Actions of Motor Neurons and Leg Muscles in Jumping by Planthopper Insects (Hemiptera, Issidae)

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ABSTRACT

To understand the catapult mechanism that propels jumping in a planthopper insect, the innervation and action of key muscles were analyzed. The large trochanteral depressor muscle, M133b,c, is innervated by two motor neurons and by two dorsal unpaired median (DUM) neurons, all with axons in N3C. A smaller depressor muscle, M133a, is innervated by two neurons, one with a large-diameter cell body, a large, blind-ending dendrite, and a giant ovoid, axon measuring 50 μm by 30 μm in nerve N5A. The trochanteral levator muscles (M132) and (M131) are innervated by N4 and N3B, respectively. The actions of these muscles in a restrained jump were divisible into a three-phase pattern. First, both hind legs were moved into a cocked position by high-frequency bursts of spikes in the levator muscles lasting about 0.5 seconds. Second, and once both legs were cocked, M133b,c received a long continuous sequence of motor spikes, but the two levators spiked only sporadically. The spikes in the two motor neurons to M133b,c on one side were closely coupled to each other and to the spikes on the other side. If one hind leg was cocked then the spikes only occurred in motor neurons to that side. The final phase was the jump movement itself, which occurred when the depressor spikes ceased and which lasted 1 ms. Muscles 133b,c activated synchronously on both sides, are responsible for generating the power, and M133a and its giant neuron may play a role in triggering the release of a jump. J. Comp. Neurol. 518: 1349–1369, 2010.

INDEXING TERMS: Indexing terms: Auchenorrhyncha; Fulgoromorpha; motor patterns; giant neurons

Jumping is an extreme form of locomotion that can move an animal rapidly away from a dangerous predator, increase the speed of locomotion, or launch an animal into flight. It requires large forces to be generated by the muscles and concomitant specializations of the neural, muscular, and skeletal machinery. Moreover, in insects with short legs the acceleration must be applied in such a short time that it may be beyond the capability of striated muscles. Different strategies must then be adopted to generate the forces slowly while storing energy, and then release the stored energy rapidly in a catapult-like action. In the champion insect jumpers, the froghoppers (Hemiptera, Cercopidae) the rapid movements of the hind legs accelerate the body at 5,400 m s^{-2}, to a takeoff velocity of 4.7 m s^{-1}, so that the insect experiences a force equivalent to 550 times gravity (Burrows, 2003, 2006a). This jumping performance requires an energy output of 136 μJ and a power output of 155 mW. Planthopper insects (Hemiptera, Issidae), which are the subject of this article, match, and in some aspects exceed this performance (Burrows, 2009). In its best jumps, a male *Issus* accelerates in 0.8 ms to a takeoff velocity of 5.5 m $s^{-1}$and is subjected to a force of 719*g* (225 mN), or more than 700 times its body weight. This requires an energy output of 303 μJ, and a power output of 388 mW, a performance that could only be achieved if a jump were propelled by a catapult mechanism.

Other insects that jump must also solve similar problems because their legs generally do not provide sufficient leverage over the short times that the body must be accelerated. In fleas, the jumping mechanisms have been inferred from the kinematics of the movement and the anatomical arrangements of the muscles, but storage of energy in pads of the elastic protein resilin before the jump and its sudden release are implicated (Bennet-Clark and Lucey, 1967; Rothschild and Schlein, 1975; Rothschild et al., 1975). Larger insects such as locusts (Godden, 1975; Heitler and Burrows, 1977; Burrows, 1995) also use a cat-

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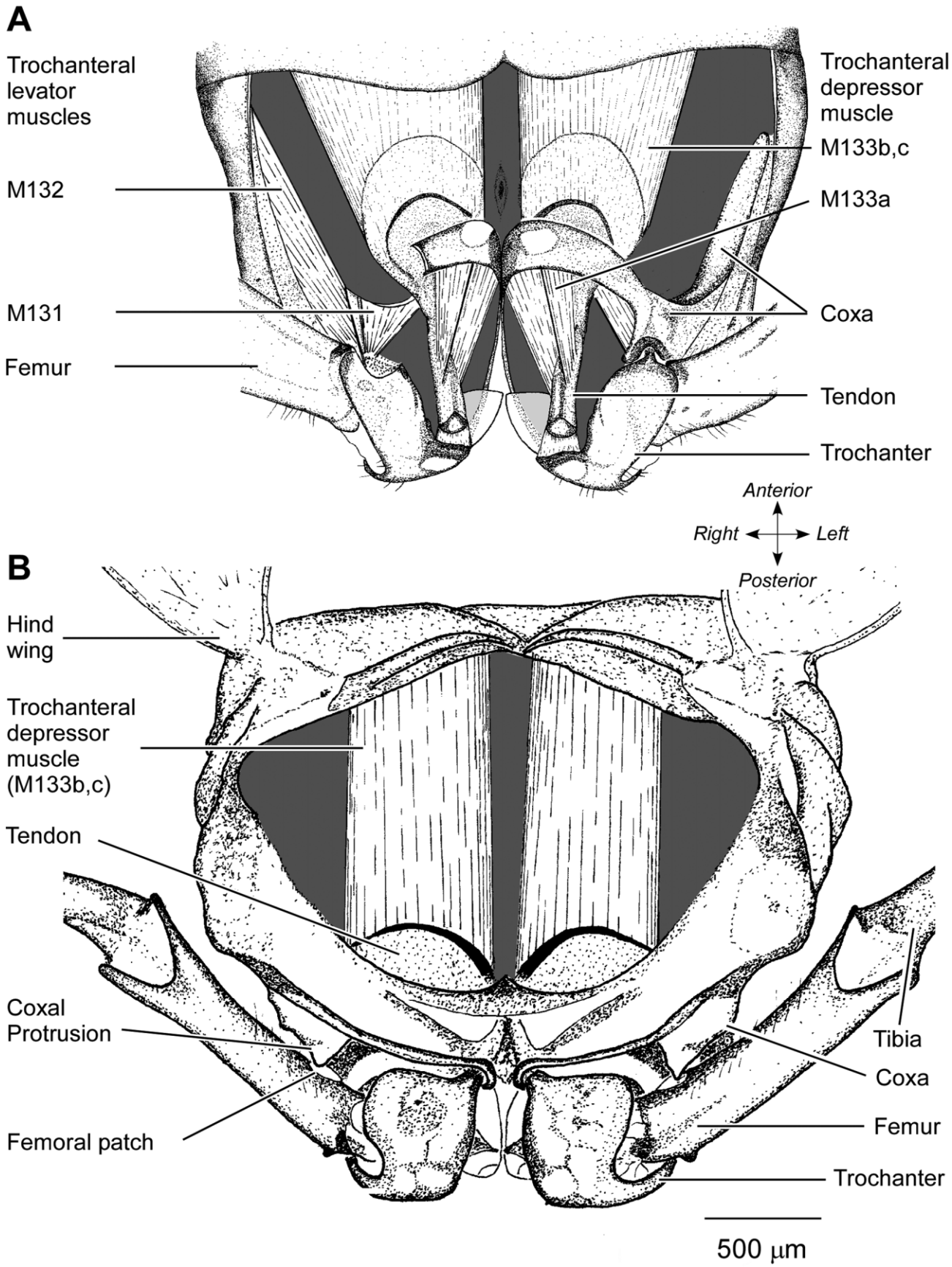
A
Trochanteral levator muscles
M132
M131
Femur
Trochanteral depressor muscle
M133b,c
M133a
Coxa
Tendon
Trochanter
Anterior
Right
Left
Posterior
B
Hind wing
Trochanteral depressor muscle (M133b,c)
Tendon
Coxal Protrusion
Femoral patch
Tibia
Coxa
Femur
Trochanter
500 μm

Figure 1

apult mechanism when propelling their jumps by rapid extension of their hind tibiae. About half of the required energy is stored in distortions of the femoral cuticle and the rest in distortions of semi-lunar processes at the femorotibial joints (Bennet-Clark, 1975; Burrows and Morris, 2001). Bush crickets (Burrows and Morris, 2003) and crickets (Hustert and Gnatzy, 1995) do not precede a jump with a long co-contraction of the muscles, because the leverage of the legs is sufficient for a jump to be generated with a direct muscle contraction.

The overall picture to emerge, therefore, is that powerful and rapid jumping requires a slow contraction of the main power-producing muscles, restrained by the action of other muscles, often aided by mechanical skeletal specializations. Similar mechanisms for generating rapid and powerful movements are found in the prey capture strike of mantis shrimps (Crustacea, Stomatopoda) (Burrows, 1969; Patek et al., 2004; Patek and Caldwell, 2005) and in rapid jaw movements of ants (Gronenberg et al., 1993; Gronenberg, 1995).

What are the properties of the neurons and the motor patterns in which they participate that generate jumping? Froghoppers (Hemiptera, Cercopidae) and leafhoppers (Hemiptera, Cicadellidae) contract their jumping muscles well in advance of a jump to generate sufficient force by using a three-phase motor pattern (Burrows, 2007a,c). First, the hind legs are fully levated so that in froghoppers a mechanical locking device on each hind leg is engaged. Second, both trochanteral depressor muscles contract without causing the hind legs to depress. In froghoppers this period can last several seconds with the spikes in the left and right depressor muscles tightly synchronized, but in leafhoppers the period is much briefer and the spikes on the two sides are independent. In froghoppers, the energy developed by the prolonged contractions is stored in the pleural arches of the internal skeleton, which are made of a composite of elastic resilin and much stiffer cuticle (Burrows et al., 2008). The storage mechanisms in leafhoppers are not known. Finally, the rapid and synchronous depression movements that propel the jump last less than a millisecond in froghoppers and a few milliseconds in leaf hoppers. In these insects the power is generated by trochanteral depressor muscles in the thorax so that the hind legs are light and can therefore be accelerated rapidly. In locusts, a three-phase motor pattern also generates a jump, but now involves a co-contraction between the flexor and extensor tibiae muscles (Burrows, 1995; Heitler and Burrows, 1977) and mechanical arrangements at the femoro-tibial joint (Heitler, 1974) that allow the small flexor to restrain the much large extensor so that energy can be stored.

Planthoppers differ substantially from other jumping insects, even their relatives the froghoppers and leafhoppers, in the arrangement of their jumping muscles and the associated joints of the hind legs. To reveal the neural mechanisms that underlie their jumping performance, this study determined the innervation pattern of the trochanteral depressor and levator muscles that control jumping and the anatomy of their motor neurons in the central nervous system. The large power-producing trochanteral depressor muscles are each innervated by two motor neurons with a conventional morphology. Recordings from these muscles show that they contract for extended periods in advance of a restrained jump and receive motor spikes synchronously on both sides. One of the neurons innervating the small coxal part of the depressor muscle has a giant axon and an unusual morphology. Its possible role in jumping is discussed.

Figure 1. Drawings of the depressor and levator muscles of the hind trochantera of *Issus*. **A:** Ventral view following removal of the cuticle from the metathorax and hind coxae, and part of the ventral wall of the right coxa and trochanter. Each trochanteral depressor muscle consists of two parts: the largest part (M133b/c) is contained entirely within the metathorax and the smaller part (M133a) is in the coxa. Levator muscle M132 arises from a lateral projection of the coxa and the smaller M131 from the lateral coxal wall. **B:** Posterior view with the abdomen removed to show the large metathoracic parts of the trochanteral depressor muscles.

MATERIALS AND METHODS

Adult planthopper insects, *Issus coleoptratus* (Fabricius, 1781), of either sex were collected on their host plants, ivy (*Hedera*), during August and September in Aachen, Germany. *Issus* belongs to the order Hemiptera, suborder Auchenorrhyncha, superfamily Fulgoroidea, and family Issidae.

The morphology of the muscles and the proximal joints of the hind legs were examined in live animals and in those preserved in either 50% glycerol or 70% ethanol. Drawings of the muscles were made with the aid of a drawing tube attached to a Leica MZ16 stereo microscope. Individual color photographs were taken with a Nikon DXM1200 digital camera attached to the same microscope.

To reveal the innervation pattern of the nerves supplying the muscles moving the trochantera of the hind legs, the metathoracic neuromere of the fused thoracic and abdominal ganglion was isolated in Vaseline and stained with 0.5% nickel chloride overnight at 6°C. Nickel staining was developed using rubeanic acid (1 drop of a saturated alcoholic solution added to 1 mL saline) (Sakai and Yamaguchi, 1983).

For light and transmission electron microscopy tissues were fixed in 2.5% glutaraldehyde, 2.0% formaldehyde, and 0.025% $CaCl_2$ in 100 mM cacodylate buffer (pH 7.2). Tissue was postfixed for 1 hour in 1% osmium tetroxide in phosphate-buffered saline (PBS), washed in distilled water

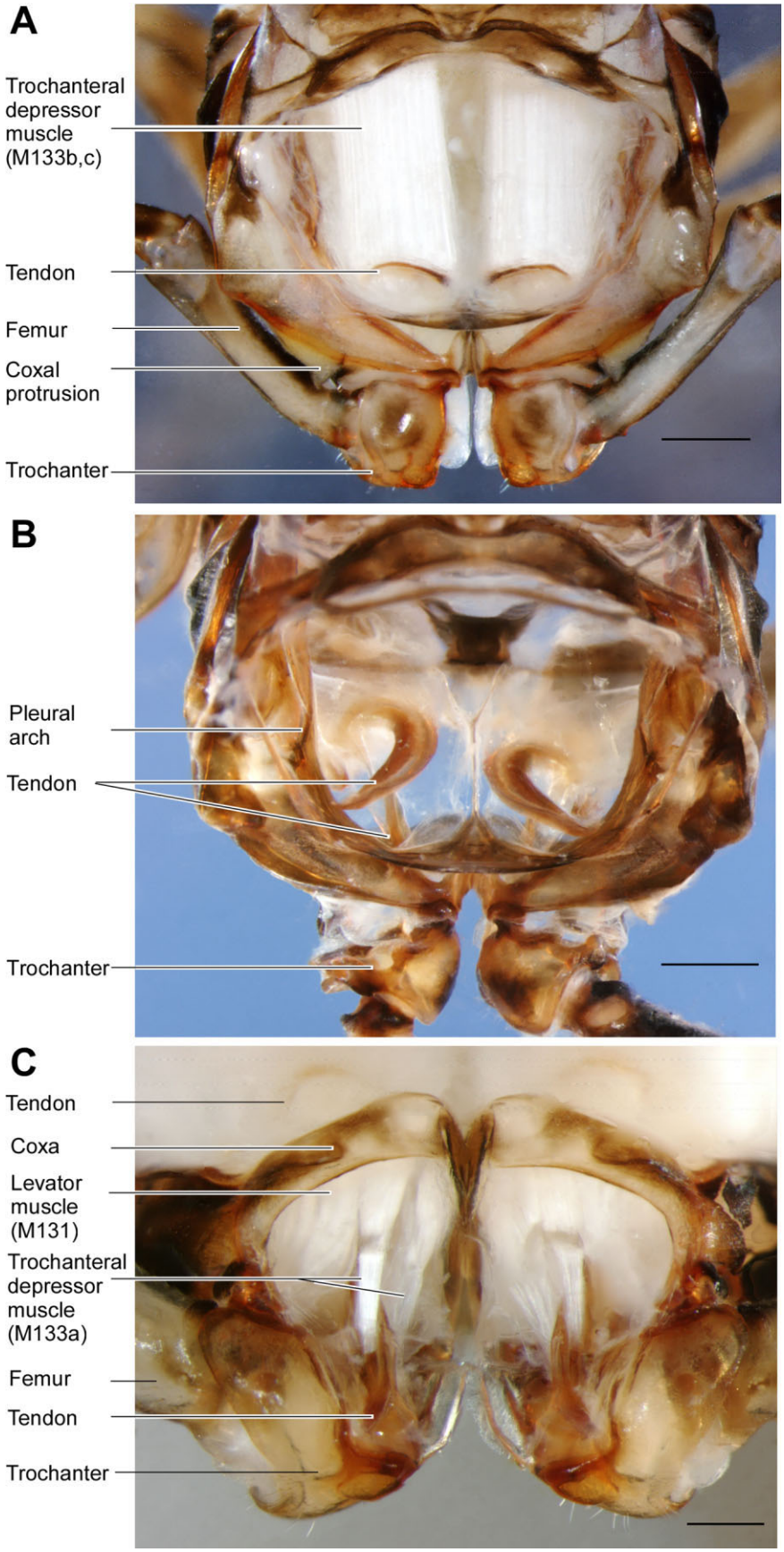
A
Trochanteral depressor muscle (M133b,c)
Tendon
Femur
Coxal protrusion
Trochanter
B
Pleural arch
Tendon
Trochanter
C
Tendon
Coxa
Levator muscle (M131)
Trochanteral depressor muscle (M133a)
Femur
Tendon
Trochanter

Figure 2

(3 times for 15 minutes), and stained for 1 hour with 2% uranyl acetate in ethanol. The tissue was then dehydrated in an ascending alcohol series followed by two 30-minute periods in propylene oxide and a 16-hour period in a mixture of propylene oxide and epoxy resin (Epon, Serva, Heidelberg, Germany). After washing in Epon (two times for 2 hours) the samples were embedded and polymerized for 48 hours at 57°C. Semithin sections (1 μm) of the nerves for light microscopy and ultrathin sections were cut on a Reichert OmU3 ultramicrotome and examined in a Zeiss EM10C microscope (Zeiss, Germany). Semithin sections were counterstained in 0.5% methylene blue, 0.5% azur II, and 1% borax in distilled water.

Neurons supplying the trochanteral depressor muscles of the hind legs were stained by backfilling lateral nerves N3A/C, N5A, N3B, and the branches of N4 that innervate levator muscle 132. These nerves were exposed in the thorax and the stump of a particular nerve, cut close to its muscles, and isolated in a Vaseline well that was filled with distilled water for 3–5 minutes. The water was then replaced with a 5% solution (w/v in distilled water) of neurobiotin (Vector Laboratories, Burlingame, CA). The insect was maintained for 2–4 hours at room temperature, or overnight at 6°C. The fused ganglion was then dissected and fixed for 1–2 hours in 4% formaldehyde in distilled water at room temperature, or overnight at 6°C. The fixative was then washed out for 2 hours in PBS containing 0.25% Triton X-100 (PBS-Tx) and a ganglion was incubated in streptavidin tagged with CY3 (Jackson ImmunoResearch, West Grove, PA) dissolved in PBS-Tx at 1:2,000 for 48 hours. Finally, a ganglion was washed briefly in PBS-Tx, dehydrated in an ascending series of isopropanol, mounted, and cleared in methyl salicylate. The results described are based on 12 successful stains of N5A, 9 of N3A/C, 3 of N4, and 3 of N3B.

Digital images of stained neurons in whole ganglia were obtained with a Leica TCS SP2 confocal laser-scanning microscope using a 20× multi-immersion objective with an aperture of 0.7 and the green line (543 nm) of the He/Ne laser. Leica Confocal Software was used to merge stacks of digitized images and to make 3D reconstructions and stereo images.

Figure 2. Trochanteral depressor muscles and tendons. **A:** The thoracic parts (M133b/c) of the left and right trochanteral depressor muscles viewed posteriorly after removal of the overlying flexible cuticle. Both hind legs are in the fully levated (cocked) position with the coxal protrusions engaged with the dorsal femora. **B:** Posterior view of the metathorax to show the tendons of the trochanteral depressors after removal of the muscle fibers by boiling in 5% potassium hydroxide. **C:** Ventral view of the hind coxae to show the coxal part (M133a) of the trochanteral depressor muscles inserting on the main tendons before they in turn insert on the trochantera, and the small levator muscle (M131). Scale bars = 500 μm A,B; 200 μm in C.

We used the same nomenclature for the nerves and muscles (Snodgrass, 1929, 1935; Campbell, 1961) as those used for a locust (Bräunig, 1982) and froghopper hind leg (Bräunig and Burrows, 2008). This nomenclature allows easy comparison between insects, without inferring homologies. For example, in the locust N3A and N3C describe nerves that emerge separately from a thoracic ganglion, but in *Issus* they emerge together as N3A/C, separate further distally, but innervate comparable structures.

To record the electrical activity of muscles generating jumping, an *Issus* was restrained on its back in Plasticene with the hind legs free to move. All recordings are thus from restrained or fictive jumps. Rapid and coordinated movements of the two hind legs occurred spontaneously or could be induced by the same mechanical stimuli used to promote unrestrained jumping. As in froghoppers (Burrows, 2006a) and leafhoppers (Burrows, 2007b), the sequence of movements of the hind legs was the same as that observed in unrestrained jumping. Pairs of 20 μm or 30 μm steel wires, insulated but for their tips, were inserted through small holes in the sternal plates or soft membrane of the metathorax or the hard anterior, ventral cuticle of the coxa and into the trochanteral depressor and levator muscles of the hind legs. The positions of the electrodes could either be seen through the flexible cuticle or their positions were confirmed by dissection at the end of the recording session. Movements of a hind leg were recorded at the same time as the electrical activity of the muscles by capturing images at rates of 250 s^{-1} with a Photron Fastcam 1024 PCI camera (Photron, Marlow, Bucks, UK) attached to a stereo microscope and viewing the insect ventrally. The captured images were stored on one computer. The electrical signals from the muscles were amplified with a bandwidth of 50 Hz to 5 kHz and then digitized at a sampling rate of 16.7 kHz with a CED (Cambridge Electronic Design, Cambridge, UK) interface running Spike 2 v. 5 or v. 7 software. These data were then written directly to a second computer. The data files of images and muscle recordings were synchronized on the two computers to a resolution of 4 ms, by feeding a 0.5 ms long electrical pulse to a separate channel of the CED interface and that simultaneously triggered a light flash of a miniature LED in the visual field of the camera. Eighty-four restrained jumps by 9 *Issus* were analyzed in which both muscle recordings and images of the movements were captured. A further 169 restrained jumps by 15 different *Issus* were also analyzed in which the timing of the jump was indicated by a brief movement artifact in the recordings. Intracellular recordings were made from pairs of fibers in muscles M133b/c of both sides, and from individual fibers of M131 with glass microelectrodes filled with 2M potassium acetate and with resistances of 30–40

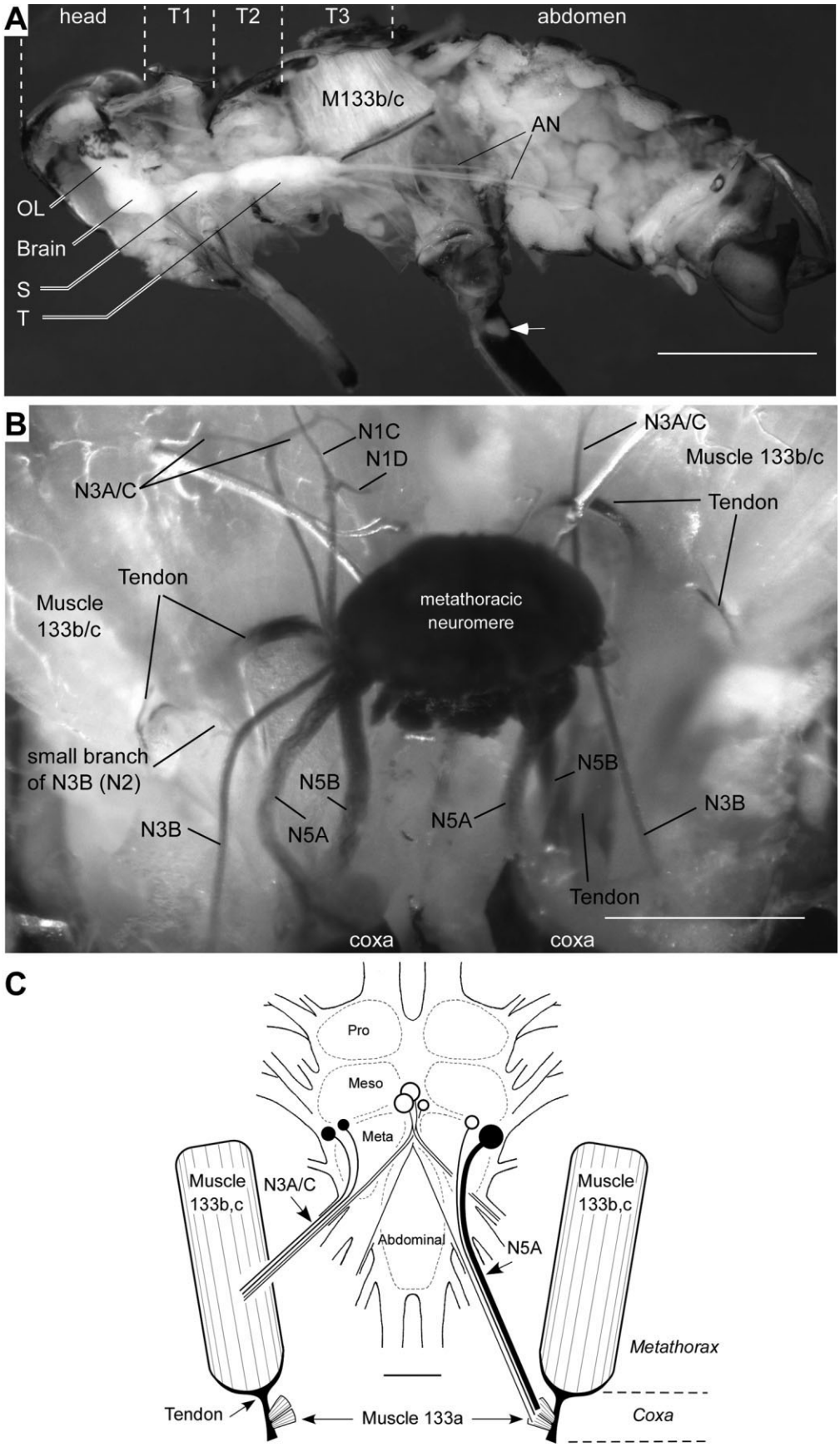
A
head
T1
T2
T3
abdomen
M133b/c
AN
OL
Brain
S
T
B
N1C
N1D
N3A/C
N3A/C
Muscle 133b/c
Tendon
Tendon
Muscle
133b/c
metathoracic
neuromere
small branch
of N3B (N2)
N5B
N5B
N5A
N5A
N3B
N3B
Tendon
coxa
coxa
C
Pro
Meso
Meta
Muscle
133b,c
N3A/C
Abdominal
N5A
Muscle
133b,c
Metathorax
Tendon
Muscle 133a
Coxa

Figure 3

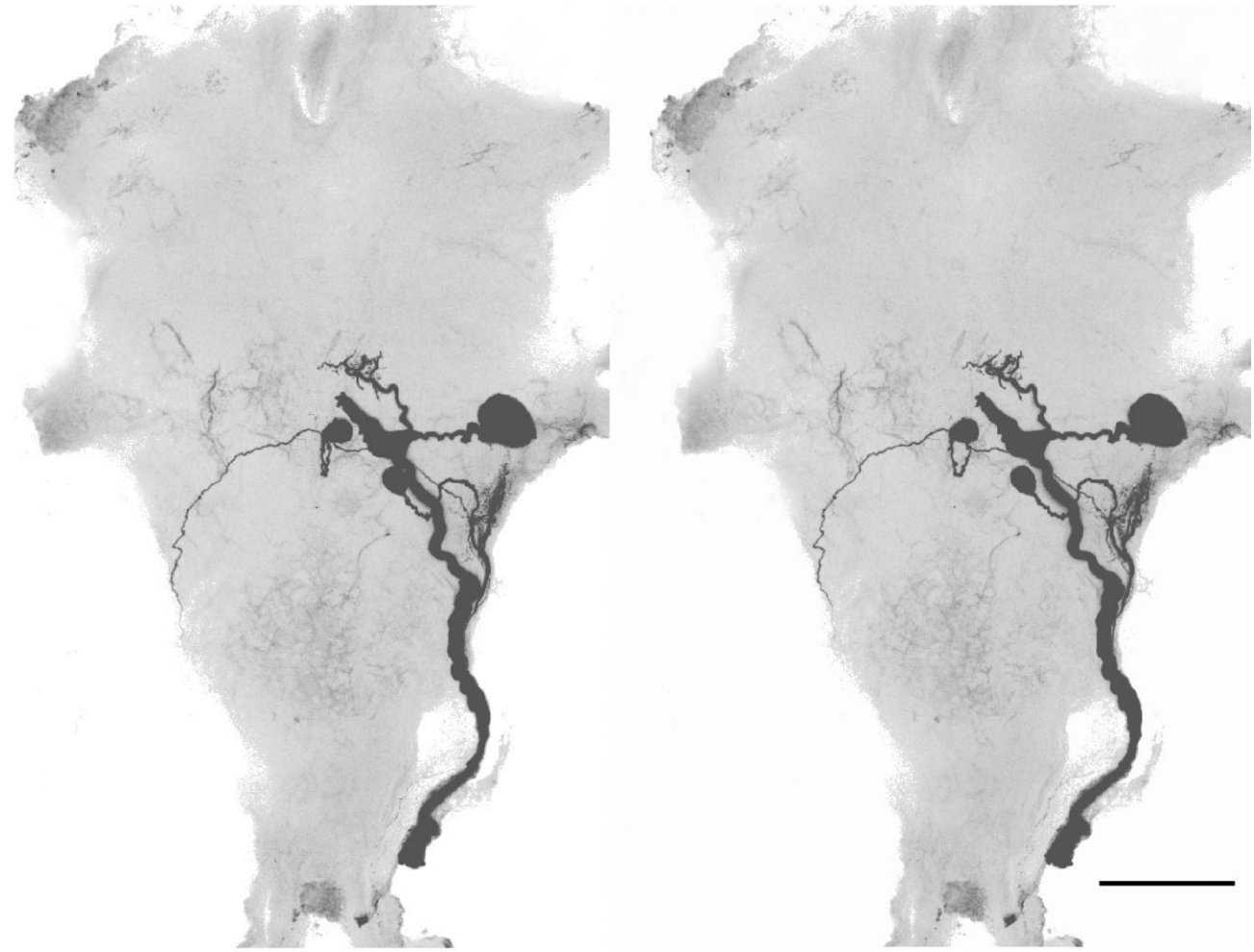

Figure 4. A stereo pair of images of the neurons supplying the coxal part of a hind trochanteral depressor muscle (M133a), stained with neurobiotin introduced through N5A. The stereo image shows the fused thoracic ganglion viewed dorsally. A neuron with a large, ventral, and lateral cell body has two anteriorly pointing branches, one with fine side branches and the second of large diameter and ending blindly. This neuron also has a giant axon. A second neuron with a dorsal and more medial cell body, and a DUM neuron with an axon in both left and right N5A were also stained. Scale bar = 100 μm.

Figure 3. Nerves of the metathoracic neuromere of the fused ganglion in the thorax that innervate trochanteral depressor muscles of the hind legs involved in jumping. This ganglion contains the neuromeres of all thoracic and abdominal segments. **A:** Photograph of the whole central nervous system as seen in a longitudinal section of the body close to the midline. AN, nerves to the abdomen; OL, optic lobes of the brain; S, subesophageal ganglion; T, fused thoracic and abdominal ganglion; T1-3, thoracic segments. The white arrow indicates a small, white patch of cuticle on the dorsal hind femur that engages with a coxal protrusion when the hind leg is fully levated. **B:** Nerves of the metathoracic neuromere, viewed ventrally, after staining with 1% nickel chloride and rubeanic acid. M133b/c and its large tendon are visible on either side of the metathorax with the overlying branches of nerves 3 and 5. **C:** Drawing of the fused ganglion, viewed ventrally, to show the position of the cell bodies and paths of the axons of the motor neurons that innervate a trochanteral depressor muscle. The motor neurons innervating the large M133b/c are shown on the left and those innervating the small M133a on the right. Dorsally located cell bodies are shown as open circles and lateral and ventrally located ones by filled circles. Scale bars = 1 mm in A; 500 μm in B; 100 μm scale bar in C refers only to the ganglion.

Mohm. All recordings were made at room temperatures of 20–25°C.

Canvas (ACD Systems of America, Miami, FL) was used to assemble all the figures, to adjust brightness and contrast in photographs, and to make line drawings.

RESULTS

The rapid movements of the hind legs that propel *Issus* in a jump are powered by large, bilaterally symmetrical trochanteral depressor muscles of the metathoracic segment (Figs. 1, 2). The corresponding muscles of the front and middle legs are much smaller.

Morphology of trochanteral muscles and tendons

The trochanteral depressor muscle of a hind leg consists of two parts that both insert on the same large, complex

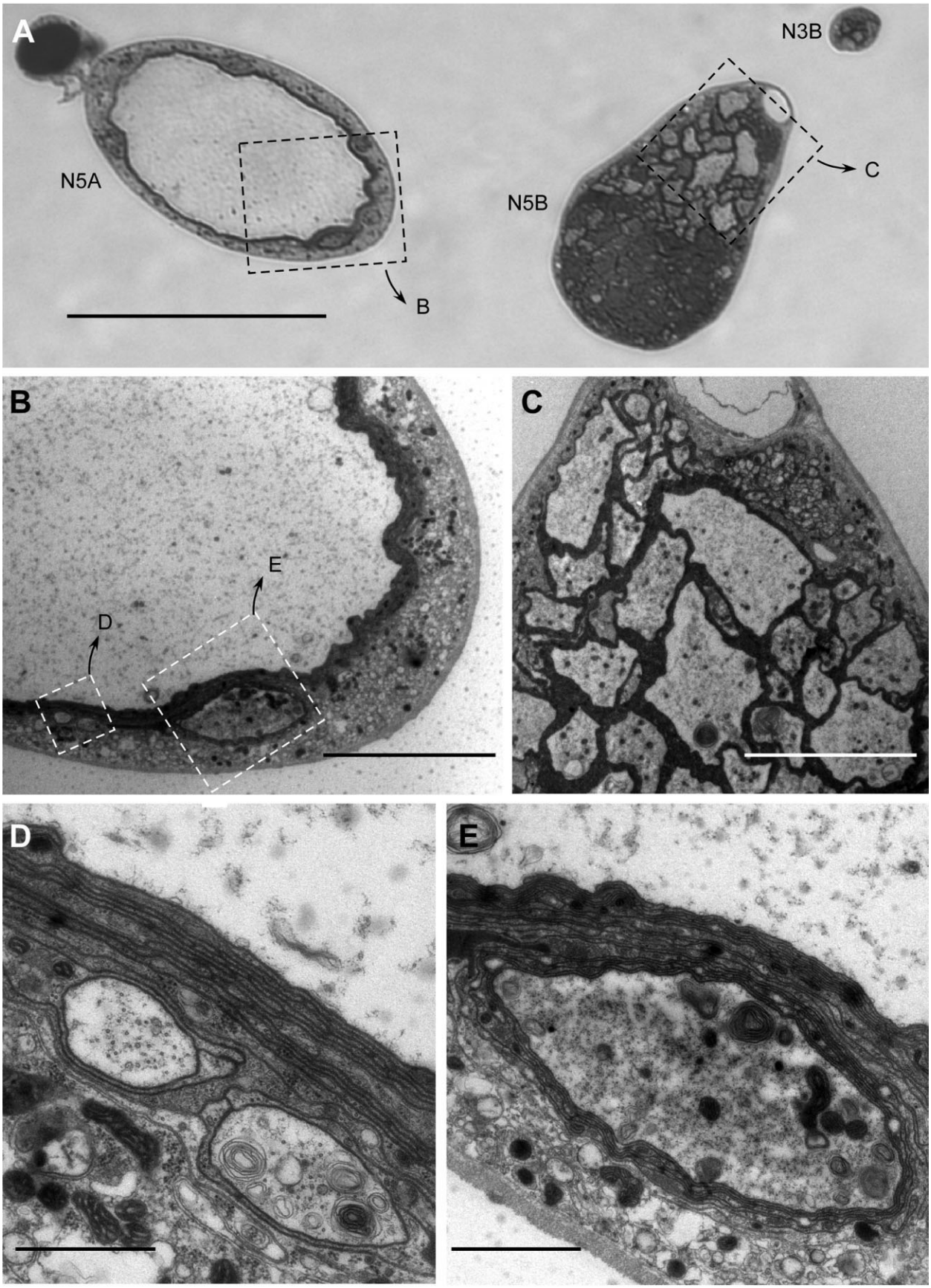

Figure 5

tendon (Figs. 1, 2). The first and largest part of the muscle (M133b/c) occupies most of the space in one-half of the metathorax, extending from close to the midline laterally, and from the ventral to the dorsal surface. It consists of many parallel fibers that arise from the cuticular partition between the meta-and mesothoracic terga and insert on an umbrella-shaped part of the tendon within the thorax (Figs. 1A,B, 2A).

The second part of the muscle (M133a) is much smaller and is contained entirely within a coxa (Figs. 1A, 2C). Its fibers are arranged in two bundles: one arises from the anterior ventral wall of a coxa close to the midline and the other from the medial wall of the coxa. Both insert around the cylindrically shaped region of the tendon within the coxa.

In the froghopper *Philaenus* there is also a third, small thoracic part of the muscle (M133d). In *Issus*, this muscle is represented only by a small ligament that connects from the sternal apodeme to the depressor tendon.

The tendon of a trochanteral depressor muscle extends from the metathorax through a coxa to insert on the ventral and medial wall of a trochanter (Figs. 1A, 2A–C). The large umbrella-shaped area of the tendon is sclerotized and stiff, and part is transparent but also stiff (Fig. 2B). The tendon then tapers into a cylindrically shaped, sclerotized region that enters the ipsilateral coxa, receives the fibers from muscle 133a, and then inserts on the trochanter. As the coxo-trochanteral joint is depressed and levated this stiff tendon therefore moves anteriorly and posteriorly through both a coxa and the metathorax. These movements are visible in a live *Issus* through the transparent membrane that covers the sternal region of the metathorax.

There are two trochanteral levator muscles of a hind leg (Figs. 1A, 2C). The fibers of M132 arise from a lateral, anteriorly, and dorsally directed process of the coxa that is fused with the pleural wall of the thorax, and extend through a coxa to insert laterally on the trochanter. M131 is smaller and is restricted to a coxa, arising at its lateral wall and again inserting laterally on the trochanter (Figs. 1, 2C).

Figure 5. Ultrastructure of the axons innervating trochanteral depressor muscle (M133a). **A:** Semithin sections through metathoracic N5A, N5B and N3B close to the coxa. A single giant axon occupies most of N5A. **B:** An expanded portion of N5A from A (dashed box) viewed with the transmission electron microscope (TEM), to show small-diameter axons at the edge of the giant axon. **C:** TEM section through a part (dashed box in A) of N5B at the same magnification as in B to show the largest axons in this nerve for comparison with the giant axon. **D:** The glial wrapping of the giant axon and the profiles of two small diameter profiles from the region in B indicated by the dashed box. **E:** The glial wrapping of the giant axon and the axon of the other motor neuron innervating M133a. Scale bars = 50 μm in A; 10 μm in B,C; 1 μm in D; 2 μm in E.

Nerves supplying the hind legs

The central nervous system consists of the brain and optic lobes, subesophageal ganglion, and a fused ganglion in the thorax which is a fusion of the pro-, meso-, and metathoracic neuromeres and all the abdominal neuromeres (Fig. 3A). This ganglion lies ventrally between the two trochanteral depressor muscles. The muscles and joints of the hind legs are supplied by nerves of the metathoracic neuromere of which the following lateral nerves could be recognized (Fig. 3B).

Nerve 1 has two branches that could be traced. N1D emerges medially and runs dorsally between left and right M133b/c to supply the dorsal longitudinal muscles of the hind wings. N1C continues anteriorly and laterally over the ventral surface of M133b/c to innervate sense organs at the wing base and in the wing itself.

Nerve 3 has three major branches. Branches 3A and 3C emerge together from the ganglion and run laterally for 500 μm before separating from each other. Branch A continues to run laterally to innervate small dorsoventral muscles in front of the pleural arch (not shown in Fig. 1). The much larger branch C innervates M133b/c (Fig. 3C). Nerve 3B runs posteriorly from the ganglion to enter the coxa. One branch innervates levator muscle M131 and a second continues further distally to fuse with N5B, as in other insects. A small nerve was sometimes observed to run parallel to nerve 3B, but its targets could not be discerned. It might correspond to nerve 2 in locusts and froghoppers.

Nerve 4 emerges from nerve 5B some distance from the ganglion. We named it N4 rather than as a branch of 5B by analogy with locusts and froghoppers because the only difference that we can find is that here nerve 4 does not emerge as a separate nerve from the ganglion, but emerges from nerve 5 further distally. Two branches of this nerve innervate trochanteral levator muscle M132 and another small branch innervates dorsoventral muscles in the thoracic cavity.

Nerve 5 divides into two branches close to its emergence from the ganglion. The more dorsal branch, N5B, enters the coxa and then continues into the more distal segments of a hind leg. The more ventral branch, N5A appeared transparent in a live *Issus* and innervates M133a in the coxa (Fig. 3C).

Neurons innervating the trochanteral depressor muscle

M133a

Backfilling N5A revealed the cell bodies of three neurons supplying the small part of the trochanteral depressor muscle in the coxa (M133a) (Figs. 3C, 4, 6B). The largest cell body with a diameter of 40–45 μm was ventral and

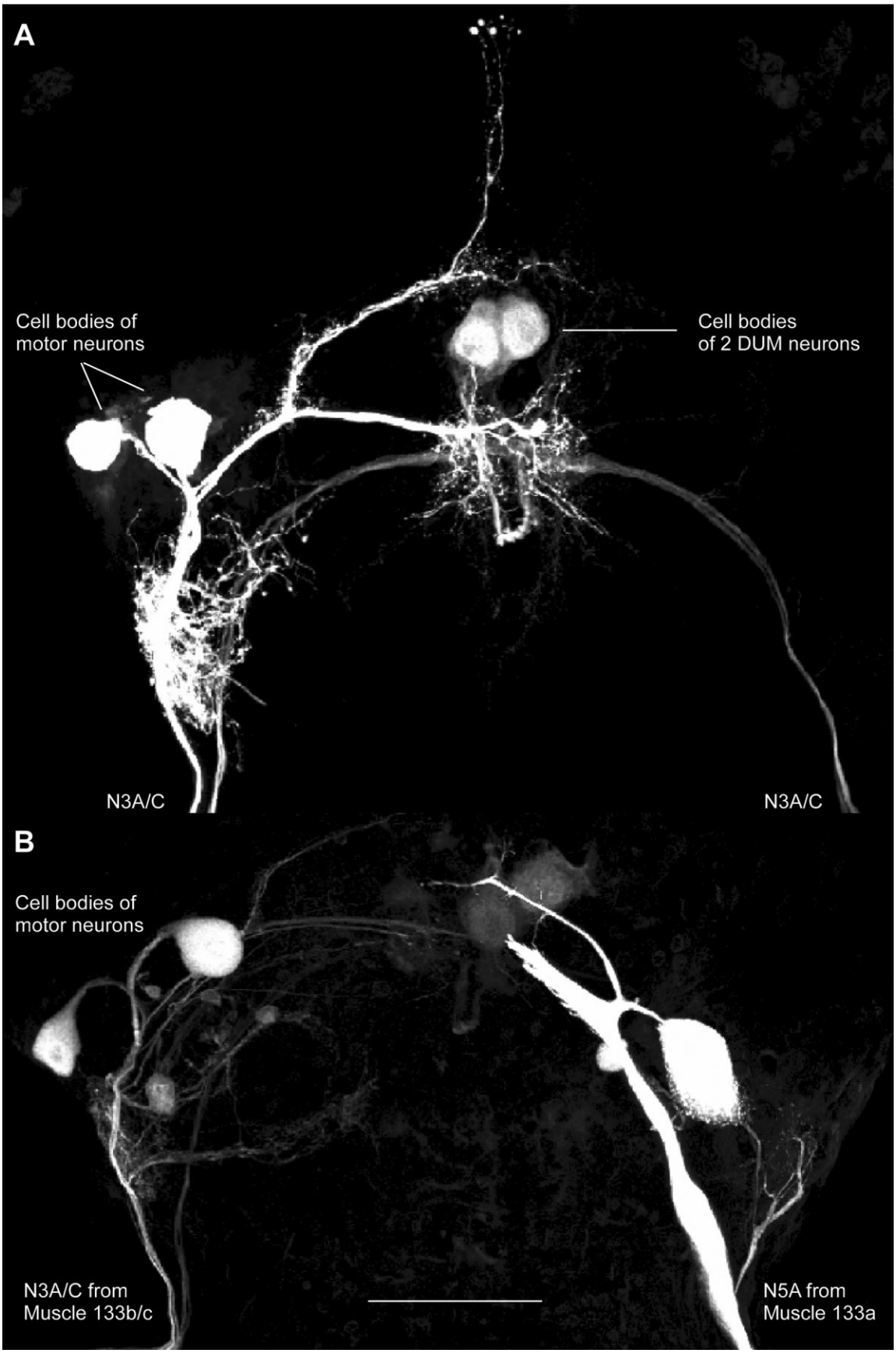
A
Cell bodies of
motor neurons
Cell bodies
of 2 DUM neurons
N3A/C
N3A/C
B
Cell bodies of
motor neurons
N3A/C from
Muscle 133b/c
N5A from
Muscle 133a

Figure 6

lateral. The primary neurite from this cell body ran dorsally and medially and gave off one side branch that projected ventrally and anteriorly. From this side branch emerged a series of smaller branches of differing but small diameters, with some approaching the anteroposterior midline of the ganglion. After giving rise to this single side branch, the primary neurite expanded enormously. From this expanded region a large but tapering and apparently blind ending process projected about 50 μm anteriorly and ventrally. It bore no fine branches. The expanded primary neurite then ran laterally and posteriorly to enter N5 as a large-diameter axon. Within nerve 5A this process was always oval in profile with its longest axis measuring 50 μm and its shortest axis 30 μm as seen in semithin, plastic sections (Fig. 5A), or in thin sections examined with the transmission electron microscope. It was the largest-diameter process in the whole fused ganglion and within N5A it occupied virtually all of the cross-sectional area. Its diameter was some 5 times larger than that of any of the other axons in N5B to the hind leg (Fig. 5C). The only other profiles that could be seen accompanying the giant axon in N5A were two axons with diameters of less than 2 μm (Fig. 5B,D), and a third with a diameter of 4–5 μm (Fig. 5B,E). The giant axon itself was wrapped by a thicker layer of glial cells than the other axons of this nerve and N5B (Fig. 5B–E).

The second smaller cell body had a diameter of 20 μm and was dorsal and lateral. It had the more conventional features of a motor neuron (Fig. 4). The primary neurite ran ventrally and gave rise to an array of fine branches in the lateral region of neuropil on the same side of the ganglion as the axon which entered N5A. Its axon was presumably the 4–5 μm profile seen in sections of this nerve (Fig. 5A,B,E).

The third cell body was dorsal and at the midline. It had an axon in both the left and right N5A and arrays of neurites in both left and right halves of the metathoracic neuromere. It therefore has all the characteristics of a dorsal unpaired median (DUM) neuron. Its axon was presumably one of the two very small diameters axons in N5B (Fig. 5B,D).

Figure 6. Neurons in the metathoracic neuromere (viewed ventrally) supplying the trochanteral depressor muscle. **A:** Neurons of the right trochanteral depressor muscle M 133b/c stained with neurobiotin introduced through the right N3A/C. The two lateral cell bodies belong to motor neurons, and the two medial ones to DUM cells. **B:** Double labeling of the right N3A/C and the left N5A in the same *Issus*. Neurobiotin introduced through the right N3A/C stained neurons from the main part of the muscle (M133b/c), and through the left N5A stained neurons from the small coxal part of muscle (M133a). On the right smaller somata are visible that have axons in N3A and supply other thoracic muscles. Scale bar 100 μm.

M133b/c

Backfilling N3A/C revealed the cell bodies of four neurons supplying the large part of the trochanteral depressor muscle in the thorax (M133b/c) (Figs. 3C, 6A,B). Two were lateral and ventral and had diameters of 25–30 μm. Their primary neurites followed a parallel path within the ganglion and their patterns of branches were similar, so that the following description applies to either neuron. About 50 μm from the cell body the primary neurite gave rise to a prominent branch that projected medially and anteriorly and which in turn had numerous short and small-diameter side branches (Fig. 6A). This branch then bifurcated with one prominent branch projecting anteriorly and the second medially. The first anterior branch divided further with some of its branches extending to the midline and others a further 150 μm anteriorly. The second medial branch extended to the midline where arrays of fine branches were formed in neuropils both ipsilateral and contralateral to its axon and cell body. It would therefore be expected that the branches of the two sets of neurons innervating M133b/c on the left and right sides of the body would be in the same areas of neuropil in these regions.

Two other cell bodies with diameters similar to those of the motor neurons were dorsal and medial. Both had axons in the left and right N3A/C and arrays of branches in the left and right halves of the ganglion. They therefore have all the morphological characteristics of DUM neurons. Smaller-diameter cell bodies were sometimes also stained (Fig. 6B) that supply small dorsoventral muscles in front of the pleural arch.

To directly compare the sizes of motor neuron somata and fiber diameters we also filled nerves 5A and 3A/C simultaneously on the opposite sides of five *Issus* (Fig. 6B). Such stains clearly show that one of the neurons stained via nerve 5A had a much larger diameter soma and axon than the other motor neurons.

Action of the muscles and motor neurons during jumping

The pattern of spikes recorded from trochanteral depressor and levator muscles during a restrained jump indicated three phases of action.

First, both hind legs were fully levated by high-frequency bursts of activity in the two levator muscles so that the femora were drawn forwards and closely apposed to the coxae. The white patches of cuticle on the dorsal surface of the femora engaged with the coxal protrusions (Figs. 1B, 3A) (Burrows, 2009). This phase lasted for several hundred milliseconds.

Second, motor neurons innervating the trochanteral depressor muscles M133b/c on both sides of the body spiked continuously, but the trochantera of the hind legs did not move and thus remained fully levated about the

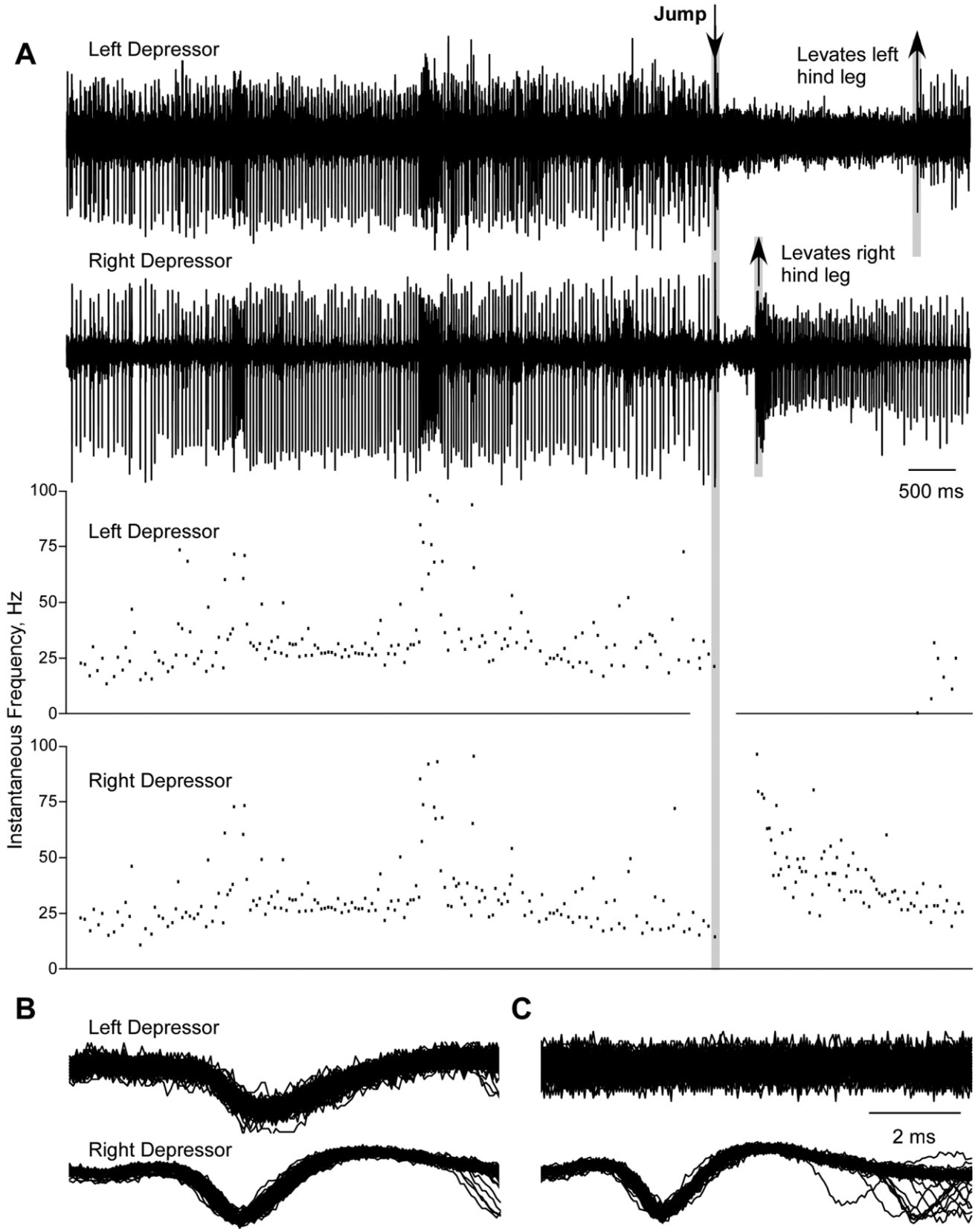
A
Jump
Left Depressor
Levates left hind leg
Right Depressor
Levates right hind leg
500 ms
Instantaneous Frequency, Hz
100
75
50
25
0
Left Depressor
Right Depressor
B
Left Depressor
Right Depressor
C
2 ms

Figure 7

coxae. During this phase the levator muscles spiked only sporadically and not in a pattern that was repeatable from jump to jump. This phase could last for many seconds.

Third, the trochanteral depressor motor neurons stopped spiking and the energy stored by the prolonged contractions of the depressor muscles powered the rapid and simultaneous depression movements of both hind legs that propel the jump. This phase lasted less than 1 ms.

Depressor Muscles

All successful recordings were made from the large parts (M133b/c) of the trochanteral depressor in the thorax. No activity could be recorded from the small part (M133a) of the depressor in the coxa, despite using the same methods (myogram and intracellular recordings) that revealed the activity of the small levator within the coxa.

Once both hind legs had been levated, a continuous sequence of spikes was recorded in depressor muscle 133b/c (Fig. 7A). These spikes were not recorded in legs that were not fully levated and thus positioned in readiness for a jump. In some recordings the spikes appeared to result from the action of a single motor neuron but in others (e.g., Fig. 7A) the contribution of a second motor neuron closely synchronized to the spikes of the first neuron was implicated. The period from the start of a continuous sequence of spikes in both left and right depressors M133b/c until a jump was released lasted 48 $\pm$ 3.3 s (mean $\pm$ SEM, range 6–134 s in 76 jumps by nine *Issus*). During this time the spikes occurred at average frequencies of about 25 Hz, although could rise to brief instantaneous frequencies of 100 Hz, and the overall frequency could rise before a jump was released. All jumps observed were preceded by several seconds of this continuous spike activity.

Recordings from the depressor muscles of both sides during restrained jumping showed close coupling between the frequency trends (Fig. 7A) and even between the individual spikes on the two sides. In sequences lasting several seconds, every spike on one side was accompanied by a spike on the other (Fig. 7B) and the differing intervals between successive spikes were reflected on both sides in synchronous shifts in their instantaneous spike frequencies. On rare occasions in these extended sequences a spike would be present on one side that was not recorded on the other. Such occurrences also showed that the synchrony of the majority of spikes was not simply caused by crosstalk between the two sets of recording electrodes. Furthermore, complete independence between the depressor spikes on the two sides could occur, particularly after a jump when one hind leg was levated again before the other (Fig. 7C).

To understand the coupling between the motor neurons, intracellular recordings were made from fibers in these muscles on the two sides of the body during the sustained contractions when the hind legs were cocked (Fig. 8). The recordings showed a continuous sequence of excitatory post synaptic potentials (EPSPs) in the fibers of the muscle on one side that were closely synchronized with those on the other side (Fig. 8A,B). In some fibers the EPSPs appeared to be of consistent amplitude, indicating the action of a single motor neuron. Occasionally two summating EPSPs occurred (Fig. 8A,B) and an occasional EPSP failed on one side (Fig. 8A). In fibers in other *Issus*, pairs of EPSPs occurred more frequently and were often present on both sides at the same time (Fig. 8B). Histograms of the intervals between successive EPSPs were bimodal, with the small peak at short intervals representing the occurrence of pairs of EPSPs and the larger peak at longer intervals representing the period between the more frequent single EPSPs, or between the pairs themselves (Fig. 8C,D). These data indicate that the spikes of the two motor neurons to the depressor on one side were closely coupled so that they spiked at the same time. They also indicated that the four motor neurons, two to each side, were also closely coupled. If such coupling were not present both within and between sides, then the uncoupled spikes should lead to a histogram in which all intervals between the two observed peaks occurred. To determine whether the pairs of EPSPs on one side were caused by the action of one or both motor neurons, successive EPSPs were superimposed triggered by the rising phase of a single EPSP (Fig. 8E). The second EPSP sometimes occurred only 1–2 ms after the first, indicating that it must have been caused by a spike in the closely coupled second motor neuron. Other second EPSPs occurred with a longer latency that suggested they could have been caused by a second spike of either motor neuron.

The precision in the coupling between the left and right motor neurons was demonstrated by superimposing EPSPs of both sides while triggering from those on one side (Fig. 8F). The EPSPs on the opposite side occurred with an increased variability in timing of only 0.6 ms compared to the triggering EPSPs. The same experimental procedure, however, also showed that the coupling was not obligatory

Figure 7. Actions of motor neurons to the left and right trochanteral depressor muscles (M133b/c) during a jump. **A:** Before a jump, both left and right depressor muscles spiked continuously (recorded extracellularly on upper two traces). The same frequency trends occurred in both as revealed by the instantaneous frequency plots. The spikes in both muscles stopped at the time of the jump. The right hind leg was levated 400 ms after the jump and its depressor spikes then resumed while those in the left depressor restarted only when the left hind leg levated again. **B:** Synchrony (141 spikes superimposed) of depressor spikes on the two sides before a jump. **C:** Asynchrony of spikes (89 spikes of the right muscle superimposed) after a jump. The superimposed sweeps in B and C were triggered by spikes in the right depressor.

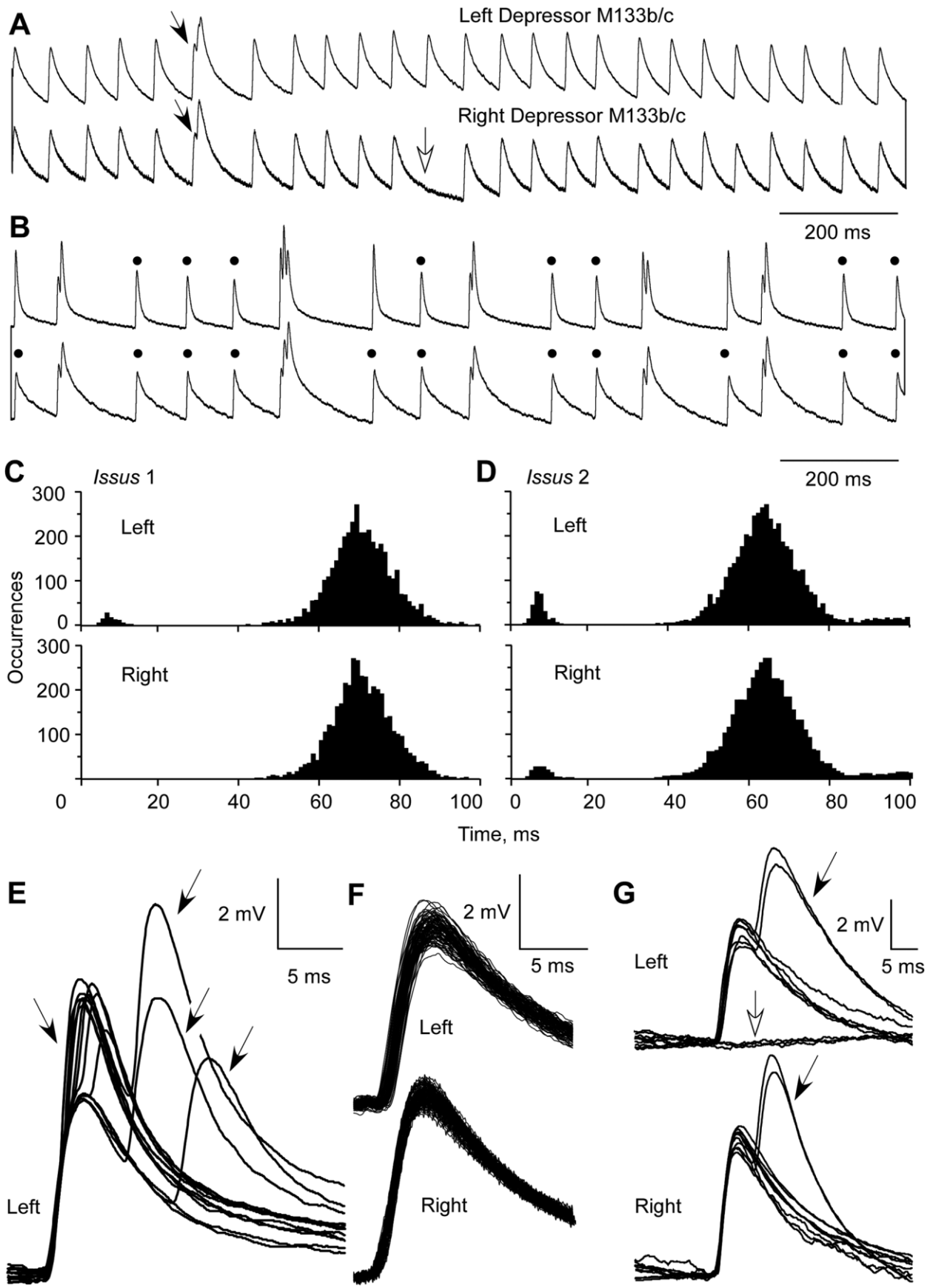
A
Left Depressor M133b/c
Right Depressor M133b/c
B
200 ms
C
Issus 1
Left
Right
Occurrences
300
200
100
0
D
Issus 2
200 ms
Left
Right
0
20
40
60
80
100
Time, ms
E
2 mV
5 ms
Left
F
2 mV
5 ms
Left
Right
G
2 mV
5 ms
Left
Right

Figure 8

because an EPSP would sometimes fail on the opposite side (Fig. 8G). Stimulating a muscle on one side with myogram electrodes does not evoke an EPSP in the depressor muscle on the opposite side, suggesting that the neurons to both sides are independent and are not linked by electrical synapses.

Levator muscles

Levation of the two hind legs into their cocked position was an essential prerequisite for restrained jumping and was achieved either by moving both hind legs at the same time, or more usually independently and with varying intervals between their actions.

In the period preceding a restrained jump during which the depressor M133b/c spiked continuously, recordings from the small levator M131 in the coxa showed that it spiked only sporadically (Fig. 9A–C). The pattern of this activity was not consistent in successive jumps by the same or different *Issus*. The activity consisted of a series of spikes at low frequency, groups of spikes at higher frequency, and more rarely a few spikes before a jump occurred. A consistent feature of the action of this levator, however, was a high-frequency burst of spikes in what were apparently a few different motor neurons after the jump occurred. These were usually accompanied by a levation of the hind legs to their fully levated position and a consequent resumption of the continuous spike activity in the depressor (Fig. 9A–C). On other occasions the levation was only partial and was not accompanied by a resumption of depressor activity. Intracellular recordings from individual fibers revealed EPSPs of different amplitudes that suggest the presence of at least three motor neurons (Fig. 9D).

Similar activity occurred in the larger levator (M132) that extended into the thorax (Fig. 10). Before the jump when the depressor was spiking continuously, occasional spikes occurred either singly or in brief bursts (Fig. 10A), but again not in a repeatable pattern in different jumps and in different *Issus*. After a jump a burst of spikes occurred that was associated with a return to full or partial levation. This muscle was only accessed by myogram wires and not by intracellular electrodes. The bursts after a jump were much briefer than the preceding depressor activity but their duration was variable resulting in visibly different rates of levation. The bursts lasted 597 ± 92.2 ms (mean ± SEM, range 92–1784 ms in 27 jumps) and appeared to result from the actions of a single motor neuron for the following reasons. First, the changing amplitude of the spikes could be represented by a continuous function throughout a burst. Second, the spikes showed no indication of summation or drift between them, which would be expected of similar patterns of spikes in two motor neurons. Third, none of the frequencies observed fell outside the expected range of capabilities of a single neuron; if there were two loosely coupled neurons there might be very short intervals between some of their successive spikes. The spikes occurred in a group of 65–70 at frequencies of about 100 Hz, followed by 2–6 spikes at frequencies below 50 Hz (Fig. 10B–D). The intervals between spikes of the initial burst were consistent during levations following a series of jumps by the same *Issus* (Fig. 10E).

Figure 8. Synchrony and failure of motor spikes to the left and right trochanteral depressor muscles (M133b/c) when the hind legs are cocked in preparation for jumping. **A,B:** Simultaneous intracellular recordings from fibers of the left and right muscles. A: Most of the EPSPs were of similar height and occurred at the same time on each side. A double EPSP (filled arrows) occurred once, and one EPSP failed on the right side (open arrow). B: A second *Issus* in which more double EPSPs were interspersed between the single ones (black dots). **C,D:** Histograms of the intervals between 5,000 successive EPSPs in the two muscles from two *Issus*. The bimodal peaks indicate the occurrence of the double EPSPs and that events on the two sides are tightly coupled. **E:** Superimposed sweeps triggered by the smaller EPSP show the time of occurrence of a second EPSP in a pair (filled arrows). **F:** Synchrony of EPSPs on the two sides when the 103 overlaid sweeps were triggered by the right EPSP (lower traces). There is greater jitter in the time of occurrence of the left EPSP. **G:** Ten superimposed sweeps triggered by the right EPSPs (lower traces) show that EPSPs may fail on the left (open arrow), and that double EPSPs can occur synchronously on both sides (filled arrows).

DISCUSSION

Jumping movements of the hind legs of the planthopper *Issus* are powered by two huge and complex trochanteral depressor muscles. The largest part (M133b,c) of one of these muscles occupies most of one-half of the metathorax, whereas a small part (M133a) is restricted to a coxa, but both insert on the same complex tendon. M133b,c is innervated by two motor neurons with axons in N3C, each with a similar array of branches restricted to one-half of the metathoracic neuromere, and by two DUM neurons. M133a is innervated by two presumed motor neurons, one with a large-diameter, ventral cell body and a giant axon in nerve N5A, the other with a smaller, dorsal cell body, and by one DUM neuron. The levator muscles are innervated by a number of motor neurons that have not been stained as individuals.

Number of neuromeres in the fused thoracic ganglion

All the neurons described here originate in the metathoracic neuromere of a ganglion in the thorax of *Issus*. This ganglion results from a fusion of the three thoracic neuromeres with all the abdominal neuromeres and thus differs from the ganglionic fusion that occurs in other groups of bugs (Hemiptera). In froghoppers (Hemiptera, Auchenorrhyncha, Cercopidae) the three thoracic neuromeres re-

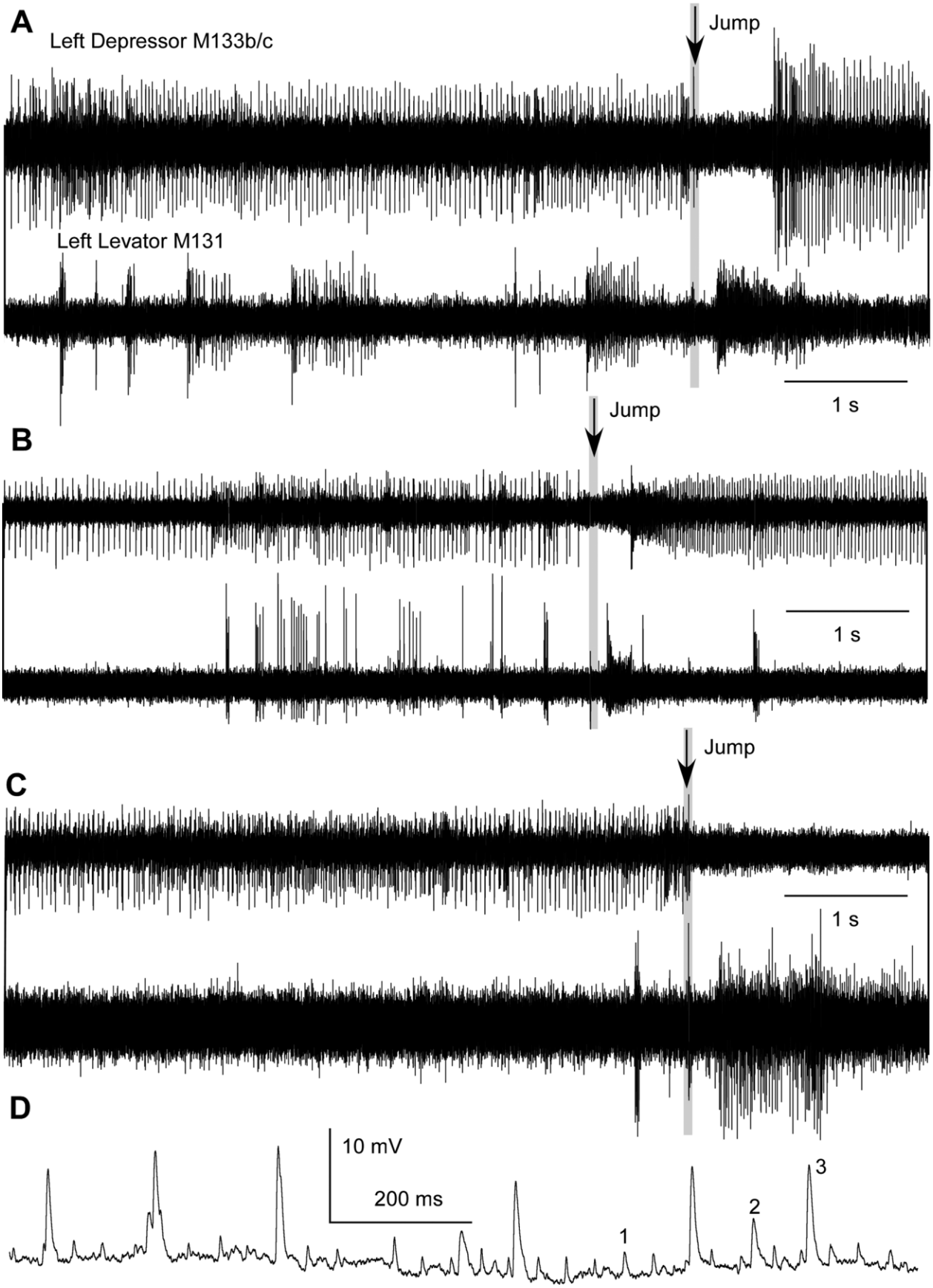
A
Left Depressor M133b/c
Jump
Left Levator M131
1 s
B
Jump
1 s
C
Jump
1 s
D
10 mV
200 ms
1
2
3

Figure 9

main separate from each other (Bräunig and Burrows, 2008). In other jumping bugs such as leafhoppers (Hemiptera, Auchenorrhyncha, Cicadellidae) and treehoppers (Hemiptera, Auchenorrhyncha, Membracidae) (Pflugfelder, 1937), and other bugs such as bedbugs (*Cimex*, Hemiptera, Heteroptera, Cimicidae) (Singh et al., 1996) and *Rhodnius* (Hemiptera, Heteroptera, Reduviidae) (Orchard et al., 1989; Wigglesworth, 1959), the prothoracic neuromere is separate from the fused meso-, metathoracic, and abdominal neuromeres.

Muscles and neurons

The main power-producing part of the trochanteral depressor muscle within the thorax of the froghopper *Philaenus* consists of two parts (Burrows, 2007c): M133b consists of pinnate sheets of fibers, while M133c consists of a large mass of parallel fibers. By contrast, in *Issus* all the fibers are arranged in parallel and insert on a complex umbrella-shaped tendon. In *Issus*, as in froghoppers (Bräunig and Burrows, 2008) and locusts (Bräunig, 1982), two motor neurons with small-diameter cell bodies and branches restricted to one-half of the metathoracic neuromere innervate this muscle through N3C. In froghoppers, there is also a small thoracic part of the muscle (M133d) which has the largest-diameter axon of any innervating all parts of the trochanteral depressor muscle. In *Issus*, this part of the muscle is represented only by a small ligament linking the sternal apodeme to the depressor tendon and for which we have not found any innervation.

The coxal part of the muscle (M133a) is innervated by N5A containing two motor neurons in both froghoppers and planthoppers. In locusts, M133a is innervated by a slow motor neuron with a dorsal cell body and a fast motor neuron with a ventral cell body (Bräunig, 1982; Siegler and Pousman, 1990). In locusts and froghoppers there is nothing distinctive about the larger, fast motor neuron with a ventral cell body, either in terms of the diameter of its axon or the branches from its primary neurite within the ganglion. By contrast, in *Issus* this neuron has just one neurite with conventional smaller-diameter side branches in the metathoracic neuromere. The only other central process from the primary neurite is unusual in ending blindly. The primary neurite is the largest-diameter process in the ganglion and leads directly to an axon with an oval profile measuring 50 μm by 30 μm, which is much larger than other motor axons to the hind leg. This means that the large axon ranks as a giant neuron (defined as of a diameter that sets it apart from all other axons in its nervous system).

Insect motor neurons are not known to have axons with diameters approaching that seen in this *Issus* neuron. For example, in cockroaches the diameters of the largest motor axons to leg muscles (in nerve N6Br4) reach only 20 μm (Pearson et al., 1970). In locusts the motor neuron with the largest cell body (the fast extensor tibiae) has an axon of only 15 μm (Gwilliam and Burrows, 1980). In the ant *Odontomachus* the axon of the motor neuron innervating the trigger muscle involved in rapid movements of the jaws is 15–21 μm (Gronenberg, 1995). Similarly, examples of insect sensory neurons with large-diameter axons are few. The largest sensory axon recorded in a locust, the stretch receptor from a wing hinge, has a diameter of 11–14 μm (Altman and Tyrer, 1977). Axons from sensory hairs on the mandibles that can initiate rapid jaw movements in ants have diameters of 15–20 μm (Gronenberg et al., 1993; Gronenberg and Tautz, 1994). A very small number of insect interneurons have large-diameter axons in the connectives linking the ventral chain of ganglia. These neurons have diameters ranging from 6–8 μm in *Drosophila* (Power, 1948) to 16–31 μm in the abdominal connectives in the cockroach *Periplaneta americana* (Daley et al., 1981) and 40–60 μm at the caudal end of the metathoracic ganglion (Castel et al., 1976). The overriding assumption for these interneurons is that their large-diameter axons speed conduction velocity of signals and therefore reduce processing time in pathways where rapid response is of the essence. What stands out is the proportionately huge size of the axon in *Issus* relative to its body size; *Issus* is only a few millimeters long, whereas cockroaches are much larger insects measuring several centimeters. Comparable sized insects such as *Drosophila* do not have such large axons.

Why should this presumed motor neuron in *Issus* be so large? The length of the axon from the thoracic neuromere to M133a is 400–500 μm (depending on body size; males are smaller than females). The relationship between axon diameter and conduction velocity for insect neurons is given by:

$$Velocity\ in\ m\ s^{-1} = 10^{(0.74\ log\ (diameter\ in\ \mu m) - 0.416)}$$

where 0.416 is the intercept of the line shown in fig. 8 of Pearson et al. (1970).

Extrapolating from this indicates that spikes will have a conduction velocity of 4.8 m s^{-1} and a journey time of 0.1 ms. Thus, having an axon diameter 3–6 times greater than

Figure 9. Activity of trochanteral levator muscle 131 during restrained jumping. **A–C:** Paired myogram recordings were made from depressor M133b/c and levator M131 in three *Issus*. The levator generated a burst of spikes at the end of each jump, but no consistent pattern occurred during the continuous activity in the depressor preceding a jump. In A and B both hind legs levated again at the end of the jumps, but in C full levation was delayed. **D:** Intracellular recording from a muscle fiber in M131 revealed three amplitudes of EPSP (1–3) that indicate innervation by at least three motor neurons.

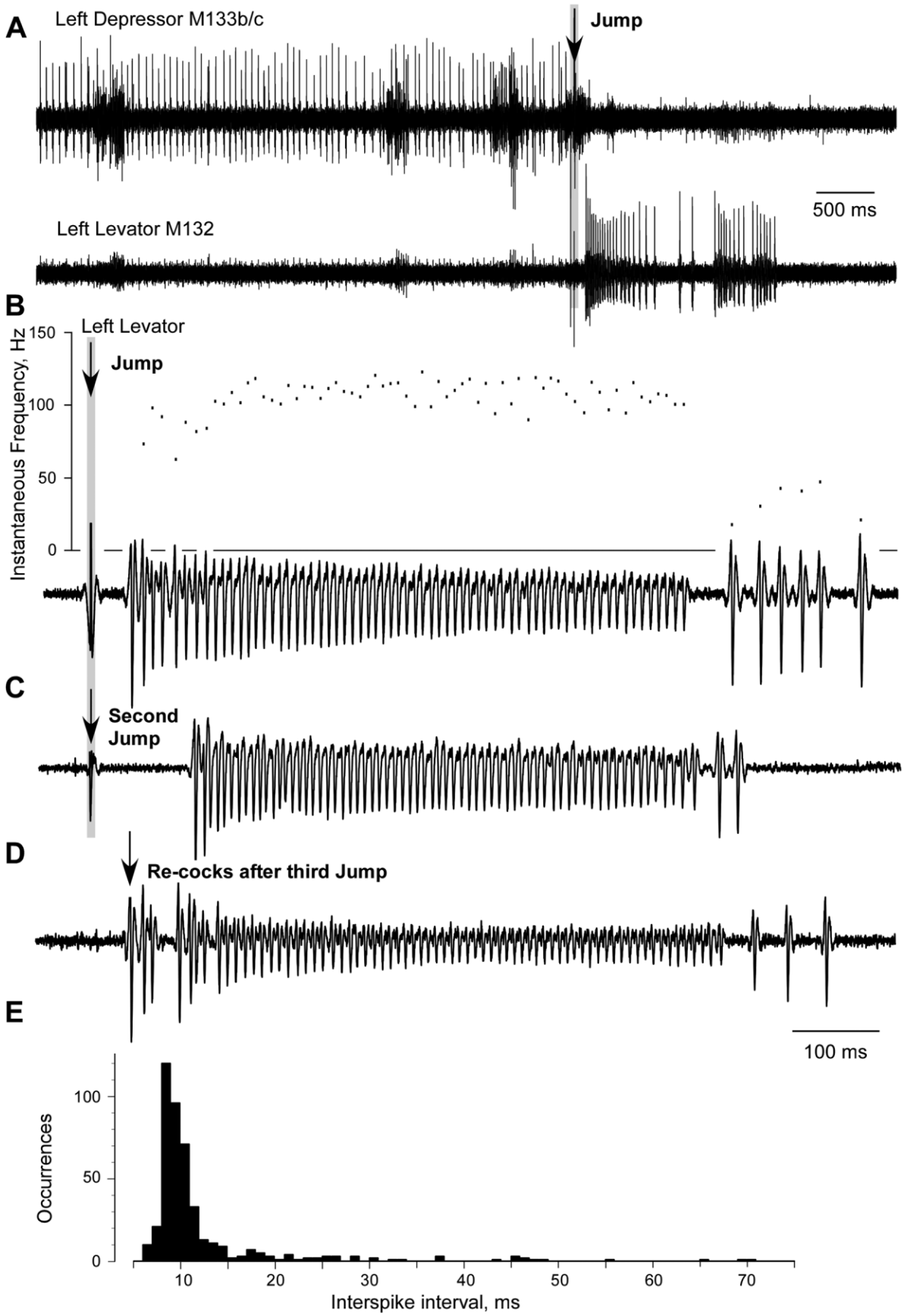
A
Left Depressor M133b/c
Jump
500 ms
Left Levator M132
B
Left Levator
Jump
Instantaneous Frequency, Hz
150
100
50
0
C
Second Jump
D
Re-cocks after third Jump
100 ms
E
Occurrences
100
50
0
10
20
30
40
50
60
70
Interspike interval, ms

Figure 10

other motor neurons reduces the journey time of the spikes to the muscle by 0.13–0.28 ms. The assumption must be that there is some premium to reducing the transit time of signals in this neuron by these small amounts. These calculations are all based on the assumption that it is a motor neuron and a spiking neuron. It is possible that it may be nonspiking sensory neuron, similar to those in crustaceans (Bush, 1981), with its size again reflecting a need to transmit signals rapidly or with little attenuation.

What role might this neuron and the small thoracic part of the depressor muscle play in jumping? In fleas it has been suggested that the contraction of a small coxal muscle shifts the line of action of the main power producing trochanteral depressor muscle to allow it to depress a hind leg for jumping (Bennet-Clark and Lucey, 1967). In *Issus* there is no evidence that the line of action of the depressor goes over the center of the pivot in advance of a jump. Attempts in this investigation to record from this muscle have not revealed any activity that is linked to the jumping motor pattern. The same methods, however, did reveal the activity of the small levator muscle in the coxa. However, muscle M133a might act in some way as a trigger for jumping, acting either as a muscle generating force or as one acting as a muscle receptor organ, with the large-diameter axon needed to get the signals to or from the muscle rapidly so that both hind legs are released at the same time.

Motor patterns during restrained jumping

Issus uses a three-phase motor pattern in restrained jumping, similar to that used by froghoppers (Burrows, 2007c) and leafhoppers (Burrows, 2007a) in their catapult-propelled jumps. First, a short but high-frequency burst of spikes to the two trochanteral levator muscles moves the hind legs into their fully levated position. Second, the motor neurons to the large part of the trochanteral depressor muscle spike continuously for many seconds but the hind legs remain stationary. The levator muscles spike sporadically indicating that they provide some co-contraction to the much larger depressor during this period. Third, the spikes in the depressors and levators stop abruptly and the hind legs depress in less than 1 ms to propel a jump.

Figure 10. Action of trochanteral levator muscle M132 during jumping. **A:** Simultaneous recording from the left depressor muscle (M133b/c) and the left levator muscle (M132). The jump was followed by a burst of high-frequency spikes in the levator, and in this example by a second burst as the hind leg still failed to recock fully. **B–D:** Bursts of levator spikes during three jumps on an expanded time scale; the first two levations occurred soon after a jump, but the third was delayed. The first levator burst is shown with an instantaneous frequency plot of its spikes. **E:** Histogram of the intervals between spikes in levator M132 after five jumps.

In froghoppers the contraction of the trochanteral depressor muscles during phase 2 is also prolonged but much shorter (mean of about 3 sec) than in *Issus* (Burrows, 2007c). In leafhoppers, phase 2 lasts on average only 80 ms (Burrows, 2007a). The minimal duration of the muscle activity should be determined by the time that it takes to store sufficient energy to propel a jump. This would suggest that in leafhoppers sufficient force to act against the long hind legs could be achieved in a short period, even though when using a catapult mechanism leg lengths have no influence on takeoff velocity, but do alter acceleration times and hence ground reaction forces (Burrows and Sutton, 2008). The much longer period in *Issus* compared even to froghoppers suggests that preparedness for jumping takes precedence over the energy costs of contracting the muscles for such a long time. In free and natural jumping, *Issus* can respond quickly to external stimuli by jumping, but then we do not know how long in advance the depressor muscles have been contracting. Holding the hind legs cocked for long periods allows jumps to be triggered quickly by a variety of stimuli without having to wait for energy to be stored. This suggests a mechanism for initiating a jump that either transiently increases the force in the large depressor muscle above a certain threshold, reduces the sporadic activity of the levators and hence their contribution to a co-contraction, or triggers a jump through the action of another, as yet unknown mechanism.

What prevents the hind legs from depressing during the prolonged contraction of the depressor muscles? In froghoppers the engagement of the coxal and femoral protrusions, both of which are covered with microtrichia, provides a physical barrier to depression, perhaps acting in conjunction with the mechanical advantages of the lever arms of the levator and depressor muscles (Burrows, 2006b). In leafhoppers there are no protrusions on either a coxa or femur that could provide a physical barrier to extension (Burrows, 2007a), so that any restraint must result from the lever arms of the muscles. In *Issus*, there is a coxal protrusion covered in microtrichia but it engages with a smooth and only slightly raised surface on the dorsal femur. Unless there are frictional forces acting between these two structures, they would not seem to provide a restraint to the prolonged contraction of the trochanteral depressor muscle. The lever arms of the depressor and levator muscles are again implicated.

Spike synchrony between the two sides

The two motor neurons to the large depressor muscles on one side of the body are anatomically distinct from the two that innervate the same muscle on the opposite side. Nevertheless, recordings from the left and right depressor muscles show that their spikes can occur in two distinct states of coupling. First, when both legs are in their cocked

position, spikes in the two motor neurons innervating one side are tightly coupled to each other and to spikes in the two motor neurons to the muscle on the other side. Differences in timing vary by no more than 0.6 ms, but occasional EPSPs can fail on one side or the other, indicating that the coupling is not obligatory. Second, when the hind legs are used in movements other than jumping, or when only one hind leg has been moved into its cocked position, the spikes on the two sides can show complete independence of action. Synchrony of the spikes to M133b/c between the two sides also occurs in froghoppers during the same phase of the jumping motor pattern (Burrows, 2007c) but not in leafhoppers (Burrows, 2007a). In frog- and planthoppers, neurobiotin, which can cross gap junctions (Vaney, 1991), does not stain motor neurons on one side of the ganglion when introduced into motor neurons on the other side. Furthermore, stimulating the muscle on one side with myogram wires does not evoke an EPSP in the muscle on the other side. These lines of evidence suggest that electrical coupling between the left and right motor neurons does not occur. The explanation for the synchronous spikes in both froghoppers and planthoppers must therefore lie in a strong but common synaptic drive to the motor neurons on both sides. The independence of action between the two sides could be explained by their respective motor neurons receiving independent synaptic inputs. The occasional absence of spikes or EPSPs on one side during synchronous activity could be caused by branch point failures on that side, or by different spike thresholds to the common synaptic drive on the two sides.

Unresolved jumping mechanisms

Three further questions are raised by this study about the control of jumping in *Issus*. First, contractions of the power-producing muscles well in advance of a jump implies that energy must be stored. Where and how is this energy stored? Is it stored in the metathoracic pleural arches (which are well developed in *Issus*) in a manner similar to the mechanism in froghoppers (Burrows et al., 2008)? Second, the synchronization of spikes to the trochanteral depressor muscles of the left and right hind legs would not alone appear sufficient to provide the precision of coupling between the movements of the two hind legs that is observed behaviorally. Are there other mechanical mechanisms to ensure synchrony? An analysis of energy storage and leg synchronization mechanisms will be addressed in a subsequent article. Finally, current work is directed toward analyzing the extraordinary neuron with a giant axon that supplies the small coxal part of the trochanteral depressor muscle and whether its action influences the release of a jump.

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LITERATURE CITED

Altman JS, Tyrer NM. 1977. The locust wing hinge stretch receptors. I. Primary sensory neurones with enormous central arborizations. J Comp Neurol 172:409–430.

Bennet-Clark HC. 1975. The energetics of the jump of the locust *Schistocerca gregaria*. J Exp Biol 63:53–83.

Bennet-Clark HC, Lucey ECA. 1967. The jump of the flea: a study of the energetics and a model of the mechanism. J Exp Biol 47:59–76.

Bräunig P. 1982. The peripheral and central nervous organization of the locust coxo-trochanteral joint. J Neurobiol 13:413–433.

Bräunig P, Burrows M. 2008. Neurons controlling jumping in froghopper insects. J Comp Neurol 507:1065–1075.

Burrows M. 1969. The mechanics and neural control of the prey capture strike of the Mantid shrimps *Squilla* and *Hemisquilla*. Z vergl Physiol 62:361–381.

Burrows M. 1995. Motor patterns during kicking movements in the locust. J Comp Physiol A 176:289–305.

Burrows M. 2003. Froghopper insects leap to new heights. Nature 424:509.

Burrows M. 2006a. Jumping performance of froghopper insects. J Exp Biol 209:4607–4621.

Burrows M. 2006b. Morphology and action of the hind leg joints controlling jumping in froghopper insects. J Exp Biol 209: 4622–4637.

Burrows M. 2007a. Anatomy of hind legs and actions of their muscles during jumping in leafhopper insects. J Exp Biol 210: 3590–3600.

Burrows M. 2007b. Kinematics of jumping in leafhopper insects (Hemiptera, Auchenorrhyncha, Cicadellidae). J Exp Biol 210: 3579–3589.

Burrows M. 2007c. Neural control and co-ordination of jumping in froghopper insects. J Neurophysiol 97:320–330.

Burrows M. 2009. Jumping performance of planthoppers (Hemiptera, Issidae). J Exp Biol 212:2844–2855.

Burrows M, Morris G. 2001. The kinematics and neural control of high speed kicking movements in the locust. J Exp Biol 204: 3471–3481.

Burrows M, Morris O. 2003. Jumping and kicking in bush crickets. J Exp Biol 206:1035–1049.

Burrows M, Sutton GP. 2008. The effect of leg length on jumping performance of short and long-legged leafhopper insects. J Exp Biol 211:1317–1325.

Burrows M, Shaw SR, Sutton GP. 2008. Resilin and cuticle form a composite structure for energy storage in jumping by froghopper insects. BMC Biol 6:41.

Bush BMH. 1981. Non-impulsive stretch receptors in crustaceans. In: Roberts A, Bush, BMH, editors. Neurones without impulses. Cambridge, UK: Cambridge University Press.

Campbell JI. 1961. The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratorioides*. Proc Zool Soc Lond 137:403–432.

Castel M, Spira ME, Parnas I, Yarom Y. 1976. Ultrastructure of region of a low safety factor in inhomogeneous giant axon of the cockroach. J Neurophysiol 39:900–908.

Daley D, Vardi N, Appignani B, Camhi JM. 1981. Morphology of the giant interneurons and cercal nerve projections of the American cockroach. J Comp Neurol 196:41–52.

Godden DH. 1975. The neural basis for locust jumping. Comp Biochem Physiol 51A:351–360.
Gronenberg W. 1995. The fast mandible strike in the trap-jaw ant *Odontomachus*. II. Motor control. J Comp Physiol [A] 176: 399–408.
Gronenberg W, Tautz J. 1994. The sensory basis for the trap-jaw mechanism in the ant *Odontomachus bauri*. J Comp Physiol [A] 174:49–60.
Gronenberg W, Tautz J, Holldobler B. 1993. Fast trap jaws and giant neurons in the ant *Odontomachus*. Science (NY) 262: 561–563.
Gwilliam GF, Burrows M. 1980. Electrical characteristics of the membrane of an identified insect motor neurone. J Exp Biol 86:49–61.
Heitler WJ. 1974. The locust jump. Specialisations of the metathoracic femoral–tibial joint. J Comp Physiol 89:93–104.
Heitler WJ, Burrows M. 1977. The locust jump. I. The motor programme. J Exp Biol 66:203–219.
Hustert R, Gnatzy W. 1995. The motor program for defensive kicking in crickets: performance and neural control. J Exp Biol 198:1275–1283.
Orchard I, Lange AB, Cook H, Ramirez JM. 1989. A subpopulation of dorsal unpaired median neurons of the blood-feeding insect *Rhodnius prolixus* displays serotonin-like immunoreactivity. J Comp Neurol 289:118–128.
Patek SN, Caldwell RL. 2005. Extreme impact and cavitation forces of a biological hammer: strike forces of the peacock mantis shrimp *Odontodactylus scyllarus*. J Exp Biol 208:3655–3664.
Patek SN, Korff WL, Caldwell RL. 2004. Deadly strike mechanism of a mantis shrimp. Nature 428:819–820.
Pearson KG, Stein RB, Malhotra SK. 1970. Properties of action potentials from insect motor nerve fibers. J Exp Biol 53:299–316.
Pflugfelder O. 1937. Vergleichendanatomische, experimentelle und embryologische Untersuchungen uber das Nervensystem und die Sinnesorgane der Rhynchoten. Zool Stuttgart 34:1–102.
Power ME. 1948. The thoracico-abdominal nervous system of an adult insect, *Drosophila melanogaster*. J Comp Neurol 88: 347–409.
Rothschild M, Schlein J. 1975. The jumping mechanism of *Xenopsylla cheopis*. Exoskeletal structures and musculature. Philos Trans R Soc Lond B 271:457–490.
Rothschild M, Schlein J, Parker K, Neville C, Sternberg S. 1975. The jumping mechanism of *Xenopsylla cheopis*. III. Execution of the jump and activity. Philos Trans R Soc Lond B 271:499–515.
Sakai M, Yamaguchi T. 1983. Differential staining of insect neurons with nickel and cobalt. J Insect Physiol 29:393–397.
Siegler MVS, Pousman CA. 1990. Motor neurons of grasshopper metathoracic ganglion occur in stereotypic anatomical groups. J Comp Neurol 297:298–312.
Singh RN, Singh K, Prakash S, Mendki MJ, Rao KM. 1996. Sensory organs on the body parts of the bed-bug *Cimex hemipterus* Fabricius (Hemiptera, Cimicidae) and the anatomy of its central nervous system. Int J Insect Morphol Embryol 25:183–204.
Snodgrass RE. 1929. The thoracic mechanism of a grasshopper, and its antecedents. Smithson Misc Collect 82:1–111.
Snodgrass RE. 1935. Principles of insect morphology. New York: McGraw-Hill.
Vaney D. 1991. Many diverse types of retinal neurons show tracer coupling when injected with biocytin or neurobiotin. Neurosci Lett 125:187–190.
Wigglesworth VB. 1959. The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). II The central ganglia. Q J Micros Sci 100:299–313.