INNERVATION OF COXAL DEPRESSOR MUSCLES IN THE COCKROACH, PERIPLANETA AMERICANA

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INTRODUCTION

Peripheral inhibition is well known in crustacean muscle (reviews by Wiersma, 1961, and Atwood, 1968) but has been clearly demonstrated in insect muscle only in the past few years (Usherwood & Grundfest, 1965; Usherwood, 1968; Pearson & Bergman, 1969; Miller, 1969). Two types of peripheral inhibitory motoneurones have been found in crustaceans, the first type being specific inhibitory neurones which innervate only one muscle or group of functionally identical muscles, and the second being common inhibitory neurones which innervate a number of functionally different muscles. In crustaceans many muscles have dual inhibitory innervation, receiving one specific inhibitory axon and one axon branch of a common inhibitory neurone. By contrast there have been only two reports of multiple inhibitory innervation of insect muscle, namely dual inhibitory innervation of the posterior coxal levator muscle of the cockroach (Pearson & Bergman, 1969) and triple inhibitory innervation of a coxal depressor muscle in the cockroach (Iles & Pearson, 1969). In both of these muscles one of the inhibitory axons was a branch of the common inhibitory neurone described by Pearson & Bergman (1969) whereas the other inhibitory axons have not, until now, been classified as either specific or branches of other common inhibitory neurones. We will present evidence that all these inhibitory axons to the metathoracic coxal levator and depressor muscles are branches of three different common inhibitory neurones.

The innervation of the mesothoracic coxal depressor muscles (135D, 135E, 136 and 137; notation of Carbonell, 1947) has been described from a number of electro-physiological investigations (Becht & Dresden, 1956; Becht, 1959; Becht, Hoyle & Usherwood, 1960; Usherwood, 1962). All these studies have reported that muscles 136 and 137 are innervated by a single common fast axon. There is less agreement on the innervation of muscles 135D and 135E. Usherwood (1962) reported that two fast and two slow axons supply these muscles whereas earlier Becht (1959) found them to be innervated by one fast, at least one slow and possibly inhibitory axons.

Dresden & Nijenhuis (1958) found histologically that the mesothoracic coxal depressor muscles are innervated by five motor axons. In the metathoracic segment where the musculature is homologous, nerve 571 also contains five motor axons whose diameters, initial branching and discharge patterns correspond to those of the mesothoracic segment (Pearson & Iles, 1970). The largest metathoracic axon has been classified as fast, and the other four as one slow and three inhibitory (Iles & Pearson, 1969; Pearson & Iles, 1970). Unfortunately neither Becht's (1959) nor Usherwood's
K. G. Pearson and J. F. Iles (1962) results allow a clear classification of the five mesothoracic axons. Even so both these workers considered that there was more than one slow axon to the coxal depressor muscles, while Usherwood (1962) reported that at least two of the axons to be fast. If the functional properties of the five axons are the same in both segments then these conclusions about the mesothoracic axons are not consistent with the classification of the metathoracic axons (see above).

The aims of this study were first to re-examine the functional properties of the motor axons supplying both the mesothoracic and the metathoracic coxal depressor muscles, and secondly to determine the distribution of these axons to the various muscles.

PREPARATIONS AND METHODS

(1) Anatomy

The four metathoracic coxal depressor muscles have been numbered 177D, 177E, 178 and 179 by Carbonell (1947). These muscles are innervated by axons contained in nerve 5ri (Pipa & Cook, 1959). Muscles 177D and 177E are two branches of the main depressor muscles, 177, and share a common apodeme to the trochanter with the other three branches (177A–C) of this muscle. Branches A and C attach to the pleuron and branch B to the sternum; these three branches are innervated by axons in nerve 4 (Pipa & Cook, 1959). Muscle 178 is a broad flat muscle lying dorsally in the coxa (Text-fig. 1) while muscle 179 is located ventromedially in the coxa. These two muscles each have individual apodemal attachments to the trochanter; the apodemes of muscles 178 and 179 lying dorsally and ventrally respectively to that of muscle 177.

Becht (1959) has reported for the mesothoracic segment that muscles 135D and 135E (which correspond to muscles 177D and 177E in the metathoracic segment) each have two distinct parts. This is shown in Text-fig. 2. (Throughout this paper we refer to the parts of these muscles with small letters and use capital letters when referring to the two parts together.) Muscle 135d' is a slender muscle running from the medial part of the coxal rim directly to the distal end of the apodeme of muscle 135, and is quite separate from muscle 135d. The two muscle branches 135e and 135e' lie close to each other but are easily recognized by a difference in colour (135e appears paler) and by the fact that a large trachea runs between them. We have found the same anatomical divisions in the metathoracic muscles 177D and 177E. To be consistent with Becht's labelling of the mesothoracic muscles, we will refer throughout this paper to the various divisions of these muscles as 177d, 177d', 177e and 177e' (see Text-fig. 2).

The metathoracic posterior coxal levator muscles, 182, and its mesothoracic homologue, 140, each have four branches (A–D, Text-fig. 1) which are innervated by nerves 6Br4 and 5r3. Nerve 6Br4 branches many times as it progresses along muscle 182 and eventually joins nerve 5r3.

The labelling and electrical identifications of the axons in nerves 6Br4 and 5r1 have been described in previous papers (Pearson & Bergman, 1969; Pearson & Iles, 1970; Pearson, Stein & Malhotra, 1970).
Preparations and dissection

Adult male cockroaches, *Periplaneta americana*, were used in all experiments. Animals were lightly anaesthetized with carbon dioxide, decapitated and pinned ventral side up on a cork board.

(a) Intracellular recordings

Intracellular recordings were made from single muscle fibres in all the metathoracic coxal depressor muscles (177D, 177E, 178 and 179) and parts of the posterior coxal levator muscle (182), as well as from all the corresponding muscles in the mesothoracic segment (135D, 135E, 136, 137 and 140). The following dissections were used to expose the different nerves and muscles.

*Muscle 177E (135E).* The coxa was first rotated to expose its dorsal surface. The soft cuticle between the abdomen and the dorsal coxal rim was removed and the remoter muscles 174 (129), 175 (130), and 176 (131) were detached from the dorsal coxal rim. Nerves 6A, 6Br2 and 6Br3 were cut, and nerve 6Br4 was cut at its distal branch point. The end of nerve 6Br4 was lifted in a pair of forceps, pulled medially and laid across a small piece of black paper on the centre of the abdomen. The coxa was then returned to its normal position (ventral side up) leaving nerve 6Br4 exposed. Recordings from this nerve allowed the activity in the common inhibitory neurone to be monitored.
Part of muscle 177E (135E) and nerve 5r1a were exposed by removing a piece of the ventromedial coxal cuticle as shown approximately for the leg on the right of Text-fig 1. A single recording electrode was placed under nerve 5r1a to monitor the activity of the motor axons in this nerve.

Text-fig. 2. Subdivisions of the mesothoracic muscle 135D and 135E and the corresponding metathoracic muscles 177D and 177E, ventral view. Muscles 135D and 177D have two quite distinct parts, d and d', d' attaching to the trochanter apodeme close to the trochanter, while part d has a distributed attachment along the same apodeme. The two parts e and e' of muscles 135E and 177E are less distinct but can be recognized by a difference in colour (part e is paler) and by the fact that a large trachea runs between them (not shown in the above diagram).

Muscle 177D (135D). The dissection was very similar to that described for muscle 177E (135E). The only difference was that a larger part of the ventromedial coxal wall was cut away and the whole of muscle 179 (137) was removed to expose the underlying muscle 177D (135D). Recordings were again made from nerve 5r1a.

Muscles 178 (136) and 179 (137). Small pieces of cuticle were removed above these muscles. Nerve recordings were not made when investigating these muscles because it was found that they were innervated by only one axon (see Results). This axon was activated by stimulation of nerve 5.

Muscle 182 (140). The coxa was rotated to expose its dorsal surface and a small piece of cuticle was removed from above the part of the muscle under study. Nerves 5r1b and 6Br4 were exposed and placed on recording electrodes.

(b) Tension recording

The three separate apodemal attachments of muscles 177 (135), 178 (136) and 179 (137) on the trochanter allowed preparations to be made in which the tension could be easily recorded from any one. The medial coxal cuticle was removed close to the
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trochanter to expose all three apodemes. The apodemes of the muscles not being studied were cut near the trochanter. The proximal tip of the trochanter was cut between the attachment of the muscle under study and the coxa-trochanter joints. The small piece of trochanter cuticle still connected to the muscle was attached to an RCA 5734 tension transducer.

In the present experiments the tensions produced in branches A, B and C of muscle 177 (135) were not of interest; so, to ensure that they did not contribute to the tension when recording from muscles 177D (135D) and 177E (135E), nerve 4 was cut close to the ganglion. No attempt was made to record separately the tensions in muscles 177D (135D) and 177E (135E) or either of the two subdivisions of each of these muscles.

(3) Recording equipment

The glass microelectrodes used for intracellular recording were filled with 0.5 M-K₂SO₄ or 1 M potassium acetate and mounted on a flexible silver-silver chloride wire, the flexibility allowing stable intracellular recordings during small movements. Signals were amplified using a Medistor A-35 Electrometer amplifier. The reference electrode was a silver wire placed in the saline bathing the preparation. Extracellular nerve records were made using either monopolar or bipolar 75 μm silver wire electrodes. All nerves on recording electrodes were coated with petroleum jelly to prevent drying. Isleworth type A101 pre-amplifiers were used to amplify the nerve action potentials. All experiments were recorded on magnetic tape using a Thermionic Products T3000 four-channel tape recorder.

(4) Histology

Nerve 5r1 was fixed for 3–4 h in glutaraldehyde in 0.1 M phosphate buffer, left overnight in buffer and postfixed in 2% osmium tetroxide in buffer. The nerves were then dehydrated in ethanol and embedded in Araldite. Thin sections were stained for 2 h with uranyl acetate and counter-stained with 0.2% lead citrate.

RESULTS

(1) Axons in nerve 5r1

In an earlier study Pearson & Iles (1970) reported that nerve 5r1 of the metathoracic segment contains five motor axons and from the amplitude of the extracellularly recorded action potentials estimated their diameters to be 28 μm, 13 μm and three of 5 μm. Histological sections of nerve 5r1, Pl. 1, show that the largest five axons in this nerve have diameters of about 24 μm, 16 μm and three of about 6 μm. The reasonable agreement between the measured diameters of the five largest axons and the estimated diameter of the five motor axons strongly suggests that the five largest axons seen in the histological sections correspond to the five motor axons. We have labelled these five motor axons as follows: the largest, D₁; the second largest, D₂; and the three smaller, D₃, D₄ and D₅. One of the three small axons is a branch of the common inhibitory neurone described by Pearson & Bergman (1969) (Pearson & Iles, 1970). Since the branch of this inhibitory neurone in nerve 6Br4 has been labelled axon 3 (Pearson & Bergman, 1969) we will refer to the branch in nerve 5r1 as axon D₃.
Axons $D_1$ and $D_2$ have similar discharge patterns, distribution and properties (see below) and cannot be distinguished consistently in different preparations.

Pl. 1 also shows that nerve 5r1 contains a large number of other axons all with diameters less than about 4 $\mu$m. The possibility immediately arises as to whether any of these smaller axons are also motor. The following three observations make this possibility appear unlikely. First, in recordings from the cut end of nerve 5r1 only five motor axons have been found to be active, these corresponding in diameter to the five largest axons (see above). We have never observed action potentials which could arise from the smaller axons in this nerve. Secondly, we have never observed any junctional potentials in single fibres of the different coxal depressor muscles not correlated with action potentials in one of the five largest axons. Thirdly, all the responses (mechanical and electrical) elicited on stimulation of nerve 5 are readily explained in terms of either single or combined activity in the five larger axons. Dresden & Nijenhuis (1958) in histological studies on nerve 5r1 in the mesothoracic leg also found that this nerve contained five motor axons, these five axons having diameters similar to those given above for the metathoracic leg. Furthermore, they found that this nerve contained a number of smaller axons which they classified as sensory axons arising from the medial coxal wall. Pipa & Cook (1959) also reported that a branch of nerve 5r1 runs to the coxal wall. In comparing our results with the observations of Dresden & Nijenhuis (1959) and of Pipa & Cook (1959) we have concluded that nerve 5r1 contains only five motor axons (these axons all supplying the coxal depressor muscles) and a large number of small sensory axons which probably arise from receptors in the coxal wall.

(2) Axon $D_f$

Axon $D_f$ could be activated in some intact animals by stimulation of the cercus. The unreliability of this method of activation, and the large movements produced by cercal stimulation in the intact animal, made this preparation unsuitable for studying the properties of axon $D_f$. Since this axon is the largest in nerve 5r1, selective activation should be obtained by electrically stimulating nerve 5. This was tested by stimulating nerve trunk 5 close to the ganglion and recording from nerve 5 r1. Axon $D_f$ was found to have the lowest threshold in about 80% of preparations. For frequencies of stimulation of 20-100/s there was a large range of stimulus amplitudes over which the axons did not follow stimuli 1:1 (cf. Wilson, 1964). This suggests that caution is required when interpreting the muscle responses to tetanic stimulation of its nerve.

Selective single activation of axon $D_f$ produced a powerful twitch contraction in muscle 178 and in muscle 179. The duration of these twitch contractions were quite short, being in the range of 20-35 ms. Similar observations were made on the corresponding mesothoracic muscles (136, 137), in agreement with Usherwood (1962). Intracellular recordings from single fibres of the four muscles 136, 137, 178 and 179 were very similar. The resting potential in the fibres of these muscles varied from $-50$ to $-70$ mV. The excitatory junctional potential, e.j.p., elicited by axon $D_f$ usually showed a positive overshoot of up to 20 mV. The duration of the e.j.p. was about 5 ms. The responses observed in single fibres of muscles 136 and 137 were similar to those seen by Becht, et al. (1960).

With repetitive stimulation of axon $D_f$ there was an antifacilitation of the e.j.p. as
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The recovery time was rapid in a fresh preparation but increased after a number of tetani had been given. Often after a prolonged high-frequency tetanus there was never complete recovery. The tension in these muscles also decreased throughout a tetanus and usually there was never complete recovery of the mechanical response. An interesting finding was that the antifacilitation of the e.j.p. did not parallel the fall in tension throughout a tetanus. The decline in tension was noticeable at frequencies of stimulation of about 10/s whereas the antifacilitation of the e.j.p. in the fibres which we have recorded from did not commence until the frequency was greater than about 50/s. Becht et al. (1960) and Usherwood (1962) also reported the antifacilitation of the electrical and mechanical responses in muscles 136 and 137.

![Text-fig. 3. Antifacilitation of the e.j.p. in a single fibre of muscle 179 on repetitive stimulation of axon Df. Part (a) shows the time course of this antifacilitation for a 2 s tetanus of 100/s. Note that the recovery was almost complete within 1 s of the end of the tetanus. In part (b) the e.j.p. are shown on a faster time scale at various times throughout the tetanus. As the amplitude decreased the duration becomes longer. The e.j.p. shown on the left in part (b) was that elicited before the tetanus was given.]

When the stimulus strength was increased to be suprathreshold for the four smaller motor axons in nerve 5r1, no changes were observed in the shape of the e.j.p. produced by axon Df in any of the fibres of muscles 178 (136) and 179 (137) from which recordings were made. Furthermore no spontaneous junctional potentials have been seen in any fibre when axons Dp, D1, Dz and Ds were spontaneously active but axons Df inactive. These findings indicate that none of the four smaller motor axons innervate these muscles although the possibility remains that they innervate fibres not penetrated. Since the mechanical responses in muscles 178 (136) and 179 (137) to suprathreshold stimulation, of all five axons were identical to those observed when axon Df was stimulated alone, we conclude that these muscles are not innervated by any of the four smaller axons in nerve 5r1. This is in agreement with the histological findings of Dresden & Nijenhuis (1958) for muscles 136 and 137.

Becht (1959) reported that muscles 135d' and 135e' both gave visible twitch contractions on stimulation of mesothoracic nerve 5. Correspondingly we have observed that stimulation of metathoracic nerve 5 gives twitch contractions in muscles 177d' and 177e'. Furthermore, visual observations indicated that the threshold for the twitch contractions in these muscles was identical to that for eliciting twitch contractions
in muscles 178 and 179. These observations therefore suggested that axon $D_f$ innervates not only muscles 178 and 179 but also muscles 177d' and 177e'. To test this possibility we simultaneously recorded from single fibres of either muscle 178 or muscle 179 and single fibres of either muscle 177d' or muscle 177e'. If a single fast axon ($D_f$) innervates all these muscles then there should be a 1:1 correspondence of e.j.p.'s in single fibres of any pair of these muscles when the stimulus intensity is adjusted to be just threshold, activating the axon on about 50% of the occasions that the stimulus is applied. On the other hand if there is more than one fast axon to these muscles then with just threshold stimuli, constant 1:1 activation would not be expected. The results of an experiment in which recordings were made simultaneously from single fibres of muscles 177e' and 179 are shown in Text-fig. 4, and it can be seen that there is a 1:1 correspondence in the evoked e.j.p. Similar results were obtained with all pair combinations of the four muscles. Thus we conclude that axon $D_f$ innervates all four muscles, 177d', 177e', 178 and 179. Similar results were obtained in the corresponding mesothoracic muscles.

\[\text{Text-fig. 4. Simultaneous intracellular recordings from single fibres of muscles 177e' and 179 showing the e.j.p. elicited by a just threshold stimulus to axon } D_f.\]

(a) 1:1 correspondence between the e.j.p. elicited in each of the muscles indicating that axon $D_f$ innervates both these muscles. (b) The time course and amplitudes of the e.j.p. produced by axon $D_f$ in both muscles are very similar. Time scales: (a) 1ms, (b) 10 ms.

The e.j.p.'s recorded from muscle 177d' and from the proximal end of muscle 177e' were very similar to those recorded in muscles 178 and 179. The amplitude of the e.j.p. in muscle 177e' decreased towards the trochanter and was only 10–20 mV in amplitude near its apodermal attachment to the trochanter which indicated that the neuromuscular junctions of axon $D_f$ on muscle 177e' are possibly located close to the coxal rim. All fibres from which recordings were made in muscles 177d' and 177e' were innervated by axon $D_f$. The anti-facilitation of the e.j.p. to repetitive stimulation seen in muscles 178 and 179 also occurs in muscles 177d' and 177e'. There is also an irreversible decline in the mechanical response in muscles 177d' and 177e' after a series of tetani to axon $D_f$. Usherwood (1962) reported that the twitch response in muscles 135D and 135E could only be detected in fresh preparations. Our findings agree and indicate that one of the fast axons described by Usherwood is axon $D_f$. We have found no evidence for a second fast axon to muscles 135D and 135E contrary to Usherwood's (1962) finding.

(3) Axon $D_s$

Axon $D_s$ discharges in high-frequency bursts in all preparations except the neurally isolated ganglion, where it is usually inactive (Pearson & Iles, 1970). In those preparations in which axon $D_s$ was active large movements made intracellular recording difficult. Fortunately, however, in about 20% of the experiments in which nerve 5
was electrically stimulated, axon $D_a$ had a lower threshold for activation than the larger axon $D_f$, and it was in these preparations that the properties of axon $D_a$ were studied.

Using threshold stimuli and simultaneous intracellular recordings from single fibres of various pairs of muscles (cf. Text-fig. 4) axon $D_a$ was found to innervate every fibre of muscles 177d', 177d, 177e and 177e', but not muscles 178 and 179. There were consistent differences in the amplitude and duration of the e.j.p. elicited by activity in axon $D_a$ in the different muscles. A series of these e.j.p.'s for the four muscles is shown in Text-fig. 5, which also shows e.j.p.'s for the corresponding mesothoracic muscles. The duration of the e.j.p. is clearly longer in muscles 177d and 177e than in muscles 177d' and 177e'. The e.j.p. durations in muscles 177d and 177e were usually 30–60 ms, although some lasted for more than 100 ms. By contrast the duration of the e.j.p.'s in muscles 177d' and 177e' was considerably shorter, usually being 10–20 ms. These findings correlate well with what is known of the other types of innervation of these muscles; muscles 177d' and 177e' are also innervated by the fast axon $D_f$ but not by inhibitory axons, and muscles 177d and 177e are innervated by inhibitory axons but not by axon $D_f$ (see below).

![Text-fig. 5. Excitatory junctional potentials elicited by single impulses in axon $D_a$ in fibres of muscles (a) 177d' and 175d', (b) 177d and 175d, (c) 177e and 175e and (d), 177e' and 175e'. The top two rows are from fibres of the four parts of muscle 177 and the bottom row from fibres of the corresponding parts of muscle 175. The amplitude scale is 25 mV for (b) and (c), and 10 mV for (a) and (d). The time scale is 25 ms for all the potentials except that from muscle 135e (bottom of c) where it is 50 ms. Note that the potentials in columns (b) and (c) are very similar and have a larger amplitude and longer duration compared to those potentials shown in columns (a) and (d).](image-url)

The amplitudes of the e.j.p.'s also vary in the different muscles. In muscles 177d and 177e the e.j.p. amplitude was usually 10–20 mV but could sometimes be as large as 30 mV. The amplitudes in muscles 177d' and 177e' were 5–15 mV for muscle 177d' and 2–6 mV for muscle 177e'.

One further difference in the e.j.p.'s between the different muscles was that on repetitive stimulation there was considerable facilitation of the e.j.p. in muscles 177d' and 177e' (Text-fig. 6) but only slight facilitation in muscles 177d and 177e. In muscles 177d' and 177e' the amplitude of the e.j.p. could increase by a factor of about five, and sometimes in muscle 177d' an all-or-none active response appeared to be elicited by each impulse in a tetanus. The large facilitation in the e.j.p. of the slow
axon to the coxal depressor muscles can also be seen in extracellular recordings from these muscles (Iles & Pearson, in preparation). One point of interest is that repetitive stimulation of axons \(D_f\) and \(D_s\) has opposite effects on the e.j.p.'s they elicit in single fibres of muscles 177d' and 177e', the e.j.p. from axon \(D_f\) showing antifacilitation while that from axon \(D_s\) shows facilitation. All the above results were also obtained in the homologous mesothoracic muscles.

Bursts of activity in axon \(D_s\) produced strong graded contractions in muscles 177D and 177E. These mechanical responses will be described in a following paper (Iles & Pearson, in preparation). Similar responses have been obtained in the corresponding mesothoracic muscles, 135D and 135E. Therefore, the axon we have labelled \(D_s\) probably corresponds to the low-threshold slow axon described by Usherwood (1962). We have found no evidence for the second slow axon to muscles 135D and 135E for which Usherwood (1962) had inconclusive evidence.

Text-fig. 6. Facilitation of the e.j.p. elicited on a single fibre of muscle 177c' by repetitive stimulation (50/8) of axon \(D_s\) (a) before stimulation, (b) near the beginning of the tetanus, (c) when facilitation was maximal.

\(4\) Axons \(D_1, D_2\) and \(D_3\)

We have found no way of selectively stimulating axons \(D_1\) and \(D_2\), and to investigate the properties of these two axons, as well as those of axon \(D_3\), we simultaneously recorded extracellularly from nerves 5ria and 6Br4 and intracellularly from single fibres of the various coxal depressor muscles. This allowed the spontaneously occurring action potentials in the different axons to be correlated with electrical events in single muscle fibres. To prevent excessive movement these experiments were carried out in segments with the anterior and posterior nerve cord connectives cut.

Impulses in any of the three axons \(D_1, D_2\) or \(D_3\) produced hyperpolarizing, or inhibitory junctional potentials (i.j.p.'s) in the fibres of muscles 177d and 177e (Text-fig. 7). No depolarizing junctional potentials were ever observed to be correlated with activity in any of the axons \(D_1-3\) and none of the other coxal depressor muscles was found to be innervated by them. The latter finding is interesting because all these other muscles are innervated by the fast axon \(D_f\). In Text-fig. 7 the i.j.p.'s are shown for two fibres of muscle 177e both of which were innervated by all three axons. In both muscles 177d and 177e all the fibres penetrated received at least one inhibitory axon. About 40% were innervated by all three; about 50% by two, one of which was usually the axon branch of the common inhibitory neurone, i.e. axon \(D_3\); and the remainder (10%) by one, which again was usually axon \(D_3\). The amplitudes of the i.j.p.'s varied from less than \(-1\) mV up to \(-10\) mV. In the majority of those fibres receiving axon \(D_3\) and at least one of the other inhibitory axons, the amplitude of the i.j.p. produced by axon \(D_3\) was the largest (Text-fig. 8b).

Similar results were also found in the corresponding mesothoracic muscles 135d
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and 135e. Text-fig. 8 shows simultaneously recorded i.j.p. from single fibres in these two muscles, each fibre having triple inhibitory innervation.

Axon $D_3$ is a branch of a common inhibitory neurone, another branch of which is contained in nerve 6Br4. Stimulation of nerve 6Br4 therefore selectively activated

Axon $D_3$ is a branch of a common inhibitory neurone, another branch of which is contained in nerve 6Br4. Stimulation of nerve 6Br4 therefore selectively activated

Axon $D_3$ and thus allowed the mechanical effect of this axon to be investigated. Activity in axon $D_3$ resulted in a decrease in tension during spontaneous contractions produced by activity in axon $D_s$ (Text-fig. 9). We have found no way of selectively activating axons $D_1$ and $D_2$ and consequently have been unable to investigate their mechanical effect. However, apart from the differences in i.j.p. amplitudes and the number of fibres innervated by these two axons compared to axon $D_3$, we have found nothing to suggest that the mechanical effect of axons $D_1$ and $D_2$ would be qualitatively different from that of axon $D_3$.

Becht (1959) showed that as the strength of a repetitive stimulus to nerve 5 was increased, muscle 135E initially contracted and then relaxed (fig. 14 in Becht (1959)). We have made the same observations in both the metathoracic muscles 177D and 177E, and the mesothoracic muscles 135D and 135E. Our interpretation of this result is that initially axon $D_s$ is selectively activated to produce a graded contraction and an increased stimulus then activates the smaller inhibitory axons $D_{1-3}$ resulting in a
decrease in tension. Intracellular recordings from single fibres of muscles 177d and 177e show that as the stimulus strength to nerve 5 is increased beyond the threshold for activation of axons $D_{1-3}$, the depolarizing e.j.p. of axon $D_1$ is abolished and the

Text-fig. 8. Triple inhibitory innervation of single fibres in muscles 135d and 135e. The top traces are records from nerve 6Br4, and the middle and bottom traces are from single fibres of muscles 135e and 135d respectively. I.j.p.'s from the axon $D_1$ are indicated by the dots under each set of records. Activity in this axon was identified by the 1:1 correspondence of the i.j.p.'s and impulses in a small axon in nerve 6Br4 (axon 3). The two arrows under each set of records indicate i.j.p.'s from two further inhibitory axons. In (a) these were distinguished by a difference in amplitude, while in (b) the time interval between the two i.j.p.'s ($\sim 13$ ms) was considerably less than the minimum time interval of 30 ms seen between successive impulses in any one of the inhibitory axons in an isolated ganglion preparation. Note the 1:1 correspondence of the i.j.p.'s in the two muscles indicating both are innervated by all three inhibitory axons. The large spike in the record from nerve 6Br4 is from axon 5 while the spike from axon 4 is a little larger than that from axon 3 (notation of Pearson & Bergman, 1969).

Text-fig. 9. Inhibition of the spontaneous contractions produced by activity in axon $D_1$ in muscles 177D and 177E on stimulation of the common inhibitory neurone (axon $D_1$) at 50/8 (black bars). The second stimulation period coincided with a spontaneous fall in tension as indicated by the slower subsequent increase.

response becomes predominantly hyperpolarizing as the three inhibitory axons are activated. In some preparations it is possible to distinguish different threshold levels for the three inhibitory axons.
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Since there is no clear evidence for the existence of specific inhibitory neurones to the leg muscles of insects (Pearson & Bergman, 1969) it is of interest to consider whether the inhibitory axons $D_1$ and $D_2$ could be classified as such. If these two axons are specific to the coxal depressor muscles 177d and 177c, then there should be no 1:1 correspondence between their activity and activity in any axon in another nerve trunk. However, in both the meso- and metathoracic segments simultaneous recordings from nerves 5r1b and 5r3 showed that all three inhibitory neurones have axon branches in both nerves as indicated by the 1:1 correspondence of the action potentials recorded from each nerve (Text-fig. 10).

This finding suggested that the posterior coxal levator muscle, 182, is innervated by all three inhibitory axons via nerve 5r3. To test this possibility further, nerve 6Br4 was cut and recordings were taken simultaneously from nerves 5r1b and 6Br4, and intracellularly from single fibres of muscle 182A. Hyperpolarizing junctional potentials corresponding to activity in axons $D_1$, $D_2$ and $D_3$ were found in many fibres of muscle 182A, examples of which are shown in Text-fig. 11. Thus we have concluded that neither axon $D_1$ nor axon $D_2$ is specific to the coxal depressor muscles 177d and 177e.

The two common inhibitory neurones, of which axons $D_1$ and $D_2$ are branches, appear to have a less widespread distribution than the third common inhibitory neurone which is the one described by Pearson & Bergman (1969). In fact we have been unable to find branches of the first two common inhibitory neurones leaving any of the other ipsilateral nerve trunks of the ganglion, i.e. nerve trunks 2, 3, 4 and 6. We have not attempted to determine whether either of these two neurones has branches in the connectives, or has a branch in nerve 5 beyond nerve 5r3 which could innervate muscles in the femur and tibia.

We have also found that all three inhibitory axons in nerve 5r1 to the mesothoracic muscles 135d and 135e are branches of three different common inhibitory neurones, each having a similar distribution to the corresponding neurone in the metathoracic segment.

**DISCUSSION**

(1) Properties and distribution of motor axons

Our electrophysiological measurements on both the mesothoracic and metathoracic segments have shown that nerve 5r1 contains five motor axons. This agrees with the histological findings of Dresden & Nijenhuis (1958) for the mesothoracic nerve. We
have labelled the largest of these axons \( D_f \), the next largest \( D_s \) and the three smallest \( D_1, D_2, \) and \( D_3 \). The distribution of these five axons to the various coxal depressor muscles found in this investigation is shown in Text-fig. 12. It is very similar to that described by Dresden & Nijenhuis (1958) for the mesothoracic segment but with one important difference, this being that axon \( D_f \) also innervates muscles 135d' and 135e'.

**Text-fig. 11.** Intracellular recordings from single fibres of muscle 182A showing that all three inhibitory neurones innervating the coxal depressor muscles 177d and 177e also innervate the posterior coxal levator muscle 182A. (a) i.j.p.'s correlated with impulses in the branch of the common inhibitory neurone in nerve 6Br4 (axon 3). These are marked by the lines between the two traces. The other small spike in the record from nerve 6Br4 is from axon 4 and the large spike from axon 5 (notation of Pearson & Bergman, 1969). A second i.j.p. is also seen in the intracellular record from muscle 182A. (b) i.j.p.'s correlated with impulses in axons \( D_1 \) and \( D_2 \) but not with impulses in axon \( D_3 \). Axon \( D_3 \) is identified by the 1:1 correspondence with impulses in axon 3 of nerve 6Br4.

**Text-fig. 12.** Distribution of the five motor axons \( D_1, D_2, D_3, D_4, \) and \( D_f \) to the mesothoracic and metathoracic coxal depressor muscles.
Innervation of cockroach leg muscles

(Dresden & Nijenhuis reported that the largest axon innervated only muscles 136 and 137). Becht (1959) has reported, and we have confirmed, that muscles 135d' and 135e' are innervated by one fast axon and one slow axon. He also reported that muscles 136 and 137 only receive one fast axon, this axon having similar properties to those we have described for axon Df. Becht did not attempt to determine whether the twitch responses in muscles 135d' and 135e' and in muscles 136 and 137 were elicited by activity in a single fast axon or in two separate axons. Similarly Usherwood (1962) found twitch responses in the two groups of muscles but again did not determine whether only one fast axon was responsible.

The slow graded contractions produced in muscles 135D and 135E by repetitive activity in axon Ds were very similar to those found by Becht (1959) and Usherwood (1962) for a slow axon to these muscles. Thus from the present study, and from these previous two studies, there seems no doubt that muscles 135D and 135E (and the corresponding metathoracic muscles 177D and 177E) are innervated by at least one slow axon and one fast axon, the fast axon innervating divisions d' and e' but not divisions d and e. The major discrepancy between our findings and the earlier work is that Usherwood (1962) reported that muscles 135D and 135E were also innervated by a second fast axon and possibly by a second slow axon. Becht (1959) also considered that there might be more than one slow axon, but his results could be explained by the non 1:1 following of axon Ds when stimulated at high frequencies.

The finding of triple inhibitory innervation of muscles 177d and 177e (and the corresponding mesothoracic muscles 135d and 135e) is of interest because, as far as we are aware, this situation has not previously been found in any other animal. The similarity in the discharge patterns of these three inhibitory axons suggests they have a common function (Iles & Pearson, 1970) but it is not clear why all three are necessary. Axon Ds, which is a branch of the common inhibitory neurone described by Pearson & Bergman (1969), innervates the majority of fibres in branches d and e of muscles 135 and 177, whereas the other two inhibitory axons, D1 and D2, have a less widespread distribution. No consistent method of identification for axons D1 and D2 has been found, which has meant that we have been unable to determine whether there is a difference in the distribution of these two axons. If there is a difference in this distribution and if each inhibitory neurone can operate partially independent of the other two, then quantitatively different (although qualitatively similar) effects could be produced by each neurone. Thus the triple inhibitory innervation perhaps allows a greater flexibility in the inhibitory input to the coxal depressor muscles. At present, however, there is no evidence to indicate that each inhibitory neurone can operate independently, while a close study of the discharge patterns of the three inhibitory neurones has shown a strong tendency for all three to discharge at almost the same instant (Iles & Pearson, in preparation).

Another notable feature of the inhibitory innervation of the coxal depressor muscles is that there are no inhibitory axons to any fibres receiving the fast axon Df. Thus it may be concluded that the inhibitory axons function to modify in some way the contractions produced by the slow axon Ds but not those produced by the fast axon Df. Consistent with this conclusion is that stimulation of the inhibitory axons does not reduce the contractions produced by axon Df (unpublished observations). Similar findings have been reported for the extensor tibiae muscle of the locust (Usherwood...
& Grundfest, 1965) and leg muscles of crustaceans (Wiersma & Ellis, 1942; Atwood, 1967) where activity in inhibitory axons has little effect on phasic contractions produced by fast axons.

(2) Common inhibitory neurones

There is at present no conclusive evidence for the existence of specific inhibitory axons to leg muscles in insects. Atwood, Smyth & Johnston (1969) reported a single inhibitory axon innervating the extensor tibiae muscle of the cockroach, but this axon cannot be regarded as a specific inhibitory axon until it is shown not to be a branch of either the common inhibitory neurone described by Pearson & Bergman (1969) or of either of the two common inhibitory neurones of which axon D1 and D2 are branches, or even perhaps of other common inhibitory neurones. Pearson & Bergman (1969) suggested that i.j.p. they recorded in fibres of muscles 182C and 182D not correlated with activity in any of the axons in nerve 6Br4 could have been produced by activity in a specific inhibitory axon contained in nerve 5r3. The present finding that branches of two further common inhibitory neurones are contained in nerve 5r3 makes this suggestion very unlikely. Most probably the uncorrelated i.j.p. recorded in muscles 182C and 182D were the result of activity in either one of these other two common inhibitory neurones.

The finding that all three axons, D1, D2 and D3, are axon branches of three different common inhibitory neurones shows a major difference between the inhibitory innervation of crustacean and insect muscle. In crustaceans no muscle has been described which is innervated by more than one common inhibitory neurone, the usual pattern being one specific inhibitory axon and one axon branch of a common inhibitory neurone. The function of either specific or common inhibitory neurones to leg muscles in crustaceans is poorly understood, as is the function of the common inhibitory neurones to the leg muscles of insects. However, in a following paper (Iles and Pearson, in preparation) we show that one possible function of the three common inhibitory neurones to the coxal depressor muscles is to give a more rapid relaxation of contractions produced by bursts of activity in axon D8.

SUMMARY

1. The innervation of the mesothoracic and metathoracic coxal depressor muscles has been investigated using intracellular microelectrodes.
2. Five motor axons have been found to innervate the coxal depressor muscles; the largest is a fast axon, the second largest a slow axon and the three smallest are all inhibitory axons.
3. The fast axon innervates the anterior and posterior coxal depressor muscles as well as parts of the coxal branches of the main leg depressor muscle. The slow innervates all fibres in the coxal branches of the main leg depressor muscle. The three inhibitory axons innervate only those parts of the coxal branches of the main leg depressor muscle which are not innervated by the fast axon.
4. The three inhibitory axons were found to be branches of three different common inhibitory motoneurones.
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REFERENCES


EXPLANATION OF PLATE

Pl. 1. Cross-section of metathoracic nerve S11. The five largest axons have been outlined. These five largest axons very probably correspond to the five motor axons identified by electrophysiological methods, the largest being a fast axon, the second largest a slow axon and the three smaller all inhibitory. Also seen in this section are a large number of other axons almost all of which have diameters less than 4 \( \mu \text{m} \). These are probably sensory axons arising from receptors in the coxal wall.