Force feedback reinforces muscle synergies in insect legs

Sasha N. Zill [a, *], Sumaiya Chaudhry [a], Ansgar Büschges [b], Josef Schmitz [c]

[a] Department of Anatomy and Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25704, USA
[b] Department of Animal Physiology, Zoological Institute, Biocenter Cologne, University of Cologne, 50923 Cologne, Germany
[c] Department of Biological Cybernetics, University of Bielefeld, 33501 Bielefeld, Germany

ARTICLE INFO

ABSTRACT

The nervous system solves complex biomechanical problems by activating muscles in modular, synergist groups. We have studied how force feedback in substrate grip is integrated with effects of sense organs that monitor support and propulsion in insects. Campaniform sensilla are mechanoreceptors that encode forces as cuticular strains. We tested the hypothesis that integration of force feedback from receptors of different leg segments during grip occurs through activation of specific muscle synergies. We characterized the effects of campaniform sensilla of the feet (tarsi) and proximal segments (trochanter and femur) on activities of leg muscles in stick insects and cockroaches. In both species, mechanical stimulation of tarsal sensilla activated the leg muscle that generates substrate grip (retractor unguis), as well as proximal leg muscles that produce inward pull (tibial flexor) and support/propulsion (trochanteral depressor). Stimulation of campaniform sensilla on proximal leg segments activated the same synergistic group of muscles. In stick insects, the effects of proximal receptors on distal leg muscles changed and were greatly enhanced when animals made active searching movements. In insects, the task-specific reinforcement of muscle synergies can ensure that substrate adhesion is rapidly established after substrate contact to provide a stable point for force generation.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Activation of muscles in modular groups with similar biomechanical actions (synergists) is viewed as a basic mechanism in motor control (Ting and Macpherson, 2005; De Groote et al., 2013). The use of muscles in synergist groups simplifies the regulation of limbs with multiple degrees of freedom (Bernstein, 1967) and may be reflected in the organization of pre-motor elements in the nervous system (Hart and Giszter, 2010). Similar synergies can be utilized in diverse behaviors, such as standing and walking, but the magnitude and timing of muscle activation is adaptable and depends upon the behavior and context, i.e. it is task specific (Chvatal and Ting, 2013).

Sensory receptors of the limbs that monitor forces and movements could readily contribute to the generation and adaptation of muscle synergies (Safavynia and Ting, 2013; Duysens et al., 2013). Some types of sense organs, such as receptors that monitor muscle forces and loads, have been shown to have widespread effects on motor neurons to leg muscles (Eccles et al., 1957; Harrison et al.,

1983). However, the specific contribution of force feedback to use of muscles as synergists is poorly understood.

The present experiments have studied how force feedback in insects contributes to activation of muscle synergies in substrate grip, a behavior that involves the coordinated activation of muscles at different leg joints. In all insects, substrate contact occurs through adhesive and frictional structures on the feet (tarsi) (reviews Gorb, 2001, 2008; Labonte et al., 2014) but it is established and actively maintained by a discrete pattern of activation of leg muscles at different leg joints as synergists (Wile et al., 2008; Zill et al., 2014). Previous studies have shown that forces exerted in substrate grip are detected by campaniform sensilla, sense organs that detect strains in the exoskeleton (Zill et al., 2010). Experiments in stick insects have demonstrated that campaniform sensilla of the tarsus (foot) can provide positive feedback to the muscle that directly generates substrate grip (Zill et al., 2014). However, it has been not clear how these effects are integrated with force signals from campaniform sensilla of other leg segments and how they contribute to the requisite muscle synergies.

In the present study, we characterized the effects of campaniform sensilla of the feet (tarsi) and proximal segments (trochanter and femur) on activities of leg muscles in stick insects and

* Corresponding author. Tel.: +1 304 696 7384; fax: +1 304 696 7290.
 E-mail address: sensillum@aol.com (S.N. Zill).

cockroaches. These experiments were designed to test the hypothesis that the effects of force detecting sense organs of the feet are integrated with force generation for support of body load through activation of the same muscle synergies. The tarsal campaniform sensilla in both species have been identified and characterized but their motor effects were only studied in stick insects (Zill et al., 2014). Many groups of campaniform sensilla are found on the proximal leg segments, the trochanter and femur (Schmitz, 1993). Previous studies in cockroaches have suggested that the sensilla of proximal segments can strongly activate distal leg muscles: in 'pegleg' experiments, the leg is severed or denervated in the mid femur (Noah et al., 2004). Animals still use the leg stump in walking and show 'phantom' bursting in the tibial flexor and extensor muscles if the stump is pressed against the substrate, even though the distal muscles produce no tensions or movements. Those results suggested that the proximal sense organs can contribute to the activation of distal leg muscles, as was shown in earlier studies of stick insects (Akay et al., 2001). In addition, stick insects are advantageous in that extensive previous research has shown that the effects of leg sense organs are not constant but can be experimentally changed according to the behavior of the animal (Bässler and Büschges, 1998; Hellekes et al., 2012). Overall, our results support the idea that inputs of sense organs that detect forces in different leg segments are integrated, in part, through the activation of common muscle synergies.

2. Methods

Adult male cockroaches (Fig. 1A, *Periplaneta americana*) were obtained from Carolina Biological Supply and fed puppy chow (Purina). Adult female stick insects (Fig. 1B, *Carausius morosus*) were obtained from breeding colonies at the Universities of Bielefeld and Cologne.

2.1. Preparations for recording and mechanical stimulation

The methods of mechanical stimulation of tarsal campaniform sensilla have been previously described (Zill et al., 2010, 2014). Animals were restrained and all proximal segments (up to the second or third tarsal segment) of the left middle leg (stick insects) or right hind leg (cockroaches) were supported and constrained by staples (Fig. 2A, B). The distal tarsal segment and pretarsus were free to move. Myographic activities were recorded with pairs of 50 micron silver wires. The wires were placed in the proximal coxa to record the depressor, mid femur to monitor the flexor and proximal tibia to record the retractor unguis muscle (recordings of the femoral part of the retractor were complicated by cross talk). Forces were imposed on the tarsus using a probe with strain gauges mounted on a piezo-electric crystal (Fig. 2A, B; Zill et al., 2010). Voltage waveforms were generated from pre-recorded sequences using a Cambridge Electronics laboratory interface (power 1401mkII, CED Ltd., Cambridge, UK).

2.2. Forces applied to the depressor muscle insertion

Forces were applied to the insertion of the Trochanteral Depressor muscle using a computer-controlled motor (as previously described, Zill et al., 2012) The shaft of a minuten pin was attached to the armature of the motor and the sharp end was inserted into the cuticle distal to the attachment of the depressor muscle tendon (Fig. 4A). In both cockroaches and stick insects, the proximal, ventral part of the trochanter is reinforced by an internal cuticular buttress (Zill et al., 2000, 2012), creating a small compartment distal to the muscle insertion. The tip of the pin was inserted through ventral cuticle into this compartment. Forces were applied to the pin and insertion point to mimic depressor
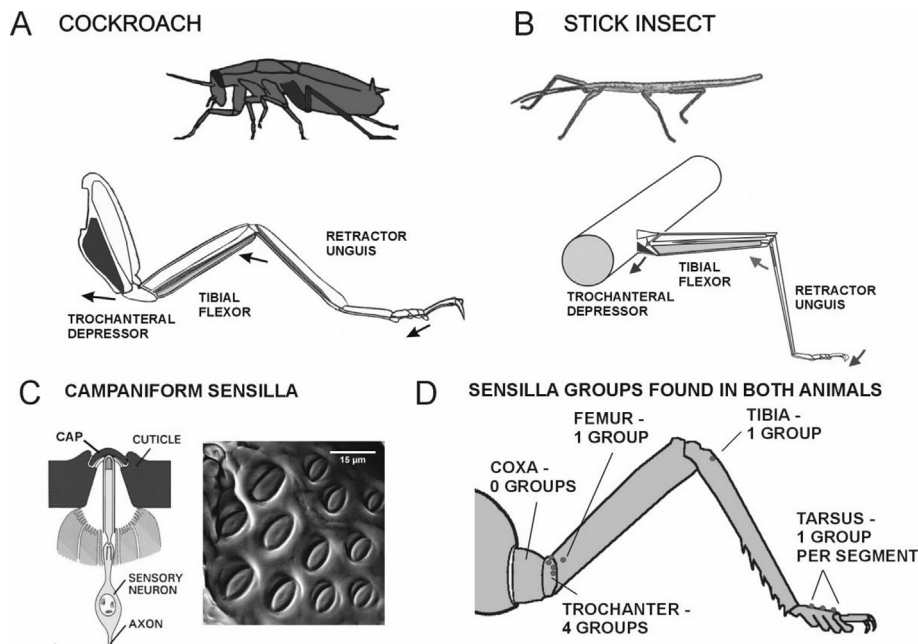

Fig. 1. Homologous leg muscles used in substrate grip and force detecting sense organs in cockroaches and stick insects – A. Cockroach leg muscles used in substrate grip – Diagram of cockroach hind leg. The tarsus is pressed against the substrate by the action of the retractor unguis muscle. Adhesion is enhanced and maintained by the tibial flexor and trochanteral depressor muscles. B. Homologous muscles in stick insect (middle leg) – The same muscles (retractor, flexor and depressor) are activated in substrate contact and adhesion in stick insects. C. Campaniform sensilla – Forces in the exoskeleton are detected by campaniform sensilla through attachments to cuticular caps in the exoskeleton. The sensilla are arranged in groups with consistent orientations of the oval shaped caps (scanning electron micrograph – stick insect trochanteral campaniform sensilla imaged by Annelie Exter, University of Bielefeld). D. Distribution of groups of campaniform sensilla – Diagram showing groups campaniform sensilla on the legs. A similar (common) arrangement of sensilla is found in both stick insects and cockroaches.
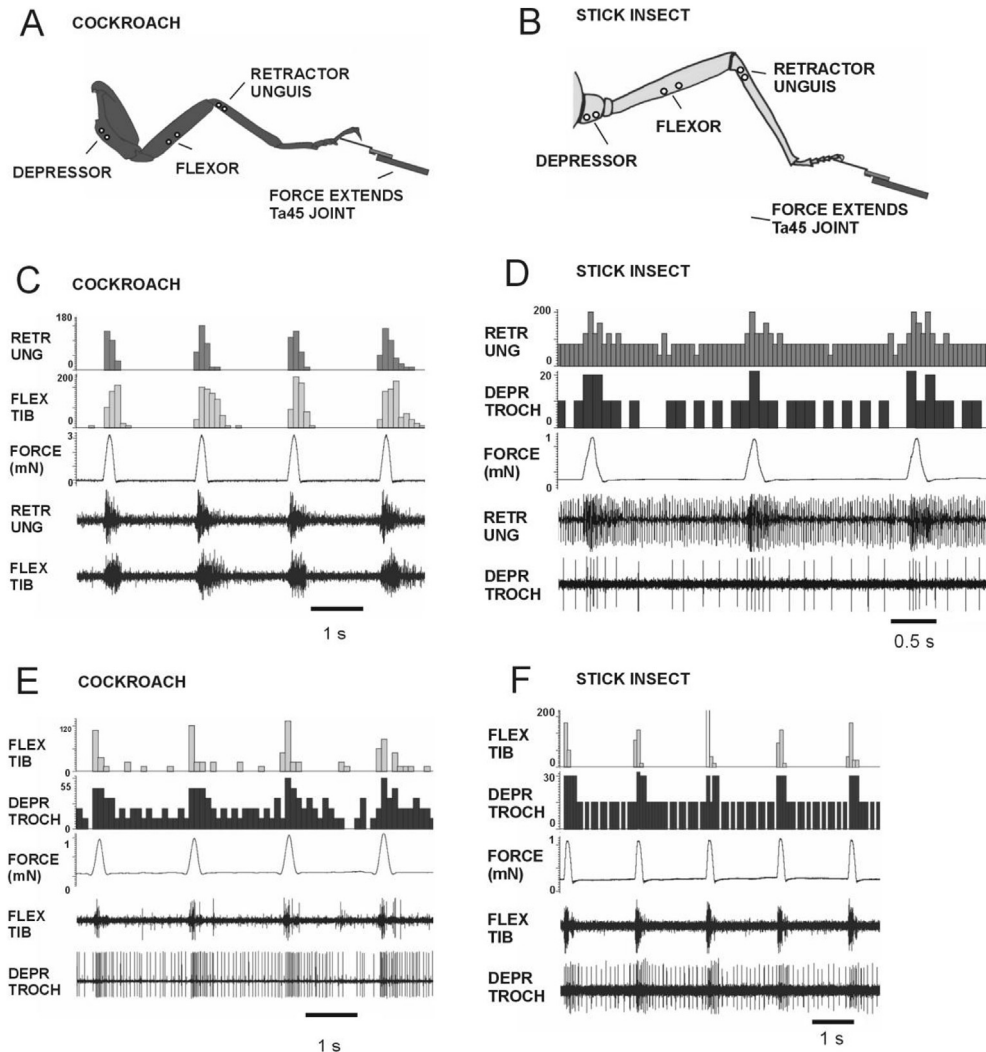
Fig. 2. Campaniform sensilla of the tarsus have widespread effects on leg muscles in cockroaches — A. and B. Preparations — Tarsal campaniform sensilla were activated by extending the joint between the fourth and fifth tarsal segments (Ta45 joint) with other leg segments immobilized. Motor activities were monitored in the retractor unguis, tibial flexor and trochanteral depressor muscles (recorded as pairs). C. Force applied to the cockroach tarsus produced simultaneous activation of the retractor unguis (Retr Ung) and tibial flexor (Flex Tib) muscles. D. Recording in a stick insect showing excitation of the retractor unguis and the slow motor neuron to the depressor trochanteris muscle (Depr Troch). E and F. In both cockroaches (E) and stick insects (F), stimulation of the tarsal campaniform sensilla produced consistent firing in the tibial flexor and depressor trochanteris muscles.

muscle contractions. Voltage waveforms were applied to the motor from the CED interface (Cambridge Electronic Design). The plane of pull of the motor was carefully adjusted so that it produced smooth depression movements when unopposed by the force probe at the femur.

2.3. Pegleg preparation

In these experiments, the distal leg was removed at or distal to the femoro-tibial joint (Fig. 4A). To insure that the joint was denervated, we also severed the leg nerves in the distal femur through a small incision in the cuticle (Noah et al., 2004). A mixture of paraffin oil and petroleum jelly was placed on the cut end of the leg and the incision to limit bleeding and dehydration. Myographic activities were recorded as described above. Activities of campaniform sensilla were monitored in the coxa with pairs of wires placed adjacent to the main leg nerve (Zill et al., 1999).

2.4. Inducing active searching movements in stick insects

The legs were first restrained with staples and the left middle leg was severed in the distal femur. Forces were applied to the middle leg trochanter that emulated contraction of the depressor muscle with movement resisted. The restraints on the front legs were then removed to induce searching and attempted righting responses. These bouts often occurred repeatedly in stick insects between rest periods. Searching could also be induced by gently touching the abdomen or cerci. Attempts to apply similar techniques in cockroaches were unsuccessful (animals only showed intense and prolonged struggling and then often became unresponsive).

2.5. Data analysis

All nerve and muscle recordings, force signals and stimulating voltages were digitized using Spike2 software (Cambridge Electronics). Data were analyzed using Spike2 scripts and Excel

spreadsheets. Statistical tests were performed in SigmaStat and data were plotted in Sigmaplot.

3. Results

3.1. Leg muscles and force detection

Stick insects and cockroaches use similar mechanisms to establish and maintain substrate adhesion (Fig. 1). The tarsi (feet) produce adhesion/grip with the substrate by frictional and adhesive pads (euplantulae and arolia) and through grasping claws. Adhesion is directionally specific (anisotropic) and is actively maintained by muscle forces that pull the tarsus toward the animal, a process known as distributed inward grip. Tarsal engagement occurs through the actions of specific leg muscles (Fig. 1A, B): the leg and tarsus are lowered by the depressor (trochanteral extensor) muscle, the pretarsal claws and arolium are engaged by the retractor unguis muscle and the tarsus is pulled toward the animal by the tibial flexor muscle to establish adhesion and grip.

Campaniform sensilla monitor the forces generated by the leg muscles when the substrate is contacted and movement is resisted (Fig 1C, D). Groups of campaniform sensilla are found at discrete locations in the leg in a pattern that is similar in both stick insects and cockroaches. The most proximal segment, the coxa, has no groups of campaniform sensilla. Four groups are present on the trochanter, one group is found on the femur, the tibia and each of the tarsal subsegments.

3.2. Activation of leg muscles by tarsal campaniform sensilla in cockroaches and stick insects

The effects of tarsal campaniform sensilla in cockroaches and stick insects were tested by applying forces to the fifth tarsal segment with all other tarsal joints immobilized (Fig. 2A, B). Activities of leg muscles were recorded as pairs. Fig. 2C–F shows recordings during tests in which forces were applied to the tarsus using brief half sine waveforms. In the cockroach, repetitive mechanical stimulation of the sensilla produced bursts in the retractor unguis, tibial flexor and trochanteral depressor muscles (Fig. 2C, E). Activity in the tibial extensor muscle was inhibited during the period of flexor excitation (N = 4, data not shown). We obtained similar recordings in stick insects, including myographic recordings of the Trochanteral Depressor muscle which had not previously been studied. Those tests showed regular activation of the same group of leg muscles (Fig. 2D, F).

Fig. 3 shows cumulative histograms of the motor effects of the tarsal sensilla in cockroaches (Fig. 3A) and stick insects (Fig 3B). In both animals, motor neuron firing increased after the onset of the stimulus and decreased during the declining phase. In these tests the mean latencies to onset of muscle bursting were in the range of 30–45 msec (Cockroach Latencies − Depressor 41.0 ± 89.1 msec. 388 repetitions in 3 animals; Flexor 29.6 ± 23.4 msec, 152 repetitions in 3 animals; Retractor − 39.5 ± 22.2, 93 repetitions in 3 preparations; Stick insect Latencies − Depressor − 41.4 ± 22.0 msec 337 repetitions in 3 preparations; Flexor 35.0 ± 12.6 msec 163 repetitions in 2 preparations recorded with depressor Retractor latency 43.1 ± 15.2 msec, 166 repetitions in 2 preparations recorded with depressor). Thus, in both animals the tarsal sensilla enhanced the muscle synergies that act to establish and maintain substrate adhesion.

3.3. Activation of cockroach distal leg muscles by campaniform sensilla on proximal leg segments

How are the motor effects of tarsal sensilla integrated with effects of receptors of proximal leg segments? We tested the effects of feedback from the trochanter and femur in cockroaches by applying forces at the insertion of the depressor muscle using 'pegleg' preparations (Fig. 4) as previous studies suggested that signals from campaniform sensilla contributed to 'phantom' bursting of tibial muscles (Noah et al., 2004). When movement was resisted, these forces activated the tibial flexor muscle and inhibited the tibial extensor (Fig. 4A, C). Flexor discharges were greatly reduced or absent when the forces were not resisted (Fig. 4B, E) while activity in the tibial extensor was variable, often showing transient excitation during the leg movement. Fig. 4D plots the mean discharges in the tibial flexor muscle when movement was resisted (n = 193 tests in N = 3 animals). Flexor motor neurons (large and medium amplitude potentials) showed intense bursting at short latency after the onset of resisted forces. The mean latency of the large units in the tests plotted in Fig. 4D was 21.7 ± 12.1 msec. Fig. 4F is a comparable histogram of the flexor muscle in tests in which force was not resisted (n = 134 tests in N = 3 same animals). Leg movements produce little or no excitation of the flexor. These studies imply that the interjoint effects on the flexor were due to stimulation of force receptors, not sense organs encoding joint position or movement.

We were able to directly confirm this in cockroaches by recording activities of the trochanteral campaniform sensilla during application of forces to the depressor muscle (Fig. 5A–C). Our previous experiments and modeling studies demonstrated that loads applied to the distal femur in the plane of movement of the coxo-trochanteral joint were encoded by trochanteral Group 3 campaniform sensilla (Zill et al., 1999; Kaliyamoorthy et al., 2005). We therefore ablated all groups of sensilla except Group 3 and cut the main leg nerve (n5) to eliminate motor discharges. Intense firing of units of large and small amplitude occurred when the forces applied at the depressor insertion were resisted (Fig 5A) but were absent when the force probe was removed and leg the moved freely (Fig. 5B). We confirmed that these discharges were derived from the Group 3 by ablation of the cuticular caps, which eliminated the recorded sensory activities (Fig. 5C). In addition, we tested whether the Group 3 sensilla mediated the excitatory effects on the tibial flexor muscle (all sensilla groups and motor innervation were intact in these tests). We found that discharges in the tibial flexor muscle to forces applied at the depressor insertion were effectively eliminated by ablation of the Group 3 sensilla (Fig. 5D).

We also tested the effects of feedback from proximal receptors on the tibial part of the retractor unguis muscles. Tests using forces applied to the depressor insertion in cockroaches were complicated by repetitive movements of the femorotibial joint by the intact tibial muscles (this method was successful in stick insects). We therefore tested effects in the retractor unguis by selective stimulation of the cuticular caps of Group 3 sensilla (Fig. 5E–G). Surprisingly, in some preparations stimulation of the cuticular caps of single Group 3 sensilla could elicit spiking activity in the retractor (Fig. 5E). In these tests, the mean latency from the sensory discharge to the onset of large potentials in the retractor was 20.7 ± 11.7 msec (n = 200 repetitions in N = 2 preparations). In stable preparations, repetitive bursts in the retractor were elicited by cap stimulation (Fig. 5F). Fig. 5G is a cumulative histogram of retractor activity normalized to the onset of movement of the piezoelectric crystal that drove the cap stimulator (n = 312 repetitions in N = 3 preparations). These experiments showed vigorous excitation of the retractor unguis and confirmed that the Group 3
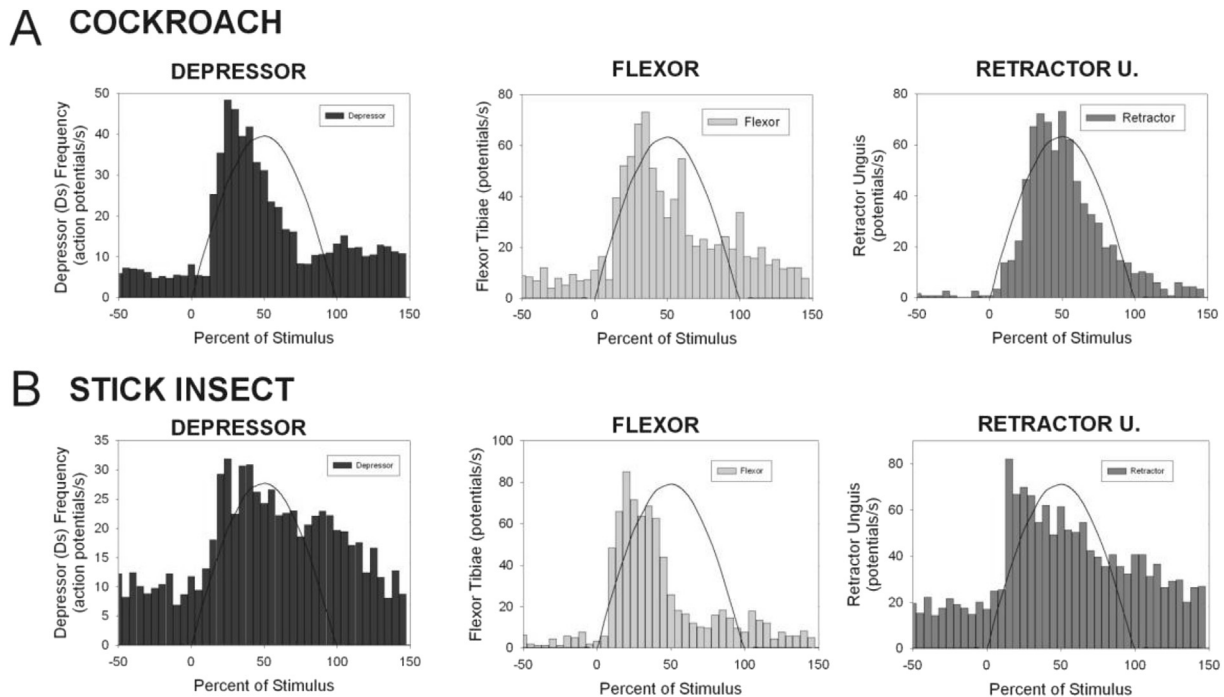
Fig. 3. Cumulative histograms of motor responses to tarsal sensillum stimulation − A. Histograms of motor responses in cockroaches in the depressor trochanteris (left), tibial flexor (middle) and retractor unguis muscles (Depressor n = 411 tests in N = 3; Flexor n = 152 tests in N = 3, Retractor n = 93 tests in N = 3). B. Histograms of motor responses to sensory stimulation in the same muscles in stick insects (Depressor = n 353 tests in N = 4; Flexor 126 tests in N = 2 recorded simultaneously with depressor; Retractor n = 151 tests in N = 2 recorded simultaneously with the depressor). In both animals, tarsal campaniform sensilla activated the muscles that act as synergists in producing substrate grip.

trochanteral campaniform sensilla could mediate interjoint effects on muscle synergies.

3.4. Effects of depressor muscle force on leg muscles in stick insects

We were able to perform similar experiments in stick insects in animals that were showing active searching movements. Our previous studies in stick insects showed that the effects of trochanteral campaniform sensilla on the Depressor muscle can show sign reversal when animals made active leg movements (evoked by touching the abdomen, Zill et al., 2012). We adapted the stick insect preparation to systematically induce searching movements in the front legs (Berg et al., 2013). We first tested responses at rest (Fig 6A) and then released the restraints on the front legs to elicit searching movements (Fig. 6B). At rest, increased depressor force transiently inhibited firing in the depressor (Fig. 6C). When searching was induced in the front legs, the effects of depressor force reversed and produced intensely discharges in the slow depressor motor neuron (Fig. 6D). Depressor excitation could often be elicited for some time after apparent searching movements had ended (see also Fig. 8D; Bässler, 1976). Fig. 6E and F show cumulative histograms of responses of animals at rest (Fig. 6E, n = 290 repetitions in N = 3 animals) and after inducing front leg searching in the same preparations (Fig. 6F, n = 135 trials in N = 3 animals). Thus, the effects of force receptors on the depressor show sign reversal during active searching movements.

Does similar plasticity occur in the effects of the proximal receptors on the muscle synergies used in substrate grip? When force was resisted, depressor force produced strong excitation in the tibial flexor muscle when searching movements were induced in the front legs (Fig. 7A). Fig. 7B is a cumulative histogram of flexor firing when depressor force was resisted after inducing searching (large and small sized potentials counted in n = 299 repetitions in N = 3 preparations). The flexor discharges often showed large

potentials associated with recruitment of intermediate and fast units (mean latency 36.4 ± 14.0 msec in the tests shown in Fig. 7B). Flexor excitation was absent or greatly reduced when the resistance was removed and the leg moved freely (Fig. 7C, D; histogram n = 142 repetitions in the same animals, N = 3). Recordings of activities from the tibial extensor (data not shown, N = 5) showed consistent inhibition of the slow tibial extensor motor neuron during the period of flexor excitation (similar to findings of Akay et al., 2001; Akay, 2002).

We also evaluated the effect of front leg searching on the evoked flexor discharge by comparing tests before and after inducing searching movements in the front legs. Fig. 7E shows a recording of tibial flexor activity in which force was first applied at rest and then searching was initiated by releasing the restraints on the front legs. The flexor responses in the middle legs are greatly enhanced in the period following the onset of searching (see also Akay et al., 2001). Fig. 7F is a cumulative histogram of average flexor frequency recorded during the stimulus (half sine wave form) both at rest and after the initiation of searching (all size potentials counted, n = 66 tests at rest, n = 133 tests after searching, N = 5 animals). There is a large and significant increase in frequency following the onset of front leg searching (paired t-test, P = 0.002). Thus, the effects of the campaniform sensilla upon the tibial flexor show plasticity and increase in the gain of the response but not the reversal in sign seen in the trochanteral depressor.

As previous studies showed that femoral campaniform sensilla could mediate interjoint effects on the flexor muscle (Akay et al., 2001), we tested (N = 4) whether flexor discharges could be elicited to resisted forces applied to the depressor insertion after prior ablation of the femoral campaniform sensilla (technical limitations prevented tests before and after ablations in the same animal). These animals showed an overall reduction in spontaneous and elicited flexor activity but some responses persisted in the flexor muscle to forces applied at the depressor muscle

insertion (data not shown). The latency to the onset of flexor potentials was somewhat longer after ablation of the femoral campaniform sensilla (mean latency 45.58 ± 22.3 msec, n = 80 tests in N = 4 animals). Further studies are planned using stimulation of cuticular caps to test whether the trochanteral campaniform sensilla contribute activation of the flexor muscle (as was shown in cockroaches).

Are the interjoint effects on muscle synergies tonic or transient? We tested the extent of tonic coupling of the flexor muscle to force inputs by applying forces to the depressor insertion using ramp and hold waveforms. These tests were often problematic due to movements of the animal during the sustained stimuli. Fig. 8A is a recording of flexor responses in a test in which the animal showed no apparent movement. The flexor fires intensely to the force increase during the ramp phase but the response adapts completely during the hold phase. Similar results are seen in the cumulative histogram in Fig. 8B as motor firing rapidly adapted to baseline levels (n = 150 repetitions in N = 4 animals). Thus, the effects of force receptors on the muscle synergies are only phasic and do not produce tonic coupling to the sensory signals.

We also were able to utilize forces applied to the depressor insertion that were resisted in the femur to evaluate the effects of the proximal receptors on the retractor unguis muscle (Fig. 8C). In these preparations, the leg was severed in the middle of the tibia and recordings were obtained from the tibial part of the retractor immediately distal to the femoro-tibial joint (stick insects did not show the repetitive joint movements seen in cockroaches). Fig. 8D shows a recording of activities in the retractor unguis and depressor muscles in the period of time after searching movements were elicited in the front legs. Repeated force application elicited bursts in both muscles. Fig. 8E is a cumulative histogram of the responses of the retractor (n = 83 repetitions from N = 3 animals). The retractor shows a large increase in firing after the onset of force application. The latency to firing of the large retractor units was 32.2 ± 21.5 msec (n = 67, N = 3). Thus, activation of the proximal receptors could, in sum, enhance the activities of the muscle synergies stick insects use in substrate grip.

4. Discussion

These experiments have shown that, in both stick insects and cockroaches, 1) campaniform sensilla on the tarsi activate the muscles (retractor unguis, flexor tibiae, depressor trochanteris) that act as synergists to generate and enhance substrate grip; 2) receptors located on proximal leg segments that detect resisted forces
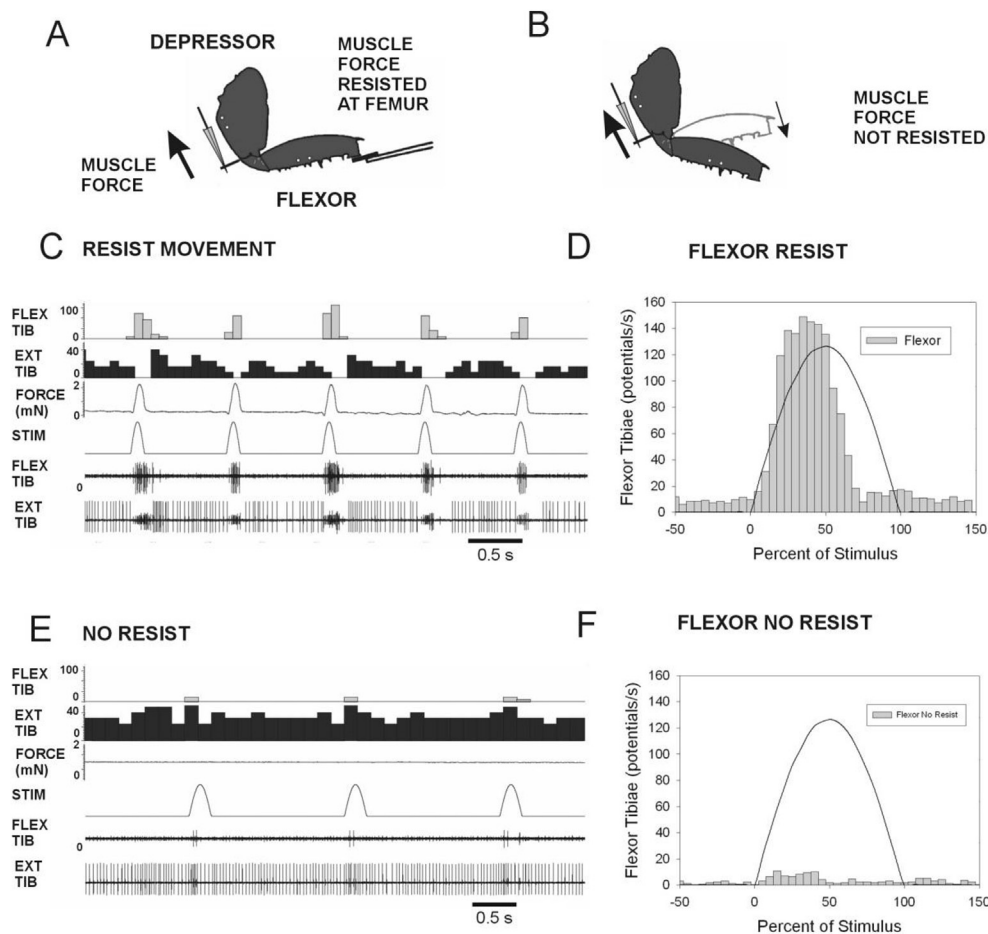

Fig. 4. Effects of depressor muscle force on the tibial muscles in the cockroach leg – A. 'Pegleg' preparation – The metathoracic leg was severed at the femorotibial joint and all nerves were cut in the femur. Forces were applied to pull on the insertion of the trochanteral depressor muscle. Movement was resisted and forces were measured in the distal femur. Myographic activities were recorded from the tibial flexor (Flex Tib) and extensor (Ext Tib) muscles. B. Movement not resisted – Mechanical stimuli produce unimpeded extension of the coxo-trochanteral joint when the force probe was removed. C. Activities of tibial muscles with movement resisted – Recordings of activities of the tibial muscles showed bursts in the tibial flexor and inhibition of the extensor muscles. D. Cumulative histogram of flexor muscle firing shows a rapid rise in discharge after onset of the stimulus. E. Activities with movement not resisted – Bursts in the flexor muscle were greatly reduced or absent. Variable discharges persisted in the tibial extensor. F. Histogram of flexor muscle during unresisted movement. Only small and delayed firing occurred in the flexor muscle.

of the depressor muscle activate the same muscle synergies (summary Fig. 9). In addition, studies in stick insects showed that effects of proximal receptors systematically change according to the behavior of the animal. When animals make active searching movements with the front legs, effects of depressor force change from inhibition to excitation of depressor motor neurons while excitatory effects in the tibial flexor are enhanced but not 'reversed' in sign. These results are discussed below in the context of previous findings about force detection and the mechanisms that generate synergistic muscle actions.

4.1. Substrate adhesion and the effects of tarsal campaniform sensilla on leg muscles

Adequate friction and adhesion with the substrate is essential to use of a leg in posture and locomotion (Gorb, 2008). Tarsal campaniform sensilla of cockroaches had strong and widespread effects on activities of motor neurons to the muscles (retractor, flexor, depressor) that generate and enhance substrate grip. We have also confirmed and extended our previous study in stick insects and shown that they excite the same muscle synergies. The excitatory
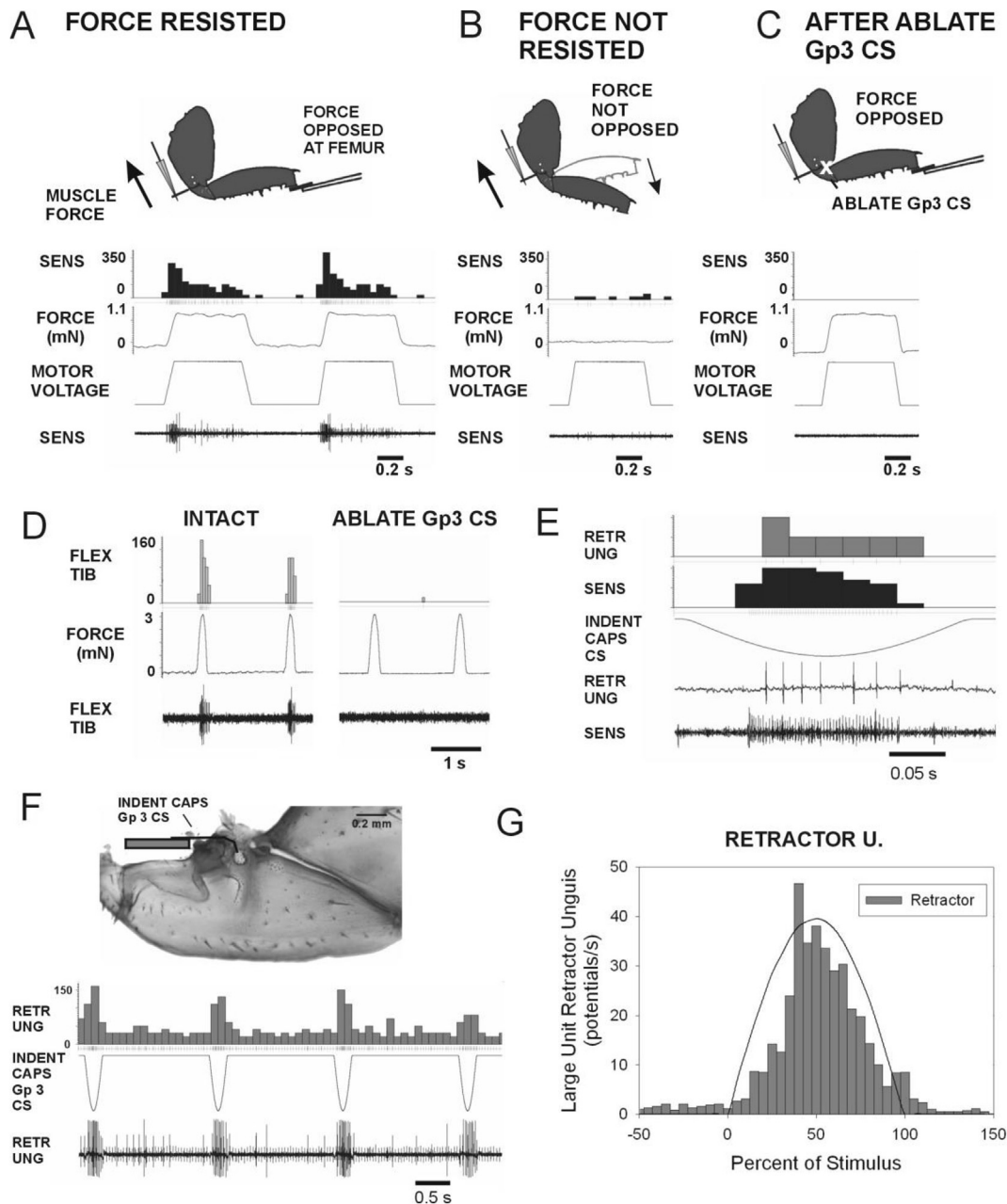

Fig. 5. Campaniform sensilla encode depressor muscle force and activate the retractor unguis – A. Preparation as in 4A but all nerves cut proximal to the coxa to record sensory activities in the distal coxa. Forces applied at the depressor apodeme produced bursts of sensory activities in units of large and small amplitude when movement was resisted. B. No discharges occurred when forces are not resisted. C. Ablation of the cuticular caps of Group 3 campaniform sensilla (Gp3 CS) eliminated the sensory discharge. D. Effect of ablation on flexor activities – Preparation as in 4A. Forces applied to the depressor insertion elicited bursts in the tibial flexor. Bursts did not occur after ablation of the Group 3 campaniform sensilla. E and F. Stimulation of Group 3 campaniform sensilla with a fine wire elicited a sensory bursts and firing of the retractor unguis (E). Repeated cap stimulation produced regular bursting in the retractor. G. Cumulative histogram of retractor firing to stimulation of caps of Group 3 campaniform sensilla. The histogram was averaged to the voltage applied to the cap stimulation (see delay in E).

effects of tarsal sensilla upon the retractor unguis can be considered to provide local positive feedback, as the receptors in both species have been shown to be activated by resisted forces from retractor muscle contractions (Zill et al., 2010, 2014). We also found that excitation of the tibial flexor muscle was accompanied by inhibition of the tibial extensor. Activation of the tibial flexor would therefore generate forces to pull the tarsus toward the animal. Pulling forces can enhance adhesion due to the surface structures of tarsal pads (pulvilli) and the orientation of the pre-tarsal claws (Labonte and Federle, 2013; Labonte et al., 2014; Ichikawa et al., 2014). In addition, insects and other arthropods can use pulling forces in opposite legs to generate adhesion without displacement of the body, in a pattern called distributed inward grip and (Wile et al., 2008; Wohlfart et al., 2014). The balance of pulling forces enables animals to stand on and traverse non-horizontal substrates. The excitatory effects on depressor muscle activity should generate force to press the leg and tarsus against the substrate and enhance grip by producing increased friction and adhesion (Quimby et al., 2006; Clemente et al., 2009; Schmitz et al., 2015). We have not, as yet, been able to test effects in the trochanteral depressor and levator muscles simultaneously in non-dissected preparations. However, previous studies in stick insects have shown that other

tarsal receptors activate the same muscle synergies (Fisch, 2007), including excitation of the depressor and inhibition of the trochanteral levator.

4.2. Effects of campaniform sensilla located on proximal segments on muscle synergies in cockroaches: 'phantom' bursting in pegleg walking

Forces applied to the depressor muscle insertion were used to mimic muscle contractions, as previous studies have shown that this method generates discharges in campaniform sensilla when movement is impeded (Zill et al., 2012, 2013). In cockroaches, resisted forces elicit vigorous firing of the tibial flexor. In addition, stimulation of the caps of the Group 3 campaniform sensilla (that encode resisted depressor forces) excited the retractor unguis muscles. While activities of the retractor unguis have not previously been characterized in freely moving cockroaches, recordings of activities of the tibial muscles during slow walking showed that the tibial flexor muscle fires early in the stance phase, followed by a period of co-contraction with the tibial extensor (Krauthamer and Fourtner, 1978; Watson et al., 2002). The prolongation of flexor firing and co-contraction was dependent upon sensory inputs, as it
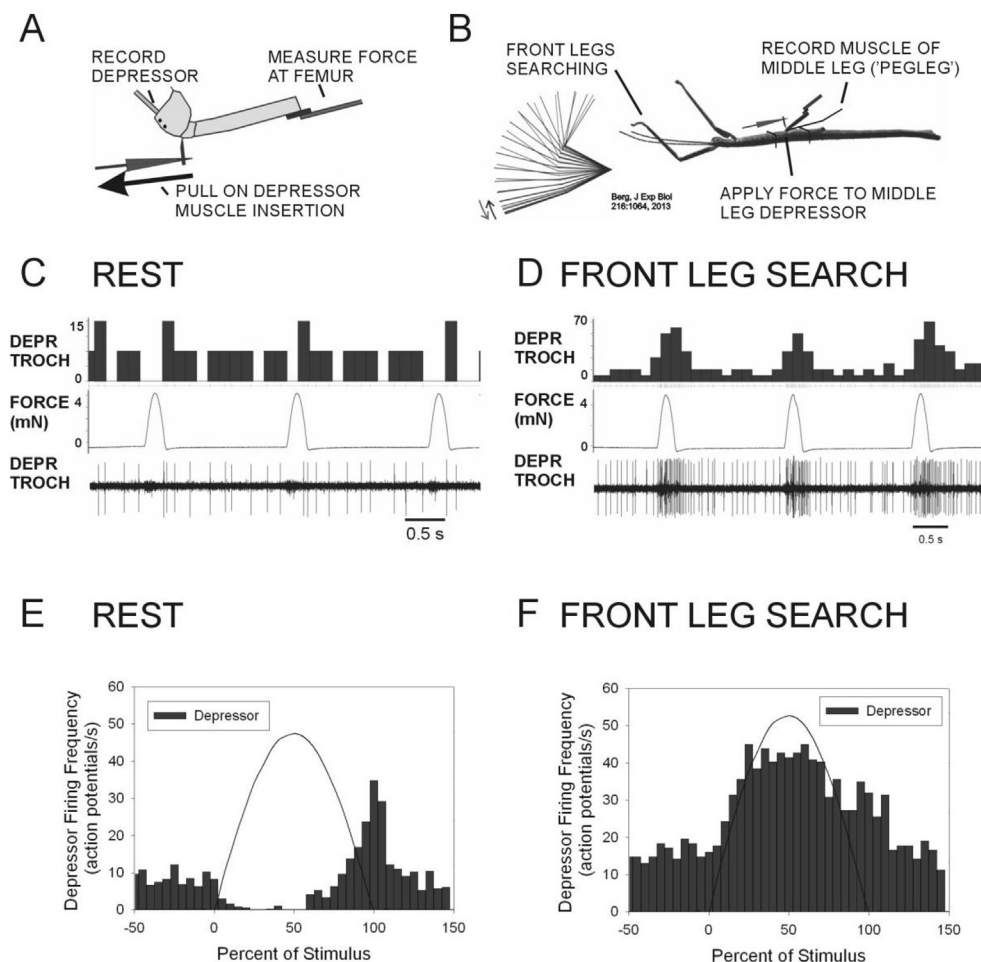

Fig. 6. Reversal of effects of depressor force on the slow depressor motor neuron during front leg searching movements in stick insects – A. Preparation – Forces were applied to depressor muscle insertion the middle leg and resisted on the end of the femur. Depressor muscle activity was monitored in the coxa. B. Effects of the sensilla were first tested with the front legs restrained. The front legs were then free and made active searching movements while the effects of forces were tested in the middle legs (inset of front leg searching movements redrawn from Berg et al., 2013). C. Response at rest – Resisted forces produced transient inhibition in the slow depressor motor neuron in resting animals. D. Searching movements – The same stimulus produced excitation of the depressor after inducing searching movements. E. Cumulative histogram of responses at rest – The depressor was inhibited during the stimulus but showed transient rebound excitation after force application. F. Histogram of responses after inducing searching movements – The depressor showed consistent excitation that slowly declined after force application.

was absent when the leg did not make contact with the substrate (Krauthamer and Fourtner, 1978, Fig. 5).

The effects of proximally located campaniform sensilla on the tibial muscles also provides a mechanism for the observation of 'phantom' bursting in 'pegleg' experiments (Noah et al., 2004). In those studies, the leg was severed (or all nerves and muscle tendons cut) in the distal femur. The 'pegleg' was still used in walking if it contacted the substrate and movement was resisted. Recordings from the tibial muscles showed 'phantom' bursting in the tibial muscles when the leg or stump was pressed against the substrate (by the intact coxal muscles), even though the tibial muscles produce no tensions or movements. Only irregular firing at low levels occurred if the leg or stump did not contact the surface. The discharges of the trochanteral campaniform sensilla, demonstrated in the present experiments, could readily facilitate the occurrence of 'phantom' discharges in the tibial flexor muscle when leg movements are resisted.

4.3. Diverse mechanisms of effects of campaniform sensilla in stick insects

We were able to test the effects of feedback from forces applied to the depressor muscle insertion in stick insects, both at rest and during active movements. Previous studies in stick insects showed that the effects of trochanteral campaniform sensilla on the depressor muscle could show sign reversal when animals were made 'active' by touching the abdomen (Zill et al., 2012). We found that reversal in sign of effects on the depressor also occurred in the
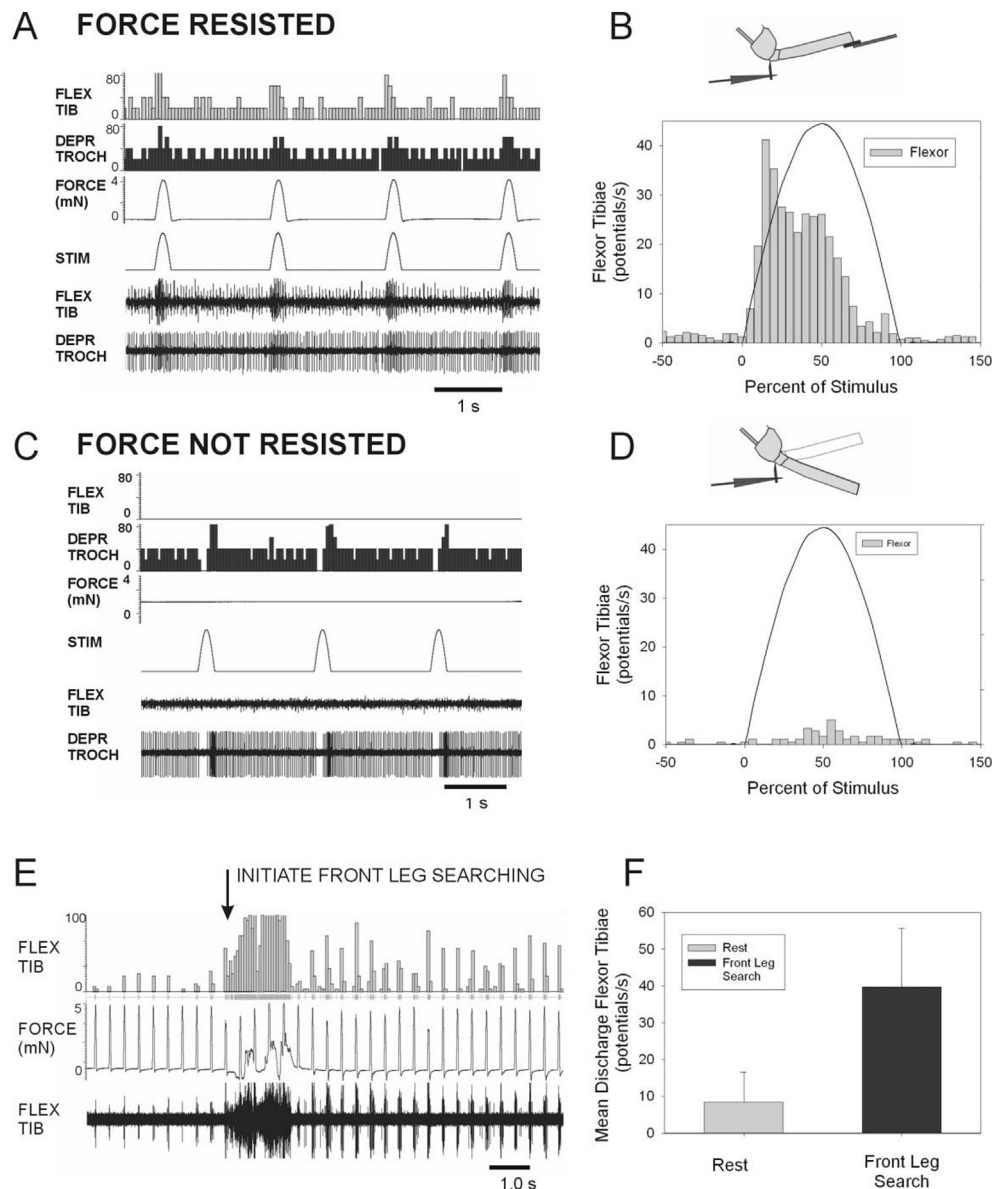

Fig. 7. Effects of depressor force on flexor muscle activities in the stick insect tibial flexor muscle — A. Effects of resisted forces — during searching movements of front legs, both the flexor and depressor muscles showed excitatory responses to resisted forces applied to the trochanter. B. Histogram of tibial flexor discharges (large and small potentials included) showed rapid excitation that declined as forces decrease. C. Responses to unresisted forces — Flexor responses were absent when resistance was removed and the coxo-trochanteral moved freely. D. Cumulative histogram of flexor muscles responses — Only small discharges occur in some preparations. E. Effect of inducing searching movements on flexor muscle activities — In this sequence, the animal was at rest and then searching movements were elicited by removing restraints on the front legs. The responses in the flexor muscle to resisted forces were greatly increased after initiation of searching. F. Plot of flexor responses at rest vs. after inducing searching — The mean frequency of recorded potentials greatly increased when active searching movements were elicited.

middle legs when active searching movements were induced in the front legs of stick insects (Bässler et al., 1991; Berg et al., 2013). At rest, increased depressor force transiently inhibited firing in the depressor. The effects reversed and increased force enhanced depressor firing immediately following bouts of front leg searching. Although front leg movements were not monitored in the present experiments, the excitatory effects in the depressor could be prolonged and continue after large movements of the front legs ceased (see also Bässler, 1976). Stepping movements of front legs have been demonstrated to entrain rhythmic activities in legs of caudal segments (Borgmann et al., 2009). Reflex reversal in effects of the femoral chordotonal organ of the middle legs have also been shown to be enhanced by movements of the front legs (Hellekes et al., 2012). Reversals of the motor effects of the trochanteral

campaniform sensilla also occur during walking when the direction of progression is changed (Akay et al., 2007). The effects of many types of proprioceptive sense organs have been shown to be task specific, in both invertebrates and vertebrates (Duysens et al., 2000; Mu and Ritzmann, 2008).

We also found that increased depressor force produced excitation in the tibial flexor and retractor unguis muscles in stick insects when force was resisted, but not when the leg is free to move. Tests applied before and after front leg searching showed that the effects on the flexor muscle were enhanced by active movements but did not change in sign. Previous experiments by Akay et al. (2001) studying the effects of joints movements showed that levation of the femur often induced flexor discharges accompanied by a decrease or inactivation of tibial extensor activity. Akay et al. (2001)
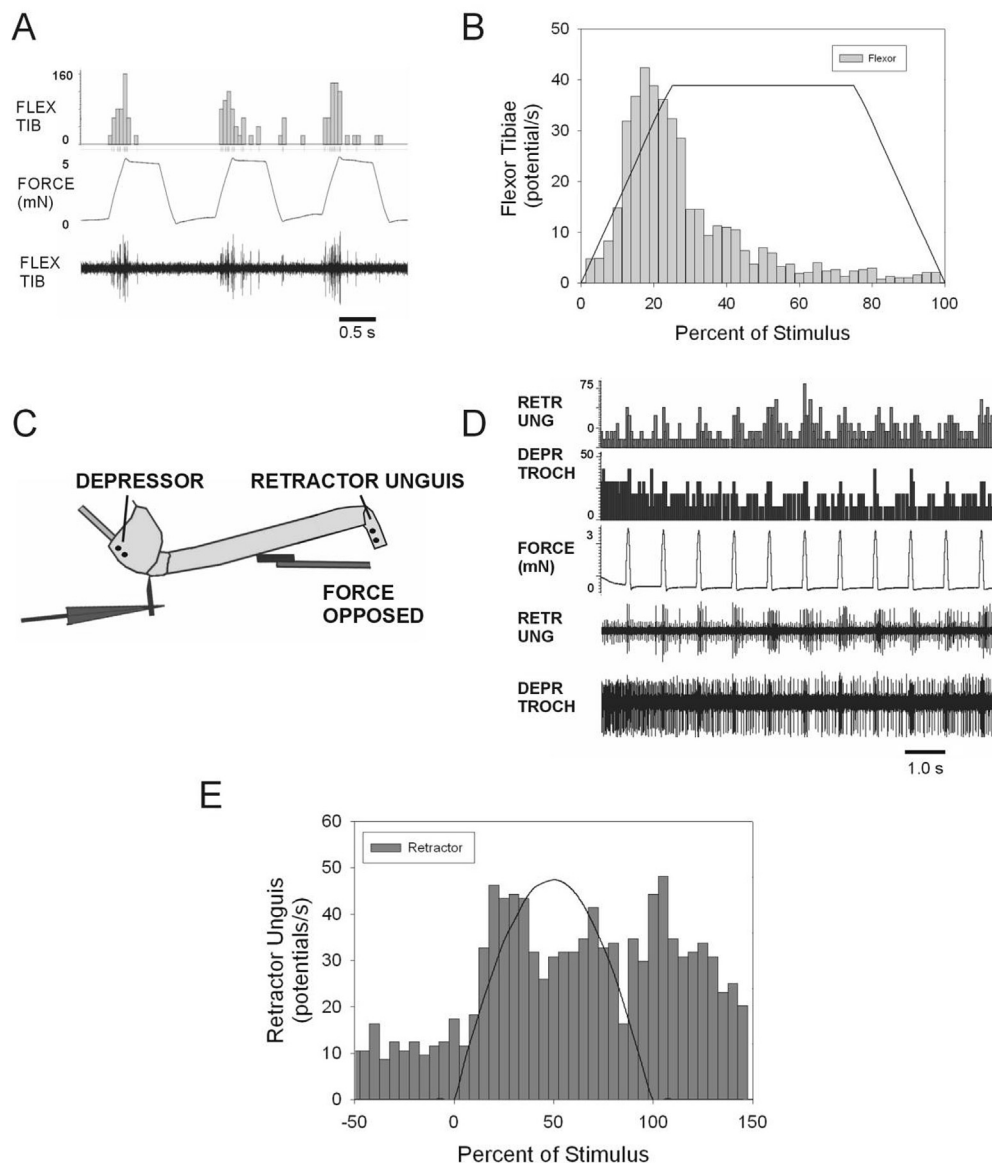

Fig. 8. Response adaptation and effects on stick insect retractor unguis muscle — A. Activity of the flexor muscle was recorded during searching movements of the front leg. Forces were imposed that mimicked depressor muscle contractions using ramp and hold waveforms. The flexor muscle responds vigorously during the ramp but shows considerable adaptation in the hold phase. B. Cumulative histogram of responses of flexor responses using ramp and hold waveforms. The responses were not sustained through the 1 s hold phase. C. Preparation — Force was applied at the insertion of the trochanteral depressor muscle and resisted in the femur. Activity of the retractor unguis muscle was recorded in the proximal tibia (the femoro-tibial joint was intact). D. Forces applied to the depressor insertion produced increased firing in the retractor unguis and depressor muscles immediately following a bout of searching movements in the front legs (note elevated firing at the start of the sequence). E. Cumulative histogram of retractor unguis during searching — Retractor firing increases in response to the force increment and remained irregularly sustained following the force application.
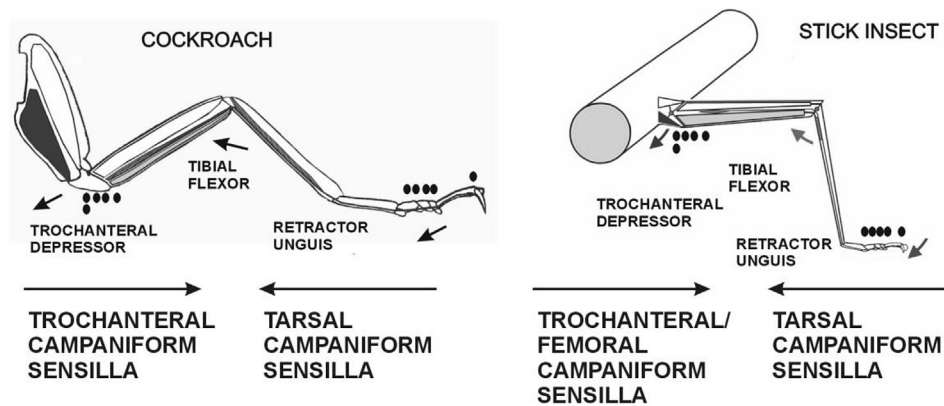
SUMMARY: FORCE FEEDBACK REINFORCES MUSCLE SYNERGIES

Fig. 9. Summary: Force feedback reinforces muscle synergies — In both stick insects and cockroaches, tarsal campaniform sensilla enhance the activities of the muscles used in substrate grip (retractor unguis, trochanteral depressor, tibial flexor). Resisted forces exerted by the trochanteral depressor muscle excite campaniform sensilla in the proximal leg and activate the same muscle synergies. See text for discussion.

also found that joint movements of the femur had only a weak influence on the activity of extensor and flexor motor neurons in animals at rest but became much more pronounced when animals were made 'active' by touching the abdomen with a paintbrush (see also Bässler et al., 1991; Hess and Büschges, 1997). The sign of the activation of the flexor to coxo-trochanteral joint movement did not reverse. A series of experiments showed that the inhibition of extensor activity persisted after ablation of sense organs of the trochanter but was eliminated by ablation of the femoral campaniform sensilla (Akay et al., 2001).

No previous studies in stick insects have reported activation of the retractor unguis to stimulation of the trochanteral or femoral campaniform sensilla. In stick insects, intracellular recordings from retractor unguis motor neurons during single leg stepping showed a sustained depolarization during stance (Fischer et al., 2001). Retractor unguis activity is also strongly influenced by the femoral chordotonal organ and the muscle is excited in active reactions (Nothof and Bässler, 1990). Recordings of the retractor muscle in freely walking locusts and beetles showed high frequencies of activity following substrate contact that were sustained at a lower levels during the stance phase (Laurent and Hustert, 1988; Bußhardt and Gorb, 2013, 2014).

4.4. Specific groups activated on the trochanter/femur

In cockroaches, we showed that the Group 3 trochanteral campaniform sensilla could mediate the effects of depressor forces on distal muscles: Group 3 sensilla encoded forces applied at the depressor muscle insertion when movement was impeded but do not discharge during unresisted leg movements. Ablation of the Group 3 sensilla could eliminate the discharge in flexor muscle to depressor forces. We also showed that stimulation of the caps of the receptors could drive activities in the retractor unguis. Group 3 sensilla have previously been shown in physiological and modeling studies to be activated by loads applied in the direction of joint flexion in the plane of movement of the coxo-trochanteral joint (Zill et al., 1999; Kaliyamoorthy et al., 2005).

In contrast, studies in the stick insect showed that the reinforcement of muscle synergies was strongly dependent on signals from the femoral campaniform sensilla. Previous studies have shown that mechanical stimulation of the femoral sensilla produced excitation of the tibial flexor and inhibition of the extensor (Akay et al., 2001). Schmitz et al. (2015) have also recently studied

the sense organs that affect motor neuron firing in the stance phase in a single leg preparation using a 'foot in the hole' paradigm. They found that activation of the trap door produced a delay in activation of the tibial flexor muscle. A change in timing of the flexor muscle was also found after ablation of the femoral campaniform sensilla. They did not quantify the changes in magnitude of the bursts, so direct comparison with the present study is not possible. In the present study, we found that flexor coupling was substantially reduced but not invariably eliminated after ablation of the femoral campaniform sensilla, implying that the trochanteral sensilla may also influence tibial muscle activities in stick insects. Further studies are planned using stimulation of cuticular caps to identify the specific groups that could mediate these effects.

One factor that could readily contribute to the relative importance of the different groups of sensilla is the structure of the joint between the trochanter and femur. In stick insects, trochanterofemoral joint is fused and forces are directly transmitted to the femur (Bässler, 1983). In cockroaches and many other insects, the articulation between the trochanter and femur is a hinged joint and cyclical changes in the joint angle occur in walking (Frantsevich and Wang, 2009; Bender et al., 2010). However, the specific responses of the femoral campaniform sensilla have not been determined and further studies are planned to characterize the groups in both cockroaches and stick insects.

4.5. Mechanisms underlying muscle synergies in insects

The interjoint effects demonstrated in the present study support the idea that inputs from the campaniform sensilla that detect forces can reinforce muscle synergies. Receptors that monitor forces may be particularly important in enhancing appropriate muscle synergies as a multi-jointed leg transmits force as a linked chain and the effect of torques at individual joints are, to some extent, interdependent (ex. Prilutsky and Gregor, 1997; McGowan et al., 2010).

Many studies have shown that sensory feedback plays an important role in determining interjoint coordination in walking. In stick insects, interneurons that generate walking are thought to form oscillators that can function independently at each joint (Büschges, 1995; Büschges and Borgmann, 2013). Sensory feedback produced by joint movements, muscles forces and loads can serve to determine the phase of activation of muscles as synergists (Hess and Büschges, 1997, 1999; Akay et al.,

2004; Büschges and Gruhn, 2008). Recent models have been able to elegantly formulate these effects as analog inputs, similar to neuronal activities (Daun-Gruhn and Büschges, 2011; Toth et al., 2015).

The present study has shown that the effects of campaniform sensilla on muscle synergies can occur in animals that are not walking but showing searching movements. However, several factors suggest that the reinforcement of muscle synergies shown in the present study do not function as 'chain reflexes' or simple sensory switches. Campaniform sensilla encode muscle forces only when movement is resisted (Zill et al., 2004, 2012). Application of load alone does not generate sensory discharge but instead depends upon the presence of muscle activation, which can occur without prior reflex activation (Quimby et al., 2006). In addition, latencies of interjoint effects are relatively long, suggesting that they act through interneurons rather than by direct effects upon motor neurons. Most effects also showed considerable adaptation so that sensory signals could effectively reinforce centrally driven activities but not independently sustain motor discharges. Overall, the system showed considerable plasticity and task dependence that resulted in precise but complex changes in sign and gain in the effects of force inputs.

These findings instead support the idea that activation of muscles as modules is dependent upon properties of premotor interneurons rather than not direct or fixed reflex activation. Few studies have examined the interneuronal processing of signals from campaniform sensilla in insects (Elson, 1987; Newland and Emptage, 1996; Akay, 2002). In addition, the mechanisms that couple local interneurons are not well understood and a systematic organization of premotor elements, similar to the motor 'primitives' of vertebrates, has not been identified in invertebrates (Hart and Giszter, 2010).

However, previous studies have identified interneurons that can affect the activities at more than one joint (Büschges, 1995; Rosenbaum, 2013). Recent work has elegantly demonstrated that some interneurons may be particularly important in searching for substrate grip (Berg, 2014; Berg et al., 2015). One non-spiking premotor interneuron, I4, is known to provide excitatory synaptic drive to the muscles synergies that produce substrate grip (retractor, depressor, flexor) as well as inhibitory synaptic drive to the tibial extensor (Sauer et al., 1996; Büschges, 1995). In addition, NSI I4 has also been shown to be a part of the network that generates rhythmic leg movements (Büschges, 1995). Non-spiking interneurons have been shown to produce task specific changes in gain of sensory effects on motor output (Hellekes, 2012). The effects of campaniform sensilla on these interneurons and the pre-motor mechanisms that generate muscle synergies are important areas for future investigation.

In sum, all data in the present study support the idea that effects of force receptors are not monotonic but can be selectively tuned according to the specific forces they encode. Rather than occurring as fixed or invariant connections, utilization of interneuronal pathways and modulatory mechanisms can allow force signals to be used flexibly and adaptively (Hess and Büschges, 1997; Clarac, 2008; Duysens et al., 2013). Similar mechanisms may be present in both vertebrates and invertebrates.

Acknowledgments

References

Akay, T., Bässler, U., Gerharz, P., Büschges, A., 2001. The role of sensory signals from the insect coxa-trochanteral joint in controlling motor activity of the femur-tibia joint. J. Neurophysiol. 85, 594–604.

Akay, T., 2002. The Role of Sensory Signals for Interjoint Coordination in Stick Insect Legs (Carausius morosus and Cuniculina impigra) (Ph. D. thesis). University of Cologne.

Akay, T., Haehn, S., Schmitz, J., Büschges, A., 2004. Signals from load sensors underlie interjoint coordination during stepping movements of the stick insect leg. J. Neurophysiol. 92, 42–51.

Akay, T., Ludwar, B.C., Göritz, M.L., Schmitz, J., Büschges, A., 2007. Segment specificity of load signal processing depends on walking direction in the stick insect leg muscle control system. J. Neurosci. 27, 3285–3294.

Bässler, U., 1976. Reversal of a reflex to a single motoneuron in the stick insect, Carausius morosus. Biol. Cybern. 24, 47–49.

Bässler, U., 1983. Neural Basis of Elementary Behavior in Stick Insects. Springer, Berlin.

Bässler, U., Büschges, A., 1998. Pattern generation for stick insect walking movements: multisensory control of a locomotor program. Brain Res. Rev. 27, 65–88.

Bässler, U., Rohrbacher, J., Karg, G., Breutel, G., 1991. Interruption of searching movements of partly restrained front legs of stick insects, a model situation for the start of a stance phase? Biol. Cybern. 65, 507–514.

Bender, J.A., Simpson, E.M., Ritzmann, R.E., 2010. Computer-assisted 3D kinematic analysis of all leg joints in walking insects. PLoS One 5 (10), e13617.

Berg, E., 2014. . Adaptive Motor Control: Neuronal Mechanisms Underlying (Targeted) Searching Movements (Ph. D. thesis). University of Cologne.

Berg, E., Büschges, A., Schmidt, J., 2013. Single perturbations cause sustained changes in searching behavior in stick insects. J. Exp. Biol. 216, 1064–1074.

Berg, E.M., Hooper, S.L., Schmidt, J., Büschges, A., 2015. A leg-local neural mechanism mediates the decision to search in stick insects. Curr. Biol. http://dx.doi.org/10.1016/j.cub.2015.06.017.

Bernstein, N., 1967. The Coordination and Regulation of Movements. Pergamon Press, New York.

Borgmann, A., Hooper, S.L., Büschges, A., 2009. Sensory feedback induced by front-leg stepping entrains the activity of central pattern generators in caudal segments of the stick insect walking system. J. Neurosci. 29, 2972–2983.

Büschges, A., 1995. Role of local nonspiking interneurons in the generation of rhythmic motor activity in the stick insect. J. Neurobiol. 27 (4), 488–512.

Büschges, A., Gruhn, M., 2008. Mechanosensory feedback in walking: from joint control to locomotor patterns. Adv. Insect Physiol. 34, 193–230.

Büschges, A., Borgmann, A., 2013. Network modularity: back to the future in motor control. Curr. Biol. 23, 936–938.

Bußhardt, P., Gorb, S.N., 2013. Walking on smooth and rough ground: activity and timing of the claw retractor muscle in the beetle Pachnoda maginata peregrina (Coleoptera, Scarabaiedae). J. Exp. Biol. 216, 319–328.

Bußhardt, P., Gorb, S.N., 2014. Ground reaction forces in vertically ascending beetles and corresponding activity of the claw retractor muscle on smooth and rough substrates. J. Comp. Physiol. A. http://dx.doi.org/10.1007/s00359-014-0896-0 (Published on line: ISSN 0340-7594).

Chvatal, S.A., Ting, L.H., 2013. Common muscle synergies for balance and walking. Front. Comput. Neurosci. 7, 48. http://dx.doi.org/10.3389/fncom.2013.00048.

Clemente, C.J., Dirks, J.-H., Barbero, D.R., Steiner, U., Federle, W., 2009. Friction ridges in cockroach climbing pads: anisotropy of shear stress measured on transparent microstructure substrate. J. Comp. Physiol. A 195, 805–814.

Clarac, F., 2008. Some historical reflections on the neural control of locomotion. Brain Res. Rev. 57, 13–21.

Daun-Gruhn, S., Büschges, A., 2011. From neuron to behavior: dynamic equation-based prediction of biological processes in motor control. Biol. Cybern. 105, 71–78.

De Groote, F., Jonkers, I., Duysens, J., 2013. Task constraints and minimization of muscle effort result in a small number of muscle synergies during gait. Front. Comput. Neurosci. 8, 115. http://dx.doi.org/10.3389/fncom.2014.00115.

Duysens, J., Clarac, F., Cruse, H., 2000. Load regulating mechanisms in gait and posture, comparative aspects. Physiol. Rev. 80, 83–133.

Duysens, J., De Groote, F., Jonkers, I., 2013. The flexion synergy, mother of all synergies and father of new models of gait. Front. Comput. Neurosci. 7, 14. http://dx.doi.org/10.3389/fncom.2013.00014.

Eccles, J.C., Eccles, R.M., Lundberg, A., 1957. Synaptic actions on motoneurons caused by impulses in Golgi tendon organ afferents. J. Physiol. 138, 227–252.

Elson, R.C., 1987. Interneuronal processing of inputs from the campaniform sensilla of the locust hindwing. J. Comp. Physiol. A 161, 761–776.

Fisch, K., 2007. Investigations on the Role and Function of Tarsal Sensory Signals in the Stepping in the Middle Leg of the Stick Insect Carausius morosus (Master's thesis). University of Cologne, Germany.

Fischer, H., Schmidt, J., Haas, R., Büschges, A., 2001. Pattern generation for walking and searching movements of a stick insect leg: 1. intra- and interjoint coordination of motor activity in a single middle leg preparation. J. Neurophysiol. 85, 341–353.

Frantsevich, L., Wang, W., 2009. Gimbals in insect legs. Arthropod Struct. Dev. 38, 16–30.

Gorb, S.N., 2001. Attachment Devices of Insect Cuticle. Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 1–305.

Gorb, S.N., 2008. Biological attachment devices: exploring nature's diversity for biomimetics. Philos. Trans. R. Soc. A 366, 1557–1574.

Harrison, P.J., Jankowska, E., Johannisson, T., 1983. Shared reflex pathways of group I afferents of different cat hind-limb muscles. J. Physiol. 338, 113–127.

Hart, C.B., Giszter, S.F., 2010. A neural basis for motor primitives in the spinal cord. J. Neurosci. 30, 1322–1336.

Hellekes, K., 2012. . Task-specific Modulation of a Proprioceptive Reflex in a Walking Insect (Ph. D. thesis). University of Cologne.

Hellekes, K., Blincow, E., Hoffmann, J., Büschges, A., 2012. Control of reflex reversal in stick insect walking: effects of intersegmental signals, changes in direction, and optomotor-induced turning. J. Neurophysiol. 107, 239–249.

Hess, D., Büschges, A., 1997. Sensorimotor pathways involved in interjoint reflex action of an insect leg. J. Neurobiol. 33, 891–913.

Hess, D., Büschges, A., 1999. Role of proprioceptive signals from an insect femur-tibia joint in patterning motoneuronal activity of an adjacent leg joint. J. Neurophysiol. 81, 1856–1865.

Ichikawa, T., Toh, Y., Ohkubo, K., Nishino, H., 2014. Microscopic analysis of mechanosensory system monitoring the dynamic claw actions in the tenebrionid beetle *Zophobas atratus*. Zoomorphology 133, 273–284.

Kaliyamoorthy, S., Zill, S.N., Quinn, R.D., 2005. Force sensors in hexapod locomotion. Int. J. Robot. Res. 24, 563–574.

Krauthamer, V., Fourtner, C.R., 1978. Locomotory activity in the extensor and flexor tibiae of the cockroach, *Periplaneta americana*. J. Insect Physiol. 2, 813–819.

Labonte, D., Federle, W., 2013. Functionally different pads on the same foot allow control of attachment: stick insects have load-sensitive "heel" pads for friction and shear-sensitive "toe" pads for adhesion. PLoS One 8 (12), e81943. http://dx.doi.org/10.1371/journal.pone.0081943.

Labonte, D., Williams, J.A., Federle, W., 2014. Surface contact and design of fibrillar 'friction pads' in stick insects (*Carausius morosus*): mechanisms for large friction coefficients and negligible adhesion. J. R. Soc. Interface 11 (94), 20140034 doi: 10.1098.

Laurent, G., Hustert, R., 1988. Motor neuronal receptive fields delimit patterns of motor activity during locomotion of the locust. J. Neurosci. 8, 4349–4366.

McGowan, C.P., Neptune, R.R., Clark, D.J., Kautz, S.A., 2010. Modular control of human walking: adaptations to altered mechanical demands. J. Biomech. 43, 412–419.

Mu, L., Ritzmann, R.E., 2008. Interaction between descending inputs and thoracic reflexes for joint coordination in cockroach. I. Descending influence of thoracic sensory reflexes. J. Comp. Physiol. 194, 283–298.

Newland, P.L., Emptage, N.J., 1996. The central connection and actions during walking of tibial campaniform sensilla in the locust. J. Comp. Physiol. A 178, 749–762.

Noah, J.A., Quimby, L., Frazier, S.F., Zill, S.N., 2004. Walking on a 'peg leg': extensor muscle activities and sensory feedback after distal leg denervation in cockroaches. J. Comp. Physiol. A 190, 217–231.

Nothof, U., Bässler, U., 1990. The network producing the 'active reaction' of stick insects is a functional element of different pattern generating systems. Biol. Cybern. 62, 453–462.

Prilutsky, B.I., Gregor, R.J., 1997. Strategy of muscle coordination of two- and one-joint leg muscles in controlling an external force. Motor Control 1, 92–116.

Quimby, L., Amer, A., Zill, S.N., 2006. Common motor mechanisms support body weight in serially homologous legs of cockroaches in posture and locomotion. J. Comp. Physiol. A 192, 247–266.

Rosenbaum, P., 2013. Motor Flexibility: Neuronal Control of Walking Direction and Walking Speed in an Insect (Ph. D. thesis). University of Cologne.

Safavynia, S.A., Ting, L.H., 2013. Long-latency muscle activity reflects continuous, delayed sensorimotor feedback of task-level and not joint-level error. J. Neurophysiol. 110, 1278–1290.

Schmitz, J., 1993. Load-compensating reactions in the proximal leg joints of stick insects during standing and walking. J. Exp. Biol. 183, 15–33.

Schmitz, J., Gruhn, M., Büschges, A., 2015. The role of leg touchdown for the control of locomotor activity in the walking stick insect. J. Neurophysiol. 113 (7), 2309–2320.

Ting, L.H., Macpherson, J.M., 2005. A limited set of muscle synergies for force control during a postural task. J. Neurophysiol. 93, 609–613.

Toth, T.I., Grabowska, M., Rosjat, N., Hellekes, K., Borgmannm, A., Daun-Gruhn, S., 2015. Investigating inter-segmental connections between thoracic ganglia in the stick insect by means of experimental and simulated phase response curves. Biol. Cybern. 109 (3), 349–362.

Watson, J.T., Ritzmann, R.E., Pollack, A.J., 2002. Control of obstacle climbing in the cockroach, *Blaberus discoidalis*. I. Motor activities associated with joint movement. J. Comp. Physiol. A 188, 55–69.

Wile, G.D., Daltorio, K.A., Diller, E.D., Palmer, L.R., Gorb, S.N., Ritzmann, R.E., Quinn, R.D., 2008. Screenbot: walking inverted using distributed inward gripping. In: Proceedings of the IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS'08), Nice, France, pp. 1513–1518, 2008 IEEE/RSJ.

Wohlfart, E., Wolff, J.O., Arzt, E., Gorb, S.N., 2014. The whole is more than the sum of all its parts: collective effect of spider attachment organs. J. Exp. Biol. 217, 222–224.

Zill, S., Chaudhry, S., Exter, A., Büschges, A., Schmitz, J., 2014. Positive force feedback in development of substrate grip in the stick insect tarsus. Arthropod Struct. Dev. 43, 441–455.

Zill, S., Chaudhry, S., Schmitz, J., Büschges, A., 2012. Force encoding in stick insect legs delineates a reference frame for motor control. J. Neurophysiol. 108, 1453–1472.

Zill, S., Chaudhry, S., Büschges, A., Schmitz, J., 2013. Directional specificity and encoding of muscle forces and loads by stick insect tibial campaniform sensilla, including receptors with round cuticular caps. Arthropod Struct. Dev. 6, 455–467.

Zill, S.N., Frazier, S.F., Neff, D., Quimby, L., Carney, M., DiCaprio, R., Thuma, J., Norton, M., 2000. Three dimensional graphic reconstruction of the insect exoskeleton through confocal imaging of endogenous fluorescence. Micros. Res. Tech. 48, 367–384.

Zill, S.N., Keller, B.R., Chaudhry, S., Duke, E.R., Neff, D., Quinn, R., Flannigan, C., 2010. Detecting substrate engagement: responses of tarsal campaniform sensilla in cockroaches. J. Comp. Physiol. A 196, 407–420.

Zill, S.N., Ridgel, A.L., DiCaprio, R.A., Frazier, S.F., 1999. Load signalling by cockroach trochanteral campaniform sensilla. Brain Res. 822, 271–275.

Zill, S., Schmitz, J., Büschges, A., 2004. Load sensing and control of posture and locomotion. Arthropod Struct. Dev. 33, 273–286.