Pattern Generation for Walking and Searching Movements of a Stick Insect Leg. I. Coordination of Motor Activity

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INTRODUCTION

In legged organisms, locomotor patterns are generated by a close interaction between central rhythm-generating networks in the nervous system and sensory information about actual movements of the appendages and changes in body posture and equilibrium (e.g., Bässler 1983; Cruse 1990; Grillner 1981; MacPherson et al. 1997; Orlovsky et al. 1999; Pearson 1995; Wendler 1964). The motor pattern of each leg emerges from interactions of sensory signals from the leg with central rhythm-generating networks governing the action of individual leg joints (summaries in Büschges and El Manira 1998; Clarac 1991; Pearson 1993, 1995) and from sensory signals and central commands coordinating the movements of adjacent leg joints to produce a functional locomotor pattern (invertebrates: Bässler 1993; Bässler and Büschges 1998; El Manira et al. 1991; Hess and Büschges 1999; vertebrates: Orlovsky et al. 1999). The resulting walking pattern is cyclic and consists of a stance phase in which the leg is on the ground and generates propulsion of the organism relative to the substrate and a swing phase or a return stroke in which the leg returns to its initial position to start the next stance.

The movements, joint forces and motor patterns of individual legs in walking insects have been analyzed during walking in considerable detail (e.g., Burns 1973; Cruse 1976; Dean and Wendler 1984; Delcomyn and Usherwood 1973; Duch and Pfüger 1995; Godden and Graham 1984; Graham 1972; Graham and Bässler 1981; Graham and Epstein 1985; Hoyle 1964; Pearson and Franklin 1984; Reingold and Camhi 1977; Runion and Usherwood 1968; Schmitz and Hassfeld 1989; Tryba and Ritzmann 2000a,b; Watson and Ritzman 1998a,b). In the stick insect, as in other species, two main gaits are known for locomotion: the tripod gait and the tetrapod gait (for summary, see Bässler 1983; Cruse et al. 1995). Various approaches, such as amputation experiments and experiments perturbing ongoing leg movements, have revealed the mechanisms that coordinate the relative timing of leg movements (e.g., Cruse and Knauth 1989; Dean and Wendler 1983; Foth and Bässler 1985a,b; Graham 1977; Wendler 1964). Six different coordinating mechanisms have been identified that contribute to the generation of stepping patterns during walking (recent summary in Cruse et al. 1995). For example, when the middle leg is in swing, the front leg will not initiate a swing and when the posterior leg starts a stance, the anterior neighbor can start a swing (summary in Cruse 1990). The proper execution of the coordinating mechanisms relies on information about the actual phase of movement of each leg and its loading in the walking cycle, provided by sense organs on the legs (e.g., Cruse et al. 1984; summary in Cruse et al. 1995).

How are signals that serve intersegmental coordination fed into the segmental neuronal networks of an individual leg and what are the neuronal mechanisms that affect patterning of
motoneuronal activity in a single leg? To be able to tackle these questions, we first need to know the coordination pattern of the joints of a single walking leg in the absence of coordinating influences from other legs. At present, very little information is available on this issue. This is because investigations on walking pattern generation in the stick insect and other insect species were mostly carried out in preparations with several legs present, i.e., when intersegmental coordinating mechanisms were expressed, with only a few exceptions (e.g., Bässler 1986, 1993; Bässler et al. 1991; Karg et al. 1991). The present study focuses on this question by using a stick insect preparation in which the walking system is reduced to an “isolated single middle leg.” This single-leg preparation is an adaptation of a preparation developed for the front leg of Cuniculina impigra (Bässler 1993; Karg et al. 1991). Bässler and coworkers used the front leg preparation for behaviorally oriented projects on movement control (e.g., Karg et al. 1991). We have chosen the middle leg because the neuronal networks controlling the motoneurons of this leg are known in greater detail than for the front leg (summaries in Bässler and Büschges 1998; Büschges et al. 2000). We describe the activity patterns of the motoneuron pools innervating the muscles of the coxa-trochanteral (CT), the femur-tibia (FT), and tibia-tarsal (TT) joints during walking and searching movements. The coordination of motor activity between the different leg joints was determined, and differences in the relative timing of activity patterns in searching and walking movements were analyzed. In addition, we investigated the influence of the walking and searching movements in distal leg joints on the motor activity generated in the restrained and deafferented most proximal leg joint, the thoraco-coxal joint (TC joint). Further investigations will make use of this preparation and its detailed quantitative description to elucidate the neuronal mechanisms of intrajoint and intersegmental coordination. The companion paper (Schmidt et al. 2000) describes the use of this preparation to describe the intracellular generation of motoneuronal activity patterns in the locomotor cycle.

**Methods**

*Animals and preparations*

All experiments were carried out on adult female stick insects, Cuniculina impigra Redtenbacher (syn. Bacillum impigra Brunner) obtained from a colony maintained at the Institute for Zoology, University of Colorado. The experiments were performed at room temperature (20–22°C) under dimmed-light conditions.

After removing all legs at the level of mid-coxa except for the left mesothoracic leg, the animal was fixed dorsal side up with insect pins or dental cement (Protemp, ESPE) on a foam platform with the coxa of the remaining leg situated exactly at the edge of the platform. The coxal stumps of the severed legs were fixed to the platform. The subcoxal (TC) joint was fixed with dental cement at an angle of approximately 90° to the thorax. Thus leg movements were only possible in the transverse plane. The distal leg joints, i.e., the CT, the FT, the tibia-tarsus joint, and the tarsal segments were all free to move. The mesothoracic scutum was opened dorsally by removing the tergum. The gut was left intact and placed outside of the cavity. Fat and connective tissue were removed, and the mesothoracic ganglion including the proximal basis of the lateral nerve roots was placed on a wax-coated ganglion holder. Connective tissue surrounding the ganglion was fixed to the platform with small cactus spines from Nopalea deflcta.

The lateral nerves of the removed contralateral leg were crushed as were the lateral nerves nl2, nl4 and nl5 (nomenclature after Marquardt 1940) of the left middle leg supplying coxal muscles of the leg. For intracellular recordings, the ganglion was treated with Pronase E (Merck Chemicals) for 30–90 s, and the thoracic cavity was washed several times and filled with Ringer solution (Weidler and Diecke 1969) following established procedures (Büschges 1989).

**Recording of leg movements**

A treadband, similar to the one used by Bässler (1993), was placed underneath the middle leg. Its direction of movement was aligned with the projection of the longitudinal axis of the femur. The adhesional friction of the treadband was low so that the leg was able to move the band at rates similar to those observed in intact walking, ranging from 0.5 to 2 s (see also Bässler 1993; Karg et al. 1991). Sequences of walking (and searching) movements of the middle leg were elicited by slightly touching the abdomen with a soft brush or by a brief air puff to the antennae or the abdomen. In some initial experiments (n = 4), the leg movements were filmed with a video system (50 frames/s, SONY DVC101e and JVC HR-D 530E6) to provide a basic description of the leg movements (Figs. 1A and 3A). In a later set of experiments (n = 5), the movements of the tibia (for walking movements) or the femur (for searching movements) were monitored by an optical detector (e.g., Elsner 1977; Foith and Graham 1983) in synchrony with the electromyographic (EMG) recordings (see following text) from femoral and tibial muscles (Figs. 1B and 3A).

**Electrophysiology**

Activity of the muscles driving the CT and FT joint was monitored by inserting low-resistance EMG wires (50-µm copper wire, insulated except for the tip). For EMG recordings from tibial muscles, i.e., extensor tibiae and flexor tibiae, the activity of both antagonists was monitored by a common bipolar EMG recording (cf. Weiland et al. 1986). In these recordings, potentials from the antagonists could be easily distinguished either on the basis of their activity with flexion or extension movements of the tibia elicited by activating the animal or by the different reflex activation of extensor and flexor tibiae muscles to imposed movements of the tibia at rest. In addition, intracellular recordings from motoneurons of both muscles verified their clear antiphase activity during walking and searching movements (see Schmidt et al. 2001 for details). Walking muscles in stick insects consist of individual muscle fibers that are only loosely coupled to each other by connective tissue. It is not possible to distinguish single motor units from EMG recordings of muscles which are innervated by more than two motoneurons, such as the flexor tibiae and the levator trochanteris (Debrodt and Bässler 1989; Hess and Büschges 1997; Storrer et al. 1986). Hence we did not distinguish individual muscle potentials of those muscles (but see Duch and Pfleger 1995) but rather evaluated their summed activity. In those muscles that are innervated by two excitatory motoneurons, i.e., the depressor trochanteris and the extensor tibiae (Bässler and Storrer 1980; Schmitz 1986; Storrer et al. 1986), the largest muscle potentials can be clearly attributed to the activity of the fast motoneurons, i.e., the fast extensor tibiae (FETi) and the fast depressor trochanteris (FDTr).

The activity of the motoneurons supplying the protractor coxae and the retractor coxae muscles of the TC joint was monitored by extracellular hook-electrode recording (Schmitz et al. 1988) from the lateral nerves nl2 and nl5, respectively (nerves labeled according to Marquardt 1940). The activity of the motoneurons innervating muscles moving the tarsal segments, i.e., the levator and depressor tarsi and the retractor unguis, was monitored by intracellular recordings from the neuropilar regions of their motoneurons in the mesothoracic ganglion. Intracellular recordings were made using an SEC-10 L amplifier (NPI Electronics, Tamm, Germany) thin-walled glass microelectrodes (Since Products) filled with a tip solution of 3 M...
KAc/0.05 M KCl (electrode resistance, 15–25 MΩ) were used. In general, leg motoneurons were identified by eliciting movements of the tarsus or its segments on being depolarized with injected current pulses surpassing the action potential threshold (see also Wolf 1992).

**Data evaluation and statistics**

Intracellular recording, EMG recordings, injected current, leg-position signals, and voice track were stored on an eight-channel digital audio tape recorder (Biologic DAT 1800 or DRA 800). For evaluation, the data were either displayed on a Yokogawa DL2300 chart recorder or they were analyzed off-line on a Pentium II personal computer using Spike 2 software (Cambridge Electronics). A/D conversion was performed by a CED 1401plus interface (Cambridge Electronics). Statistical analysis was performed with Apple KaleidaGraph for Windows, StatView, and MS Excel after the criteria described in Sachs (1978) and Batschelet (1981). In the text, \( N \) gives the number of animals, \( n \) gives the sample size.

**RESULTS**

**Motor pattern of active walking and searching movements of the middle leg**

WALKING MOTOR PATTERN OF THE MUSCLES SUPPLYING THE FT AND CT JOINT. Usually, brief tactile stimulation with a fine paint brush or a short wind puff on the abdomen was used to induce continuous walking sequences of several steps, although walking movements (Fig. 1A) were occasionally generated spontaneously. A step cycle (Fig. 1, A and B) was characterized by two phases, stance and swing. The power stroke, during which the treadband was pulled toward the animal will be referred to as stance (see also Bässler 1993), was generated by a strong activation of the flexor tibiae muscle. In the subsequent phase, the return stroke, the leg was lifted off the treadband, the FT joint was extended by activity of the extensor tibiae muscle and the leg returned to its distal starting position. This phase will be referred to as swing (see also Bässler 1993). Because the leg only moves in the transversal plane, the relationship between joint extension versus flexion and swing and stance is more typical of front legs during intact walking than it is of middle legs (see DISCUSSION for details). In many walking episodes, a short pause in activity was detectable at the times of transition between extensor and flexor activity (FIG. 1B, ▲). The average duration of the pause was 29.8 ± 29.60 (SD) ms for the transition from stance to swing, and 112 ± 108 ms for the transition from swing to stance (\( N = 14, n = 122 \)). The duration of these pauses did not change with step cycle duration (\( P > 0.05, \) data not shown).

The cycle duration of a step was defined as the time from the...
start of flexor tibiae activity to the start of the next flexor burst of the following step based on the activity in the tibial EMGs. Walking episodes varied considerably in duration and usually consisted of four to six steps. A histogram of the measured step cycle durations is shown in Fig. 1C, and the mean step cycle duration was $1,515 \pm 481\text{ ms}$ ($N = 20, n = 195$). During walking, the duration of both flexor tibiae (stance) and extensor tibiae (swing) muscles were significantly correlated with cycle period ($Fig. 1D, P < 0.01$) with swing duration in general longer than stance duration. This indicates that increasing or decreasing walking speed was achieved by changing both the duration of swing and stance.

During walking movements, the activities of trochanteral and tibial muscles were coupled to each other (Fig. 2). Typical muscle activity patterns generated in the FT joint (flexor and extensor tibiae) and the CT joint (depressor and levator trochanteris) during walking monitored by simultaneous EMG recordings are shown in Fig. 2A. Activity of large units in the levator EMG started during the second half of stance and persisted into leg swing. Sometimes activity of smaller units in the levator EMG (Fig. 2A, middle) were also detectable outside the mass activity in the levator muscle throughout the step cycle. Activity of the large unit in the depressor trochanteris muscle, arising from the FDTr, was confined to late swing and early stance, i.e., the transition from swing to stance.

The burst duration of the large units of the levator trochanteris muscles changed in relation to cycle period (Fig. 2B), while in most preparations, no such correlation was found for the large units of depressor trochanteris activity, representing activity of the fast depressor trochanteris (FDTr) motoneuron. Average burst onset, the mean burst duration and the motor pattern of FT- and CT-joint muscles during walking is given in Fig. 2C. Fast depressor and large levator units of the trochanter always showed alternating activity. However, co-contractions of small levator units together with depressor activity and occasional activity of the slow depressor motoneuron along with the large levator units was observed (as indicated in Fig. 2C, □). The onset of main levator activity occurred during mid-flexor activity, the main depressor activity started in the middle of the extensor activity and lasted into the first third of flexor activity of the following step.

SEARCHING MOTOR PATTERN OF THE MUSCLES SUPPLYING THE FT AND CT JOINT. When the treadband was removed, the middle leg performed stereotype sequences of movements that resembled searching movements previously described for the single foreleg preparation by Karg et al. (1991). Searching movement sequences were reconstructed from video recordings and are shown in Fig. 3A. The search cycle consists of two phases, trochanteral depression in combination with FT flexion and trochanteral levation in combination with FT extension (Fig. 3Aii). A histogram of the cycle periods observed is given in Fig. 3Aiii. The mean cycle period of the population investigated was $1,039.8 \pm 458.9\text{ ms}$ ($N = 12, n = 129$).

Activities of tibial and trochanteral muscles were clearly coordinated during searching movements. This is exemplified by typical EMG recordings of CT- and FT-joint muscle antagonists during searching movements in Fig. 3B. The large units of the depressor trochanteris (DprTr) and large units of the levator trochanteris (LevTr) as well as the flexor tibiae and extensor tibiae exhibited clearly alternating activity. During searching, burst lengths of the large units of the muscles moving the FT joint and the CT joint were significantly correlated with cycle period ($P < 0.05$, Fig. 3, C and D). How-

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**FIG. 2.** Coordination of motor activity generated in the femur-tibial (FT) and coxa-trochanteral (CT) joints during walking movements. A: simultaneous EMG recordings of the depressor trochanteris (DprTr, top), the levator trochanteris (LevTr, middle), and both the flexor tibiae and the extensor tibiae (Flex/Ext, bottom) in a typical experimental animal performing continuous step sequences. smu, small motor units; lmu, large motor units. B: plot of the burst length of CT-joint muscles (LevTr, DprTr) as a function of cycle period. $r$, average coefficient of correlation ($n = 72, N = 8; P < 0.05$). Levator burst length was significantly correlated in 5 of the 8 investigated animals with the correlation coefficient $r$ ranging from 0.499 to 0.987 ($P < 0.05$, $n = 7–17$; - - - , the regression for the pooled data). No significant correlation was found for the burst length of the large depressor unit, i.e., fast depressor trochanteris (FDTr); burst length significantly correlated in only 2 of 8 animals, $r = 0.623, n = 17$, and $r = 0.863, n = 13; P < 0.05$; - - - - , the regression for the pooled data). C: plot of the average activity of the tibial and trochanteral muscles in the walking cycle. Parameters were normalized to the period. $\bullet$, the average duration of activity, error bars on the left indicate the SD of the mean latency to the antagonist of the joint, the error bars to the right represent the SD of the mean burst duration. □, activity of small units in the EMG that was observed in some experiments outside the mass activity in the levator EMG throughout the locomotor cycle detectable.
ever, the burst length of the extensor tibiae and the levator trochanteris were more strongly related to cycle duration than their joint-specific antagonists. This is obvious from the steeper slopes of the regression lines and the higher coefficient of correlation in Fig. 3, C and D. This indicates that the frequency of continuous search cycles is mainly determined by the duration of FT extension and CT levation. The average phase of activity of the muscles and the coordination pattern between FT and CT joints during searching movements is shown in Fig. 3E. The onset of activity in levator trochanteris and extensor tibiae occurred almost simultaneously. The onset of levator trochanteris activity was barely leading, on average, by 3.44 ± 33.5 ms only \((N = 8, n = 109\), data not shown).

**WALKING AND SEARCHING PATTERNS OF MOTONEURONS SUPPLYING TARSAL JOINTS.** The tarsus, including the pretarsus composed of the arolium and claws, is moved by three muscles, the levator tarsi (LevT), the depressor tarsi (DprT), and the Retractor unguis (RetU) muscle. The latter is tripartite and consists of one femoral and two tibial muscles (Radnikow and Bässler 1991). The activity of the motoneurons innervating these muscles was recorded intracellularly from their neuro-

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**FIG. 3.** Aii: schematic drawing of leg movement during searching in a single middle leg as revealed from video film. The numbers give the sequence of frames that the images were taken from. The circle on the femur denotes the location of the reflection foil (Scotch3M) for measuring femoral movement by an optical detector. Aiii: recording of the femur position (bottom, measured by an optical detector) simultaneously with EMG recorded Ext muscle activity during searching movements. Note that the extensor muscle is active during levation of the femur. Aiii: histogram showing the distribution of cycle periods observed in 12 preparations. B: simultaneous EMG recordings of the DprTr (top), the LevTr (middle), and the Flex/Ext (bottom) activities during a searching sequence. EMG potentials on the top trace are truncated. C: plot of the burst length of the tibial muscles as a function of cycle period. \(r\), average coefficient of correlation \((P < 0.05; n = 119, N = 8)\). The relationship was significant in all investigated animals with the correlation coefficient ranging from 0.874 to 0.994 for the extensor and from 0.625 to 0.889 for the flexor; \(n = 12–17\) per investigated animal \((P < 0.05)\). D: plot of the burst length of CT-joint muscles as a function of cycle period. \(r\), coefficient of correlation for the pooled data \((N = 8; n = 119, P < 0.05)\). Levator activity was correlated with cycle period in all of the 8 animals investigated with particular \(r\) ranging from 0.823 to 0.979; \(P < 0.05; n = 15–17\) per animal; depressor activity was correlated with cycle period in 7 of 8 animals \((r\) from 0.715–0.873, \(P < 0.05)\). E: plot of the mean activity of the tibial and trochanteral muscles during searching movements. Data normalized to cycle period. For details of presentation, see Fig. 2C.

**pilar processes.** EMG recordings from the main part of the retractor unguis muscle in the femur are not possible due to its pilar processes. EMG recordings of the LevT and DprT would have impaired the leg movements due to the long EMG wires (4–6 cm) necessary for the distal segments of the leg.

The activity of LevT motoneurons \((N = 3)\) was related to leg swing during walking. LevT motoneurons were maximally activated during swing (Fig. 4A) with lower activity observed during stance. The membrane potential of LevT motoneurons was most negative during the transition from stance to swing. Motoneurons innervating the DprT generally exhibited opposite activity patterns \((N = 4)\) with maximal activity during stance (Fig. 4B). Similarly, motoneurons innervating the RetU muscle \((N = 8)\) exhibited maximal activation during stance (Fig. 4C). Activation of RetU and DprT in the walking cycle on average slightly preceded the beginning of mass activity in the flexor tibiae during stance (see Fig. 4D).

During searching, the activity of tarsal motoneurons was coupled to the activity of motoneurons innervating the CT and FT joint. LevT motoneurons were generally depolarized su-
prathreshold during FT extension and consequently during levation of the trochanter (Fig. 5A). Repolarization and inactivation occurred during FT flexion and could last into the extensor phase of the next searching cycle (Fig. 5A, △). In RetU motoneurons, rhythmic depolarizations were observed in antiphase with extensor activity, with their maximal depolarization occurring at the start of flexor activity. RetU motoneurons repolarized during FT extension (Fig. 5B). In contrast to the LevT motoneurons, the motoneurons supplying the DprT were activated in both phases of the cycle (Fig. 5C) outlasting both the extensor and the flexor phase of the searching cycle.

Coupling of motor activity in the CT, FT, and TT joint during walking and searching movements as a function of cycle period

During walking, there was no significant correlation between extensor onset and cycle period, as defined from flexor onset to flexor onset (P > 0.05, Fig. 6Ai), indicating that the phase of extensor onset was independent of walking speed. Similarly, the phase of onset in activity of the muscles moving the CT joint, i.e., the LevTr and the DprTr, was constant over the range of cycle periods investigated relative to the onset of extensor tibiae activity (P > 0.05; Fig. 6Ai). This indicates a phase-constant coordination of CT and FT joint during consecutive steps in the single-leg preparation.

In searching, the onset of activity in the extensor tibiae muscle changed in relation to cycle length (P < 0.05, Fig. 6Bi), resulting in an earlier onset of extensor activity during longer searching cycles. As expected from the preceding results, the activation of the LevTr did not change in relation to extensor onset (P > 0.05, Fig. 6Bii), and it did not change with cycle period. In contrast, activation of the DprTr depended on cycle period measured relative to the onset of tibial extensor activity, in a way that it was delayed with increasing cycle period (P < 0.05, Fig. 6Bii). In summary, in contrast to the phase-constant pattern of muscle activation in the CT joint and the FT joint during walking, interjoint coordination during searching did depend on cycle period [but showed a simultaneous onset of the tibial extensor and LevTr muscles (see also Fig. 3E)].

The phase of onset of tarsal motoneuron activity during walking is plotted in Fig. 6Aiii as a function of cycle period. The onset of activity in DprT and RetU in relation to flexor onset was observed to be independent of the cycle period (P > 0.05). In contrast, motoneurons innervating the LevT muscle exhibited a significant shift in their phase of activation with changing cycle period (P < 0.05). Thus for LevT motoneurons, there was no fixed coupling of activity onset in relation to the tibial muscle activation.

A general difference in the motor patterns generated either during walking or during searching movements concerned the duty cycle, i.e., the relative duration of the muscle activity as percentage of the cycle length, of the muscle antagonists of the CT and FT joint in the walking and searching cycle. The major
difference in both patterns was the duty cycle of tibial muscles in the movement cycle, being for the extensor muscle on average about 75% in searching and 60% during walking and about 25% for the flexor muscle during searching and 40% during walking (not shown).

Activity of the motoneuron pools of the TC joint during walking and searching movements

In the isolated middle leg walking preparation, the most proximal leg joint, the TC joint was not only immobilized but also deafferented and de-efferented by cutting or crushing the lateral nerves nl2, nl4, and nl5. Lateral nerve nl2 carries the axons innervating the protractor coxae muscle (ProCx), and lateral nerve nl5 innervates the retractor coxae muscle (RetCx) (Graham and Wendler 1981). Extracellular recordings from the nerves nl2 and nl5 and EMG recordings of tibial muscle activity during continuous walking sequences revealed that the activity of the motoneurons innervating coxal muscles was tightly coupled to the motor program generated in the distal leg joints (Fig. 7A). The motoneurons of both pools (ProCx and RetCx) exhibited clearly alternating activity in the walking cycle. During stance, activity of RetCx motoneurons occurred simultaneously with activity in the flexor tibiae muscle. A short delay between the start of activity in the flexor tibiae and the start of activity in retractor coxae motoneurons was frequently observed (Fig. 7Ai; see also 7C). During swing, i.e., activity of the tibial extensor muscle, ProCx motoneurons were active. The activity of protractor motoneurons slightly outlasted leg swing and was terminated at the beginning of stance (Fig. 7, A and C). This pattern of activity in coxal motoneuron pools was observed in 93% of the step cycles ($N = 6, n = 58$). Activity of ProCx motoneurons with swing phase and activity of RetCx motoneurons coincident with stance phase (Fig. 7, Ai and C) shares similarities with the leg motor pattern generated in intact “forward” walking animals (e.g., Büschges et al. 1994; Graham and Bässler 1981; Schmitz and Hassfeld 1989). In the remaining 7% of the step cycles recorded, alternating activity in TC motoneurons was observed as well. In these step cycles RetCx motoneurons were found to be synergistically active with the extensor tibiae, and ProCx motoneurons were active together with the flexor tibiae muscle. These steps resembled “rearward” walking (not shown). However, this rearward walking pattern was never steadily maintained and never lasted for more than two step cycles.

During walking, the burst length of ProCx and RetCx activity was significantly correlated with cycle period ($P < 0.05$, Fig. 7Bi) as was observed for the muscles of the CT and FT joints (see Fig. 2). Furthermore the onset of activity in ProCx and RetCx muscles in relation to the onset of activity in the flexor tibiae muscle did not change with step cycle duration ($P > 0.05$, Fig. 7Bii). Therefore the phase difference between the activation of TC motoneuron pools did not change when cycle period was changing ($P > 0.05$). The resulting motor pattern is schematically shown in Fig. 7C. This finding was further confirmed by intracellular recording from neuropilar processes of coxal motoneurons. These recordings revealed (ProCx; $N = 3$; RetCx; $N = 4$) that the motor output generated during walking in coxal motoneurons was due to a strong modulation of the motoneuronal membrane potential around its resting membrane potential in the walking cycle (Fig. 7Aii).

During searching movements, the activity pattern of ProCx and RetCx motoneuron pools was different as it was not related to the movements cycle of the leg (Fig. 8, A and B). In the majority of animals tested either RetCx or ProCx motoneuron pools could exhibit tonic activity that was sustained over consecutive search cycles, whereas little or mostly no activity was observed in the particular antagonist (Fig. 8A). During
ongoing searching sequences, activity could sometimes switch from one motoneuron pool to the other (Fig. 8A). Occasionally, slight modulations in the overall activity of the coxal motoneuron pools were observed (Fig. 8B) as also seen in front legs performing searching movements (Bässler et al. 1991). Intracellular recordings from the coxal motoneurons revealed a tonic activation of individual motoneurons during the performance of searching movements (Fig. 8C).
DISCUSSION

The present investigation provides a description of motor patterns generation in the stick insect middle leg, in a preparation in which influences from movements of the other legs were excluded. There are three main results of our experiments.

1) Without neighboring legs, the middle leg is able to generate stereotype walking and searching movements. Coordination of motor activity in adjacent leg joints during walking movements was found to be independent of cycle duration and hence phase-constant, while motor patterns during searching exhibited considerable dependence on cycle duration.

During walking, the duration of both stance and swing depended on step cycle duration, similar to the pattern seen in tripod gait (see below). 3) During walking and searching movements, motoneuron pools supplying the TC joint were also activated, even though this joint was deafferented and de-efferented. During walking there was a tight phase-constant coupling of coxal motoneuron activity with the movements generated in the distal leg joints.

Motor patterns for walking movements of a single walking leg

During walking movements of the single middle leg, the activity of all leg muscles was driven in a cyclic manner...
generating consecutive stance and swing phases (Fig. 9A). The cycle periods averaged $1.515 \pm 0.481$ s and thus are in the same range (0.3–2 s) as those reported for the intact stick insect (Bässler 1983). Walking movements were characterized by a stereotyped coordination between the motoneuron pools of the joints that were free to move, i.e., CT, the FT, and the tarsal joints. The basic pattern of activity in the leg muscles was the following: during stance flexor tibiae motoneurons were active together with depressor tarsi and retractor unguis motoneurons and the treadband was pulled toward the animal. Levator trochanteris motoneurons were activated late in the stance phase. During late stance, levator muscle activity assists the other stance phase muscles (flexor, retractor unguis) in pulling the treadband toward the animal. The activation of levator trochanteris motoneurons was maximal when the leg was lifted off the treadband, i.e., at the transition from stance to swing. This stance-to-swing transition was characterized by the offset of flexor activity and the onset of activity in extensor tibiae and levator tarsi motoneurons. This switch in motoneuronal activity caused lifting of the leg off the treadband and extending the FT joint toward its initial position prior to the former stance phase. In late swing, activation of depressor motoneurons contributed to setting down the leg on the treadband and the next step cycle started.

In addition to the observed range of cycle periods, one similarity of the single middle leg preparation to the intact walking stick insect was the bi-phasic structure of walking movements with sequential stance and swing modes. Differences to intact straight walking occur in the operating range of FT-joint angle during stance and swing. Excursions in FT joint are smaller for the middle leg in straight walking than in the

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**FIG. 8.** A: activity of the muscles supplying the immobilized and de-efferented TC joint (top) and the tibial muscles (bottom) during a sequence of consecutive search cycles. Please note that neither ProCx nor RetCx motoneuron pools showed alternating activity correlated with the searching motor pattern in tibial muscles. Each of the antagonists was active for some time throughout consecutive cycles while the particular antagonist was not active. B: activity recorded from the levator trochanteris muscle (top) and protractor nerve nl2 (3rd trace) muscles during searching movements together with a position signal from the femur (bottom). The 2nd trace from top shows an instantaneous frequency plot of protractor activity (BIN width: 100 ms). Please note the slight modulation in activity of protractor motoneurons related to the searching motor pattern. C: intracellular recording of a RetCx motoneuron during searching. Please note that the retractor motoneuron is tonically depolarized during the searching sequence.

**FIG. 9.** Schematic drawing of the average motoneuron activity (normalized to the period) for the leg motoneuron pools investigated during active walking (A) and searching movements (B) in the middle leg. Gray bars or hatched bars in B indicate tonic activity of motoneurons.
single middle leg preparation. This derives from the fact that in straight walking of intact animals stance and swing leg excursions are mainly due to movements of the coxa, i.e., in the horizontal plane of the TC joint (Cruse et al. 1995), while in the single leg preparation they are generated by flexion and extension of the FT joint. In this respect, the coordination of leg muscles in the walking cycle of the preparation used here is more similar to forward walking in the front leg (see Bässler 1983, 1988, 1993; Cruse 1976). As in the single middle leg, the front leg stance is generated predominantly by FT-joint flexion and swing by FT-joint extension (Bässler 1993). Furthermore a similar coordination pattern occurs in the intact walking system e.g., an inner leg during curve walking (Jander 1982; Rixe and Dean 1995). A similar reciprocity in activity of femoral muscles, as observed in the single middle leg preparation was also reported for walking at shorter cycle periods of the cockroach (Krauthamer and Fourtner 1978).

In the single middle leg preparation, the duration of both stance and swing depended on cycle period. Such a dependence is seen in the intact stick insect walking with a tripod gait (see also Bässler 1983; Cruse et al. 1995; Graham 1985). There is, however, a difference between these two situations. The proportion of swing in the single middle leg preparation is around 50% and thus significantly larger than in the intact animal walking in tripod gait, in which swing covers around 20% of the cycle (cf. Bässler 1983; Graham 1985). Several factors might contribute to this difference. In the intact stick insect, the transition from swing to stance and vice versa is under control of segmental and intersegmental sources: sensory signals from the TC joint of the same leg (Bässler 1977; Dean and Schmitz 1992) affect the transition from stance to swing and vice versa and coordinating signals related to the movement phase or loading of neighboring legs affect both transitions (Cruse 1990). It is quite conceivable that coordinating influences do affect the duration of the swing phase of the middle leg by shortening it and by lengthening stance phase in intact walking. The marked quantitative changes occurring with respect to the proportion of leg swing in the step cycle thus may arise from these absent constraints. As such, the neuronal system generating middle leg movements appears to operate in a “free-run mode” in the single leg preparation. This offers a variety of approaches to investigate neuronal mechanisms underlying coordination.

Two other influences have to be taken into account as well. First, load signals from the same leg, i.e., the middle leg, and the mesothoracic segment that are known to affect the generation of the stepping patterns in walking may differ in the single middle leg preparation (e.g., Bässler 1977; Schmitz et al. 1995; Wendler 1964). Such local load signals are not necessarily restricted to the same leg but can also arise from the other legs via mechanical coupling within the walking system. Such signals are absent in the preparation and thus load information about the status of the thorax is altered (e.g., Delcomyn 1991; Ridgel et al. 2000; Schmitz 1993; Zill and Moran 1981). Second, the TC-joint of the middle leg is not free to move. Sensory information from the CT-joint provides relevant information about the actual phase of the leg in the walking cycle (e.g., Bässler 1977; Cruse et al. 1984). Both aspects may also affect the activity motor pattern of coxal motoneurons so that it differs from the intact walking stick insect. As such Graham and Wendler (1981) have reported that in the middle leg the initial 30% of the stance phase of the middle leg are generated during ongoing co-activation of protractor and retractor coxae motoneurons. In the single leg preparation, we did not observe such a phase of co-activation of coxal motoneuron pools in the walking cycle. Both the lack of specific load signals as well as the lack of proprioceptive information from the coxa may contribute to this pattern.

Motor patterns for searching movements of a single walking leg

Searching movements of the single middle leg were composed of trochanteral depressor and tibial flexor activity in conjunction with activity in retractor unguis motoneurons (Fig. 9B). Subsequently trochanteral levator, tibial extensor and levator tarsi motoneurons activity started together and tarsal depressor motoneurons were activated throughout the full search cycle. In contrast to walking, the searching motor pattern exhibited considerable dependence on cycle period. The most prominent characteristic in searching movements was the simultaneous onset of tibial extensor and trochanteral levator activity. Finally, there was no coupling of motor activity in the thoraco-coxal joint to the searching cycle.

The searching movements generated in the middle leg and their underlying motor patterns reported here are very similar to searching movements generated in the stick insect front leg (cf. Bässler 1993; Karg et al. 1991). Recently data were presented for freely searching front legs (Dürre 1999) reporting that the stick insect leg tends to change the position of its thoraco-coxal joint independent of the ongoing searching movements of the CT and FT joints. This result is in accordance with our finding that the coxal motoneuron pools were mostly tonically active during searching sequences with activity sometimes switching from one motoneuron pool to the other.

Interjoint coordination in stick insect walking legs

Recording motoneuronal activity of the CT-motoneuron pools during walking revealed that both the protractor and the retractor coxae motoneuron pools exhibited rhythmic activity coupled to the ongoing motor pattern. The coordination mostly resembled the one known for movements of the front legs during forward walking (e.g., Bässler 1983; Graham 1985) where during stance, retractor coxae motoneurons were active and during swing, protractor coxae motoneurons were active. The occurrence of patterned activity in TC-motoneuron pools during walking is interesting because this joint was de-effereented and deafferented. The question emerges as to how the activity of the motoneurons of the TC joint is coupled to the walking motor pattern. From previous investigations, it has become clear that proprioceptive signals from one leg joint contribute to patterning of motoneuronal activity in adjacent leg joints (Bässler 1993; Hess and Büschges 1999). It thus appears quite conceivable that sensory signals reporting movements and/or forces from distal leg joints during walking may affect the premotor networks of the TC joint so that their activity is coupled to the ongoing motor pattern. Similar results have been reported for the action of proprioceptive signals in the walking system of the crayfish (e.g., El Manira et al. 1991). The best candidate sense organs for such signals would be those that report ground contact of the leg, such as tarsal sense
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