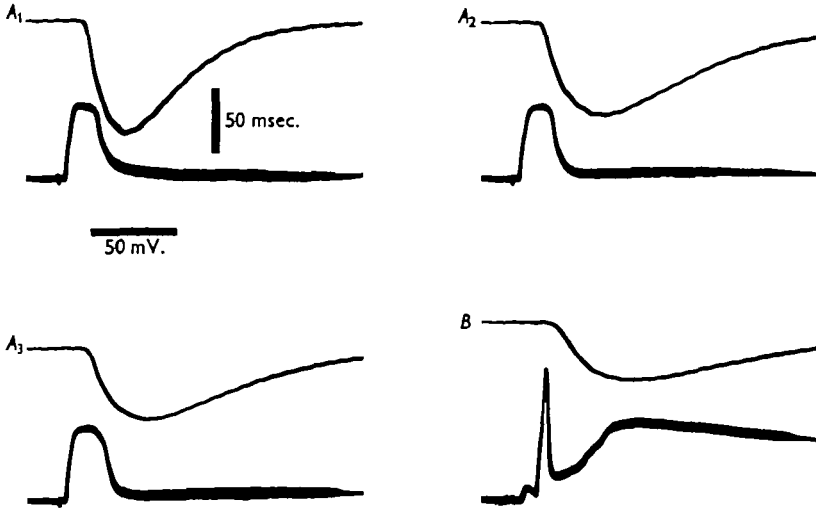


Text-fig. 7. Responses to repetitive stimulation of the central giant fibres. 4–5 connective stimulated above giant fibre threshold at 0.5/sec.; the microelectrode is in a fibre of *A*₁₁, XVIII. *A*, *B*, *C* and *D* show the first, fourth, seventh and tenth responses. The arrows mark the inflexions produced by diminution of the motor giant e.j.p.

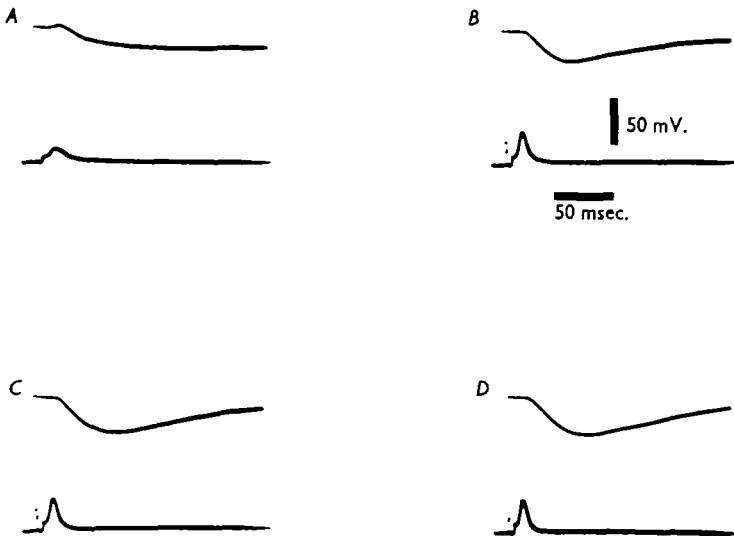
the non-giant motor axon. In any case it is clear that although the non-giant e.j.p. was capable of eliciting a secondary response without substantial contribution from the motor giant e.j.p., the total amplitude of depolarization was larger when both motor axons were participating in the reflex discharge. In other cases it has been observed that the 'compound' junctional potentials evoked reflexly after the motor giant has been rested may evoke double secondary responses, whereas the non-giant axon can produce only one.

These results would predict that a series of stimuli to the central giant fibres should produce a series of contractions which decline in effectiveness over the first few stimuli until the motor giant axon is no longer adding its substantial increment of junctional potential to all of the muscle fibres in that segment. Text-fig. 8 shows an experiment in which tension in a large bundle of oblique muscle *A*₁₁, XVIII in segment 3 was measured along with intracellular potentials from a fibre in it. The nerve cord was stimulated once every 2 sec., after the preparation had initially been rested for 5 min. *A*₁, *A*₂, *A*₃ and *B* are the first, third, fifth and seventh responses. The peak tension developed showed a pronounced decrease after the first stimulus, and the mechanical response became somewhat more prolonged. The muscle fibre penetrated in *A*_{1–3}

exhibited a junctional potential with a small electrogenic component. During the repetitive stimulation, the early phase of this junctional potential became slightly attenuated with respect to the second. In *B* the electrode moved into another muscle fibre, in which the early junctional potential due to the motor giant impulse had



Text-fig. 8. Combined intracellular recording (lower trace) and tension measurement (upper trace) from a bundle of *A*_{II}, XVIII fibres. *A*₁, *A*₂, *A*₃ and *B* are the first, third, fifth and seventh responses to stimulation of the 3-4 connective at 0.5/sec., at an intensity just above the giant-fibre threshold. The potential record from the fibre in *A*₁₋₃ correlates rather poorly with the tension decline, but between *A*₃ and *B* the microelectrode penetrated a new fibre which showed reduction of the early junctional potential of the motor giant.



Text-fig. 9. Combined intracellular recording (lower trace) and tension measurement (upper trace) from a bundle of *A*_{II}, XIX fibres. In this instance the cord stimulus activated (via the giant fibres) *only the non-giant motoneurone*, transmission at the motor giant synapse having been interrupted. Stimulation at 0.5/sec.; first, third, fifth and tenth responses shown in *A*, *B*, *C* and *D*.

declined markedly. Although the heterogeneity of fibres within any crustacean muscle makes intracellular events difficult to correlate with whole-muscle tension, such experiments do demonstrate that the expected decrease in peak tension does occur, and that it is accompanied by selective reductions in the post-junctional effects of the motor giant axon.

The drop in effectiveness of repeated stimuli due to antifacilitation of the motor giant junctions is in part counteracted by the tendency of non-giant junctions to show facilitation. Text-fig. 9 shows a combined tension-potential measurement like that in Text-fig. 8; in this case, however, the motor giant synapse had failed centrally and only the non-giant e.j.p. was being evoked by cord stimulation. Repetitive stimulation at 0.5/sec. followed a 6 min. rest; *A*, *B*, *C* and *D* are alternate responses. The sub-threshold e.j.p. facilitated to produce a secondary response, and this accompanied an increase in the peak tension reached.

DISCUSSION

General features of the innervation

It is apparent that the twitch flexors of the abdomen differ markedly from the muscles of the distal limb, which have heretofore formed the basis for the comparative neuromuscular physiology of decapod crustacea. Dual excitatory innervation is common in limb muscles; but normally, 'slow' and 'fast' axons may be distinguished according to the time course and facilitation of the e.j.p.s they produce. The present muscles, on the other hand, exhibit extraordinarily large junctional potentials with superimposed secondary responses upon stimulation of either excitatory axon; each of these responses may be of an overshooting amplitude (70–80 mV.) without facilitation. These muscles are therefore clear—and, so far, unique—examples of fibres with a dual, exclusively fast innervation.

Among muscles previously described they most closely resemble the closer of *Pachygrapsus* (Hoyle & Wiersma, 1958). The proximal region of that muscle was classified as 'Group II' by Hoyle and Wiersma because some of the fibres in it responded only to the fast axon with brief e.j.p.s that evoked spikes; other fibres responded only to the slow axon with large, long-lasting e.j.p.s, but never produced spikes. The slow abdominal flexor muscle described in the second paper (Kennedy & Takeda, 1965) might be considered analogous to the latter category of fibres, and the oblique muscles to the former; but both abdominal muscles differ from those of the limb in having a very much more complex innervation.

Motor giant e.j.p.s

The most surprising finding of these experiments is that the motor giant e.j.p.s show an antifacilitation (Bullock & Horridge, 1965) unparalleled in neuromuscular systems. The mechanism of this decline in effectiveness, as well as its behavioural significance, requires explanation. While no direct information is available one might speculate that rapid depletion of the stored transmitter is involved. If the wide distribution and consequent frequent branching of the motor giant axon results in relatively fine motor endings, one might expect them to have a smaller storage capacity for transmitter. On the other hand, an entirely different transmitter with different rates of synthesis and supply might be involved.

Behavioural role of the motor giant system

The oblique abdominal flexor muscles are, *par excellence*, an escape system; they provide the entire power output for backward swimming via the 'tail flip' reflex. So firmly is this response associated with escape that it is the usual example given for the adaptive significance of central giant fibres. The discovery of the fast, electrotonic excitation of the motor giant axon by central giants (Furshpan & Potter, 1959) seemed consistent with the notion that the entire pathway was adapted for minimal delay; but the picture is complicated by the fact that other, non-giant motoneurons *innervating the same muscle fibres* were also activated by the central giants via more conventional junctions (Takeda & Kennedy, 1964).

The present results explain the apparent paradox. The routes are not in fact entirely duplicate; the decrement in post-junctional response to the motor giant is so rapid that following a few tail flips at the normal swimming rate of 5-8 contractions/sec. (Eckert, 1961) the motor giant axon is not participating in the response at all. Though the non-giant axons are able to deliver a long series of twitches during sustained swimming, these contractions are not as powerful as the first few, especially the very first one.

The motor giant system must therefore be thought of as having an especially exclusive 'escape' function. It contributes almost nothing to continuous swimming, but generates powerful extra thrust for the first few strokes that must accelerate the animal away from its position of rest. The extra response appears to be produced in two ways: first, the contribution of a small amount of additional depolarization above that which is available from non-giant impulse alone; second, by prolongation of the duration of the compound junctional event so that in some muscle fibres paired secondary electrogenic responses may be produced by each discharge of the central giant fibre.

Finally, it should be noted that the main flexor system may operate in reflex actions *not* mediated by the central giant fibres. Non-giant motoneurons receive complex input from root and cord elements (Takeda & Kennedy, 1964), and these may thus be used selectively in controlling other types of movement.

The role of inhibition

Though direct information is lacking it seems unlikely that even with appropriate timing the inhibitor axon could bring about a substantial reduction in the peak tension developed during a twitch evoked by the central giants. This conclusion is based upon the rather small effects of stimulation of the inhibitory nerve upon excitatory depolarization, and also upon the observation that the tension evoked by a third-root stimulus is not noticeably reduced when an inhibitory impulse is included in the discharge. It is possible that a high frequency of inhibitor discharge during activation of the excitor axons might achieve a substantial attenuation, but it is difficult to imagine how, or for what purpose, such a discharge could be triggered centrally to *precede* the activation of the 'escape' system. In fact, there seems little reason to postulate, as a function of peripheral inhibition, graded reduction in tension of muscles such as the ones under study here, which normally twitch but presumably are never tetanized. Usually there is little reason to require a full range of variation in twitch power output; where such a need does exist it can be taken care of by varying the number of units

participating or, where that is not possible on account of size, by varying the interval between paired discharges (Wilson, 1964). The very organization of the 'tail-flip' system, with its primary dependence upon impulse activity in a single set of central channels, exemplifies the usual operation of the main flexors as an all-or-none system.

If this is indeed true the presence of a widespread peripheral inhibitory axon requires some other explanation. A clue may be provided by the frequent occurrence of junctional potential-spike responses of long duration in oblique muscle fibres. The swimming response depends, of course, upon the alternation of extension with flexion. The extensors are much less powerful, and their action occupies about two-thirds of each cycle (Eckert, 1961). However the rhythm of swimming contractions is set centrally, it seems probable that the ultimate rate limit is partially imposed by the time at which tension in the flexors can be cut off so that the extension can begin. Under these conditions the inhibitor could play a critical role—not in reducing peak tension, but in actively repolarizing the muscle fibres so as to abolish residual tension. A somewhat analogous function will be proposed in the second paper (Kennedy & Takeda, 1965) for the inhibitory supply to the slow flexor system.

SUMMARY

1. The flexor musculature of the crayfish abdomen is divided into two systems: a set of tonic superficial muscles, and a complex series of massive flexor muscles that produce powerful twitches but never exhibit tonic contractions. The muscle types are histologically differentiated, and also separately innervated: the main flexors receive ten large motor axons, and the slow superficial muscles six smaller ones.

2. Fibres of the main flexor muscles studied are almost all triply innervated; each receives endings from (a) the 'motor giant' axon, (b) one of several specific non-giant motor axons, and (c) a common inhibitor.

3. Excitatory junctional potentials (e.j.p.s) due to motor giant and non-giant axons are similar and large; each may trigger secondary, active 'spikes', thus often producing post-junctional responses of 100 mV. or more. The responses differ in that the motor giant e.j.p. shows a dramatic decrease upon repetitive stimulation, whereas that due to non-giant motor axons exhibits some facilitation.

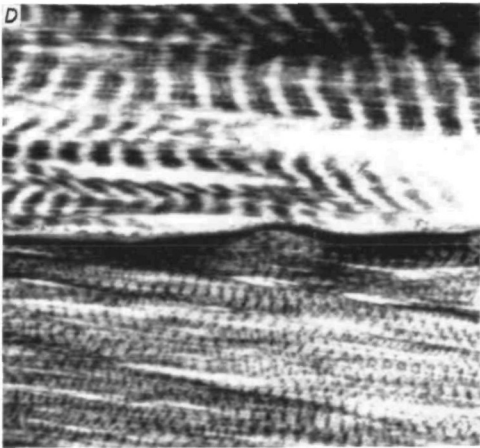
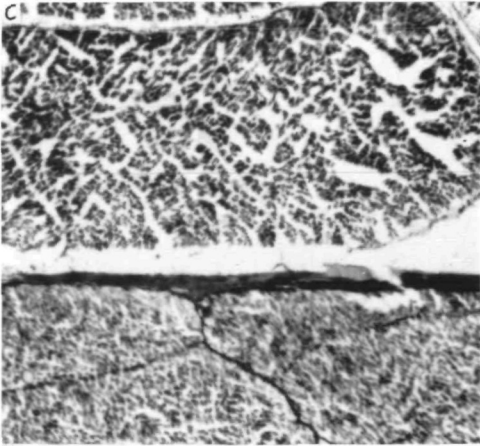
4. Activity in the central giant fibres drives both motor axons. The response to both, when the motor giant system is fully rested, is slightly larger than that to either alone; when activated by stimulation of the central giant fibre the junctional potentials are evoked asynchronously due to differences in central reflex time, and double spiking in the muscle fibres sometimes results. Upon repeated stimulation the response to the giant is reduced to a very low level; this is accompanied by a decrease in the tension developed in successive reflexly evoked twitches. The motor giant system thus apparently functions to provide additional tension for the first few 'flips' in a series of swimming movements during escape.

5. Impulses in the inhibitor axon, even at the optimal interval, reduce the amplitude of excitatory post-junctional potentials by only a small amount; their effect in shortening duration is more notable. It is postulated that the peripheral inhibitor functions to cut short excitatory depolarizations and hence to terminate lingering tension that might oppose subsequent reflex actions.

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EXPLANATION OF PLATE

- A. Cross-section of the main third root, segment XVII, just after its first branch.
- B. Cross-section of the bundle innervating the superficial flexor muscle, taken from the same section as A.
- C. Cross-section of the superficial (above) and oblique (below) flexor muscles.
- D. Longitudinal section of the superficial (above) and oblique (below) flexor muscles.

A and B, Masson's trichrome; C and D, silver (Rowell, 1963). 5 μ paraffin-embedded sections.

