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DISCHARGE PATTERNS OF NEURONES SUPPLYING TONIC ABDOMINAL FLEXOR MUSCLES IN THE CRAYFISH

BY WILLIAM H. EVOY,* DONALD KENNEDY AND DONALD M. WILSON†

Department of Biological Sciences, Stanford University, Stanford, and Department of Zoology, University of California, Berkeley

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INTRODUCTION

To understand the central nervous control of behaviour it is necessary to inspect cell-to-cell interactions at a number of transition points: sensory axon to central neurone, central neurone to motoneurone, motoneurone to muscle fibre. The restricted number of elements found in arthropod nervous systems offers some encouragement that in such animals this procedure can be carried out at the level of single, individually identified cells. The abdomen of the crayfish presents, in addition to these general advantages, certain special ones. It performs a set of fairly simple but precisely controlled movements, and the physiology of the nervous system operating it has been intensively studied (Wiersma, 1961; Kennedy, Evoy & Fields, 1966).

Each abdominal segment in the crayfish (and in other Macrura as well) is equipped with thin sheets of superficial ventral muscles having long (10 μ) sarcomeres. These are responsible for all postural flexion (Kennedy & Takeda, 1965*a*, *b*). The superficial flexors of each side are innervated by six efferent axons, five of them excitatory and one inhibitory; these neurones show different levels of spontaneous activity, which may be modulated by a variety of natural stimuli. The muscle fibres rarely exhibit all-or-none spikes and are subjected to a continuously varying motor barrage, which results in smooth temporal variations in tension. During reflexes that evoke activity in the antagonistic slow extensor muscles, this motor activity is suppressed by central inhibition, and the peripheral inhibitor is excited at the same time; thus, in contrast to the situation in some crustacean motor systems (Bush, 1962; Wilson & Davis, 1965), reflex inhibition is achieved more by central than by peripheral means.

The innervation of these efferent neurones, and the properties of their neuromuscular junctions, have been analysed (Kennedy & Takeda, 1965*b*). It has also been shown that the motoneurones can, to some extent at least, be selectively excited by central or afferent stimuli, so that the task of tension maintenance is not equally shared among them. To make this conclusion applicable to reflex situations and to understand the significance of the neuromuscular organization, it is necessary to have detailed information about the motoneurones themselves—in particular, discharge patterns characteristic of each, and the influence these have on the development of tension in

* Present address: Laboratory for Quantitative Biology, Department of Biology, University of Miami, Coral Gables.
† Present address: Department of Molecular Biology, University of California, Berkeley.

the muscles. Since the abdominal segments move in a correlated fashion rather than as a set of independent elements, it would also be desirable to know how closely the assembly of motoneurones and muscles is replicated from segment to segment, and how co-ordination of efferent discharge across and between segments is achieved. Finally, in order to understand the central nervous control of abdominal posture one must know the location of the cell body and synaptic apparatus of individual motoneurones, and the sites of origin of their spontaneous and evoked activity.

The present paper gives such information about the slow flexor control system; it is a sequel to that of Kennedy & Takeda (1965*b*), and provides a basis for current studies on the action of single central neurones in controlling abdominal position.

METHODS

Crayfish (*Procambarus clarkii*) were collected locally and maintained in aerated tanks at room temperature; for a few experiments lobsters (*Homarus americanus*) were flown from the east coast and held in cold sea-water aquaria. All physiological preparations consisted of animals pinned out ventral-side up in a shaped, paraffin-filled Perspex chamber. The details of preparation were similar to those given earlier (Takeda & Kennedy, 1965*a*, *b*); all nerve recording from superficial third (flexor) and second (extensor) roots was carried out with fine bipolar wire leads in an oil layer, and up to four such records could be displayed and recorded simultaneously. To record tension a flap of connective tissue on which a medial superficial flexor bundle was inserted was gripped in fine forceps attached to the pin of an RCA 5734 transducer. In some experiments single central interneurones were used to supply excitation to the flexor inhibitor axon; these were isolated by dissection from the 5–6 abdominal connective, lifted on to platinum wire electrodes in oil, and stimulated with trains of brief (0·1 msec.) RF-isolated pulses from an electronic stimulator. In experiments where antidromic stimuli were delivered to the superficial or main third root, the stimulating electrodes were also micromanipulated platinum hooks, and they and the region of the root around them were drawn into the oil layer. The nerve distal to the electrodes was cut or crushed to eliminate artifacts or sensory feedback from contraction, and the input volley was frequently monitored by a proximally located pair of recording electrodes. Methods for the computation of phase histograms are discussed in Results.

RESULTS

Size, location and general discharge characteristics of efferent axons

In the third abdominal segment the six efferent elements have been numbered from 1 to 6 in order of increasing size (Kennedy & Takeda, 1965*b*). In a general way the size of the axons is related to their discharge characteristics. Table 1 shows average discharge frequencies in two different experiments for the four spontaneously active axons in the nerve innervating the superficial muscles of segments 2 and 3. The records used for these measurements were made in intact preparations in which uncontrolled sensory stimulation undoubtedly contributed to the excitation of motor elements. When all roots to the ganglia were cut the ratio of activity in the units was approximately maintained; but the frequencies of all of them became more regular.

In general, as Table 1 shows, the 'spontaneous' discharge frequencies of the excitatory axons were higher in ganglion 3 than in ganglion 2. This gradient of excitability tends to persist when the ganglia are isolated from one another.

The source of the impulses belonging to these six amplitude classes was assessed by cutting central connexions. The superficial branch of each abdominal third root leaves the abdominal connective at a point about two-fifths of the way caudal from one ganglion to the next. If the connective is severed caudal to this exit point, between it and the next posterior ganglion, the smallest spike (unit no. 1) disappears from the record; if the cut is made rostral to the exit only spikes from axon no. 1 remain. This procedure shows that the nerve supply to the slow flexors of a given segment is bisegmental in origin; it also provides a means of studying the effect of one isolated motor element.

Table 1. *Average discharge frequencies*

	Unit number			
Ganglion no.	1	2	3	4
2*a*	18·2	7·8	2·5	6·1
b	8·2	7·8	4·0	2·8
3*a*	28·3	9·5	8·2	8·0
b	10·5	18·4	6·5	7·3

Axon no. 1 is readily inhibited by a variety of segmental stimuli. These include promotion of the pleopods, manipulation of the anterior ventral inter-segmental rib and mechanical stimulation of a region of soft ventral exo-skeleton overlying the origin of the slow flexor muscles of that segment. Inputs from dorsal hairs are only weakly excitatory. Some extrasegmental sources are effectively excitatory, especially flexion of the uropods and telson. Extension of these appendages is inhibitory.

Axon no. 2 resembles axon no. 1 except in anatomical location and frequency of spontaneous discharge. Its frequency often is lower, falling below that of nos. 3 and 4 in the same ganglion, and its inputs are not distinct from those of the intermediate-sized excitors.

Axon nos. 3 and 4 are lower than no. 1 in discharge frequency and less regular. They are strongly excited by stimulation of tactile hairs on the dorsum of the segment and on the caudal appendages; like the other flexor motoneurones, they are inhibited by telson extension and by pleopod protomion. In general their segmental inputs are not so predominantly inhibitory as those to no. 1.

Axon no. 5 is the peripheral inhibitor; its properties will be discussed below.

Axon no. 6 is the largest excitatory nerve fibre supplying the superficial flexor muscles; it normally shows little or no spontaneous activity, though in preparations with rostrally sectioned nerve cords it may discharge regularly at low frequency (L. Kahan, unpublished observations). When active it often fires in doubles or triples, separated by relatively long intervals. It has already been pointed out that axon no. 6 produces a high level of neuro-muscular facilitation; the interval produced by this pattern of discharge is well adapted to utilize this property. The propensity to discharge in this way is not dependent upon properties of the peripheral axon membrane. Threshold determinations were made for the second of a pair of test shocks delivered to the fibre, and no supernormality attributable to post-spike depolarization

could be detected. If the properties of the axon truly reflect those of the spike-initiating locus in the ganglionic neuropil, then the discharge mode must be attributed to other causes, such as the pattern of arriving input. The number of impulses per burst usually increases as the inter-burst interval shortens.

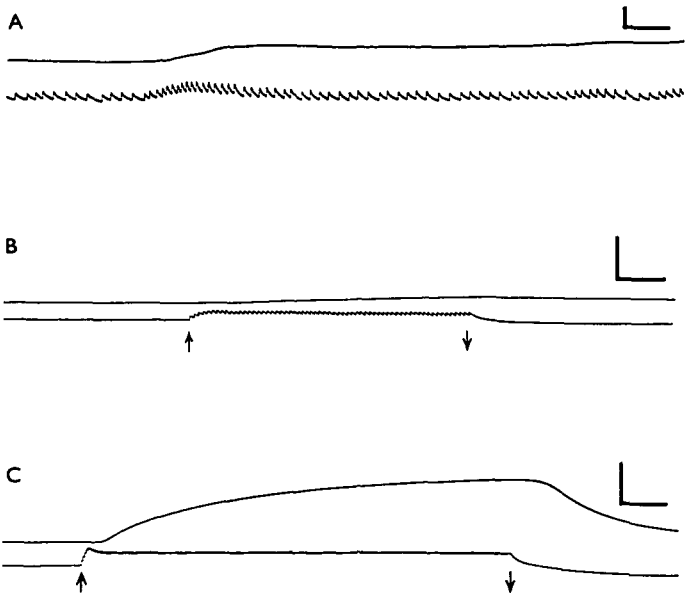

Fig. 1. Conversion of membrane potential to tension in slow flexor muscle fibres. Tension (top traces) recorded from a bundle of medial fibres; intracellular records (lower traces) from one of them. A, Isolated activity of still centrally connected axon no. 1; calibrations, 10 mV., 0·5 sec. B, C, Electrical stimulation (arrows) of an intermediate-sized axon at 20 and 60/sec, respectively, in another preparation. Calibrations: 50 mV., 0·5 sec. Relative tension gains in A, B and C were 10, 2 and 1.

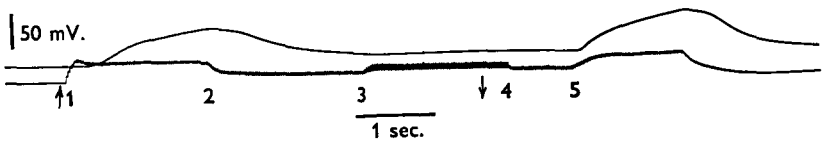

Fig. 2. Tension (upper trace) from a bundle of medial slow flexor muscle fibres; intracellular record (lower trace) from one of them. The motor nerve bundle was stimulated electrically at about 40/sec., with increasing (upward arrow) and then decreasing (downward arrow) intensity. The numbers below the record indicate points at which new motor elements were recruited or dropped out (see text).

Tension development

The difference in the junctional effects produced by the five excitatory axons (Kennedy & Takeda, 1965*b*; Kennedy, 1966), together with the previously described irregularities in their distribution, suggested that each one might contribute in a special way to the maintenance of tension under various conditions. For this reason we measured the tension in medial bundles of slow flexor muscle fibres while simultaneously recording junctional potentials with a microelectrode in one of them. Figs. 1 and 2 illustrate E.J.P.'s and tension development in response to repetitive stimulation of different efferent axons. Fig. 1*a* shows that axon no. 1, which was isolated by cutting

the nerve cord rostral to the exit point of the superficial third root, can produce substantial tension by itself. Increases in its discharge frequency, resulting from spontaneous fluctuations or from natural stimulation of the uropods and telson, caused facilitation and summation of junctional potentials and generated a relatively slow increment in tension. The larger motoneurones (nos. 3, 4 and 6) produced more tension at equivalent frequencies, but in no case was tension development rapid. Fig. 1 (*b* and *c*) shows responses to stimulation of one of the intermediate-sized axons. Tension development was strongly dependent upon frequency; that produced at 60/sec. (*c*) reached a level more than five times higher than that resulting from stimulation at 20/sec.

Single stimuli, even when applied to all excitatory axons at once, produced no tension whatever. The muscle fibres clearly depend for tension development upon sequences of impulses in the motor axons innervating them. Even at the highest motor-nerve frequencies observed in most reflex discharges (*c.* 100/sec.) the onset of tension development had a latency of several hundred milliseconds, and peak tension was not reached for several seconds.

In other experiments the trains of inhibitory impulses were delivered alone and followed by motoneurone stimulation at various frequencies. It was not possible to test all the motor axons in this way, but none of the experiments involving axon no. 6 and axons nos. 3 and 4 revealed any enhancement of contraction due to preceding inhibitor activity.

In this experiment one of the intermediate axons had the lowest threshold; at (2) in the record the inhibitory axon was activated and produced a marked drop in tension. Another motor fibre, whose threshold was exceeded at point (3) on the record, added little tension in the presence of concurrent inhibitor activity. Decreasing the intensity (arrow) simply reversed the sequence, showing that discharge in the inhibitory axon had little or no 'conditioning' effect upon the responses to excitatory stimulation (cf. Hoyle, 1966*b*).

The role of peripheral inhibition

When the peripheral inhibitory axon is electrically stimulated simultaneously with the excitors, E.J.P.'s and tension produced by the latter are both reduced (Kennedy & Evoy, 1966, and Fig. 2). This fact, however, is not helpful in explaining the function of peripheral inhibition in the slow flexor system, since observations of discharge under a variety of conditions (Takeda & Kennedy, 1965*b*; Kennedy, Evoy & Fields, 1966) have shown that the inhibitory axon is almost exclusively active during periods when the excitors have been centrally inhibited. This finding is consistent with the absence of the presynaptic component of peripheral inhibition (Kennedy & Evoy, 1966).

Several types of experiment were undertaken to determine whether activity in the inhibitory axon had any effect upon tension, relaxation rate, or the response to subsequent excitation. Tension development was measured in response to repetitive stimulation of an excitatory axon; no change in its time-course could be detected when the excitation followed a period of inhibitory nerve stimulation (Fig. 2).

It was postulated earlier that impulse trains in the peripheral inhibitor axon, immediately following reflex suppression of motor out-flow, might accelerate the repolarization of the muscle fibres and thus terminate residual tension (Kennedy & Takeda, 1965*b*; Kennedy & Evoy, 1966). Though slightly faster repolarization can

sometimes be seen in intracellular records, Fig. 3 shows that this is not reflected in a significantly faster rate of tension decline. The experiment utilized central interneurones that supply co-ordinated commands for extension (Kennedy *et al.* 1966; Evoy, 1966). Such fibres, when dissected from a central connective and stimulated at 100/sec, produce central inhibition of all the excitatory axons to the slow flexors, and drive the peripheral inhibitor at high frequency. In Fig. 3B such a central command fibre was stimulated while the motor nerve discharge was monitored *en passant* and tension was simultaneously recorded. In Fig. 3A motor discharge was

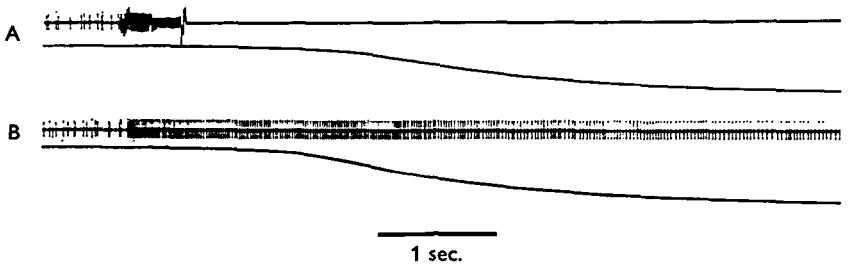

Fig. 3. Central and peripheral inhibition. Lower traces, tension in medial slow flexor bundle; upper traces, extracellular record from the superficial third root supplying the slow flexors. In A the activity in the excitatory fibres was interrupted by cutting the nerve (artifact). In B, an earlier experiment with the same preparation, a command interneurone isolated from the 5–6 connective was stimulated at 100/sec to produce activation of the peripheral inhibitor along with central suppression of the excitatory discharge.

interrupted suddenly (at the point marked by the artifact) by cutting the superficial branch of the third root. In the two cases the background frequencies of the excitatory nerves were approximately the same, and the steady tensions likewise matched; one of the two procedures simply cut off excitatory activity, whereas the other one also produced high-frequency inhibitor discharge during the silent period. As the records show, the rate of tension decline was not noticeably influenced by the presence of peripheral inhibition.

Since in some muscles membrane hyperpolarization can produce relaxation even in the absence of concurrent excitatory bombardment, i.e. at 'resting' membrane potential (Hoyle, 1966*a*), we sought to determine whether the tension exerted by superficial muscle fibres at rest might be influenced by activity in the peripheral inhibitory axon. Inhibitory trains were therefore delivered to the muscle at a wide range of initial lengths. In no case was there a reduction in tension. These experiments also make it unlikely—though not impossible—that inhibitory impulses have an influence upon the stretch resistance of the muscle fibres.

Trans-ganglionic interactions

In records of spontaneous or naturally evoked discharges in the peripheral inhibitor axon, taken simultaneously from the two sides of a single ganglion, a large number of coincident impulses are seen (Kennedy & Evoy, 1966). We have made a quantitative analysis of this phenomenon by preparing phase histograms for long series of discharges. Such histograms, illustrated in Fig. 4*b–d* and in several subsequent figures, evaluate the temporal relationships between the discharge of any pair of units which are firing at approximately the same frequency. The phase of discharge in one unit

relative to that in another is calculated as the ratio of two intervals—the cross interval or latency from the spike in the first unit to the spike in the second, divided by the inter-spike interval for the first unit. Thus a phase of 0·5 indicates that the spike in the second unit fell half-way between two spikes in the first, whereas phase values of 0·0 or 1·0 indicate coincidence. The histograms have been computed from data measured on an Oscar K digitizer and entered on punched cards. Methods and computer programs for carrying out such analyses have been described earlier (Wyman, 1965).

Fig. 4 is an analysis of the discharge of four simultaneously recorded flexor inhibitor axons—those from each side of ganglion 3 and from each side of ganglion 2. During

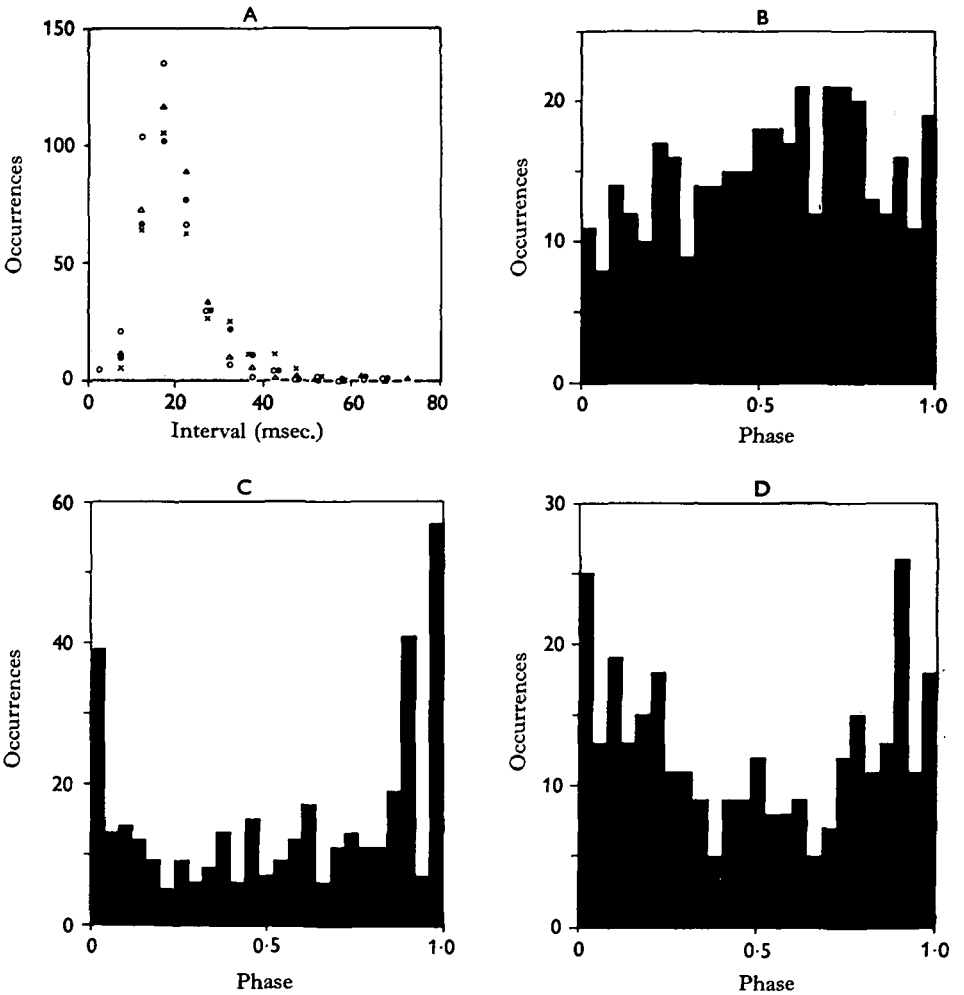

Fig. 4. A, Interval histogram for the discharges of four peripheral inhibitory axons in two adjacent ganglia. Open circles, ganglion 2 left; closed circles, ganglion 3 left; triangles, ganglion 2 right; crosses, ganglion 3 right. Average frequencies for these units were, respectively, 53·8, 48·7, 43·0 and 46·0/sec. B, Phase histogram showing the correlation of discharges in the inhibitor of ganglion 2 left with those in the inhibitor of ganglion 3 left. C, D, Phase histograms showing discharge correlations for the two inhibitors from ganglion 2 (C) and ganglion 3 (D). Intact preparation; peripheral inhibitors driven reflexly by telson extension.

the period of recording, repetitive discharge in the inhibitors was evoked by extending the telson. Fig. 4 gives interval histograms for each axon; they have peaks at the same interval value and show similar distributions. Fig. 4*b* shows that there is no significant phase correlation between the axons from two different ganglia. There is, however, a strong tendency for phase correlation between the inhibitor axons coming from two sides of the *same* ganglion. This is expressed most strongly in the second ganglion (4*c*), and less so in the third (4*d*).

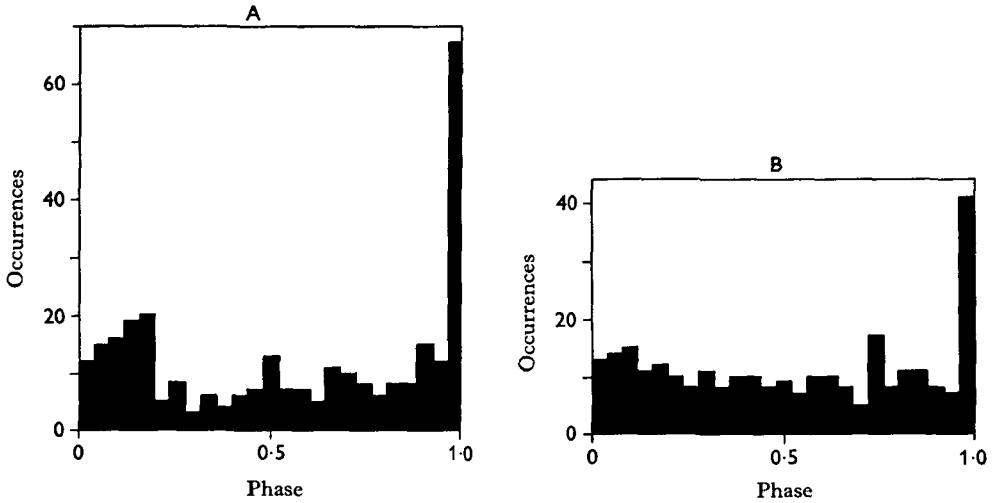

Fig. 5. Phase histograms showing the correlation of discharge between the two peripheral inhibitory axons from ganglion 1 (A) and from ganglion 2 (B). Activity in the inhibitory axons was evoked by stimulating an extension command fibre, isolated from the 5–6 connective, at 100/sec. Average frequencies (in impulses/sec): G_1 left, 38·8; G_1 right, 36·4; G_2 left, 42·6; G_2 right, 31·3.

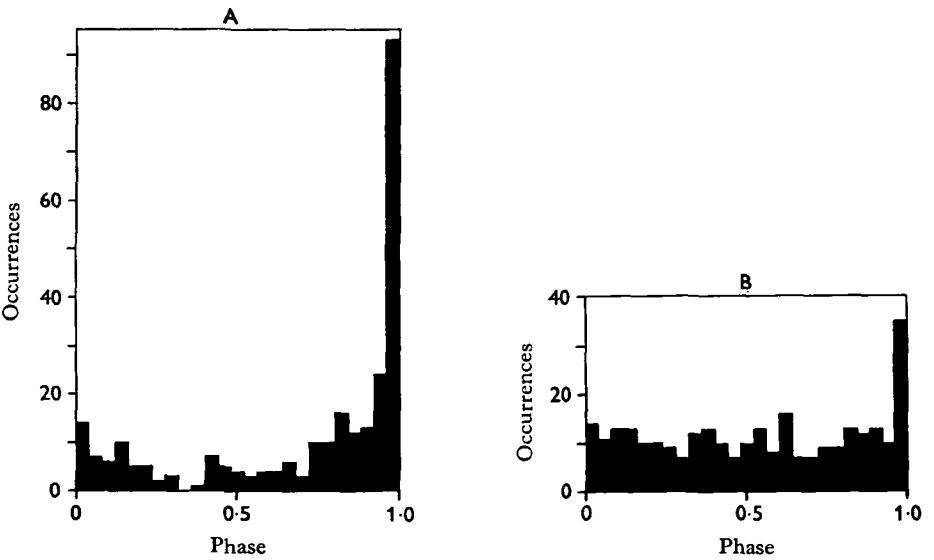

Fig. 6. Phase histograms for discharges of the inhibitory axons from ganglion 1 (A) and ganglion 2 (B); details as in the preceding figure, but data from a different preparation. Frequencies: G_1 left, 28·8; G_1 right, 32·8; G_2 left, 27·6; G_2 right, 28·9.

Similar results were obtained when the inhibitory axon was driven by electrical stimulation of single central 'command' interneurones that promote extension (Evoy, 1966; Kennedy *et al.* 1966). It was of particular interest to see whether different central routes of activation would produce different phase relationships in the output. Figs. 5 and 6 show phase histograms for the slow flexor inhibitors from both sides of ganglia 1 and 2 in two different preparations. As in Fig. 4, the more anterior ganglion exhibited the tighter cross-coupling. The strong phase correlations in this ganglion clearly indicate a short coupling time-constant between the inhibitory axons. The effect is largely restricted to a single rectangle in the phase histogram, indicating

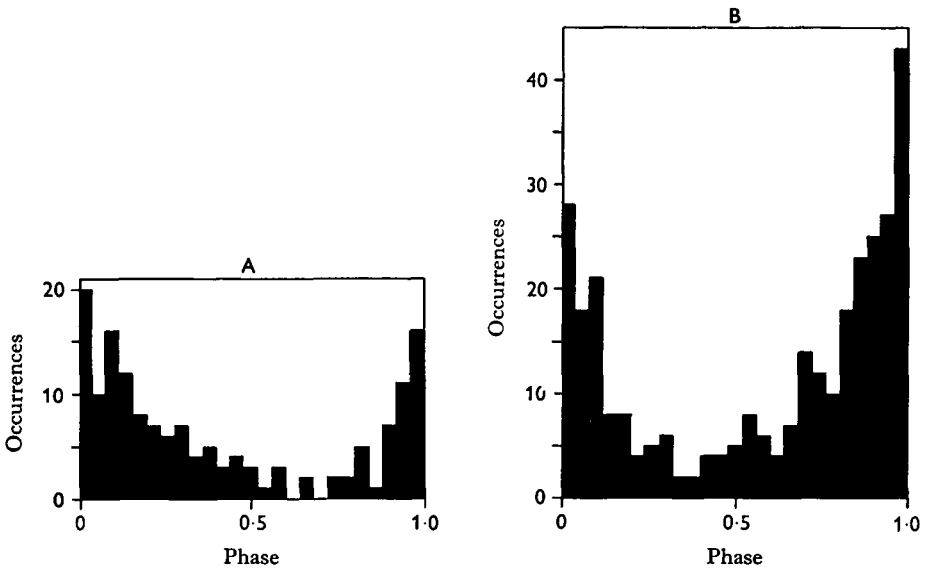

Fig. 7. Identical with Fig. 6 except that a different command fibre in the 5–6 connective was stimulated. The difference in the form of the two histograms for the first (A) and second (B) ganglia from those shown in Fig. 6 is in part due to differences in command fibre effectiveness. See text for details. Frequencies: G_1 left, 6·4; G_1 right, 9·7; G_2 left, 13·1; G_2 right, 13·1.

that it has a duration of approximately 3 msec. Fig. 7 shows the analysis for a second command fibre in the same preparation used in Fig. 6. The phase histograms are quite different, but these differences are partly due to the relatively high discharge frequencies produced in ganglion 2 compared with ganglion 1. There is a purely statistical predilection for stronger phase correlations to emerge in higher-frequency discharges than in lower-frequency ones: even if the coupling time-constant were invariant with frequency its effect would extend over a wider range of phase values at higher frequencies. For this reason the differences between Figs. 6 and 7 may not indicate a fundamentally different mode of connexion of the two command fibres. Higher-frequency discharge also promotes synchrony for physiological reasons. The record of Fig. 8 was obtained in response to a very phasic extension of the uropods and telson; the bilateral pair of inhibitory axons discharged in nearly perfect synchrony when the frequency reached the unusually high value of 200/sec. A high level of excitability in both cells would have the effect that a low-amplitude coupling signal from either cell would be above threshold for the other.

Similar analyses were made for flexor inhibitor axons in the lobster (Fig. 9), for the peripheral inhibitors to the slow extensor muscles in the crayfish (Fig. 10), and for the paired accessory nerve axons which supply inhibitory input to the dorsal stretch-receptor neurones in the crayfish (Kuffler & Eyzaguirre, 1965). The latter sets of cells

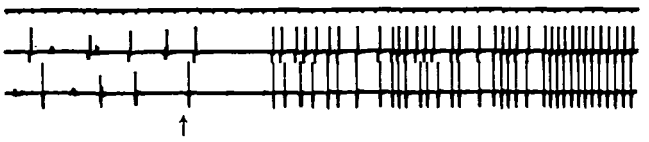

Fig. 8. Bilateral phase coupling of inhibitory axons, ganglion 2. At the arrow the telson was depressed; as the frequency of discharge in the peripheral inhibitors increases, the synchrony becomes more precise. Time-marks, 10 msec.

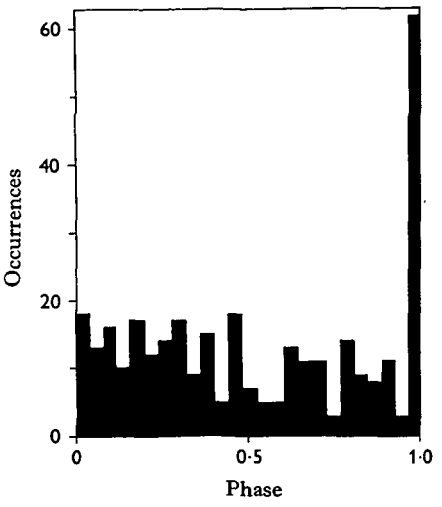

Fig. 9

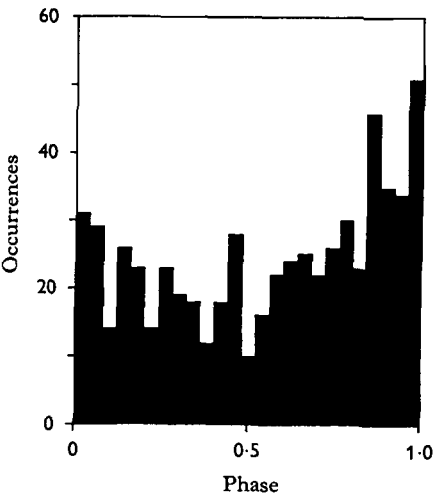

Fig. 10

Fig. 9. Phase histogram for discharges of the two peripheral inhibitory axons to the slow flexors in the third ganglion of the lobster. Frequencies: left, 60·3; right, 59·5.

Fig. 10. Phase histogram for discharges of the two extensor inhibitor axons of the third ganglion in the crayfish. Records were made from the two peripherally crushed second roots in an otherwise intact preparation. Frequencies: left, 10·1; right, 8·5.

can be identified in records from the dorsal nerve, a branch of the second ganglionic root that innervates the dorsal slow extensor musculature (Fields, 1966; Fields, Evoy & Kennedy, 1967). As shown in Fig. 9, the phase correlation between slow flexor inhibitors in the lobster resembles that in crayfish. The phase relationship between crayfish extensor inhibitors was much weaker, though present (Fig. 10), and that between the accessory nerves was not detectable at all.

We have evaluated the interaction between bilateral pairs of excitatory axons to the slow flexors in the same way. In Fig. 11 interval and phase histograms are given for axon no. 1. This motoneurone (see above) has its cell body in the ganglion posterior to the root entry, and was isolated for study by sectioning the nerve cord above the third roots of ganglion 3. The interval histograms show that the spontaneous frequency on each side was fairly constant, with a mean value of about 16 impulses/sec (cf.

Table 1). The phase relationships (11 C) were random. Similar results have been obtained from axon no. 2 (see Fig. 12*d*), indicating that the relatively tonic small axons lack the characteristic coupling seen in the peripheral inhibitor. The intermediate-sized motor axons, nos. 3 and 4, do show some bilateral phase correlation (Figs. 12*a*, *c*), though its time-constant is larger than that for the inhibitors. Serially homologous axons on the same side of two adjacent ganglia, however, display no phase correlation (Fig. 12*b*).

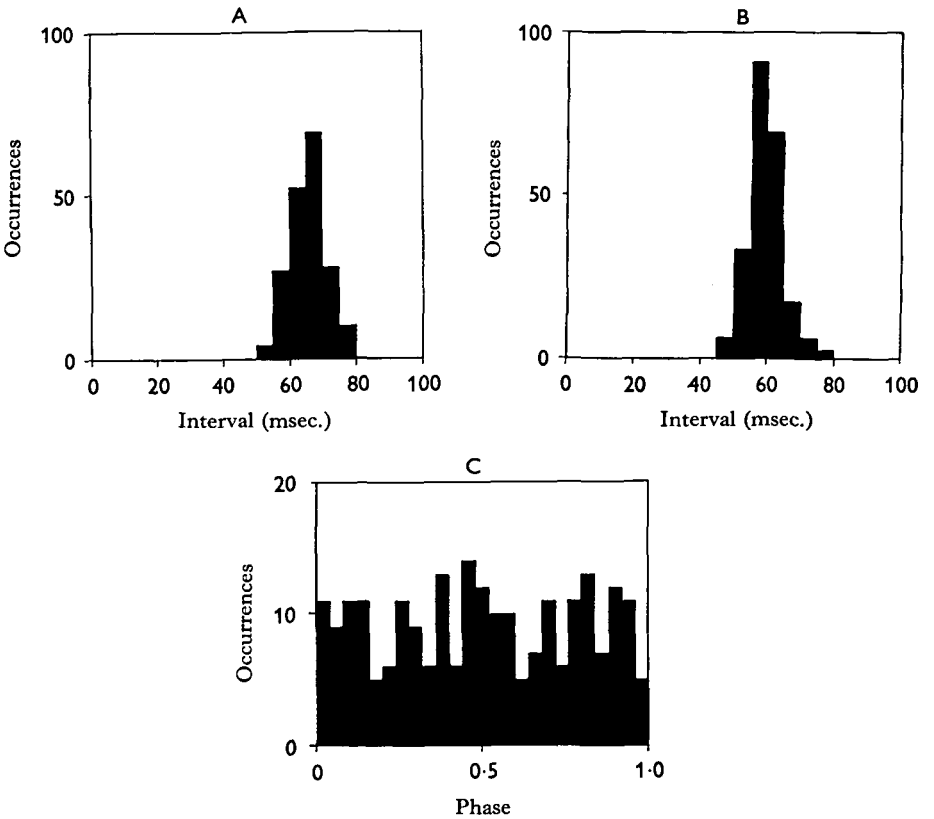

Fig. 11. Analysis of the discharges of slow flexor motoneurone no. 1, recorded from the slow flexor roots of ganglion 2 after sectioning the nerve cord just below ganglion 2 to interrupt the other efferent elements. A, Interval histogram for units on the right side; the mean interval for 203 values was 66 msec., standard deviation 5·0 msec. B, Same, left side; $N = 226$, mean 60 msec., standard deviation 5·1 msec. C, Phase histogram for the discharge correlation of the right motoneurone with the left.

The phase relations of axons nos. 3 and 4 between and within ganglia are given in more detail in Fig. 13. In these experiments axons nos. 3 and 4 were compared with their homologues in adjacent ganglia (Fig. 13*a*, *b*), with each other in the same root (Fig. 13*c*, *d*), and with non-homologues in adjacent ganglia (Fig. 13*e*, *f*). The results may be summarized as follows: (1) there is a tendency for phase coupling in the intermediate-sized motoneurones; (2) this tendency is especially strong between serially homologous axons on the same side of adjacent ganglia; (3) it is weaker in non-homologues within the same ganglionic root; (4) it is weakest of all between

non-homologues in adjacent ganglia. In no case is the coupling as strong or as brief as that between bilaterally paired homologues (cf. Fig. 12*a*, *b*).

Antidromic activation

In order to determine whether coupling connexions actually occur at the motoneurone level one flexor root was stimulated antidromically to test for the presence of connexions with the contralateral one. The superficial branch of the third root contains only the six efferent axons that innervate the slow flexor muscles (Kennedy & Takeda, 1965*a*, *b*). Electrical stimuli delivered to a peripherally severed root thus produce

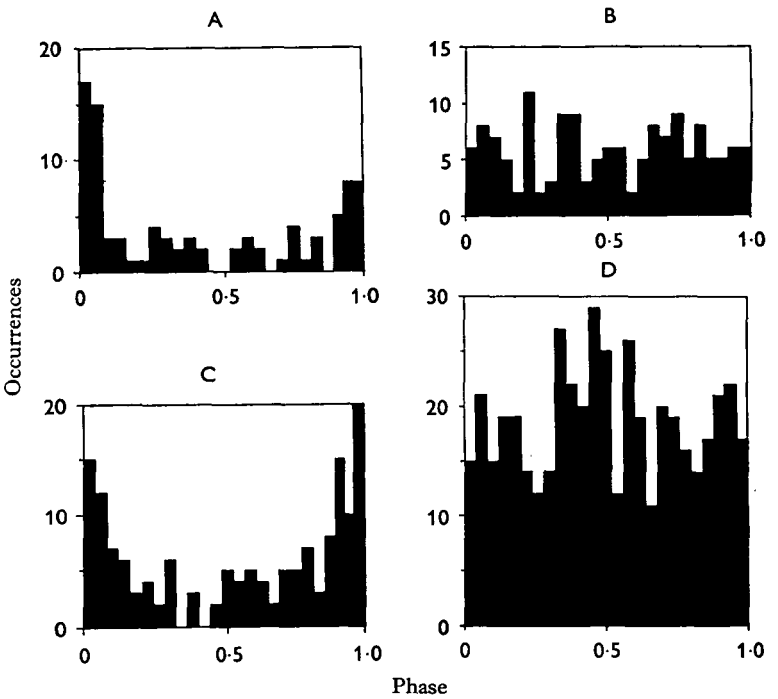

Fig. 12. Phase histograms for discharge correlations between homologous and non-homologous slow flexor motoneurones on the left and right sides of ganglion 3. A, Phase comparison of axon no. 3, left upon axon no. 3, right. B, Phase comparison of axon no. 4, left upon axon no. 3, left. C, Phase comparison of axon no. 4, left, upon axon no. 4, right. D, Phase comparison of axon no. 2, right upon axon no. 2, left. Frequencies: unit no. 3 left, 2·9; no. 3 right, 3·1; no. 4 left, 5·0; no. 4 right, 4·9; no. 2 left, 13·9; no. 2 right, 14·9.

antidromic impulses in these fibres without accompanying sensory activation. In some experiments the antidromic volley so produced was monitored by placing recording electrodes more proximally on the root; recording electrodes were also placed on the contralateral second and superficial third roots.

A typical result is shown in Fig. 14, in which the responses of the contralateral extensor nerve are shown as well as those in the contralateral superficial third root. High-frequency antidromic stimulation of the slow flexor efferents produced an acceleration of discharge in the intermediate-sized axons of the contralateral flexor root; a unit in the second root, identified as the extensor inhibitor, was also driven. When the procedure was reversed, so as to stimulate the contralateral superficial third

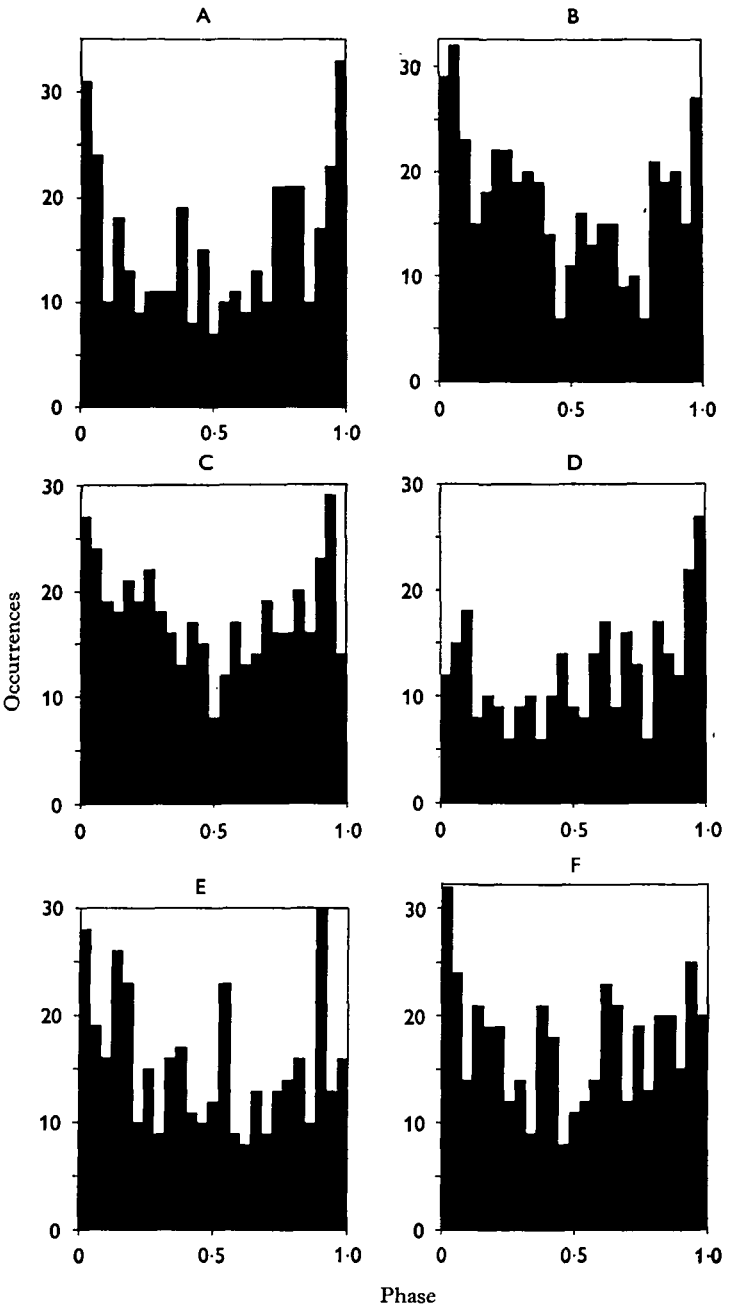

Fig. 13. Phase histograms showing the correlation of discharges between the intermediate-sized slow flexor motoneurones (nos. 3 and 4) on the same side in two adjacent ganglia. The phase comparisons are as follows: A, No. 3, ganglion 3 upon no. 3, ganglion 2. B, No. 4, ganglion 3 upon no. 4, ganglion 2. C, No. 3, ganglion 3 upon no. 4, ganglion 3. D, No. 3, ganglion 2 upon no. 4, ganglion 2. E, No. 3, ganglion 3 upon no. 4, ganglion 2. F, No. 4, ganglion 3 upon no. 3, ganglion 2. Frequencies: unit no. 3, ganglion 2, 2·9; no. 3, ganglion 3, 5·8; no. 4, ganglion 2, 4·8; no. 4, ganglion 3, 6·7.

root and record from the root originally stimulated, an identical result was obtained (Fig. 14*b*).

Experiments in which the antidromic input volley was monitored showed that stimuli graded so as to activate only the largest axon (no. 6) produced increases in the discharge frequencies of intermediate-sized axons contralaterally and of the extensor inhibitor recorded in the second root of the same ganglion. Several attempts were made to stimulate the peripheral inhibitor axon selectively, but these were not successful.

The results of these experiments indicate that a very weak form of coupling exists between the efferent axons leaving a particular ganglion, and that this coupling is *not* restricted to bilaterally homologous pairs. Whether it depends upon non-specific electronic interactions or upon synapses of recurrent collaterals is not clear. We have attempted to determine whether generalized 'field' influences between parallel motor elements are involved by testing the effects of antidromic stimuli delivered to the fast

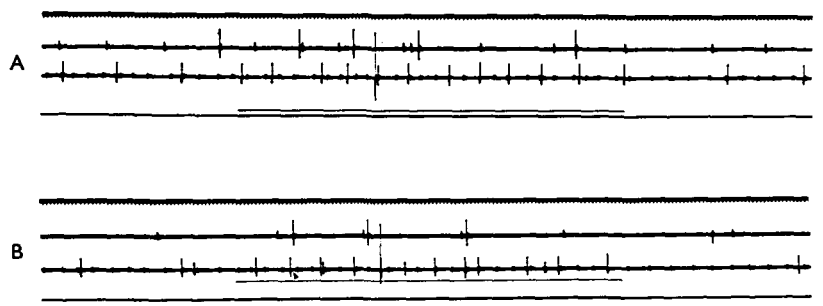

Fig. 14. Responses of slow flexor and extensor efferents to antidromic stimulation of the homologous axons in the contralateral superficial third root. Traces, from top to bottom in each record, are: time-mark (10 msec.), record from distally crushed left second root, record from superficial third root, and stimulus monitor. In A, the right superficial third root was stimulated at 300/sec. and the third trace record is from the left side; this procedure was reversed in B.

flexor motor axons which supply the main flexor muscles via the deep branch of the third root. Ten large efferent axons comprise this root, and they lie adjacent to the slow flexor axons in the rostral portion of the interganglionic connective (Takeda & Kennedy, 1964). The effectiveness of the stimuli in producing antidromic impulses was monitored by an electrode placed on the nerve cord between the third root and the ganglion rostral to it. Such stimuli, delivered at the same frequencies used to test contralateral antidromic interaction between the superficial third roots, failed to affect the discharge of either ipsilateral or contralateral slow flexor motoneurones. Since the much larger main flexor motoneurones should be more effective external current generators than the slow ones, this result argues against a broadly generalized 'field' effect upon the sites of spontaneous impulse discharge in the ganglionic neuropil.

DISCUSSION

The analysis of discharge frequencies in the several slow flexor motoneurones, of the junctional potentials they produce, and of their capacity to initiate tension development reinforce the conclusions reached earlier about this motor system (Kennedy & Takeda, 1965*b*). The motoneurones show a tendency toward spontaneous discharge

which is inversely related to their size, reminiscent of a phenomenon already noted in mammalian motoneurones (Henneman, Somjen & Carpenter, 1965*a*, *b*). Each motoneurone—even axon no. 1, which has the smallest innervation field and the smallest average amplitude of junctional potential—can contribute measurable tension at its own characteristic level of spontaneous discharge in unstimulated preparations. Since extensor motoneurones are less active under these conditions the normal posture of the animal involves a state of considerable flexor tone. This may seem a paradoxical situation since the extensors are the anti-gravity muscles; but several factors may help to explain it. First, the normal gradient of flexor excitability is low rostrally and high caudally; thus the tendency toward extension is greatest in the most anterior segments, which have the greatest mechanical advantage. Second, an extensor motoneurone is involved, with the slow dorsal muscle receptor organ, in a length-stabilizing reflex loop which tends to maintain posture against any flexor tendency (Fields, 1966). Third, the slow extensor muscles are heavier and stronger than the flexors and probably exert substantially more tension for a given level of motor outflow.

The conversion of membrane depolarization to tension in the muscle fibres has an extremely long time-constant; single impulses in all motor axons are without effect, and over most of the motor frequency range the tension at a given moment reflects impulse frequency over the preceding several seconds. This is entirely consistent with the behaviour of tonic, coarsely striated muscles in crustacean limbs (Atwood & Dorai Raj, 1964; Atwood, Hoyle & Smyth, 1965). The individual motoneurones do not participate equally in producing whole-muscle tension; their contribution depends upon the extent of their innervation, the average size of junctional potentials they produce, the degree of facilitation and antifacilitation shown by the latter, and the frequency at which they are normally active. It is extremely difficult to apportion among this wealth of variables the responsibility for tension maintenance at different levels of excitation. Several general statements, however, seem applicable. First, the tendency of the smaller fibres, especially axon no. 1, to discharge spontaneously makes them responsible for a large fraction of the flexor tone found in an undisturbed animal. Second, the extreme degree of facilitation shown by the junctional potentials in axon no. 6, the functionally appropriate tendency of this motoneurone to fire in bursts, and its relatively high threshold, all suggest that it is the most important element in strong reflexes which evoke comparatively rapid movements. Axons no. 3 and no. 4 are intermediate in discharge properties, as well as in size, and it is difficult to make any important distinctions between them. Third, other experiments (Evoy, 1966; Evoy & Kennedy, 1967) show that central interneurones may selectively activate these intermediate-sized axons or axon no. 6. The animal thus employs the special properties of its motor elements in generating movements of the appropriate extent and time-course. The functional role of the peripheral inhibitor to the slow flexor muscles is still in doubt. It can provide substantial increases in the membrane conductance of muscle fibres, and when stimulated concurrently with motoneurones it can reduce or even eliminate the tension produced by the latter. Under normal conditions, however, the intact central nervous system never employs the peripheral inhibitor to counteract ongoing excitation; and we have been unable to demonstrate that inhibitor bursts during the silent period of the excitors affect tension in any significant way. It may be that in some special reflexes, which we have never encountered, the inhibitor is used

to oppose motor impulses. If that is so then two new paradoxes arise. First, why would the inhibitor not utilize the more effective mechanism of presynaptic inhibition? This is the dominant means by which reduction of excitatory junctional potentials is achieved in the claw opener system (Dudel & Kuffler, 1961), where the peripheral inhibitor is used to counteract simultaneous excitation (Bush, 1962; Wilson & Davis, 1965). The presynaptic inhibitory component, however, is missing in the slow flexor system (Kennedy & Evoy, 1966). Second, why is it that all of the central interneurones that produce extensor motor outflow and suppress that to the flexors also drive the peripheral inhibitor? Since the central programme seldom permits simultaneous excitatory and inhibitory outflow, it seems difficult to avoid the inference that peripheral inhibition must accomplish something in an unexcited muscle. For example, inhibitory discharge during the silent period might have a slight effect upon the rate at which residual flexor tension declines, or might decrease the resistance of these muscles to passive stretch by the antagonistic movement. These proposals are tentative, and hardly constitute a satisfactory resolution.

In view of the tonic, highly smoothed nature of the transformation from motor nerve impulses to tension in the slow flexor muscles, it is surprising to find strong bilateral phase relationships between the inhibitory axons and weaker ones involving the excitors. There is good reason to suppose that coupling between the inhibitory axons is achieved by means of low-resistance, electrotonic connexions between the partner cells. D. D. Potter & M. Otsuka (personal communication) have been able to identify the somata of three inhibitory neurones in an abdominal ganglion of the lobster; these are known, by intracellular stimulation experiments, to innervate fast flexors, slow flexors and extensors respectively. By simultaneous intracellular recording from bilateral homologues, and by resistance measurements, they have shown that the members of each pair are electrotonically coupled, but not sufficiently tightly to ensure 1:1 transmission. The somata of all these cells are contralateral to the roots through which their axons leave, as was shown earlier for several of the fast flexor efferents in crayfish ganglia (Takeda & Kennedy, 1964). It is a reasonable supposition that the electrotonic junctions occur at the point of decussation, which occupies a long commissural tract and would provide considerable opportunity for membrane contact between the crossing partners (Takeda & Kennedy, 1964; Kendig, 1967).

The interactions between excitors pose a different sort of problem. In no case is the tendency toward synchrony between bilateral homologues as strong as in the inhibitors. Furthermore, coupling might be due to common presynaptic input, which would produce phasing tendencies independent of any cross-connexion at the motoneurone level. Such a mechanism is suggested by the weak phase correlation shown between serially homologous intermediate-sized excitors in adjacent ganglia. Since the correlation is substantially stronger across the ganglion, however, a more direct interaction is indicated, and this is supported by the effects of antidromic stimulation. The latter procedure also reveals a possible third mode of connexion, involving non-homologues in the same ganglion and operating at the motoneurone level.

It is difficult to see a physiological role for any phasing mechanism between motoneurones in view of the extremely tonic nature of the muscles involved. Rather, the connexions must be regarded as outcomes of a basic plan of ganglionic organization in motor systems; they presumably serve useful purposes in faster systems. The coupling

between inhibitory axons is probably related to the fact that they cross; since some of the fast flexor motoneurones have contralaterally placed somata (Takeda & Kennedy, 1964) it is reasonable to suppose that tonic flexor motoneurones may also. Unfortunately, there are few other investigations dealing with synergistic, bilaterally symmetrical, motor systems with which to compare this result, though 'cross-talk' between central premotor giants has been analysed in annelids (Bullock, 1953; Wilson, 1961; Hagiwara, Morita & Naka, 1965) and arthropods (Wiersma, 1947; Watanabe & Grundfest, 1961). If a phasing system between bilaterally placed premotor interneurones is absent, an easy way to promote synchrony of motor discharge is to provide for decussation of the axons. Since it is difficult to think of other advantages in having cell bodies contralaterally placed, it may well be that the opportunity for electrotonic synchronization was a critical factor in the phylogenetic origin of decussation.

Antidromic cross-excitation of motoneurones may be mediated by such electrotonic junctions at crossing points; but since there are also effects upon the extensor inhibitor axon and between non-homologues, additional factors must also be involved. The lack of specificity is reminiscent of the weak electrotonic interactions recently found in vertebrate motoneurones (Grinnell, 1965; Nelson, 1966). Since the antidromic effect is restricted to functionally related elements, it would be necessary to propose that electrical interactions operate at close range within a very well-defined tract of fibres in the neuropil. An alternative would be that recurrent collaterals distribute sparsely to contralateral and antagonistic motor elements, or to some common presynaptic driver controlling a group of these (Kennedy *et al.* 1966). In either case the effect is an extremely weak one, and under physiological conditions would play only a very minor role in balancing excitation between the two sides.

The third mechanism underlying phase correlation is common presynaptic input. Here, the analysis of phase relationships between homologues and non-homologues in adjacent ganglia has been helpful in confirming proposals about the selectivity of central interneurones that control the abdominal position system. Since antidromic stimulation experiments have revealed no motor connexions between ganglia, any phase relationship between axons in adjacent ganglia is probably due to coincidence of arriving input. It is significant that homologous axons in adjacent ganglia show more phase correlation than non-homologues on the *same side* of a single ganglion, and much more than non-homologues in adjacent ganglia. This result must mean that there is considerable segregation of the central inputs to the different motor axons; for example, axon no. 3 shares more input with its serial homologue in the next ganglion than it does with non-homologous neighbours in its own. Such input specificity was earlier proposed on the basis of responses to natural stimuli (Takeda & Kennedy, 1965*b*), and command fibres that select between axon no. 6 and the two intermediate-sized axons have been found (Evoy, 1966). The present results show that input pathways extend the specificity of their connexion even to two motoneurones of nearly identical size.

SUMMARY

1. The discharge patterns of tonic flexor motoneurones in the crayfish abdomen have been investigated by simultaneous recording from several nerve roots. The five flexor motoneurones supplying each segment are serially homologous, smaller units

have higher discharge frequencies, and the excitability of a given unit is generally higher in more caudal ganglia.

2. Even the smallest axons are capable of generating substantial muscle tension at their spontaneous discharge frequencies. Tension development is extremely tonic. Single motor impulses are without effect, latencies are long, and the frequency/tension relation is steep.

3. The inhibitory axon to each flexor discharges during 'extension' reflexes, but has no visible effect upon relaxation time, or upon the response to subsequent excitation. Inhibitory impulses do not relax previously unexcited muscle.

4. Phase histograms for the discharge of pairs of homologous or non-homologous efferent axons across a single ganglion, within the same root, and between adjacent ganglia have revealed several coupling mechanisms. One is mediated by short time-constant electrotonic junctions between bilaterally paired inhibitory axons. The second involves weaker, more generalized interactions of longer time-constant between non-homologous axons. The third is brought about by common presynaptic sources of excitation. Inputs tend to be common for homologues in adjacent ganglia rather than for non-homologues in a single ganglion, a finding consistent with the selectivity shown by central interneurones in their effects upon motoneurones.

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