Interjoint Coordination in the Stick Insect Leg-Control System: The Role of Positional Signaling

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Bucher, Dirk, Turgay Akay, Ralph A. DiCaprio, and Ansgar Büsches. Interjoint coordination in the stick insect leg-control system: the role of positional signaling. J Neurophysiol 89: 1245–1255, 2003; 10.1152/jn.00637.2002. Interjoint coordination is essential for proper walking behavior in multi-jointed insect legs. We have shown previously that movement signals from the femur-tibia (FT) joint can shape motor activity of the adjacent coxa-trochanter (CT) joint in the stick insect, Carausius morosus. Here, we present data on the role of position signals from the FT-joint on activity generated in motoneurons (MNs) of the CT-joint. We show that the probability of occurrence of stance (with depression in the CT-joint) or swing movements (with levation in the CT-joint) at the start of walking sequences is influenced by the angle of the FT-joint in the resting animal. We tested the influence of FT-joint angle on pharmacologically induced rhythmic activity of CT-joint depressor (DprTr) and levator (LevTr) MNs. The burst duration, mean spike rate within bursts, and duty cycle for each MN pool were found to depend on FT position. For LevTr MNs, these parameters progressively increased as the FT-joint was moved from extension to flexion, and the opposite was true for DprTr MNs. The cycle period of CT-MN rhythmicity also depended on FT position. In addition, we sometimes observed that the motor output shifted completely to one MN pool at extreme positions, suggesting that the central rhythm-generating network for the CT-joint became locked in one phase. These results indicate that position signals from the FT-joint modulate rhythmic activity in CT-joint MNs partly by having access to central rhythm generating networks of the CT-joint.

INTRODUCTION

Locomotion in animals with multi-jointed limbs requires the coordinated action of several appendages and their segmented limbs. The control of the actual motor output encompasses three levels: intrajoint control, interjoint control, and intersegmental coordination between adjacent limbs (for reviews, see Clarac 1991; Cruse et al. 1995; Grillner 1981; Orlovsky et al. 1999; Pearson 1995; Stein and Smith 1997). Sensory signaling from leg proprioceptors plays a pivotal role in these tasks. It provides information about ongoing limb movement and forces generated by the limbs and is utilized in generating a functional locomotor pattern (for review see Bässler and Büsches 1998; Grillner 1981; Orlovsky et al. 1999; Wendler 1964). Considerable information has been gathered on the role that phasic sensory signals play in the organization of the overall motor output for walking. Signals from leg proprioceptors in response to movement serve two functions in sculpting the locomotor output: modification of the strength of motoneuron activity during the locomotor cycle and direct effects on central rhythm-generating networks of the leg motor-control system (for reviews, see Marder and Bucher 2001; Pearson 1995, 2000). In the first case, sensorimotor pathways allow the modulation of spike frequency and burst duty cycle within the framework established by the rhythmic input from premotor pattern-generating networks, whereas in the second case, sensory feedback plays a role in determining the phase transitions of the locomotor cycle. Specific emphasis has been put on the role of proprioceptive information in generating the locomotor output of multi-jointed limbs in walking systems that contain loosely coupled neuronal networks governing individual leg joints such as the stick insect walking system (Bässler and Büsches 1998; Orlovsky et al. 1999). Proprioceptive signals have also been shown to play a critical role in interjoint motor patterning in other arthropod motor systems (Vedel and Clarac 1979; El Manira et al. 1991).

Phasic sensory signals evoked by movements of individual segments of the stick insect leg effect the central rhythm-generating networks of the adjacent leg joint and thus can influence interjoint coordination (Akay et al. 2001; Hess and Büsches 1999). For example, movement signals from the primary movement transducer of the femur-tibia (FT) joint, the femoral chordotonal organ (ICO), participate in the patterning of motoneuron activity in the adjacent proximal leg joint, the coxa-trochanter (CT) joint (Hess and Büsches 1999). Flexion movement signals from the FT-joint are capable of inducing transitions in activity from depressor to levator motoneuron pools, whereas the reverse is the case for extension movement signals occurring during levator activity.

However, the ICO encodes not only information about the dynamics of movement (velocity and acceleration) but also about static joint angle, i.e., position (stick insect: Büsches 1994; Hofmann and Koch 1985; Hofmann et al. 1985; for review, see Field and Matheson 1998). A previous investigation by Hess and Büsches (1999) focused primarily on the role of phasic movement signals, but no specific information is available on the role that ICO position signals serve with respect to interjoint control during the generation of locomotor activity in trochanteral motoneurons (MNs). Evidence that positional information is important for the generation of func-

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tional walking movements is presently restricted mainly to the behavioral level (e.g., Bässler 1977; Cruse 1985a,b; Dean and Schmitz 1992).

We have investigated the role of positional information from one joint of the stick insect leg, the FT-joint of the middle leg, in controlling the generation of rhythmic motor activity in the adjacent CT-joint. Indications that positional information plays an important role in interjoint control between the FT-joint and the CT-joint are derived from experiments conducted by Graham and Bässler (1981). They permanently reversed the sign of sensory feedback from the fCO by attaching the receptor apodeme to the tendon of the flexor tibia muscle, a muscle that moves in antiphase to the fCO apodeme in the intact animal. As a result of this “crossed receptor apodeme” operation, the stick insect was no longer able to generate functional locomotor movements with the modified leg. In a later study, Bässler (1993) showed that the effect of sign reversal in the FT-joint directly resulted from the influence of the altered sensory input from the fCO onto the segmental motor control system of the middle leg. During the generation of locomotor movements, the operated middle leg usually adopts a so-called “saluting” posture. The tibia was maximally extended (with the fCO signaling full flexion), and the trochanter/femur was maximally elevated. Significantly, this “saluting” posture was initiated by an active flexion of the FT-joint (Fig. 1A). This implies that sensory signals from the FT-joint signaling a flexed FT-joint angle can induce and maintain activation of the levator trochanteris MNs in the adjacent CT-joint. The question arises as to how this influence is mediated. Positional information from the FT-joint could modulate the magnitude of motor activity in the adjacent CT-joint via sensorimotor pathways or by directly affecting the central rhythm-generating networks of the adjacent leg joint. The stick insect walking system is particularly useful for addressing this question as sensory signaling can readily be reduced to a single sense organ and a single sensory modality, and the effect on fictive locomotor patterns can be analyzed. Furthermore, the central rhythm-generating networks (CRGs) for different leg joints interact only weakly with each other in the isolated ganglion (Büschges et al. 1995), and the feedback from sense organs to CRGs of each joint is therefore likely to impose its effect in an independent manner. We investigated the specific influence of position signals from the fCO at the FT-joint on rhythmic CT-joint MN activity induced by application of pilocarpine (Büschges et al. 1995) in an isolated mesothoracic ganglion that was deafferented except for the fCO.

METH ODS

Electrophysiological experiments were performed at room temperature (20–22°C) on adult stick insects, Carausius morosus, from a colony maintained at the University of Cologne. Behavioral experiments were performed on adult stick insects, Cuniculina impigra, from a colony at the University of Cologne.

Behavioral experiments in the single leg preparation

The experimental animals were fixed to a foam platform with only the left middle leg remaining. The subcoxal joint of the middle leg was fixed with dental cement at an angle of 90° to the thorax according to the established procedures (Fischer et al. 2001). The middle leg was positioned on a treadband at different FT-joint angles.

After several minutes, when the animal was quiescent and maintaining a stable posture, the antennae or abdomen were lightly touched with a small brush to activate the animal and to induce “walking-like” movement sequences of the leg. These single leg movements have been shown to be due to the coordinated activity of MNs and muscles that closely resembles many aspects of the motor pattern generated during a tripod gait in the intact walking animal (Fischer et al. 2001). Experiments were videotaped from the front and repeated several times at different FT-joint angles (camera: JVC TK-C1380; s-VHS recorder: Panasonic 5600). Afterward, the video sequences of the experiments were evaluated frame by frame to identify the initial movement of the leg when the animal became active. The start of the walking sequences was classified as either stance-like, swing-like, or indeterminate. Stance was defined as flexion of the FT-joint with the tarsus pulling the treadband toward the animal, swing was defined as elevation of the femur coupled with a simultaneous extension of the tibia.

FIG. 1. Femur-tibia (FT)-joint angle and limb movement. A: Graham and Bässler (Bässler 1993; Graham and Bässler 1981) reversed signaling from the femoral chordotal organ (fCO) by attaching the receptor apodeme to the flexor tendon. As a result of this “crossed receptor apodeme” operation, the stick insect was no longer able to generate functional locomotor movements with the flexor tendon. As a result of this “crossed receptor apodeme” operation, the stick insect was no longer able to generate functional locomotor movements, and the operated middle leg was mostly kept in a so-called “saluting” posture. The tibia was maximally extended (with the fCO signaling full flexion), and the trochanter/femur was maximally elevated. Significantly, this “saluting” posture was initiated by an active flexion of the FT-joint (Fig. 1A). This implies that sensory signals from the FT-joint signaling a flexed FT-joint angle can induce and maintain activation of the levator trochanteris MNs in the adjacent CT-joint. The question arises as to how this influence is mediated. Positional information from the FT-joint could modulate the magnitude of motor activity in the adjacent CT-joint via sensorimotor pathways or by directly affecting the central rhythm-generating networks of the adjacent leg joint. The stick insect walking system is particularly useful for addressing this question as sensory signaling can readily be reduced to a single sense organ and a single sensory modality, and the effect on fictive locomotor patterns can be analyzed. Furthermore, the central rhythm-generating networks (CRGs) for different leg joints interact only weakly with each other in the isolated ganglion (Büschges et al. 1995), and the feedback from sense organs to CRGs of each joint is therefore likely to impose its effect in an independent manner. We investigated the specific influence of position signals from the fCO at the FT-joint on rhythmic CT-joint MN activity induced by application of pilocarpine (Büschges et al. 1995) in an isolated mesothoracic ganglion that was deafferented except for the fCO.

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Preparation for electrophysiology

The animals were mounted dorsal side up on a foam platform with the forelegs and hindlegs cut off and the middle legs fixed along the longitudinal axis of the body. The left middle leg was cut at the level of mid-tibia. The femur was fixed with dental cement (Protemp, ESPE) onto a foam rim pointing slightly upward at an angle of ~30°. The FT-joint was then fixed with dental cement at an angle of ~120°.

The thorax of the animal was opened by a sagittal cut along the dorsal midline of the meso- and metathorax. The thoracic cavity was filled with stack insect saline (Büsschges 1977; Weidler and Diecke 1969). Connective tissue and some internal leg muscles were removed to allow access to the leg nerves. Care was taken to leave as much of the tracheal system intact as possible.

Stimulation of the femoral chordotonal organ (fCO)

The femur was opened, and the muscles and tracheae were removed to expose the apodeme of the fCO, which was then fixed in the clamp of an electromechanical stimulus device (Büsschges 1989). The apodeme was cut distally and moved over a range of lengths, with an amplitude of 300 μm corresponding to a tibia movement of 60° (Weiland et al. 1986). Elongation of the fCO corresponds to flexion of the tibia, whereas relaxation of the fCO corresponds to extension of the tibia. The fCO was stimulated either with a step-like stimulation program with holding positions of the CO apodeme equivalent to FT angles or with very slow ramps between 30° and 180°. This range of FT-joint angles covers the normal working range of the joint in the stick insect middle leg during straight walking (Cruse and Bartling 1995).

Electrophysiological recordings

The activities of MNs were recorded extracellularly with monopolar hook electrodes (Schmitz et al. 1988) from the lateral nerves that carry the axons of MNs innervating the muscles of the CT-joint. These motor nerves (named after Marquart 1940) are C1, carrying the MN axons to the levator trochanteris muscle, and C2, carrying the MN axons to the depressor trochanteris muscle. The nerves were cut or crushed distal to the recording site. The ganglion was deafferented and deinnervated except for the fCO by cutting or crushing all other lateral nerves. For pharmacological experiments, the connectives anterior and posterior of the mesothoracic ganglion were also cut.

For intracellular recordings, the mesothoracic ganglion was prepared according to the established procedures (Büsschges 1990). Recordings were made using thin-walled glass microelectrodes, filled with a solution of 2 mol/l KAc and 0.05 mol/l KCl (electrode resistance: 15–20 MΩ). Intracellular recordings, using an intracellular amplifier (SEC-10L; NPI, Tamm, Germany), were made from the ipsilateral neuropil region of the mesothoracic ganglion that contains the arborizations of leg MNs (Storrer et al. 1986).

Pharmacological experiments

Stock solutions of 10⁻² mol/l pilocarpine (Sigma Chemicals) in saline were prepared in advance and were diluted with saline to their final concentrations prior to application. The saline was removed from the thorax of the experimental animal and replaced by the drug solution with final concentrations in the range of 1 × 10⁻⁴ to 1 × 10⁻³ mol/l (Büsschges et al. 1995). Only animals in which a stable rhythm with clear alternation between depressor and levator activity was produced within several minutes were used.

Data analysis

Electrophysiological signals were recorded and analyzed on a PC using Spike2 software (Cambridge Electronic Design, version 3.14). Intracellular and extracellular recordings were sampled at 14 kHz and movement signals at 500 Hz. Raw extracellular traces were converted to spike times using a threshold crossing and sorted into fast and slow depressor trochanteris (f- and sDprTr) MNs. This was straightforward for these two MNs in all cases because the fDprTr has a much larger amplitude in extracellular recordings of nerve C2 and additional signals (e.g., the common inhibitor 1 MN, CI1) are much smaller than the sDprTr (Hess and Büsschges 1997). However, it was not possible to distinguish among the nine levator trochanteris MNs (Hess and Büsschges 1997) based on their amplitudes in extracellular recordings of nerve C1. Therefore spike times were determined for the whole MN pool, using a simple threshold function that is insensitive to summation of simultaneous spikes of different MNs.

Start and end times of bursts were determined semi-automatically by programs written in the Spike2 script language. Recordings from ramp-and-hold stimulation experiments were edited so that the initial alternating bursts were excluded from the evaluation to exclude phasic reflex responses to movement (Hess and Büsschges 1997, 1999). fCO-responses to movement can also reset rhythmic activity, and it sometimes took one to three cycles after moving the apodeme for the rhythm to become stable again. In some instances, there was no burst of depressor or levator MNs between two bursts of the antagonist. These cycles were excluded for analyses that required determination of cycle periods of bursting activity, so that only complete cycles were used.

Data analysis and plots were rendered using Spike2, Statview 5.0 (SAS Institute) and PlotIt 3.2 (SPE); layout editing was performed using Canvas8 (Deneba). Means were compared using a t-test (Dixon and Massey 1969; Sachs 1997). Means were regarded as significantly different at P < 0.05. Data from ramp stimulation were evaluated using regression analysis. N = number of experiments; n = sample size.

RESULTS

In the walking cycle of the stick insect leg, activation of the levator trochanteris muscle after cessation of activity in the depressor trochanteris muscle underlies the transition from stance to swing (e.g., Graham 1985). To determine whether position signals from the FT-joint affect activation or inactivation of these CT-joint muscles, we first investigated whether FT-joint position affects the phase in which the stick insect middle leg starts a walking sequence.

Five animals were filmed during the start of walking sequences with the middle leg on a treadmill (Fig. 1B). The initial movement of the leg after tactile stimulation was recorded and classified as stance, swing, or indeterminate. Stance was defined as flexion of the FT-joint with the tarsus pulling the treadmill toward the animal, and swing was defined as elevation of the femur with a simultaneous extension of the tibia. With the FT-joint at a starting angle of <80° in the resting animal, initial movements were usually swing movements (74.9%, N = 5, n = 78). With an FT-joint angle between 80° and 100°, initial movements were usually swing (55%, N = 5, n = 52) versus stance movements (32%, N = 5, n = 52). Finally, with the FT-joint at an angle >100°, the initial movement was predominately stance (89%, N = 5, n = 64).

From these experiments, it was clear that there is a correlation between flexed angles of the FT-joint in the resting animal and the execution of a swing movement at the initial onset of locomotor activity, whereas extended angles correlate with the execution of a stance movement. The CT-joint angles in the resting animal differed only slightly with different FT-joint angles (5–15°). Taken together with the previous findings of...
Bässler and Graham (Bässler 1993; Graham and Bässler 1981), these results provide evidence that positional information may play a role in interjoint coordination when the locomotor system is active.

**Effect of fCO stimulation in the resting animal**

The antagonistic muscles of the CT-joint are innervated by separate nerves. Nerve C1 carries the MN axons to the levator, and C2 carries the MN axons to the depressor (Storrer et al. 1986). The depressor trochanterus muscle is innervated by only two excitatory MNs, the f- and sDprTr. Both MNs were classified with respect to morphology and their responses to fCO stimulation in the resting animal by Hess and Büschges (1997). Responses of the fDprTr to fCO signals in the inactive animal are subthreshold, whereas the sDprTr responds with an increase (to relaxation/joint extension) or decrease (to elongation/joint flexion) in tonic firing. The levator trochanterus is innervated by nine excitatory MNs that have been characterized as fast (2), semifast (4), and slow (3) on the basis of their physiological properties (Hess and Büschges 1997). Levator MNs tend to exhibit only phasic responses to fCO stimulation. Hess and Büschges (1997) found that all but one of the nine excitatory MNs received excitatory synaptic input from elongation (joint flexion) of the fCO. Only two MNs showed responses to position signals from the fCO in the resting animal. Five MNs were excited by movement signals during fCO relaxation (joint extension). Both the depressor trochanterus and the levator trochanterus are innervated by the common inhibitor 1 (Schmitz 1986; Storrer et al. 1986), which was, however, not studied here.

We found similar results in mesothoracic ganglia that were deafferented except for the fCO (Fig. 2). Depressor MN activity decreased with a concurrent increase in levator MN activity at flexed joint angles (Fig. 2A). Intracellular recordings from CT-MNs, sDprTr (Fig. 2B), and fDprTr (not shown) in the isolated mesothoracic ganglion revealed a position-dependent modulation of the resting potential of similar magnitude to that found by Hess and Büschges (1997) in a less reduced preparation in the resting animal. However, we only observed suprathreshold levator activation due to fCO relaxation in one experiment (see DISCUSSION).

In addition, we observed that the phasic interjoint reflex response of trochanteral MNs caused by velocity signals from the fCO, previously described by Hess and Büschges (1997), exhibited a marked dependency on FT-joint position (plots of mean frequency in Fig. 2A). There was little or no tonic activity in levator MNs at rest (in the experiment shown in Fig. 2A, only 1 unit is tonically active at 40°). Phasic activation was greater for flexion stimuli of a given amplitude when starting at more elongated fCO lengths, compared with shorter lengths. This enhanced response consists of an increased frequency (approximately fourfold increase in peak firing rate from 160 to 400°) and duration of MN activation as well as recruitment of additional units (Fig. 2A). There was a similar increase in the strength of the phasic inhibitory influence of fCO elongation signals on the tonic activity of sDprTr (Fig. 2). A slight position dependency of the initial response of CT MNs with fCO relaxation was also observed, but this response was highly variable and much less pronounced (not shown).

**Effect of fCO stimulation during pilocarpine-induced rhythmic activity**

We investigated the influence of position signals from the FT-joint on activity in CT-joint MNs during pilocarpine-induced rhythmic activity. Application of pilocarpine to the isolated mesothoracic ganglion elicits rhythmic alternating bursts of activity in antagonistic MN pools of the leg joints, including the CT-joint (Büschges et al. 1995). The motor pattern is strictly alternating for each leg joint with very little (if any) overlapping activity of DprTr and levator trochanterus (LevTr) MNs. However, pilocarpine-induced rhythmicity always exhibits some variability, specifically with respect to cycle period and burst duration. Furthermore, significant changes in these parameters could occur over time in a given preparation. This made extensive quantitative analysis impossible in some preparations, although the basic findings were similar for all of them. For ramp-and-hold stimulation, we restricted our statistical analysis of burst parameters to 4 of 23 experiments, where cycle period and burst duration at a given equivalent FT-joint angle did not change significantly for periods of 20–40 min.

There are a number of indications that the central rhythm-generating networks that control the different joints of the stick insect leg function largely independently in the absence of coordinating influences from sensory feedback and possibly from descending pathways (reviewed in Bässler and Büschges 1998). Therefore, although application of pilocarpine to the isolated mesothoracic ganglion elicits rhythmic bursts of alternating activity in antagonistic leg MN pools, there is little coordination between the activity of MN pools serving different leg joints (Büschges et al. 1995). Coordinated bursting of MNs of different joints is generated only occasionally in these preparations during a burst in fDprTr or in the fast extensor tibiae MN (FETi). These coordinated interjoint sequences have been termed spontaneous recurrent patterns (SRPs) by Büschges et al. (1995). In the present investigation, we only recorded intrajoint activity of CT-MNs, and SRPs could therefore not be identified. However, given that SRPs are typically characterized by long bursts in fast MNs occurring at irregular intervals, and these were not observed here, the regular occurrence of SRPs can be excluded.

Figure 3A shows a recording from an experiment in which different FT-joint angles were mimicked with ramp-and-hold movement of the fCO during pilocarpine-induced rhythmic motor activity. In the rhythmic preparation, as in the resting animal, depressor activity decreased with more elongated fCO positions, corresponding to flexed FT-joint angles, while levator MN activity increased. The fDprTr was never activated in a reliable fashion and even remained silent for long periods in some experiments (e.g., Figs. 3 and 5). The probability of recruitment of the fDprTr appeared to be higher at fCO lengths corresponding to more extended FT-joint angles (e.g., Fig. 6), but due to the irregularity of recruitment, this could not be verified statistically. Figure 3B presents the statistical evaluation of burst parameters from the four selected experiments. The mean rate of MN activity within bursts, and the burst duration, exhibited a clear dependence on maintained FT-joint angle. Similarly, the duty cycle (burst duration/cycle period) of MN activity changed with the position of the FT-joint. The duty cycle of levator MN pool activity decreased from flexed
to extended positions, whereas the reverse was true for the duty cycle of depressor MN activity. All these parameters did not appear to change with a constant slope. The differences between the mean values at 80 and 120° were not significant and the larger (and significant) changes appeared at the extremes of fCO joint angle. Furthermore, individual experiments showed a dependence of cycle period on FT-joint position in some parts of the records, indicating that longer cycle periods correlated with more flexed FT-joint angles, although this could not be confirmed statistically for the four animals evaluated quantitatively. In other experiments, rhythmic activity ceased at extreme joint angles, indicating that position-sensitive fCO afferents have access to central rhythm generating networks (see following text).

Disruption of rhythmic activity at extreme joint angles

Figure 4A shows an example where rhythmic bursting stopped at the minimum and maximum FT-joint angles of 40 and 160°. At 40°, there was no suprathreshold activity seen in DprTr recordings, and the LevTr MNs fired tonically, although there was still some modulation of the tonic activity. We used autocorrelation analysis of the spike trains from ramp-and-hold stimulation to determine if this modulation of spike frequency was caused by underlying rhythmic input with characteristics comparable to rhythmic activity seen at other joint angles. Figure 4B shows two examples of autocorrelograms. Left presents data from a LevTr recording in a very regular and stable preparation at different FT-joint angles. Rhythmicity is very
regular between 40 and 120° and shows little change in cycle period (mean period = 1.51s) as indicated by the first positive and negative peaks. However, at 160° (black line), the rhythm was less stable, the first harmonic peak is broadened and reduced in relative amplitude. Qualitatively similar changes were observed in 80% of all experiments evaluated. Right presents data from a LevTr recording in a preparation in which rhythmicity ceased at extreme FT-joint angles. This was observed in 20% of the preparations evaluated (N = 5). No suprathreshold activity was generated at 160° in levator MNs and at 40° levator MN activity became tonic. At 40°, there are no significant peaks in the autocorrelogram at t < 0, indicating that there was no periodic modulation of spiking activity.

**Slow ramp stimulation**

The experiments described in the preceding text have shown that rhythmicity in trochanteral MNs is strongly affected by positional signals from the fCO. Two lines of evidence indicate that position signals from the fCO affect central rhythm-generating networks of the CT-joint: in parts of individual experiments, cycle period changed with fCO position and in a portion of the experiments, rhythmicity ceased at extreme FT-joint angles (Fig. 4). To further evaluate the effect of position signals on the CT-joint rhythm generator, we employed an experimental protocol that produced slow continuous changes in positional signals during the expression of a rhythmic motor output (N = 6). Very slow ramp stimuli (extension: 6.32°/s flexion: 3.64°/s) were used to determine if positional signals from the FT-joint have an effect on the cycle period of CT-joint MN rhythmicity (Fig. 5). Figure 5A shows an example of pilocarpine-induced rhythmic activity in CT-joint MNs during ramp stimulation of the fCO. As in experiments with ramp-and-hold stimulation, rhythmic activity in depressor and levator MNs was modulated by fCO length. With rather flexed FT-joint angles, rhythmicity was biased toward levator activity (with respect to spike frequency and burst duration), while it was the reverse for extended angles (see Fig. 5A, a and b, insets). However, as in the previous set of experiments, rhythmicity ceased in some experiments at extreme angles, and MNs of one pool became completely silent while MNs of the antagonistic pool were tonically active (not shown). This was always true for two of the six experiments and occurred occasionally in two other experiments. The remaining two experiments showed alternating activity at every joint angle. In the four experiments that (at least in part) maintained alternating activity over the whole range of FT angles, the cycle period of the trochanteral MN rhythmicity was altered by the slow ramp stimulation (Fig. 5). This was verified by plotting cycle period versus FT-joint position (Fig. 5B). The cycle period of the
rhythmic activity significantly increased in three of four experiments as the joint was moved from extended angles to more flexed angles and decreased again at extended angles. These results verify that fCO position signals can influence the central rhythm-generating networks driving the MN pools of the adjacent CT-joint.

Influence of position signals on motoneuronal membrane potential oscillation during rhythmic activity

To determine how position signals mediate the changes observed in motoneuronal rhythmicity, we recorded intracellularly from trochanteral MNs. We focused on the fDprTr that was recruited primarily at extended FT-joint angles (Fig. 5A) and recorded from its neuropilar arborizations (Fig. 6).

In the inactive animal, slight changes in membrane potential occurred in fDprTr that were dependent on fCO position (not shown) with the membrane potential being slightly more depolarized (~3 mV) at relaxed fCO positions compared with elongated positions. These changes were of a similar amplitude as those shown in Hess and Büschges (1997, their Fig. 3D). However, slow ramp stimulation during pilocarpine-induced rhythmic activity revealed marked changes in the amplitude of membrane potential modulation (Fig. 6) that were dependent on fCO position. The amplitudes of membrane potential modulation were several times larger at extended FT-joint angles compared with flexed ones and occurred from a fairly stable baseline membrane potential (dashed line). This indicates that position signals modulate the strength of the rhythmic input to the MN in a phase-dependent manner, by either gating the connections from central rhythm-generating networks (CRGs) to the MNs or directly affecting the activity of the CRGs (see DISCUSSION).

DISCUSSION

We analyzed the influence of positional signaling from the fCO, the primary movement transducer of the FT-joint, on rhythmic locomotor activity of MNs of the adjacent proximal CT-joint. Previous studies of the leg motor-control system indicated that position signals from the FT-joint can affect motor activity of the CT-joint (Graham and Bässler 1981). When sensory feedback from the FT-joint of the middle leg was permanently inverted by attaching the receptor apodeme of the fCO to the flexor tendon, so that flexion of the tibia was signaled as extension and vice versa, both postural control and walking movements were altered. When the femoral chordotonal organ signaled flexion in the FT-joint (in response to an extension of the tibia), the CT-joint was elevated and held in an elevated position for as long as the “flexion” signal from

FIG. 4. Disruption of rhythmicity of CT-MNs at extreme FT joint angles. A: in some experiments, activity in 1 antagonist ceased at extreme angles, whereas the other became tonically active. Note that small signals in C1 and C2 recordings at 160 and 40° are not from trochanteral MNs. There was no suprathreshold activity of dDprTr in this experiment. B: autocorrelations of levator activity at different FT-joint angles. Data were normalized to the mean number of spikes per bin at a given angle. Plotted are the relative counts of intervals (bin size: 40 ms) between every spike in the data file and every other spike within a range of ±6 s. The results are presented as line plots to make comparisons between different angles easier. The 1st positive and negative peaks different from 0 represent the cycle period, earlier and later peaks are harmonics of the cycle period. In animals with very regular activity (left), very little modulation of cycle frequency is seen and the plots for 40, 80, and 120° are practically indistinguishable. However, at 160° (weakest levator activity), the rhythm is less regular. In animals with less regular activity (right), rhythmicity sometimes ceases at extreme FT-joint angles. In the example shown, the LevTr MNs are silent at 160° and tonically active at 40°. Rhythmicity at 80 and 120° is not as regular as in the left as can be seen from the broader peaks and smaller relative amplitudes. Autocorrelograms show that frequency modulations during the tonic activity at 40° do not correspond to rhythmicity seen at 80 and 120°. The very small tonically active units in the recordings are slow motoneurons and the C11 motoneuron in nerve C1. C11 activity is also present in the C2 recording.
the FT-joint was maintained. The transient portion of this interjoint influence, i.e., the initial elevation of the trochanter with FT-joint movement, can be attributed to input from movement (velocity and acceleration) signals from the FT-joint to the neural networks controlling the CT-joint. As Hess and Büschges (1997, 1999) have shown, two qualitatively different mechanisms contribute to these interjoint influences. Movement signals from the FT-joint modulate CT-joint MN activity via intercalated sensorimotor pathways on the basis of direct and polysynaptic ("reflex-like") (see Marder and Bucher 2001) neuronal pathways from fCO sensory neurons onto CT-joint MNs (Hess and Büschges 1997). In addition, movement signals from the FT-joint have access to the CRGs that produce alternating motor activity in the two antagonistic CT-joint MN pools (Hess and Büschges 1999). However, as phasic movement signals from the fCO will decrease in strength once the "salute posture" is established, these studies do not explain the persistent influence of fCO inputs to the CT-joint when animals with a crossed receptor apodeme maintain the CT-joint in the elevated position. In an initial set of experiments, we found indications that position signals from the FT-joint affect locomotor activity in the CT-joint in the intact animal. By eliciting leg movements at different initial FT-joint angles in a single

**FIG. 5.** A: stimulus ramps with very shallow slopes show that the cycle period of rhythmicity depends on FT position. This is shown in detail in the insets a and b. B: cycle period was significantly correlated with FT position. The data plotted derive from 1 of 4 experiments. Three of these experiments showed significant correlation (see Table 1).

**FIG. 6.** Intracellular recording of the fDprTr during rhythmic activity and slow ramp stimulation. Positional signaling predominantly induces changes in the amplitude of membrane oscillations as opposed to shifts in the resting potential.
middle leg preparation, we found a clear correlation between FT-joint angle and the direction of the movement generated at the CT-joint (Fig. 1). We subsequently investigated the influence of position signals from the FT-joint on motor activity in the CT-joint in detail.

Positional signaling from the fCO onto CT-MNs in the resting animal

Hess and Büschges (1997) have shown influences of both movement and position of the fCO onto CT-joint MNs in the resting animal, and our findings verified these reports with respect to the influence of position signals. fCO position modulates tonic activity of the sDprTr, with spike frequency increasing with more extended FT-joint angles. In both s- and fDprTr, we found small, but maintained, shifts in the baseline membrane potential that depend on fCO position [sDprTr: Fig. 2B; fDprTr: Hess and Büschges (1997)]. We also verified the position dependency of tonic activity that has been reported for slow and semi-fast levator trochanteris MNs by Hess and Büschges (1997). However, levator activation by relaxation of the fCO (mimicking extension of the FT-joint) as described by Hess and Büschges (1997) was observed in only one animal. This could be because in this study we used angular steps of a smaller amplitude (40°) as compared with 60° in the previous study.

A marked feature that we observed in quiescent preparations was the position-dependency of the phasic reflex responses in levator MNs (Fig. 2A). Activation of levator MNs elicited by flexion signals from the fCO becomes stronger with more flexed starting angles. A similar position dependency of phasic reflex responses has been reported for intrajoint resistance reflexes in various arthropod legs (stick insect: Bässler 1965; Schmitz 1985; crab: Bush 1962; Spirito et al. 1972; locust: Field and Burrows 1982; Field and Coles 1994). At least for the locust fCO, this position dependency is thought to arise from a decrease in the number of phasic afferents that respond to joint movement at larger FT angles (Field and Coles 1994).

During postural control in the stick insect leg motor system, the observed interjoint influence of position signals from the FT-joint on trochanteral MNs is likely to contribute to height control in standing (for discussion, see Hess and Büschges 1997). The CT-joint plays a pivotal role for the control of body height over the substrate (Cruse et al. 1992, 1993). Although phasic velocity and acceleration fCO afferents are major inputs to normal resistance reflexes, positional inputs would be expected to have a greater influence when the perturbations are relatively slow. Changes in tonic activity of the MNs supplying the CT-joint caused by position signals from the FT-joint could help to keep the body at a fairly constant distance from the substrate by counteracting changes in height due to different FT-joint angles. A decrease in height above ground caused by extended positions of the FT-joint would result in a stronger activation of the trochanteral depressors (which would lift the body), whereas an increase in body height caused by more flexed positions would result in an increase in activity of trochanteral levator MNs, which would bring the body closer to the ground.

Positional signaling from the fCO to CT-MNs during pilocarpine-induced rhythmic activity

The activity of trochanteral MNs is affected by position signals from the fCO during rhythmic motor activity. The mean spike rate within bursts, burst duration, and duty cycle are modulated by the FT-joint angle (Fig. 3). Mean spike rate, burst duration, and duty cycle of depressor MNs increased with fCO signals reporting more extended FT-joint positions, whereas the reverse was true for the LevTr MNs. These effects could be solely explained by a modulation, i.e., alteration of the magnitude of the motor output, similar to findings in the resting animal. A tonic modulation of the baseline membrane potential, both by afferents and intercalated interneurons, would bring MNs closer or further away from spiking threshold, and superimposed rhythmic inputs of constant amplitude would result in changes in burst duration and spike frequency within the burst. However, intracellular recordings from the depressor MNs revealed that fCO-mediated modulation was manifest predominantly as a change in the amplitude of membrane potential oscillation during rhythmic activity (Fig. 6), and marked tonic shifts in baseline membrane potential were not apparent. This could potentially be due to a large electrotonic distance between the recording site in the neuropil and the sites of synaptic inputs from afferents and intercalated interneurons onto the MN. However, the lack of a marked shift in baseline membrane potential correlated with fCO position, together with the large changes in amplitude of membrane oscillations during pilocarpine-induced rhythmicity with fCO position, clearly indicates that tonic inputs from position-sensitive fCO afferents to CT-MNs can at best play only a minor role in mediating the position-dependent effects described here.

The mechanisms responsible for the modulation of the amplitude of rhythmic depolarizations in depressor MNs have not been investigated in detail. In principle, four mechanisms could be involved. 1) fCO afferents signaling FT-joint angle could access the CRGs responsible for rhythmic activity in the CT-joint directly and thus modulate timing and strength of input to the MNs. 2) Gating mechanisms could restrict direct influences from sensorimotor pathways on the MNs to specific cycle phases set by the CRGs, especially the depolarization phase. 3) Afferent signaling could presynaptically modulate the connections of CRGs to MNs. 4) The input resistance of trochanteral MNs could be modulated by positional signaling from the fCO and thus change the response of the MNs to a given synaptic input.

However, a number of findings clearly indicate that positional signaling from the fCO has direct access to the CRG controlling rhythmic activity of the CT-joint. First, in some preparations, rhythmic activity ceased at extreme joint angles (Fig. 4), with one antagonist falling silent and the other being tonically active. Autocorrelation analysis revealed no evidence of residual rhythmic modulation in the tonic motor activity, suggesting that the CRG of the CT-joint became locked in one phase of its cycle. Second, by applying very slow ramps to the fCO, we have shown that the cycle period of the rhythm in CT-joint MNs changes as a function of FT-joint angle. These effects can only be explained if the activity of the CRG is modulated by positional signaling from the fCO. In the light of these results and data from previous studies
(Bässler 1993; Graham and Bässler 1981; Hess and Büssinges 1997, 1999), it is now clear that both position and movement signals from the FT-joint take part in patterning the activity of MNs of the adjacent CT-joint, both at rest and in the active animal. A comparable influence was not observed for the reverse direction, i.e., from the CT-joint onto the FT-joint (Akay et al. 2001) nor from FT-joint and CT-joint onto the most proximal thoraco-coxal joint (Gerharz 1999; Akay and Büssinges, unpublished observation).

There are two possible functions that interjoint position signals may perform in the locomotor system during the execution of active leg movements. First, during walking, modulation of CT-joint MN activity by FT-joint position could control the height of the animal above the substrate by regulating the angular range of movements around the CT-joint. For example, during active leg movements, alternating FT-joint flexion and extension of a given amplitude can occur around more flexed or more extended mean FT-joint angles. Movements in the range of more extended FT angles would bias the CT-joint at a more depressed angle by increasing the activity of trochanteral depressors, while trochanteral levator activity would be increased at flexed FT-joint angles, thereby serving to maintain the height of the animal above the substrate. Second, position signals from the fCO could also assist in the timing of step phase transitions in the movement cycle of individual legs in concert with velocity information during tibial movement (Hess and Büssinges 1999). In the front legs, flexion and extension of the FT-joint is the major component of the stance and swing phase, respectively (Cruse and Bartling 1995). At the beginning of the stance phase in the middle legs, FT-joint flexion occurs at relatively more flexed positions of the FT-joint, whereas FT-joint extension occurs at relatively more extended FT-joint angles during the swing phase. In specific walking situations, such as for an inner leg in curved walking or for the leading leg in sideward walking, this relationship can be even more pronounced (for discussion, see Bässler 1993; Fischer et al. 2001).

During walking, position signals from the FT-joint could facilitate phase transitions at the CT-joint by increasing the probability of the initiation of levator activity with more flexed FT-joint angles and increase the probability of depressor activity with more extended FT-joint angles. The influence of FT-joint position appears to be more pronounced at the extreme ends of the range of angles used in our experiments (Fig. 3B, 40 and 160°). These angles are close to the limits of the normal working range of the FT-joint in the stick insect middle leg during straight walking (Cruse and Bartling 1995), where step phase transitions are very likely to occur. Therefore an increased probability of switching the activity of the CT-joint to the antagonist at these angles would seem even more appropriate. These considerations relate in general to behavioral experiments conducted by Cruse (1985a,b). He investigated the influence of leg position on determining the start of leg swing for the hindleg, partially in attempting to identify interactions of position and load signals from the leg. In his investigation, Cruse presented evidence that position information from the leg is relevant for determining its stance-swing transition during walking.

We found that position signals from the fCO affect the cycle frequency of the ongoing rhythm in trochanteral MNs, with increased flexion of the FT-joint angles causing an increase in cycle period. Apart from the basic conclusion that positional signals from the FT-joint affects the CRG controlling the CT-joint (see preceding text), there is no obvious functional conclusion that can be drawn concerning this interaction. Similar results have been reported for the influence of position signals from the cat hindlimb on the cycle period of fictive locomotor rhythms with flexion signals increasing the cycle period of fictive motor patterns in the hindlimbs of Clonidine-treated spinal cats (Pearson and Rossignol 1991) (see also following text).

Role of positional signaling in walking pattern generation in other systems

Our present findings on the stick insect walking system provide some interesting parallels with studies of vertebrate walking. In chronic spinal cats, preventing the hip from reaching an extended position inhibits the onset of the swing phase (Grillner and Rossignol 1978). In the decerebrate cat, position signals were found to affect the activity of the CPG network for walking. During fictive locomotor activity in the cat lumbar spinal cord, sensory signals related to hip position are able to modify rhythmic motor activity in intensity, duty cycle, and cycle period (Pearson and Rossignol 1991). Furthermore, sustained maintenance of a flexed hip position was able to arrest the fictive locomotor rhythm. A similar importance of hip position for the initiation of the swing phase has recently been reported for stepping movements in human infants (Pang and Yang 2000).

Limb position has also been shown to affect the generation of the swimmeret rhythm in the crayfish. Sustained retraction of the swimmeret could slow the motor rhythm, whereas maintained retraction or protraction of the swimmeret could, in some instances, completely inhibit an ongoing rhythm (West et al. 1979). Similarly, in the gill ventilation system of crustaceans, injection of DC current into the nonspiking afferents of the proprioceptive oval organ can stop or change the rate of the ventilatory rhythm as does fixing the position of the gill baiier (DiCaprio 1999).

In summary, the present results further underline the significance of proprioceptive signaling from the FT-joint for shaping CT-joint activity in the stick insect walking system. Timing as well as magnitude of CT-joint motor activity is under substantial influence from the FT-joint arising from sensory signals from the fCO. These signals include information about movement as well as position of the FT-joint. Our results have identified mechanisms by which positional signaling may play a role in coordinating the dynamic range of movements in adjacent leg joints and can assist the generation of step phase transitions.

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