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Local Innervation Patterns of the Metathoracic Flexor and Extensor Tibiae Motor Neurons in the Cricket *Gryllus bimaculatus*

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ABSTRACT—To elucidate neural mechanisms underlying walking and jumping in insects, motor neurons supplying femoral muscles have been identified mainly in locusts and katydids, but not in crickets. In this study, the motor innervation patterns of the metathoracic flexor and extensor tibiae muscles in the cricket, *Gryllus bimaculatus* were investigated by differential back-fills and nerve recordings. Whereas the extensor tibiae muscle has an innervation pattern similar to that of other orthopterans, the flexor has an innervation unique to this species. The main body of the flexor muscle is divided into the proximal, middle and distal regions, which receive morphologically unique terminations from almost non-overlapping sets of motor neurons. The proximal region is innervated by about 12 moderate-sized excitatory motor neurons and two inhibitory neurons while the middle and distal regions are innervated by three and four large excitatory motor neurons, respectively. The most-distally located accessory flexor muscle, inserting on a common flexor apodeme with the main muscle, is innervated by at least four small excitatory (slow-type) and two common inhibitory motor neurons. The two excitatory and two inhibitory motor neurons that innervate the accessory flexor muscle also innervate the proximal bundles of the main flexor muscle. This suggests that the most proximal and distal parts of the flexor muscle participate synergistically in fine motor control while the rest participates in powerful drive of tibial flexion movement.

Key words: insects, orthoptera, femoral muscles, back-fills

Abbreviations: AAF: anterior branch of AF, AF: accessory flexor nerve, AFM: accessory flexor muscle, APF: anterior branch of PF, CI: common inhibitor, DF: distal flexor nerve, DFM: distal flexor muscle, DUM: dorsal unpaired median neuron, MF: middle flexor nerve, MFM: middle flexor muscle, PAF: posterior branch of AF, PF: proximal flexor nerve, PFM: proximal flexor muscle, PPF: posterior branch of PF.

INTRODUCTION

The metathoracic flexor and extensor tibiae muscles in orthopteran insects have been thoroughly studied as a model comparable to vertebrate limb muscles (review: Zill, 1993). They participate in walking (Burns and Usherwood, 1979; Cruse, 1980), jumping (Heitler, 1974; Heitler and Burrows, 1977), kicking (Burrows, 1995; Hustert and Gnatzy, 1995) and postural reflexes (Field and Burrows, 1982; Field and Coles, 1994). The extensor tibiae muscle is especially developed for jumping, occupying almost 88% of the cross section of the femur in the locust, *Schistocerca gregaria* (Bennet-Clark, 1975). It has only two excitatory motor neurons, one inhibitory motor neuron and one modulatory neuron. This number and combination are well conserved in the three pairs of legs (Wilson, 1979; Theophilidis, 1983) and in several families of orthopteroid insects: locusts (Hoyle and Burrows, 1973), katydids (Theophilidis, 1983) and stick insects (Godden, 1972).

On the other hand, the flexor tibiae muscle is much smaller than the extensor in volume yet it receives innervation from a large number of excitatory motor neurons, making it one of the most complexly innervated muscles in the Arthropods. Hoyle (1955) suggested in the locust that the flexor muscle is compartmentalized into five to six parts and that the different compartments are separately innervated. This was recently confirmed by intracellular recordings, in which a set of motor neurons supplies only a restricted array of muscle fibers (Sasaki and Burrows, 1998). Although the basic structure of the orthopteran flexor tibiae muscle is similar among species (Theophilidis and Burns, 1983), the number of excitatory motor neurons innervating this muscle

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appears to differ among different pairs of legs in the same species or the same legs in different species: 12 in the locust mesothoracic leg (Theophilidis and Burns, 1983), at least nine in the locust metathoracic leg (Philips, 1980), 14 to 15 in the stick insect mesothoracic leg (Debrodt and Bässler, 1989) and at least 10 in the katydid metathoracic leg (Theophilidis and Dimitriadis, 1990).

Theophilidis and Burns (1983) proposed that the flexor motor neurons are functionally differentiated to participate in various behavioral patterns. Indeed, different flexor tibiae motor neurons are recruited in the postural resistance reflex when the speed of the passive tibial extension is changed (Field and Burrows, 1982; Theophilidis and Burns, 1990; Newland and Kondoh, 1997). The flexor activity patterns show marked differences during horizontal walking, vertical climbing and upside down walking (Duch and Pflüger, 1995). However, it is still not known how each muscle compartment participates in tibial motor control. As it is plausible that the different numbers of the flexor motor neurons in different species reflect different functional modifications of the muscle compartments, a comparative study could uncover detailed functions of this muscle complex.

The cricket *Gryllus bimaculatus* prefers to live among rocks and has a different escape strategy from the locust. Instead of jumping, it runs fast (Tauber and Camhi, 1995) and crawls into small interstitial spaces, where it exhibits a sudden freezing (thanatosis) due to the restrained conditions (Nishino and Sakai, 1996). Hence, it is not surprising that the leg motor neurons of the cricket have innervation patterns which may reflect this unique behavioral pattern, and which are modified from the locust innervation pattern. In this study, almost all flexor and extensor tibiae motor neurons in the cricket were reliably identified by back-fill stainings and nerve recordings. The results show that certain modifications unique to the cricket occur in the flexor tibiae muscle rather than in the extensor, leading to a deeper understanding of functions of each compartment of the flexor tibiae muscle.

MATERIALS AND METHODS

Animals

Male and female crickets *Gryllus bimaculatus* DeGeer of 3–15 days after imaginal molt were used. Over 120 crickets were used for the experiments.

Anatomy of leg muscles

Crickets were cold-anesthetized on iced water for one hr and fixed ventral-side-up on a beeswax plate. The ventral thorax was opened to give access to the main leg nerve 5 (see Laurent and Richard, 1986). To stain peripheral motor nerves in the femur, Nerve 5 (N5) was cut at the level of the coxa and its peripheral cut end placed into the tip of a tapered polyethylene capillary tube filled with a mixture (23:2) of 0.25 M nickel (II) chloride hexahydrate (Merck) and 0.25 M cobalt (II) chloride hexahydrate (Merck). The preparation was left in a moist chamber at 5°C for 24 hr. The leg was then cut off at the proximal end of the coxa and fixed on a beeswax plate filled with cricket saline (see Nishino and Sakai, 1997) with the anterior side of the femur facing up. The femoral muscles were exposed by removing the whole anterior cuticle and reacting with rubeanic acid for 20 min. The leg was then immersed in 50% ethanol for 5 min, fixed in 4% formaldehyde solution for 3 hr, dehydrated in an ethanol series and cleared in methyl salicylate. Some of stained muscles were further divided into quadrants to facilitate absorption of the developer base and then processed by conventional silver intensification (Bacon and Altman, 1977).

Back-fill staining of motor neurons

A cold-anesthetized cricket was fixed ventral-side-up on a beeswax plate. To prevent leg movement, the posterior halves of the femur and tibia were embedded in plasticine. After the removal of the anterior cuticle over the metathoracic femur, an arbitrarily selected motor nerve was cut and its proximal cut end placed into the tip of a tapered polyethylene capillary tube filled with a mixture (23:2) of 0.25 M nickel (II) chloride (Merck) and 0.25 M cobalt (II) chloride hexahydrate (Merck). Extra care was taken not to stretch the nerve when handled as stretching nerves causes dye-leakage and stains extra number of neurons. The preparations were left in a moist chamber at 4°C for up to 72 hr. The metathoracic ganglia were removed, immersed in 50% ethanol for 5 minutes, fixed in alcoholic Bouin's for 3 hr and then processed by the conventional silver intensification. This pre-fixation by ethanol was crucial to avoid the deposition of silver granules on the surface of the ganglion during intensification and to promote intensification. The intensified ganglia were cleared in methyl salicylate, then photographed and sketched using a microscope drawing tube.

In order to investigate overlapping motoneuronal innervation of muscle compartments, differential staining was achieved by immersing the cut ends of two nerve bundles into separate glass electrodes, one filled with 1% solution of dextran plus tetramethyl rhodamine (abbreviated to TMR, 3000 MW, Molecular Probes) and the other with 1% solution of dextran plus fluorescein (abbreviated to FITC, 3000 MW, Molecular Probes). After the incubation in the moist chamber at 4°C for 48–72 hr, the metathoracic ganglia were fixed in 4% formaldehyde solution, dehydrated in alcohol series, cleared in methyl salicylate and observed under a confocal laser microscope (Zeiss, LSM510). The ganglia were manually cut transversely with a razor blade to check the numbers of somata stained. Multiple scanning mode was used to excite FITC and TMR differentially. The size of each neuronal soma was presented as the mean value of the long and short axes.

Electrophysiology

The extracellular recordings were made from motor nerve branches in minimally dissected animals which were restrained in plasticene. The leg from which recording was made was fixed so that the tibia and tarsus were free to move. The spike activities were recorded with suction electrodes, stored in DAT recorder and analyzed on Lab View ver. 6I. The height and wave form of spikes were used as criteria to discriminate different units.

Terminology

The author refers to Hoyle (1978) for the naming of motor neurons, Laurent and Richard (1986) for naming of nerves originating from the ganglion and Williamson and Burns (1978) for detailed nomenclature of femoral nerves.

RESULTS

Anatomy of the flexor and extensor tibiae muscles

The main body of the flexor tibiae comprises 12 muscle bundles (Fig. 1A) which all insert onto a common central apodeme (Sasaki and Burrows, 1998) specialized distally as an enlarged cushion unique to the crickets (Hustert and Gnatzy, 1995). The cushion tapers distally and attaches to

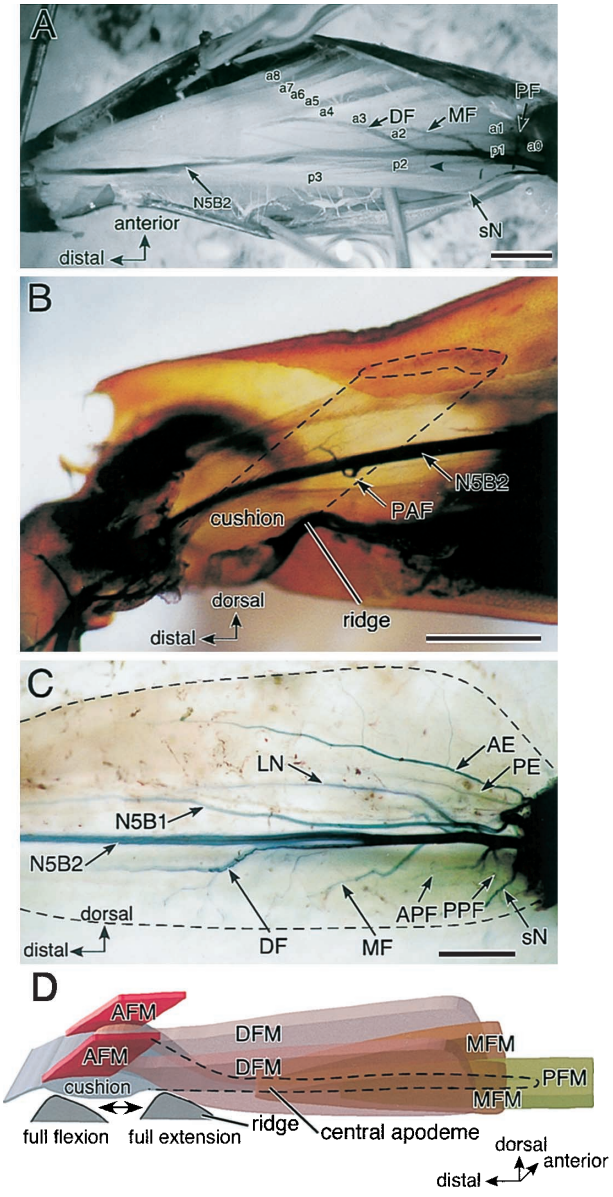

Fig. 1. Motor innervation patterns in the flexor- and extensor tibiae muscle of the metathoracic leg. **A**: Photomicrograph of the main flexor tibiae nerves overlaid on the primary muscle bundles (a0-8, p1-3) viewed ventrally. Nerves innervating the main body of the flexor muscle comprise the proximal flexor nerve (PF), middle flexor nerve (MF) and distal flexor nerve (DF), which innervate proximal, middle and distally located muscle fibers. Note that a small branch diverging from the DF (arrow head) innervates the single muscle bundle, p3. **B**: Photomicrograph of the posterior accessory flexor muscle innervated by the posterior branch of the accessory flexor nerve (PAF). The outline of the muscle is indicated by broken lines. The dorsal attachment site of the accessory flexor muscle is pigmented (encircled by broken line) while the ventral attachment site is attached to the cushion enlargement. **C**: Photomicrograph of main muscle and nerve branches viewed anteriorly. The extensor nerve bifurcates into the anterior branch (AE) and posterior branch (PE). LN: lateral nerve; sN: sensory nerve. **D**: Three-dimensional representation of the flexor muscle compartments innervated by different nerves. The muscle bundles innervated by PF, MF, DF and AF are represented as PF muscle bundles (PFM), MFM, DFM and AFM, respectively. The approximate position of the cuticular ridge against the cushion enlargement when the tibia is positioned from tibial full flexion to full extension (see Hustert and Gnatzy, 1995) is also shown. Broken lines indicate central apodeme. Scale bars, 1 mm.

the proximal tibia. There is asymmetry between the anterior- and posterior halves of the muscle: the former comprises eight muscle bundles (a1-a8) and the latter comprises only three (p1-p3). The proximally located three muscle bundles, anterior bundle 0, 1 (a0, 1) and posterior bundle 1 (p1) merge into one another and form a fusiform-like bundle inserting almost axially onto the central apodeme. a0 has a proximal origin in the trochanter while the a1 and p1 in the proximal femur. The remaining bundles (a2-8 and p2,3) are pinnate-type that has discrete insertion points in the distal femur (Fig. 1A). At the distal end is a pair of 5 to 6 bundles set apart from the main body of the muscle, inserting onto the cushion at an angle of about 45°, that forms the accessory flexor muscle (Fig. 1B). The accessory flexor muscle in the cricket is relatively large proximo-distally (outlined by broken lines, Fig. 1B) compared to the locust (Heitler, 1974).

Rather then innervated by many short nerves as in locusts (Theophilidis and Burns, 1983; Sasaki and Burrows, 1998) or katydids (Theophilidis and Dimitriadis, 1990), the flexor tibiae muscle in the cricket is simply innervated by four distinct branches from N5B2 that supply discrete regions of the muscle. Thus, each nerve is reliably identifiable among individuals. As in the locust mesothoracic legs (Theophilidis and Burns, 1983), the main body of the cricket flexor muscle is divided into the proximal, middle and distal regions, that occupy about 25%, 25% and 50% in volume of the main muscle, respectively. They receive innervation from the proximal flexor nerve (PF), middle flexor nerve (MF) and distal flexor nerve (DF), respectively (Fig. 1C). More detailed innervation patterns are given as follows. Immediately after diverging from N5B2, PF bifurcates into the anterior branch (APF) innervating bundles a0 and a1, and the posterior branch (PPF) innervating bundle p1. MF originates distally to PF and provides several side-branches to innervate bundles a2-4 and p2. DF originates distally to MF and provides a single branch innervating the most posterior muscle bundle, p3 (arrow head, Fig. 1A) and then subdivides some side-branches to innervate the most anterior three bundles, a5-8. The accessory flexor nerve (AF) diverges from N5B2 in the distal femur and bifurcates into the anterior branch (AAF) and posterior branch (PAF) to innervate the pair of the accessory flexor bundles (Fig. 1B). The distal insertion sites and angles of all flexor muscle bundles reflect three-dimensional arrangements of the muscle bundles: those innervated by PF, MF, DF and AF insert progressively into more distal-lateral points on the apodeme/cushion complex at deeper angles (Fig. 1D).

The nerve innervation pattern of the extensor tibiae muscle in the cricket is almost identical to that in the locust *Schistocerca gregaria* (Burns, 1973; Evans and O'shea, 1978). The muscle bundles forming the extensor tibiae are all pinnate-type. Immediately after entering the femur, the extensor nerve diverging from nerve 5B1 (N5B1) bifurcates into the anterior extensor nerve branch (AE) and the posterior extensor nerve branch (PE, Fig. 1C). AE innervates the proximo-anterior part of the main body of the muscle while

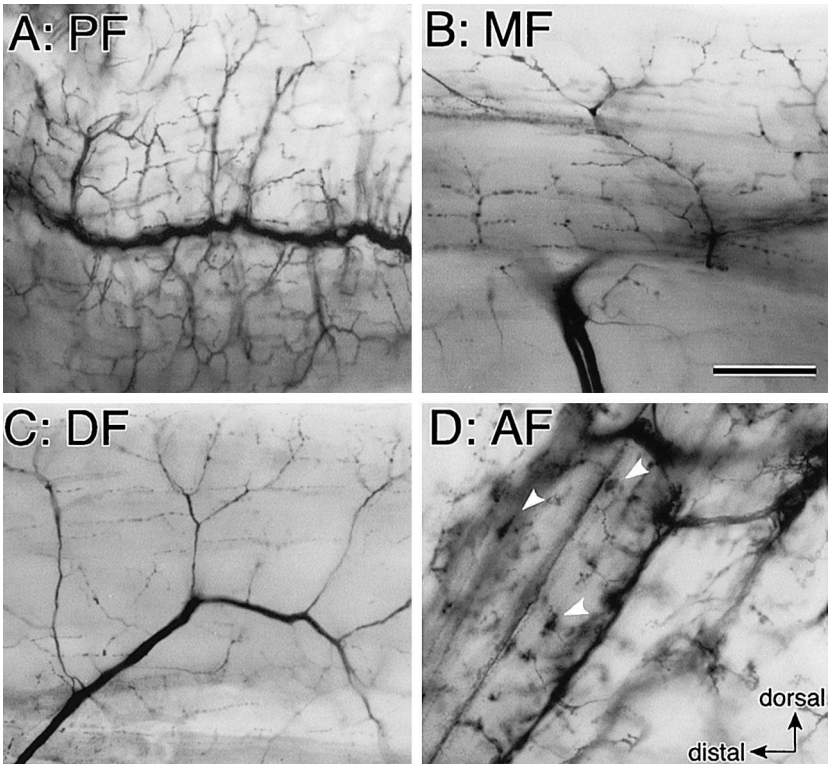

Fig. 2. Terminal specializations of the PF (A), MF (B), DF (C) and AF (D). They exhibit different terminal branching decorated with different numbers and sizes of varicosities. Scale bar, 100 μm.

the PE innervates the postero-distal part of the main muscle and the accessory extensor muscle.

The silver-intensification revealed that each flexor nerve branch exhibits unique terminal specializations (Fig. 2). PF showed dense terminal arborizations with small terminal varicosities distributed along their length (Fig. 2A). MF and DF basically had a similar branching pattern, giving rise to thin long side-branches decorated with small varicosities along their length, although MF appeared to provide finer branching than DF (Fig. 2B, C). The branching pattern of AF was apparently similar to that of PF but the density of arborization was more modest. The terminal varicosities of AF tended to cluster into petal-like large varicosities irregular in size (white arrow heads, Fig. 2D).

Innervation patterns of motor neurons in the flexor and extensor tibiae muscles

Back-fills from the distal cut ends of flexor and extensor nerve branches revealed that each nerve contains axons of particular sets of efferent neurons (Fig. 3). The excitatory and inhibitory motor neurons were discernible based on soma location, dendritic fields and axonal trajectory, as in wetas (Hoyle and Field, 1983) or in locusts (Hale and Burrows, 1985). The exciters had somata belonging to the antero-lateral neuromere of the metathoracic ganglion and send axons into the middle part of N5. The two inhibitors, which exhibited GABA-like immunoreactivity (Nishino, unpublished observation) as those in locusts (Watson, 1986), had somata close to the midline of the ganglion (broken lines, Fig. 3) and sent axons into the medial part of N5. The dendritic arborizations of the inhibitors were medio-dorsal and segregated from those of the exciters, allowing inhibitors to be drawn separately (Fig. 3G). The anterior inhibitor (AI) and the posterior inhibitor (PI) supplied additional axonal branches to innervate unidentified coxal- and tibial muscles, thus could be defined as “common inhibitory motor neurons (CIs)”.

Back-fills from APF (n=6) or PPF (n=8) stained 10–12 excitatory motor neurons and two inhibitory motor neurons in the metathoracic ganglion (12 neurons were stained in Fig. 3A, B). Back-fills from the PF (n=4) also stained similar numbers of neurons (data not shown), indicating that an identical set of neurons bifurcates to innervate this pair of muscle bundles. The position of somata varied among individuals, making it difficult to subdivide the pool of flexor exciters into anterior-, lateral- and posterior groups as in locusts (Hoyle and Burrows, 1973). Also it was difficult to trace single axonal projections into each particular nerve branch. Nevertheless, the back-fills from PF always revealed one large soma in the base of N1 (arrows, Fig. 3A, B), which could be uniquely identified as a neuron that supplies PF exclusively.

Back-fills from MF (n=7) and DF (n=8) consistently stained three and four excitatory motor neurons, respectively (Fig. 3C, D). The somata of neurons that send axons to the MF were similar in size and tended to aggregate in

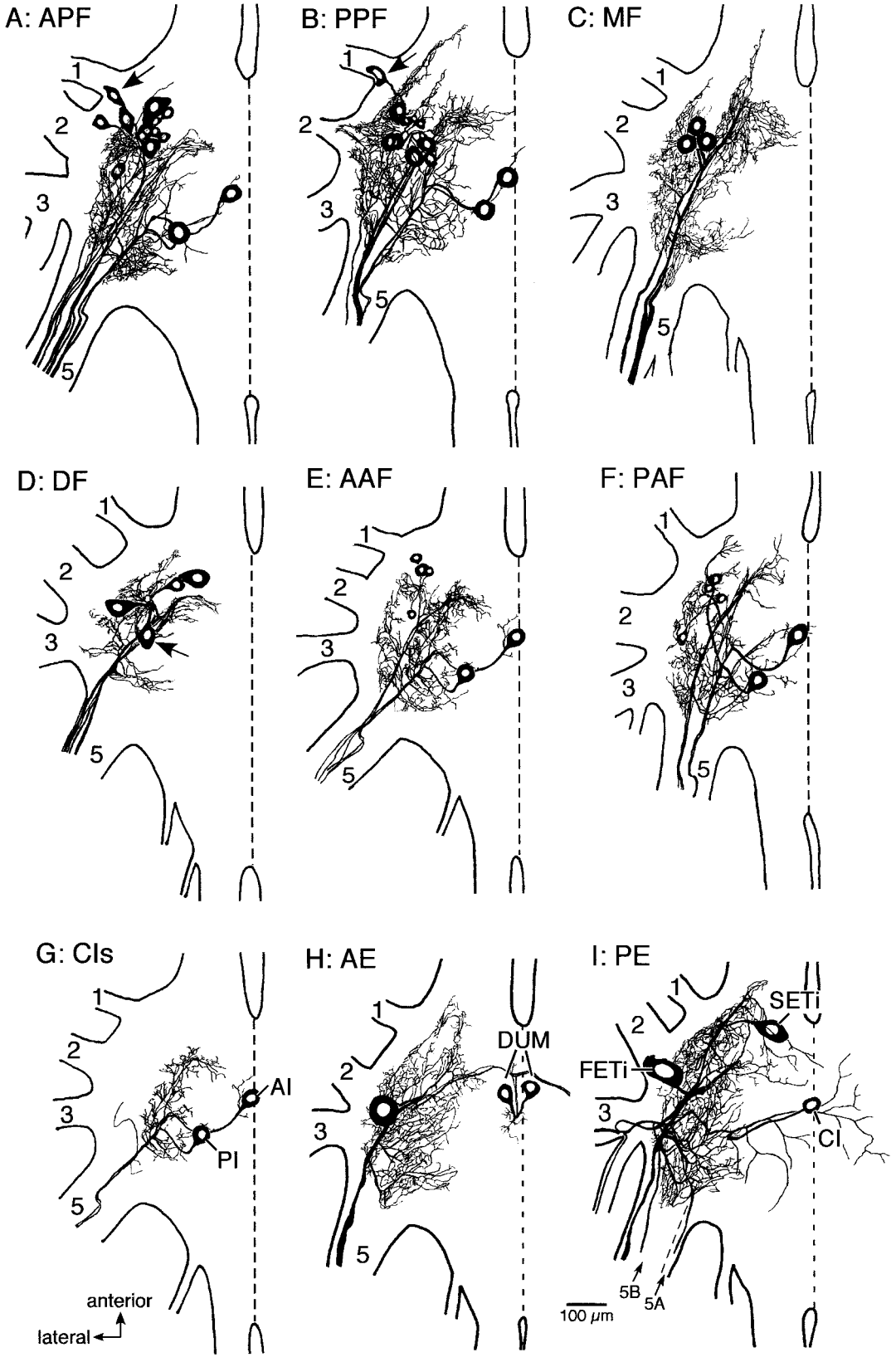

Fig. 3. Camera lucida drawings of the flexor and extensor motor neurons back-filled from different motor nerve branches in the metathoracic flexor tibiae muscle. All motor neurons are silver-intensified. APF: anterior branch of the proximal flexor nerve. PPF: posterior branch of the proximal flexor nerve. AAF; anterior branch of accessory flexor nerve. PAF: posterior branch of the accessory flexor nerve. AI: anterior inhibitor; PI: posterior inhibitor; CI: common inhibitor; FETi: fast extensor tibiae; SETi: slow extensor tibiae. The visible nerve roots are indicated as numbers. Broken lines indicate midline of the ganglion. Scale bar, 100 μm.

one place (Fig. 3C). The neurons sending axons to DF comprised one smaller and three larger somata. Two out of eight back-fills from DF stained two DUM neurons faintly, suggesting that the DUM neurons may send tiny axons into DF. Back-fills from DF sub-branch supplying p3 (see arrow head in Fig. 1A) stained one large neuron that was the most

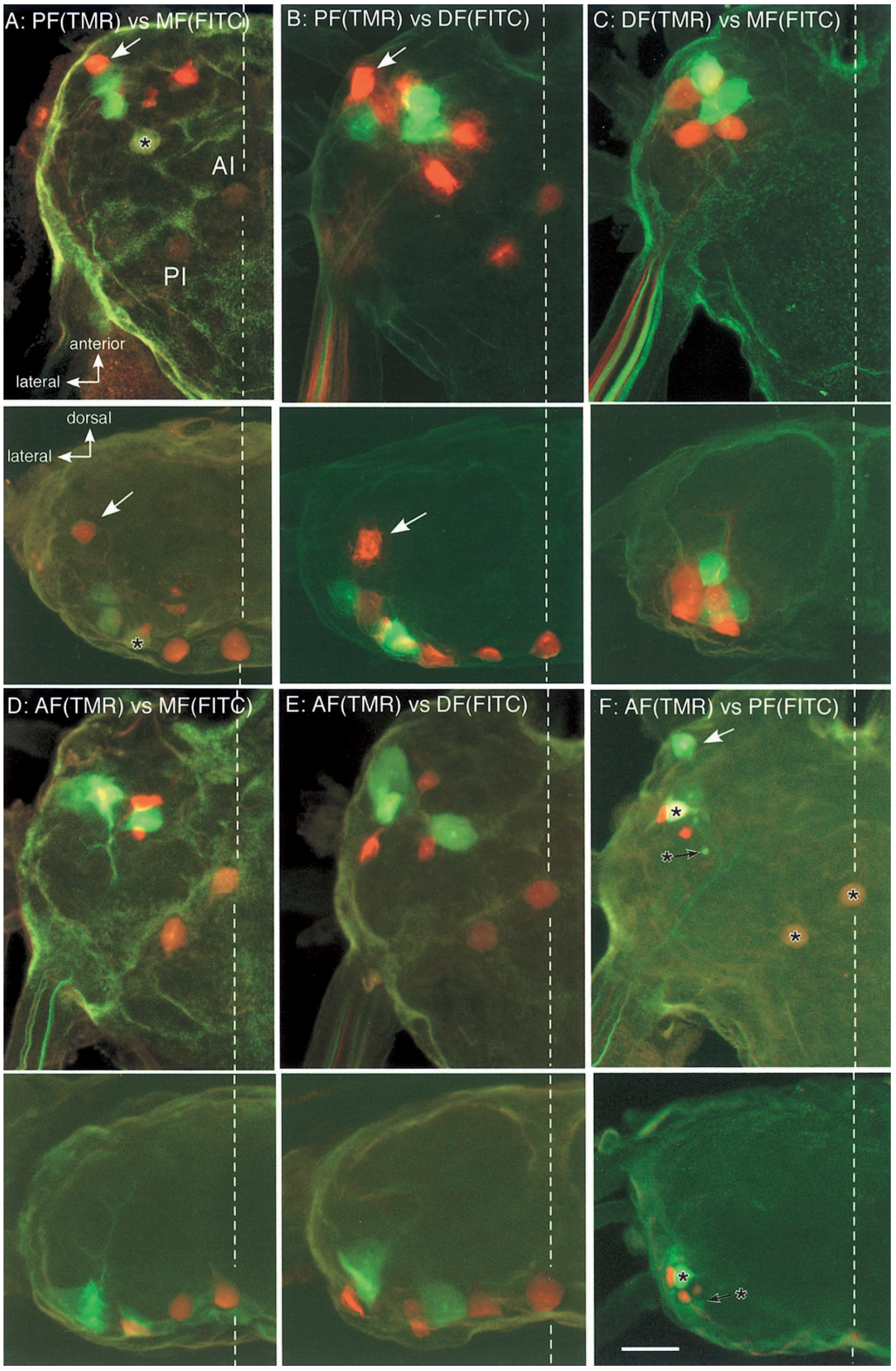

Fig. 4. Differential back-fills of two flexor nerve branches using dextran plus tetramethyl rhodamine (TMR) and dextran plus fluorescein (FITC). Arrows in A, B and F indicate an motoneuronal soma that sends an axon specifically to the proximal flexor nerve (PF). Asterisks in A and F indicate motoneuronal somata stained with both TMR and FITC. Scale bar, 100 μm.

posterior soma (arrow in Fig. 3D) of four neurons supplying the main trunk of DF (n=4). Back-fills from the anterior branch (AAF, n=6) and posterior branch (PAF, n=5) stained four to six small exciters and two inhibitors (n=12, Fig. 3E, F). Back-fills from AF (n=4) also stained similar number of neurons (data not shown), suggesting that an identical set of neurons bifurcates to innervate this pair of muscle bundles.

Back-fills from AE (n=3) stained one fast excitatory motor neuron (FETi) and two dorsal unpaired median (DUM) neurons, while those from PE (n=3) stained one FETi, one slow excitatory motor neuron (SETi) and one common inhibitory motor neuron (homologous to CI1, Hale and Burrows, 1985). The axons of the FETi and SETi traveled through N5 and N3, respectively (Fig. 3I). The axon of the inhibitor trifurcated to travel through N3, 5A and 5B (Fig. 3I)

Overlapping degree of sets of flexor motor neurons

In order to determine whether the four discrete regions of the flexor tibiae muscle receive overlapping innervation from the above motor neurons, differential staining using two fluorescent dyes (FITC and TMR) was applied to all possible pairs of nerves (Fig. 4).

The results show that the proximal-, middle- and distal regions of the main flexor muscle receive almost non-overlapping innervation from sets of motor neurons (Fig. 4A-C). Only one soma was excited by both filters set for detecting FITC or TMR, when neurons supplying PF and MF were differentially stained (asterisk, Fig. 4A), suggesting that this neuron sends axons into both PF and MF. This was also confirmed by the finding that back-fills from MF always stain a single axon travelling into PF and *vice versa* (Nishino, unpublished observation). No evidence of overlapping between neurons sending axons into PF and DF was detected (Fig. 4B). Despite their similar branching patterns (see Fig. 2B,C), neurons supplying MF and DF had no overlap (Fig. 4C). Also no overlap occurred between neurons supplying AF and MF (Fig. 4D) or between neurons supplying AF and DF (Fig. 4E). However, most compellingly, at least four out of six neurons supplying AF (Fig. 4F, indicated by asterisks) overlapped with those of PF, meaning that two exciters and two inhibitors supply axons to both proximal region of the main flexor muscle and to the accessory flexor muscle.

In the locust, all excitatory flexor motor neurons are categorized into slow-, intermediate- and fast-type motor neurons according to their physiological properties. The slow-type fires tonically to maintain muscle tonus, while the fast-type fires very briefly but leads to a strong twitch contraction. The intermediate-type fires in longer-lasting bursts with progressively declining frequency during visible movements (Hoyle and Burrows, 1973; Hoyle 1980). These slow-, intermediate- and fast-type neurons have small, moderate and large-sized somata, respectively, and thus they can be

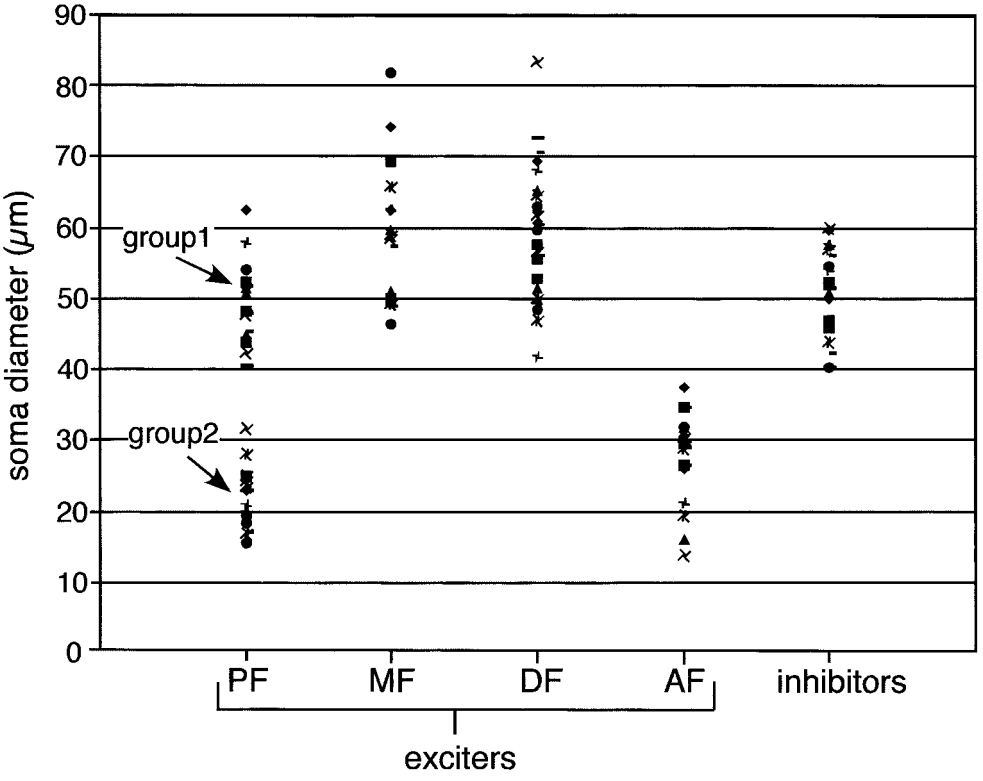

Fig. 5. Quantitative measurements of somata of the flexor motor neurons supplying flexor nerve branches. Data derived from five PF back-filled-, five MF back-filled-, six DF back-filled-, five AF back-filled specimens are used. Inhibitors supplying PF and AF were separately plotted from exciters. Different shapes of points represent samples from different individuals.

roughly divided into three types based on soma size (Burrows and Hoyle, 1972; Philips, 1980). Quantitative measurements of the flexor motor soma diameters were made from five PF, five MF, six DF and five AF back-filled samples (Fig. 5, inhibitors are separately plotted). They showed a variation in soma size between individuals, making it difficult to classify each neuron into slow, intermediate and fast motor neurons using a morphological criterion only. Nevertheless, the pool of exciters supplying PF tended to have smaller somata than those supplying MF or DF, and they form roughly two clusters comprising those with moderate-sized (group1) and small-sized somata (group2) rather than forming a continuous distribution. The excitatory motor neurons supplying the accessory flexor muscle had only small somata.

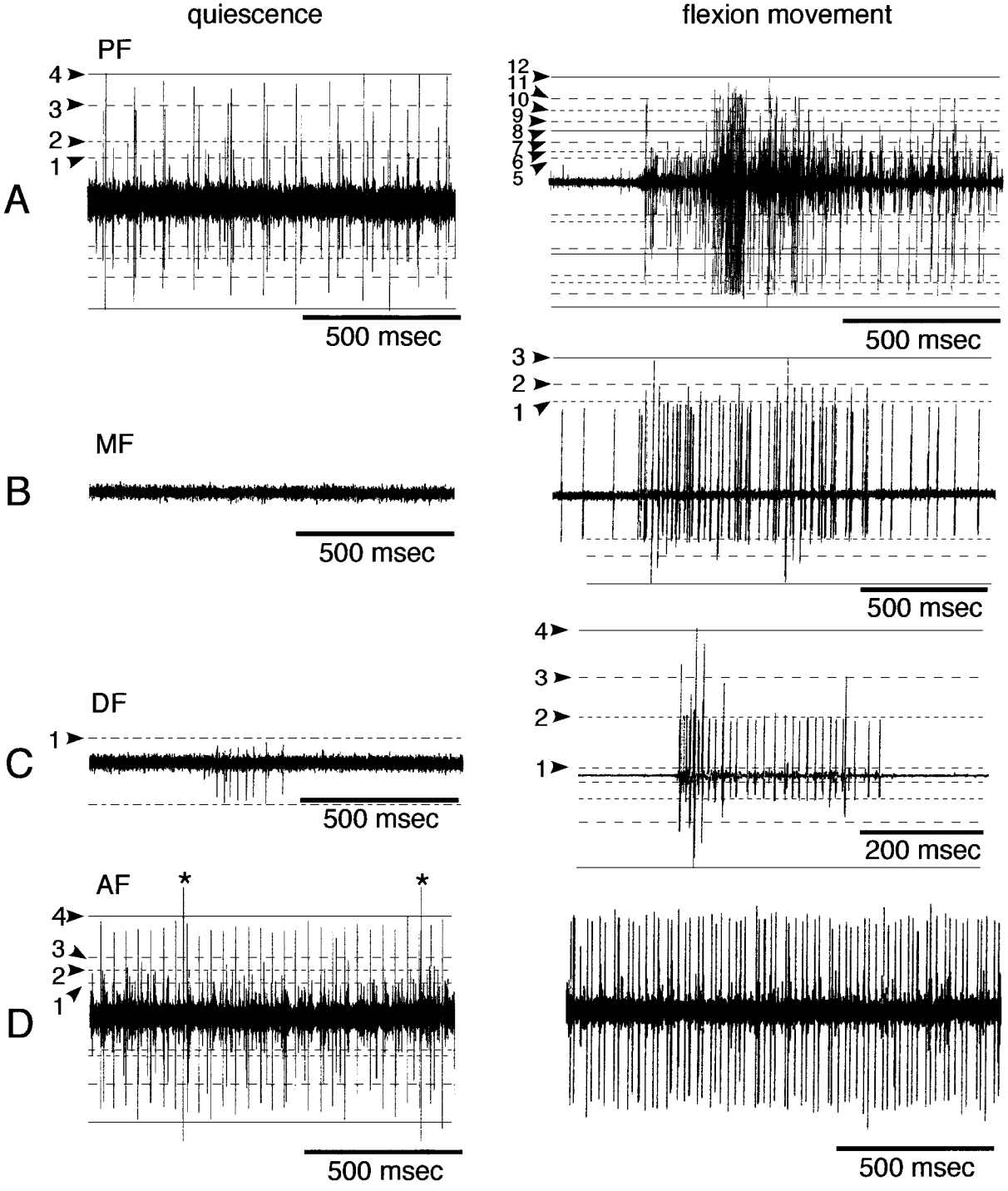

Fig. 6. Extracellular recordings from single flexor nerve branches during quiescence and flexion movement. Different motorneuronal units discriminated based on spike height and spike wave form are indicated as numbers. Asterisks indicate tentative inhibitors characterized by low frequency discharge compared to exciters.

Extracellular recordings from single nerve were in good agreement with above morphological observations. Recordings from PF showed that four units were continuously active during quiescence, while at least eight units are additionally activated when the tibia flexed (Fig. 6A). Neurons supplying MF were completely silent during quiescence but two out of three units exhibited sustained bursts declining progressively and lasting for 0.5-3 seconds when the tibia flexed slowly (Fig. 6B). Neurons supplying DF were inactive during quiescence except when occasional activation of one small unit coincided with ventilatory movements (arrow, Fig. 6C). This small unit also exhibited a slowly adapting discharge during flexion movements. The remaining three units were very large (note large signal and noise ratio in Fig. 6C) and showed a brief burst lasting for 60-300 msec only when the tibia moved rapidly (Fig. 6C). Nerve recordings from AF revealed four small, tonically active units (largest one occasionally stopped when the tibia was maintained in an extended position) and two large units that were active sporadically at 0.5-1Hz (Fig. 6D). This agreed well with the firing characteristics of slow exciters and inhibitors in locusts, respectively (Burrows, 1973; Burrows and Horridge, 1974; Hale and Burrows, 1985). When the tibia flexed, all the exciters discharged at high frequency (Fig. 6D).

DISCUSSION

So far, simultaneous recordings from motor neurons and isolated muscle fibers have been used to search for junction potentials that match the evoked motor spikes and indicate neuron types and numbers supplying single muscle fibers (Theophilidis and Burns, 1983; Sasaki and Burrows, 1998). In this study, two kinds of back-fills and electrophysiological recordings from respective motor nerves tentatively revealed all neurons sending axons to the flexor and extensor tibiae muscles. However, it will be difficult to provide detailed innervation patterns (e.g. motor innervation pattern on single muscle fiber) because all motor neurons running in a main nerve do not necessarily send axons to the subbranches, as exemplified by an innervation pattern made by DF (Fig. 3D). Nevertheless, both general orthopteran and cricket-specific features were detectable in the extensor and flexor tibiae muscles. Furthermore, insertion sites and insertion angles of the muscle compartments were also provided (Fig. 1D), allowing inference about how each compartment is served for tibial flexion in cricket-specific behaviors.

Motoneuronal innervation of the extensor tibiae muscle is similar in crickets and other orthopteran insects (Fig. 3). AE and PE contain axons of different sets of efferent neurons, the former of which has one FETi and two DUM neurons while the latter has one FETi, one SETi and one CI neuron. This matches roughly the morphological features of the locust extensor tibiae muscle in which DUMETi axon accompanies FETi while CI always innervates the muscle fibers innervated by the slow type motor neuron (Titmus, 1981). However, it is also plausible that each nerve branch contains additional axons of small diameters not revealed by backfilling.

The basic structure of the flexor tibiae muscle seems to be similar among orthopteran insects: the main body of the muscle is divided into the proximal, middle and distal regions (Fig. 1; Theophilidis and Burns, 1983). This anatomical feature seems to represent its embryonic development in which the flexor tibiae muscle is derived from three muscle pioneers (Ball and Goodman, 1985). However, the number of nerves supplying the flexor muscle in the cricket was much smaller than that of the locusts (Theophilidis and Burns, 1983; Sasaki and Burrows, 1998) or katydids (Theophilidis and Dimitriadis, 1990), suggesting simpler motoneuronal innervation in crickets. Indeed, the three muscle compartments forming the main flexor had simple innervation by almost non-overlapping sets of motor neurons except for one motor neuron innervating both middle and distal regions (Fig. 4). Presumably these compartments are controlled independently, since intracellular studies in locusts indicate no evidence for either direct or indirect cross excitation or inhibition between any of the different flexor neuron sets (although they appear to be driven by many common sources of synaptic inputs) (Hoyle and Burrows, 1973). The proximal region innervated by PF has a large number of motor neurons that consist of mainly intermediate- and slow-type exciters, in addition to one or two fast-type candidates and two inhibitors (Figs. 3A, B, 5, 6A). Morphological and physiological data collectively suggest that the middle region innervated by MF has one fast-type and two intermediate-type neurons and the distal region innervated by DF has three fast- and one intermediate-type neurons (Figs. 3C, D, 6B, C).

Thus, the main flexor muscle exhibits a progressively slow to fast innervation trend distally, which has never been reported in other orthopterans. This is also confirmed by the morphological observation that the PF, MF and DF exhibit progressively simpler terminal morphologies distally (Fig. 2). Titmus (1981) suggested that terminals of fast-type motor neurons have simply elongated, tubular form decorated with small, rounded varicosities while those of slow type neurons have more complex profiles.

There are certain similarities in motoneuronal innervation of the main flexor tibiae muscle between crickets and locusts. First, the proximal region of the muscle is innervated by the largest number of motor neurons and the distal region by the fewest. Second, innervation from the intermediate-type neurons are restricted to the proximal region of the main muscle (Sasaki and Burrows, 1998). Third, the three fast-type neurons collectively innervate the whole muscle (Sasaki and Burrows, 1998). However, there is no evidence that slow type motor neurons innervate the middle or distal regions of the main muscle in the cricket (Figs. 3–6) although two out of three slow-type neurons innervate almost exclusively the distal half of the main muscle in the locust (Sasaki and Burrows, 1998).

Instead, the slow nature is concentrated to the most dis-

tally located muscle bundles, that is, accessory flexor muscle which is particularly developed in the cricket (Fig. 1B). Back-fills and physiological recordings from AF suggested that the cricket accessory flexor muscle is innervated by at least four exciters (possibly three slow- and one intermediate type neurons) and two inhibitors. The two out of four exciters and two inhibitors also innervated the most proximal part of the main muscle (Fig. 4F). This is in sharp contrast to the locust accessory flexor muscle that has only one slow-type out of nine excitatory motor neurons (Sasaki and Burrows, 1998). Complex motor innervation of the accessory flexor muscle was also reported in the tettigoniid, *Decticus albifrons*, where a cross section of the AF revealed thirteen small axons (Theophilidis and Dimitriadis, 1990). Although accurate determination of the total number of motor neurons supplying the cricket flexor tibiae muscle is difficult because numbers of stained neurons supplying PF were somewhat different in the two back-fill procedures using heavy metals and dextrans, the range is estimated to be 19 to 21, the largest so far reported in orthopteran insects.

Then a question arises why crickets are equipped with such a high number of excitatory neurons, especially enriched with intermediate- and slow-types. Is this relevant to cricket-specific behaviors? One reason would come from the relative importance of the flexor tibiae muscle compared to the extensor tibiae muscle for static posture control. The resting cricket rarely maintains the metathoracic tibia beyond a femoro-tibial joint angle of 90° (Nishino, unpublished observation). Indeed, myographic recordings showed that the SETi activity is completely silent during normal standstill state on the horizontal substrate except occasional excitations with ventilatory movements (Nishino *et al*., 1999), whereas SETi is continuously active in the resting locust (Burns and Usherwood, 1979; Hoyle, 1980). Thus, the static posture to resist gravity in the cricket must be primarily controlled by the combined activities of slow-type exciters and common inhibitors in the flexor muscle. The high numbers of intermediate-type and slow-type exciters must be served for fine motor control of the static posture.

The second reason may be explained by the dynamic motor control unique to the cricket. The proximal region of the muscle inserting in line onto the central apodeme must be responsible for initial tibial flexion in any movement because this compartment has maximum effective leverage in more extended positions of the tibia (a femoro-tibial joint angle of ca. 40°) than other muscle compartments (Hustert and Gnatzy, 1995). The homologous region of the tettigoniid possess a fatigue-resistant property (Theophilidis and Dimitriadis, 1990), suggesting that the proximal region is the most frequently used part for static posture control as well as movement initiation. The middle and distal regions of the main muscle, innervated by non-overlapping sets of motor neurons must be recruited progressively only when rapid, powerful tibial flexion is required because fast-type exciters are active only in rapid movements (Fig. 6C; Field and Burrows, 1982; Nishino *et al*., 1999). As these regions insert onto the cushion at slightly larger angles than the proximal region, they effectively work when the tibia is in a flexed position (Hustert and Gnatzy, 1995). Thus, ongoing tibial flexion movement is accelerated by synergistic action of the effective leverage and recruitment of fast-type exciters. The progressive recruitment of the high number of exciters must explain the agility and flexibility in the cricket locomotion: the cricket runs faster than the locust and change the running speed more smoothly (Gras and Hörner, 1992).

Finally the accessory flexor muscle acts to stabilize the tibia at an almost fully-flexed position in the pre-kick period that precedes the actual jump or kick (Hustert and Gnatzy, 1995). As the proximal part of the muscle and the accessory flexor muscle are innervated by largely overlapping sets of motor neurons, one might speculate that simultaneous contraction of two segregated muscle compartments having insertions at different angles may disturb the smooth slide of the flexor apodeme. However, this problem may be solved because the accessory flexor, compared to the proximal flexor bundles, is innervated mainly by small motor neurons that must have lower conduction velocities of action potentials and also cause slower contraction in the flexor muscle. This co-contraction may be functional with the outer specialization of the cuticle ridge unique to crickets (Hustert and Gnatzy, 1995). At a femoro-tibial joint angle of 0°, the isometric contractions of the proximal region of the main muscle and the accessory flexor muscle pull the cushion proximally and dorso-proximally, thus enabling it to fit into the ridge at the distal edge (see Fig. 1D) and store catapult-energy for a subsequent kick or jump.

This restricted innervation pattern in the cricket flexor tibiae muscle provides a clear advantage for future study of actual roles of muscle compartments in various behavioral contexts, by recording neural activities from each nerve in the unrestrained condition.

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