REFLEX CONTROL OF ABDOMINAL FLEXOR MUSCLES
IN THE CRAYFISH

II. THE TONIC SYSTEM

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In the crayfish abdomen most of the power for the flexor twitches employed in swimming is supplied by the massive oblique muscles which are innervated by large motor axons from the third root. All of the delicate flexor control of the abdomen is vested in a thin, superficial sheet of muscle fibres which have the histological characteristics of ‘slow’ muscle and are innervated by a special, independent supply of small motor nerves (Kennedy & Takeda, 1965). These forty or so muscle fibres and half-dozen axons comprise a reflex apparatus of exquisite complexity, both with respect to innervation pattern (which ranges from single to sextuple for single muscle fibres) and to central modulation of efferent discharge.

Two sorts of information about this system have been sought in the present experiments. The first involves the way in which the muscle fibres are innervated by the motor axons. Sampling the responses of different fibres to activity in the six axons should yield useful information about whether certain innervation combinations are common or rare, whether motoneurones of special function (e.g. the peripheral inhibitor) selectively accompany others, and whether there are specific correlations between the properties of a muscle fibre and the identity of its innervation. In a sense, this approach is directed at learning about the development of patterns of connexion between two populations of cells.

The second kind of question involves, instead, the nature of the central reflex control of a polyneuronally innervated group of muscle fibres. While a good deal of information exists about the junctional effects of single-axon stimulation upon different muscle fibres (e.g. Hoyle & Wiersma, 1958a), we cannot now correlate this with any knowledge of the normal firing patterns of these neurones during reflex action. It might be asked, for example, whether axons that produce strongly facilitating junctional potentials in most muscle fibres tend to have rather different discharge patterns from those that produce non-facilitating ones. We do not even know whether the several different axons that innervate a given muscle—or muscle fibre—have independent sources of central activation.

One very important specific question about reflex control in crustacea concerns the role of peripheral inhibition. Among the variety of hypotheses about the function of this mechanism a prominent one has always been the straightforward view that it is the primary agent responsible for reciprocal reflex inhibition of muscles during

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contraction of their antagonists. Bush (1962), who discusses this problem at length, has provided an elegant demonstration that this idea is correct for the opener muscle of the crab claw; suggestive evidence had been provided earlier by Eckert (1959) for the same muscle. In that preparation, interestingly, the reflex excitation of the peripheral inhibitor is unaccompanied by any decrease in the discharge rate of the excitor; on the contrary, the two rates are positively correlated (Wilson & Davis, 1965), showing that the entire burden of reflex inhibition is accomplished peripherally. The opener muscle is, however, unusual in that it is supplied by only a single excitatory axon. The slow abdominal flexors studied in the present experiments are probably more typical of 'fine' reflex systems in having a complex polyneuronal innervation. It will be shown that in fact they are reflexly inhibited in almost precisely the opposite way from that found by Bush for the claw opener, bearing out Bush's own prediction (1962, 1963) that central inhibition would have the greater importance in most systems.

**METHODS**

The method of preparation and the stimulating and recording techniques were in general similar to those described in the previous paper (Kennedy & Takeda, 1965). The ventral superficial muscles were exposed by cutting away a flap of exoskeleton from the caudal portion of abdominal sternites 2, 3 or 4. The thin posterior branch of the third root could be located running caudo-laterally to the medial edge of the superficial muscles. For some experiments, this branch was raised on a pair of platinum electrodes for stimulation; in others, it was lifted on to a single silver hook and then raised into an oil layer for capacity-coupled recording against the earthed bath. With this recording arrangement reflex responses to a variety of natural stimuli could be recorded. When direct electrical stimulation of the motor axons was used instead, the root was cut centrally and spontaneous activity thereby eliminated. Sufficient slack is present in the nerve to permit en passant recording by the former method without damage to the motor axons; even though 1–2 mm. of the posterior branch were in the oil layer, impulses were conducted through to the muscles for upwards of 2 hr. Where failure did occur it was quickly detectable as a shift in waveform of the efferent impulses from diphasic to monophasic.

All muscle recording was accomplished, as previously, with 'floating' intracellular microelectrodes. The fibres of the superficial sheet are large and form a nearly uniseriate layer; it was often possible to penetrate a series of fibres from the medial to nearly the lateral edge of the muscle, and to note their location accurately. Individual spikes identifiable in the nerve discharge could be compared, in dual-beam oscilloscope records, with the junctional events in a large number of muscle fibres sampled during a single experiment. Tension measurements were sometimes also made, using very small sectors of the sheet containing, at most, three or four muscle fibres.

**RESULTS**

*Electrical responses of the superficial fibres*

As was demonstrated in the preceding paper (Kennedy & Takeda, 1965) the superficial muscles are responsible for all graded abdominal flexion; further, they show histological features characteristic of other slow crustacean muscle fibres, and are
innervated by a special bundle of small motor nerves that exhibit a high degree of spontaneity. It is therefore not surprising that the main feature of electrical activity recorded intracellularly from these muscle fibres in an intact preparation was a continuous flux of membrane potential caused by summatting, facilitating junctional potentials (Fig. 1A, C₂).

Secondary active responses were never common in superficial muscle fibres; in most they did not occur at all. Several examples of active responses and their relation to tension development are shown in Fig. 1. Single spikes or trains sometimes occurred, as in Fig. 1A, superimposed upon summated depolarization due to junctional activity when the latter was sufficiently high. Repetitive electrical stimulation of excitatory axons with the root cut centrally, as in Fig. 1B, duplicated the effect. In B₁ an abortive active response was evoked; in B₂, where the frequency of stimulation was higher, full spikes were generated on the plateau. The frequency of spiking was limited, because the repolarizing action of the spike made necessary a new cycle of summation to regain the plateau.

Correlated tension and potential measurements on very small bundles of medially located muscle fibres are shown in Fig. 1, C₁ and C₂. A background level of junctional potentials was occasionally interrupted by larger depolarizations composed of summated j.p.s and active responses (early portion of both sweeps). These large depolarizations were associated with large increments of tension; but the fluctuations in the later part of C₁ illustrate that, even when spiking occurred, tension was a continuous function of membrane potential and could be varied effectively even by low levels of junctional potential activity (cf. Orkand, 1962; Atwood, 1963a).
Since the main mechanism of tension gradation appears to be through the level of depolarization attained by junctional potentials, it is necessary to assess the relative importance of summation and facilitation in accomplishing reduction of membrane potential. In the case shown in Fig. 1 B, for example, summation clearly played the most important role in depolarization; facilitation was not marked, but the duration of e.j.p.s was very long, and each added to depolarization from the previous one even at motor discharge frequencies of 10/sec. Fig. 2 illustrates that for other axons facilitation may be of considerable significance. In A1 and A2 the effect of stimulating the lowest threshold excitatory axon in the bundle is shown. The first e.j.p. was vanishingly small, but augmentation was rapid and the final amplitude ratio of facilitated to non-facilitated e.j.p.s was at least 6:1. In a number of muscle fibres sampled with repetitive...
stimulation of the motor nerve the most dramatic facilitation was shown by an axon with especially low threshold. Fig. 3 illustrates responses of a muscle fibre to stimuli of gradually increasing \((A_1)\) and then decreasing \((A_2)\) intensity delivered to the nerve bundle at a constant frequency of \(10/\text{sec}\). Three distinct levels of e.j.p. were shown; the transitions were blurred by facilitation during the increase in intensity, but appeared sharply during the decrease. No inhibitory innervation was apparent in this fibre. The lowest-threshold excitatory axon, however, exhibited a somewhat larger facilitation ratio than did the other two. In Fig. 3B, only the decreasing-intensity phase of a similar experiment on a different muscle fibre is illustrated. In this case two excitatory axons exhibited higher voltage thresholds than the inhibitor; for this reason they dropped out before the latter, which gave hyperpolarizing potentials (end of record). The properties and thresholds of the excitatory axons agreed with those of the two highest threshold units in \(A\), and the threshold for the inhibitor was such as to suggest that it is intermediate in size between the lowest-threshold axon in \(A\) and the others. This question will be discussed below, in connexion with the assignment of junctional events to specific axons.

The fact that the inhibitor is the largest axon innervating some muscle fibres permitted an analysis of the properties of inhibitory junctional potentials (i.j.p.s) without physical isolation of the inhibitory axon. In general, the results agreed with those obtained by others on limb muscles (Fatt & Katz, 1953\(a\); Hoyle & Wiersma, 1958\(b\)). Because the membrane potential of any muscle fibre undergoes such constant variation in intact preparations, the effect of inhibitory impulses could be viewed at various levels of the membrane potential. As expected, the amplitude of hyperpolarization of an i.j.p. increased at lower values of membrane potential. Typically, i.j.p.s were hyperpolarizing at the ‘resting’ potential level, i.e. in the absence of any excitatory activity; responses to single stimuli were frequently small, but showed considerable facilitation when evoked repetitively (Fig. 2B). The amplitudes of hyperpolarization attained in such experiments suggest that the reversal potential for the i.j.p. in these muscles is at least 5–10 mV. above normal ‘resting’ potential.

**Patterns of innervation**

Simultaneous recording from pairs of muscle fibres revealed a strong influence of regional factors in the distribution of motor axons. In order to be certain that a single microelectrode penetration was an adequate sampling method for the input to a single muscle fibre, several paired recordings were made from the rostral and caudal ends of the same fibre. The potential variations recorded in this way were virtually superimposable under a variety of stimulus conditions in intact preparations; it seems safe to conclude that in this muscle as in others (Fatt & Katz, 1953\(a\)) junctional potentials are fully distributed along the fibres.

Fig. 4\(A\) is a simultaneous comparison of spontaneous activity in two adjacent fibres near the medial edge of the superficial muscle. It may be seen from the records that the innervation was fully congruent: every junctional potential in one fibre was accompanied by one in its neighbour. The responses differed, however, in two respects. First, the ratios of junctional potential amplitudes showed marked differences. Though most of the responses \((A_1\) and \(A_2)\) were of nearly equal amplitude in the two fibres, that due to one axon—the middle e.j.p. in \(A_2\) —was very much larger in the more
lateral fibre (lower trace). Secondly, the fibres did not always generate active responses at the same time. The spike shown in the upper trace at the end of A3 was accompanied by only a very small active response in the other fibre, whereas at another time (near the beginning) the active response was larger in the lower trace. In part, such differences depended on the arrival of sequences of impulses which produced somewhat larger e.j.p.s in one fibre than in the other, and in part they involved apparent variations in the inherent ability of similarly innervated muscle fibres to produce electrogenic responses.

In Fig. 4B the upper-trace record is from the same medial fibre as in Fig. 4A; the other electrode had been moved most of the way to the lateral edge of the superficial muscle. The differences between this distant pair are much more pronounced. Not only did the ratios of e.j.p.s from shared axons vary; each fibre received an excitatory axon which evoked no e.j.p. at all in the other one, and the fibre in the lower trace exhibited pronounced hyperpolarizing i.j.p.s. In addition, the latter fibre never produced secondary responses.

It should be emphasized that these regional differences (discussed further below) are statistical in character. There is no break in innervation corresponding to the morphological separation of the superficial flexors into medial and lateral heads (Pilgrim & Wiersma, 1963), and we doubt that there is any important functional difference between the two heads.

Though of course not all adjacent pairs of fibres receive duplicate innervation, the pairs of fibres illustrated are typical in showing the close similarity of neighbouring
Reflex control of abdominal muscles in crayfish. II

cells and the tendency of widely separated ones to differ. The most dramatic instances of variation between adjacent fibres occurred along a dorsal/ventral gradient rather than a lateral one: where medial fibres occurred directly above and below one another, differences in innervation were usually noted. Usually, the more dorsal fibres tended to receive inhibitory supply as well as a richer excitatory innervation. For a more detailed analysis of the pattern of axon distribution, however, a different method was required. Fig. 5 illustrates the principle underlying the technique used. The efferent

Fig. 5. Intracellular recordings from superficial muscle fibres (upper traces) and simultaneous en passant recording from the intact superficial branch of the third root innervating the muscle (lower traces). Columns A and B are from two neighbouring muscle fibres about 2/5 of the distance from the medial to the lateral edge of the superficial muscle. The records were taken during natural stimulation of the intact animal; selected sweeps are shown in order to represent each motor axon. Diagonal lines are used to show the junctional event produced by each spike the first time it appears, but are not drawn for subsequent appearances. Dotted line indicates no effect. Voltage calibrations in this and succeeding figures refer to intracellular trace only. The responses of fibre no. 6 have been retouched for photographic purposes in this and the following figure.

bundle, still connected to the intact central nervous system, was looped over a recording electrode located in an oil layer just above the saline bath, so that impulses in the six axons could be recorded as they passed on their way to the muscle. Simultaneously, an intracellular electrode (upper trace) monitored the activity in a single muscle fibre. A series of muscle penetrations could thus be made, and the junctional events in each one correlated with the spikes in the nerve discharge. A large number of sweeps were recorded for each muscle fibre, usually while a variety of natural stimuli (stroking
carapace hairs, bending the telson, etc.) were delivered to the animal in order to ensure that each different efferent spike would appear several times in the records.

The distribution of spike amplitudes in the efferent bundle was extremely consistent from preparation to preparation. Numbers were assigned to the six spikes, beginning with the smallest as no. 1, and proceeding in order of increasing amplitude to no. 6. We make the assumption in what follows that these assignments actually apply to the fibres themselves, and are consistent between preparations. The amplitude distribution agrees very well qualitatively with the diameter distribution measured from cross-sections. Such sections (of which that in plate I of the previous paper is a mediocre example, chosen for size comparison with the main third root from the same section) regularly show one large axon, three of middle size with one slightly larger than the other two, and two of small but distinguishably different diameter. Because of the clear size separation between most spikes, and because of the distinctive action of some (e.g. no. 5 was inhibitory), only one pair of spikes—nos. 3 and 4—are sufficiently similar to allow one to be identified as the other in different preparations. Even though that possibility cannot be excluded, these two fibres can be distinguished easily throughout a single experiment.

In Fig. 5, the correlations between spikes numbered on the above basis and junctional events are shown for two muscle fibres, recorded successively in the same preparation. They were selected to document the complexity of innervation as well as to serve as an example of the method, so both represent comparatively difficult cases. In the four sweeps shown for each fibre each different spike is labelled and a line is drawn to indicate the junctional event in the muscle trace for which it was responsible. The same spike may appear later (or earlier) in the records without a label; it will be noted that it always produces the same junctional event in the muscle fibre. The dotted line from spike no. 6 in $A_4$ indicates that it produced no junctional potential.

The four traces shown in the figure for each muscle fibre were selected from a large number because, in combination, they allow a complete demonstration of the innervation in a minimum space. Examination of a large number of such records, usually thirty or more, allowed any ambiguities to be resolved. In most cases, for example, paired discharges of a given axon could be found, so that a possible junctional potential could be looked for under conditions where it would be facilitated; and the search for hyperpolarizing j.p.s in response to spike no. 5 was carried out carefully in sweeps where that axon discharged while the fibre was considerably depolarized by e.j.p.s. Despite these procedures a number of muscle fibres had to be dropped from the analysis because there was doubt concerning the effect of one or more of the six axons. The statements to be made below are based largely on a sample of seventy-seven muscle fibres, taken from five different preparations. In each of these, sampling was carried out in different regions of the muscle.

Table 1 summarizes the numerical distribution of axons to muscle fibres. It is evident that the muscle fibres are quite heterogeneous with respect to the number of axons they receive; all possible numerical combinations occur, but most fibres are innervated by an intermediate number of axons, and both extremes are rare. The inhibitor, furthermore, appears to innervate selectively those muscle fibres which also receive a highly polyneuronal excitatory innervation. This may, however, have its explanation in the regional factors to be discussed below.
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Since the vast majority of muscle fibres receive combinations of two or more excitatory axons, it might be supposed that these combinations would have special significance in terms of the discharge properties of the axon or the characteristics of the junctions, and that therefore there would be strongly preferred pairings between specific axons. Table 2 is a matrix which allows this idea to be tested; it gives the actual number of combinations found in the sample between all possible axons. The number found is compared with the number that would be expected if combination were entirely at random (i.e. with the calculated product of the frequencies of the two contributors to the combination). The subscripts in parentheses under the spike numbers give the total number of innervations found for each axon.

Table 1

<table>
<thead>
<tr>
<th>Number of excitatory axons</th>
<th>Number of muscle fibres</th>
<th>Percentage receiving inhibitor</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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Table 2

<table>
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<th>3(10)</th>
<th>4(10)</th>
<th>5(10)</th>
<th>6(10)</th>
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<td>18</td>
<td>30</td>
<td>30</td>
<td>15</td>
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<tr>
<td>(exp. 10)</td>
<td>(exp. 12)</td>
<td>(exp. 18)</td>
<td>(exp. 20)</td>
<td>(exp. 13)</td>
<td>(exp. 17)</td>
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<tr>
<td>9</td>
<td>26</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>(exp. 18)</td>
<td>(exp. 25)</td>
<td>(exp. 37)</td>
<td>(exp. 16)</td>
<td>(exp. 23)</td>
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<td>9</td>
<td>16</td>
<td>26</td>
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<td>(exp. 23)</td>
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Several conclusions may be drawn from the table. First, the excitatory axons do not share the task of innervation equally: no. 3 serves over 80% of the muscle fibres, nos. 6 and 4 over 60%, and nos. 1 and 2 connect with less than one-fourth. Secondly, the inhibitor is by no means common to all fibres; it innervates less than half of them. Thirdly, most combinations between axons appear to be made randomly: their frequency in the sample is very near that to be expected on the basis that the connexions are independent events. The most obvious exceptions to this can be summarized by the following statements: axon no. 5 combines with nos. 1 and 2 about twice as often as expected, and with no. 6 less often than expected; no. 6 combines with nos. 1 and 2 only half as often as expected; and nos. 1 and 2 tend to combine more often than expected.

It is questionable, however, whether these departures from randomness need be attributed to some precise developmental mechanism which ensures that certain axons grow and innervate together while others ‘avoid’ each other. The regional distribution of innervating axons suggests a simpler explanation. Table 3 summarizes
the approximate location, along a medial-to-lateral gradient, of the individual innervation relationships found in two of the experiments from Table 2, in which a large number of muscle fibres with a wide distribution were sampled. It is quite clear that axons nos. 1, 2 and 5 tend not to distribute to the lateral portion of the muscle, whereas nos. 4 and 6 tend to be more concentrated laterally. While this regional concentration does not explain all of the non-randomness exhibited in Table 2, it makes it unnecessary to invoke complex explanations for the affinity of no. 5 with no. 1 and of no. 1 with no. 2, or for the low correlation between no. 6 and the medially located axons. In addition, it accounts nicely for the observation (Table 1) that muscle fibres with a diverse polyneuronal excitatory innervation have a high probability of receiving peripheral inhibition.

### Table 3

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>Medial</th>
<th>Area</th>
<th>Lateral</th>
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<td>b</td>
<td>c</td>
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<tr>
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<td>4</td>
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<tr>
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</table>

**Effects of different axons**

From Figs. 4 and 5, it is evident that the junctional events produced by different axons in the same muscle fibre differ in several respects, and that the same axon may also produce quite different responses in the several muscle fibres it innervates. This finding duplicates the experience of others (Furshpan, 1955; Hoyle & Wiersma, 1958a); but the present situation affords the opportunity of correlating these features with such properties of the axon as its size and its central inputs. The following account summarizes what we know of the characteristics of each axon.

**No. 1.** The smallest fibre in the bundle; it usually shows a moderate level of activity, and produces small junctional potentials in those medial fibres it innervates.

**No. 2.** Nearly as small as no. 1, this fibre has the highest rate of spontaneous activity. It usually produces small junctional potentials but may, as in Fig. 5A, produce relatively large ones. It innervates the medial portion of the sheet.

**No. 3.** This axon innervates most fibres in all regions of the muscle, and usually produces large junctional potentials of long duration that show modest facilitation if any. It normally shows a high rate of spontaneous activity, even when the ganglion of origin is isolated.

**No. 4.** This axon shares with no. 3 the main burden of innervation; near no. 3 in size, it also distributes to all regions of the muscle. It tends to show faster-rising junctional potentials than no. 3, and a considerable level of spontaneous activity. In the classical terminology it would probably be called ‘fast’. Nevertheless, no. 4 has been observed to produce facilitating e.j.p.s in one muscle fibre and antifacilitating ones in an adjacent one. Both nos. 3 and 4 are activated most effectively by tactile stimulation of abdominal segments, or by stimulation of the telson and uropods.
No. 5. The inhibitory axon distributes selectively to the medial portion, and produces hyperpolarizing junctional potentials; it is discussed elsewhere.

No. 6. The largest excitatory axon, no. 6, is more densely represented in the lateral half of the sheet. It differs from the others in always producing very small j.p.s which usually facilitate dramatically. Its main sources of excitation are extra-ganglionic. It shows a low degree of spontaneity in isolated ganglia, but where spontaneous impulses occur they are very often paired. The most effective natural stimuli are to rostrally located tactile hairs, especially those on the carapace and thoracic appendages; the axon shows a pronounced tendency to fire in phasic bursts.

This account confirms that there are real differences between the central driving systems for these motor axons, a point which will be further documented below. There are also differences in the temporal behaviour of the junctional potentials evoked by a given axon, the most consistent one being the relatively strong facilitating tendency of the e.j.p.s evoked by no. 6. This is correlated with a 'bursty' mode of discharge, so that the facilitation property is normally taken advantage of in reflex actions. By contrast, the discharge pattern of nos. 3 and 4 is relatively more tonic.

**Reflex actions**

Figs. 6 and 7 document some of the points of the preceding section in a somewhat different way. In Fig. 6 the responses of two muscle fibres located in the medial (A) and lateral (B) portions of the superficial sheet are shown with their accompanying nerve discharge. In each run of record the natural stimuli most effective for each were employed. During the two periods of high membrane potential in A, the telson was passively moved to its full extent; it was then flexed, inducing depolarizations again. In B hairs of the carapace were stroked periodically. The two muscle fibres are fairly characteristic representatives of the common extremes of the innervation spectrum. The medial one (A) received from nos. 3 and 4; these axons produced nearly identical junctional potentials of large size which did not facilitate but instead tended to decrease in amplitude when repetitively evoked. This behaviour was prob-
ably only partially due to antifacilitation; it may also have resulted from the fact that successive e.j.p.s were being evoked at lower and lower values of membrane potential. Fairly constant depolarization, and hence tension, was maintained by the spontaneous discharge even without deliberate natural stimulation. Following the inhibition evoked by telson extension, high frequency activity returned; the amplitude of the membrane potential change from full inhibition to ‘ambient’ excitation was at least 25 mV. The lateral fibre in B, by contrast, received major excitatory input only from no. 6 and was not innervated by the inhibitor. The membrane potential was relatively invariant in the absence of deliberate stimulation because those axons exhibiting most endogenous activity did not connect with the fibre. Stimulation of the carapace hairs evoked phasic bursts of activity in no. 6; these in turn produced strongly facilitating e.j.p.s in the muscle fibre. Although intermediate patterns appear these two types of muscle fibres are quite common, and we refer to them for convenience as type I and type II fibres respectively. The contrast in their behaviour indicates that the task of maintaining tonic tension in the superficial muscle is not equally shared among its fibres. Instead, some are almost continuously poised in the ‘working’ range of membrane potential, whereas others contribute only occasionally in response to natural stimuli specifically capable of exciting their own major efferent axon.

In Fig. 7 responses to electrical volleys are shown, again with simultaneous nerve and muscle recording. In $A_1$, the intensity of a single stimulus to the first and second
roots together was increased. The nerve response indicates that this mixed input activated most of the efferent axons; but by far the most effectively driven unit was axon no. 4, which produced repetitive discharges at the intensities shown. The response in the muscle fibre to axon 4 was an e.j.p. that showed considerable facilitation. The presence of such facilitation, and its interval sensitivity, produced a considerable amplification of volley strength in the response of the muscle; the depolarization resulting from the shorter, lower frequency train in \( A_1 \) was only one-third as great as that following the longer, higher-frequency train in \( A_4 \).

There was a considerable difference between the responses produced from electrical stimulation of ipsilateral first and second roots. Stimulation of the first root alone evoked activity in no. 4 similar to that just discussed; the second root activated some excitatory efferents, but its most obvious action was to drive the inhibitor. Repetitive stimulation, in most preparations, produced a tonic activation of no. 5, accompanied by suppression of activity in all other efferent axons; this was often followed by long (but variable) after-effects. Fig. 7A shows a 90/sec. stimulus applied to the second root. After an initial depolarizing response attributable to activation of an excitatory axon by the first few stimuli the excitatory junctional potentials stopped and the membrane was concomitantly hyperpolarized. Even after cessation of stimulation no. 5 continued to discharge at a slow rate; activity in the excitatory axons returned slowly, and the e.j.p.s gradually returned to their former amplitude—presumably through facilitation, and perhaps also through a slow restoration of normal membrane conductance. The identity of the afferent second-root component responsible for producing this 'inhibitory state' in the ganglion is not known. The effect is rather smoothly graded in intensity, has a higher threshold than that for single-shock driving of excitatory axons, and cannot be directly evoked by stimulation of the receptor organs in the dorsal muscles of the same segment.

**Reflex inhibition**

Figs. 6A and 7B are also typical examples of the ways in which reflex inhibition of the slow flexor muscle is accomplished upon extension of the telson or by electrical stimulation of the second root; it also frequently was exerted 'voluntarily' by the animal. When brought about in these ways discharge in the peripheral inhibitor always accompanied complete central suppression of activity in all five excitatory axons. The frequency of inhibitor discharge during such periods varied considerably from one preparation to another, but central inhibition of the excitors was always powerful and effective.

All of the available information leads to the conclusion that the major mechanism of reflex inhibition in the superficial flexors is central rather than peripheral. First, less than half the fibres receive the peripheral inhibitor; secondly, the discharge rate in the inhibitory axon is often not very high, even when suppression of excitatory outflow is complete.

The reciprocal discharge of inhibitor and excitor axons (which may not hold for all peripheral routes of reflex inhibition) naturally raises questions about the central mechanisms employed to achieve it. The present experiments have not provided an answer, but make one possibility highly unlikely. In several experiments intracellular recording from slow flexor-muscle fibres was carried on while the nerve bundle...
innervating the muscle was stimulated repetitively. Such stimulation, which could be
graded in intensity to secure antidromic activation of the inhibitory axon, produced
no alteration in the spontaneous discharge frequency of the motor axons. If one can
assume invasion of all regions of the motoneurone (cf. Takeda & Kennedy, 1964), it may
be concluded that reciprocity is not achieved by inhibitory collaterals from the peripheral
inhibitor to the motor axons serving the same muscle. Whatever inhibitory cross-
connexions exist would then be located at a level presynaptic to the motoneurones.

**DISCUSSION**

Several features of these muscles are at variance with the classical picture built up
largely from studies on limb muscles. In the latter it has been the rule to find slow
and fast responses generated by the different junctional events produced by different
motor axons in the same set of muscle fibres, and/or by the actions of electrically
differentiated or specially innervated muscle fibres scattered through the same muscle.
In the flexors of the abdomen, however, the separation is complete, involving a duplex
innervation as well as anatomically distinct sets of muscles.

The terms 'fast' and 'slow', originally applied to the types of contraction obtained
from certain limb muscles when different motor axons were stimulated, are of
doubtful utility in the present system. The obliques perform as 'twitch' muscles
regardless of which excitatory axon drives them; the superficial fibres exhibit slow
contractions in response to activity in all of their motor nerves. Within the slow
system a variety of types of junctional potentials is found; following the terminology
of Hoyle & Wiersma (1958a), which stresses the importance of facilitation, these could
be divided into relatively 'fast' and 'slow' categories. Under such a system axon
no. 4, which frequently evokes large and relatively fast-rising e.j.p.s, would be called
fast; and axon no. 6, which always produces initially small e.j.p.s with pronounced
facilitation, would be called slow. Acceptance of such a classification, however,
simply produces further contrasts with the limb muscles. Normally, the thick axon
supplying a limb muscle is the fast axon, and the thinner the slow; axon no. 6,
however, is the largest in the bundle innervating the slow flexor. Furthermore, the
inhibitor to limb muscles is normally thinner than any excitatory axons; in the present
case, the inhibitor is the second largest of the six.

The electrical properties of the muscle fibre membrane, not directly studied here,
may also relate to the classification problem. Some of the slow flexor muscles (e.g.
'type I') resemble the type B fibres of the *Carcinus* 'closer' studied by Atwood
(1963a), and others (type II) may correspond to his type C. But electrical differences
between muscle fibres, even if present, do not correspond in the present case to a clear
fast/slow dichotomy in innervation. The same lack of correlation has been noted by
Atwood (1963b) for other crustacean muscles.

Other difficulties also attend the codification of post-junctional events in terms of
specific motor nerves. Size of junctional potential is clearly not consistent; a pair of
axons that produce e.j.p. amplitudes having a 2:1 ratio in one muscle fibre may
exhibit a reversal of that ratio in a nearby fibre. Rise-times of e.j.p.s may be somewhat
more reliable, but are by no means completely so. Tendency to facilitate, which has
been heavily relied upon in the past, is not at all consistent in the present system: in
some experiments a particular axon has shown opposite temporal properties even in
nearly adjacent fibres. These variations suggest that the phenomena involved depend not upon some property of the nerve cell as a whole, but upon attributes which might vary from one set of efferent terminals to another. Among these latter, the most promising would seem to be size or geometry of the terminals, and the density with which they occur upon the muscle fibre. The former characteristics would be decisive for either of the mechanisms that might determine the temporal 'recovery cycle': the extent to which the amplitude or extent of invasion of efferent spikes is altered by previous activity; or the amount of available (or mobilizable) transmitter per terminal. It would be expected that some axons might show consistency in some or all of these properties. Thus, large axons might have many branches, and hence be able to innervate densely; or smaller axons might be expected to have relatively fine terminals where they innervate densely, and therefore to lose transmitter relatively rapidly and exhibit antifacilitation. It is also possible that in a given axon there might be variation in these properties from region to region.

In complexity of innervation the slow flexor muscles exceed even the main flexor muscle of the carpopodite, which receives four excitatory axons (Wiersma & Ripley, 1952). The latter muscle was analysed by Furshpan (1955) in terms of combinations of axons upon single muscle fibres, and his results afford an opportunity for comparison of the two systems. The majority of fibres in this limb muscle receive either two or three axons (26 and 29% respectively); another substantial group receives a single neuron (38%), and very few (7%) receive all four. The abdominal slow flexor (Table 1) likewise shows a relative abundance of doubly- and triply-innervated fibres, but differs in the extreme rarity of singly- innervated ones. Though fibres receiving four and five excitatory axons are also rare, they do occur, usually in combination with the inhibitor.

It is possible that the number of muscle fibres innervated by the inhibitor axon has been underestimated. Atwood (1964) has recently shown that in some muscles 'phasic' fibres (those showing active electrogenic membrane responses) undergo only slight conductance changes in response to stimulation of the inhibitory nerve compared to that exhibited by 'tonic' (non-spiking) fibres. The distinction is hardly applicable to the present system, but it is nevertheless possible that weak inhibitory responses (of the type shown by Atwood's 'phasic' fibres) could have been missed, especially in some lateral fibres; it is difficult to see, however, what functional role could be played by such a weak inhibitory innervation. The more apt comparison between Atwood's results and the present experiments is between his phasic fibres and our fast flexor fibres; in both cases impulses in the inhibitor had a small effect. The slow flexor fibres that do have i.j.p.s produce large ones, like Atwood's tonic fibres.

The complexity of the innervation pattern of slow flexor muscles, and the fact that specific excitatory axons have preferred routes of reflex excitation, suggested that particular axons might form sets within which there was an appropriate matching of function, and that such sets would provide predominant combinations in innervation. This expectation was not realized. On the contrary, the frequency of most possible combinations is approximately that to be anticipated on the basis of random association, and the few exceptions are readily explained on the grounds of regional restrictions in the distribution of certain axons. Thus precise developmental mechanisms are not required to achieve a specific pattern of connexions; the observed pattern could be brought about by a group of axons that grow out into the muscle (for a distance.
determined in part perhaps by their diameter), and branch repeatedly to innervate muscle fibres with variable density. There is no evidence for correlative influence between specific sets of neurones in this process, though of course the major branch points may show the congruence characteristic of crustacean motor axons.

Since specific, preferred innervation combinations are apparently not arranged for, what is the functional organization of this motor system? The motor axons have different sources of central activation and different discharge patterns, and from this group muscle fibres 'sample' an average of two or three. The sample, though nearly random, is somewhat regionally weighted; lateral muscle fibres are relatively likely to receive no. 6 and very unlikely to get nos. 1 or 2, for example. The muscle thus shows broad regional differences; these may be due to variations in the electrical properties of fibres as well as to the presence of certain motor nerves in particular areas. More than this, it also exhibits a functional heterogeneity even very locally, due to incomplete sampling of a heterogeneous population of motor nerves by the muscle fibres. The burden of tension maintenance thus shows a tendency to shift among the fibres according to the source of reflex activation. Moreover certain fibres carry a higher overall load due to their innervation. The fibres of many crustacean muscles, including the slow abdominal flexors, are relatively scattered, with their origins and/or insertions rather distant from one another; and it may be that the broad regional differences that we have noted relate to slight differences in action or mechanical advantage. For example, the lateral fibres of the superficial muscles shorten considerably less than do medial ones. Such regional specializations, however, do not explain the more local variations emphasized in this discussion.

The discharge patterns of motor axons observed in these experiments suggest that there is a matching between the normal mode of firing of the nerves and the properties of their junctions with the muscle fibres. The most direct demonstration of this relationship is in axon no. 6, which normally discharges in bursts and frequently, even in isolated ganglia, produces clustered spontaneous impulses. The junctions formed by no. 6 are unique in that they always require facilitation, and the input to the axon is clearly organized to make use of this property. The prevalence of paired discharges is characteristic of other motor systems in arthropods as well; a detailed analysis is presented by Wilson & Davis (1965) for the opener axon.

Finally, the reflex role of the peripheral inhibitor axon deserves special attention. The present results, which indicate that central suppression of excitatory outflow is the chief mechanism of reciprocal inhibition, are in sharp contrast to those obtained on the opener muscle of the claw (Eckert, 1959; Bush, 1962; Wilson & Davis, 1965). The latter muscle is supplied by a single excitatory axon and a single inhibitor; when a proprioceptive closer reflex is initiated via the P-D organs, a train of impulses is triggered in the opener inhibitor (Bush, 1962). This event is not accompanied by reduction in the excitatory nerve discharge; in fact, that axon also increases its impulse frequency. A careful analysis of this situation has been presented by Wilson & Davis (1965), who have shown that the balance between discharge frequencies in the two axons is employed as a tension regulating device. Central stimulation that causes opening also excites both axons, but drives the excitatory one at a higher rate than the inhibitory; a reversed E/I frequency ratio results from peripheral stimulation of the P-D proprioceptive organs, which evokes closing. The slow flexor system has been
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subjected, in our experiments, to as wide a variety of stimuli as possible; yet a similar mode of control has never been revealed. On the contrary, the discharge rates of excitatory and inhibitory axons are reciprocal under nearly all circumstances.

Since the same central events that activate discharge in the peripheral inhibitor also turn off excitatory outflow, it is necessary to postulate some function for the inhibitor other than that of counteracting concurrent excitation. One reasonable proposal is that, in many muscle fibres at least, the duration of e.j.p.s is sufficiently long to require active means of restoring 'resting' membrane potentials after the cessation of excitatory bombardment. The inhibitor axon most frequently innervates muscle fibres that have a rich excitatory innervation and therefore tend to show a high degree of ongoing activity; these are the very fibres which would be most likely to exhibit lingering depolarization (and hence residual tension).

Regardless of what function is put forward for the inhibitor axon it is quite clear that it can only achieve that function through changing the conductance of the postsynaptic membrane. The usefulness of presynaptic inhibition is restricted to those systems in which inhibitory and inhibited neurones are concurrently active; and thus, in effect, central reciprocity between peripheral inhibitor and excitators may be a substitute for similar interactions in the periphery. It is of interest in this connexion that the only demonstration of presynaptic inhibition at crustacean neuromuscular junctions (Dudel & Kuffler, 1961) has been in the walking-leg homologues of the claw opener, where exciter and inhibitor axons are not reciprocally controlled centrally (Bush, 1962).

**SUMMARY**

1. Fibres from the tonic, superficial abdominal flexor muscles in the crayfish receive a complex, highly polyneuronal innervation from among five motor axons and one inhibitor. All efferent nerve fibres show some degree of 'spontaneous' activity.

2. The muscle fibres therefore exhibit a constant flux of membrane potential, and hence of tension, in intact preparations. Depolarization is the result of facilitation and/or summation of junctional potentials of various amplitudes, and in some fibres of superimposed electrogenic responses. Neighbouring fibres tend to show similar innervation patterns, more distant ones dissimilar ones.

3. No useful distinction may be made between 'fast' and 'slow' motor axons. A given axon may produce junctional potentials of very different amplitudes (and somewhat different rise-times) in neighbouring muscle fibres while another exhibits a precisely reciprocal relationship. The largest axon produces facilitating junctional potentials in all the muscle fibres it innervates, but others may exhibit facilitation in one muscle fibre and antifacilitation in another.

4. Most muscle fibres are innervated by two or three excitatory axons; fibres with single, quadruple or quintuple motor innervation are relatively rare. There is a pronounced tendency for fibres with a rich excitatory innervation to receive the inhibitor as well. The innervation is not shared equally among motor axons: one serves over 90% of the muscle fibres, and two others 20% or less. Statistical analysis of the combinations of motor axons serving muscle fibres reveals that these are apparently random, with all variations from randomness accountable on the grounds of broad regional differences in distribution.
5. The motor axons are selectively activated by specific reflex inputs. Since muscle fibres receive, on the average, only a restricted sample of the available motor supply, it follows that they participate differentially in different reflex actions. Evidence is presented that the firing pattern of motor nerves is appropriate for the temporal properties of their neuromuscular junctions.

6. Reflex inhibition is accomplished by central inhibition of all excitatory motor outflow, accompanied by reciprocal firing in the inhibitor axon. This and the fact that less than half the muscle fibres receive inhibitory innervation demonstrate that, in contrast to the one other crustacean system analysed, reflex inhibition is primarily a central event. Peripheral inhibition in the slow flexor system must serve mainly as a device to achieve repolarization and thus terminate contractions. Such action necessarily depends upon post-synaptic rather than presynaptic mechanisms.

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