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COXAL DEPRESSOR MUSCLES OF THE COCKROACH AND THE ROLE OF PERIPHERAL INHIBITION

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INTRODUCTION

In a previous paper (Pearson & Iles, 1971) the pattern of innervation of the coxal depressor muscles of the cockroach *Periplaneta americana* was described. On the basis of microelectrode recordings these muscles could be classified into three types: (i) those receiving one 'fast' axon, (ii) those with one 'slow' and branches of three different common inhibitory neurones, and (iii) those whose fibres received both the 'fast' and 'slow' axons but no inhibitory innervation.

The mechanical properties of the mesothoracic muscles of the first type (136, 137, notation of Carbonell, 1947) have been studied with both isotonic (Becht, 1959; Becht & Dresden, 1956) and isometric (Becht, Hoyle & Usherwood, 1960; Usherwood, 1962) techniques. Nerve stimulation was found to result in a single twitch response of the muscle which antifacilitated at frequencies above 10/s. This is consistent with innervation by a single fast axon, and we have found the same result in the metathoracic homologues (178, 179, Pearson & Iles, 1971).

The position with regard to the mechanical properties of the second and third types is more confused. Becht (1959) discovered that the mesothoracic muscles 135D, E were each anatomically divisible into two parts of differing colour. Two of these, 135d', e' gave twitch responses to nerve stimulation, and the others, 135d, e were darker in colour and showed slow responses. Inhibition was found in 135e and possibly in 135d. We have shown that 135d', e' (and also the metathoracic homologues 177d', e') receive one fast and one slow axon; and 135d, e (177d, e) receive the same slow axon and three inhibitory axons (Pearson & Iles, 1971). This is consistent with Becht's mechanical results.

These findings are not entirely supported by the observations of Usherwood (1962). From records of the isometric tension generated by the whole muscle complex 135D, E it was concluded that innervation was by two fast and possibly two slow axons, but not by any inhibitory axon.

The experiments described in the present paper were in part designed to re-investigate the mechanical properties of muscles 135(177) D, E and to find a correlation with the results from microelectrode studies (Pearson & Iles, 1971).

The coxal depressor muscles are active during walking in the cockroach and the discharge of the excitatory and inhibitory axons has been studied (Pearson & Iles,

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1970). We have investigated the mechanical effects of such patterns of discharge order to elucidate the functional role of peripheral inhibition which in insects has hitherto been unclear (Hoyle, 1966, 1969). These results have been briefly described elsewhere (Iles & Pearson, 1970).

METHODS

Anatomy

Experiments were performed on both the metathoracic (177D, E) and mesothoracic muscles (135D, E) of adult male cockroaches *P. americana*. Each of these muscles is divided into two parts: 177 (135) d, d′, and 177 (135) e, e′. We use capital letters when referring to the two parts together. The dissection for exposing the muscles and related nerves has been described (Pearson & Iles, 1971) and this will be referred to as the *in situ* preparation. In some experiments the leg was severed from the thorax proximal to the coxal rim and pinned dorsal side uppermost in a bath of saline. Records uninfluenced by respiratory movements of the whole animal could then be obtained. The saline used consisted of the inorganic constituents of that described by Treherne (1961), with the addition of 40 mM glucose and 80 mM glycine. The increased osmolarity produced by the glucose and glycine was found necessary for the preservation of twitch responses of the muscles.

Tension recording

The apodemal attachments to the trochanter of the coxal depressor muscles 178 (136) and 179 (137) were severed. The remaining apodeme of muscle 177 (135) and some connected trochanter cuticle was attached to an RCA 5734 tension transducer. To ensure that branches A–C of muscle 177 (135) did not contribute to the recorded tension their innervating nerve trunk (nerve 4) was cut. This left a preparation of muscles 177 (135) D plus E (which were extended to their maximum natural length), and the innervating nerve branch 5r1 (Pipa & Cook, 1959) of nerve 5. In some experiments all but the part d′ was cut away from the transducer. It was not found possible to record tension from part e′ alone.

Nerve stimulation

The excitatory axons to muscles 177 (135) D, E were activated by electrical stimulation of nerve 5 proximal to branch 5r1. Of the three inhibitory axons supplying the muscles, one, the common inhibitor of Pearson & Bergman (1969), sends branches in the ipsilateral nerve trunks 3–6 and could be activated by stimulating nerve 6Br4 in *in situ* preparations. All three peripheral inhibitors (but not the excitatory axons) have branches in nerve 5 distal to 5r1 and could be activated by stimulation at this site. These arrangements of electrodes are given in Fig. 1.

Electrical recording

Electromyograms were recorded with 85 μm diam. copper wires insulated to the tip. Intracellular and nerve recording methods have been described previously (Pearson & Iles, 1971). In *in situ* preparations activity of the common inhibitory neurone was monitored by recording electrodes on nerve 6Br4 (Fig. 1).

Statistics

Nerve discharge patterns were recorded on magnetic tape. For detection of interactions between different neurones a BIOMAC 1000 (Data Labs. Ltd) computer* was used to construct backward cross-interval density histograms between pairs of spike trains and interval histograms for each train alone. A digital computer (Oxford University KDF9) calculated the first backward recurrence time densities from the interval histograms and these estimates were compared with the cross-interval histograms to determine departures from independence (Perkel, Gerstein & Moore, 1967).

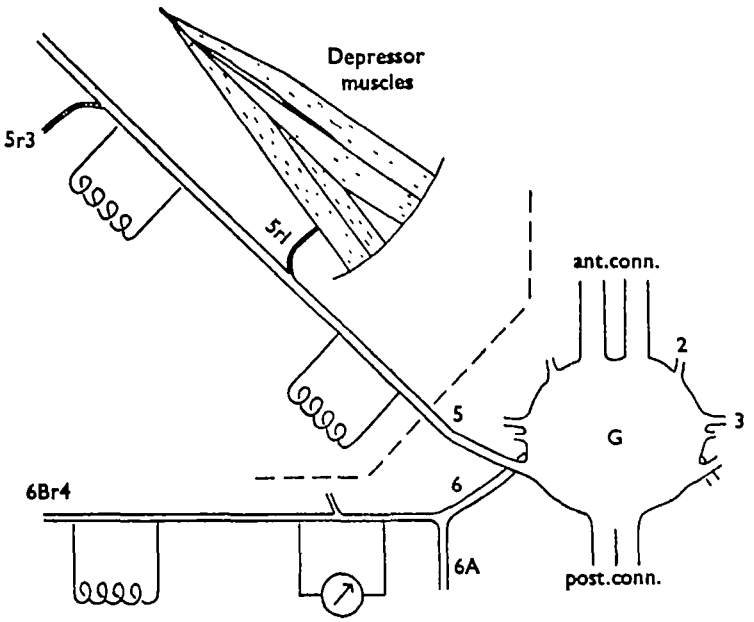

Fig. 1. Diagram of the preparation showing the position of stimulating and recording electrodes. ant.conn., anterior connectives; post.conn., posterior connectives; G., ganglion; 2–6, numbering of nerve trunks. The isolated leg preparation consists of the part distal to the broken line.

RESULTS

Axons in nerve 5r1

The five motor axons in nerve 5r1 to the coxal depressor muscles (Dresden & Nijenhuis, 1958) have been characterized (Pearson & Iles, 1971). The largest (diameter *c.* 24 μm) has been labelled D_f, the second largest (16 μm) D_s, and the three smallest (6 μm) D_{1-3}. The branch of the common inhibitory neurone described by Pearson & Bergman (1969) in this nerve was labelled D_3. Activity in D_f was associated with a large E.J.P., and active membrane response in all fibres of muscles 178 (136), 179 (137), 177 (135) d', e'. Axon D_s was found to innervate all fibres of muscles 177 (135) D, E and produce small E.J.P.s. The axons D_{1-3} produced hyperpolarizing I.J.P.s in muscles 177 (135) d, e, all fibres receiving at least one of these inhibitory axons.

Since it was not found possible to monitor electrical activity in nerve 5r1 and record

* On loan from the U.K. Medical Research Council.

tension in 177 (135) D, E at the same time, it was important to know which axons we activated by stimulation of nerve 5. This was studied in a separate series of experiments with stimulation of nerve 5 and recording from branch 5r1. In most preparations the largest axon (D_f) had a lower threshold than D_s, which in turn was more easily excited than D_{1-3}. In about 20% of preparations, however, D_s had a lower or equal threshold to D_f, and also in about 20% D_s and D_{1-3} did not have distinguishable thresholds For stimulation frequencies greater than 20/s there was a range of intensities over which axons did not fire 1:1 with the stimulus. This behaviour must be taken into account when interpreting the muscle responses to nerve stimulation.

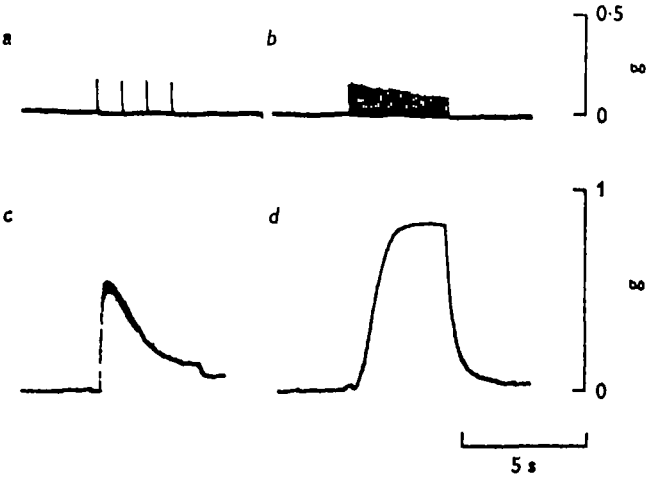

Fig. 2. Tension response of muscle 177d' to nerve 5 stimulation at (*a*) 5/s, (*b*) 10/s, (*c*, *d*) 100/s. (*a–c*) are the response to stimulation of axon D_f alone. Note the anti-facilitation of twitches and tetanus at 10 and 100/s respectively; (*d*) is the response to stimulation of axon D_s after fatigue of the response to D_f.

Responses of muscle 177 (135) *d'*

In most preparations just-threshold stimulation of nerve 5 produced small twitch responses in this muscle which antifacilitated at frequencies above about 10/s (Fig. 2*a–c*). With stimulation at 100/s a tetanus of 5 s duration almost completely fatigued the response, full recovery taking several minutes. An increase in stimulus intensity at this frequency revealed a slow response which did not fatigue (Fig. 2*d*). In some preparations the fast and slow responses could not be separated or were reversed, presumably because axon D_s had an equal or lower threshold to axon D_f. Continued increase in stimulus intensity did not alter the response. This is consistent with the conclusion from microelectrode experiments that all fibres of this muscle receive both axons D_s and D_f but not any of the inhibitory axons D_{1-3}.

We have not succeeded in recording the tension of muscle 177 (135) e' alone, but since its innervation is identical to d' (Pearson & Iles, 1971), the responses are likely to be similar.

Responses of muscles 177 (135) d, e

It was not possible to record from parts d and e alone: d' could be cut from the apodeme, but the remaining response included that from part e' which could not be

cleanly removed. Threshold stimulation of nerve 5 produced a small twitch response which direct observation showed to originate in part e'. This response did not interfere with subsequent measurements as it could be almost completely abolished by a preliminary high-frequency tetanization.

Increased stimulus amplitude brought in a slow response which was accompanied by the appearance of small E.J.P.s in fibres of 177 (135) d, e (Fig. 3) characteristic of activity in axon D_s. The relationship between stimulus frequency and tension was sigmoid with little detectable response below 10/s and reaching a maximum at about 150/s. Tension could be varied continuously over a small range by changes in stimulus intensity. Intracellular recording showed that this was due to axon D_s not following the stimulus 1:1, and does not imply the presence of more than one excitatory axon. Relaxation after a tetanus was slow, generally taking more than 300 ms to complete

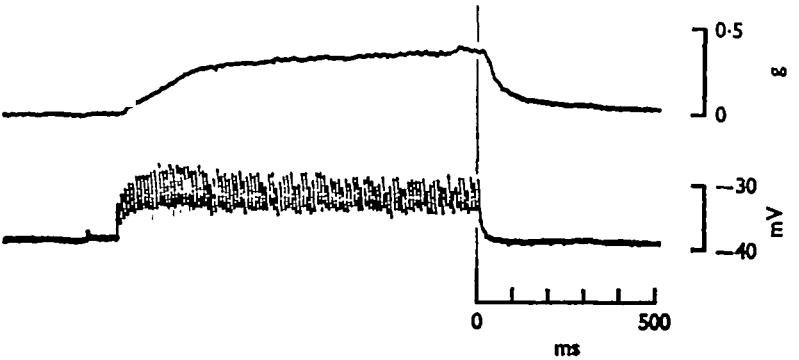

Fig. 3. Response of muscles 135d, e, e' to stimulation of axon D_s at 100/s. Upper trace, tension; lower trace, intracellular record from a single fibre of muscle 135d showing partial summation of the E.J.Ps produced by axon D_s. The vertical line passes through the last stimulus artifact. Note that the relaxation time was about 500 ms compared with membrane repolarization complete in 50 ms.

(as observed by Usherwood, 1962), a typical value for slow insect muscles. In all fibres from which intracellular recordings were made membrane repolarization was more rapid than relaxation of the whole muscle. Further increase in stimulus amplitude (at 100/s) caused a fall in tension to around 40% of its previous value (cf. Becht, 1959). In *in situ* preparations in which records were taken from nerve 6Br4 this reduction was found to coincide with activation of the widespread common inhibitory neurone (D_3).

These results are consistent with the finding from microelectrode experiments that muscles 177 (135) d, e receive the slow excitatory axon D_s and the inhibitory axons D_{1-3}. The tension remaining with maximal stimulation is probably largely the contribution of muscle e' which does not receive inhibition (Pearson & Iles, 1971).

Response of muscles 177 (135) *D, E to natural motoneuronal activity*

These muscles are at least partly responsible for producing the leg-depression movements during walking. In intact walking animals E.M.G. recording with implanted electrodes showed that axon D_s became active (frequency between 50 and 200/s) in bursts lasting 30 to over 1000 ms corresponding to the depression phase of

leg movement. Axon D_f occasionally fired a few spikes near the beginning of high intensity D_s bursts. The duration of the levation phase, when D_s was silent, was between 50 and 250 ms. This pattern of activity in axon D_s (which is similar in both mesothoracic and metathoracic segments) persists in dissected preparations (Pearson & Iles, 1970). In such preparations muscle tension can be recorded, and it was found that muscles 177 (135) D, E relaxed completely after the D_s burst even when the levation period was as short as 50 ms (Fig. 4). This rapid relaxation would ensure minimal resistance to the levation movements. This is in contrast to the situation with electrical stimulation of D_s at comparable frequencies where relaxation was incomplete after 300 ms. One difference between these two experiments is that during spontaneous activity all three inhibitory axons become active immediately after the D_s burst, i.e. during the

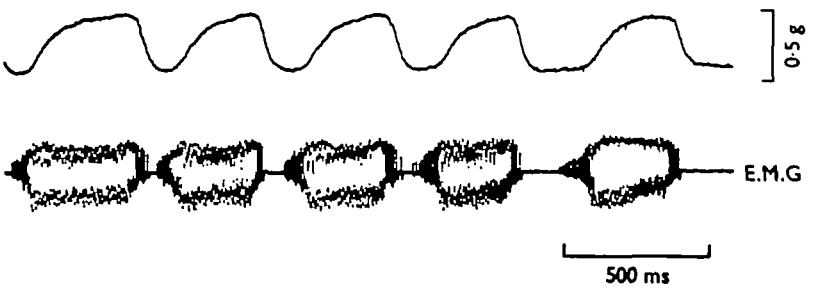

Fig. 4. Contractions in muscles 177D, E in response to spontaneous activity of axon D_s. Upper trace, tension; lower trace, electromyogram. Only a few individual electrical responses to single impulses in D_s can be discerned on this time scale. The electrodes were close to part d′ of the muscle and so considerable facilitation is evident in the electrical record. Note that the relaxation time was about 80 ms (cf. Fig. 3).

levation phase (Pearson & Iles, 1970). (For levation durations of less than 100 ms the combined frequency of D_{1-3} reaches 180/s, falling to about 80/s for durations of 200 ms.) Experiments were therefore performed to investigate the influence of inhibition on muscle relaxation.

Response of 177 (135) *d, e to inhibition*

In preliminary experiments (Iles & Pearson, 1970) inhibitory activation was produced by electrical stimulation of nerve 6Br4 in *in situ* preparations. This method, however, had two disadvantages. First, only one inhibitory axon (D_3) was stimulated, and secondly, in intact animals the inhibitory neurones were spontaneously active.

These problems have been avoided by using isolated leg preparations and activating axons D_{1-3} by stimulation of nerve 5 between branches 5r1 and 5r3. The response of e′ to axon D_f was fatigued by tetanization. When a 5 s tetanus of axon D_s (100/s) was followed by stimulation of D_{1-3} (60/s for 1 s) there was a marked increase in the rate and extent of relaxation (Fig. 5 *a*, *b*, *d*). This effect was completely reproducible in both mesothoracic and metathoracic segments. In the experiment of Fig. 5 (but not some others), inhibition alone produced a small reduction in the resting tension (Fig. 5 *c*). This preparation was bathed in saline which may have differed from haemolymph (Pichon, 1970) and so this effect may not obtain in the intact animal. Nevertheless, the effect of inhibition on relaxation was just as marked in preparations in which there was no detectable influence on resting tension.

In a simulation of the walking discharge pattern axon D_s was stimulated with 500 ms tetani repeated every 700 ms. When the inhibitory axons were stimulated during the 200 ms between D_s activity relaxation was more complete (Fig. 6*a*). When the pattern was speeded up (300 ms D_s tetani every 400 ms) relaxation was only half complete in the absence of inhibition (Fig. 6*b*). During walking the levation phase can be as short as 50 ms and inhibition is probably very important in producing a rapid relaxation of the slow depressor muscles, in order not to oppose the rapid levation movement.

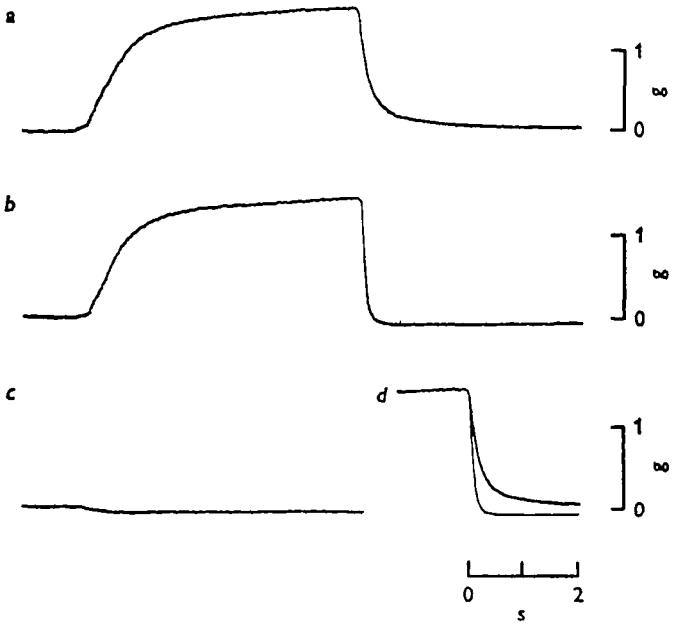

Fig. 5. Effect of inhibitory stimulation on the relaxation of muscles 177d, e, e'. Records show the tension (relative to resting tension) of the muscles for various patterns of excitatory and inhibitory stimulation. (*a*) D_s tetanus (100/s); (*b*) D_s tetanus followed by stimulation of the inhibitory axons D_{1-3} (60/s); (*c*) stimulation of inhibitory axons D_{1-3} alone; (*d*) relaxations, with and without inhibition, superimposed.

Discharge characteristics of axons D_{1-3} *and* D_s

The inhibitory axons fire at low frequencies (less than 5/s) in the resting animal and more intensely during levator axon activity (Pearson & Iles, 1970). In the latter periods spikes of axons D_{1-3} tend to occur clustered about the same time. This can be seen in nerve recordings (Fig. 7) and more clearly in cross-interval density histograms.

The first backward cross-interval density histogram is a measure of the statistical independence of two spike trains. Intervals between spikes in one train, A, and the immediately preceding spike in the second train, B, are measured. The probability of occurrence of these cross-intervals is plotted as ordinate and the cross-interval duration as abscissa. The distribution of cross-intervals for the null hypothesis of independence can be calculated from the properties of train B alone (Perkel, Gerstain & Moore, 1967). If for small intervals the measured distribution is greater than that expected for independence, spikes in trains A and B are positively correlated. This can result from either positive interaction by collaterals or a common excitatory input (Moore,

Segundo, Perkel & Levitan, 1970). Similarly, a measured distribution less than the expected for independence indicates negative coupling as a result of inhibitory collaterals or shared inhibition.

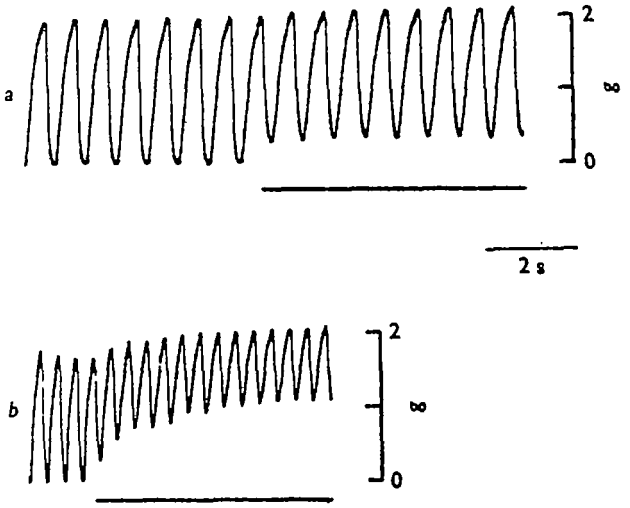

Fig. 6. Tension response of muscles 177d, e, e' during a simulation of the walking discharge pattern of axon D_s and the inhibitory axons $D_{1\text{-}3}$. (*a*) Axon D_s stimulated at 100/s for 500 ms, repeated every 700 ms; (*b*) axon D_s stimulated for 300 ms repeated every 400 ms. The inhibitory axons $D_{1\text{-}3}$ were initially stimulated (60/s) alternately with the periods of excitatory stimulation. Inhibitory stimulation was discontinued throughout the times indicated by the horizontal lines.

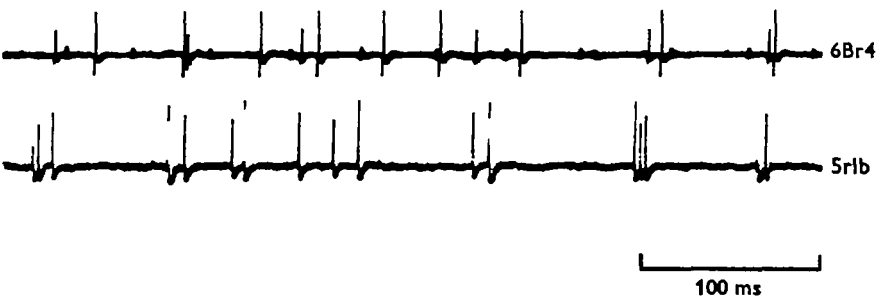

Fig. 7. Coupling of spike activity in axons $D_{1\text{-}3}$. The records are from the mesothoracic nerves 6Br4 (top) and 5r1b (bottom). Axons $D_{1\text{-}3}$ are distinguishable by their difference in spike amplitude in the record from nerve 5r1b, while activity in the inhibitory axon D_3 is recognized by the simultaneous appearance of spikes in both records. The larger spike in the record from 6Br4 is from an excitatory levator axon. Note that axons $D_{1\text{-}3}$ tend to be active at about the same instant.

The cross-interval density histograms for pairs of the inhibitory axons D_{1-3} have large peaks near the origin indicating positive coupling for a period of about 15 ms in either direction (Fig. 8*a–c*). Antidromic stimulation of axon D_3 (electrodes on nerve 6Br4) was without effect on the discharge of D_1 or D_2 but did re-set its own firing rhythm. Thus it is unlikely that there are collaterals from axon D_3 to D_1 and D_2. Pearson & Iles (1970) suggested that an interneurone which fired in bursts acted as a driver for the excitatory levator motor neurones. The same interneurone could provide a common input to D_{1-3}, producing both the positive coupling and the firing during

vator bursts. It was also postulated that the same cell inhibited the slow depressor neurone D_s. Cross-interval density histograms show that D_s tends not to fire during 30 ms before D_3, when both are firing regularly at low frequency (Fig. 8*d*). This effect is very weak but consistent with the hypothesis.

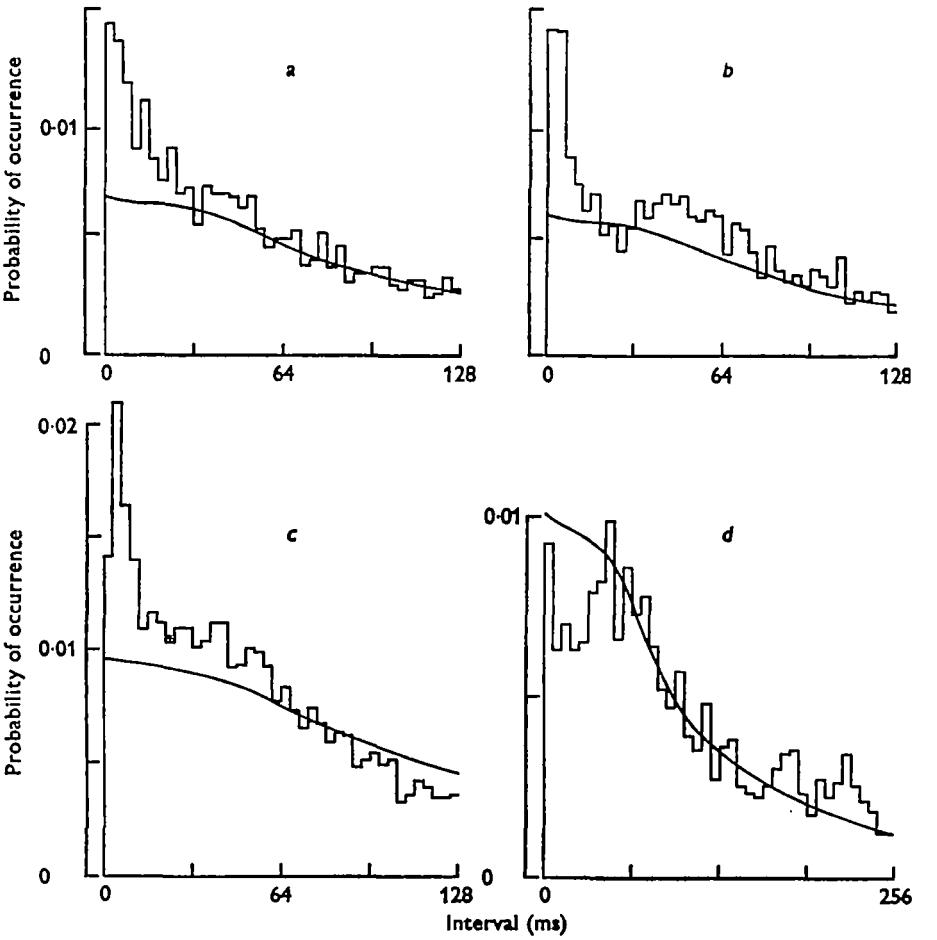

Fig. 8. Positive coupling between the spikes of the inhibitory axons $D_{1\text{-}3}$ (*a–c*), and negative coupling between axon D_s and axon D_3; shown by first backward cross-interval density histograms (see text for explanation). The solid lines are the expected distribution for the null hypothesis of independent firing. (*a*) Spikes of D_s preceding spikes of D_1; (*b*) D_s preceding D_1; (*c*) D_s preceding D_3; (*d*) D_s preceding D_3.

No statistical correlations were found between axon D_3 firing in adjacent segments or on opposite sides of the same segment. In the crayfish strong coupling between contralateral pairs of peripheral inhibitors in the same ganglion has been found (Evoy, Kennedy & Wilson, 1967), resulting from shared input and direct connexions. In the walking cockroach, legs of the same segment alternate, and hence motor neurone coupling would not be expected.

DISCUSSION

Distribution of innervation

A previous microelectrode study (Pearson & Iles, 1971) described the distribution of the five motor axons in nerve branch 5r1 to the coxal depressor muscles in both mesothoracic and metathoracic segments. The mechanical responses described in the present paper are identical for the two segments and fully compatible with the conclusions from the earlier work.

Becht (1959) appreciated that the depressor muscles 135 D, E each consisted of two parts: fast portions termed d′, e′ and slow portions (which also showed inhibition) termed d, e. We have shown that d′ and e′ receive one fast and one slow axon, and d and e the same slow axon and three peripheral inhibitors. Becht's results differ only in that he suspected that more than one slow axon supplied muscles 135 D, E and did not distinguish more than one inhibitory effect. The first difference can probably be explained by the slow axon not following 1:1 high-frequency stimulation. The three inhibitory axons we have described have very similar electrical thresholds and usually cannot be discriminated by graded stimulation. There is then no major discrepancy between the results when the limitations of using mechanical recording alone are realized.

From a study of the whole muscles 135 D, E Usherwood (1962) concluded that there was a second fast, and possibly a second slow, response, but we have been unable to confirm this.

Functional significance of inhibition

The conclusion from the experiments presented here is that inhibition ensures complete and rapid relaxation of the coxal depressor muscles during the rapidly alternating leg movements of walking. This requires first that firing of the excitatory axon should be followed by inhibitory activity. This is the case in dissected preparations (Pearson & Iles, 1970), but we have not succeeded in recording from inhibitory nerves in freely moving animals. Secondly it requires that the simulation of discharge patterns used in the experiments is an accurate one. In dissected preparations the excitatory axon D_8 fires in bursts with maximum frequency at the beginning followed by a plateau; but in intact walking animals discharge tends to be maximal at the end of the burst (possibly a result of sensory input). The constant-frequency tetanus used in the experiments is an approximation to both situations. Stimulation of nerve 5 distal to branch 5r1 synchronously activates all the peripheral inhibitors, but close coupling is also observed in the natural discharge (Fig. 7). The rate of relaxation of the muscles tends to decrease with time during an experiment but marked effects of inhibition on relaxation have been found in the freshest preparations and are likely to be important in the intact animal. The most serious objection to the hypothesis is the fact that all three inhibitory axons are branches of common inhibitory neurones which also innervate the antagonist coxal levator muscles (Pearson & Iles, 1971). The levator muscles will then receive concurrent excitation and inhibition during levator bursts. The difficulty can be resolved because inhibition has only a small effect on contractions produced by the excitatory levator axons which are active during walking, but does reduce the tension produced by another axon involved in postural control. This is

flected in the innervation patterns of individual fibres of the levator muscles (Pearson & Bergman, 1969).

The common inhibitory neurone D_3 (but not D_1 or D_2) sends branches out of the ipsilateral nerve trunks 3 to 6 and innervates many muscles. The significance of such extensive action is unknown, but this cell could control tension of many slow muscles during posture and possibly compensate for varying degrees of potassium contracture (Usherwood, 1967).

Usherwood & Runion (1970) have described the influence of an inhibitory axon, probably a branch of the common inhibitory neurone (Pearson & Bergman, 1969), on the metathoracic extensor tibiae muscle in the locust. During fast walking there was alternation of discharge of slow excitatory and inhibitory axons and inhibition increased the rate and extent of the muscle relaxation. A similar effect has been found in the locust anterior coxal adductor muscle (Hoyle, 1966) and in the first spiracle closer muscle of *Blaberus discoidalis* (Miller, 1969). This then might be a general function for peripheral inhibition in insects.

Comparison with Crustacea

The reflex responses of the dactyl opener muscles of various decapod crustacea have been studied by Bush (1960, 1962). Both the single excitatory axon to the opener muscle and its specific peripheral inhibitory axon showed tonic activity. During reflexly evoked claw closing, activity of the inhibitory axon predominated, resulting in a reduction of muscle tension, without any central suppression of the excitatory activity; in fact excitatory and inhibitory axon discharge were probably positively correlated (cf. Wilson & Davis, 1965; Spirito, 1970). The effectiveness of inhibition in reducing the opener muscle tension produced by concurrent excitation is enhanced by a presynaptic mechanism. The exclusively peripheral nature of the inhibition of this muscle can be readily understood: the opener excitatory axon is shared by the stretcher muscle of the propodite, and independent control of these muscles can only be achieved through their specific inhibitory axons. The function of the common inhibitory neurone which innervates the opener and several other claw muscles is unknown. Bush noted that the claw opener and stretcher muscles were a special situation and predicted that in muscles with unshared excitatory innervation, inhibition would have a central component, and excitatory and inhibitory axon discharge would be reciprocal. The slow flexor muscles of the crayfish abdomen are of this type (Kennedy & Takeda, 1965), and consistent with reciprocal discharge the presynaptic component of inhibition is absent.

In many slow muscles the tension generated by intense excitatory axon activity can be maintained by a much lower frequency (Wilson & Larimer, 1968; Kuffler, Laporte & Ransmeier, 1947). Pantin (1936) suggested that inhibitory discharge reciprocal with excitatory axon firing could function to speed up relaxation of such muscles when central inhibition was incomplete. In the crayfish slow flexor muscles, however, no effect of inhibition on the rate of relaxation has been found (Evoy *et al.* 1967). If muscles involved in more rapid movements were to be studied results comparable to those described for insects might be found, but the functional significance of peripheral inhibition in crustacean muscles other than those of the claw is at present unclear.

Mechanism of the inhibitory effect on muscle relaxation

Repolarization after an excitatory tetanus occurred more rapidly than relaxation of the whole muscle in all the fibres of muscles 177 (135) d, e from which we have recorded (Fig. 3). This has also been observed in single-fibre preparations of several slow invertebrate muscles (e.g. Edwards, Chichibu & Hagiwara, 1964; Dudel, Morad & Rudel, 1968), and it may be concluded that slow relaxation of a muscle is not usually the result of slow surface membrane repolarization. It is therefore unlikely that inhibition increases the rate of relaxation by speeding up repolarization of the surface membrane to the resting potential.

The effect of postsynaptic inhibition on steady tension of crayfish muscle fibres can be entirely explained by the change in membrane potential which it produces (Orkand, 1962; Atwood, Hoyle & Smith, 1965) without invoking a direct chemical influence on the muscle itself. The same is likely to be true of the effects of inhibition on relaxation. Inhibitory activity at the frequencies which produce increased rates of relaxation in insect muscle will hyperpolarize the surface membrane of the muscle fibre by up to 20 mV. If the delay between surface membrane repolarization and fall in tension results from slow repolarization of the transverse tubular system of the muscle, the effects of inhibition on relaxation could be explained in the following way. The hyperpolarization of the surface membrane produced by inhibition would speed up transverse tubule repolarization and increase the rate of fall of tension. This mechanism would operate even in fibres where hyperpolarization does not cause a reduction in resting tension. Using single, voltage-clamped fibres of the crayfish epimeralis muscle Dudel (1970) has shown that when a depolarization applied to the membrane is followed by hyperpolarization, rather than repolarization to the resting potential, the rate of relaxation is 27 % greater. In this muscle replacement of chloride in the bathing medium by an impermeant anion slows relaxation after a clamp depolarization (Dudel & Rudel, 1969). This substitution would be expected to alter the electrical properties of the transverse tubular system and suggests that repolarization of this system is a rate-limiting step in relaxation of these slow invertebrate muscles.

SUMMARY

1. Mechanical properties of the coxal depressor muscles of cockroaches have been studied and found to be compatible with the pattern of innervation determined by microelectrode recording.

2. During walking activity excitatory and inhibitory axons to the slow depressor muscles are alternately active in bursts.

3. Firings of the three inhibitory axons are positively correlated.

4. The rate of relaxation of the slow muscles is increased by activation of the inhibitory axons innervating them.

5. It is proposed that one functional significance of peripheral inhibition in insects is to increase the rate of relaxation of slow muscles used in rapid movements.

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REFERENCES

ATWOOD, H. L., HOYLE, G. & SMYTH, T. (1965). Mechanical and electrical responses of single innervated crab muscle fibres. *J. Physiol., Lond.* **180**, 449–82.

BECHT, G. (1959). Studies on insect muscles. *Bijdr. Dierk.* **29**, 1–40.

BECHT, G. & DRESDEN, D. (1956). Physiology of the locomotory muscles of the cockroach. *Nature, Lond.* **177**, 836.

BECHT, G., HOYLE, G. & USHERWOOD, P. N. R. (1960). Neuromuscular transmission in the coxal muscles of the cockroach. *J. Insect Physiol.* **4**, 191–201.

BUSH, B. M. H. (1960). Reflex responses and the role of peripheral inhibition in the thoracic limbs of Decapod crustacea. Ph.D. dissertation, Cambridge.

BUSH, B. M. H. (1962). Peripheral reflex inhibition in the claw of the crab *Carcinus maenas* (L). *J. exp. Biol.* **39**, 71–88.

CARBONELL, C. (1947). Thoracic muscles of the cockroach *Periplaneta americana. Smithson, misc. Collns* **107**, 1–23.

DRESDEN, D. & NIJENHUIS, E. (1958). Fibre analysis of the nerves of the second thoracic leg in *Periplaneta americana. Proc. K. Ned. Akad. Wet.* C **61**, 213–23.

DUDEL, J. (1970). Acceleration of relaxation by hyperpolarisation of the crayfish muscle fibre membrane. *Pflügers Arch. ges. Physiol.* **320**, 152–67.

DUDEL, J. & RUDEL, R. (1969). Voltage controlled contractions and current-voltage relations of crayfish muscle fibres in chloride-free solutions. *Pflügers Arch. ges. Physiol.* **308**, 291–314.

DUDEL, J., MORAD, M. & RUDEL, R. (1968). Contractions of single crayfish muscle fibres induced by controlled changes of membrane potential. *Pflügers Arch. ges. Physiol.* **299**, 38–51.

EDWARDS, C., CHICHIBU, S. & HAGIWARI, S. (1964). Relation between membrane potential changes and tension in barnacle muscle fibres. *J. gen. Physiol.* **48**, 225–34.

EVOY, W. H., KENNEDY, D. & WILSON, D. M. (1967). Discharge patterns of neurones supplying tonic abdominal flexor muscles in the crayfish. *J. exp. Biol.* **46**, 393–412.

HOYLE, G. (1966). Functioning of the inhibitory conditioning axon innervating insect muscles. *J. exp. Biol.* **44**, 429–53.

HOYLE, G. (1969). Comparative aspects of muscle. *An. Rev. Physiol.* **31**, 43–84.

ILES, J. F. & PEARSON, K. G. (1970). Peripheral inhibition and muscle relaxation. *J. Physiol. Lond.* **210**, 20–22*P*.

KENNEDY, D. & TAKEDA, K. (1965). Reflex control of abdominal flexor muscles in the crayfish. II. The tonic system. *J. exp. Biol.* **43**, 229–46.

KUFFLER, S. W., LAPORTE, Y. & RANSMEIER, R. E. (1947). The function of the frog's small nerve motor system. *J. Neurophysiol.* **10**, 395–408.

MILLER, P. L. (1969). Inhibitory nerves to insect spiracles. *Nature, Lond.* **221**, 171–3.

MOORE, G. P., SEGUNDO, J. P., PERKEL, D. H. & LEVITAN, H. (1970). Statistical signs of synaptic interaction in neurons. *Biophys. J.* **10**, 876–900.

ORKAND, R. K. (1962). Chemical inhibition of contraction in directly stimulated crayfish muscle fibres. *J. Physiol., Lond.* **164**, 103–15.

PANTIN, C. F. A. (1936). On the excitation of crustacean muscle. IV. Inhibition. *J. exp. Biol.* **13**, 159–69.

PEARSON, K. G. & BERGMAN, S. J. (1969). Common inhibitory neurones in insects. *J. exp. Biol.* **50**, 445–72.

PEARSON, K. G. & ILES, J. F. (1970). Discharge patterns of coxal levator and depressor motoneurones of the cockroach *Periplaneta americana*. *J. exp. Biol.* **52**, 139–65.

PEARSON, K. G. & ILES, J. F. (1971). Innervation patterns of coxal depressor muscles in the cockroach *Periplaneta americana*. *J. exp. Biol.* **54**, 215–232.

PERKEL, D. H., GERSTEIN, G. L. & MOORE, G. P. (1967). Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys. J.* **7**, 419–40.

PICHON, Y. (1970). Ionic content of haemolymph in the cockroach *Periplaneta americana*. *J. exp. Biol.* **53**, 195–210.

PIPA, R. & COOK, E. (1959). Studies on the hexapod nervous system. I. Peripheral distribution of the thoracic nerves of the adult cockroach *Periplaneta americana*. *Ann. ent. Soc. Am.* **52**, 695–710.

SPIRITO, C. P. (1970). Reflex control of the opener and stretcher muscles in the cheliped of the Fiddler Crab *Uca pugnax*. *Z. vergl. Physiol.* **68**, 211–28.

TREHERNE, J. E. (1961). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach *Periplaneta americana*. *J. exp. Biol.* **38**, 315–22.

USHERWOOD, P. N. R. (1962). Nature of fast and slow contractions in the coxal muscles of the cockroach. *J. Insect Physiol.* **8**, 31–52.

USHERWOOD, P. N. R. (1967). Insect neuromuscular mechanisms. *Am. Zool.* **7**, 553–82.

USHERWOOD, P. N. R. & RUNION, H. I. (1970). Analysis of the mechanical responses of metathora extensor tibiae muscles of free-walking locusts. *J. exp. Biol.* **52**, 39–58.

WILSON, D. M. & DAVIS, W. J. (1965). Nerve impulse patterns and reflex control in the motor system of the crayfish claw. *J. exp. Biol.* **43**, 193–210.

WILSON, D. M. & LARIMER, J. L. (1968). The catch property of ordinary muscle. *Proc. natn. Acad. Sci. U.S.A.* **61**, 909–16.