

## THE MULTISEGMENTAL MOTOR SUPPLY TO TRANSVERSE MUSCLES DIFFERS IN A CRICKET AND A BUSHCRICKET

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### Summary

Most abdominal sternites of the cricket *Gryllus bimaculatus* and the bushcricket *Decticus albifrons* are bridged by a transverse muscle (TM) which supports expiratory movements. In the cricket, ventilatory contractions are controlled both within each segment, by a bilateral pair of excitatory motoneurons in the abdominal ganglion supplying the left and right halves of the TM independently, and intersegmentally, by peripheral collaterals of homologous motoneurons from adjacent segments. The axons of these motoneurons run in the ipsilateral paramedian nerve. This unique divergence of excitatory motoneurons to different muscles also results in massive convergence of excitatory inputs from different ganglia, especially on the TMs of the middle abdominal segments. TM contraction rates are increased by this intersegmentally divergent and convergent motor supply, especially in the middle abdominal segments.

In bushcrickets, each transverse muscle in segments 3–7 is innervated bilaterally by four pairs of neurons: (i) two pairs of contralateral excitatory motoneurons with axons that diverge, supplying two adjacent muscles; (ii) one pair of contralateral excitatory neurons found in the second anterior ganglion and (iii) a pair of median inhibitory neurons in the segmental ganglion. Transverse muscles 2 and 8 receive reduced innervation. The excitatory motoneurons generate slow excitatory postsynaptic potentials (EPSPs), which must sum to cause muscle contractions. During ventilation, contralateral paired transverse motoneurons fire at similar frequencies, thus synchronizing the contractions of the left and right halves of the muscle so that the whole muscle acts as a single unit.

### Introduction

The transverse muscles of the cricket abdomen are serially homologous muscle bundles that span bilaterally between the left and right edges of the sternites in abdominal segments 2–8 (DuPorte, 1920; Ford, 1923; Richards, 1963; Huber, 1960).

Key words: insect muscle, innervation, intersegmental, convergence, bushcricket, cricket, *Decticus albifrons*, *Gryllus bimaculatus*.

They run dorsally across the ventral nerve cord and are among the few skeletal muscles that bridge the midline of the body without a median insertion on a cuticular structure (Voss, 1905).

The tension and contraction of these compact muscles control the width of each sternite and its degree of U-shaped bending. The transverse muscles also keep the abdominal organs from pressing ventrally onto the nerve cord, and their activation pattern is usually synchronized with expiratory dorsoventral compression during abdominal pumping (Hustert and Schneider, 1986). At the location of the transverse muscles of crickets, other orthopteran insects have similar (bushcrickets) or different hyperneural muscle systems in the sternal region of their abdomen: locusts and mole crickets have a ventral diaphragm consisting of an almost continuous network of delicate muscle fibres (Albrecht, 1953), whereas cockroaches have a longitudinal system of hyperneural muscles (Shankland, 1965). The inter-species difference in hyperneural muscle systems is more extreme than in other homologous muscle systems described for orthopterans; for example, the rostral, tergal, sternal or appendage muscles. Comparisons of the nervous and motoneurone supply have often shown a very similar *Bauplan* for homologous motor systems in all orthopteran insects. We expected a similar situation for hyperneural muscle systems, but the innervation of the locust diaphragm (Peters, 1977; Yang and Burrows, 1983) and the cockroach system (Hertel and Penzlin, 1986) differed from each other and from that of crickets (Hustert and Schneider, 1986).

A preliminary study (Hustert and Schneider, 1986) has demonstrated an extraordinary peripheral branching pattern for single motoneurons in the transverse muscles of crickets: branches diverge to transverse muscles in several segments, and several motoneurons converge from different ganglia onto single transverse muscles. This design may either be a primitive form of intersegmental integration based on the convergence of efferents or a specific system of peripheral integration based on efferent redundancy that cooperates with central control of motoneurons in the central nervous system (CNS).

In bushcrickets, the innervation pattern of transverse muscles is quite different because, typically, they are innervated by four bilateral pairs of neurones originating from different neighbouring ganglia. One pair are inhibitory neurones whereas the branches of another pair of motoneurons diverge, supplying transverse muscles of two adjacent segments (Consoulas, 1990). The developmental significance of the peripheral and intersegmental collaterals has not been assessed here.

In this paper, as a first step in a general comparison of transverse muscle systems, we studied the motor supply of the apparently similar muscular systems of the cricket *Gryllus bimaculatus* and of the bushcricket *Decticus albifrons*.

### Materials and methods

Crickets, *Gryllus bimaculatus* (DeGeer) (Grylloidea, Orthoptera), were taken from a laboratory culture (Zoologisches Institut der Universität Göttingen). Bushcrickets *Decticus albifrons* (Fabricius) (Tettigonioidea, Orthoptera) were collected from fields in the Peninsula of Halkidiki and from fields in Vergina (the capital of ancient Macedonia)

during the summer months. Dissections were started after chilling the animals to 2–4°C for anaesthesia. In experiments requiring the suppression of ventilatory activity, the neck connectives were severed. After the wings and legs had been removed, the animal was pinned down ventrally and its abdomen was dissected by dorsal longitudinal incisions. Reproductive organs and the gut were dissected from the interior and the tergites were gently pinned out laterally so that the abdomen could be filled with cricket saline without leakage. In experiments using *D. albifrons*, the body cavity was filled with physiological saline (in mmol l<sup>-1</sup>: NaCl, 140; KCl, 5; CaCl<sub>2</sub>·2H<sub>2</sub>O, 5; NaHCO<sub>3</sub>, 4; MgCl<sub>2</sub>, 1; saccharose, 140; and Tes buffer, 5; pH7.0) Transverse muscles (TM), nerves and ganglia were then accessible for stimulation, for extracellular recording with hook and suction electrodes, for intracellular recording with glass microelectrodes, and also for neuroanatomical tracing by conventional cobalt backfilling from nerve stumps, followed by silver intensification (Bacon and Altman, 1977; Hustert, 1978).

The neural connections extending from each segmental TM to TMs in other segments were studied by stimulating antidromically from the motoneurone branches using surface suction electrodes placed on several TM fibres or hook electrodes placed on the appropriate peripheral nerves. Suprathreshold stimuli produced twitching in every TM showing a common motor innervation. These results were reaffirmed by centrifugal cobalt filling of the TM motoneurons selectively *via* the dorsal nerve close to its ganglionic nerve root. In crickets, the intersegmental motoneurone collaterals through the paramedian nerves to other segments were then traced in silver-intensified wholemounds of the abdominal sternal regions. In bushcrickets, the ganglionic sheath was treated with 1% protease (Sigma XIV) for 1 min before intracellular recording and staining of neurones. Glass microelectrodes were filled with cobalt hexamine chloride (resistance 50–80 MΩ). To monitor the muscle tension generated by a spontaneously active TM, one of its insertions, including a small area of the membrane, was cut off, denervated ipsilaterally and attached to the probe of an isometric tension transducer. The other end remained intact.

#### *Nomenclature*

Ganglia were numbered according to the number of their corresponding abdominal segment (AG6, ganglion of the sixth abdominal segment). In *G. bimaculatus*, the bilateral dorsal and ventral nerves are termed d and v respectively (Edwards and Palka, 1974). TM7 is the transverse muscle and 7d is the dorsal nerve of the seventh segment. In *D. albifrons*, the nomenclature of Cambell (1961) was applied with some modifications to the nerve branches of the abdominal system.

### **Results**

#### *Morphology and function of the transverse muscles*

The transverse muscles bridge the sternites in the anterior half of abdominal segments 2–7 in *G. bimaculatus* (Fig. 1A) and 2–8 in *D. albifrons* (Fig. 1B). Their muscle fibres connect directly between left and right sternal insertions across the midline. In male crickets, the TMs of each segment remain separate in segments 2–8. The only link

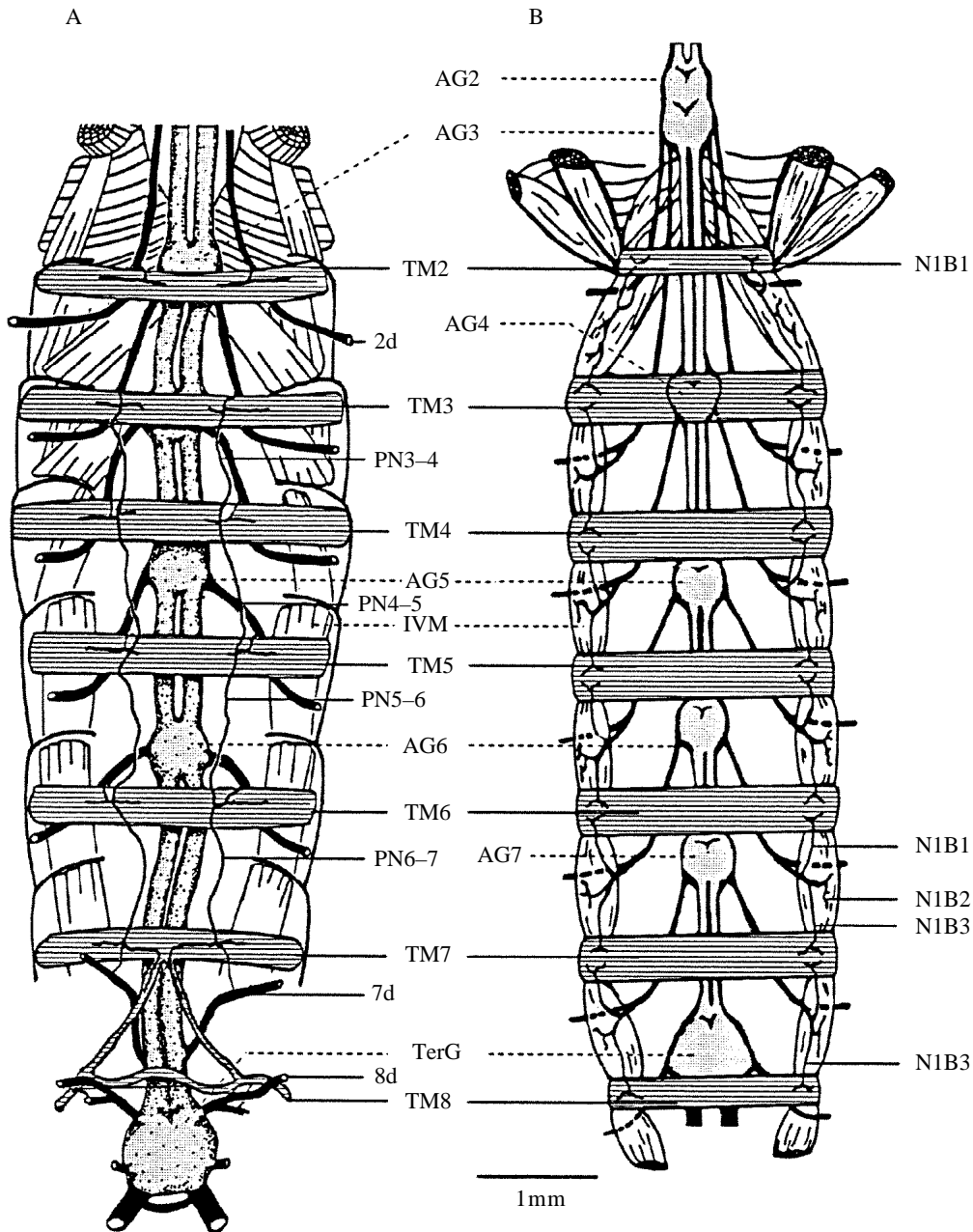


Fig. 1

between transverse muscles by intersegmental muscle strands is present between the seventh and eighth abdominal segments of females (Fig. 1A). From their very fine TM8, a pair of lateral oblique muscle bundles branches anteriorly and converges on the middle of TM7 (Fig. 1A). This connection resembles the pattern of oblique intersegmental

Fig. 1. Dorsal view of abdominal sternites in the pregenital segments of a female cricket (A) and the bushcricket (B). The transverse muscles (TM2–TM8) bridge the abdominal segments just dorsal to the ventral intersegmental muscles and the abdominal CNS. (A) The cricket CNS consists of the free abdominal ganglia (AG3–AG6) and the terminal ganglion (TerG), which comprises neuromeres from the seventh to the eleventh abdominal segments. Only the dorsal nerve roots (2d–8d) extending from the ganglia and their first branches to the transverse muscles are shown. Between the third and eighth transverse muscles, the paramedian nerves (PN3–4 to PN6–7) connect to TMs of different segments. (B) In the bushcricket CNS, the neuromeres supplying the second and third abdominal segment are fused into one separate ganglion; the following ganglia are single and the terminal ganglion includes the eighth neuromere. One bilateral pair of nerve roots exits from each ganglion. A branch (N1B2) of the main tergal nerve (N1B) supplies the lateral sternal muscle. An anterior branch (N1B1) to the segmental transverse muscle and a posterior branch (N1B3) split off.

muscle strands in the ventral diaphragm of locusts and, in some respects, the hyperneural muscle of cockroaches (Richards, 1963; Shankland, 1965).

Transverse muscle contractions in the cricket and the bushcricket narrow the sternites, which bulge ventrally at the same time (Fig. 2A,C). Sternite bending in the cricket is eased by two flexible sutures some distance from the segmental longitudinal midline (Gnatzy and Hustert, 1989). When the TMs relax, the sternites extend again as a result of elastic recoil in these sutures. In the bushcricket, with its less sclerotized sternite, this is achieved by lateral recoil of the previously compressed tergites. TMs contribute to the compression phase of expiratory dorsoventral pumping (Fig. 2B,D) by decreasing the width and volume of the segments. They also prevent the sternites from being pulled too far laterally by the lateral tergoventral muscles (Fig. 2A,C). During strong expiration, the serial arrangement of left dorsoventral, transverse sternal and right dorsoventral muscles can stabilize or even narrow the width of each segment. At the same time, sternal bending protects the CNS from being compressed by a filled gut and by genital organs lying dorsally in the abdomen. One major transverse muscle function, therefore, is to support expiration (Hustert and Schneider, 1986).

#### *Nervous supply of the transverse muscles and paramedian nerves*

##### *Ipsilateral restriction of innervation*

Visual inspection under the light microscope, and serial longitudinal sections, reveals that transverse muscle fibres bridge the segmental midline without a morphological discontinuity in the median region. The nervous supply, in contrast, never crosses the segmental midline. This strictly hemisegmental extent of the motoneurons of each muscle is seen both in peripheral cobalt fills (Fig. 3B) and in electrophysiological recordings (Fig. 3A,C). Only attenuated excitatory postsynaptic potentials (EPSPs) can be recorded from the contralateral side of any muscle fibre in the vicinity of the midline (Fig. 3A). In *D. albifrons*, where inhibitory innervation is present, TM6 muscle fibres receive converging inputs from both left and right inhibitory neurones in only a small area around the midline (Fig. 3C).

##### *Homologous motoneurons in the two species*

In *G. bimaculatus*, there is only one bilateral pair of excitatory motoneurons from

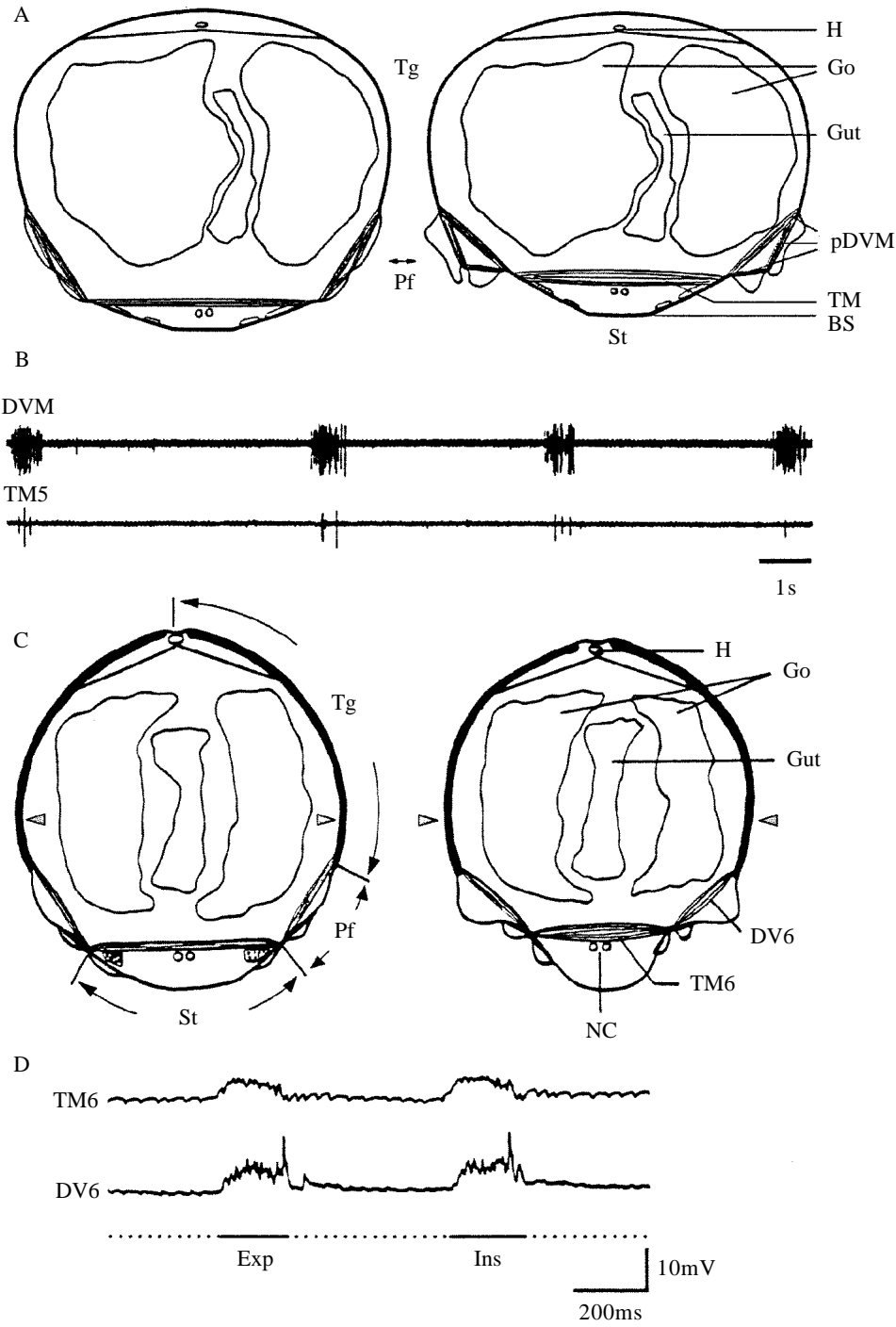


Fig. 2

Fig. 2. Function of transverse muscles in the abdomen of the cricket and the bushcricket. (A) Schematic cross sections of a cricket fifth abdominal segment showing the relaxed state (left) and the expiratory state (right). The dorsal movement towards the tergites (Tg), bending of the pleural fold (Pf) and bending of the sternite (St) are caused by contraction of the pleural dorsoventral muscle (pDVM) and the transverse muscle (TM). The sternite buckles mainly at the sutures (BS). H, Go and Gut indicate the positions of heart, gonads and gut, respectively. (B) Myograms from the fifth transverse muscle (TM5) and a pleural dorsoventral muscle (DVM) recorded chronically during ventilation in freely moving crickets. (C) Schematic cross sections of a bushcricket's sixth abdominal segment. When the muscles are relaxed (left), the abdomen extends in the sternal (St) and pleural (Pf) regions. This determines the lateral extension of the tergites (Tg, arrowheads). Narrowing of the segment (right, arrowheads) is caused by contraction of the dorsoventral muscles (DV6) in conjunction with the transverse muscle (TM6) that overlies the nerve chord (NC). The position of heart (H), gonads (Go) and gut (Gut) are indicated. (D) Recording from fibres of the transverse muscle (TM6) and the dorsoventral muscle (DV6) during inspiratory (Ins) and expiratory (Exp) phases of ventilation in a bushcricket.

each ganglion to its corresponding segmental TM. They can be backfilled from the axon in the nerve branch connecting to the dorsal nerve (Fig. 4A). One of the motoneurons innervating the TMs in *D. albifrons* is homologous to the cricket transverse motoneurons (Fig. 4B). In both cases, the motoneurone somata lie ventrally in the anterior region of the contralateral hemiganglion. The primary neurite crosses the midline through the anterior dorsal commissure and gives rise to extensive dorsal branches which extend far into the more posterior neuropiles of both hemiganglia (Fig. 4A,B).

Exceptions to this strictly segmental pattern occur in the cricket, where the two fused ganglia contain abdominal neuromeres (Fig. 4A). (i) In the metathoracic ganglion, which contains the neuromeres for the first and second abdominal segments, the TM2 motoneurons develop additional anterior dorsal branches that extend into the first abdominal neuromere. (ii) In the terminal ganglion, the TM7 motoneurons (supplying both TM7 and the rudimentary TM8) extend their posterior dorsal branches into the neuromere of the eighth abdominal segment.

#### *Intersegmental features in Gryllus bimaculatus*

The transverse muscles from the third to the seventh segments are linked by the left and right paramedian nerves (Fig. 1A). This is the only nervous supply other than the segmental supply *via* the dorsal nerve. The intersegmental paramedian nerves only contain intersegmental collaterals of excitatory motoneurone axons that branch to the segmental transverse muscle and send collaterals anteriorly, posteriorly or in both directions to one or more successive transverse muscles (Fig. 5). The intersegmental collaterals connect to most, if not all, fibres of every muscle they reach, but the density of contacts seems to decrease at increasing distance from the segment of motoneurone origin.

The intersegmental divergence of the TM motoneurons through the paramedian nerves in the middle abdominal segments circumvents the CNS functionally and connects the TMs of the third to eighth segments by a separate pathway. As a consequence, several intersegmental motoneurone branches, in addition to the segmental ones, converge on

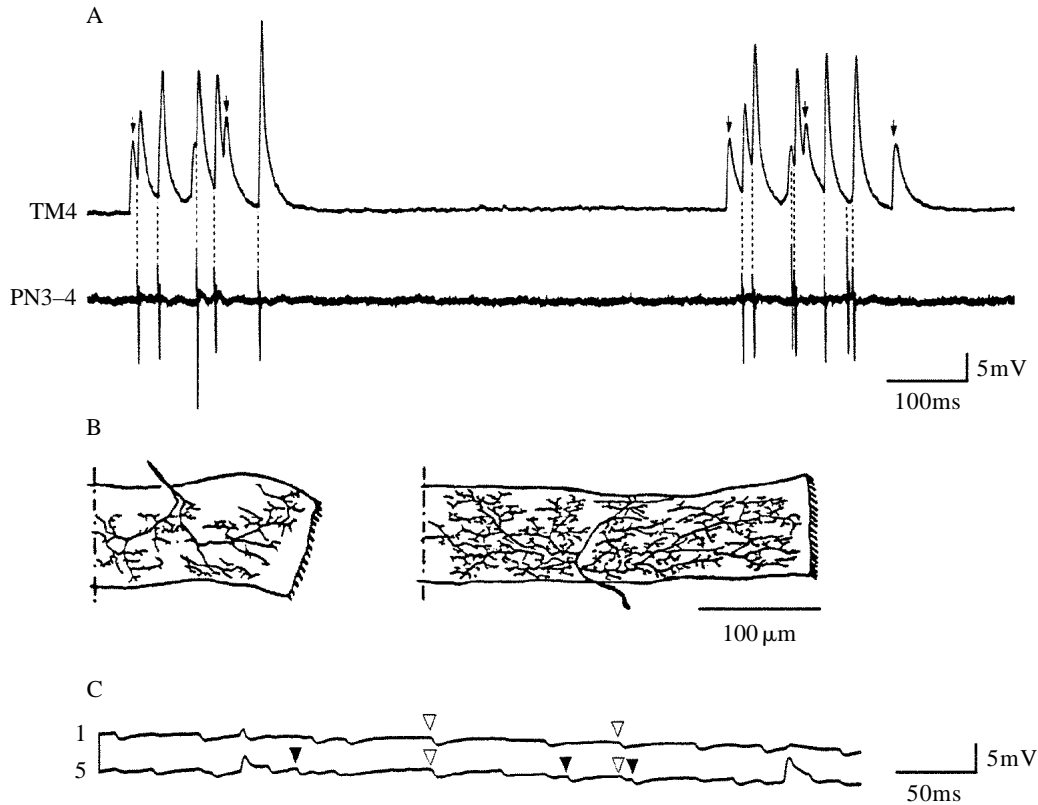


Fig. 3. (A) Recording from a cricket TM4 fibre close to the midline (upper trace) shows EPSPs from the ipsilateral side and corresponding (dotted lines) nerve impulses of the only two efferent units of PN3–4. Additional attenuated EPSPs from the contralateral side (arrows) appear only in recordings made very close to the midline. These EPSPs are very small or absent when recording further from the midline. (B) Details of the innervation of the right part of a bushcricket TM6 after cobalt infusion. The muscle is supplied from nerve branch 1B3 anteriorly (left, muscle relaxed) and from branch 1B1 posteriorly (right, muscle stretched). (C) Paired intracellular recordings from the right half (upper trace) and from the midline (lower trace) of one TM6 fibre in a bushcricket. IPSPs generated by the ipsilateral inhibitory neurone appear to be in synchrony (open triangles); those from the contralateral inhibitor are seen only in the midline (filled triangles).

each transverse muscle (TM3–TM7) (Fig. 5). Conduction speeds in these axons are  $0.35\text{--}0.4\text{ ms}^{-1}$ .

Stimulation of the terminal branches of motoneurons from a single muscle demonstrates that muscles innervated by the same motoneurone contract in synchrony. Neuroanatomy and electrical stimulation showed that the innervation patterns of the transverse muscles include (i) exclusively heterosegmental motoneurons to TM8 from the neighbouring segmental neuromere (seventh abdominal neuromere) in female crickets; (ii) a bilateral homosegmental supply to TM2 from its second abdominal neuromere in the metathoracic ganglion; and (iii) supply by the homosegmental and three



additional heterosegmentally converging pairs of motoneurons to TM6, totalling eight different motoneurons for this single transverse muscle. This complex intersegmental innervation pattern raises the question of how efferents to these muscles are coordinated in the abdomen of crickets.

#### *Additional motoneurons in Decticus albifrons*

The innervation pattern in the bushcricket *D. albifrons* differs from that in the cricket. Each of the five adjacent mid-abdominal transverse muscles TM3–TM7 is innervated bilaterally by both an anterior and a posterior pair of nerve branches. Transverse muscle 6 (TM6), for example, is supplied by the pair of N1B1 branches of the sixth abdominal ganglion (AG6) and by the pair of N1B3 branches arising from the next anterior ganglion (AG5) (Fig. 1B). TM2, the most anterior transverse muscle, is supplied only by N1B1 branches of the second abdominal ganglion, whereas the most posterior muscle (TM8) receives only the N1B3 branches from the seventh abdominal ganglion (Fig. 1B). The sixth transverse muscle was selected as typical of a mid-abdominal segment. Backfilling of posterior nerve 1B1 of TM6 (see inset in Fig. 6) reveals median and contralateral neurones in the sixth abdominal ganglion. The contralateral neurone is homologous to one of the cricket neurones, as described above. Backfilling of the anterior N1B3 branch reveals a contralateral motoneurone in each of the next two anterior ganglia (AG5 and AG4, Fig. 6). One motoneurone, which is homologous to the cricket transverse muscle motoneurons, has an axon that bifurcates in different directions *via* N1B1 to the segmental TM and *via* N1B3 to the posterior TM on the same side (see inset of Fig. 6).

The axons of contralateral motoneurons located in the fifth and fourth abdominal ganglia, innervating the right half of TM6 (see inset in Figs 6, 7), pass through N1B3. This makes it possible to record their action potentials extracellularly (Fig. 7A), to identify the motoneurons physiologically (Fig. 7) and to stain them intracellularly. Paired recordings reveal that the tonically activated neurone is located in the fourth abdominal ganglion in a medio-posterior position (Fig. 7B). Its cell body lies ventrally, its main dendritic field dorsally, while its axon descends through the connective to the next posterior ganglion (AG5) from where it branches to the segmental muscle (TM6) *via* N1B3. This tonic motoneurone is rarely active during normal or strong ventilation, but it is active during shallow ventilation or after the connectives have been severed anterior to the fourth abdominal ganglion. By homology with cricket transverse motoneurons, the bushcricket's anterior contralateral motoneurone innervates TM5 and TM6, and its large-amplitude action potentials may be recorded from N1B3 (Fig. 7A). It fires in a typical bursting pattern during expiration (Fig. 7C).

Cobalt fills and stimulation of peripheral nerves 1B1 and 1B3 of all of the segments revealed the complete innervation plan of all transverse muscles in *D. albifrons* (Fig. 5B).

#### *Activity and coordination of the Gryllus bimaculatus transverse motoneurons*

Myograms from a transverse muscle and a dorsoventral ventilatory muscle in the same abdominal segment showed that the transverse muscles are active mainly during expiratory pumping movements and are relatively inactive in the intervening periods (Fig. 2B). The muscles usually relax during inspiration and during the ventilatory pause.

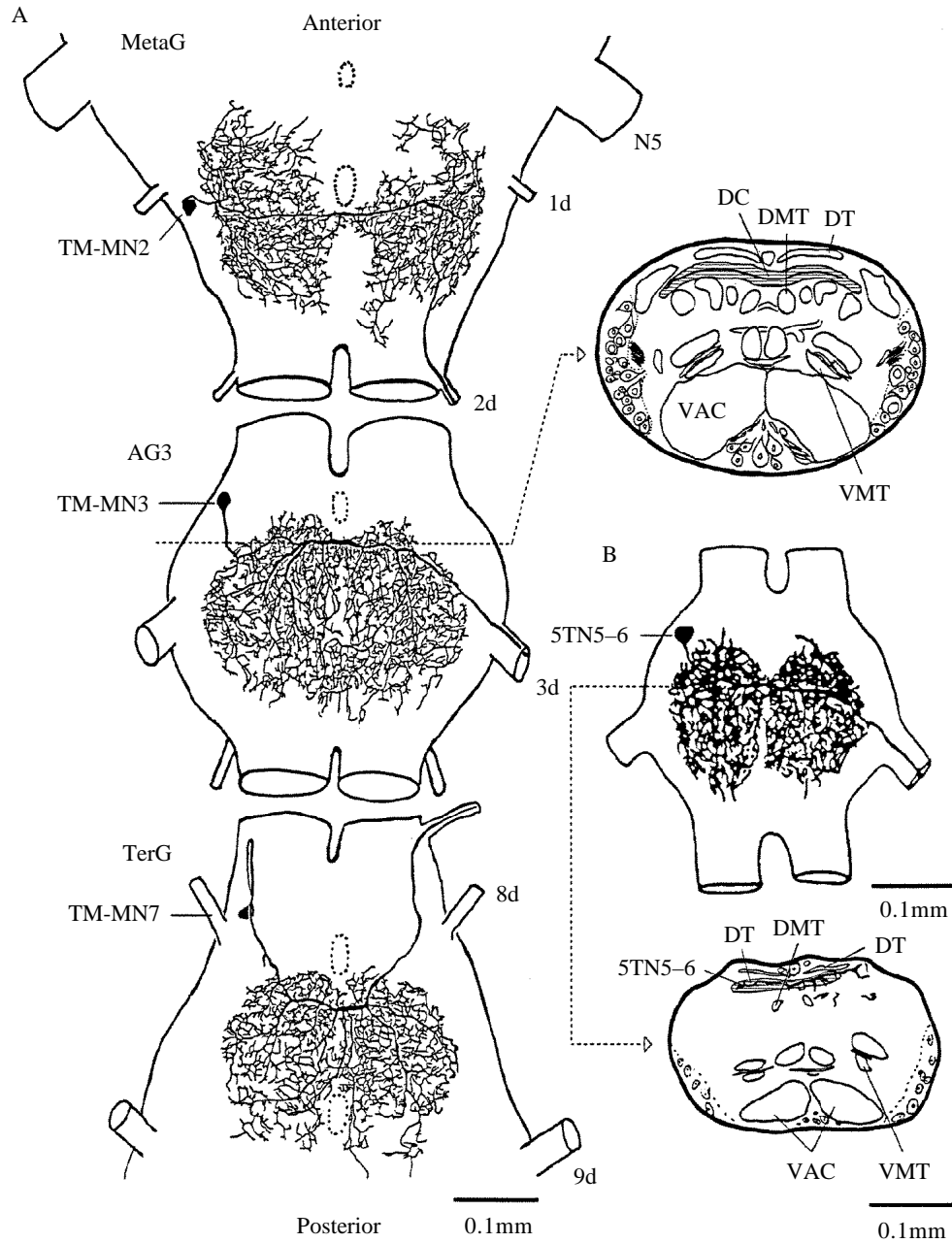


Fig. 4

During natural ventilation, the expiratory bursts are well synchronized in all abdominal ganglia. The convergent bursts of motor output therefore superimpose at each TM of this area in reasonable synchrony (Fig. 8), so there is no effective time lag between the efferent bursts transmitted from the metathoracic centres through the segmental ganglion

Fig. 4. (A) Location and central projections of cricket transverse muscle motoneurons (TM-MN2 to TM-MN7) in their corresponding segmental ganglion or neuromere. The typical pattern for all unfused abdominal ganglia is shown for the third abdominal ganglion (AG3), with a contralateral ventral soma, postero-dorsal branching in both hemiganglia, and a primary neurite traversing through the anterior dorsal commissure (DC in the right-hand inset of the ganglionic cross section, drawn at the level of the arrow from AG3. DMT, dorso-median tract; DT, dorsal tract; VMT, ventro-median tract; VAC, ventral 'association centre'). The axon leaves the ganglion through the dorsal nerve root (3d). The projection pattern of the motoneurone to the second transverse muscle extends into both the first and second abdominal neuromeres of the metathoracic ganglion (MetaG). In the anterior neuromeres of the terminal ganglion (TerG), the motoneurone of the seventh transverse muscle branches into both the seventh and eighth neuromeres. (B) The homologous excitatory motoneurone (5TN5-6) of the bushcricket in the fifth abdominal ganglion stained intracellularly with cobalt. The inset below shows a cross section at the level indicated by the arrow with the motoneurone's neurite in the dorsal commissure (tracts named as in inset A). Some other branches of the neurone have been omitted for clarity.

and those signals that have travelled intersegmentally for a longer distance through the paramedian nerves. The overall frequency of incoming motor impulses is highest in TM6 because it has the greatest convergence of motoneurons. The intersegmental convergence on muscles causes an increased frequency of incoming motor spikes. Additionally, during expiratory bursts, temporal and spatial summation, and possibly mutual facilitation of PSPs by different motoneurons, occur on multiply innervated transverse muscles.

Only EPSPs were recorded from any cricket TM. Their amplitudes at different frequencies of motoneurone discharge (both from a single or from several converging motoneurons) often showed signs of facilitation (Fig. 9A,D), but occasionally a slight decrease in amplitude was seen (Fig. 9C). In simultaneous extracellular recordings from the paramedian nerve and intracellular recordings from transverse muscles innervated by just two ipsilateral efferents, large differences in the amplitude of EPSPs caused by two motoneurons could be demonstrated (Fig. 9A).

#### *Activity and coordination of the Decticus albifrons transverse motoneurons*

In *D. albifrons*, the entire set of transverse muscles contracts rhythmically to cause expiration. Most of the muscle fibres are controlled by bursts of EPSPs during expiration and IPSPs during inspiration (Fig. 10A). During regular ventilation many EPSPs summate in TM5 fibres (Fig. 10A), but during shallow ventilation the EPSPs may be separated into three amplitudes (Fig. 10B). In neighbouring TMs, subsets of EPSPs correspond 1:1 because they are generated by the same bifurcating neurone, i.e. the anterior contralateral neurone of the fifth abdominal ganglion. Nerve 1B3 contains only the axons of the two excitatory contralateral motoneurons (Fig. 7). Paired recordings from nerve branch 1B1 and the ipsilateral TM5 fibres reveal (i) that the large action potentials recorded from N1B1 generate one of the two group of EPSPs (Fig. 10D); (ii) that EPSPs without corresponding action potentials are generated by the motoneurone that enters *via* nerve branch 1B3 (Fig. 10D); and (iii) that the smaller-amplitude action potentials generate IPSPs (Fig. 10 C,D). From these recordings and the total number of

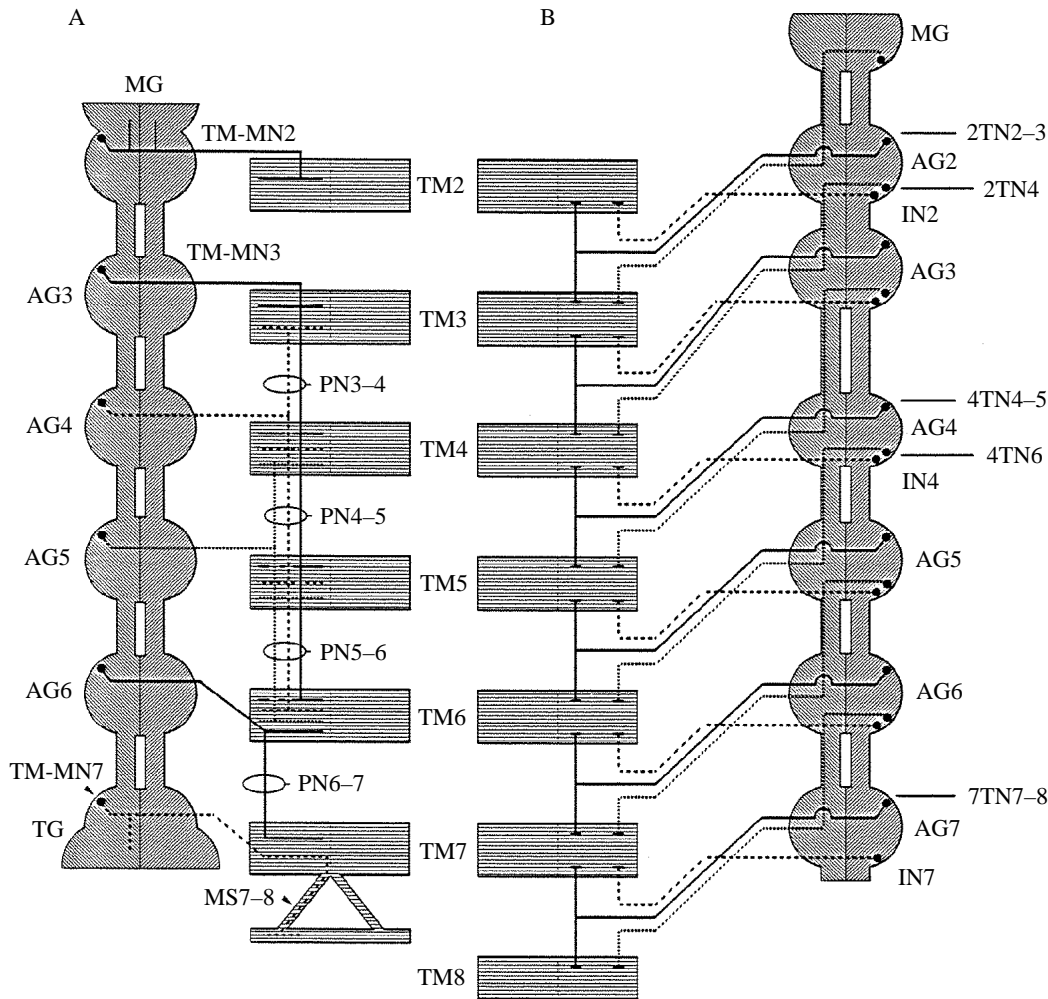


Fig. 5. Schematic comparison of the motoneurone supply to the transverse muscle system (TM2–TM8) of a cricket (A) and a bushcricket (B). For clarity, the innervation is shown for just one half of each muscle (TM2–TM8). The CNS, which contains the neurone somata, naturally lies below the TMs, but is indicated laterally. The types of motoneurones in the cricket (TM-MN2 to TM-MN7) and in the bushcricket (1TN3 to 6TN8, 2TN23 to 7TN7–8, IN2 to IN7) are labelled for one anterior, one posterior and one middle ganglion. The ipsilateral paramedian nerves (PN3–4 to PN6–7) cross the segmental borders to adjacent segments. MS7–8, muscle strand connecting TM7 and TM8.

effluent neurones to TM6, it is obvious that the posterior and anterior contralateral neurones are excitatory and the median ones are inhibitory.

The excitatory bifurcating motoneurones are continuously active, and their firing pattern and coordination may be recorded from their efferent axons in the appropriate N1B3 branches. Recordings from left and right N1B3 branches show that, although the action potentials do not correlate 1:1, they discharge at similar frequencies during

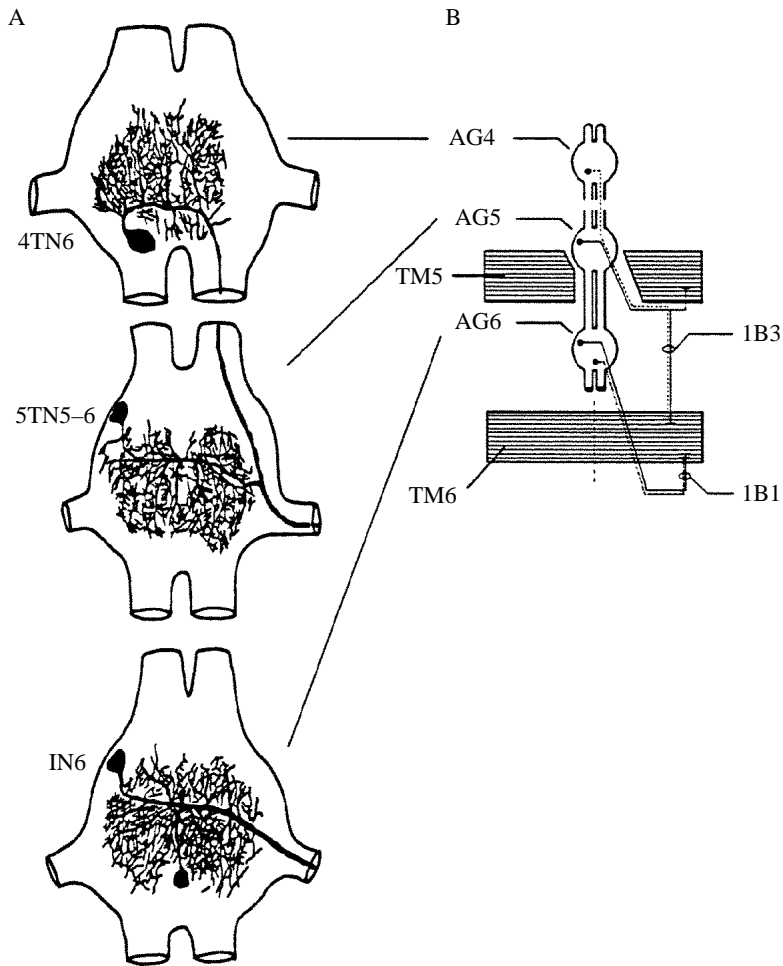


Fig. 6. Origin of efferent neurones converging on one half of TM6 in the bushcricket (B) revealed by cobalt backfilling from the nerve branches to TM6 (A). Backfilling of 1B1 reveals two neurones in AG6. Two other neurones are stained in the two anterior ganglia (AG5 and AG4) after backfilling from branch 1B3. Neurones 4TN6 and IN6 innervate only TM6, whereas 5TN5-6 also innervates TM5.

ventilatory bursts (Fig. 11A). The effect of a bifurcating motoneurone on the contractions of TM was studied in the sixth transverse muscle. One side of TM6 was attached to a tension transducer and both N1B3 branches and the right N1B1 branch were cut off so that it was possible to study the effect of the only remaining active contralateral bifurcating motoneurone. The rhythmical contractions of the muscle followed the rhythmical activation of this neurone alone and correlated well with the summing EPSPs (Fig. 11B). Single EPSPs did not induce contractions here but may increase the tension of the muscle between the expiratory phases.

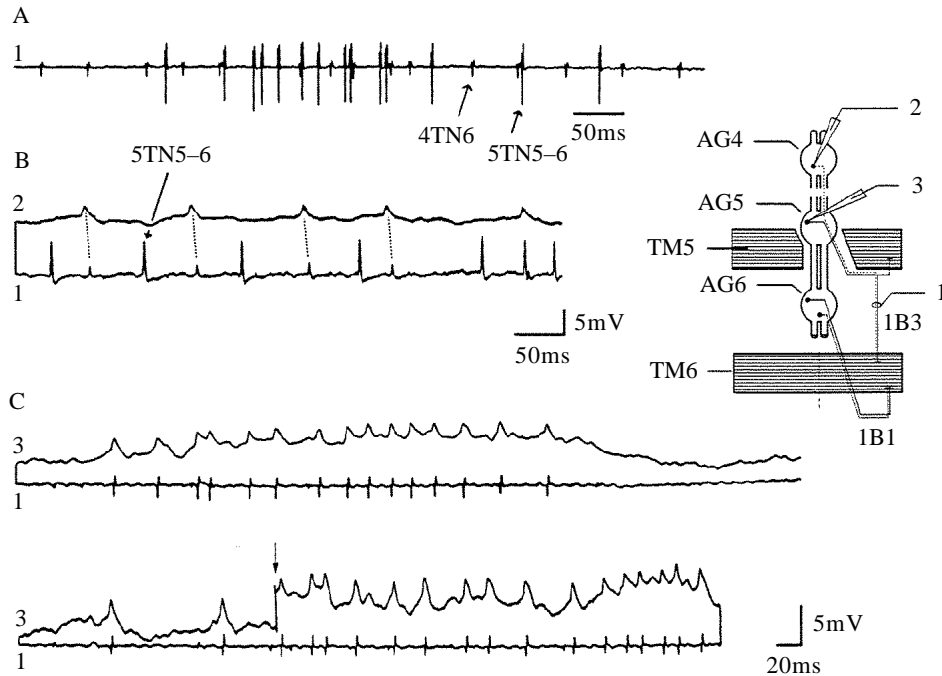


Fig. 7. Characterisation of the motor supply to the bushcricket's TM6. (A) The activity of two motor units (4TN6 and 5TN5-6) in nerve 1B3. (B) Paired intra- and extracellular recordings from the soma of 4TN6 and branch 1B3 (stippled lines and sites 2 and 1 in inset). This tonic motoneurone does not usually discharge during ventilation, but it is active after the connectives anterior to the fourth abdominal ganglion have been severed. One 5TN5-6 spike is marked by an arrow. (C) Ventilatory bursts seen in the discharge of motoneurone 5TN5-6 in paired intra- and extracellular recordings (solid lines and sites 3 and 1 in inset). In the lower trace, intracellular depolarisation (at the arrow) increases the discharge rate.

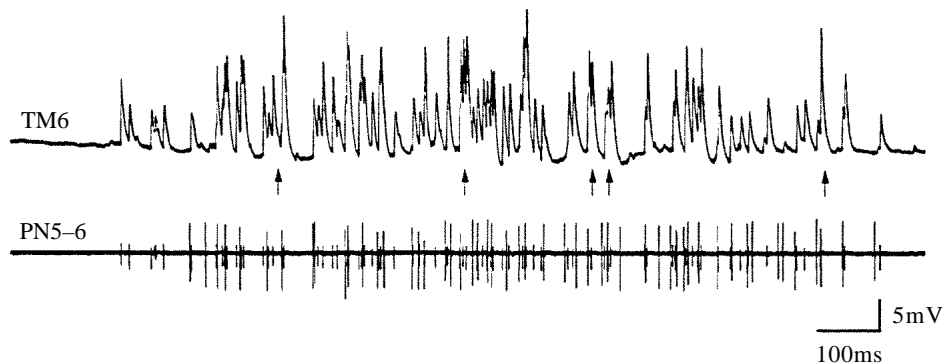


Fig. 8. Convergence and EPSPs elicited by several efferent axons converging on the sixth transverse muscle (TM6) via the anterior paramedian nerve (PN5-6) during intense ventilatory bursts. Note the frequent summation of EPSPs from different sources (arrows).

## Discussion

### *Comparative aspects*

Topographical criteria characterize the transverse muscles of *G. bimaculatus* and *D. albifrons* as being homologous to other hyperneural muscle systems of orthopteran sternites, such as the ventral diaphragm of the locust (Albrecht, 1953) or the hyperneural muscles of the cockroach (Shankland, 1965). However, the muscular structures of the cricket and bushcricket systems show remarkable differences and similarities in their morphology, insertion sites, dimensions and position in relation to the nerve cord. Differences are also seen in the way these muscles are innervated. Yang and Burrows (1983) have suggested that the ventral diaphragm of the locust, in the first abdominal segment, is innervated by six pairs of neurones whereas the other segments each have two pairs of motoneurones located in two adjacent ganglia (Peters, 1977; M. Ferber, personal communication). The hyperneural muscles of cockroaches are innervated by an unknown number of neurones with axons in the median nerve (Hertel and Penzlin, 1986). Since there is no information available about the origin and development of the above systems, one could claim that, although these muscles occupy similar positions in the abdominal segments, they are not homologous and therefore have different innervation. This would not apply to the differences in the innervation patterns of the transverse muscle systems of the cricket *G. bimaculatus* and the bushcricket *D. albifrons*. In both species, these muscles fulfil the morphological criteria of homology because (i) both occupy the same position in the segment, running across the nerve cord, (ii) both are supplied by nerve branches running through the tergal nerve of each abdominal ganglion; and (iii) both have the same function, participating in ventilatory pumping movements of the abdomen. If these serially homologous muscles have evolved from a common ancestor, one would expect that they should be innervated by homologous neurones. The results presented here only partly confirm this hypothesis. In *G. bimaculatus*, six pairs of serially homologous excitatory motoneurones innervate the transverse muscles, while their axons diverge to supply different (up to four) transverse muscles. In *D. albifrons*, each transverse muscle, with the exception of the first and last, is innervated by three pairs of excitatory and one pair of inhibitory neurones. The axon of only one type of excitatory motoneurone divides to innervate two adjacent transverse muscles. This motoneurone appears to be homologous to the cricket motoneurone as far as the location, intraganglionic architecture and type of EPSPs generated in the fibres are concerned. This neurone, therefore, seems to be the only one common to the innervation of both systems. The inhibitory neurones specifically and exclusively innervate the transverse muscles. Four other groups of segmental muscles in *D. albifrons* (ventral and dorsal intersegmentals and first and second dorsoventrals) are innervated by different inhibitors (Consoulas, 1990; Consoulas and Theophilidis, 1992). Moreover, inhibitory innervation has never been described in other hyperneural muscle systems.

Because of the presumed close common ancestry of the cricket and bushcricket lines, it is not possible to evaluate which system is more advanced. Further clues may be gained from the TM system in 'primitive' crickets, such as Stenopelmatidae (Jerusalem crickets), that receives inhibitory input on TM muscle fibres as do bushcrickets (C.

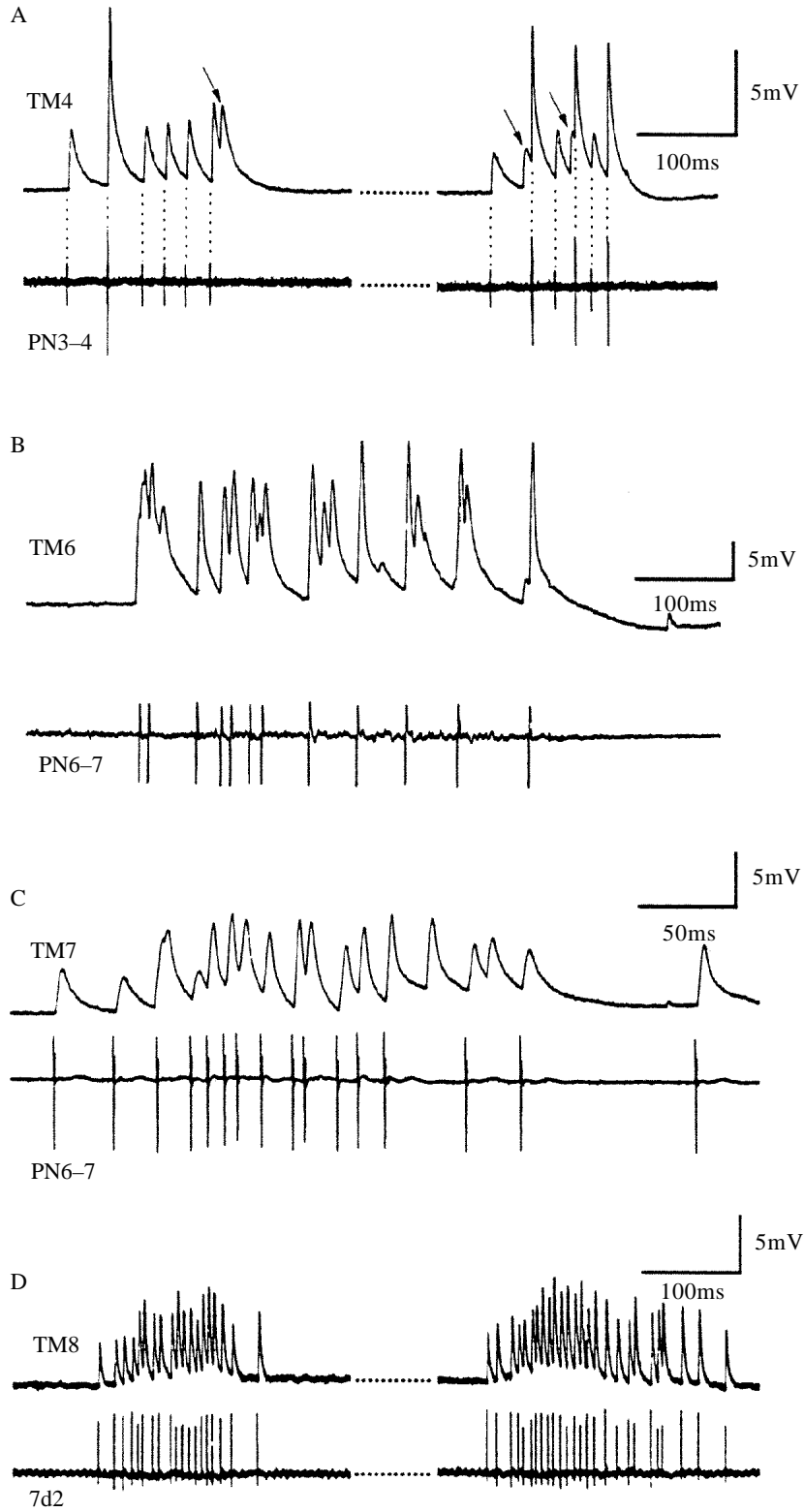


Fig. 9



Fig. 9. Different types of efferent convergence and EPSP amplitudes in fibres of several cricket transverse muscles. (A) Recordings from TM4 (three motoneurons per half) and its anterior paramedian nerve (PN3–4) containing two efferents from the third and fourth abdominal ganglia and corresponding EPSPs (dotted lines). Arrows indicate EPSPs elicited by axons in the posterior nerve. (B) Recordings from TM6 (four motoneurons per half) and its posterior paramedian nerve, which contains just one axon (PN6–7). EPSPs summate with those from anterior motoneurons. (C) Recordings from TM7 (two motoneurons per half) and its anterior paramedian nerve (PN6–7, one axon). (D) Recordings from TM8 (one motoneurone per half) and the nerve branch arising from the seventh dorsal nerve (7d2) that also supplies TM7.

Consoulas and R. Hustert, unpublished observation). This animal has morphological features of both bushcrickets (fused second and third abdominal ganglion separate from the metathoracic ganglion and just one lateral nerve root from each ganglion) and crickets (clavate hairs on the cerci) (C. Consoulas and R. Hustert, unpublished observations).

The differences in the TM structure and innervation in crickets and bushcrickets may be required by differences in the functional design of their anatomy: *G. bimaculatus* has a completely sclerotized sternite, *D. albifrons* has only muscular attachments sclerotized in

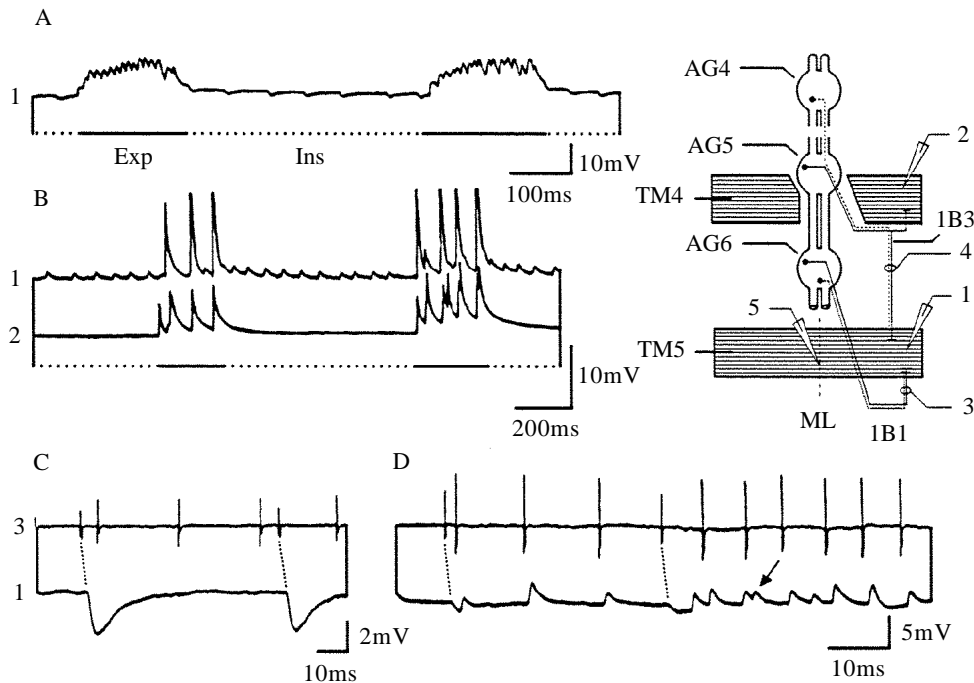


Fig. 10. The occurrence of EPSPs during expiration (solid lines) and IPSPs during inspiration (dotted lines) in transverse muscles of the bushcricket (numerals on the recordings indicate electrode positions shown in the inset). (A) Recordings of EPSPs and IPSPs from TM5. (B) Paired recordings from TM4 and TM5, showing partly synchronized discharge (from motoneurone 4TN4–5). (C,D) Paired intra- and extracellular recordings from TM5 and N1B1 relating IPSPs in TM5 to their origin from the inhibitory neurone 5TN6 (dotted lines). In D, an arrow marks one of the EPSPs arising from an axon in nerve branch 1B3.

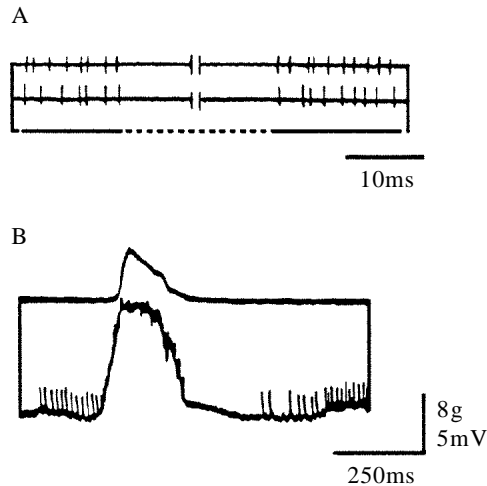


Fig. 11. Ventilatory effects in the bushcricket. (A) Synchronisation of discharge during expiration (solid line) seen in extracellular recordings from left and right motoneurons 5TN5-6 innervating TM6. (B) Intracellular recording (lower trace) from the left end of a TM6 fibre and a tension recording (upper trace) from the right end of a muscle attachment to the same fibre during an expiratory burst. Contractions are seen only during higher-frequency summing EPSPs.

this region of the abdomen. In bushcricket, active ventilation is achieved mainly by narrowing the abdominal segments in the pleural region and, to a lesser extent, by lifting the sternite region. Both types of ventilatory movements require strong involvement of the TMs during expiration to pull the tergal regions towards each other. This is favoured by the less sclerotized sternites of *D. albifrons*.

#### *Natural functions*

We have analysed the basic functions of the transverse muscles and of their peculiar nervous supply in the orthopterans *G. bimaculatus* and *D. albifrons*. Their role in shaping and stabilizing the sternite during ventilation is obvious, for they usually contract in phase with the expiratory dorsoventral muscles. During expiration, the dorsoventral expiratory muscles in a segment and the interconnecting transverse muscle make up a muscular sling that, in addition to bending and lifting the sternite, stabilizes or narrows the tergite.

In crickets, the expiratory discharge rates in ventilatory motoneurons that arise from each abdominal ganglion are not strictly proportional to each other, indicating that, for the expiratory and transverse muscles, there is no common final output in a segment. Similarly, transverse muscle discharge can differ to some extent from one segment to another. The rate of contraction in a segment may also depend on internal conditions, such as the volume of haemolymph or the presence of more solid material (gut, reproductive organs) that requires adaptability in the compression rate of a segment during ventilation. These specific needs in each abdominal segment may vary according to the variable volumes of the gut contents and genital products. The gut and gonads do

not end at the segmental borders and so proprioceptors should regulate each segment and its vicinity. Correspondingly, in most insects, many primary mechanosensory afferents in the abdomen extend into several ganglia (Zawarzin, 1924; Hustert, 1978). In contrast, most afferents from the legs reach the ipsilateral hemisegment only (Pflüger *et al.* 1989).

In bushcrickets, which have a very soft sternal region, pressure caused by ventilation and the weight of gut and reproductive organs spreads directly to most of the other segments. This may require a high degree of synchrony of discharges at the common final output to all transverse muscles. The morphological (body shape) and functional (the way the abdomen pumps during ventilation) differences observed between the two species could reflect the differences found in the innervation pattern of the transverse muscles.

#### *Sensory control in the Gryllus bimaculatus transverse muscle system*

Stress on the sternites is monitored by campaniform sensilla located in the cuticle and by the chordotonal organ that spans its flexible sutures (Gnatzy and Hustert, 1989). Additionally, pleural chordotonal organs of cricket segments, homologous to those of locusts (Hustert, 1974), can record the strain on the extending pleural folds when the segment expands dorsoventrally (R. Hustert, unpublished results). Since most abdominal afferent axons extend into the segmental and neighbouring ganglia, direct proprioceptive information about the volume of a segment is available for local control and also diverges for the coordination of transverse and other muscles in neighbouring segments. This may result in a lower 'drive' for a transverse muscle contraction in a segment that contains bulky tissue than for a neighbouring normal segment, even if high motor output converges from other ganglia. A comparable mechanosensory control function is achieved by the multipolar sensilla of the locust ventral diaphragm, which respond more strongly when the diaphragm is loaded (Hustert, 1975). In that system, the transverse sternal muscles (ventral diaphragm) contract during inspiration and are not synchronous with expiration as are the TMs in crickets (Fig. 2).

#### *Integration at the peripheral level*

Direct motoneurone supply to different muscles of insects has so far only been described for common inhibitory neurones (Hale and Burrows, 1985). Several examples are known of individual insect wall muscles being innervated by several excitatory motoneurones converging from two adjacent ganglia or neuromeres (Lewis *et al.* 1973) or for visceral musculature (Klemm *et al.* 1986) and genital muscles (Hustert and Topel, 1986; Heckmann and Hustert, 1989; Hustert, 1980). However, convergence from several segmental ganglia is unusual. Could there be a specific functional reason for this? An explanation is required for the high convergence of the segmental plus intersegmental motoneurone supplies onto the transverse muscles of most abdominal segments, especially in crickets. The rate and strength of muscle contraction are increased by this convergence: several motoneurones discharging in the same expiratory bursting period produce a higher frequency of EPSPs in a TM than if only the segmental efferents are active in bilateral synchrony. In bushcrickets, single EPSPs in a TM are not able to elicit contractions. In crickets, the large gut and genital contents present in the middle abdominal segments require a larger variability in segmental volume and a larger range of

motor control during expiratory compression of these segments. The high convergence of motoneurons onto the transverse muscles in the fourth, fifth and sixth abdominal segments supports this view.

*Why is there a specific peripheral motoneurone pathway?*

Why should specific peripheral pathways bridging several segments be required for transverse muscle motoneurons? Why do these motoneurone collaterals not use the CNS as a pathway? Only developmental reasons come to mind. Collateral branching of the motoneurons within the CNS and through the connectives may not be possible during development. Developmental conditions and/or functional requirements apparently favour collateral sprouting in the periphery. As a result, the TM motoneurons that diverge to different segments in the periphery can receive synaptic inputs only in their segmental ganglion of origin.

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