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INNERVATION PATTERN OF A POOL OF NINE EXCITATORY MOTOR NEURONS IN THE FLEXOR TIBIAE MUSCLE OF A LOCUST HIND LEG

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Summary

The flexor tibiae muscle of a locust hind leg consists of 10–11 pairs of fibre bundles in the main body of the muscle and a distal pair of bundles that form the accessory flexor muscle, all of which insert onto a common tendon. It is much smaller than the antagonistic extensor tibiae muscle and yet it is innervated by nine excitatory motor neurons, compared with only two for the extensor. To determine the pattern of innervation within the muscle by individual motor neurons, branches of the nerve (N5B2) that supplies the different muscle bundles were backfilled to reveal somata in the metathoracic ganglion. This showed that different muscle bundles are innervated by different numbers of excitatory motor neurons. Physiological mapping of the innervation was then carried out by intracellular recordings from the somata of flexor motor neurons in the metathoracic ganglion using microelectrodes. Spikes were evoked in these neurons by the injection of current, and matching junctional potentials were sought in fibres throughout the muscle using a second intracellular electrode.

Each motor neuron innervates only a restricted array of muscle fibres and, although some innervate a larger array than others, none innervates fibres throughout the muscle. Some motor neurons innervate only proximal fibres and others only more distal fibres, so that the most proximal and most distal bundles of muscle fibres are innervated by non-overlapping sets of motor neurons. More motor neurons innervate proximal bundles than distal ones, and there are some asymmetries in the number of motor neurons innervating corresponding bundles on either side of the tendon.

Individual motor neurons cause slow, fast or intermediate movements of the tibia, but their patterns of innervation overlap in the different muscle bundles. Furthermore, individual muscle fibres may also be innervated by motor neurons with different properties.

Key words: motor neuron, muscle, innervation, locust, *Schistocerca gregaria.*

Introduction

Arthropod muscle is innervated by many fewer motor neurons than is vertebrate muscle, so that force is controlled more by changes in the frequency of motor spikes and by differences in the intrinsic properties of the muscle fibres than by recruitment of motor units. For some insect muscles, the excitatory innervation consists of a single motor neuron. Small muscles in the leg of a locust, such as the coxal adductor and the levator tarsi, and even the large tergosternal muscle that moves the wing in flight are each innervated by one motor neuron. In most insect muscles, no more than 2–3 excitatory motor neurons control the force, aided by inhibitory and neuromodulatory neurons, but for particular muscles that move the head (Shepheard, 1974), the abdomen (Tyrer, 1971), a wing (Neville, 1963) or a leg (Pearson and Bergman, 1969) the number is larger. Why there are different sizes of motor neuron pools has never been satisfactorily explained.

Nowhere is the contrast in complexity of innervation more stark than for the two muscles within the femur of a leg, contractions of which control movements of the tibia. The flexor tibiae muscles are innervated by as many as eight motor neurons in cockroaches (Dresden and Nijenhuis, 1958), by 14–15 in stick insects (Debrodt and Bässler, 1989; Storrer *et al.* 1986) and by at least nine excitatory (Phillips, 1980) and two inhibitory (Hale and Burrows, 1985) motor neurons in the hind leg of a locust. In contrast, the antagonistic extensor tibiae has only two excitatory motor neurons and one inhibitor, innervating a muscle that in the locust hind leg has a cross-sectional area 88 % larger (Bennet-Clark, 1975) and a total mass five times greater than those of the flexor.

The cell bodies of the flexor motor neurons that innervate a hind leg of a locust belong to one of the eight groups of motor cell bodies in one half of the metathoracic neuromere (Siegler and Pousman, 1990). Within this group, they are enwrapped by glial cells together with the cell bodies of some 15 other leg motor neurons. Moreover, their primary neurites are also bundled together and wrapped in glia as they follow

the same course through the neuropil towards the origin of nerve 5 (Phillips, 1981). All the flexor motor neurons therefore have a similar shape, with individual neurons differing in the details of their branching, but not in a way that has yet allowed unequivocal characterisation of individual neurons on these anatomical criteria alone. The pool of flexor motor neurons has been further subdivided into three groups (anterior, lateral and posterior) each containing three motor neurons (Hoyle and Burrows, 1973) although, in practice, variation in position precludes the use of this feature as a reliable indicator of identity. Within the whole pool are motor neurons that produce slow contractions of the muscle and are often tonically active, those that produce fast contractions and are active more phasically, and those that have intermediate properties.

Hoyle (1955) suggested that the flexor tibiae muscle in a hind leg of a locust was made up of 5–6 different parts inserting onto a common tendon and that the different parts were separately innervated. In the flexor muscle of a hind leg of the orthopteran *Calliptamus*, some motor neurons innervate only the proximal part where the fibres have the longest sarcomere lengths, whereas others innervate only the more distal fibres (Theophilidis and Dimitriadis, 1990). A similar pattern of innervation also occurs in the middle leg of the locust. Here, the flexor muscle is larger than its antagonistic extensor, and Theophilidis and Burns (1983) suggest, on gross morphological grounds, that it is divisible into three parts: a proximal part consisting of a single bundle of fibres, a middle part consisting of a pair of muscle bundles (one bundle anterior, the other posterior) and a distal part consisting of 8–10 pairs of muscle bundles. On the basis of measurements of force from these different parts and the identification of neurons from the amplitudes of their spikes in extracellular recordings from nerve branches, they suggest that the whole muscle is innervated by 12 excitatory motor neurons. They also provide some evidence for independent innervation of the different parts of the muscle by individual excitatory motor neurons, with 5–6 neurons innervating the proximal part, 7–8 the middle part and 9–10 the distal part. For example, one motor neuron innervates only the proximal bundle, a second the proximal and middle bundles but not the distal ones, and a third innervates all the parts. It is, however, difficult to identify the motor neurons with certainty using these methods and, consequently, to define the innervation pattern for all of them and for all fibres in all parts of the muscle.

In contrast to these findings, Phillips (1980) concluded that all the excitatory motor neurons innervated fibres throughout the flexor muscle of a locust hind leg. This conclusion was based on experiments in which she stimulated an individual motor neuron with an intracellular electrode in its cell body and recorded the evoked junctional (synaptic) potentials in various muscle fibres. It was also supported by the finding that the nerve branches to different regions of the muscle contained similar numbers of axon profiles and showed similar numbers of increments in the amplitude of their extracellular compound spike when the entire main leg nerve (N5) was stimulated electrically. From force measurements of proximal and distal parts of the muscle and from the ultrastructure of individual fibres, she also concluded that the muscle consists of proximal phasic fibres with shorter sarcomere lengths grading continuously into more tonic fibres with longer sarcomeres distally.

To try and resolve the present conflicting picture of its pattern of innervation, we have re-examined the flexor tibiae muscle in a hind leg of the locust. We have made detailed recordings of the distribution of junctional potentials in the different fibres throughout the muscle evoked by intracellular stimulation of individual motor neurons within the metathoracic ganglion. We show that each of the nine motor neurons has a distinctive pattern of innervation so that each innervates only a specific region of the muscle.

Materials and methods

Mature adult locusts, *Schistocerca gregaria* (Forskål), were taken from our crowded laboratory culture and mounted ventral surface uppermost in Plasticine. The left hind leg was fixed so that the tibia and tarsus were free to move. The ventral cuticle of the femur was removed to expose the flexor tibiae muscle. The thorax was also opened to expose the meso- and metathoracic ganglia, which were then stabilised on a wax-coated platinum platform. The thorax and the exposed muscle were then perfused continuously with saline (Usherwood and Grundfest, 1965) at 20–22 °C. The sheath of the metathoracic ganglion was treated with protease (Sigma type XIV) for 1–2 min to facilitate the penetration of the somata of flexor tibiae motor neurons with glass microelectrodes. The electrodes used to record intracellularly from motor neurons and muscle fibres were filled with 2 mol l^{-1} potassium acetate and had resistances of 40–80 MΩ. In some experiments, electrodes used to record from somata were filled with 0.1 mol l^{-1} hexammine cobaltic chloride. Simultaneous intracellular recordings were made from (a) the somata of two different flexor tibiae motor neurons, (b) the soma of one motor neuron and a flexor muscle fibre, or (c) two muscle fibres. Data were recorded on an FM tape recorder for later display on a Gould TA240 thermal chart recorder.

Intracellular recordings in the metathoracic ganglion were identified as originating in a flexor tibiae motor neuron if they met the following criteria. First, the presence of a monosynaptic excitatory postsynaptic potential (EPSP) caused by an antidromic spike in the fast extensor tibiae (FETi) motor neuron (Burrows *et al.* 1989). These spikes were evoked by stimulation of the axon terminals of FETi with a pair of stainless-steel wires, 50 μm in diameter and insulated except at their tips, inserted into the extensor tibiae muscle. Second, spikes evoked in the impaled flexor tibiae motor neuron by pulses of depolarising current caused spikes that evoked flexion movements of the tibia. Third, spikes in an impaled motor neuron, evoked by intracellular injection of current or occurring during evoked tibial movements, matched junctional potentials recorded intracellularly from fibres in the flexor tibiae muscle.

Within the pool of flexor tibiae motor neurons, individual neurons could be classified as fast, slow or intermediate according to their threshold for spike initiation when depolarising current was injected (fast motor neurons required larger currents to make them spike) and according to the type of tibial movement they caused. Neurons were arbitrarily given the numbers 1–9, where neurons 1–3 were slow motor neurons, 7–9 fast motor neurons and 4–6 intermediate motor neurons. Anatomical features also helped to characterise a neuron as a flexor but did not give reliable identification of individual neurons; the position of the soma was not a reliable indicator of identity, nor was the morphology of its neuropilar branches as revealed by the intracellular injection of cobalt from electrodes filled with 0.1 mol l^{-1} hexammine cobaltic chloride (Brogan and Pitman, 1981) followed by silver intensification (Bacon and Altman, 1977). In experiments where recordings were made sequentially from the somata of flexor motor neurons in the same locust, the following procedure was adopted to ensure that different neurons were sampled. First, the soma of a motor neuron was penetrated and its innervation pattern in the muscle was plotted. The muscle electrode was removed and was then used to penetrate the soma of a second motor neuron in the ganglion. If spikes evoked in the first motor neuron could not be recorded by the second electrode, and *vice versa*, then it was concluded that the electrodes were in the somata of two different flexor motor neurons. The innervation pattern of the second motor neuron was then plotted. For subsequent flexor motor neurons, the procedure was repeated with the additional precaution of placing the electrode at a site in the ganglion distant from those where previous recordings had been made.

To estimate the number of motor neurons innervating particular bundles of fibres within the flexor muscle, the cut ends of small branches of nerve 5B2 (N5B2) (nomenclature of Campbell, 1961) were immersed in 0.1 mol l^{-1} hexammine cobaltic chloride contained within a Vaseline well. The preparation was left for 20–24 h at room temperature to allow the cobalt to diffuse into the metathoracic ganglion. The ganglion was then removed from the locust, and the cobalt was precipitated as a sulphide. The ganglion was then fixed in formaldehyde, silver-intensified, dehydrated and cleared in methyl salicylate.

The innervation pattern of a particular motor neuron within the flexor muscle was initially surveyed by observing the contractions evoked when it was made to spike. A detailed picture was obtained by sequential sampling of different muscle fibres both within and between the different bundles of fibres, to search for junctional potentials that matched the evoked motor spikes. Fibres from the whole muscle were sampled in each experiment but with particular emphasis on the anterior bundles. In a typical experiment, such a survey involved the penetration of 10–20 fibres within each bundle, so that at least 200 fibres in total were sampled in each locust. If the same pattern of innervation was found in different locusts, then it was assigned to the same motor neuron unless it could be established by recordings in the same locust (see above) that they were distinct neurons. According to the different patterns of innervation, the motor neurons were then numbered 1–9. The results are based on recordings from 47 locusts.

Results

Anatomy

The fibres of the flexor tibiae muscle in a hind leg are 6–7 mm long and insert on the tendon at a shallow angle of less than 10 ° (Fig. 1A). The muscle fibres that form the main body of the muscle are grouped into 10–11 pairs of bundles in the main body of the muscle and all insert onto a common central tendon (Fig. 1B). At the distal end is a pair of small bundles set apart from the rest of the muscle, but inserting on the same tendon, that forms the accessory flexor muscle. All the muscle bundles are innervated by small side branches of N5B2.

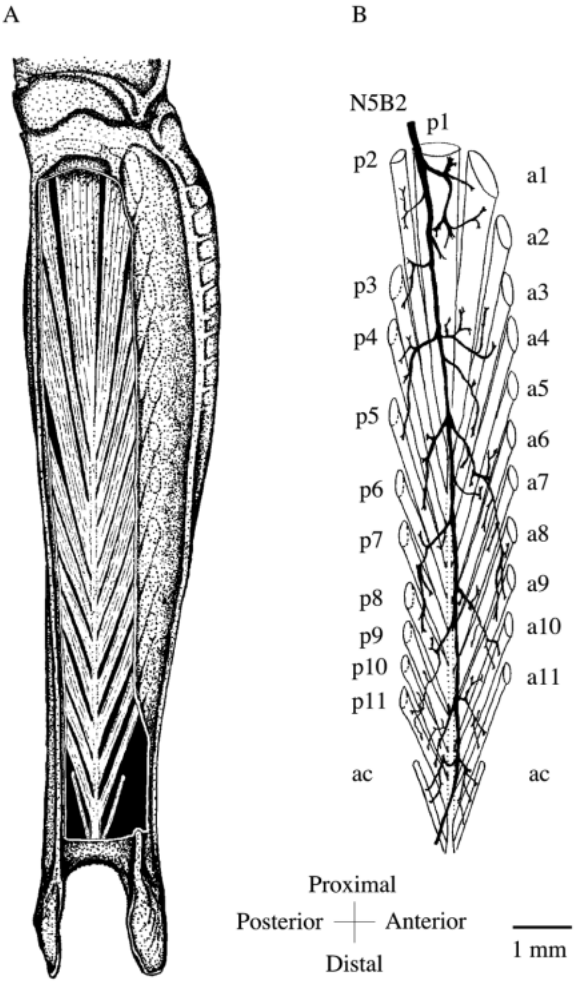

Fig. 1. The morphology of the flexor tibiae muscle in a left hind leg of a locust. (A) The muscle was exposed by removal of part of the ventral cuticle of the femur. (B) The muscle drawn in isolation to show the arrangement of the paired anterior (a1–11) and posterior (p1–11) muscle bundles and the accessory flexor (ac) bundles. The branches of N5B2 that innervate the different bundles of fibres are drawn from one locust.

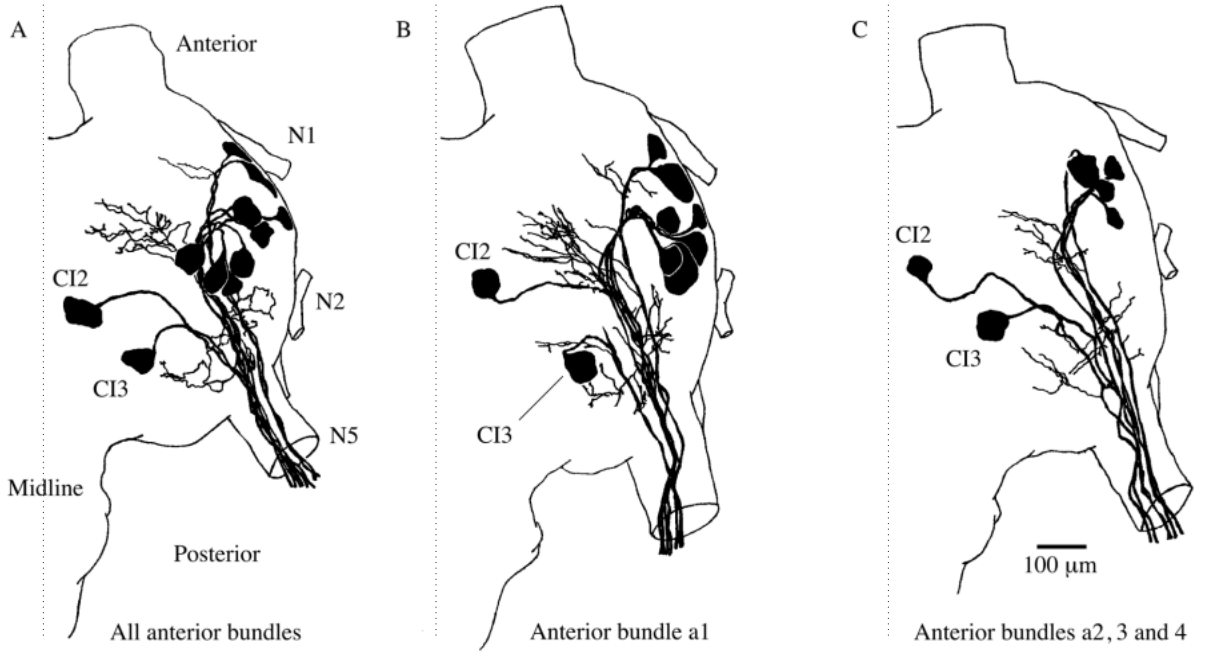

Fig. 2. Cell bodies of flexor tibiae motor neurons in one half of the metathoracic ganglion. Lateral nerves N1, N2 and N5 and one anterior connective are drawn. (A) The cell bodies revealed by backfilling the branches of N5B2 that supply all the anterior bundles of muscle fibres. (B) The cell bodies that supply anterior bundle a1. (C) The cell bodies that supply anterior bundles a2, 3 and 4. CI2 and CI3 are common inhibitory motor neurons.

Backfilling the whole of N5B2 from the proximal point where it supplies all the anterior bundles of the flexor tibiae muscle towards the central nervous system revealed between 11 and 15 cell bodies in the metathoracic ganglion of four different locusts (Fig. 2A; Table 1). The more medial position of two of these cell bodies sets them apart from the rest and identifies them as common inhibitory motor neurons (CI2 and CI3; Hale and Burrows, 1985; Watson *et al.* 1985). The remaining more laterally placed cell bodies are excitatory motor neurons and often fall into three subgroups – anterior, lateral and posterior – although the positions are not constant from locust to locust. In some preparations, more cell bodies were stained than can be explained by the number of neurons that innervate the flexor tibiae muscle, suggesting that cobalt may have leaked into other axons within the whole of nerve 5. Backfilling the smaller nerve branches to individual muscle bundles was less prone to such problems. Backfilling the nerve branch that innervates anterior bundle 1 revealed the cell bodies of the two common inhibitors and seven more anteriorly and laterally placed cell bodies, with the more posteriorly placed cell bodies unstained (Fig. 2B). Backfilling the branches that supply anterior bundles 2, 3 and 4 again revealed the two common inhibitory motor neurons, but only four excitatory motor neurons in an anterior and lateral location (Fig. 2C). We were unable to stain the motor neurons to the more distal muscle bundles, presumably because the cobalt failed to travel the large distances from the axon terminals to the metathoracic ganglion.

Innervation pattern

The nine excitatory motor neurons consist of three that cause slow movements of the tibia (labelled here as neurons 1–3), three that cause fast movements (neurons 7–9) and three that cause movements with intermediate characteristics (neurons 4–6). The patterns of innervation of these motor neurons were revealed by injecting current into a cell body and correlating the evoked motor spikes with junctional potentials recorded in the sampled muscle fibres (Fig. 3A–C). Less current was needed to evoke spikes in slow motor neurons than in fast motor neurons, and the frequency of these spikes was usually higher. Each of the motor neurons innervates a restricted array of fibres within the muscle; some innervate a larger region of

Table 1. *Number of cell bodies stained by backfilling the flexor branch of N5B2*

Muscle bundles innervated	Total number of somata stained	Number of common inhibitors CI2 and CI3	Number of excitatory motor neurons	Sample size
a1	8–9	1–2	7	6
a2, a3 and a4	5–6	1–2	4	5
All anterior bundles	11–15	1–2	9–13	4

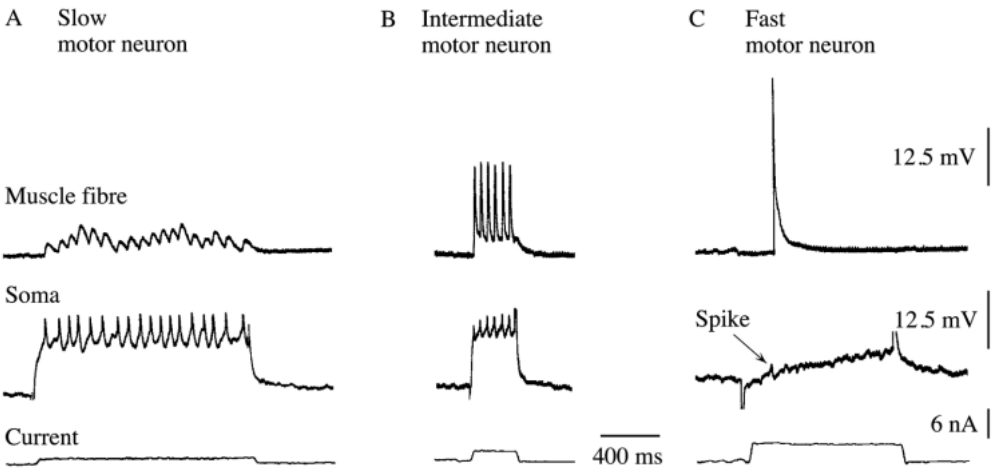

Fig. 3. Correlating spikes in the cell bodies of flexor motor neurons with junctional potentials in muscle fibres. (A) Injection of depolarising current into the soma of a slow motor neuron evokes a sequence of spikes and matching junctional potentials in a muscle fibre. (B) A current pulse injected into an intermediate motor neuron evokes spikes and matching junctional potentials in a muscle fibre. (C) A large current evokes only a single spike in a fast motor neuron and a single junctional potential in a muscle fibre. Substantial currents were needed to make the motor neurons generate spikes, necessitating overcompensation of the bridge circuit to display the voltage changes.

the muscle than others, but none supplies fibres throughout the muscle. The innervation patterns for all the motor neurons are summarised in Fig. 4.

The proximal part of the muscle is innervated by the largest number of motor neurons and the distal part by the fewest. The number of neurons revealed by these physiological methods for the more proximal bundles corresponds to the number of cell bodies of excitatory motor neurons revealed by backfilling.

Proximal bundles (a1, p1) are supplied by a total of seven excitatory motor neurons, with motor neuron 2 (slow) and motor neuron 8 (fast) being the two that are absent. Muscle bundles a2 and 3 are innervated by three motor neurons. Muscle bundle a4 is innervated by five motor neurons. Muscle bundle a5 is innervated by four motor neurons. Bundles a6–9 are innervated by the same three motor neurons, but only two of these innervate the most distal bundles a10, 11. The accessory flexor is innervated by at least one motor neuron that also innervates other bundles in the main body of the muscle. Only four motor neurons were tested to see whether they innervate the distal accessory flexor muscle.

On this basis, therefore, motor neurons 5 and 6 innervate only the most proximal fibres and motor neurons 2 and 8 innervate only the more distal fibres. Motor neuron 9 has a similar distribution in anterior bundles and for the restricted number of posterior bundles that we sampled. Motor neurons 1, 4 and 7 innervate up to anterior bundle 5 but do not extend more distally, whereas motor neurons 2 and 8 innervate only the distal anterior bundles.

Of all the flexor motor neurons, a fast one (motor neuron 8) has the most widespread pattern of innervation; it supplies all the posterior bundles except p1 and all the anterior bundles except a1–3 and some muscle fibres of a4. In contrast, intermediate motor neuron 5 has the most restricted distribution in that it supplies only the most proximal bundles (a1, p1). Fast motor neuron 9 also has a restricted distribution in that it innervates only bundles a1 and p1,2 in our restricted sampling of the posterior bundles. Some motor neurons such as 5 and 6 have very similar innervation patterns. For these motor neurons, it was necessary to establish that they are distinct motor neurons and not merely the same motor neuron sampled in different locusts. When a motor neuron with such an innervation pattern was encountered, a second electrode was placed in the cell body of another flexor motor neuron and its pattern of innervation was plotted. In this way, it could be demonstrated directly that two motor neurons can have the same pattern of innervation.

There are some asymmetries between the anterior and posterior bundles but, in general, the two parts of the muscle show a similar pattern of innervation. For example, both the first anterior and the first posterior bundles are innervated by the same seven motor neurons. In contrast, while the second posterior bundle is also innervated by seven motor neurones, the second anterior bundle is innervated by only three. These patterns of innervation were present in all the locusts that we sampled. Differences in the number of motor neurons innervating corresponding bundles in the distal parts of the muscle may well be due to our less complete sampling of posterior bundles. There are, however, asymmetries in the gross distribution of the nerve branches (Fig. 1B), and in *Calliptamus* there are fewer axon profiles in branches to posterior muscle bundles than in those to anterior bundles (Theophilidis and Dimitriadis, 1990).

No evidence has been found to suggest that there are restricted regions of the muscle that are innervated exclusively by fast, slow or intermediate motor neurons. One slow motor neuron (motor neuron 1) innervates only proximal muscle bundles, whereas a second (motor neuron 2) innervates only

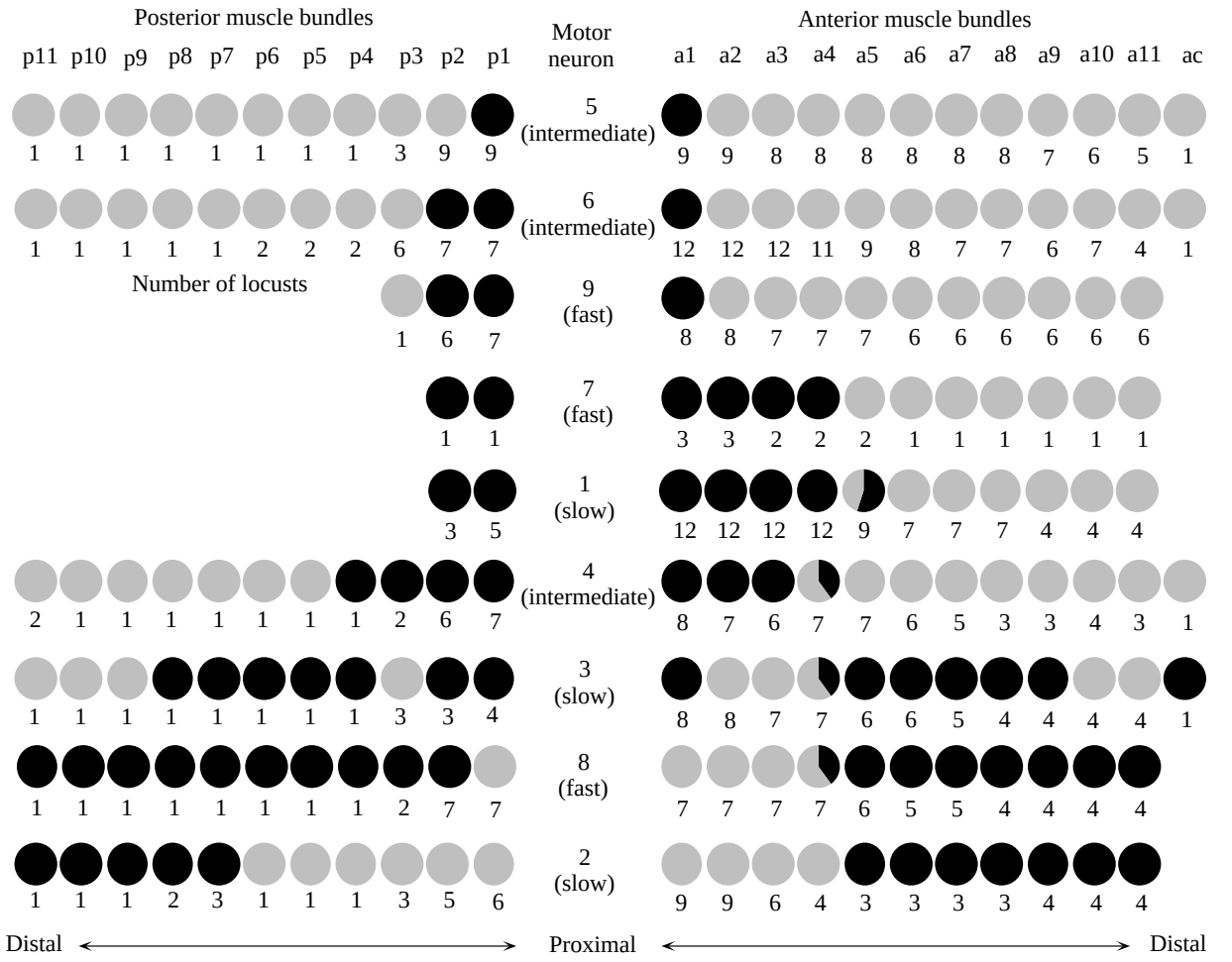

Fig. 4. The innervation pattern of nine excitatory motor neurons. The columns show the bundles of muscle fibres and the rows the different motor neurons (neurons 1–9) arranged according to the proximo-distal distribution of the muscle fibres that they innervate. The most proximal muscle bundles are shown in the centre of the diagram, and the distal ones at the left and right sides. The filled circles indicate that all muscle fibres tested within a bundle were innervated, the partially filled circles indicate the proportion of fibres sampled that were innervated, and the grey circles that none of the fibres tested was innervated. The absence of a circle indicates that this muscle bundle has not been sampled. The numbers beneath each circle indicate the number of locusts in which a particular bundle was sampled.

more distal fibres, but the third (motor neuron 3) has a more widespread distribution. Two of the fast motor neurons (motor neurons 7 and 9) innervate only proximal fibres and the third (motor neuron 8) innervates all but the most proximal fibres. All of the intermediate motor neurons (motor neurons 4–6) innervate only the proximal muscle bundles.

Fast, slow and intermediate types of motor neuron therefore overlap in their patterns of innervation. This is particularly obvious for bundles a10 and a11 that are innervated by only two motor neurons, one of which is slow (motor neuron 2) and one fast (motor neuron 8). Individual muscle fibres themselves can be innervated by more than one motor neuron that can be slow or fast as judged by the movements of the tibia that they cause (Fig. 5). For example, in individual fibres of bundle 7, junctional potentials can be recorded from both slow and fast motor neurons (Fig. 5A,B). Muscle fibres in anterior and posterior bundles 7 and fibres in anterior bundle 10 are innervated both by motor neuron 8 and by a slow motor neuron (Fig. 5A–C).

Discussion

Innervation patterns of the flexor motor neurons

This paper demonstrates by direct physiological measurements, supported by anatomical observations, that each member of the pool of nine excitatory flexor tibiae motor neurons innervates only a restricted array of muscle fibres. None has been found to innervate fibres throughout the muscle. Some motor neurons only innervate fibres in the proximal part of the muscle, whereas others only innervate more distal fibres. Some motor neurons innervate only a small region of the muscle, whereas others have a more extensive field of innervation. This pattern of innervation means that the most proximal muscle bundles and the most distal ones are each

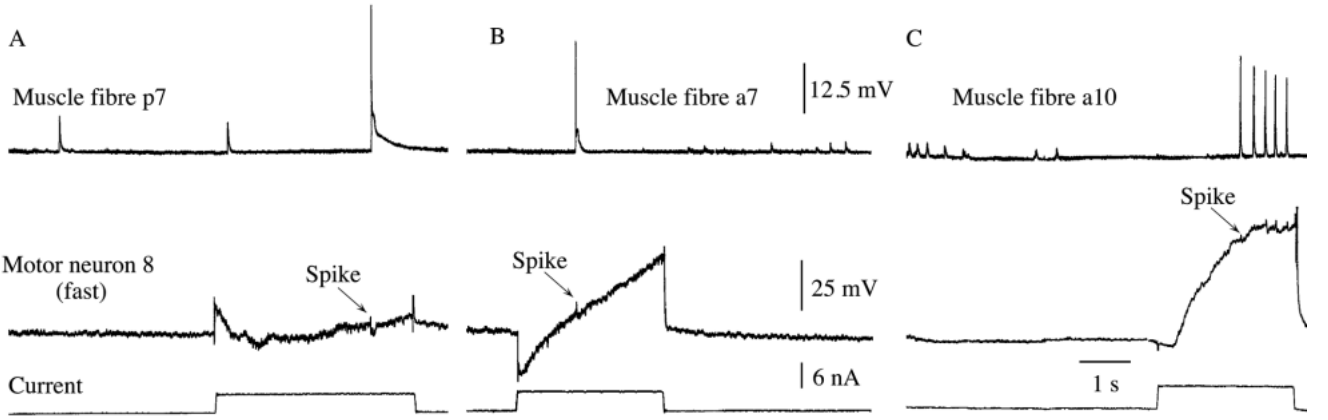

Fig. 5. Individual muscle fibres are innervated by more than one motor neuron. Motor neuron 8 was stimulated intracellularly to evoke spikes recorded in its soma and junctional potentials in muscle fibres. (A–C) The individual muscle fibres sampled in three of the bundles innervated by this motor neuron were also innervated by other motor neurons. As in Fig. 3, the bridge circuit is overcompensated.

innervated by distinct subsets of the flexor motor neuron pool. Only for bundles in the middle of the muscle is there any overlap in the innervation between those motor neurons that innervate the anterior proximal region and those that innervate the anterior distal region of the muscle. Five of the flexor motor neurons do not overlap at all in their field of innervation, and of the other four motor neurons the overlap extends to no more than a few bundles of fibres in the middle of the muscle. Individual motor neurons that cause slow, fast and intermediate movements of the tibia overlap in their patterns of innervation, and an individual muscle fibre can be polyneuronally innervated by motor neurons with different properties.

Our results differ from those of Phillips (1980), who concluded that the terminals of each motor neuron were distributed throughout the muscle. Phillips provided no data on the patterns of innervation derived from the same methods that we have used – stimulation of individual motor neurons by electrodes inserted into their cell bodies. Instead, she bases her conclusions on one figure of two ‘widely separated’ muscle fibres with common junctional potentials evoked by electrical stimulation of the whole of N5 that contains the axons of all nine excitatory motor neurons bundled together. We suggest that such common junctional potentials occurred because different motor neurons with the same threshold for spike initiation were activated together when their axons were stimulated electrically. Such experiments cannot shed light on the innervation patterns of individual motor neurons.

Our results are, however, in agreement with the suggestion of Hoyle (1955) that the flexor muscle consists of different parts that are separately innervated. They are also in agreement with the innervation pattern suggested for the flexor muscle in the middle leg of the locust (Theophilidis and Burns, 1983) and for the hind leg of another orthopteran *Calliptamus* (Theophilidis and Dimitriadis, 1990). In these other muscles, there is evidence that individual motor neurons do not innervate fibres throughout the muscles, but are restricted to certain regions.

If we take all of this evidence together, it seems reasonable to conclude that the innervation of the flexor tibiae muscles in different legs of different orthopterans conforms to the same plan: each motor neuron innervates only a restricted subset of fibres, but an individual muscle fibre may be innervated by several motor neurons.

Development of the flexor muscle

Could developmental processes determine the pattern of innervation of the flexor muscle? Each muscle bundle in the leg is formed during development from an individual muscle pioneer cell of mesodermal origin (Ball *et al.* 1985), but the processes that lead to the formation of the extensor and flexor tibiae muscles are different (Ball and Goodman, 1985*a*,*b*). The extensor develops from a single pioneer cell which fuses with surrounding mesodermal cells to form a giant syncytial cell, the supramuscle pioneer, containing hundreds of nuclei. This then splits into a series of multinucleate cytoplasmic bridges that divide to form the precursors for the different bundles of muscle fibres. The flexor, in contrast, develops from three pioneer cells that do not fuse into a supramuscle pioneer. One of the original pioneers dies while additional pioneers are added symmetrically, each of which divides to produce a particular bundle of muscle fibres. Combining these results with those presented here indicates that the innervation pattern of a particular motor neuron may be linked to a particular array of muscle pioneer cells. Furthermore, the differences in development of these two antagonistic muscles might suggest that they have a different evolutionary origin, with the larger number of initial pioneers for the flexor suggesting that it had its origins as more than one muscle. This in turn might explain why the flexor has nine excitatory motor neurons while the extensor has only two. The distribution of the nine excitatory flexor motor neurons to the different muscle bundles offers some support for this idea, in that some motor neurons innervate only proximal muscle bundles and others only the more distal bundles.

Why are there so many motor neurons to the flexor muscle?

The explanation for the large number of motor neurons to a flexor tibiae muscle may lie in a number of additional factors: a subdivision of function among members of the motor pool, a subdivision of action by different parts of the muscle by virtue of different innervation patterns or intrinsic differences in the properties of the muscle fibres, or a combination of all factors. Particular neurons of a motor pool may be reserved for specific actions (Loeb, 1985). In insects, for example, specific slow motor neurons may be used to control limb muscles for reflex responses while others are used for generating locomotory movements (Zill and Moran, 1982), and in crustaceans different members of a motor pool may act differently because of the different sensory inputs they receive (Skorupski *et al.* 1992). Hoyle (1964) also suggested that the different motor neurons to the flexor muscle in the hind leg of a locust might be used differently in different movements; the same neurons would be used in most movements but some would be restricted to the less frequently used postures. Flexor tibiae motor neurons that have large spikes when recorded in extracellular myograms (they are thus probably the fast motor neurons) are active at each step during horizontal walking, are active occasionally during vertical climbing, but are inactive when a locust is walking upside down (Duch and Pflüger, 1995). The dominant feature of simultaneous recordings from two or more flexor tibiae motor neurons is, however, the correspondence between many of their synaptic inputs (Burrows, 1996). The pool of flexor motor neurons thus appears to be driven by many common sources of synaptic inputs with their inherent membrane properties determining their spike response and hence their order of recruitment. There are, however, important exceptions. Campaniform sensilla on the tibia of the middle leg directly excite fast flexor tibiae motor neurons of the same leg but do not excite slow motor neurons (Newland and Emptage, 1996). Imposed movements of the apodeme of the femoral chordotonal organ in a hind leg to simulate movements of the femoro-tibial joint lead to responses in both flexor and extensor motor neurons that are caused in part by the direct connections made by the sensory neurons with the motor neurons (Burrows, 1987). Fast flexor motor neurons are excited by fast movements but not by slow movements, whereas the opposite is true for the slow motor neurons (Field and Burrows, 1982). Thus, although the different flexor motor neurons receive many synaptic inputs in common, each has specific dynamic responses to movements of the femoro-tibial joint (Newland and Kondoh, 1997). Such differential activation of members of the motor pool could perhaps lead to differential contraction of parts of the muscle that are independently innervated by the particular motor neurons. The mechanical consequences of this are, however, not easy to predict given that all the muscle fibres attach to a common tendon and at similar angles. The small distal accessory bundles would not seem capable of generating force independently as their fibres share innervation with those in other distal bundles. The contractions of the main body of the muscle may, however, deform the femoral cuticle in different ways and this may be detectable by separate arrays of sensory neurons which would, in turn, lead to different effects by virtue of their different connections within the central nervous system.

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