

# Rapid mechano-sensory pathways code leg impact and elicit very rapid reflexes in insects

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*Accepted 13 May 2003*

## Summary

The temporal sequence of mechanoreceptor input arriving at the motoneuron level in the central nervous system (CNS) after distal mechanical contact was studied for the locust middle leg. Different types of afferent information from potential contact areas after selective stimulation showed propagation times of no less than 8 ms from mechanosensory hairs, campaniform sensilla (CS) and spurs of the distal leg segments. Impact of the same mechanical stimuli, even if very delicate, elicits strain that is transferred in less than 1 ms *via* the cuticle and stimulates proximal CS on the trochanter and femur. These propagate the afferents that code distal leg contact in about 1 ms to the CNS, where they connect mono- and polysynaptically to motoneurons of the depressor

trochanteris system. The elicited excitatory postsynaptic potentials (EPSPs) contribute to rapid efferent commands, since single EPSPs already rise near firing threshold of the motoneurons. The short delays in this mechano-neuronal-muscular pathway from the tip of a leg to the neuromuscular synapses (5–7 ms) can very rapidly raise muscle tension in the trochanteral depressors at new leg contacts during locust landing and locomotion. At substrate contact, proximal leg CS contribute to very rapid motor responses supporting the body.

Key words: mechanosensory afferent, leg impact, timing, motor response, landing, locomotion, locust, *Schistocerca gregaria*.

## Introduction

In standing insects, continuous muscular activity of the leg muscles must support the body in order to keep it off the ground or other substrates. When insects walk or land after jumping or flying, increased muscular tension must compensate almost instantly for the rise of load after a leg contacts the substrate. During walking, the muscles that press the legs against the ground must be activated at the transition from the swing to the stance phase of stepping with minimal delay. As shown for walking locusts (Laurent and Hustert, 1988), mechanoreceptors of the tarsus usually record the first leg contact that terminates the swing phase, but afferent signals from locust tarsal sensilla proceed to the central nervous system (CNS) in 8–14 ms. Further synaptic transmission in the CNS that could initiate motor reflexes requires about 2 ms when transferred directly to motoneurons. Their efferent commands are propagated to the neuromuscular end plates within 1–5 ms, and muscle tension can only increase after another 5–20 ms, depending on the contractile status of the muscle. This sequence of delays leads to extremely long response times and is incompatible with the need of grasping for substrate when landing after jumping or flight. During walking on uneven ground and at higher speeds (step frequencies up to 9 Hz; Burns, 1973), smooth swing-to-stance transitions could be impeded if the locust relied solely on the pathways from tarsal sensilla. For step-by-step movement

control, sensorimotor transfer from mechanoreceptors to target muscles should not exceed 10 ms.

Previously, it was hypothesised (Wilson, 1965) that insects stepping at high frequencies, such as cockroaches or flies, might only use an integrated signal from all leg afferents for the sensory control of walking rather than the detailed signals of contact, load and joint angles in each leg during a step. By contrast, Jindrich and Full (2002) postulate that deviations of a running cockroach from its path are compensated mainly by viscoelastic components in the skeletomotor system of the legs. This mechanism would prevail over sensorimotor responses, with delays in the range of 10–15 ms for cockroach motor responses, as found by Ridgel et al. (2001) after stimulation of tibial campaniform sensilla (CS). However, if leg contact is monitored by proximal CS, the delay for reflex support and load compensation may be reduced sufficiently to allow neural properties to affect movement even at relatively rapid walking speeds. To test this hypothesis, we studied the timing and cooperation of distal and proximal mechanoreceptors for the middle legs of locusts, which basically perform a rowing-type movement about an axis transverse to the body during walking (Hustert, 1983). In these legs, the depressor muscles support the ipsilateral half of the body regularly when the animal walks in tripod gait. As an indicator of the speed of sensory information processing and of reflex convergence, we selected

motoneurons of the tripartite depressor trochanteris muscle (M103; Snodgrass, 1929). This muscle is in close proximity to the CNS and is most important for keeping the body above ground and therefore should receive the earliest afferent commands available from the periphery.

### Materials and methods

Experiments were performed on adult locusts (*Schistocerca gregaria* Forskål) of either sex taken from our crowded laboratory colony. Animals were restrained ventral side up in Plasticine™. The middle leg could be restrained independently and positioned for adequate access to the different mechanoreceptors. Parts of the ventral cuticle were removed in order to expose the meso- and metathoracic ganglia as well as thoracic nerves and muscles.

#### Intracellular recordings

A wax-coated steel platform was used to stabilize the meso- and metathoracic ganglia. The ganglionic sheath of the mesothoracic ganglion was treated for 2 min with a 1% (w/v) solution of protease (Sigma type XIV) to facilitate penetration of the ganglion with glass microelectrodes.

Microelectrodes were either filled with 2 mol l<sup>-1</sup> potassium acetate or 1 mol l<sup>-1</sup> lithium chloride when used for later staining with Lucifer yellow (only in the tips), giving a tip resistance of 40–80 MΩ.

The dye was applied iontophoretically by 500 ms pulses of negative current at 1 Hz. Motoneurons were identified by correlating the spikes recorded intracellularly from neuropilar processes, while extracellular potentials were recorded from the efferent nerve 3C2 with a pair of 50 μm steel wires. The M103d fast motoneuron could also be identified as eliciting visible twitch-contractions of M103d upon stimulation (nomenclature of thoracic nerves according to Campbell, 1961).

#### Afferent spike recordings

Spikes from single tibial hair sensilla were recorded by placing a saline-filled microelectrode over the cut shaft of the trichoid sensillum (Hodgson et al., 1955). Afferent spikes from the exteroceptive campaniform sensilla (CS) at the base of each tibial spur were recorded with hook electrodes at the peripheral nerves 5B3 (anterior row of spurs) or 5B4 (posterior row of spurs) located just beneath the ventral cuticle close to the receptors (Mücke, 1991).

In order to record afferent discharges selectively *via* the proprioceptive CS on various leg segments, an electrolytically sharpened tungsten wire was carefully pushed through the dome-shaped structure of the sensillum to make contact with the receptor haemolymph.

Trochanteral groups of CS were recorded in isolated legs with suction electrodes from the proximal stumps of their afferent nerve (5B2a), in which at least one large trachea was opened to the air at the saline surface, while the persistent pumping of the myogenic accessory leg heart of the trochanter

(Hustert, 1999) maintained saline flow in the leg. This expands viability of the preparation from several minutes to several hours.

#### Motoneuron identification

Backfills of motor nerves were made to reveal the innervation of the mesothoracic depressor trochanteris muscle and central branching pattern of each motoneuron. After removal of the thoracic ganglia from the animal, the cut end of the particular nerve was placed in a miniature Vaseline™ well, filled with a near-saturation solution of 3000 M<sub>r</sub> dextrane conjugated with the fluorescent dyes fluorescein isothiocyanate (FITC) or tetraethyl-rhodamine-isothiocyanate (TRITC) (obtained from Molecular Probes Europe, Leiden, The Netherlands). The preparations were immersed in saline and incubated for 24 h at 4°C to allow diffusion of the dye throughout the neurons. After dissecting out the ganglia, they were fixated in 4% paraformaldehyde, dehydrated in ethanol and, after clearing in methyl salicylate, were viewed under a Leitz Aristoplan epifluorescence microscope and drawn or photographed from whole mounts.

#### Receptor stimulation

All receptors were stimulated mechanically. In the case of the hair sensilla, a blunt microelectrode was glued to a piezoelectric tongue driven by a function generator and mounted onto a micromanipulator. Ramp-like deflections were used to stimulate the hair sensilla. The tibial spurs were deflected by a minuten pin fixed to the piezoelectric tongue. The proprioceptive CS were stimulated directly by applying pressure perpendicular to the surface of the cuticle close to the receptor with a minuten pin. In some cases, the tungsten electrode itself was pushed carefully towards the sensillum to elicit spikes.

In order to define delays between afferent spike generation of two different receptor types, e.g. a tibial spur and a trochanteral CS, a two-channel function generator driving two piezoelectric devices was used for the exact timing of receptor stimulation.

#### Conduction measurements

Afferent conduction times to the CNS were measured by recording extracellularly from a peripheral site of the particular nerve close to the mechanoreceptor and at the main leg nerve 5 where it enters the ganglion. Central latencies could thus be estimated by subtracting this time of spike propagation from the overall delay to the postsynaptic potential (Laurent and Hustert, 1988). In most cases, signal averaging was used.

#### Mechano-sensory conduction

Delays between impact-like tension changes onto the tarsus and first afferent spikes in the proximal CS were measured in middle legs, excised carefully at the thoracocoxal joint. The leg was positioned as in the standing animal, with the coxa, trochanter and femur horizontal and the tibia vertical. Only the coxa was fixed on a small platform ventrally, and dorsal parts

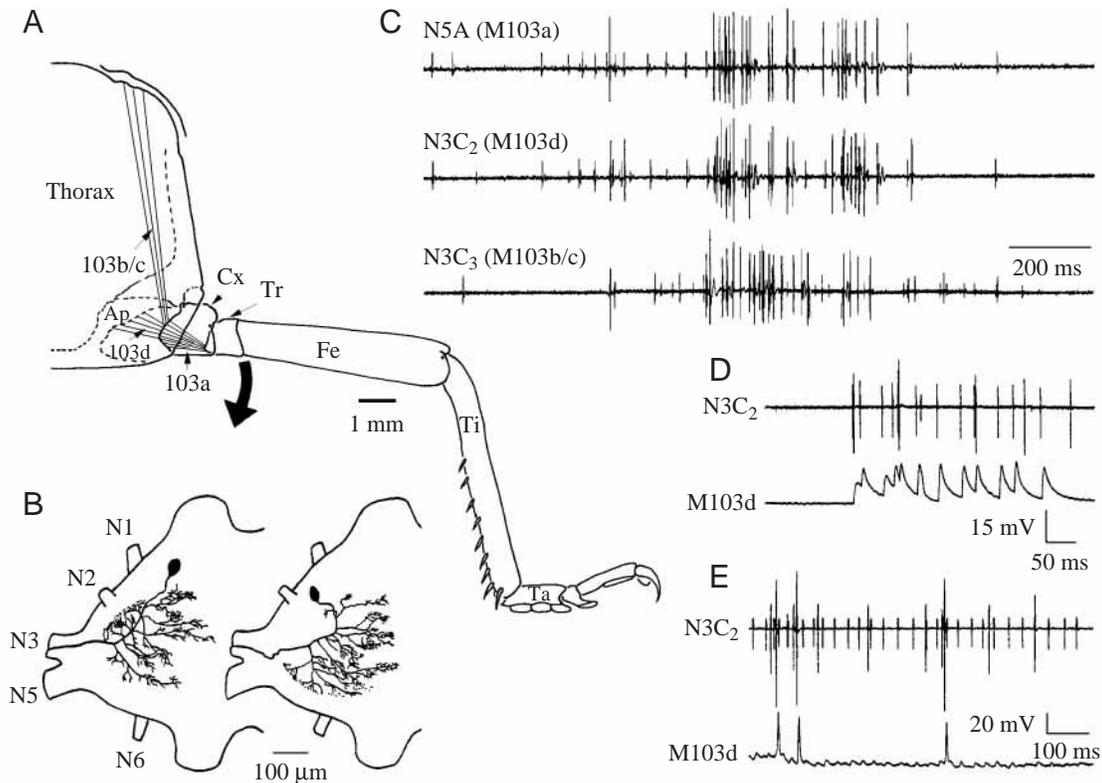


Fig. 1. Features of the depressor trochanteris system. (A) Schematic view of the three main parts of the depressor trochanteris (M103a, M103d and M103b/c) in a cross-section of half of the locust mesothorax. Cx, coxa; Tr, trochanter; Fe, femur; Ti, tibia; Ta, tarsus. (B) Morphology of the fast and slow depressor motoneurons of M103d, viewed in mesothoracic hemiganglia. The nerves are numbered. (C) Simultaneous recordings from the three thoracic nerves that supply the M103 as a whole. Upon stimulation by touching the ventral coxa with a small brush, the motor patterns appear similar, with some tonically active units of small amplitude and bursts of larger amplitude spikes occurring upon stimulation. (D) Intracellular recording of summing excitatory junction potentials (EJPs) in a fibre of M103d resulting from efferent spikes (small amplitudes) recorded from nerve N3C<sub>2</sub>. (E) Intracellular recordings of EJPs in a fibre of M103d evoked by efferent spikes (lower trace) of the fast-depressor motoneuron (upper trace; large amplitude potentials) recorded at nerve N3C<sub>2</sub>.

of the coxa and trochanter levator muscles were removed. The tendon of the trochanter depressor was also pinned with a minuten pin to the platform. This avoided dorsal excursions of the leg when mechanical stimuli directed dorsally at the tarsus were applied by a piezoelectric bender from below.

#### Force measurements

Measurements of the time required for the transfer of force from the tip to the base of a leg were also performed on a fresh, isolated middle leg in the natural positions of still stance. The ventral coxa was mounted onto a force transducer while forces from the tarsus were applied *via* one pad (pulvillus). A piezoelectric tongue (bimorphic piezoceramic strip; Valvo PXE70; Valvo, Hamburg, Germany) with a minuten needle extending from its moveable end was mounted on another force transducer. The strain produced by the piezoelectric tongue during ramp-like deflection (generated by a function generator) was monitored when the minuten pin indented the highly elastic tarsal pulvillus. By mounting the device on a micromanipulator, the tip of the pin could touch the tarsal pulvilli very delicately so that just the area around one of the

canal sensilla (Kendall, 1970) was indented by the stimulus. This strain was sufficient to be recorded *via* the whole leg as a force at the coxa-attached transducer.

#### Recording and analysis

Recordings were displayed on a digital oscilloscope (Hitachi, Fukuoka, Japan) and stored on magnetic tape for later computer analysis by Neurolab 7.0 (Hedwig and Knepper, 1992) and Datapac 2000 (RUN Technologies, Mission Viejo, CA, USA) software.

#### Results

The depressor trochanteris muscle of the middle leg (M103; Snodgrass, 1929) was selected for this study of motor responses as it provides support during the stance phase of walking (Burns, 1973) and compensates for leg impacts on substrates. It comprises three major parts (Fig. 1A), which originate separately (1) on the scutum (M103b/c), (2) ventrally on the pleural apodeme (M103d) and (3) on the ventral coxa (M103a). All parts insert distally on the ventral rim of the

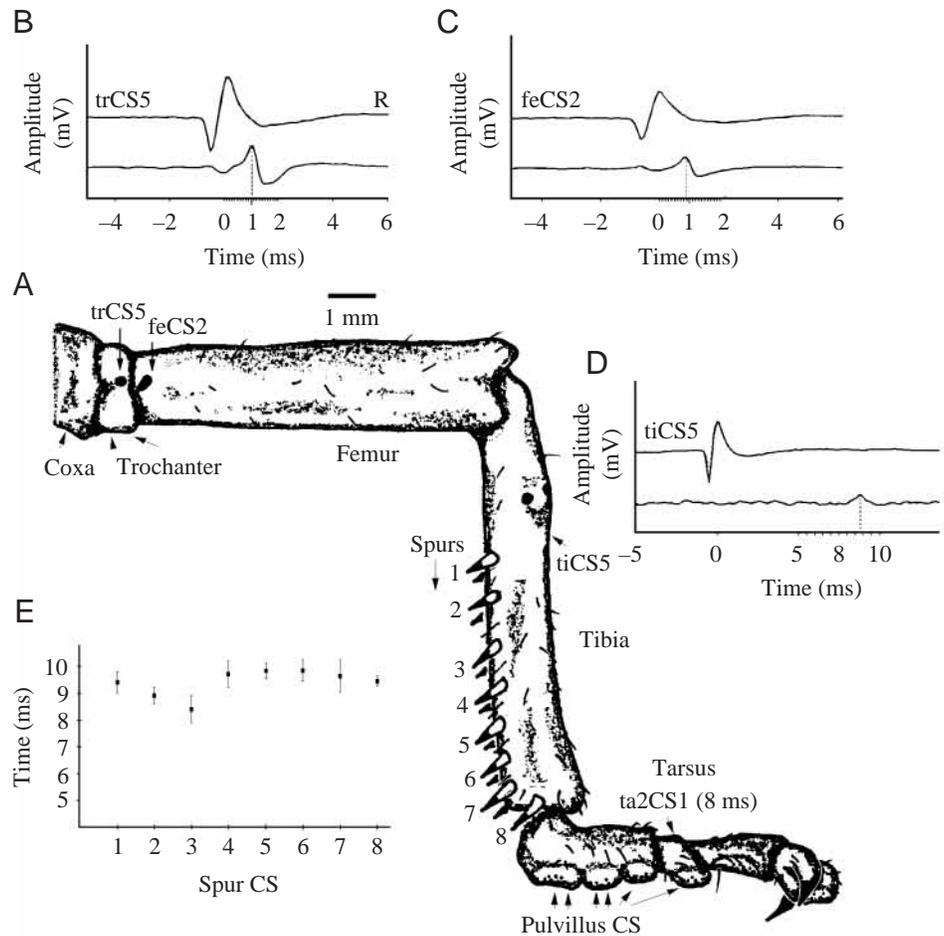


Fig. 2. Afferent conduction times of different mechanoreceptors on the segments of the middle leg of *Schistocerca gregaria*. Signal averages (64 sweeps) are triggered by the action potential from the vicinity of the soma (upper trace) and afferent in the main leg nerve root at the ganglion (lower trace). (A) Posterior overview of leg segments and location of some of their mechanoreceptors. (B) Averages axonal conduction time of campaniform sensilla (CS) afferents from the posterior trochanteral group (trCS5). (C) Averages axonal conduction time of CS afferents from the posterior femoral group (feCS2). (D) Axonal conduction time of CS afferents from the two distal tibial CS (tiCS5). (E) Comparison of axonal conduction times of tibial spur CS afferents (1–8) from the posterior row of receptors (means  $\pm$  S.D.;  $N=5$ ).

trochanter or its proximal tendinous extension. These different parts of the muscle are also supplied by separate efferent nerve branches that carry similar but not identical efferent patterns to the different parts of M103 (Fig. 1C). For our study of time-course and convergence of reflexes, the pleural part, M103d, was selected since its two excitatory motoneurons (Fig. 1B) are most suitable for recording intracellularly while stimulating identified leg mechanoreceptors; in these ‘fast’ and ‘slow’ motoneurons, the membrane potentials usually lie near or above firing threshold for action potentials that clearly show corresponding excitatory junction potentials (EJPs) in most of the muscle fibres (Fig. 1D,E). In addition, M103d also receives innervation from the modulatory neuron DUM3,4,5 (Duch et al., 1999), which also supplies all other branches of M103, and from the inhibitory neuron CI<sub>1</sub>, which supplies only parts M103a and M103d.

#### Afferent conduction times in the leg

In order to see which afferent signals of mechanosensory neurons are the first to reach the CNS after the mechanical contact, conduction times from mechanosensory cells to the CNS were compared for different parts of the leg. Three types of receptors can encode leg contact directly or indirectly (Fig. 2A): (1) tarsal and tibial mechanoreceptive hairs, being bent by touch, (2) a campaniform sensillum (CS) at the base

of each moveable tibial spur and (3) CS, singularly or in groups, on the cuticle of all leg segments but the coxa. All record load or strain from nearby or further away, e.g. when the tarsus or tibia contact the substrate. During locust walking or landing, the tactile hairs that completely cover the tibia or tarsus are often the first to encounter the substrate in structured terrain. Afferent conduction times to the CNS are at least 8 ms and even more than 12 ms from the distal hairs (Fig. 2) as the rather distal long unguis hairs, that have monosynaptic connections to motoneurons of the depressor tarsi (Laurent and Hustert, 1988). None of these seem suitable to convey information on substrate contact rapidly to the CNS. The CS of spines from different locations on the tibia may also be the first sensilla to encounter mechanical contact. Their afferents reach the CNS with a very similar delay of about 8–9 ms, since their individual conduction times compensate for their proximo-distal position on the tibia (Fig. 2E). The CS that are located on the leg cuticle at varying distances to the thorax mainly record strain between the body and the substrate due to gravity or muscular tension (Hustert, 1985). The most proximal of these are the trochanteral and femoral CS, which show the shortest conduction times to the CNS (Fig. 2B,C; in the range of 1 ms).

This observation led us to pursue the hypothesis that, after leg impact on distal segments, the proximal CS afferents could

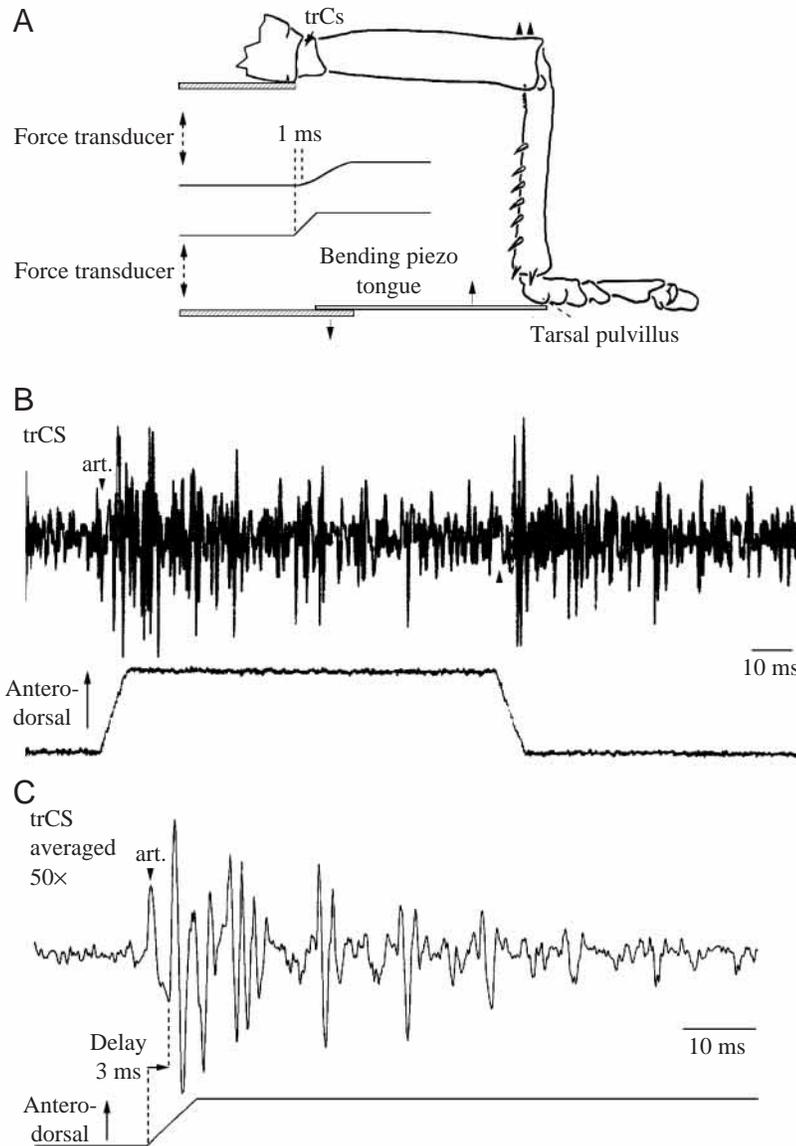


Fig. 3. Force transfer from the tarsus to the coxa of a locust middle leg. (A) A piezo-ceramic tongue bends during a 2 ms ramp (and hold) voltage step and indents the tarsus at one pulvillus with a force of 0.1 mN locally. The connected force transducer records the tension (lower trace in the centre) where the stimulus is applied, while the upper transducer, with the coxa glued onto it, records the transferred tensile force (upper trace in the centre; averaged 64 $\times$ ) after a latency of less than 1 ms. Arrows indicate direction of forces. (B) Recording afferents with a suction electrode from nerve stump from the antero-dorsal groups of trochanteral sensilla trCS1–3 while applying an antero-dorsally directed ramp stimulus (arrow) of approximately 0.1 mN to a proximal (posterior) pulvillus. (C) Recording as in B but over a larger time-scale and averaged 64 $\times$ . The first afferent spikes arise 3 ms after the start of ramp stimulus [arrowheads mark electrical artefacts (art.) from the ramp generator].

depressor motoneurons. In response to the same stimulus, mechanosensory afferents arrive  $\geq 4$  ms later from more distal locations on the leg (Fig. 2).

#### Sensory-motor connections

Central timing and cooperation of afferents from selected single proximal and distal sensilla were studied by recording the postsynaptic responses in the fast and the slow motoneuron of the trochanter depressor M103d.

(1) Afferents from CS of the posterior femur (feCS2; Hustert et al., 1981) and the dorsal tibia (tiCS5; Mücke, 1991) could be recorded from their terminal nerve branches and thus be correlated with events in the M103d motoneurons. The feCS2 afferents have a short central latency below 2 ms before eliciting excitatory postsynaptic potentials (EPSPs) in both slow and fast M103d motoneurons (2.6 ms from the periphery to EPSPs, including the 0.9 ms for peripheral conduction along the peripheral axon). This strongly suggests monosynaptic connections. Spikes are elicited readily in the fast depressor (Fig. 4A), while the EPSPs in the slow depressor remain generally below firing threshold (Fig. 4B). By contrast, afferents from tiCS5 cause inhibition in the fast M103d motoneuron (Fig. 4D) with a central latency of 6 ms (not shown), indicative of a polysynaptic connection.

(2) High frequency (phasic) afferents from CS at the base of posterior spurs elicit EPSPs but rarely summate to reach the spiking threshold of a motoneuron (Figs 5A, 6). The central delay of 3.5 ms before EPSPs arise in both the fast and slow M103d motoneurons indicates polysynaptic connections. By contrast, responses from the anterior spurs were observed only once as low EPSP amplitudes in a slow M103d motoneuron (not shown).

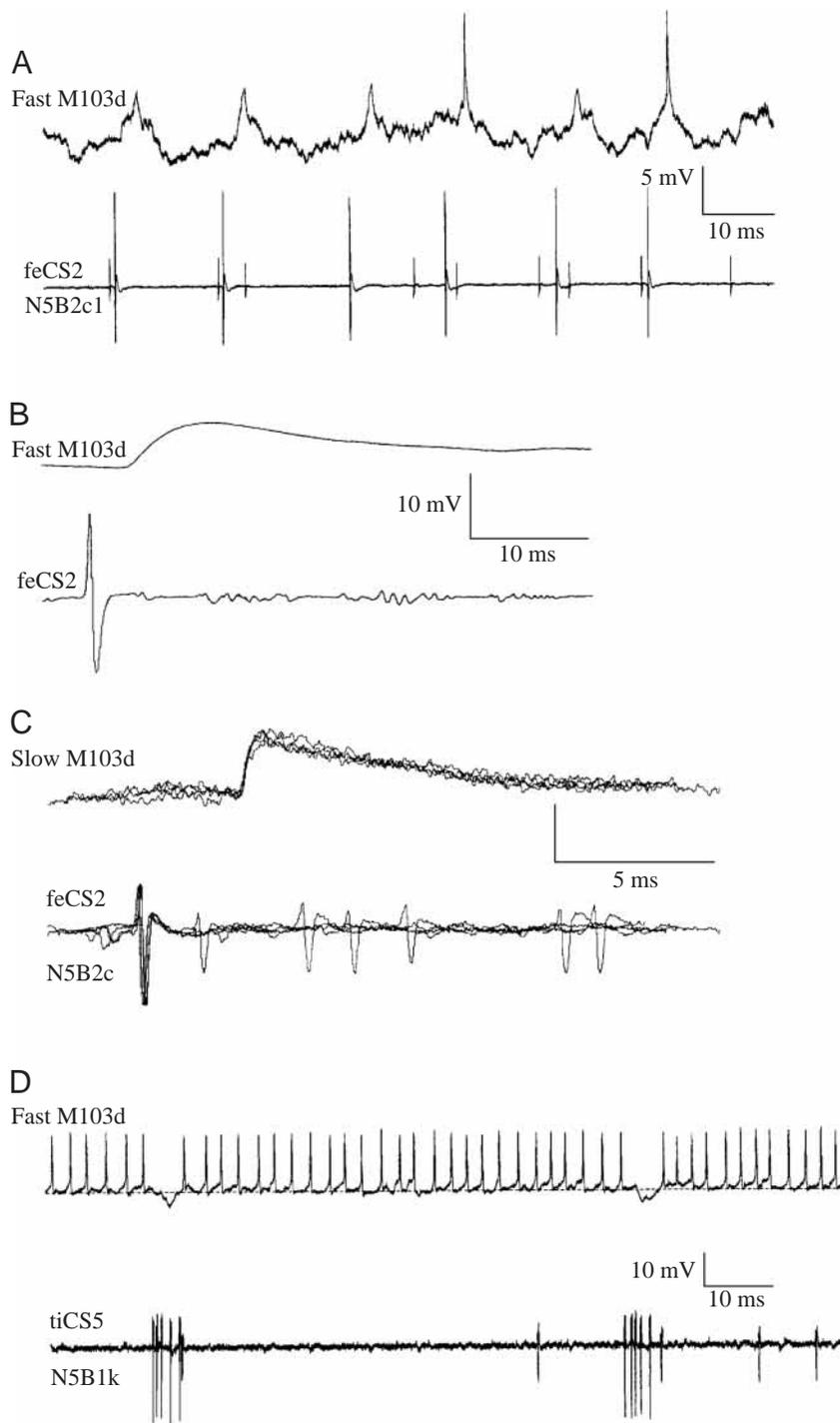
(3) The afferents from tactile hairs between the posterior tibial spurs elicit EPSPs in the slow M103d motoneuron regularly, but their central latency of 5 ms indicates

be the first of all sensory inputs from the leg to reach the CNS upon leg-to-substrate contact due to cuticular strain conducted rapidly in the leg.

#### Force transfer and sensory responses upon leg contact

The transfer of force to proximal leg segments upon slight indentation of a tarsal pulvillus occurs within 1 ms (Fig. 3A). Proximally, such forces are recorded by trochanteral and femoral CS. Responses of trochanteral CS can be recorded readily from their afferent nerves while natural force transfer to the sensilla from the distal leg occurs. The first afferent responses are elicited about 3 ms after the onset of a dorsally directed ramp stimulus applied to the tarsus (Fig. 3B,C). The signals from the CS reach the CNS 3–4 ms after stimulus onset and form the most rapid pathway indicating that forces are applied to the leg.

The first incoming afferents from the proximal CS can initiate the first efferent responses to leg impact in the leg



polysynaptic transmission (Fig. 5B), delayed by 8.6 ms afferent conduction time in the periphery (Fig. 2). High-frequency bursts of afferents from a single hair can elicit efferent spiking in the motoneuron.

#### *Cooperation of afferents*

Converging inputs onto the M103d motoneurons were elicited by stimulating selectively a single posterior spur and the feCS2, since this may reflect the situation of recording

Fig. 4. Postsynaptic effects of femoral and tibial campaniform sensilla (CS) on the activity of the motoneurons of muscle M103d. (A) Spikes of a CS (feCS2; lower trace) are followed 1:1 by depolarisations or even spike generation in the fast-depressor motoneuron (upper trace). (B) Averaged data from A: the constant central delay of 2 ms indicates a direct connection. The amplitude of the excitatory postsynaptic potential (EPSP) is approximately 7 mV (signal average of 64 sweeps). (C) Four superimposed sweeps in the slow-depressor motoneuron (upper trace) triggered from a CS afferent (feCS2; recorded at nerve 5bc2). The EPSPs are evoked after a constant central delay of 1.5 ms. (D) Bursts of spikes (large potentials; lower trace) from a CS (tiCS5; recorded in nerve 5B1k) on the dorsal tibia inhibit spike generation in the fast-depressor motoneuron (upper trace).

primary substrate contact by a tibial spur exiting its sensory neuron and, by high-speed propagation of tension in the leg cuticle, the feCS2 sensilla as well (Fig. 3). When single afferents from these mechanoreceptors produce overlapping EPSPs in the M103d fast motoneuron they elicit spikes (Fig. 6A,C), which cannot be achieved by their isolated single EPSPs. So, for single afferent spikes, only a precise timing of their onset in the periphery within a range of 4–7 ms delay from spur to feCS discharge could elicit motoneuron spikes reliably. A comparable convergence occurs on the M103d slow motoneuron.

#### **Discussion**

For insect walking movements and landing after being airborne, the long delay between substrate contact of a leg's tarsus or tibia and the arrival of the resulting mechanosensory input at the CNS raises the question of adequate timing of the reaction to the first substrate contact. Sensory information should reach the CNS with sufficient speed to organize the body's altered support by leg muscles very rapidly.

We have shown for locusts that when a leg makes contact with a substrate the impact causes a shock wave of strain in the leg that progresses from the tarsus proximally in the cuticle – or often from the tibia in structured terrain (Laurent and Hustert, 1988) or on stems (Hassenstein and Hustert, 1995) – and arrives at the body in less than 1 ms. This wave is far ahead of action potentials conducted axonally from distal mechanoreceptors that were stimulated directly by the same contact. The most proximal mechanoreceptors able to detect this shock wave are CS of the proximal femur and

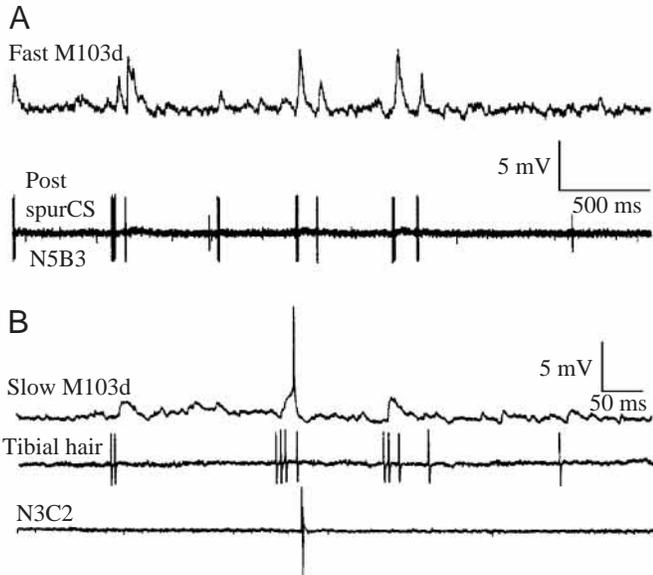


Fig. 5. Afferents from distal mechanoreceptors causing excitatory postsynaptic potentials (EPSPs) in slow- and fast-depressor motoneurons of muscle M103d. (A) Spikes from a posterior spur afferent evoke EPSPs in the fast-depressor motoneuron. A central latency of 4 ms (mean not shown here) indicates a polysynaptic connection. (B) Tibial hair afferents cause EPSPs in the slow-depressor motoneuron with a central latency of 5 ms, indicating polysynaptic connectivity.

trochanter, which code and conduct this information within 1 ms to synapses in the CNS. Motoneurons of the depressor trochanteris system receive these afferents directly and can immediately release their efferent commands that increase muscle tension and thereby keep the body off the ground. This rapid reflex takes 5–7 ms from the time of mechanical contact to the first efferent potentials arriving at the depressor trochanteris; in summary (1) 1 ms or less for conduction of force in the leg cuticle, (2) 1–2 ms for raising the receptor potential to spiking threshold in the CS, (3) 1 ms for the sensory afferent conduction to the CNS, (4) 1–2 ms for the central delay and (5)  $\leq 1$ ms for efferent conduction in the motoneuron to the neuromuscular synapse. The delay until muscle tension rises after neuromuscular transmission depends on the prevailing tension of the muscle.

The most obvious use of this pathway for a locust is when landing on a substrate with the legs after jumping or flight. It should be noted that the proximity of the depressor trochanteris muscles to the CNS also contributes to very low reflex times.

In cyclic movements, such as walking of cats and cockroaches (Gorassini et al., 1994; Delcomyn, 1973), anticipation of the forthcoming substrate contact of a leg starts depressor muscle activities regularly before leg impact. After substrate contact of a toe in cats, foot depressor activation increases rapidly within 10–25 ms. Timing is similar in leg depressors of running cockroaches (Watson and Ritzman,

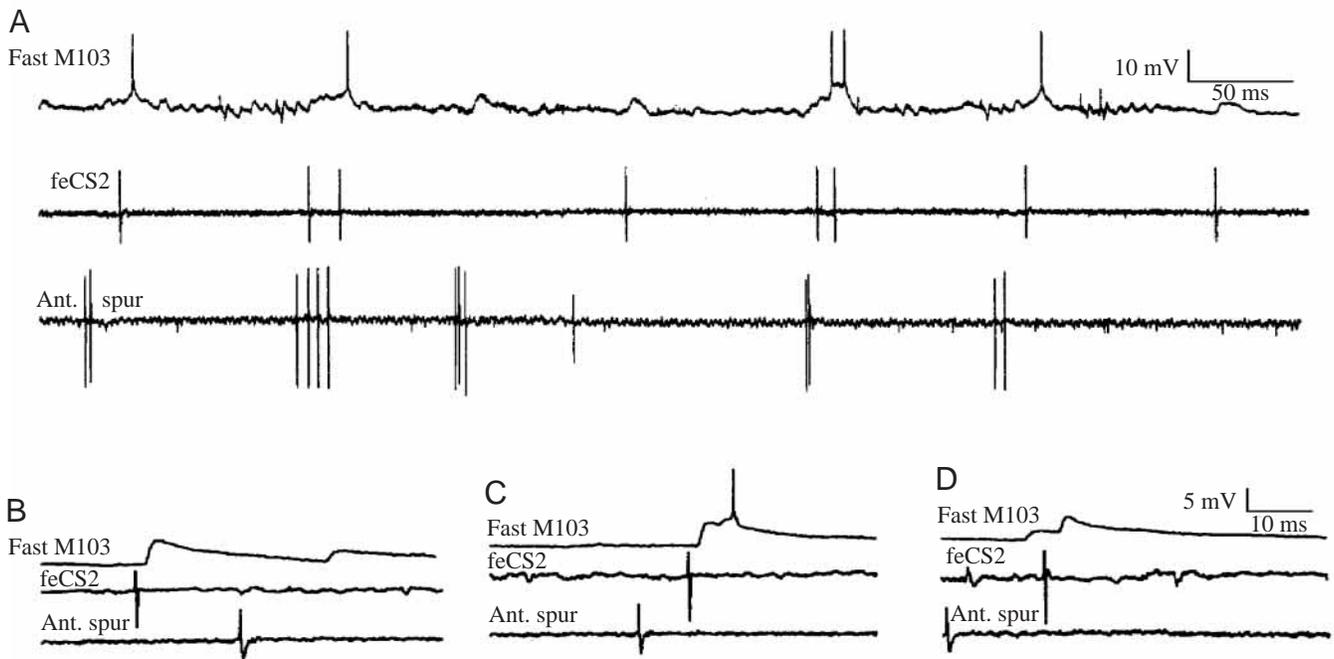


Fig. 6. Responses of the fast-depressor motoneuron of M103d to converging inputs from a femoral (feCS2) campaniform sensillum and a posterior spur afferent. (A) Single feCS2 spikes as well as short bursts of the spur afferent are followed by excitatory postsynaptic potentials (EPSPs) in the motoneuron. When the afferent spikes of both receptors reach the motoneuron at about the same time they are able to elicit spikes. (B) Individual spikes from both types of receptors arising 16 ms apart evoke only EPSPs in the fast-depressor motoneuron. Conduction delays are 2 ms for feCS2 and 12 ms for the spur afferent, and EPSP amplitudes differ. (C) When the spur afferent arises 6 ms before the campaniform sensillum, both effects summate and elicit spikes.

1998; Tryba and Ritzmann, 2000). The role of sensory feedback may differ at swing-to-stance transitions in locusts and cockroach middle legs since in cockroaches the anterior sides of the leg segments always face the substrate (steps mainly by pushing movements) while in rowing movements in locusts anterior and posterior sides often alternate in facing the substrate.

The basic problem of adequate timing of sensory input for motor control also exists during rapid cyclic movements such as the wingbeat of insects, which seems to be resolved by fast-conducting axonal pathways with monosynaptic (locusts; Burrows, 1975; Stevenson, 1997) and even electrotonic connections (flies; Fayyazuddin and Dickinson, 1996).

Walking in locusts also requires rapid switching of activity in antagonistic muscles timed by mechanoreceptors at the transitions from swing to stance phases. The rapidly conducting afferents we studied can encode tarsal or tibial contact and contribute to the transition from swing to stance.

Functionally analogous systems with strain-sensitive mechanoreceptors on proximal leg segments have been described for other animal taxa; for example, in the legs of crustaceans (Leibrock et al., 1996), arachnids (Seyfarth, 1978) and vertebrates (Conway et al., 1987).

#### *The timing of afferents arriving at the CNS after leg contact*

A major problem for fast motor responses was described for running cockroaches by Wilson (1965): after substrate contact in each step, long conduction times in afferent axons from tarsal mechanoreceptors would result in very late motor response. In the locust also, tactile afferents that monitor leg contacts directly arise from tarsal and/or tibial mechanoreceptors, many of which have many monosynaptic connections to tarsal depressor motoneurons (Burrows and Pflüger, 1988; Burrows, 1996; Laurent and Hustert, 1988). We concluded that, generally, mechanoreceptors located closer to the CNS might be responsible for the earliest arrival of afferents at the CNS after substrate contact. This requires that these mechanoreceptors respond to even a slight touch on distal leg segments conducted to them rapidly *via* the leg cuticle. An impact-released shock wave travels longitudinally in the leg and, due to the cuticular material of the leg, its speed should be  $3500 \text{ m s}^{-1}$  (as in wood; Kusch, 1976) or faster. Therefore, we could demonstrate that tarsal contact immediately leads to altered tensile forces in the proximal leg segments (Hustert, 1995; Fig. 3) and therefore also in the trochanteral CS, which respond to changing strain on the tarsus from levels of 1 mN to 5 mN (freshly moulted *vs* four-week-old adults, respectively; Wienicke, 1995). Their sensory neurons conduct with the fastest known speed of middle leg mechanoreceptor axons to the CNS, where the first afferent information on tarsal/tibial impact arrives after about 4 ms. The transfer of strain to proximal leg cuticle is at least 6 ms faster than the axonal spike transferred from distal mechanoreceptors in response to the same stimulus. Therefore, the strain-sensitive proximal CS are the first to report limb contact to CNS neurons, which in turn can organise the most rapid motor responses.

It is very unlikely that alternative pathways such as *via* the subgenual organ in the tibia or other scolopidial organs can send the first information on vibration or increasing strain caused by leg contact, since they are located near the middle of the leg and are surrounded by haemolymph in which 'vibratory' shock waves proceed 'only' at about  $1500 \text{ m s}^{-1}$  (as in water; Kusch, 1976). Nevertheless, mechanical conduction *via* cuticle and/or haemolymph out-racing afferent and interneuron pathways from the area of primary mechanical contact can occur along the insect body. Such pathways of mechanical conduction may elicit mechanosensory responses, as described for sternal and tergal chordotonal organs of the locust abdomen (Hustert, 1975) and also the rapid reflexes from the tip of the cockroach abdomen to thoracic ganglia, which are faster than reflexes mediated by their giant interneurons (Pollack et al., 1995).

#### *Cooperation of mechanoreceptor effects on depressor motoneurons*

Convergent reflexes after selective stimulation and recording of two proprioceptors simultaneously were previously shown to excite thoraco-coxal rotator (Hustert, 1983) and femoral (Jellema and Heitler, 1997) motoneurons.

Afferents from the posterior tibial spurs (rarely from anterior spurs) affect both the fast and the slow M103d motoneurons of the depressor trochanteris polysynaptically, indicating that this reflex system can subserve clasp reflexes with the lower leg segments, which are necessary for holding the locust on its substrate when landing, walking (Laurent and Hustert, 1988), climbing or turning for hiding (Hassenstein and Hustert, 1999) on rough terrain, stems, grasses or sticks. In locusts that climb stems, several spurs of a leg are stimulated at the same time so that their postsynaptic effects summate reliably (all have nearly the same conductance time to the CNS) and release efferent spikes at the motoneuron level.

The observation that anterior spurs of the middle legs do not influence the trochanter depressor motoneurons is probably due to the fact that they are normally stimulated in the late stance phase during walking when the release of the leg from the substrate is pending and when depressor activation would be antagonistic to the regular walking movements. Nevertheless, the situation may change when locusts climb up or down a stem (Hustert, 1985).

We showed that afferents from the groups of CS on the proximal leg segments activate the slow and fast-depressor trochanteris (M103d) motoneurons directly. Single femoral CS spikes drive their membrane potential near the firing threshold for action potentials, which indicates an efficient reflex coupling from the proximal leg CS. Modulations of this effect could arise from converging inputs that inhibit the motoneurons and from presynaptic influences directly on the CS afferents, as indicated by morphological studies (Watson and England, 1991).

By contrast, in phasmids (Stein and Schmitz, 1999), trochantero-femoral CS themselves influence other types of leg mechanoreceptor afferents presynaptically. If that applied also

to locusts, the proximal CS afferents arriving first at the CNS after a leg impact could diminish the excitatory efficiency of delayed afferents from distal mechanoreceptors in response to the same stimulus. The proximal CS afferents themselves, being the first to arrive in the CNS, should evade any presynaptic effects from other afferents that respond to the same leg contact. One may speculate that it may be a major advantage of PAD (primary afferent depolarisation) occurring in leg afferents that they can diminish late responses by late and less specific mechanosensory afferents during a movement.

#### Specificity of responses

For the trCS5 (Hustert, 1985) and feCS2 (similar cap orientation; Hustert et al., 1981) on the posterior face of the middle leg, the optimal stimulus is compression. This type of strain occurs when the tibia is bent posteriorly after it has rotated behind the point at which femur and body axis are perpendicular. Locusts often prefer this range of movement in middle leg stepping (Burns, 1973) so the feCS2 and trCS5 would be active throughout the stance phase. Leg motor coordination during uphill and downhill walking should be controlled specifically by the opposing groups of trochanteral CS on the anterior (trCS1) and posterior (trCS5) face of the middle leg (Hustert, 1985). The trCS1 was shown to excite the posterior rotator of the coxa strongly and it contributes to leg retraction at the transition from swing to stance in wide steps.

By contrast, inhibition of the depressor trochanteris motoneurons arises from the pair of medial CS of the dorsal tibia (tiCS5; Fig. 4D), which is comparable to its homologue in cockroaches (Ridgel et al., 1999). This reflex is similar to the polysynaptic effects from the more proximal medial CS group (tiCS3; Mücke, 1991) on the tibia onto the slow extensor tibia motoneuron in the middle leg (Newland and Emptage, 1996).

For the fine control of movements by the different afferents converging from a leg, the relative postsynaptic potential amplitudes, temporal coincidence or temporal sequence of their effects at the motoneuron level should be important.

We thank the DFG (Hu 223/10) for partial support and Dr Peter Bräunig and Mrs. Anja Becher for reading earlier versions of the English manuscript.

#### References

- Burns, M. D. (1973). The control of walking in Orthoptera. I. Leg movement during normal walking. *J. Exp. Biol.* **58**, 45-58.
- Burrows, M. (1975). Monosynaptic connections between wing stretch receptors and flight motoneurons in the locust. *J. Exp. Biol.* **62**, 189-219.
- Burrows, M. (1996). *Neurobiology of an Insect Brain*. Oxford, New York, Tokyo: Oxford University Press.
- Burrows, M. and Pflüger, H.-J. (1988). Positive feedback loops from proprioceptors involved in leg movements of the locust. *J. Comp. Physiol. A* **163**, 425-440.
- Campbell, J. I. (1961). The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratorioides*. *Proc. R. Zool. Soc. Lond.* **137**, 403-432.
- Conway, B. A., Hultborn, H. and Kiehn, O. (1987). Proprioceptive inputs reset central locomotor rhythm in the spinal cat. *Expl. Brain Res.* **68**, 643-656.
- Delcomyn, F. (1973). Motor activity during walking in the cockroach *Periplaneta americana*. *J. Exp. Biol.* **54**, 443-452.
- Duch, C., Mentel, T. and Pflüger, H.-J. (1999). Distribution and activation of different types of octopaminergic DUM neurons in the locust. *J. Comp. Neurol.* **403**, 119-134.
- Fayyazuddin, A. and Dickinson, M. H. (1996). Haltere afferents provide direct, electrotonic input to a steering motor neuron in the blowfly, *Calliphora*. *J. Neurosci.* **16**, 5225-5232.
- Gorassini, M. A., Prochazka, A., Hiebert, G. W. and Gauthier, M. J. (1994). Corrective responses to loss of ground support during walking. I. Intact cats. *J. Neurophysiol.* **71**, 603-610.
- Hassenstein, B. and Hustert, R. (1999). Hiding responses of locusts to approaching objects. *J. Exp. Biol.* **202**, 1701-1710.
- Hedwig, B. and Knepper, M. (1992). NEUROLAB, a comprehensive program for the analysis of neurophysiological and behavioural data. *J. Neurosci. Meth.* **45**, 135-148.
- Hodgson, E. S., Lettvin, J. Y. and Roeder, K. D. (1955). Physiology of a primary chemoreceptor unit. *Science* **122**, 417-418.
- Hustert, R. (1975). Neuromuscular coordination and proprioceptive control of rhythmical abdominal ventilation in intact *Locusta migratoria migratorioides*. *J. Comp. Physiol.* **97**, 159-179.
- Hustert, R. (1983). Proprioceptor responses and convergence of proprioceptive influence on motoneurons in the mesothoracic thoraco-coxal joint of locusts. *J. Comp. Physiol. A* **150**, 77-86.
- Hustert, R. (1985). The contribution of proprioceptors to the control of motor patterns of legs in orthopterous insects – the locust example. In *Insect Locomotion* (ed. M. Gewecke and G. Wendler), pp. 58-67. Berlin, Hamburg: Paul Parey.
- Hustert, R. (1995). The sequence of mechanoreceptor responses during stepping in locusts. In *Nervous Systems and Behaviour, Proceedings of the 4th International Congress on Neuroethology*. (ed. M. Burrows, T. Matheson, P. L. Philip and H. Schuppe), p. 235. Stuttgart, New York: Thieme Verlag.
- Hustert, R. (1999). Accessory hemolymph pump in the middle legs of locusts. *Int J. Exp. Morphol. Embryol.* **28**, 91-96.
- Hustert, R., Pflüger, H.-J. and Bräunig, P. (1981). Distribution and specific projections of mechanoreceptors in the thorax and proximal leg joints of locusts. III. The external mechanoreceptors: the campaniform sensilla. *Cell Tiss. Res.* **216**, 97-111.
- Jellema, T. and Heitler, W. J. (1997). The influence of proprioceptors signalling tibial position and movement on the kick motor programme in the locust. *J. Exp. Biol.* **200**, 2405-2414.
- Jindrich, D. J. and Full, R. J. (2002). Dynamic stabilization of rapid hexapedal locomotion. *J. Exp. Biol.* **205**, 2803-2823.
- Kendall, M. D. (1970). The anatomy of the tarsi of *Schistocerca gregaria* Forskal. *Z. Zellforsch.* **109**, 112-137.
- Kusch, L. (1976). *Mathematische und Naturwissenschaftliche Formeln Und Tabellen*, 4. Aufl. Essen: Verlag W. Girardet.
- Laurent, G. and Hustert, R. (1988). Motor neuronal receptive fields delimit patterns of motor activity during locomotion of the locust. *J. Neurosci.* **8**, 4349-4366.
- Leibrock, C. S., Marchand, A. R., Barnes, W. J. P. and Clarac, F. (1996). Synaptic connections of the cuticular stress detectors in crayfish: mono- and polysynaptic reflexes and the entrainment of fictive locomotion in an in vitro preparation. *J. Comp. Physiol. A* **178**, 711-725.
- Mücke, A. (1991). Innervation pattern and sensory supply of the midleg of *Schistocerca gregaria* (Insecta Orthopteroidea). *Zoomorphology* **110**, 175-187.
- Newland, P. L. and Emptage, J. (1996). The central connections and actions during walking of tibial campaniform sensilla in the locust. *J. Comp. Physiol. A* **178**, 749-762.
- Pollack, A. J., Ritzmann, R. E. and Watson, J. T. (1995). Dual pathways for tactile sensory information to thoracic interneurons in the cockroach. *J. Neurobiol.* **26**, 33-46.
- Ridgel, A. L., Frazier, S. F., Dicaprio, R. A. and Zill, S. N. (1999). Active signaling of leg loading and unloading in the cockroach. *J. Neurophysiol.* **81**, 1432-1437.
- Ridgel, A. L., Frazier, S. F. and Zill, S. N. (2001). Dynamic responses of tibial campaniform sensilla studied by substrate displacement in freely moving cockroaches. *J. Comp. Physiol. A* **187**, 405-420.
- Schmitz, J. (1993). Load compensating reactions in the proximal leg joints of stick insects during standing and walking. *J. Exp. Biol.* **183**, 15-33.

- Seyfarth, E. A.** (1978). Lyriform slit sense organs and muscle reflexes in the spider leg. *J. Comp. Physiol.* **125**, 45-57.
- Snodgrass, R. E.** (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smiths. Misc. Coll.* **82**, Nr. 2.
- Stein, W. and Schmitz, J.** (1999). Multimodal convergence of afferent inhibition in insect proprioceptors. *J. Neurophysiol.* **82**, 512-514.
- Stevenson, P.** (1997). Reflex activation of locust flight motoneurons by proprioceptors responsive to muscle contractions. *J. Comp. Physiol. A* **180**, 91-98.
- Tryba, A. K. and Ritzmann, R. E.** (2000). Multi-joint coordination during walking and foothold searching in the *Blaberus* cockroach. I. Kinematics and electromyograms. *J. Neurophysiol.* **83**, 3323-3336.
- Watson, A. H. D. and England, R. C. D.** (1991). The distribution of and interactions between GABA-immunoreactive and non-immunoreactive processes presynaptic to afferents from campaniform sensilla on the trochanter of the locust leg. *Cell. Tissue Res.* **266**, 331-341.
- Watson, J. T. and Ritzman R. E.** (1998). Leg kinematics and muscle activity during treadmill running in the cockroach, *Blaberus discoidalis*: II. Fast running. *J. Comp. Physiol. A* **182**, 23-33.
- Wienicke, A.** (1995). Funktioneller Arbeitsbereich campaniformer Sensillen auf dem Mittelbein von Feldheuschrecken. *Diplomarbeit*. Universität Göttingen, Germany.
- Wilson, D. M.** (1965). Proprioceptive reflexes in cockroaches. *J. Exp. Biol.* **43**, 397-409.