The Role of Sensory Signals From the Insect Coxa-Trochanteral Joint in Controlling Motor Activity of the Femur-Tibia Joint

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Akay, Turgay, Ulrich Bässler, Petra Gerharz, and Ansgar Büschges. 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, 2001. Interjoint coordination in multi-jointed limbs is essential for the generation of functional locomotor patterns. Here we have focused on the role that sensory signals from the coxa-trochanteral (CT) joint play in patterning motoneuronal activity of the femur-tibia (FT) joint in the stick insect middle leg. This question is of interest because when the locomotor system is active, movement signals from the FT joint are known to contribute to patterning of activity of the central rhythm-generating networks governing the CT joint. We investigated the influence of femoral levation and depression on the activity of tibial motoneurons. When the locomotor system was active, levation of the femur often induced a decrease or inactivation of tibial extensor activity while flexor motoneurons were activated. Depression of the femur had no systematic influence on tibial motoneurons. Ablation experiments revealed that this interjoint influence was not mediated by signals from movement and/or position sensitive receptors at the CT joint, i.e., trochanteral hairplate, rhombal hairplate, or internal levator receptor organ. Instead the influence was initiated by sensory signals from a field of campaniform sensillae, situated on the proximal femur (fCS). Selective stimulation of these fCS produced barrages of inhibitory postsynaptic potentials (IPSPs) in tibial extensor motoneurons and activated tibial flexor motoneurons. During pharmacologically activated rhythmic activity of the otherwise isolated mesothoracic ganglion (pilocarpine, 5 × 10−4 M), deafened except for the CT joint, levation of the femur as well had an inhibitory influence on tibial extensor motoneurons. However, the influence of femoral levation on the rhythm generated was rather labile and only sometimes a reset of the rhythm was induced. In none of the preparations could entrainment of rhythmicity by femoral movement be achieved, suggesting that sensory signals from the CT joint only weakly affect central rhythm-generating networks of the FT joint. Finally, we analyzed the role of sensory signals from the fCS during walking by recording motoneuronal activity in the single middle leg preparation with fCS intact and after their removal. These experiments showed that fCS activity plays an important role in generating tibial motoneuron activity during the stance phase of walking.

INTRODUCTION

Locomotion in legged organisms results from the interaction of central rhythm-generating networks in the nervous system with sensory organs that provide information about actual movements and forces generated by the appendages and changes in body posture and equilibrium (e.g., Bässler 1983; Bässler and Büschges 1998; Cruse 1990; Graham 1985; Grillner 1981; MacPherson et al. 1997; Orlovsky et al. 1999; Pearson 1995; Wendler 1964). In multi-jointed appendages, the emerging locomotor pattern results from the coordinated action of several joints. The control of the motor output in walking systems encompasses three different levels: intrajoint control, interjoint coordination, and intersegmental coordination (for summaries, see Clarac 1991; Cruse et al. 1995; Grillner 1981; Orlovsky et al. 1999; Pearson 1995; Stein and Smith 1997).

In insects and crustaceans, considerable detailed knowledge is available on intrajoint information processing in posture and movement control (crayfish: ElManira et al. 1991a; locust: summary in Burrows 1996; stick insect: Bässler and Büschges 1998). However, there is less known about the neuronal mechanisms responsible for interjoint information processing, specifically for the generation of coordinated activities during locomotion (e.g., Bässler 1993b; Büschges et al. 1995; ElManira et al. 1991b; Hess and Büschges 1997). In walking systems that have been reported to have a highly centralized structure, i.e., one common central locomotor pattern generator for the entire appendage, e.g., the locust (Ryckebusch and Laurent 1993) and the crayfish (Chrachri and Clarac 1990), evidence suggests that this central pattern generator organizes the motor output for all leg joints automatically. The situation, however, is more complicated in the case of walking systems that are less centrally organized and that contain neuronal networks governing the individual leg joints that are only loosely coupled, like the stick insect walking system (summary in Bässler and Büschges 1998). Here the question arises as to how the activities of adjacent leg joints are coupled together during locomotion and what role sensory and central signals might play in coordination.

A previous investigation (Hess and Büschges 1997, 1999) has shown that proprioceptive signals from one leg joint affect central rhythm generation of the adjacent leg joint when the stick insect locomotor system is active and the joint control networks are in the movement control mode (“active” behavioral state) (for definition, see Bässler 1983; Bässler and Büschges 1998). Movement signals from the femur-tibia (FT) joint e.g., flexion signals, induce specific transitions in activity of rhythm generating networks of the coxa-trochanteral (CT) joint, e.g., by eliciting levator and terminating depressor activ-
ity. Extension movements induced the opposite response. During locomotion this could facilitate the onset of leg levation due to flexion signals from the FT joint or the onset of leg depression during extension signals from the FT joint. From these studies, the question emerges as to whether such specific influences represent a common mechanism for interjoint coordination in the multi-jointed limb. Therefore we chose to investigate the influence of signals from sense organs at the CT joint on the activity of tibial motoneurons and muscles in the active stick insect. We applied levation and depression movements to the femur, while the locomotor system of the stick insect was active, i.e., in the locomotor state, and monitored changes in the activity of extensor tibiae and flexor tibiae motor neurons. Using ablation experiments, we analyzed which sense organs at the CT joint affect motoneuronal activity in tibial motoneurons and muscles. We also investigated whether the observed interjoint influences were mediated purely by “reflex-like” interactions or whether sensory signals from the CT joint have access to the central rhythm-generating premotor networks of the FT-joint. In a final series of experiments we investigated the role of this interjoint influence in controlling motor activity in the FT-joint during walking.

METHODS

The experiments were performed on adult female stick insects (Carausius morosus and Cuniculina impigra) from our breeding colonies at the University of Cologne and Kaiserslautern and were carried out under daylight conditions and at room temperature (20–22°C). Most of the experiments were carried out on both species. We did not detect any difference between C. morosus and C. impigra. In the text we specify for each given experimental protocol and figure the species used.

Preparation

EXPERIMENTS INVESTIGATING THE INFLUENCE OF FEMORAL LEVATION AND DEPRESSION. The experimental animal was fixed dorsal side up with dental cement (Protemp II, ESPE) along the edge of a foam platform with only the left middle leg left intact and fixed perpendicular to the thorax. The thoraco-coxal joint (TC joint) was immobilized with the coxa-trochanter joint (CT joint) being free to move. A small window was cut in the meso- and metathoracic tergum. The gut, fat, and connective tissue were removed to expose the ventral side. A small window was cut into the dorsal side of the thorax and the anterior and posterior connectives were also cut and pilocarpine (5 × 10⁻⁴ M) (Büschges et al. 1995) was applied in the Ringer solution (Weidler and Diecke 1969).

EXPERIMENTS WITH RESTRAINED MIDDLE LEGS. In experiments in which the influence of sensory stimulation on tibial muscle activity was investigated, the nervus cruris was left intact to allow EMG and nerve recordings in the femur. The lateral nerves, i.e., nl2, nl4, and nl5 were cut. In experiments with recordings from extensor motoneurons from nerve F2 in the femur, the nervus lateralis 3 (nl3) was left intact. The trochanteral campaniform sensilla (trCS) were destroyed with a fine insect pin (Schmitz 1993), that was heated and pushed into the cuticle at their location. The FT joint and CT joint were both immobilized with dental cement. In these experiments, femoral campaniform sensilla were selectively stimulated mechanically (see experiment shown in Fig. 8A) by means of a low-voltage piezo-electrical element (PI Physic). The femoral chordotonal organ (ICO) was stimulated as well (C. morosus), according to established procedures (Büschges 1989). In short, a small window was cut into the dorsal side of the femur. The apodeme of the ICO was exposed and then fixed in the clamp of a stimulation device, described in detail in Hofmann et al. (1985). Elongation and relaxation stimuli were applied to the ICO with an amplitude of 300 μm (equivalent to 60° movement of the FT joint) (Weiland et al. 1986).

In all experiments, the locomotor system of the experimental animal (“active” behavioral state) was activated by touching the abdomen with a small paintbrush. The active state of the locomotor system was characterized by strong bursts of activity in slow and fast motoneurons of a given motor nerve or muscle, being strictly alternating for antagonists of each joint and by rhythmic pro- and retraction movements of the coxae in the adjacent segments (see also Bässler and Büschges 1998).

EXPERIMENTS ON CENTRALLY GENERATED RHYTHMIC MOTOR ACTIVITY. For pharmacologically activated rhythmic preparations, the anterior and posterior connectives were also cut and pilocarpine (5 × 10⁻⁴ M) (Büschges et al. 1995) was applied in the Ringer solution (Weidler and Diecke 1969).

Electrophysiology

The activity of the tibial extensor motoneurons (MN) in nerves nl3 or F2 was recorded with hook electrodes (Schmitz et al. 1991). The activity of the flexor tibiae muscle was monitored by an electromyographic (EMG) recording with copper wires of 65 μm diameter. Extension movements induced the opposite response. During locomotion this could facilitate the onset of leg levation due to flexion signals from the FT joint or the onset of leg depression during extension signals from the FT joint. From these studies, the question emerges as to whether such specific influences represent a common mechanism for interjoint coordination in the multi-jointed limb. Therefore we chose to investigate the influence of signals from sense organs at the CT joint on the activity of tibial motoneurons and muscles in the active stick insect. We applied levation and depression movements to the femur, while the locomotor system of the stick insect was active, i.e., in the locomotor state, and monitored changes in the activity of extensor tibiae and flexor tibiae motor neurons. Using ablation experiments, we analyzed which sense organs at the CT joint affect motoneuronal activity in tibial motoneurons and muscles. We also investigated whether the observed interjoint influences were mediated purely by “reflex-like” interactions or whether sensory signals from the CT joint have access to the central rhythm-generating premotor networks of the FT-joint. In a final series of experiments we investigated the role of this interjoint influence in controlling motor activity in the FT-joint during walking.

FIG. 1. Schematic dorsal view of the mesothoracic ganglion of the stick insect indicating all lateral nerves and the sites of crushing or cutting (double lines). nl2–nl5, lateral nerves 2–5; ncr, nervus cruris; nup, nervus unparis; na, nervus anterior; np, nervus posterior; C1 and C2, coxal nerves 1 and 2; Tr1 and Tr2, trochanteral nerves 1 and 2; F1 and F2, femoral nerves 1 and 2.
Behavioral analysis

For behavioral analysis, the single middle leg preparation was used (Fischer et al. 2001; Karg et al. 1991). In this preparation, all legs except a middle leg of the animal were removed. The middle leg was fixed perpendicular to the thorax of the animal extending over the rim of a foam platform. The TC joint was immobilized, and the more distal leg joints were free to move. The leg performed well coordinated walking movements on a treadband or searching movements in the absence of ground contact. Sequences of walking movements were elicited by touching the abdomen with a paintbrush. We recorded the activity of tibial extensor motoneurons extracellularly from nerve ni3 and tibial flexor activity by means of EMGs from the muscle. The EMG signals were recorded also as rectified and integrated (time constant, 40 ms) records. The animals were tested in three situations, 1) control situation: the nerves innervating the muscles as well as the sense organs of the leg joints were left intact. In all of the behavioral experiments, the motor pattern was recorded in control situation first (number of experiments, \( N = 12 \)). 2) After removal of the fCS: the fCS of the leg was removed by destroying the field on the cuticle with an insect pin where the fCS is located (\( N = 9 \)). And 3) sham-operated animals: in this group, instead of destroying the fCS, we only made a small hole on the anterior side of the femur (\( N = 3 \)). Therefore any changes in motor activity in extensor and flexor MN pools due to the surgery at the femur could be monitored.

Scanning electron microscopy

The left middle legs of adult stick insect \( C. morosus \) were removed carefully to prevent damage of the TC joint. We than separated the tibia and tarsus. The preparations first were dehydrated with alcohol series (10, 30, 40, 50, 60, 70, 80, 90, and 98%), in which the preparations remained for 8 min each. The solution with 30% alcohol was applied twice as in this application the cuticle was cleaned in ultrasonic bath. After dehydration the preparations were dried and coated (SEM Coating Unit PS3, Agar Aids for Electron Microscopy) with gold (150 Å). The preparations of the legs were then inspected and analyzed with a scanning electron microscope (Hitachi, S520).

Reconstruction of serial sections of the femur

Serial sections, 10 μm thick and stained with hematoxilin-eosin, were used. Reconstructions were made from two middle legs and two hind legs of \( C. morosus \) (for details, see Bässler 1977).

Data analysis

Extracellular recordings, EMGs, and intracellular recordings were stored on a DAT-Recorder (SONY, PC 116) or on FM-tape recorder (RACAL Store 7DS). Analog-to-digital conversion was performed off-line on a CED 1401plus interface (Cambridge Electronic). The recordings were analyzed with the Spike2 software (Version 3.13). Statistical evaluation of data and plotting of graphs was done with PlotIt and Excel 97. In the text \( N \) gives the number of experiments and \( n \) gives the sample size. Differences in means of samples were tested by using the Student’s \( t \)-test (Excel 97). Means were regarded as significantly different with \( P < 0.05 \).

RESULTS

Influence of movements of the trochanterofemur on the activity of muscles and motoneurons of the FT joint

In a first set of experiments, we investigated the influence of movements of the CT joint on activity of muscles and motoneurons of the FT joint in the active animal (Figs. 2 and 3). The influence of movements of the CT joint were investigated with the thoraco-coxal (TC) and joint deafferented to exclude any indirect influences on tibial motoneuron and muscle activity. After activating the animal, extensor and flexor tibiae muscles exhibited alternating activity (Fig. 2A). The femur of...
Diff erential PST histograms for SETi and FETi showed that both exci tatory extensor motoneu rons, i.e., SETi and FETi, were affected, with the influence being stronger for FETi (Fig. 2C). In some cases, inactivation of extensor motoneurons did not outlast the end of femur leva tion (Fig. 2A, 3rd leva tion stimulus). Intracellular recordings from the neuropil processes of FETi (N = 4) revealed that inactivation induced by femur leva tion resulted from a hyperpolarization of this motoneuron (Fig. 3, A and B). Leva tion of the femur during ongoing flexor activity did not result in any change of activity (not shown). Depress ion of the femur did not elic it systematic changes in motoneuronal activity. Only occasionally, when the femur was depressed during extensor activity, a slight increase in extensor activity was observed (Fig. 2A, 1st depress ion).

This in fluence of movements of the femur on the activity of extensor and flexor motoneurons in the active animal differed clearly from the situation in the inactive, resting animal. In the inactive animal, both leva tion and depress ion of the femur regularly had only a weak influence on the activity of extensor and flexor motoneurons. Figure 3 shows this for the response of FETi and SETi to leva tion and depress ion of the femur. Both movements elicited one or two action potentials in SETi as visible from the extracellular recording of nl3 (Fig. 3A). FETi received in general small transient hyperpolarizing synaptic inputs of 1–2 mV during leva tion of the femur (N = 9; Figs. 3, A and B, and 4). In four of these recordings, we observed as well small depolarizing synaptic inputs elicited by both leva tion and depress ion of the femur (Fig. 3, A and B). In none of our recordings did we detect any change in flexor muscle activity in response to leva tion or depress ion of the fem ur (not shown) in the resting animal.

**Influence of sense organs at the CT joint on muscles and motoneurons supplying the FT joint**

To determine which sense organs are responsible for the observed interjoint in fluence, we selectively ablated sense organs at the CT joint. The ablation of sense organs measuring movements and forces at the CT joint was performed in different combinations and sequences. We then investigated the influence of leva tion and depress ion of the femur on the activity of tibial motoneurons in the active animal. Similar results were collected for both *C. morosus* and *C. impigra*.

Movement and force at the CT joint of the stick insect are measured by four different sense organs located around the CT joint.
joint: the trochanteral hairplate (trHP, Fig. 5A) located on the dorsal surface of the trochanter responds to levation movement of the trochanterofemur in relation to the coxa (Schmitz 1986a–c; Tartar 1976); the rhombal hairplate (rHP, Fig. 5B), is located on the ventral surface of the trochanter (Tartar 1976), and its hairs are bent by the joint membrane on depression of the trochanter; changes in cuticular stress at the trochanter are signaled by three different fields of trochanteral campaniform sensilla (trCS, Fig. 5, A and B) (Delcomyn 1991; Hofmann and Bässler 1982; Tartar 1976); cuticular stress at the proximal femur is detected by a field of femoral campaniform sensilla (fCS) (Hofmann and Bässler 1982; Tartar 1976) located on the posterior side of the femur close to the borderline of the trochanter (Fig. 5B). Finally, a preliminary study reports the existence of an internal levator stretch receptor organ (levSR) (Schmitz and Schöwerling 1992) that is situated inside the coxa parallel to the levator trochanteris muscle. This sense organ detects length changes of the levator trochanteris muscle, similar to the one in the locust (Bräunig and Hustert 1985).

As there was no previous information available on the innervation of the fCS, we traced their innervation in semi-thin transections (10 μm) of two middle legs and two hind legs in C. morosus (Fig. 6). From these reconstructions, it became clear that the fCSs are innervated by a side branch of nerve F2 arising in three cases proximal to the merging point of nerve nl3 (the motor root of F2) and nerve F2 (sensory root of F2) at a location close to the autotomization point at the fusion region of trochanter and femur. In one middle leg, this side branch originated just distal to the merging point of nerves nl3 and F2.

In various sets of experiments, we subsequently removed the sense organs around the CT joint either by ablation (trHP, rHP, trCS, fCS) or by cutting the innervating nerves close to the sense organ (levSR, trHP, trCS). We then activated the experimental animal and recorded the influence of femoral levation and depression on the activity of the extensor tibiae (Fig. 7, A and B). For example, in the experiment shown in Fig. 7A termination of extensor activity with femur levation was still present after removal of all sense organs (i.e., trCS, trHP, and rHP) except the fCS. After ablation of the fCS, termination of extensor activity is no longer induced by leg levation. The results of the different sets of experiments are summarized in Table 1. These experiments revealed that only removal of the fCS abolished the interjoint influence of trochanteral movements on tibial motoneuron activity (Fig. 7, A and B). The relevance of fCS signals on the interjoint influence was exemplified by removal of those prior to the experiment (Fig. 7C).

**Role of fCS signals in controlling activity of extensor tibiae motoneurons**

In further experiments, we focused on the detailed influence of signals from the fCS on extensor motoneurons. We intended to selectively stimulated the fCS by applying pressure on the femoral cuticle at their location to investigate their influence on tibial motoneuron activity. At first we recorded the activity generated by the fCS in response to application of pressure on the femoral cuticle (Fig. 8A; N = 3; C. morosus). To monitor their activity, an extracellular recording was made from the nervus cruris in the coxa while it was cut proximal to the electrode and distal to the fCS in the femur. Application of pressure to the femoral cuticle at the location of the fCS induced barrages of spikes in sensory neurons of the fCS field (top trace). This response was abolished after destroying the fCS (bottom trace), verifying that only the fCS afferents were firing in response to cuticular pressure (Fig. 8A). These experiments revealed that application of pressure to the femoral cuticle could be used as a tool to induce activity in fCS neurons and to investigate their influence on motoneurons selectively. Applying pressure to the fCS in the resting, inactive animal...
always decreased spontaneous activity in SETi, while no influence was detectable on the flexor EMG recording (arrows Fig. 8B). Intracellular recordings from FETi also revealed the inhibitory synaptic drive caused by fCS stimulation in the inactive and active behavioral state (Fig. 8C). The inhibitory influence of fCS signals was also manifested as a change in the strength of intrajoint reflex activation of extensor motoneurons by stimulation of the femoral chordotonal organ (Fig. 8, D and E), indicating that extensor motoneuron activity is determined by signals from both intra- and interjoint sensory signals.

The preceding results show that signals from the fCS affect patterning of activity of extensor tibiae motoneurons. In the light of previous findings on the role of sensory signals in interjoint information flow (Hess and Büschges 1999), the question arose as

![Image](http://jn.physiology.org/)

FIG. 7. Influence of levation and depression of the femur in the active behavioral state as a function of the sensory input from the coxa-trochanteral (CT) joint. A: removal of the trCS and trHP had no effect on the inactivation of tibial extensor activity induced by levation of the femur. ■, inactivation of the extensor activity. Please note that extensor activity is no longer terminated with femur levation after fCS were destroyed. ●, inactivation of the extensor activity. Probability of occurrence was calculated as the ratio between the number of stimuli which terminated activity in extensor motoneurons and the number of stimuli presented. C: same presentation as in B but in this experiment only the fCS were ablated and compared with the intact situation. All other sense organs on the CT joint were left intact. Data shown in A derive from C. morosus, data shown in B and C derive from C. impigra.

![Image](http://jn.physiology.org/)

FIG. 8. Influence of specific stimulation of the fCS on tibial motoneuron activity in the inactive and active stick insect (C. morosus). In these experiments, the femur was completely deafferented (except for fCS and femoral chordotonal organ (ICO)) and de-efferented (except the flexor muscles). A: recording of fCS afferent activity in the nervus cruris on mechanical stimulation of the fCS. Please note that application of pressure to the cuticle around the fCS induced sensory activity in ncr (top), which was abolished after destroying the fCS (downward trace). B: original sample recording in the inactive animal. Black bars indicates the time in which pressure was applied to the fCS. C: intracellular recording from FETi in the same experimental paradigm as shown in B. The arrowhead indicates the time at which the animal was activated by tactile stimulation of the abdomen with a small paintbrush. D: decrease of reflex activation in extensor motoneurons on stimulation of the ICO by pressure applied to the fCS in the inactive animal. Asterisks denote 2 artifacts during positioning of the stimulation probe. E: PST histogram of the average SETi activity for the experiment shown in D (binwidth: 20 ms)

### TABLE 1. Subsequent ablation of sense organs at the CT joint

<table>
<thead>
<tr>
<th>Situation/Operation</th>
<th>No. of Experiments</th>
<th>No. of Stimuli</th>
<th>Influence CT on FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>5</td>
<td>101</td>
<td>Yes</td>
</tr>
<tr>
<td>Nervus lat. 3 cut (levSR removed)</td>
<td>5</td>
<td>90</td>
<td>Yes</td>
</tr>
<tr>
<td>Nervus cruris cut</td>
<td>5</td>
<td>45</td>
<td>No</td>
</tr>
<tr>
<td>trCS removed and Nervus lat. 3 cut</td>
<td>5</td>
<td>70</td>
<td>Yes</td>
</tr>
<tr>
<td>tr HP ablated</td>
<td>3</td>
<td>51</td>
<td>Yes</td>
</tr>
<tr>
<td>Intact</td>
<td>4</td>
<td>61</td>
<td>Yes</td>
</tr>
<tr>
<td>fCS destroyed</td>
<td>5</td>
<td>72</td>
<td>No</td>
</tr>
</tbody>
</table>

CT, coxa-trochanteral; FT, femur-tibia; levSR, internal levator stretch receptor; trCS, trochanteral campaniform sensilla; trHP, trochanteral hairplate; fCS, femoral campaniform sensilla.
to whether signals from the fCS have access to the central rhythm generating networks of the FT joint. Therefore we tested the influence of signals from the fCS elicited by levation and depression of the femur on the activity of centrally generated rhythmic activity in tibial motoneurons. Relatively regular rhythmic activity can be elicited by topical application of the muscarinic agonist pilocarpine (Büsschges et al. 1995) at a final bath concentration of $5 \times 10^{-4}$ M. We stimulated the CT joint in the otherwise isolated mesothoracic ganglion (C. morosus; C. impigra) and monitored the influence of joint movements on the rhythmic activity of tibial extensor motoneurons (Fig. 9). In 57% ($n = 77$) of the trials ($N = 5, n = 135$), levation of the femur resulted in a change of rhythmic activity in extensor motoneurons. Levation of the femur in these cases led to a shortening (Fig. 9A, left) of tibial extensor activity. In about 20% of the trials, shortening of extensor activity was followed by a long interburst interval in extensor activity (Fig. 9A, right). In these cases (57%), extensor burst duration (BD, Fig. 9B, left) was correlated with the latency between extensor burst onset and the onset of the stimulus. Interestingly, not only extensor burst duration but also interburst interval (IBI) between extensor bursts was altered by the stimulus as it became more variable and increased compared with control (Fig. 9B, middle). The duration of the affected cycle depended on the time of stimulus, however, with a high degree of variability. Nevertheless cycle period was significantly correlated with the time between burst onset and stimulus onset. From generating phase response plots for these experiments (Fig. 9C), it became clear that femoral levation had a significant phase dependent influence on rhythmicity, however, with a considerable amount of variability. As judged from the regression line, femoral levation delivered early in the cycle had a general tendency of shortening the cycle, while this influence reversed to a general tendency of a slight lengthening toward higher phase values. In the remaining cases, i.e., in 43% of the trials ($N = 5, n = 135$), no change in motor activity could be detected. These results indicate that sensory influences mediated by signals from the fCS during leg levation do affect patterning of extensor motoneuron activity, however, in a rather variable and labile manner. Furthermore depression of the femur was never observed to induce any detectable changes in tibial extensor activity (not shown).

Role of fCS in walking pattern generation in the middle leg

We have shown that stimulation of the fCS inhibited extensor motoneurons and elicited activity in flexor motoneurons (e.g., Fig. 8B). In a final set of experiments, we examined the

![FIG. 9. Influence of signals from the fCS during femur levation on centrally generated rhythmic activity in tibial extensor motoneurons (initiated by bath application of $5 \times 10^{-4}$ M pilocarpine). A: in 57% ($n = 77$) of the applied stimuli ($N = 5, n = 135$) we observed a shortening of tibial extensor activity on femur levation. \( \bigcirc \), time and expected time of occurrence of burst onset in tibial extensor motoneurons (left). In 27 of these cases (20%), a long-lasting inactivation of extensors after the stimulus was induced (right). B: plot of the extensor burst duration (BD, left), interburst interval (IBI, middle), and cycle period (CP, right) as a function of stimulus time after burst onset (c) for those trials in which an influence of femur levation was observed ($N = 5, n = 77$). Inset: a scheme indicating the parameters evaluated (BD, IBI, CP, c: time between burst onset and start of stimulus). In addition, in the each graph, the mean ± SD for the parameters is given on the right-hand side (open circles). The dashed horizontal line in each graph indicates the mean value for the control cycles ($n = 87$). The horizontal top and bottom lines give their standard deviations. The thick solid lines give the regression lines for BD (corr. coeff.: 0.78) and CP (corr. coeff.: 0.59). C: reset plot showing the relation between stimulus phase and influence of the stimulation on extensor activity [data derive from the same experiments as in B ($N = 5, n = 77$)]. Inset: the paradigm of evaluation of data ($t_{mean}$: mean of control cycle period evaluated for each experiment for $7 < n < 38$ cycles), $t_{i}$: duration from onset of disturbed burst to start of stimulation, $t_{f}$: duration of disturbed cycle). Please note that despite a considerable variability, a significant correlation exists between the phase of stimulation and the influence on rhythmicity (corr. coeff.: 0.39). Data points for 1 cycle have been repeated 3 times for better clarifying the phasic influence. Open circles indicate data deriving from 1 experiment. All data presented derive from recordings in C. morosus.]
role of the fCS in controlling tibial motoneuron and muscle activity in walking. We choose to investigate this question in the single middle leg preparation (Fischer et al. 2001) (C. impigra) by ablating this sense organ and analyzing any changes in the walking motor pattern. This preparation is appropriate for such investigations because segmental mechanisms of walking pattern generation can be investigated without the influence of intersegmental coordinating influences from the other legs.

Figure 10A shows recordings from the middle leg preparation during walking with the fCS intact (Fig. 10A, left) and after their removal (Fig. 10A, right). The most obvious difference between these situations is the reduced flexor activity during stance after removal of the fCS compared with control. This is seen both in the flexor EMG as well as from the rectified and integrated EMG activity. We found such a decrease in flexor activity during stance in all animals tested (N = 9). Removal of the fCS also caused a slight but significant change in the mean flexor burst duration during walking (Fig. 10B), increasing from 1.31 ± 0.58 s (n = 486) to 1.51 ± 0.94 s (n = 475).

The decrease in flexor activity during stance was also obvious from plotting the average amplitude of the rectified and integrated EMG within a normalized burst comparing intact animals and following removal of the fCS (Fig. 10C). After removal of the fCS, the amplitude of flexor activity was significantly reduced in all bins except for the first six bins at the beginning of stance (see Fig. 10, C and D; 1st 6 bins in C and 1st bin in D). The same was true for the average normalized amplitude of flexor activity of all investigated animals for the normalized flexor burst duration (N = 9; Fig. 10D) as well as over time (N = 4; Fig. 11A). For doing so, bin values were normalized to the maximum bin value of the intact situation (“fCS intact”). No significant decrease in flexor activity, however, could be observed in sham-operated animals (N = 3, Fig. 10E). In contrast, no changes were detectable in extensor activity during leg swing, as measured by the mean spike activity of the extensor motoneurons, FETi and SETi (Fig. 11A).

We further investigated whether sensory information from the fCS plays a role in generating the step phase transitions from stance to swing and vice versa during walking on the treadmill. We compared the average latencies between the activities of the two antagonistic tibial motoneuron pools at both transitions with intact fCS and after fCS removal (Fig. 11B). For the transition from swing to stance, we measured the time between termination of tibial extensor motoneuron activity in an extracellular recording from the extensor nerve n13 and the start of tibial flexor activity on the flexor EMG. We found no significant change in latency (Fig. 11B, left) in any of the tested animals (N = 4). The same was true for the transition from stance to swing (Fig. 11B, right), although in one animal the overlap between the end of flexor and the beginning of extensor activity was significantly smaller after the removal of the fCS.

**Discussion**

We have shown that sensory signals from the CT joint affect activity of tibial motoneurons. Levation of the femur was able to inactivate tibial extensor motoneurons and to initiate activity in tibial flexor motoneurons (Fig. 2A). Ablation experiments

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**Fig. 10.** Influence of fCS ablation on the walking motor pattern in motoneurons and muscles of the FT joint. A: n13 nerve recording (top), rectified (time constant, 40 ms) flexor EMG (middle), flexor EMG (bottom) from the middle leg preparation during walking in the control animal with intact sensory supply of the middle leg (left) and after ablation of the fCS (right). Black bars indicate stance phase and white bars indicate the swing phase of the middle leg in a step cycle [determined from the activity pattern of the motoneurons of the FT joint (see Fischer et al. 2001)]. B: probability histogram of flexor burst duration during stance (binwidth: 0.5 s). There is a slight but significant (P < 0.05) increase in burst duration after ablation of the fCS (N = 9; n = 486 control, open bars; n = 475 with fCS ablated, filled bars). C: plot of the average rectified EMG amplitude in 1 animal in control conditions (open circles, n = 65) and after ablation of the fCS (filled circles, n = 57) during a normalized flexor burst. Flexor bursts were divided in 50 bins of the same duration. Notice that the amplitude of the flexor activity is significantly decreased after fCS were ablated in all bins, except for the 1st 7 bins and 1 bin in late stance (filled arrow). This was observed in all experimental animals. D: comparison of normalized rect. EMG amplitude in the 9 experiments during a normalized flexor burst (n = 486 in control, and n = 475 after ablation of fCS). Bin values for each experiment were normalized to the maximum bin value in the intact situation (“fCS intact”) of each experiment. Maximum bin value was set to 1. E: comparison of normalized rect. EMG amplitude of 3 control animals before and after a sham operation in which a hole was made in the cuticle on the anterior side of the femur (n = 96 in intact, open circles and n = 95 after sham operation, filled circles). Otherwise like C and D. No significant change was observed, except in the 4 bins marked by open arrows (see also text). Vertical lines in C–E represent the SD.
Interjoint information flow between the CT joint and the FT joint

Our results show that moving the femur influences activity of motoneurons supplying the FT joint due to inputs from the fCS and not due to movement and/or position sensitive receptors at the CT joint. The trochanteral hairplate (Bässler 1983; Tartar 1976), the rhombal hairplate (Bässler 1983; Tartar 1976), and the levator stretch receptor organ (Schöwerling 1993) did not affect patterning of activity in tibial motoneurons. However, from the design of our experiments, we cannot exclude more subtle influences of these sense organs on the magnitude of activity in motoneurons of the FT joint (e.g., Bässler 1993a). Even though our results clearly differ from previous findings on the influence of proprioceptive signals from the FT joint on motor activity in the CT joint (Hess and Büschges 1999), our present results together with previous findings suggest the following scheme for information flow between the FT joint and the CT joint: proprioceptive information is unidirectional in interjoint coordination, i.e., from the FT joint onto the CT joint. Thus our results indicate an asymmetry for the role of proprioceptive signals in interjoint coordination.

Signals from cuticular stress detecting sense organs, the fCS, affected the activity of motoneurons of the FT joint. In contrast to proprioceptive signals, which report position or movement of the joint they arise from, signals from CS are produced by strain of the cuticle (recent review in Duyens et al. 2000). As such, activity of fCS afferents, for example, could arise from forces in the CT joint elicited by femoral movements or from activation of muscles in the leg. They could as well be induced by forces generated in the FT joint or by forces at other locations on the thorax that induce torsion of the proximal femoral cuticle (see also Ridgel et al. 2000).

The presented findings differ from results reported by Bässler on the stick insect foreleg. Bässler (1993a) showed that proprioceptive signals from the trochanteral hairplate at the CT joint influenced interjoint coordination between the CT and FT joint, an influence that was not detectable in our studies on the middle leg. There are four possible factors that may account for this difference. First, the differing results may be due to differences in mechanisms of interjoint coordination between the middle leg and the foreleg and/or a differing architecture of the trochanter and femur basis in these legs. Second, influences of the trochanteral hairplate on tibial motoneuron activity may only be effective during the production of leg movements and may not be effective in reduced preparations. Third, in the experiments of Bässler (1993a), the levSR was left intact. Finally, the efficacy of proprioceptive signals from the CT joint on activity of the FT joint may depend on the actual phase of motoneuronal activity in the TC and CT joint, a possibility that was not controlled in the present investigation. At present, we have no indications that this is the factor, but this aspect will be in the focus of subsequent investigations.

Influence of femoral campaniform sensilla on FT-joint motoneurons

We have shown that when the locomotor system of the stick insect is active, signals from the fCS inhibit extensor motoneurons and activate flexor tibiae motoneurons. At present the...
neuronal pathways mediating this influence are not known. It is quite conceivable that direct connections from the afferents and pathways via intercalated interneurons are mediating this influence similar to the situation for the influence of CS signals on coxal motoneurons of stick insect (Schmitz and Stein 2000) and locust (Newland and Emptage 1996). Recent experiments indicate that in fact individual identified pathways via nonsparing interneurons of the FT-control network (for review on this network see Büschges et al. 2000) are involved in mediating the influence of fCS on the activity of tibial motoneurons (Akay and Büschges, unpublished data).

There are four fields of CS at the CT joint, three on the trochanter and one on the very proximal femur that have been treated in the past mostly as “one” sensory system (e.g., Hofmann and Bäsler 1982; Schmitz 1993; Schmitz and Stein 2000). Here we report a specific influence of one of these four fields, i.e., the fCS on the generation of motor activity. No such influence was detectable for the three fields of trCS. In this context another observation appears interesting (Gerharz 1999; Akay, Gerharz, and Büschges, unpublished observation): levation and depression of the CT joint also affected activity of motoneurons supplying the thoraco-coxal (TC) joint. However, in this case this influence from CT joint movements arose from the trCS, only. In conjunction with the present results, there appears to be a segregation of function in the CS fields at the CT joint with the fCS affecting motoneuron activity of the FT joint and the trCS affecting motoneuron activity of the TC joint.

Role of sensory signals from the campaniform sensilla in controlling motor activity during locomotion

By ablation experiments, we could show that the sensory signals from the fCS affecting tibial motoneurons influence the magnitude of activity in the flexor muscle during stance in the single walking leg preparation. These results are consistent with previous findings on the role of CS in controlling the stance phase motor output of the leg that indicated that sensory information from CS about load on the leg reinforces stance phase motor output (e.g., Cruse et al. 1983; Pearson 1972) and specifies the role of one field of CS in this functional task. Interestingly, we did not find an influence of fCS ablation on the timing of step phase transitions in the walking cycle, neither for the transition from stance to swing (e.g., Bäsler 1977; Newland and Emptage 1996) nor for the transition from swing to stance (e.g., Cruse and Bartling 1995).

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