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INNERVATION OF THE ABDOMINAL INTERSEGMENTAL MUSCLES IN THE GRASSHOPPER

II. PHYSIOLOGICAL ANALYSIS

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INTRODUCTION

It has been shown in the fourth abdominal segment of *Melanoplus differentialis* that the four median dorsal internal muscles are all innervated by branches from eight axons in the dorsal nerve (Tyrer, 1971). The effect of activity in these axons upon the muscle fibres has been investigated by recording *en passant* with external electrodes from the eight axons during spontaneous and stimulated activity, while recording from the muscle fibres with microelectrodes.

MATERIALS AND METHODS

Young adult male and female *Melanoplus differentialis* were used. The animals were lightly anaesthetized with carbon dioxide, the head, legs and wings were removed and the body was cut open along its length to one side of the mid-line. Results from ten animals which were not anaesthetized were consistent with those from anaesthetized preparations. The preparation was pinned out on a tray filled with transparent Sylgard 184 encapsulating resin (Dow Corning) and the gut was removed. Movement of the abdomen was kept to a minimum by pinning every segment to the resin. The tray was placed at an angle of 35° to the vertical in a clear plastic chamber mounted on a micro-manipulator (Fig. 1*a*). To prevent the preparation drying, the chamber contained filter paper moistened with saline (Usherwood, 1968). Occasionally the preparation was moistened with a minimum of the same saline. The preparation was viewed from the side using a Wild M4 binocular microscope and was illuminated from behind and from above (Fig. 1*a*). This arrangement has two merits. First, impaling the muscle fibres at a shallow angle to their length improved the stability of the record when the muscles contracted. Secondly, by viewing from the side the advance of the electrode could be monitored exactly. This was facilitated by dipping the electrode tip in acetate indian ink.

The connectives joining the metathoracic ganglion and the first abdominal ganglion were stimulated using a small bipolar platinum electrode (Fig. 1*b*). Single 1–5 V square pulses of 1–2 msec duration were applied using a Tektronix 161 pulse generator triggered from the oscilloscope.

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External records were obtained using a bipolar electrode made from 0·003 in. platinum wire insulated to the tip with Araldite. Although individual nerve branches to the muscles could be picked up on this electrode it was not possible to record from all the axons in any branch because of their small size (e.g. 0·3 μm; Tyrer, 1971). For this reason the electrode was placed under the dorsal nerve in the region where the pericardial membrane inserts on to the cuticle (Fig. 1*b*). At this point the nerve

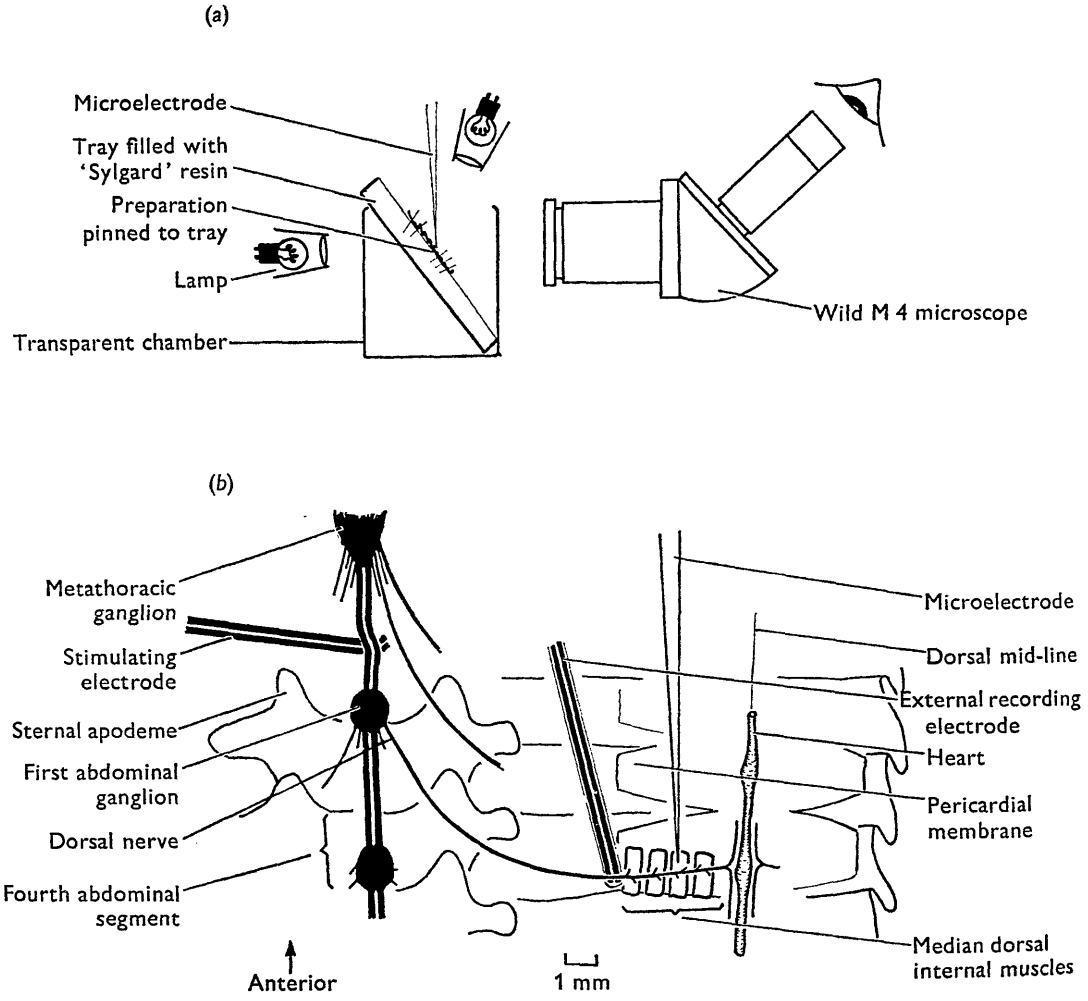

Fig. 1.(*a*) Arrangement of the preparation for recording. The preparation was viewed from the side to monitor the advance of the electrode precisely, and the muscle fibres were impaled at a shallow angle to their length. (*b*) Diagram of the abdominal segments two, three and four to show the position of the microelectrode, external bipolar electrode and stimulating electrode.

contains 21 axons (Tyrer, 1971), of which the 11 heart axons and the smaller nerve to the median external muscle are less than 1·5 μm in diameter. It is unlikely that records of their activity would be greater than the noise level (10–20 μV). The remaining nine axons are large enough to record from. Eight of these supply the internal intersegmental muscles but the ninth is the larger axon to the median external muscle. No way of distinguishing or eliminating this extraneous axon was found.

The dorsal nerve in this region lies under the dorsal trachea and is not normally visible. If the trachea is removed the preparation is short-lived, probably because the nerve dries out. With practice it was possible to pick up the nerve by probing with the electrode under the trachea and monitoring with an audio-amplifier. Good records could be obtained for 1–1½ h from a nerve picked up in this way.

Intracellular records were obtained from individual muscle fibres using glass microelectrodes and conventional recording techniques. The electrodes were filled with either 2·5 M-KCl or 0·6 M-K_2SO_4. The KCl electrodes had a resistance of 40–50 MΩ and the K_2SO_4 electrodes of 100–120 MΩ. The impaled fibres could be identified by the black marks left by the ink-coated electrode tip.

Simultaneous recordings by microelectrode and external electrodes were obtained from 41 preparations. Microelectrode records alone were obtained in 12 preparations.

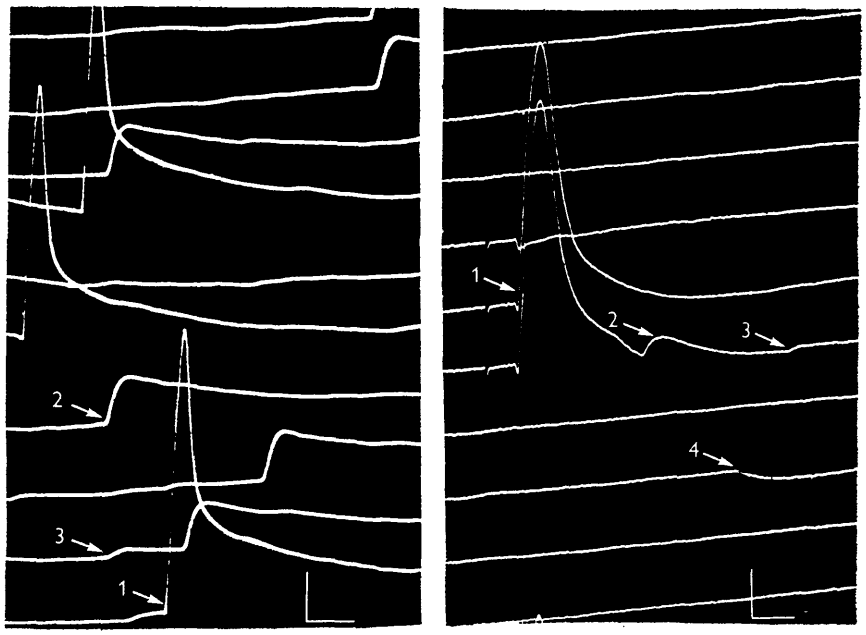

Fig. 2. Records to show the four kinds of potentials in the median internal dorsal muscles. (*a*) Typical type 1 potentials occurring spontaneously together with type 2 and type 3. The type 2 potentials have a somewhat atypical time course (see Fig. 4). (*b*) An example to show all four potentials in the same muscle fibre. The type 1 potentials have been elicited by stimulation. They have a somewhat atypical shape. The records were photographed on moving film with the camera rotated through 90° and with the oscilloscope on free run so that successive records were made one above the other. Scale: 10 msec, 10 mV.

RESULTS

Three hundred and three muscle fibres with resting potentials of over 40 mV were impaled. A range of resting potentials of 40–65 mV was obtained (mean 48 mV). Four different kinds of potential were recorded in active preparations (Fig. 2).

Type 1. Large post-synaptic potentials (PSPs) (Fig. 2*a*) giving rise to an all-or-nothing spike often with a small overshoot, similar to 'fast' responses recorded in

many insect muscles (see reviews by Hoyle, 1957, 1965; Usherwood, 1967; Aidley, 1967). These occurred spontaneously in fresh preparations and in all preparations in response to mechanical stimulation of the body wall and to electrical stimulation of the connective between the metathoracic and the first abdominal ganglion. These potentials were recorded in 93 % of the muscle fibres impaled.

Type 2. Depolarizing potentials usually between 5 and 15 mV (Fig. 3*b*) similar to 'slow' PSPs recorded in locust leg muscle (Hoyle, 1955; Usherwood & Grundfest, 1964; Grundfest & Usherwood, 1965; Usherwood, 1967). Usually these occurred in bursts of 2–3 sec duration at a frequency of 10–15/min throughout the life of the preparation. This frequency is very similar to the normal breathing movements in the intact animal. The rhythm was abolished temporarily by passing carbon dioxide over the preparation and permanently by flooding the bath with saline. The PSPs showed considerable facilitation, those occurring towards the end of the burst being $1\frac{1}{2}$–2 times as large as those at the beginning. In nine preparations type 2 potentials gave rise to a small spike response of 5–10 mV (Fig. 4*f*). Type 2 potentials were recorded in 87 % of the muscle fibres impaled.

Type 3. Small depolarizing potentials usually between 1 and 5 mV (Fig. 3*c*). The rise time was slower than the type 2 PSP and they were seen in only 40 % of the fibres impaled. They did not occur in bursts or with any regularity and no way was found in which they could be elicited selectively by stimulation. The fibres from which these potentials were recorded were scattered throughout the four dorsal muscles of the segment.

Type 4. Small potentials with a long time course (20–40 msec) which were either minutely depolarizing (up to 1 mV) or up to 5 mV hyperpolarizing (Fig. 3*d*). These were found in only 21 % of the fibres impaled and only in fibres which received type 3 innervation. As with the type 3 potentials, type 4 potentials did not occur in bursts or with any regularity and no special way of eliciting them was found.

The spikes recorded *en passant* from the dorsal nerve showed inconsistencies in size from preparation to preparation, which is to be expected in view of the variations in the sizes of the axons in different individuals (Tyrer, 1971). It was possible, however, to divide them into four categories: large (50–100 μV), medium (35–50 μV), small (25–35 μV), and very small (15–25 μV). Usually, the type 1 muscle potentials could be correlated with large spikes recorded in the dorsal nerve (Fig. 4*c*), the type 2 potentials with the medium-sized spikes (Fig. 4*g*), the type 3 with small spikes (Fig. 4*g*) and the type 4 with very small spikes (Fig. 4*h*). In a few preparations the sizes of the spikes related to the type 2 and type 3 potentials were indistinguishable (Fig. 4*e*), and occasionally the spikes producing type 1 potentials were little bigger than those producing type 2 potentials.

In fresh preparations, particularly when the connectives between the metathoracic and abdominal ganglia were stimulated electrically, it was found that the spikes tended to occur in pairs (Fig. 4*a*). A pair of large spikes, often of unequal size, preceded a volley of smaller spikes. As the preparation aged pairs of large spikes could be elicited by electrical stimulation without the ensuing volley (Fig. 4*b*) and still later large spikes could be elicited singly (Fig. 4*c*, *d*). There is evidence that the muscles contain two motor units both of which produce the type 1 response. In any one muscle fibre a type 1 potential was recorded in response to one of the nerve

spikes, but not necessarily to the first of the pair, while the other had no effect. This suggests that there are two motor nerves each producing a type 1 response in a different population of muscle fibres. Muscle fibres responsive to each spike were found scattered throughout the four dorsal muscles, so that each of the muscles

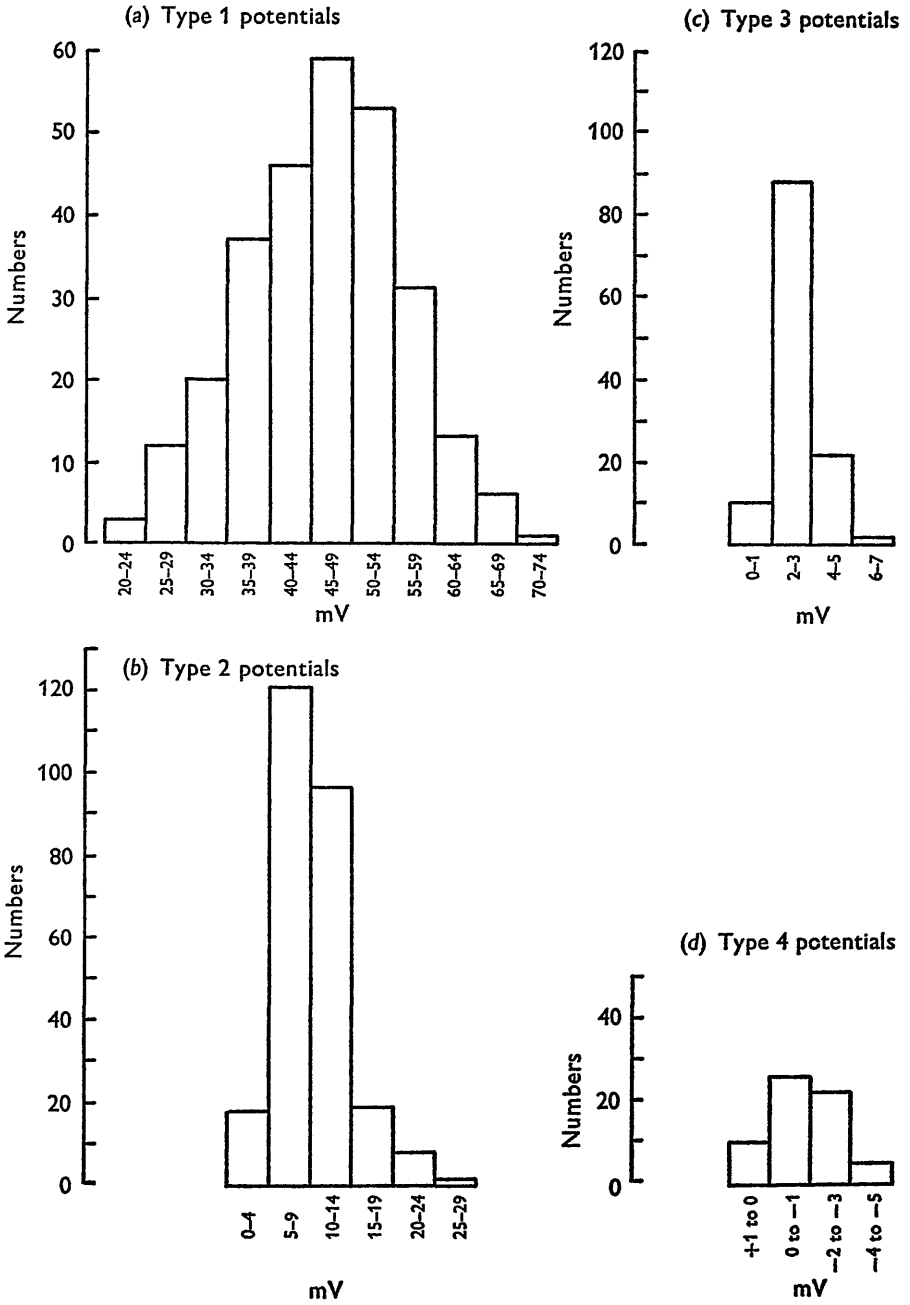

Fig. 3. Histograms to show the distribution of the sizes of the four types of PSP recorded from 53 preparations. The largest potentials in each preparation were measured.

appeared to contain a mixture of fibres innervated by each nerve. Since the two largest spikes of a stimulated volley produce the type 1 response, it is probably the two larger axons in the dorsal nerve which serve these units.

There is similar evidence that the type 2 responses are also produced by two motor neurones. During a large volley of activity in the dorsal nerve medium-sized spikes often occurred in pairs (Fig. 4*a*). Only one spike of each pair could be correlated with a type 2 potential, the other producing no response. Even where medium-sized

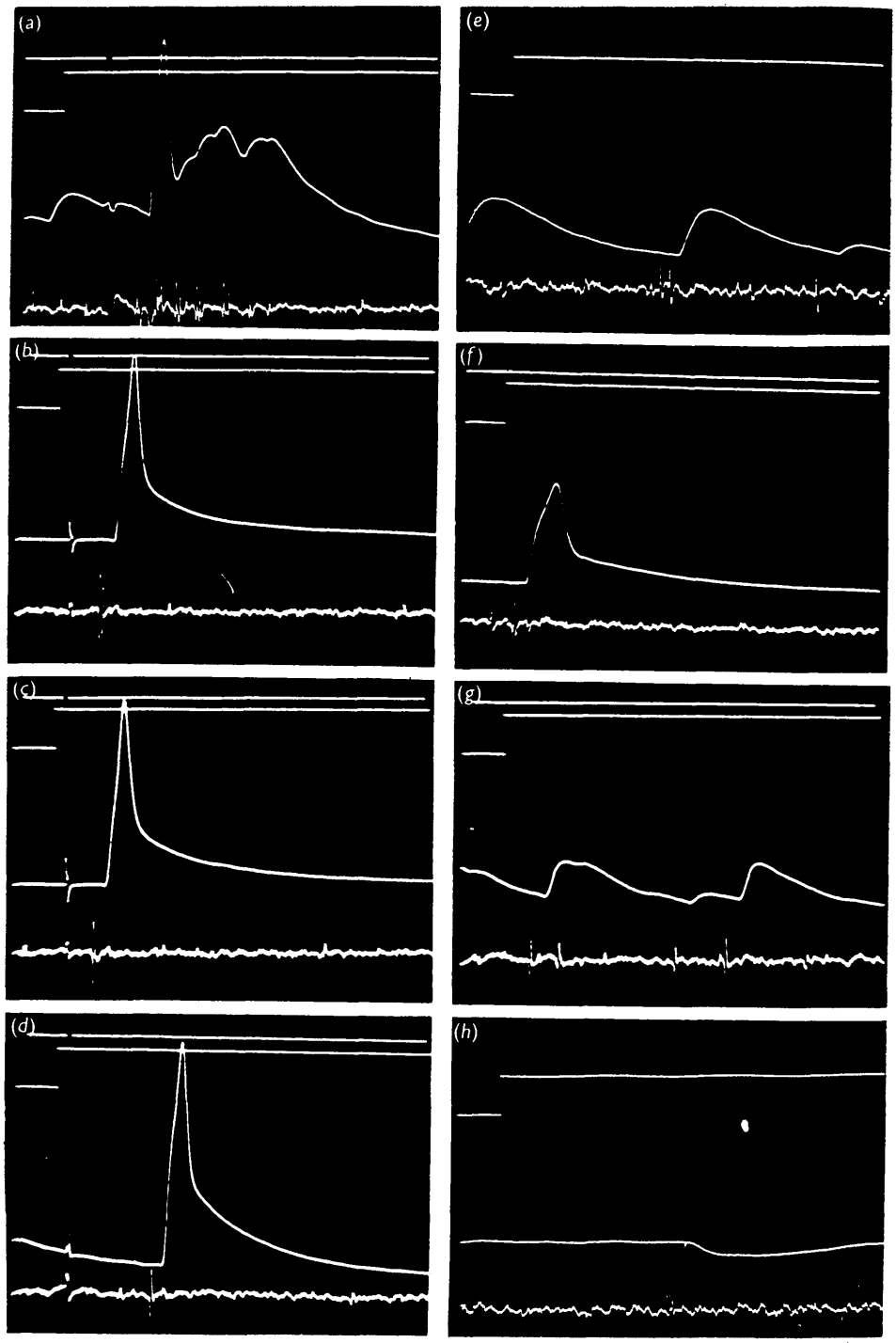

Fig. 4. For legend see facing page.

spikes did not occur in pairs, only about half of them produced a response in any one muscle fibre. Usually the spikes of a pair were the same size (Fig. 4*e*) but occasionally one was larger than the other (Fig. 4*a*).

There is some evidence that type 3 and type 4 responses are similarly produced by two motor neurones, although pairing of small and very small nerve spikes was not observed. This may have been because they were obscured by larger spikes during a volley, and because they occurred infrequently at other times. As with the type 1 and type 2 responses, however, only half of the spikes produced a potential in any one muscle fibre (Fig. 4*a*) the remainder presumably belonging to another motor unit.

DISCUSSION

It appears that there are four pairs of functionally different axons innervating the dorsal intersegmental muscles and that each axon of a pair serves a different population of fibres within the muscle. This conclusion is consistent with the anatomical arrangement of the axons innervating the muscle (Tyrer, 1971). It has not been possible to elucidate the distribution of the motor units since only one microelectrode was used.

Type 1 units are probably used in avoidance movements since they are produced spontaneously only in very fresh preparations but may be elicited at any time by electrical stimulation of the central nervous system or mechanical stimulation of the abdomen. Type 2 units appear to be concerned principally with breathing movements. The function of type 3 and type 4 units is less certain. Since the type 4 PSP is a hyperpolarizing one it seems likely that it is associated with mechanical inhibition (Usherwood & Grundfest, 1965; Hoyle, 1966; Usherwood, 1967, 1968). It may be significant that the type 4 unit was found only in fibres which received a type 3 unit. Usherwood & Grundfest (1965) observed that the inhibitory neurone of the locust extensor tibialis muscle innervated only those muscle fibres receiving the 'slow' excitatory axon. Similarly, Pearson & Bergman (1969) found in the coxal levator of the cockroach a relationship between an inhibitory fibre (axon 3) and an excitatory fibre (axon 4). They postulate that the firing rate in these two axons may

Fig. 4. Records to show the relationship between spikes recorded *en passant* in the dorsal nerve and the four types of PSP. From top to bottom: (i) stimulus trace (where present); (ii) calibration trace and zero potential for intracellular record; (iii) intracellular record; (iv) extracellular record. The height of the upward deflexion of the calibration pulse represents 5 V for the stimulus trace, 10 mV for the intracellular trace and 50 μV for the extracellular record. The horizontal portion of the pulse represents 10 msec. (*a*) A volley of spikes following stimulation correlated with a complex of type 1, 2 and 3 potentials in the muscle. A pair of large spikes of unequal size precedes several pairs of medium-sized spikes, also of unequal size. Note that only the smaller of the pairs produces a type 2 potential, the larger having no effect. (*b*) Stimulation produces an unequal pair of large spikes only, the first of which is correlated with a type 1 PSP. (*c*) Stimulation produces only the first of the pair of spikes correlated with a type 1 PSP as in (*b*). (*d*) Stimulation produces only the second of the pair of spikes correlated with a type 1 PSP in a different muscle fibre from (*b*) and (*c*). A very small spike and a type 4 PSP precede the type 1 PSP. (*e*) A pair of medium-sized spikes correlated with a type 2 PSP, the second spike having no effect. A spike of the same size is correlated with a type 3 potential. (*f*) A type 2 PSP with a small spike response. (*g*) Type 2 PSPs correlated with medium-sized spikes, type 3 with small spikes and minutely depolarizing type 4 with very small spikes. (*h*) A hyperpolarizing-type PSP correlated with a very small spike.

be involved in the maintenance of leg position when the animal is standing. Possibly the type 3 and type 4 units are concerned with postural control, but no observations relative to this could be made using the present method of immobilizing the preparation.

Most insect skeletal muscles which have been examined physiologically have been leg muscles, mainly in locusts (Hoyle, 1953, 1955, 1966; Cerf *et al.* 1959; Usherwood & Grundfest, 1964; 1965; Usherwood, 1967, 1968; Cochrane, Elder & Usherwood, 1969) and cockroaches (Pringle, 1939; Becht, Hoyle & Usherwood, 1960; Pearson & Bergman, 1969; Iles & Pearson, 1969), although Huddart (1966) has examined various Lepidoptera and Malpus (1968) the dragonfly. Most of these muscles have a relatively simple innervation, usually with two types of excitatory innervation and perhaps an inhibitory one. Spiracular muscles which have been examined (Hoyle, 1959; van der Kloot, 1963; Miller, 1969) are similarly simply innervated. It is becoming apparent, however, that many insect muscles have a much more complex innervation. Ikeda & Boettiger (1965*a*, *b*) found four types in a group of fibres in the dorsoventral fibrillar muscles in the bee and describe as many as four excitatory fibres innervating single muscle fibres in the anterior lateral region of the basalar muscle in *Oryctes*, while at least some fibres have both inhibitory and excitatory innervation. Similarly, Pearson & Bergman (1969) have found fibres in a metathoracic coxal levator muscle of the cockroach which are innervated by four different excitatory axons and two inhibitors. As many as three inhibitory axons have been demonstrated in the cockroach by Iles & Pearson (1969). Shepheard (1969) has described the neck muscles of *Schistocerca* where muscles receive up to six functionally different axons, although no fibres were found to be innervated by all six.

It seems therefore that there are many insect muscles at least as complex as the crayfish tonic abdominal flexors (Kennedy & Takeda, 1965; Kennedy, Evoy & Fields, 1966). As in the crayfish these insect muscles usually have complicated movements to perform, such as precise orientation of the head in *Schistocerca*, and variation in the angle of attack of the wing in *Oryctes*. What is unexpected is that *Melanoplus* intersegmental muscles have relatively simple functions to perform and yet have a very complex innervation. In contrast the intersegmental muscles of the caterpillar which perform similar functions are innervated by only one axon (Belton, 1969; Weevers, 1966).

SUMMARY

1. Extracellular records from the motor neurones to the median internal dorsal muscles (abdominal intersegmental) of *Melanoplus differentialis* have been made simultaneously with intracellular records from the muscle fibres.

2. Four types of PSP have been recorded intracellularly: type 1, a large PSP with a regenerative response and a small overshoot; type 2, a PSP of between 5 and 15 mV; type 3, a PSP of between 1 and 5 mV; type 4, a hyperpolarizing PSP of up to 5 mV.

3. Although there is variation in the size of the spikes recorded *en passant*, the four types of PSP can generally be correlated with large, medium, small and very small spikes.

4. Evidence is presented to demonstrate that there are two motor units for each

type of PSP in the dorsal intersegmental muscles. Each muscle appears to have a mixture of muscle fibres of each unit.

5. This is consistent with the anatomical finding of eight motor neurones supplying the muscles.

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Note added in proof

Camhi (1970 *a*, *b*) has shown that the abdomen is used for steering during flight. More recently he has shown that the dorsal intersegmental muscles are used during these steering movements (Camhi, personal communication). The complex innervation of these muscles seems more reasonable in view of the delicate control required for steering movements.

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