LOAD-COMPENSATING REACTIONS IN THE PROXIMAL LEG JOINTS OF STICK INSECTS DURING STANDING AND WALKING

JOSEF SCHMITZ

Abteilung für Biologische Kybernetik und Theoretische Biologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, FRG

Summary

The responses of retractor coxae and protractor coxae motoneurones and of the retractor coxae muscle to cuticular stress applied to the leg were investigated in standing and walking stick insects, *Carausius morosus*. The coxa of a middle or hind leg was restrained and the trochanterofemur was bent by moving the distal tip of the femur anteriorly or posteriorly, i.e. perpendicular to its normal plane of movement. The maximum amplitude used was 200 μm, which corresponds to a deflection of 0.95°, and the forces necessary to bend the trochanterofemur were between 0.29 and 2.91 mN. Thus, cuticular stress could be applied in particular directions and with controlled amplitudes within the physiological range. This cuticular stress induced direction- and amplitude-dependent reflex responses in excitatory retractor coxae and protractor coxae motoneurones. The reflexes clearly constitute a negative feedback system which continuously compensates cuticular stress in the legs of standing and walking animals. Two groups of trochanteral campaniform sensilla, the posterior group and the anterior ventral group, were shown to underlie this feedback loop. These results prove directly for the first time the important function of single groups of trochanteral campaniform sensilla in the control of posture and locomotion in stick insects. I discuss the importance of these results for the interpretation of previous findings on stick insects subjected to increased load during walking.

Introduction

Legged locomotion is particularly well suited to irregular environments but requires continuous adaptation to environmental conditions. These conditions can change slowly, as when the mass of an animal increases during maturation, or they can occur rapidly, as when irregularities in the substratum are encountered. Muscle-driven motor systems must quickly compensate for rapid changes in load such as those that occur when an animal changes from level walking to climbing or to walking downhill. Several authors have shown that, in insects, changes of load during walking influence individual leg movements (Bässler, 1977; Dean, 1985), motor output (Pearson, 1972) and the timing of steps (Foth

and Graham, 1983*a*,*b*; Dean, 1991). The campaniform sensilla (CS) located in the cuticle of the legs are known to measure cuticular stress (Pringle, 1938; Hofmann and Bässler, 1986; Delcomyn, 1991). These sense organs could well be capable of monitoring load and are thought to be involved in segmental and intersegmental compensatory mechanisms which help to establish coordinated walking under various load conditions. These hypotheses are based mainly on the results of Zill and Moran (1981*a*) and Zill *et al.* (1981), who measured the directional sensitivity of tibial CS in the cockroach and who proved that in the standing animal these tibial CS elicit direction-dependent reflexes in tibial muscles. Further support for these hypotheses comes from the investigation of Laurent and Hustert (1988), who showed that tarsal motoneurones are affected by the CS that are associated with tibial spurs. For the walking cockroach, Zill and Moran (1981*b*) showed that activity of sensory CS neurones is correlated with leg movement. The authors deduced that the activity pattern of the tibial CS could aid in load compensation and in the limitation of muscle tension through reflex effects on motoneurones during walking.

Although the stick insect is one of the best investigated model systems for terrestrial locomotion, the extent to which the CS are involved in the control of posture and locomotion is still not clear. Most recent electrophysiological investigations of the sensory neurones of the CS of the stick insect *Cuniculina impigra* have shown a complex organisation of sensory signals resulting in ambiguous information delivered to the central nervous system (Delcomyn, 1991). In contrast to the observations of Zill and his co-workers, Hofmann and Bässler (1982) reported that physiological stimulation of the trochanteral CS of the resting stick insect did not cause reflex responses in the protractor and retractor coxae muscles and caused only very weak reflexes in depressor trochanteris and extensor tibiae muscles. Ablation of all known CS in another stick insect, *Carausius morosus*, did not result in observable changes of the single leg's movement or in the coordination of the legs during level walking (H. Cruse, personal communication). In contrast, several investigations have shown that, in stick insects, changes in load are compensated during walking (e.g. Bässler, 1977; Cruse, 1976; Dean, 1985, 1991; Foth and Graham, 1983*a*,*b*). However, because of the experimental designs used in these investigations, this could also be explained by effects of velocity- and/or position-dependent feedback loops controlling the movement of the legs, and it does not prove that the CS are involved.

The present study was undertaken to prove the importance of the trochanteral CS of stick insects in the control of posture and locomotion, and to clarify the apparent contradiction in the literature concerning the reflex effects of trochanteral CS. It will be shown that two CS groups located on the anterior and posterior sides of the trochanter of the stick insect *Carausius morosus* affect the muscles acting on the leg in the anterior–posterior plane of movement. These reflexes clearly establish a servo system which continuously compensates changes in cuticular stress in both standing and walking animals.

Materials and methods

Adult female stick insects (*Carausius morosus* Br.) raised in our laboratory colony were used in the experiments. Data were obtained from 51 animals.

Preparation of restrained animals

The focus of the first three series of experiments was on the detection of reflex activity elicited in motoneurones by applying forces to the cuticle of the trochanterofemur. In these experiments, animals were totally restrained. They were pinned along the edge of a cork platform and the middle or hind leg was positioned at right angles to the body. The leg under investigation was fixed by embedding the thoraco-coxal joint and the coxa in dental glue (Protemp II, ESPE), thus fixing both to the platform and to the adjacent sternae and pleurae. Care was taken not to restrain the coxa–trochanter joint, so that the trochanter and the femur (to which it is fused) could move without obstruction. The tibia was cut away just distal to the femur–tibia joint to exclude sensory information from sense organs located in distal parts of the leg. The ganglion was exposed by cutting away the mesothoracic and the metathoracic tergum, the gut and the fatty tissue. Care was taken to leave the trachea supplying the ganglion intact.

The right hemiganglion was partly denervated, leaving intact only the main leg nerve (nervus cruris, ncr) with its trochanteral side branches (nerves Tr1 and Tr2; Bässler, 1977). The intact nerves contain the sensory neurones of the various groups of campaniform sensilla on the trochanter and the femur, of the two hairfields on the trochanter and of several mechanoreceptive hairs on the trochanter and the tibia (Bässler, 1977; Schmitz *et al.* 1991*b*). In some control experiments, in addition to the denervation described above, the femur was denervated by severing the nervus cruris and extracting the femoral chordotonal organ through a small slit cut into the proximal femur. Recordings were taken from the stumps of lateral nerves using modified Wilkens–Wolfe oil-and-hook electrodes (Wilkens and Wolfe, 1974; Schmitz *et al.* 1988).

Stimulation and data recording

Stimuli were applied to the distalmost end of the femur by a clamp attached by means of a lever to a galvanometer scanner motor (G300PD with servo-controller CCX101, Scanning Inc.). The motor was mounted on a micromanipulator which allowed manual positioning in three axes. It was driven by a function generator (Wavetek 148A) in various patterns (ramp-and-hold, sinusoidal, stepwise). The displacement of the femur clamp was adjusted to 20, 70 or 200 µm. The lever consisted of a thin piece of steel (25mm×10mm×0.2mm) inserted into a holder at the motor axis. A semiconductor strain gauge (type SR-4, BLH Electronics) was glued to each side of the steel lever. These served as force transducers to monitor the forces necessary to displace the distal tip of the femur. Forces were applied to the femur along the longitudinal axis of the body, i.e. the trochanterofemur was displaced perpendicular to its normal plane of movement. The fixed coxa and the coxa–trochanter joint provided a point against which the elastic trochanterofemur was bent anteriorly or posteriorly depending on the direction of displacement of the distal tip of the femur (see Fig. 1B). A 200 µm displacement of the tip caused an angular displacement of the trochanterofemur of 0.95˚.

All trigger signals, the position of the motor, the applied force and nerve recordings were stored on tape (eight-channel DAT-Recorder, Biologic) for later evaluation.

Ablation of groups of campaniform sensilla

The groups of campaniform sensilla lie in small pits in the relatively thick cuticle of the trochanter (Fig. 1A). Each group consists of 12–20 campaniform sensilla (Tatar, 1976; Schmitz *et al.* 1991*b*). While viewing the group to be ablated under the binocular microscope, an insect pin of appropriate diameter was gently pushed through the thin cuticle forming the bottom of the pit. This operation mechanically uncouples the sensilla from the surrounding cuticle. Except for the small hole in the pit, the trochanter remained intact.
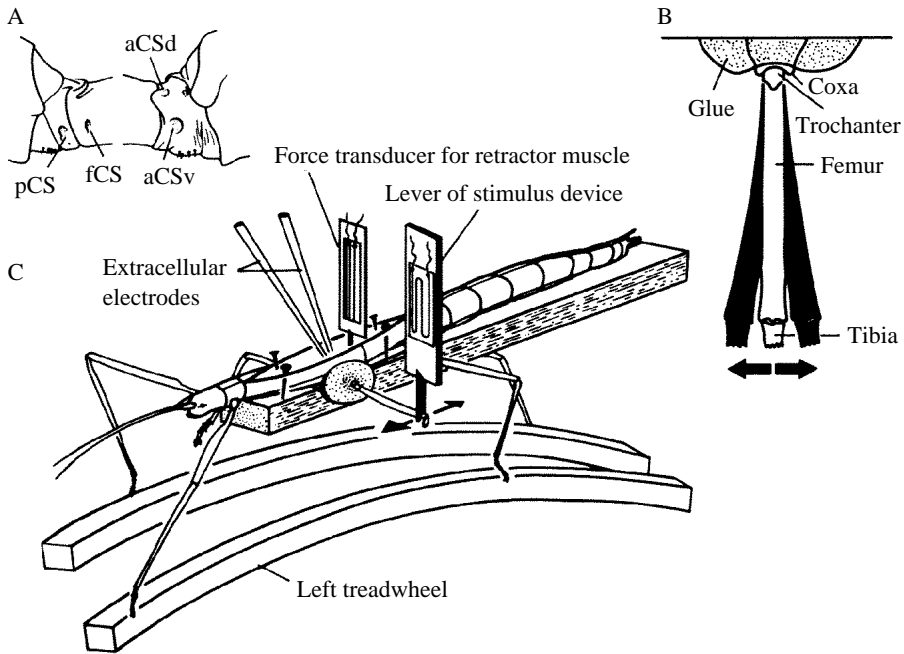

Fig. 1. (A) Schematic overview of the coxa–trochanterofemur joint of stick insects, viewed from the posterior (left) and from the anterior (right), showing the location of the different groups of campaniform sensilla on the trochanterofemur. aCSv, anterior ventral group; aCSd, anterior dorsal group; pCS, posterior group; fCS, femoral group (adapted from Bässler, 1977). (B) Dorsal aspect of a middle leg. The thoraco-coxal joint is restrained by dental glue. In order to stimulate only the CS groups responsive to cuticular stress in the anterior–posterior plane, the distal tip of the femur was slightly displaced anteriorly or posteriorly (arrows). The maximum amplitude used in the experiments was 0.95°, less than one-tenth of the amplitude shown here. (C) Sketch of the apparatus used to stimulate the trochanteral campaniform sensilla of the walking insect. The animal was opened dorsally for *en passant* nerve recordings made with oil-and-hook electrodes from the retractor coxae and the protractor coxae nerves. The thoraco-coxal joint of the middle leg under investigation was restrained (as in B) and the animal was allowed to walk with the remaining five legs on a double treadwheel. The tip of the middle leg's femur could be slightly displaced anteriorly or posteriorly with the aid of a motor-driven lever equipped with strain gauges to measure the forces necessary to bend the trochanterofemur. In some experiments, the stimulus-induced force response of the retractor coxae muscle was monitored with a force transducer clamped to the cut end of the retractor muscle apodeme.

Preparation of standing and walking animals

In a fourth series of experiments, the preparation was changed to investigate the effects of stimulation of the campaniform sensilla on force generation during standing and walking. The animals were glued to a stick of balsa wood and the coxa and the thoraco-coxal joint of the left middle leg were fixed as described above. The remaining legs were left unrestrained and the animal was placed above a double treadwheel which allowed it to stand or walk (Fig. 1C). To gain access to the lateral nerves of the left hemiganglion, the animal was opened dorsally by a longitudinal slit along the seam of the terga halves. The left and the right halves of the terga were raised and pinned in the upright position. The gut and fatty tissue around the ganglion were removed. This type of surgery performed on a ventrally fixed animal does not change the thorax geometry or disturb the joint geometry of the five free legs. The animal was still able to perform walking movements freely during the experimental sessions (Godden and Graham, 1984; Schmitz and Haßfeld, 1989; Schmitz *et al.* 1991*a*).

Recordings were obtained *en passant* from the protractor (nl2c) and/or the retractor (nl5a) nerves which, together with the main leg nerve (ncr), were left intact (the nomenclature used for the nerves is taken from Graham, 1985). In all cases, nerves na, nl3, nl4, nl2a and nl2b were cut. In some cases, nerves C1 and C2 were also cut. In experiments in which the force of the retractor coxae muscle was recorded, the common apodeme of the retractor muscles (1T-cx, la-cx; Marquardt, 1940) was also cut free from its point of insertion at the posterior rim of the coxa and clamped to a force transducer. This force transducer was similar to that described above, but the elasticity of the steel lever was such that the maximum force exerted by the retractor muscle was able to displace the tip of the force transducer by approximately 1.5mm. This allowed the muscle to shorten during contraction as much as during the normal stance movement of the leg. After the contraction, the elasticity of the steel lever stretched the muscle back to its initial length.

Data evaluation

Spikes in the recordings from each nerve were sorted by means of window discriminators into several classes according to their amplitude, stored on disk and averaged off-line to build peri-stimulus-time histograms (PSTH). The analogue data (output of the force transducers or position signal of the stimulus device) were digitized (2kHz sampling rate) and averaged (using the stimulus signal as trigger).

Results

Cuticular stress applied to the trochanterofemur elicits direction-dependent reflexes in the thoraco-coxal joint

The thoraco-coxal (TC) joint (and with it the whole leg) is moved anteriorly and posteriorly by the protractor coxae and the retractor coxae muscles, respectively. The next distal joint is the coxa–trochanter joint, which allows the trochanterofemur to be moved in a dorso-ventral plane. In the first series of experiments, restrained animals were used to investigate whether cuticular stress applied to the trochanterofemur along the

anterior–posterior axis (i.e. perpendicular to its normal plane of movement) elicits reflex responses in the protractor coxae or retractor coxae muscles.

Fig. 2A shows recordings from the protractor nerve (nl2c) made during imposed bending forces. In the example shown, the tip of the femur was displaced sinusoidally between the unloaded mid-position and a position 200 µm further anterior. Nerve nl2c contains one spontaneously active excitatory motoneurone. Its rate of activity was modulated according to the direction of the applied force. Anteriorly directed forces, most probably compressing the anterior ventral group of campaniform sensilla (aCSv) and decompressing the posterior group of campaniform sensilla (pCS), increased the discharge rate of the spontaneously active motoneurone. In addition, non-spontaneously active motoneurones were sometimes recruited. During release, activity of the protractor motoneurones declined.

Cuticular stress also elicited reflex responses in the retractor coxae motoneurones contained in nerve nl5a. In the example shown in Fig. 2B, the tip of the femur was displaced sinusoidally between the unloaded mid-position and a position 200 µm further
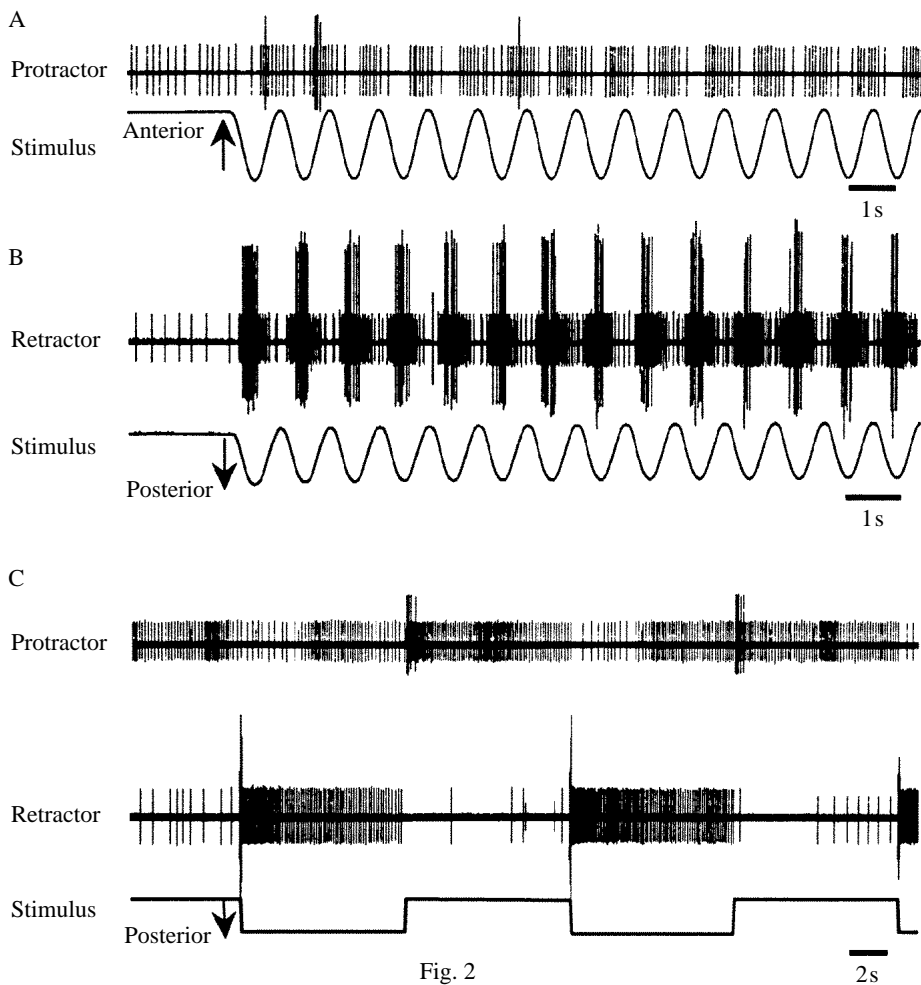

Fig. 2

posterior. The retractor nerve contains one spontaneously active excitatory motoneurone whose discharge rate was strongly increased during the posteriorly directed part of the stimulus. In addition, several non-spontaneously active motoneurones were recruited during this part of the stimulus. During release, the activities of all motoneurones declined.

The time courses of the reflex responses of the spontaneously active motoneurones were phasic-tonic, whereas the additionally recruited motoneurones responded in a purely phasic manner. This is demonstrated with several ramp-and-hold stimuli (Fig. 2C). The tip of the femur was displaced rampwise from an anterior position to a posterior position (ramp duration 10ms), held there for 10s, then bent back to the initial anterior position. This sequence was then repeated. This stimulus pattern revealed not only that the protractor and retractor motoneurones were activated by one stimulus direction but also that they were inhibited by the other stimulus direction. During the hold part of the stimulus, the response of the spontaneously active motoneurones decayed to tonic values that were different from the values observed in the unloaded mid-position. In the example shown in Fig. 2C, this is more apparent for the retractor than for the protractor motoneurones and is caused by the amplitude and the offset of the stimulus applied (see below).

Two trochanteral groups of campaniform sensilla mediate the reflex responses

The next set of experiments investigated the sense organs that mediate the reflex responses. Extensive denervation within the thorax and the coxa, denervation of the femur and ablation of the femoral chordotonal organ left the three groups of campaniform sensilla located at the trochanter (Fig. 1A) as possible mediators of the reflex response.

Applying pressure by means of a blunt probe to the trochanteral cuticle near the pits of

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Fig. 2. Responses of the protractor coxae and retractor coxae motoneurones to imposed cuticular stress applied to the trochanterofemur of the middle leg. (A) Extracellular recordings from the stump of the protractor nerve illustrate the excitation of spontaneously and non-spontaneously active motoneurones during anteriorly directed stimuli (upward deflections of the stimulus trace). Cuticular stress was produced by displacing the tip of the femur sinusoidally between the unloaded mid-position and the starting position, which was $200\,\mu m$ further anterior. (B) Extracellular recordings from the stump of the retractor nerve illustrate the vigorous excitation of several retractor motoneurones during posteriorly directed stimuli (downward deflections of the stimulus trace). Cuticular stress was applied by displacing the tip of the femur sinusoidally between the unloaded mid-position and a position $200\,\mu m$ further posterior. (C) Responses of protractor and retractor coxae motoneurones of the middle leg of one animal to ramp-and-hold stimuli. The example shown is a sequence from a longer stimulus episode. The tip of the femur was repeatedly displaced from a position $80\,\mu m$ anterior to the unloaded mid-position to a position $120\,\mu m$ posterior to the mid-position (downward deflection of the stimulus trace), held there for 10s, then bent back to the anterior position. This stimulus pattern revealed the phasic-tonic time courses of the reflex responses of both groups of motoneurones. During posteriorly directed stimuli, the retractor motoneurones are activated; during anteriorly directed stimuli, the protractor motoneurones are activated. This example also clearly demonstrates that the motoneurones are not only excited but are also inhibited in a phasic-tonic manner, depending on stimulus direction. The three examples are from different animals, so the stimulus traces are not equally calibrated.
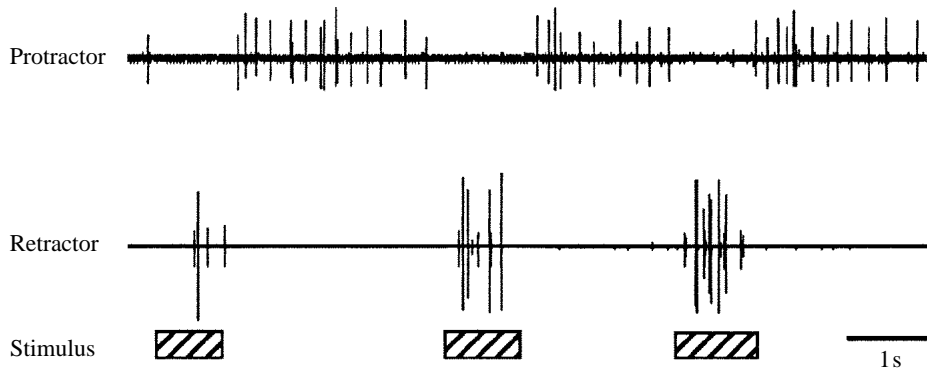
Fig. 3. Responses of protractor coxae and retractor coxae motoneurones of the middle leg to manually applied deformation of the trochanteral cuticle near the pit of the posterior group of campaniform sensilla. The example shown is a sequence from a longer episode. The stimulus was applied by hand by means of a blunt probe. Applying pressure to the cuticle inhibited the existing activity of the protractor motoneurones and elicited activity in the retractor coxae motoneurones. Release of the pressure re-established the spontaneous activity of the protractor coxae motoneurones. The times of stimulation (hatched rectangles) were reconstructed from the voice channel of the tape recorder.

the aCSv or the pCS elicited reflex responses in the protractor coxae and retractor coxae motoneurones. The recording shown in Fig. 3 was obtained from an animal which had been totally denervated, leaving intact only nerves Tr1, Tr2 and ncr. In addition, the trochanteral hair-plate, which is innervated by nerve Tr1, and the femur of this animal were totally removed. Slight deformation of the cuticle near the pCS (Fig. 3) resulted in inhibition of protractor and activation of retractor motoneurones.

Several further control experiments were performed on more intact animals (prepared as for the experiments shown in Fig. 2) in which one of the trochanteral CS groups, either the aCSv or the pCS, had been ablated prior to the experiment. Fig. 4A shows a recording from an animal in which the pCS group had been removed prior to the measurement and in which the protractor coxae motoneurones were tested. The stimulus was applied in the usual way by displacing the tip of the femur. The excitatory effects of the remaining campaniform sensilla are sufficient to elicit the normal reflex response during anteriorly

Fig. 4. Effects on the reflex response of ablation of the trochanteral campaniform sensilla groups pCS and aCSv. (A) A recording from a protractor nerve during existing sinusoidal stimulation. Prior to stimulation, the pCS had been ablated (−pCS). The first nine cycles reveal that the remaining sensilla (aCSv) are sufficient to elicit a reflex response during anteriorly directed stimuli (upward deflection of the stimulus trace). Within the stimulus cycle marked by the arrowhead, the aCSv was also ablated (−aCSv), abolishing the reflex response. Peri-stimulus-time histograms (PSTH) show the averaged reflex responses of protractor motoneurones (B) and retractor motoneurones (C) after ablation of one group of trochanteral CS (−pCS and −aCSv, respectively). (D,E) The combined ablation of both CS groups abolishes any reflex response in both groups of motoneurones (compare B with D and C with E). Each PSTH consists of 128 bins of 7.8ms. The time course of the stimulus is indicated schematically at the top of each histogram.

directed bending stimuli (Fig. 4A, the first nine cycles; Fig. 4B). If, in addition to the pCS, the aCSv was also ablated, any reflex activity in the protractor motoneurones was abolished (Fig. 4A, cycles to the right of the arrowhead; Fig. 4D).

Ablation of the aCSv had no measurable effect on the activation of retractor motoneurones during posterior bending of the femur (Fig. 4C). As in the situation with intact CS groups (Fig. 2B), posteriorly directed stimuli induced strong activation of several retractor motoneurones. The combined ablation of aCSv and pCS abolished any reflex responses in the retractor motoneurones (Fig. 4E) and, in addition, greatly reduced
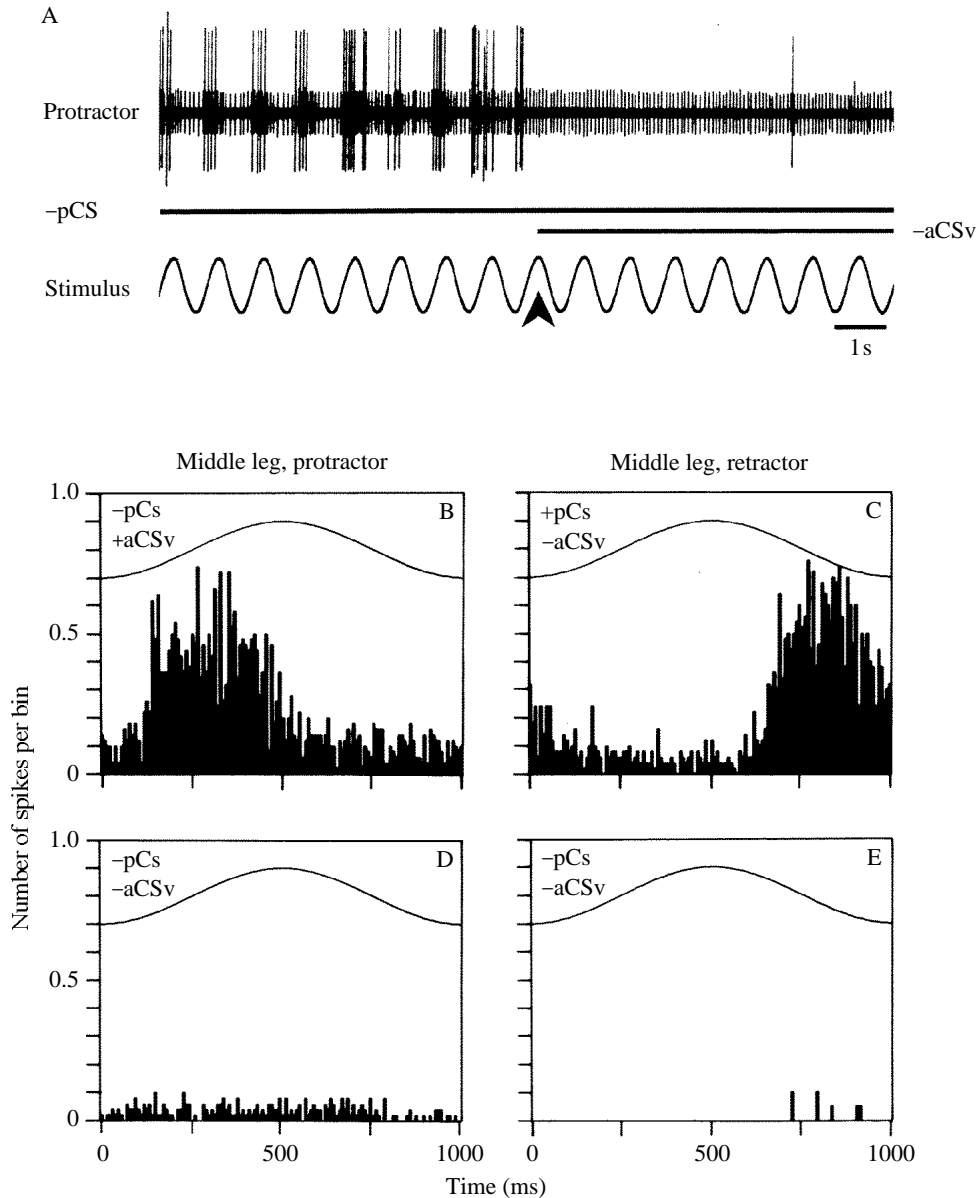

Fig. 4

the activity of the spontaneously active motoneurone. No recovery of the reflex response was observed within the next 4h.

It could not clearly be determined whether the aCSv was solely responsible for the full reflex response of the protractor motoneurones or whether the pCS was responsible for that of the retractor motoneurones. Ablation of either of these single groups of sensilla did not always result in total abolition of the reflex response of the appropriate group of motoneurones. Whether any of the campaniform sensilla of the ablated group were unintentionally left intact could not be determined, even with the aid of a scanning electron microscope.

The results demonstrate, however, that the two groups of campaniform sensilla located on the anterior (aCSv) and posterior (pCS) sides of the trochanter are sufficient to elicit reflex responses in the protractor and retractor coxae motoneurones. The other groups of campaniform sensilla and the scattered, individual campaniform sensilla located on the trochanter and femur are, alone, not capable of eliciting the reflex response. Further control experiments (not shown) revealed that neither the anterior dorsal (aCSd) nor the femoral (fCS) groups of campaniform sensilla are necessary for the reflex response, nor do they change the amplitude of the reflex response to cuticular stress applied in the anterior–posterior plane of the trochanterofemur.

Increasing cuticular stress increases the strength of the reflex response

The effects of increased cuticular load on the stress-reflex responses of the protractor and retractor coxae motoneurones were tested in two different ways: (1) the amplitude of a sinusoidal stimulus was increased in steps around the constant, unloaded mid-position; (2) the preloading of the trochanterofemur was varied while ramp-and-hold stimuli with a constant amplitude were applied.

Increased stimulus amplitudes with constant stimulus offset

Sinusoidal stimuli with amplitudes of 20, 70 or 200 μm were used to test the dependence of the motoneurones' reflex responses on stimulus amplitude. The forces applied to the distal end of the femur which were required to bend the trochanterofemur were 0.29, 1.02 and 2.91mN, respectively. The stimulus with the smallest amplitude (0.29mN) hardly modulated the spontaneous activity in the protractor and retractor motoneurones (Fig. 5A,D). Stimuli which applied peak forces of 1mN and more significantly modulated the spontaneous activity (Rayleigh test, $P<0.1\%$, nine animals) and elicited reflex responses in non-spontaneously active motoneurones (Fig. 5B,C,E,F).

Varied stimulus offset with constant stimulus amplitude

Preloading of the trochanterofemur (between −7.5 and +7.5mN) and subsequent application of stimuli of constant amplitude (1.02mN) elicited reflex responses of various amplitudes. Anterior preloading (−7.5mN) greatly decreased spontaneous activity of the appropriate retractor motoneurone (Fig. 6A). Anteriorly directed ramp-and-hold stimuli applied in this situation further decreased the remaining spontaneous activity. On release from the stimulus, the tonic firing resumed. Posteriorly directed stimuli of the same amplitude applied to the posteriorly preloaded (+7.5mN) trochanterofemur were much
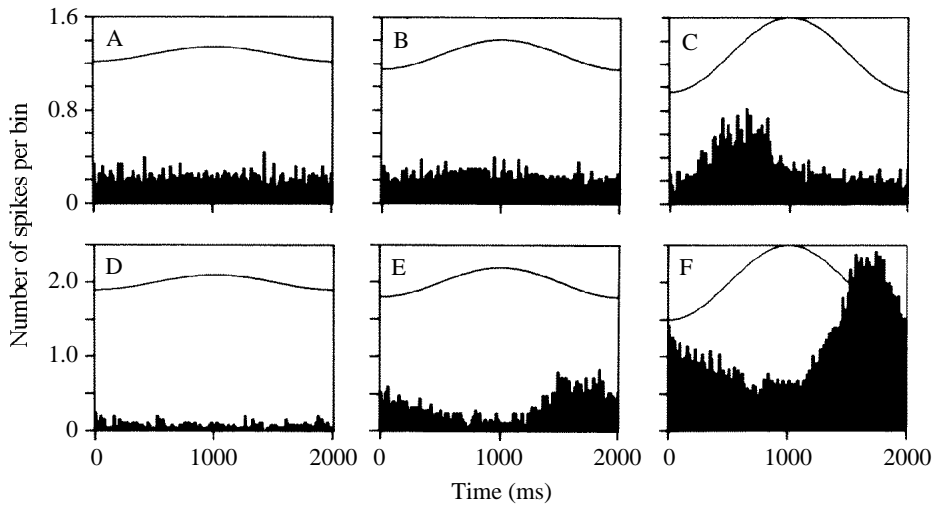
Fig. 5. Averaged reflex responses of the protractor coxae (upper row of PSTHs) and retractor coxae motoneurones (lower row of PSTHs) to sinusoidal stimulation (0.5Hz) with three different stimulus amplitudes. Stimuli which applied a peak bending force of 0.29mN to the trochanterofemur hardly modulated the existing spontaneous activity of the protractor (A) and retractor motoneurones (D). Stimulation with larger forces of 1.02mN (B,E) and 2.91mN (C,F) significantly modulated the spontaneous activity and elicited reflex responses in non-spontaneously active motoneurones. Each PSTH consists of 128 bins of 15.6ms. The time course of the stimulus is indicated schematically at the top of each PST histogram. Upward deflection indicates anteriorly directed stimulation.

more effective (Fig. 6C). Not only was the response amplitude of the spontaneously active retractor motoneurone increased, but additional motoneurones were also recruited. Stimulation around the unloaded mid-position (Fig. 6B, see also Fig. 3) elicited intermediate responses. The protractor motoneurones exhibited a behaviour antagonistic to that of the retractor motoneurones (not shown). They responded least to stimulation around a posterior preloaded position and most to stimulation around an anterior preloaded position.

Evaluation of the differences between the average activities (in spikes s^{-1}) during the two hold parts of the stimulus for each group of motoneurones and for each preloaded position tested resulted in the response curves shown in Fig. 7. This again clearly demonstrates the antagonistic behaviour of the two groups of motoneurones. It shows that anteriorly directed loads elicit strength-dependent tonic reflexes in protractor coxae motoneurones and that posteriorly directed loads elicit strength-dependent tonic reflexes in retractor coxae motoneurones.

Effects of cuticular stress on retractor coxae muscle force

Standing animal

The stimuli applied to the trochanterofemur affected excitatory motoneurones. This could easily be verified in preliminary experiments, because stimulus-induced activation
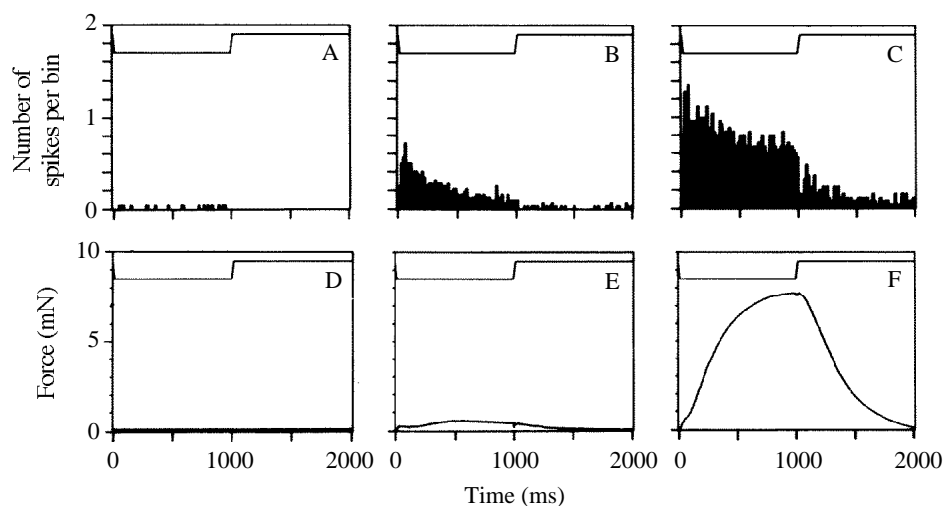
Fig. 6. Averaged reflex responses of the retractor coxae motoneurones (A–C) and the retractor coxae muscle (D–F) to ramp-and-hold stimuli of constant amplitude applied to the distal tip of the femur under three different load conditions. In the anteriorly preloaded situation (−7.5mN), the stimulus modulated only the weak spontaneous activity of one motoneurone (A) and elicited no measurable force in the retractor muscle (D). Stimuli applied in the unloaded situation (B,E) or after posterior preloading (+7.5mN; C,F) elicited intermediate and strong reflex responses, respectively, in the retractor motoneurones and in the retractor muscle. The time course of the stimulus is indicated schematically above each PSTH and each force plot. Downward deflection represents posteriorly directed stimulation.

of retractor motoneurones, for example, was accompanied by small twitches of the retractor coxae muscle. To investigate the force response of the muscle in more detail, the retractor muscle apodeme was cut from its point of insertion on the posterior rim of the coxa and the apodeme was clamped to a force transducer (Fig. 1C). The point of insertion of the protractor muscle is located deeper inside the coxa and it was not always possible to cut this muscle free without damaging it or its motor nerve. Therefore, the quantitative data presented below were obtained from the retractor muscle.

   The amplitude of the force response to ramp-and-hold stimuli was dependent on the stimulus amplitude and the preloading of the trochanterofemur. Fig. 6D–F shows the averaged force responses for three situations. When the trochanterofemur was stimulated with ramp-and-hold stimuli from the anteriorly preloaded situation the retractor muscle produced almost no force (Fig. 6D). Stimulation around the unloaded mid-position (Fig. 6E) elicited fairly weak forces, whereas stimulation in the posteriorly preloaded situation elicited strong forces (Fig. 6F). During the entire posterior-directed phase of the stimulus, the muscle force increased to as much as 8mN within 1s. During the releasing phase of the stimulus, the force declined slowly. With a stimulus repetition rate of about 0.5Hz (as shown in Fig. 6), summation of the single force responses did not occur. At higher repetition rates, the single force responses summed. This led to a high constant force level which was maintained for the duration of the stimulus. The level of this tetanic force could reach 30–40mN (not shown).
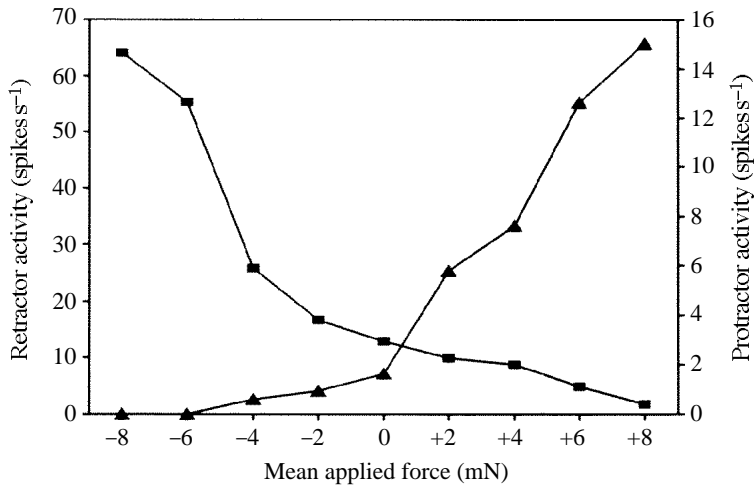
Fig. 7. Response curves, showing the dependence of the strength of the tonic part of the reflex response of the protractor (triangles) and retractor coxae (rectangles) motoneurones on imposed cuticular stress. Negative force values represent imposed cuticular stresses which bend the trochanterofemur posteriorly; positive force values represent stress acting anteriorly.

Walking animal

To test whether stimulation of the campaniform sensilla is also effective during walking, animals were mounted above a treadwheel (see Materials and methods). In a preliminary series of experiments, they were allowed to walk with all six legs, and the forces produced by the retractor coxae muscle of the left middle leg were measured. The nervous supply of the middle leg under investigation was left intact and recordings were made *en passant* from the retractor nerve. The retractor coxae muscle of this middle leg was cut and clamped to a force transducer. During walking, this middle leg was able to perform swing movements actively. The stance movement was passive because of the mechanical coupling of the legs through the treadwheel. The retractor muscle actively produced force during each stance. The peak force produced during each step was approximately 30mN.

To stimulate the campaniform sensilla of a walking animal it was necessary to restrain the middle leg under investigation. This was done in essentially the same way as in totally restrained animals. However, the other legs were left unrestrained so that the animal could freely perform walking movements with five legs while the 'standing leg of a walking animal' was in a prolonged stance phase (Bässler, 1979). The preparation is shown in Fig. 1C. During walking, the retractor coxae muscle of this middle leg produced forces whose time courses showed weak oscillations correlated with the walking movements of the other legs (Cruse and Saxler, 1980). Therefore, to avoid averaging artefacts, the parameters of the applied stimuli were carefully chosen so that the hold times were clearly shorter or longer than the step durations of the walking legs. To prevent the stimulus entraining the walking rhythm of the other legs, several short stimulus episodes (each consisting of 8–10 stimulus cycles) were randomly applied during long, continuous walking episodes.
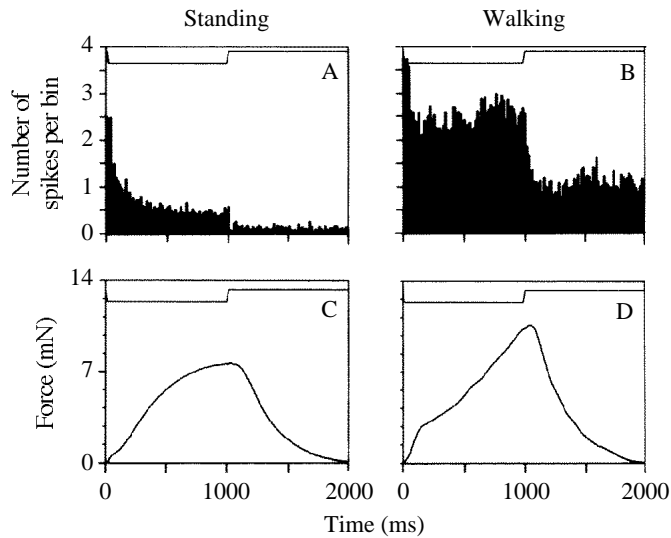
Fig. 8. Averaged reflex responses of the retractor coxae motoneurones (A,B) and the retractor coxae muscle force (C,D) to imposed cuticular stress applied to the middle leg of a standing animal (A,C) and to the 'standing leg of a walking animal' (B,D). All data were obtained from the same animal. The time course of the stimulus is indicated schematically above each PSTH and above the force averages. Downward deflection represents posteriorly directed stimulation.

Ramp-and-hold stimuli applied to the restrained middle leg of the walking animal elicited reflex responses which were superimposed on the existing walking activity. Fig. 8 compares the reflex responses of one animal during standing and walking. The time courses of the reflex responses of the motoneurones to posteriorly directed stimuli were different in the two situations. In the standing animal, these stimuli caused an increase in retractor firing rate which declined during the hold phase to a stable elevated level. This decline can be approximated by an exponential function with a time constant of about 120ms (Fig. 8A). In the walking animal, the decline is faster, having a time constant of about 30ms (Fig. 8B). In the walking animal, activity slightly increased again before the end of the hold phase (Fig. 8B). The anteriorly directed part of the stimulus decreased the activity of the motoneurones in both standing and walking animals, but the amplitude of the tonic part of the reflex response is much higher in the walking animal. The force produced by the retractor muscle increased during the entire posteriorly directed part of the stimulus and decreased during the anteriorly directed part of the stimulus. However, the peak muscle force was higher in the walking animal (compare Fig. 8C,D) where it reached about one-third of the maximum force that the retractor muscle produces during a normal step.

Discussion

The present study examines whether cuticular stress is a controlled variable in the legs of standing stick insects and whether information from trochanteral campaniform sensilla

is used to compensate for changes in load during walking. The results clearly demonstrate that, in both standing and walking animals, the trochanteral CS are part of a negative feedback system which continuously regulates cuticular stress. Stress applied in the anterior–posterior plane of the leg is measured mainly by two CS groups, the aCSv and pCS, which mediate direction- and strength-dependent reflex responses of two antagonistic muscles, the protractor and retractor coxae. Data obtained from the trochanteral CS in standing stick insects corroborate findings on the tibial CS of the cockroach (Zill *et al.* 1981). Moreover, the findings demonstrate an important function of individual groups of trochanteral CS in the walking insect.

The new results also allow a more detailed interpretation of previous experimental results obtained from stick insects subjected to increased load during walking. Bässler (1977) showed that pressure applied continuously to the trochanterofemur prevents the transition from stance to swing in the stimulated leg. Cruse (1985*c*) demonstrated that the probability of the occurrence of the stance–swing transition at a given position within the step cycle is smaller if the leg is loaded. Both authors attributed these effects to trochanteral CS groups, but this was not proved experimentally. Nevertheless, their results strongly indicate that the decrease in cuticular stress is one of the factors that triggers the transition from stance to swing. These earlier investigations did not determine whether load-monitoring CS groups have only a permissive function or whether they are continuously used to adapt leg movement during an existing step. The results reported in the present study, taken together with the results of Bässler (1977) and Cruse (1985*c*), show that information from CS is most probably used both to regulate cuticular stress during stance and to time the transition from stance to swing.

Campaniform sensilla are not only involved in negative feedback loops. For example, two single campaniform sensilla located on the proximal tibia of a locust hind leg are involved in a positive feedback loop. They both excite extensor and flexor tibiae motoneurones in order to increase the extensor force if resistance is met during walking, or in order to increase the co-contraction of both muscles when the animal is preparing for a kick (Burrows and Pflüger, 1988). However, this seems to be an adaptation to the special function of the locust's hindlegs.

Pearson (1972) observed that walking cockroaches dragging a weight increase their motor output to powerstroke muscles. Therefore, he assumed that a positive feedback signal was elicited by the increased load. Similar results were obtained from stick insects subjected to various changes in frictional or inertial load during walking (Foth and Graham, 1983*a,b*; Dean, 1991; Cruse, 1985*a*). In view of the results presented here, this positive feedback signal can be explained in terms of a stress-regulating negative feedback system. Loading the body in such a way that the load resists the body movement results in less than normal bending of the trochanterofemur during the stance movement and this is counteracted by an increase in the existing retractor coxae activity. Thus, increased motor output to the powerstroke muscles during stance is caused by the negative feedback underlying the stress control loop. Loading the body in a way that assists the body movement (e.g. walking downhill) should lead to the opposite response; i.e. decreased motor output to the retractor muscle or even activation of the antagonistic protractor muscle. Measurements of the leg movements of walking animals such as those

published by Dean (1991) can be interpreted in this way. Dean found that the velocity of the stance movement is changed only slightly by assisting torques, even if they are of considerable amplitude.

In interpreting these results one must keep in mind that not only the cuticular stress on the legs (present study) but also the velocity of the legs' movement (Cruse, 1985*b*; Schmitz, 1985) are continuously controlled during walking. Therefore, not all compensatory reactions of the animal to applied external loads can be attributed exclusively to the stress control system because acceleration or deceleration of the legs caused by external loading can also elicit responses in the velocity feedback system. The results presented here, however, prove the trochanteral CS to be well suited for compensation of load changes during walking. In experiments with the 'standing leg of a walking animal', in which the velocity control system is not phasically active, the stress feedback loop elicits phasic-tonic force responses with amplitudes of about one-third of the peak muscle force generated by the retractor muscle during a step. These results clearly show that the two trochanteral CS groups, aCSv and pCS, have strong influences on load compensation during walking. The results also justify the assumption of a load-sensitive channel, proposed in models that describe the walking movements of single legs (see review by Cruse, 1990).

How can the insect distinguish between externally applied stress and cuticular stress caused by active movement? In the experiments presented here, externally applied cuticular stress elicited open-loop feedback responses. Under closed-loop conditions, active leg movements also exert cuticular stress (Delcomyn, 1991). For example, activity of the retractor coxae muscle, as when the body is accelerated against inertia during the stance, produces a bending force which can be counteracted by the stress feedback system. This tends to decrease retractor activity or may even elicit protractor activity. The same is true in standing animals for the interaction between the stress feedback system and the position feedback system if leg position is passively changed, e.g. by a moving twig. One way of accommodating two feedback systems is to ensure that they are mostly separate and independent of each other but converge at a common integrating level (e.g. premotor interneurones or motoneurones). In this case, the resulting movement would reflect a compromise solution and would depend on the balance of strength of the two pathways. Another possibility is that an efference copy is processed simultaneously, increasing stress tolerance, i.e. the reference input to the stress servo system is increased by an intended movement. The finding of Dean (1991) that the velocity of the stance movement was held nearly constant even when large loads were applied favours the latter hypothesis.

Considering the findings in the stick insect *Carausius morosus*, it is surprising that Hofmann and Bässler (1982) reported no reflex effects on the protractor coxae and retractor coxae muscles in the stick insect *Cuniculina impigra*. One reason might be that the latter species is larger than *Carausius* and possesses much longer legs. Owing to differences in the geometry, the torques produced by the protractor coxae and retractor coxae muscles of *Cuniculina* are higher during standing and walking. Hence the threshold for eliciting reflex responses might also be higher. Because Hofmann and Bässler (1982) focused mainly on describing the physiology of the primary afferences, they might have

stimulated below or only slightly above this threshold. In addition, their method of recording muscle activation (for the subcoxal muscles they used electromyograms) might have prevented weak reflex responses from being recorded. A further reason might be that in the hindlegs of *Cuniculina*, which were the only legs investigated by Hofmann and Bässler (1982), there exists a cuticular stress-compensating mechanism which acts mainly on the coxo-trochanteral and the femoro-tibial muscles. The latter reflex pathways were described by Hofmann and Bässler (1982).

The findings obtained here for the campaniform sensilla of the stick insect *Carausius morosus* fit into a series of results obtained for other, functionally analogous, sense organs in other species, including vertebrates. In all cases, organs sensitive to load or force are involved in compensatory reactions which influence posture and locomotion. In the crab, force-sensitive mechanoreceptors (funnel canal organs) in the dactyl elicit intraleg and interleg reflexes during free walking (Libersat *et al.* 1987*a,b*; Zill *et al.* 1985). These studies demonstrate that these distal receptors affect the walking pattern and can strongly influence interleg coordination. In crayfish, the cuticular stress detector (CSD2), a force-sensitive sense organ located in the proximal part of the leg, is involved in intraleg reflexes in standing animals and might also aid proper coordination of the walking legs (Klärner and Barnes, 1986). In spiders, force-sensitive lyriform slit sense organs elicit compensatory intraleg reflexes in the standing animal (Seyfarth, 1978). The sensitivity of these organs is such that they could play a role during walking (Blickhan and Barth, 1985). In vertebrates, the Golgi tendon organs monitor loading of muscle tendons. The afferent discharge of these organs during walking would be well suited to deliver information which could be used in compensatory reactions to avoid overloading of muscles. Information from the Golgi tendon organs can also be used in the process of deciding when to end a stance and when to start a swing (Conway *et al.* 1987). The present study shows that stick insects utilize information from the trochanteral campaniform sensilla to similar advantage. The results of Bässler and Wegner (1983), obtained from partly denervated, restrained stick insects, and preliminary results from intact, walking animals (J. Schmitz, unpublished results) indicate that this information is also used to maintain proper coordination of the legs during walking.

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