Multi-Joint Coordination During Walking and Foothold Searching in the *Blaberus* Cockroach. I. Kinematics and Electromyograms

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Tryba, Andrew K. and Roy E. Ritzmann. Multi-joint coordination during walking and foothold searching in the *Blaberus* cockroach. I. Kinematics and electromyograms. *J Neurophysiol* 83: 3323–3336, 2000. Cockroaches were induced to walk or search for a foothold while they were tethered above a glass plate made slick with microtome oil. We combined kinematic analysis of leg joint movements with electromyographic (EMG) recordings from leg extensor muscles during tethered walking and searching to characterize these behaviors. The tethered preparation provides technical advantages for multi-joint kinematic and neural analysis. However, the behavioral relevance of the tethered preparation is an important issue. To address this issue, we evaluated the effects of tethering the animals by comparing kinematic parameters of tethered walking with similar data collected previously from cockroaches walking freely on a treadmill at the same speeds. No significant differences between tethered and treadmill walking were found for most joint kinematic parameters. In contrast, comparison of tethered walking and searching showed that the two behaviors can be distinguished by analysis of kinematics and electrical data. We combined analysis of joint kinematics and electromyograms to examine the change in multi-joint coordination during walking and searching. During searching, middle leg joints extended during swing rather than stance (i.e., walking) and the coordination of movements and extensor motor neuron activity at the coxa-trochanteral and femur tibia joints differed significantly during walking and searching. We also found that the pattern of myographic activity in the middle leg during searching was similar to that in the front legs during walking.

INTRODUCTION

The problem of coordinating several motor neuron pools to produce a behavior is common to many motor systems. This problem is complex as animals may use the same appendages to produce multiple behaviors and each behavior may have its own degree of variability. In walking systems, where joint oscillators exist (insect: Buschges et al. 1995; mud-puppy: Cheng et al. 1998), central or afferent influences orchestrate these oscillators to achieve appropriate inter-joint coordination during ongoing activity. Inter-joint coordination then must be reliably modified to produce multiple behaviors in a context-dependent fashion. Evidence for central and peripheral modulation of inter-joint coordination exists (Angel et al. 1996; Bassler 1993; Brunn 1998; Graham and Bassler 1981; Grillner and Zangger 1979; Hess and Buschges 1999; Robertson et al. 1985). However, few studies have begun to examine the underlying neural principals involved in control of multiple leg joints (El Manira et al. 1991; Hess and Buschges 1999; Wolf 1990). Further, intracellular analysis of neural mechanisms underlying control of inter-joint coordination in legged animals during multiple behaviors has rarely been examined (Kitmann et al. 1995). In approaching this problem, preparations that allow detailed three-dimensional kinematic analysis concurrently with intracellular and extracellular recordings would be highly advantageous. However, such data sets are technically difficult to obtain from behaving animals.

Tethered preparations provide a potential solution to this dilemma. Several studies have examined neural strategies for coordinating behaviors using semi-intact tethered preparations that allow simultaneous kinematic, intra- and extracellular analysis (e.g., crab: Hienzel et al. 1993; mollusks: Hume and Getting 1982; insects: Kitmann et al. 1995). The tether allows the animal to move relatively freely while the motor activity and kinematics can be examined in a variety of active behaviors. However, the tethered animal may not experience normal sensory inputs or display its normal behavioral repertoire. These experimental disadvantages raise the possibility that the tethered behaviors are not the same as the freely moving behaviors of interest. To address this concern and take full advantage of a tethered preparation, an investigator can compare the behavior when the animal is freely moving to that in the tethered situation (see Godden and Graham 1984; Nye and Ritzmann 1992).

We developed a tethered preparation that permits detailed kinematic analysis of joint movements and intra- and extracellular analysis of motor control while cockroaches perform a range of active behaviors. We address the issue of behavioral relevance by comparing leg kinematics and motor activity during tethered walking to that of freely moving animals. For this comparison, we used a large data set from animals walking freely on a treadmill that was obtained in a previous study (Watson and Ritzmann 1999a).

Having established the behavioral relevance of the preparation, we then examined a switch from walking to searching movements. When walking insects suffer a loss of a reliable foothold, the behavior of their legs switches from walking to "searching." The searching behavior is used to find a foothold and thereby maintain stability (Franklin and Pearson 1984). We take advantage of insects' ability to switch between walking and searching to examine multi-joint coordination during these behaviors. In this paper, we compare coordination of the coxa-trochanter (CTr) and femur-tibia (FT) joints in the front (prothoracic) and middle (mesothoracic) legs during walking and searching. The coordination of these principal joints changes between walking and searching movements. In the DISCUSSION, we present hypotheses to account for the differ-

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ences in mesothoracic walking and searching movements. In the companion paper (Tryba and Ritzmann 2000), we use intracellular analysis to begin to test these hypotheses.

METHODS

Animals

Adult male death-head cockroaches (*Blaberus discoidalis*) were used in all experiments. Cockroaches were raised in our own colony descended from 250 adult animals generously provided by Dr. Larry L. Keeley of Texas A & M University. Cockroaches were housed in 20-l plastic buckets, half filled with aspen shavings, and were held at 27°C in a 12 h light:12 h dark circadian cycle. A commercial dry chicken starter and water were provided ad libitum. Only intact, undamaged cockroaches were used.

Kinematics

We marked the ventral surface of the segments making up the body-coxa (BC) joint, the CTr joint, the anterior surface of the tibia just distal to the FT joint, and the tibia-tarsus joint with Pilot silver permanent marker ink to facilitate visualizing them against the dark thorax of the cockroach (Fig. 1, *A* and *B*). Note that in previous papers (Watson and Ritzmann 1998a) the distal joint of the coxa was referred to (incorrectly) as the coxa-femur (CF) joint because the joint between the trochanter and femur was assumed to be fused. More recent observations indicate that the trochanteral-femur joint makes small but important movements that significantly influence placement of the tarsus (foot) (Watson et al. 1998). Therefore to avoid potential confusion regarding which joint is studied, we will refer to the distal coxa joint by the more correct terminology, the CTr joint. When comparing joint angles published here with those measured during treadmill running published earlier by Watson and Ritzmann (1998a), the reader should compare our CTr data with their CF data.

Animals were tethered above an 11.5 cm × 7.5 cm × 3.0 mm glass plate made slick with microtome oil (Lipshaw Manufacturing, microtome oil No. 288). They were tethered with two (#2) insect pins each bent at a 90° angle and pushed through the animal's pronotum, lateral to each side of the animal's head (Fig. 1*C*). The other end of the pins were glued together and attached to a glass rod that was mounted on a micromanipulator. The sharp ends of the pins were placed through the apices of the dorsal "keystone"-shaped marking on the pronotum. The pronotum markings were used to direct pin placement so animals were tethered at the same relative points from animal to animal. Each hole was made slightly larger than the width of the pin to allow the animals to slide up or down on the tether to adjust their position relative to the substrate. After tethering the animals, a small droplet of cyanoacrylate glue was placed about 4 mm above the bend in the pins. This glue prevented the animal from sliding off the tether during an experiment. The ventral view of the animal was imaged via a 7.0 × 7.0 cm plane mirror mounted at a 45° angle to the glass plate and approximately 1.3 cm below it (Fig. 1*C*).

Ventral and lateral views of the running cockroach were videotaped at either 125 frames/s with a Redlake digital high-speed video (HSV) system or at 200 frames/s using a NAC 400 analog high-speed video system. The ventral and lateral projections of the CTr, FT, and BC joints of the leg of interest, as well as the anterior tip of the head and the posterior tip of the abdomen, were digitized from each video frame (see Watson and Ritzmann 1998a). The true BC, CTr, and FT joint angles in three-dimensional space were calculated from the ventral and lateral projected images (Marx et al. 1993). An earlier kinematic study provided extensive three-dimensional kinematics for mesothoracic (T2) and metathoracic (T3) legs (Watson and Ritzmann 1998a) but not for prothoracic (T1) legs. We wished to compare T1 walking versus T2 leg searching and walking joint movements. Therefore T1 leg movements were monitored in a limited number of freely moving animals using a treadmill that was described previously (Watson and Ritzmann 1998a). Prothoracic (T1) treadmill running data were collected, with a slight modification of the methods described extensively in Watson and Ritzmann (1998a). To obtain an unobstructed lateral view of the T1 coxa, about 2 mm of the lateral edge of the pronotum was trimmed off. The lateral pronotum was trimmed bilaterally to avoid unevenly reducing the weight of the animal.

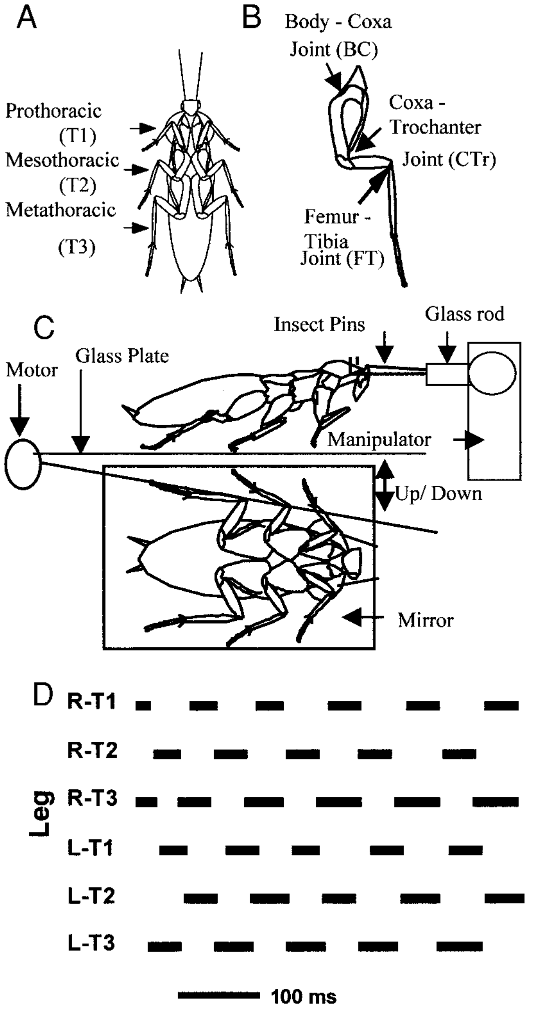

FIG. 1. *A*: *Blaberus discoidalis* illustration showing the 3 pairs of legs of the prothoracic (T1), mesothoracic (T2), and metathoracic (T3) segments. *B*: enlarged drawing of the T2 leg showing the relevant joints. The body-coxa (BC) joint, the coxa-trochanter (CTr) joint, and femur-tibia (FT) joint. Between the coxa and femur is the trochanter (as shown). For simplicity, we refer to the angle between the coxa and femur as CTr (rather than specifying the coxa-trochanteral and trochanteral-femur joints). *C*: tethered preparation set-up for simultaneous electromyography (EMGs wires not shown) and high-speed videography showing the tethered animal on a glass plate made slick with microtome oil. Raising the tether via the micromanipulator, resulted in pitching the anterior of the animal up, away from the glass plate substrate. The glass plate could also be pitched away from the anterior of the animal either by hand or using the motor. Mirror below the glass plate provided a ventral view of the animal. *D*: tripod walking stance phase duration (bars) from the right (R-) and left (L-) front (T1) middle (T2) and hind (T3) legs during tethered walking. Calibration bar = 100 ms.

Electromyograms

In experiments involving muscle activity, 50-μm bipolar electromyographic (EMG) electrodes insulated to the tip were placed in muscles of a right leg. Electrodes were placed in the main depressor muscle either of mesothoracic leg muscle 135D or prothoracic muscle 85D (Carbonell 1947). EMG electrodes were also inserted in the tibial extensor muscle (mesothoracic leg muscle 142A; prothoracic muscle 92A) (based on the numbering system of Dresden and Nijenhuis 1953) that extends the femur. The coxal depressor is innervated by one slow excitatory motor neuron (Ds) and one fast excitatory motor neuron (Df) as well as three inhibitors (Pearson and Iles 1971). EMG electrodes were also inserted in the tibial extensor muscle (142a) (Dresden and Nijenhuis 1953) that is innervated by one fast extensor (FETi) and one slow extensor (SETi) motor neuron (Atwood et al. 1969). For details of the recording sites and implantation methods, see Watson and Ritzmann (1998a). Electrodes implanted by these methods do not interfere with leg kinematics (Watson and Ritzmann 1998a). Animals having EMG wires implanted in their T1 legs also had the serrations of their mandibles trimmed off to prevent them from biting through the EMG wires with their mouth parts. EMGs were amplified and the signal digitally recorded on VHS tape using a VCR equipped with an A/D converter. Synchronizing the electrical and kinematic data was facilitated by recording an onset trigger pulse marking the start of HSV recording onto both the electrical and video records. Additionally, a timing pulse for each video frame (125 Hz) was recorded along with EMG records. The start of the electrical record coinciding with the beginning of the digitized sequence was then located by finding the correct time from the video onset trigger pulse.

Data analysis

Joint-angle records sampled at 125 Hz were smoothed using a moving boxcar averaging method of three data points each of 8 ms width. Smoothed joint-angle records were expanded by 40 times to give the same number of data points as the EMGs that were digitized at a sampling rate of 5 kHz. Relevant kinematic and EMG sequences were synchronized and merged into a common file using Data-Pac III software from Run Technologies. All data conditioning and subsequent analysis were also carried out with the Data-Pac III software.

Slow or fast motor neuron potentials were discernible in the depressor coxa and extensor tibia records used for analysis. The time of onset and termination of individual EMG spikes was determined by the time when the voltage exceeded a common threshold. The threshold was set independently for each file and was chosen to maximize the number of EMG spikes detected. Deflections that clearly resulted from movement artifact or cross-talk from other muscles were edited out by hand. Slow depressor and extensor potentials were distinguished from cross-talk due to other muscles by the broad muscle potential, the relatively larger size and the presence of a neural spike preceding each muscle potential. The burst onset and termination were defined as 0.5 ms before the first spike and 0.5 ms after the last spike in a burst (Watson and Ritzmann 1998a). The mean EMG frequency was calculated as the mean of instantaneous frequencies within a burst. The mean joint angular velocity was calculated as the regression slope of the joint-angle amplitude for the time interval between EMG spikes. The start of each joint movement was taken as 0.5 ms before the first detectable joint movement in one direction, and the end was taken as 0.5 ms before the first detectable opposing movement. Extension onset phase was calculated as the onset of extension (i.e., flexion to extension transition) relative to the peak extension at the start (0) and end (1) of each joint cycle. The tarsus touchdown and foot lift-off were determined directly from visual inspection of the video data.

Experimental protocol

Tethered cockroaches were videotaped while they generated leg movements on an oiled glass plate (Fig. 1*C*). Tethered animals running in a tripod gait could then be induced to switch to searching leg movements by altering the distance between the body and substrate. That change was accomplished by either raising the position of the tether thus pitching the animals' anterior up or pitching the substrate near the animals' anterior downward. The former method was used to collect T1 tethered data and the latter method was used to collect T2 data. T2 data were collected by the latter method as it allowed for direct comparison with subsequent intracellularly recorded data as described in the companion paper (Tryba and Ritzmann 2000). Tethered walking data were then compared with treadmill walking data collected at similar joint cycle rates.

RESULTS

The first step in establishing the behavioral relevance of our tethered preparation was to determine how similar tethered walking behavior is to free-ranging movements. Here we compare our tethered walking data to that obtained during treadmill walking at similar joint cycle rates. Comparison between pooled data from cockroaches walking on a treadmill versus walking on the tether were made from data collected at similar joint cycle rates because it is known that several variables vary with walking speed (Delcomyn and Usherwood 1973; Pearson 1972). For example, motor neuron inter-burst interval, burst duration, and frequency vary with walking rate (Delcomyn and Usherwood 1973; Pearson 1972).

T2 tethered walking

FOOTFALL PATTERN. Our study includes walking rates between about 3.8 and 7.5 Hz. At these rates (previously referred to as slow running by Watson and Ritzmann 1998a), cockroaches typically use a tripod gait during walking (Delcomyn 1971). All walking video records used for detailed analysis of single leg walking kinematics were visually examined to determine the coordination between legs. In each case, front and hind legs of one side were in-phase with the opposite side middle leg, which is consistent with a tripod gait. We further performed a detailed analysis of all six legs for 10 steps each in five animals. In each case, they used a tripod gait and an example of that analysis is shown in Fig. 1*D*.

COMPARISON OF T2 LEG CTr AND FT JOINT KINEMATICS. One of our goals in this paper is to examine the changes in T2 leg joint kinematics as the animal performs searching movements. Therefore our analysis concentrated on movements of the T2 leg. Tethered walking is qualitatively and quantitatively similar to treadmill walking in terms of T2 CTr and FT extension duration, joint-angle excursions, and maximum and minimum joint angles (Fig. 2*A*; Table 1). The combined treadmill data compared in Table 1 included 36 steps from nine animals (Watson and Ritzmann 1998a), and the tethered data represent 17 steps from three animals. In each comparison, mean values are used, and the data are from animals that did not have EMG electrodes implanted in their legs. Of the variables examined, significant differences existed only for CTr and FT flexion duration and FT mean joint angular velocity during extension (Table 1). When comparing many factors, there is a possibility that statistical tests will report false differences simply by chance. At the 0.05 confidence level, one can expect that 0.7 of 14 of such comparisons to be statistically different by chance. Thus the three differences we describe here appear to be characteristic of tethered walking. The fact that flexion duration was different in both the CTr and FT joints further sup-

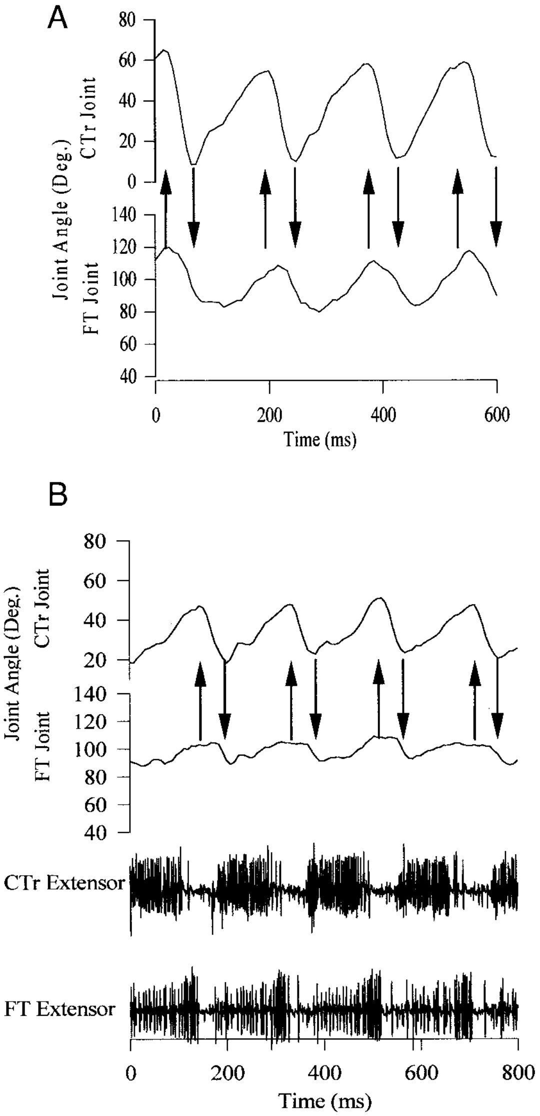

FIG. 2. *A*: mesothoracic leg CTr and FT leg joint kinematic records during tethered walking in an animal that is not wired for EMGs. Superimposed on the motion records, downward arrows indicate tarsal (foot) substrate contact. Upward arrows indicate points at which the tarsus was lifted off the treadmill. *B*: mesothoracic leg CTr and FT leg joint motion records synchronized with CTr and FT extensor electromyographic (EMG) records during tethered walking. Arrows as in *A*.

ports the notion that the differences we report here are not due to chance. The fact that 11 other parameters were not significantly different in treadmill and tethered walking suggests that tethered walking is similar (overall) to treadmill walking.

The inter-joint coordination in T2 and T3 legs was also normal in the tethered preparation. In treadmill walking, both the CTr and FT leg joints in each leg extend during the stance phase of tripod walking to propel the animal forward. While the CTr and FT joints act in concert through much of stance, the CTr joint extension onset and termination precedes that of the FT joint (Watson and Ritzmann 1998a) (Fig. 2, *A* and *B*; Table 2). For tethered walking, we found a mean CTr-to-FT extension onset delay in T2 of 15.7 ± 5.1 (SD) ms ($n = 3$ animals, 19 joint cycles; see also Table 2). This delay is within the mean and standard deviation reported for treadmill walking (Watson and Ritzmann 1998a).

TABLE 1. *Comparison of tethered and treadmill walking*

T2 Leg	Tether (Mean)	Tread (Mean)	*P* Value
CTr joint			
Cycle period, ms	134.1	146.2	0.1614
Extension duration, ms	72.0	69.8	0.3104
Mean angular extension velocity, deg/s	706.0	781.0	0.4203
Joint angle excursion, deg	49.1	46.1	0.2936
Minimum joint angle, deg	11.5	14.2	0.1382
Maximum joint angle, deg	57.9	62.0	0.3700
Flexion duration, ms	56.0	69.1	0.0018
FT joint			
Cycle period, ms	133.2	143.6	0.2534
Extension duration, ms	56.0	64.8	0.8670
Mean angular extension velocity, deg/s	385.0	489.0	0.0248
Joint angle excursion, deg	22.2	25.8	0.1490
Minimum joint angle, deg	88.1	86.4	0.1140
Maximum joint angle, deg	116.7	116.7	0.5640
Flexion duration, ms	68.2	82.5	0.0099

Comparison of tethered (tether) and treadmill (tread) walking coxa trochanter (CTr) and femur tibia (FT) joint kinematics from middle legs (T2) of cockroaches walking at similar step cycle periods (cycle period) in a tripod gait. Note the CTr and FT flexion duration and FT extension velocity were significantly different for the two groups.

T2 Ds AND SETi EMGs. The similarities in T2 CTr and FT joint extension kinematics during treadmill and tethered walking suggested that the neural control of these joints was largely unaffected by the tether. We tested this hypothesis by recording EMGs from the extensor muscles of the CTr and FT joints during tethered walking (Fig. 2*B*) and compared the results for Ds and SETi to those collected by Watson and Ritzmann (1998a) during treadmill walking. The leg joint movements in the tethered animals did not appear to be altered following

TABLE 2. *Comparison of tethered walking and searching*

T2 Leg	Walk (Mean)	Search (Mean)	*P* Value
CTr joint			
Cycle period, ms	150.4	145.3	0.6554
Extension duration, ms	80.0	40.0	<0.0001
Mean angular extension velocity, deg/s	904.0	587.0	0.0021
Joint angle excursion, deg	44.5	30.3	<0.0001
Minimum joint angle, deg	9.9	21.2	<0.0001
Maximum joint angle, deg	54.8	49.7	0.0981
Flexion duration, ms	56.0	132.0	<0.0001
Extension onset phase	0.4	0.7	<0.0001
FT joint			
Cycle period, ms	143.3	147.6	0.7436
Extension duration, ms	72.0	48.0	0.0019
Mean angular extension velocity, deg/s	363.0	1,576.0	<0.0001
Joint angle excursion, deg	22.9	66.7	<0.0001
Minimum joint angle, deg	86.5	71.5	0.0003
Maximum joint angle, deg	109.4	138.2	<0.0001
Flexion duration, ms	72.0	96.0	0.0251
Extension onset phase	0.5	0.7	0.0008
CTr-FT extension			
Onset delay, ms	15.7	−24.5	<0.0001

Comparison of tethered walking and searching CTr and FT joint kinematics from middle legs (T2) of cockroaches at similar cycle periods. Note that only the CTr maximum joint angles were not significantly different for the two groups.

implantation of EMG electrodes (see also Watson and Ritzmann 1998a).

We first looked at the relative timing of Ds and SETi burst onset. Based on the kinematics described in the preceding text, one expects that the CTr extensor (Ds) would become active prior to the FT extensor motor neuron (SETi), and this is indeed what we found (Fig. 2*B*). We graphed the Ds burst onset times as a percent of SETi burst cycle (Fig. 3*A*); each SETi burst cycle was defined by onset-to-onset times. In walking, Ds burst onset time is negative, as it precedes the SETi cycle onset by a mean of 10.07% ($n = 4$ animals, 48 cycles; Fig. 3*A*).

We next looked at the timing of T2 Ds and SETi potentials within each burst. The distribution of potentials within each burst may be altered by the tether conditions. For example, walking on a glass plate may result in very different afferent feedback than the treadmill, and the altered sensory feedback could influence the intra-burst pattern of potentials. To examine this possibility, we plotted a cumulative histogram of Ds or SETi potentials normalized across bursts (Fig. 4). The representative data shown are compiled from 13 CTr and FT joint cycles from one tethered animal. These data were also consistent with data compiled for six joint cycles taken from another tethered animal. As was the case for treadmill walking, tethered walking involved a consistent distribution of Ds or SETi potentials within a burst (Fig. 4). In this histogram 0% on the *x* axis represents the beginning of the motor neuron burst and 100% represents the end of the burst. The first 15% of the Ds burst includes a high-frequency period (Fig. 4). In contrast, SETi bursts at a high-frequency beginning at about 70% of the burst cycle (Fig. 4). This high-frequency SETi activity begins near the end of stance at a time when Ds activity markedly declines and it continues after the Ds burst terminates (Fig. 2*B*). This pattern is identical to that described for treadmill walking (Watson and Ritzmann 1998a). As cockroaches run faster, the high-frequency SETi activity at the end of stance may be accompanied by FETi motor neuron activity (Watson and Ritzmann 1998b) (Fig. 3*B*). We occasionally recorded FETi in the tethered preparation and found the placement within the SETi burst to be consistent with treadmill data (Fig. 3*B*).

RELATIONSHIP OF T2 Ds AND SETi ACTIVITY TO JOINT KINEMATICS. While the distribution of Ds or SETi potentials within a burst remained largely unchanged whether the animal was walking on a treadmill or tethered, it is possible that the relationship of the potentials relative to the joint cycle is different when the animal walks on the two substrates. Two factors that are clearly different and could be important here are the differences in friction encountered by the tarsi (foot) and the lack of inertia experienced by the tethered animals. For example, the onset of joint movement could occur earlier (or later) and/or the joint velocity could be significantly altered during tethered walking. In that case, the distribution of EMG activity relative to joint cycle may be significantly different during tethered versus treadmill walking.

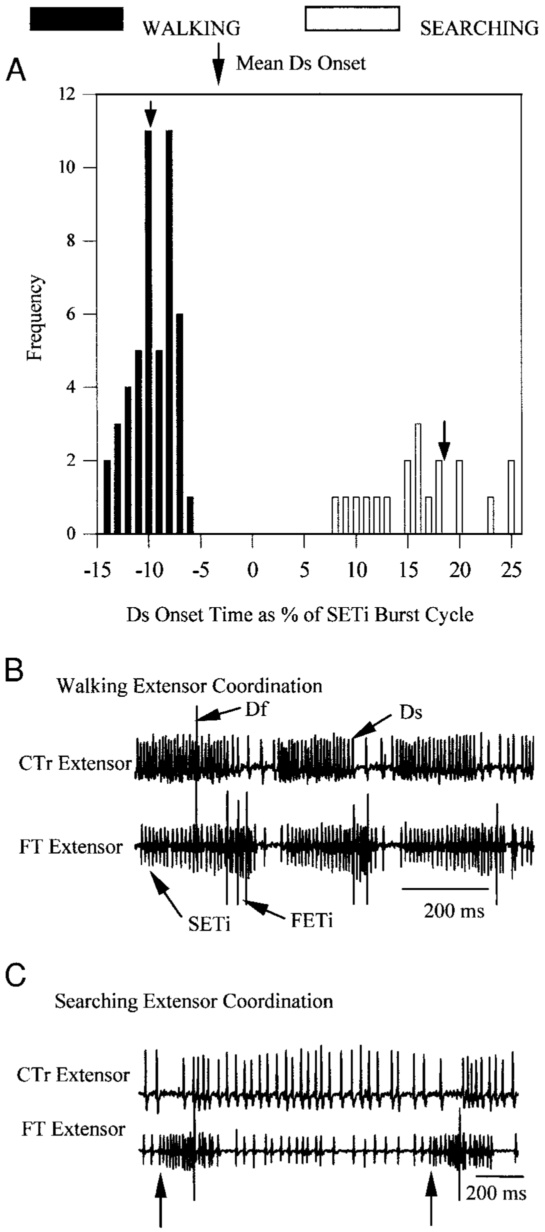

FIG. 3. *A*: histogram showing mesothoracic leg walking and searching slow depressor coxa neuron (Ds) activity onset times as a percent of the slow extensor tibia neuron (SETi) burst cycle duration. Filled bars represent walking data and open bars represent searching data. Burst cycle duration calculated as SETi onset to onset times. Downward arrows indicate mean Ds onset as percentage of SETi burst cycle. *B*: CTr and FT extensor EMG records obtained during tethered walking. EMG potentials from slow depressor motor neuron activity are marked Ds; Df indicates potentials arising from fast depressor motor neuron activity. Slow extensor tibia activity is labeled SETi, and that of fast extensor tibia is marked FETi. *C*: coxa-trochanter depressor and femur-tibia extensor EMG records showing the CTr and FT extensor coordination during tethered searching. Searching records obtained from same animal as in *B*.

To look at the relationship of muscle activity to the joint cycle, we re-plotted the EMG data, now relative to the joint kinematics. Cumulative frequency histograms of Ds or SETi motor neuron potentials within the CTr or FT joint cycle (4.4 Hz) are shown in Fig. 5, *A* and *B*. The representative data shown are compiled from 13 CTr and FT joint cycles from one tethered animal. These data were also consistent with data compiled for six joint cycles taken from another tethered animal. In contrast to the histogram plotted in Fig. 4*A*, the histograms in Fig. 5, *A* and *B*, start and end at peak joint extensions (i.e., extension-flexion transition). As was the case for T2 treadmill walking data (Watson and Ritzmann 1998a), during tethered walking, Ds and SETi markedly increase ac-

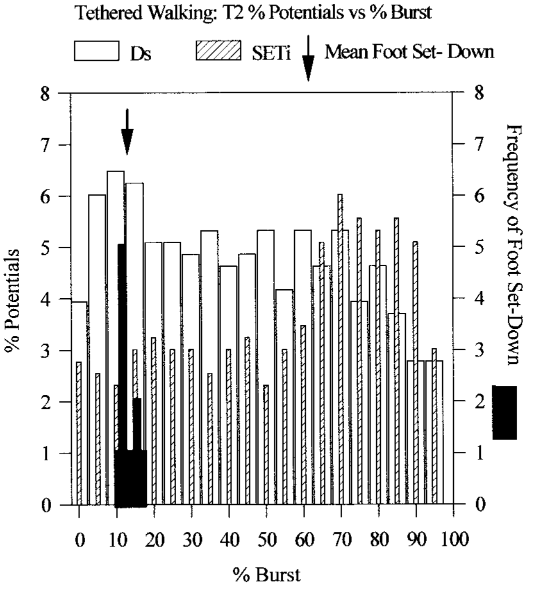

FIG. 4. CTr extensor (Ds) (open bars) and SETi (hatched bars) EMG activity from mesothoracic (T2) joint cycles in which only slow motor neurons were active during tethered walking. Data are normalized for the duration of the burst. Within each burst there is consistent occurrence of high-frequency activity. Filled bars represent frequency of occurrence of foot set down. Downward arrow indicates mean foot set down.

tivity at 35–40% of their joint cycle and activity declines at around 90% of the joint cycle (Fig. 5, *A* and *B*). The mean Ds burst onset began at about 25% of the CTr joint cycle (Fig. 5*A*) while the mean SETi burst onset began around 40% of the FT joint cycle (Fig. 5*B*). The mean CTr extension began at about 40% of the CTr joint cycle (Fig. 5*A*), as was the case during treadmill walking (Watson and Ritzmann 1998a). FT extension occurred around 47% of the FT joint cycle (Fig. 5*B*). The timing of tethered walking T2 leg FT extension onset resembled that of the T3 leg FT extension during treadmill walking onset as both began at around 48% of the FT joint cycle (Figure 5*B*). Watson and Ritzmann (1998a) did not report whether there are significant differences in the FT extension onset times for the two legs during treadmill walking.

Because motor neuron frequency markedly influences the rate of muscle contraction it should also be related to joint angular velocity. Indeed, for horizontal treadmill walking Watson and Ritzmann (1998a) found a direct relationship between mean instantaneous frequency and mean joint velocity. For tethered walking, we also found that the mean depressor or extensor motor neuron firing rate was correlated to the mean CTr or FT joint extension velocity ($r = 0.81$, $P < 0.0001$ and $r = 0.86$, $P = 0.003$, respectively; Fig. 6, *A* and *B*). Tethered CTr data shown include 20 CTr joint extensions from three animals, while FT data represent 16 joint extensions from two animals. However, the slope of the regression function was considerably lower. For tethered walking, a given mean instantaneous extensor motor neuron frequency consistently resulted in a lower mean CTr or FT joint velocity than during treadmill walking (Fig. 6, *A* and *B*). One possible explanation for this discrepancy is that during tethered walking there might be a decrease in burst duration and at a given mean EMG frequency, the muscle is not active long enough to produce similar joint velocities as occurs when the animal walks on a treadmill. We tested this hypothesis by plotting mean Ds burst duration versus mean step period; the burst duration at a given walking rate was not shorter during tethered walking compared with treadmill walking (data not shown). We will speculate further on possible reasons for this difference in slope in the DISCUSSION.

Tethered searching

SWITCHING FROM TRIPOD WALKING TO SEARCHING. Having demonstrated the behavioral relevance of tethered walking, we can now examine a context dependent change in behavior; i.e., the switch from walking to searching. In all cases examined,

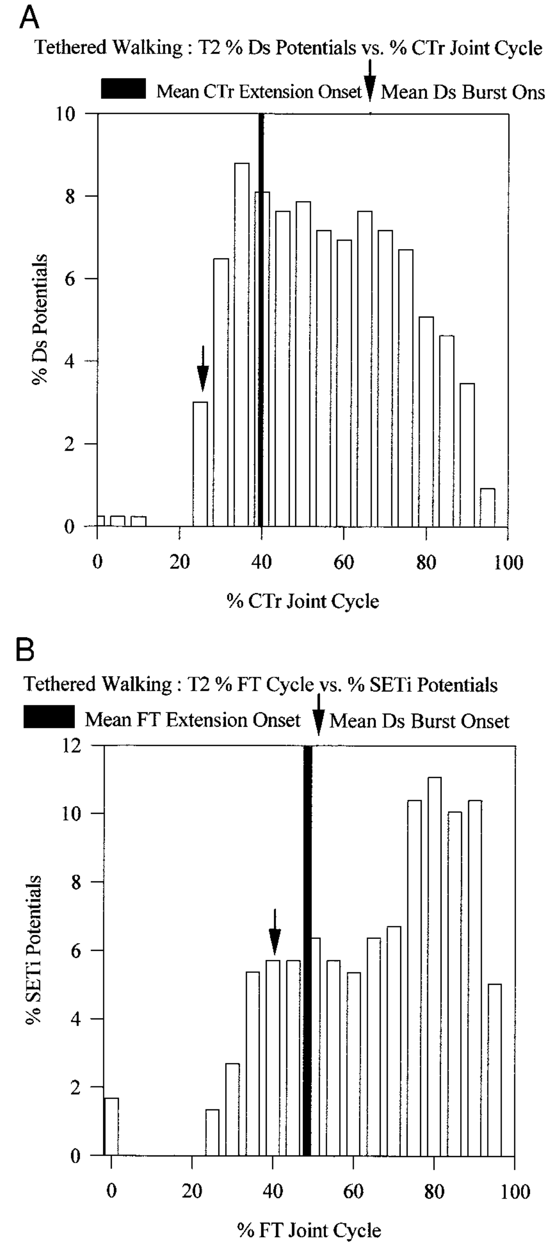

FIG. 5. *A*: CTr slow depressor (Ds) activity from mesothoracic (T2) joint cycles during tethered walking (open bars). The data are normalized to the CTr joint cycle where joint cycles begin and end at maximum extension. Filled vertical bar indicates mean CTr extension onset; downward arrow indicates the mean Ds burst onset. *B*: slow extensor tibia SETi activity normalized to the T2 FT joint walking cycle (□). Downward arrow, mean burst onset. Solid vertical bar, mean FT joint extension onset.

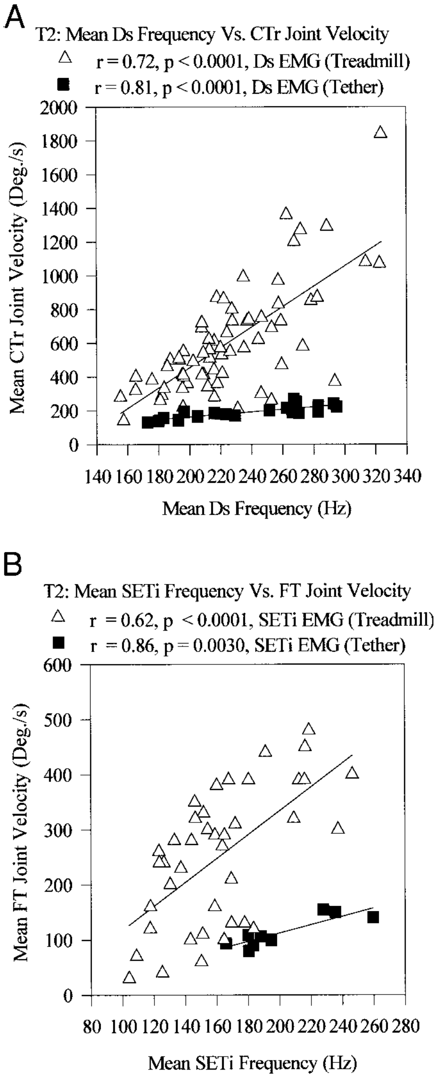

FIG. 6. *A*: mean joint extension velocity is linearly related to mean instantaneous slow motor neuron activity at each joint. Mean Ds activity vs. mean CTr extension velocity during tethered walking (filled squares) and treadmill walking (open triangles). *B*: average SETi activity vs. mean FT extension velocity during tethered walking (filled squares) and treadmill walking (open triangles).

the tethered position allowed the cockroach to make tarsal contact with the substrate for all three legs during the stance phase. The distance of the anterior end of the animal relative to the substrate could be increased by either raising the glass rod attached to a manipulator, which resulted in pitching the tethered animals' anterior upward or by dropping the glass plate on which the animal walked. In either case, this change faithfully resulted in a change in leg movements from walking to searching. That is, the animal ceased running in a tripod gait and the T1 and T2 legs engaged in searching. The T3 legs stopped cycling and remained extended. The mean CTr joint angle measured when T3 legs stopped cycling was 103.31% of the maximum CTr joint angle measured at the end of stance phase during walking (*n* = 3 animals; 19 trials). These data are indicative of the extended position of the T3 legs while the animal performed searching movements with its T1 and T2 legs. Lowering the tether or raising the substrate usually resulted in all six legs once again engaging in a tripod gait. This switch was very reproducible. Mesothoracic (T2) leg data from five animals revealed that in 100% of the trials, including 10 consecutive trials from each animal, the animals switched from walking to searching on lowering the substrate. In 86% of the trials, the animals reestablished a tripod gait following the return of the substrate to the horizontal position.

For both cats (Pearson et al. 1992; Whelan et al. 1995) and insects (Cruse 1976; Cruse and Saxler 1980), the switch from stance to swing phase during walking requires that the leg is extended and unloaded at the end of stance (see also, Pearson 1976). Therefore we examined if after the animal switched from walking to searching, the extended T3 leg would swing forward when the substrate is moved away from the animals' body. To test this, we rolled the pitched glass plate beneath the cockroach. That movement resulted in one side of the animal being closer and one side farther from the substrate. A similar effect was accomplished by placing the animal's right and left legs on two separate glass microscope slides, allowing the substrate for a T3 leg on either side to be lowered independently. When the substrate on one side was lowered (by either method), the T3 leg on the side where the animal was farther from the substrate engaged in searching, whereas the opposite T3 leg remained extended in stance (*n* = 3 animals; *n* = 12 trials).

It is possible that on pitching the glass plate down, afferent feedback from joint-angle receptors provide some of the sensory cues for the animal to switch from walking to searching. If this is the case, there should be a consistent relationship between the pitch of the glass plate and switching from walking to searching. For the T2 leg, we determined the distance that the edge of the glass plate (closest to the anterior of the animal) needed to be lowered for four animals to switch from tripod walking to searching. Each of the animals was tethered at an equivalent distance from the edge of the glass plate. For 84 trials, the mean distance was 6.61 ± 0.74 (SD) mm. While the absolute values are unique to the experimental set-up, the data indicate there was a fairly narrow range of pitch angles that induced behavioral switching despite not controlling for variables such as walking rate and rate of pitching the plate down.

In stick insects, it has been shown that searching movements can be interrupted when the leg encounters an obstacle (Bassler et al. 1991). Therefore we anticipated that if a leg performing searching movements in our preparation encountered a foothold, it would also stop searching. To test this, we introduced a 2-mm-diameter hand-held wooden dowel into the path of the tarsus while the leg searched. When the tarsus grasped the wooden dowel, the animal grasped the stick with the tarsus and stopped searching with that leg while searching persisted in other legs. Pulling the dowel away, until the animal no longer grasped the stick, resulted in the leg re-engaging in searching (*n* = 2 animals, 26 trials).

T2 LEG CTr AND FT JOINT KINEMATICS DURING SEARCHING. Unlike walking, searching may involve simultaneous extension of segmental pairs of legs (searching: Franklin and Pearson 1984; walking: Pearson 1976; Pearson and Fourtner 1975). As was the case for locust searching (Franklin and Pearson 1984), we occasionally observed nearly synchronous extension of pairs of either T1 or T2 legs during the aerial phase of a searching episode (*n* = 5 animals, 15 observations). We did not quantify the frequency of occurrence that both legs of a thoracic segment extended during the aerial phase of searching as it was not necessary for the conclusions drawn based on these data. In tethered animals, this frequency may increase due to the fact that the requirements for the legs to support the animal above the substrate are reduced compared with freely behaving animals. Nonetheless, these data suggest that left and right legs of the same segment are not as tightly coupled (in anti-phase) during searching as they are during walking.

An even more obvious difference between walking and searching is that during searching, CTr and FT extension occurs while the leg is in the aerial phase whereas during walking, extension at these joints occurs during stance. Extension during protraction allows the animal to extend the limb away from the body and toward the substrate. If the tarsus makes contact with the substrate, the limb is drawn toward the abdomen (Fig. 7*A*). The flexed leg is lifted off the substrate, protracted, extended, and then placed back down on the substrate. The searching behavior is repeated several times (Fig. 7*A*). The extension during the aerial phase allows the animals to search for a foothold over a wider area and reach higher footholds than would otherwise be possible (see also, Franklin and Pearson 1984). A closer examination of the CTr and FT joint kinematics revealed several parameters that were different from those measured for tethered walking (Table 2). The tethered walking kinematic data included 17 steps from three animals, and the tethered searching movements represent 16 steps from three animals; none of these animals was wired for EMGs (Table 2). We compared data collected only at similar mean joint cycle rates so the number of samples available for comparison was fairly limited. Nonetheless even with limited comparisons, it is clear that during tethered searching, CTr and FT joint movements are very different from tethered walking [compare kinematics from the same animal in Fig. 2*A* (walking) and Fig. 7*A* (searching)]. When we compared T2 CTr and FT extension duration, joint angle excursions, and maximum and minimum joint angles during walking with those during searching, all parameters differed significantly except the CTr maximum joint angle data (Table 2). The similarity in maximum joint angle may reflect anatomical extremes.

Among the differences between searching and walking joint kinematics, the relative timing of the CTr and FT extension and the excursions of the CTr and FT joint movements are particularly striking. The mean onset of FT joint extension during searching is phase advanced relative to that of the CTr by 24.5 ± 10.7 (SD) ms (see also Table 2). This is in contrast to walking where CTr extension precedes FT extension (Watson and Ritzmann 1998b). There is also a marked increase in the FT joint excursion and decrease in CTr excursion during searching versus walking (compare Fig. 2*A* with Fig. 7*A*; Table 2).

T2 DS AND SETi EMGS. Consistent with the advance of the FT joint movement relative to CTr movement, during the extension phase of searching versus walking, SETi activity onset precedes Ds during searching (Fig. 3*C*). The mean onset time for Ds during searching occurs at 15.76% ($n = 4$ animals, 20 cycles) of the SETi cycle, that is, after SETi activity onset (Fig. 3*A*). We also found systematic changes in instantaneous motor neuron frequency in searching relative to walking at similar joint cycle rates (CTr joint cycle period, $P = 0.081$; FT joint cycle period, $P = 0.186$). The mean and peak instantaneous SETi frequency was always higher during searching versus walking (Fig. 8, *A* and *B*). In contrast, the mean and peak Ds frequency was lower during searching than during walking (Fig. 8, *A*–*D*; $P < 0.0001$ for both mean and peak SETi and Ds values). The data shown (Fig. 8, *A*–*D*) include five SETi cycles and four Ds cycles from one animal during searching and walking. The same relation was also found in another animal for seven searching and five walking SETi cycles and six walking and six searching Ds cycles (data not shown). In contrast to tethered walking the mean depressor or extensor

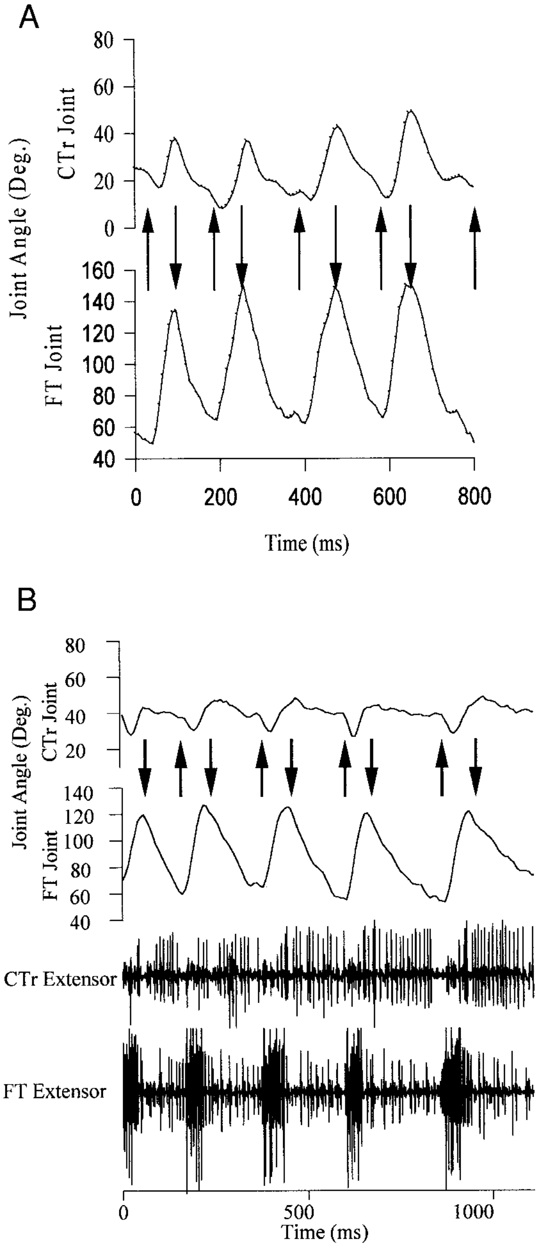

FIG. 7. *A*: mesothoracic leg CTr and FT leg joint kinematic records during tethered searching from an animal not wired for EMGs. Compare searching data in Fig. 7*A* with walking data from the same animal in Fig. 2*A*. *B*: mesothoracic leg CTr and FT leg joint motion records synchronized with CTr and FT extensor EMG records while the leg is searching for a foothold. Compare searching data in Fig. 7*B* with walking data from the same animal in Fig. 2*B*. Arrows as in Fig. 2.

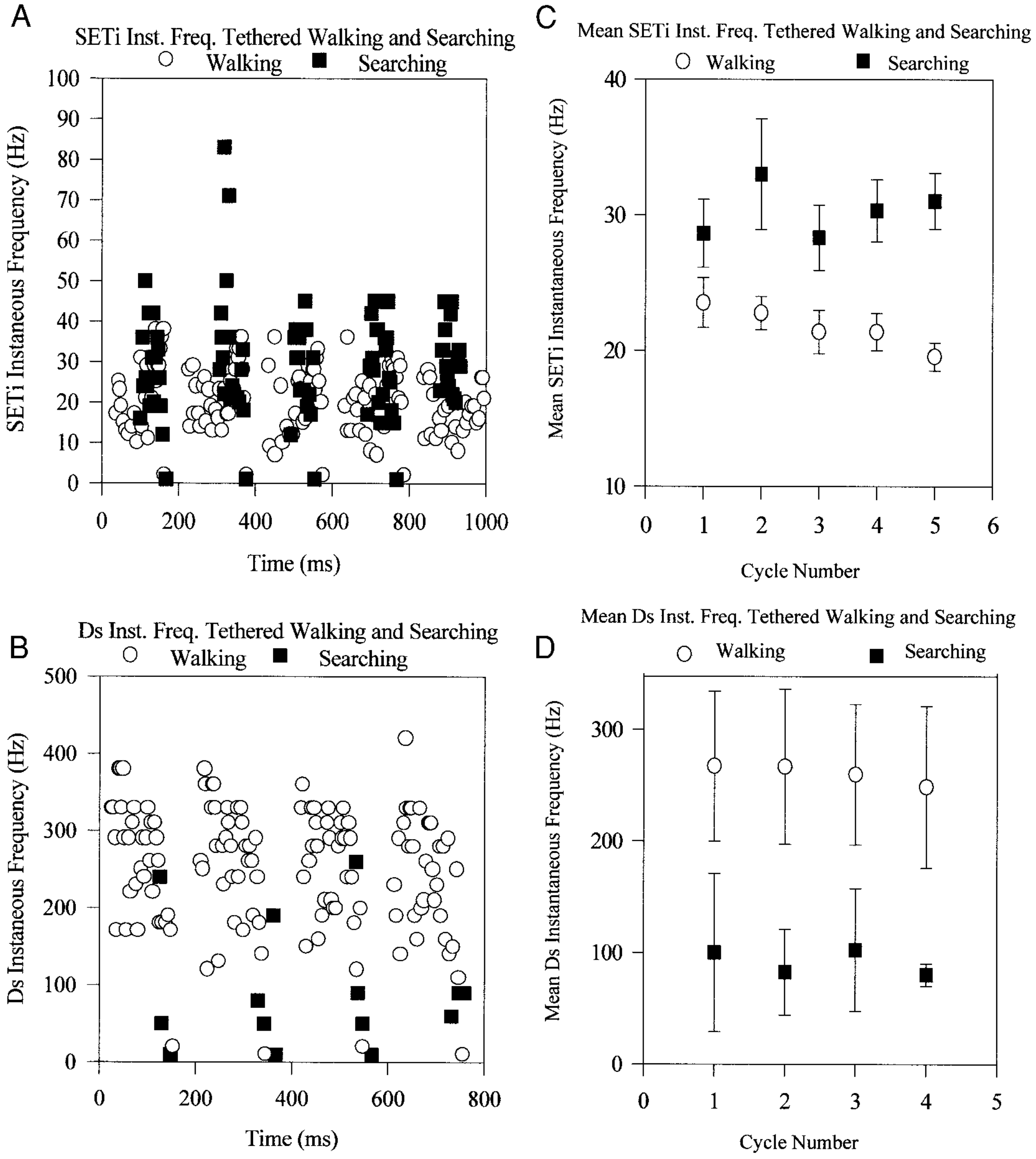

FIG. 8. *A*: peak and mean instantaneous frequency of T2 leg SETi activity are greater during searching than during walking at a similar cycle rate. *B*: Ds mean and peak instantaneous frequency are lower during searching than during walking at a similar joint cycle rate. Open circles, walking data. Filled boxes, searching data. *C*: to illustrate that the mean instantaneous frequency during each cycle of searching vs. walking do not overlap, we plotted the mean SETi instantaneous frequency and SE (bars) for each of the walking and searching cycles shown in *A*. *D*: the mean Ds instantaneous frequency and SE (bars) for each of the walking and searching cycles shown in Fig. 8*B*, illustrating that the mean instantaneous frequency for each walking or searching cycle does not overlap.

motor neuron firing rate was not directly proportional to the mean CTr or FT joint extension velocity (data not shown).

Comparison between T2 searching and T1 walking

Although the joint kinematics of the T2 legs go through considerable changes in switching from walking to searching, T2 searching has some features in common with the walking movements of the front legs. In insects, each segmental pair of legs plays a unique role during walking (Full et al. 1991). In cockroaches, hind (T3) and middle (T2) legs provide propulsive forces while front (T1) legs provide braking forces, investigate footholds, and contribute to changes in body orientation. If the movements of T1 legs during walking are similar to T2 searching movements, it is possible that during searching the T2 legs move away from their typical walking behavior and closer to the walking movements of the T1 legs. Indeed, in walking crickets, the FT joint of the T1 leg extends prior to the CTr joint during swing phase, whereas the T2 leg CTr extends prior to the FT joint during stance (Laurent and Richard 1986). The T1 legs of crickets during walking thus show similarity to the T2 legs of the cockroaches during searching.

Unfortunately, the treadmill data that we used to compare to our tethered data did not include the front legs. Thus we could not compare T2 searching data to cockroach T1 walking data without making our own observations of T1 legs during treadmill walking (Fig. 9, *A* and *B*). A complete analysis of walking and searching in the T1 legs was beyond the scope of this study. However, we were able to obtain sufficient kinematic data to confirm the similarity to cricket front leg data and to make a qualitative comparison to our T2 searching data. Recording EMGs from T1 legs during free walking presented serious technical challenges. Because of the wide range of motion of the front legs, coupled with the proximity to the head, the animal can readily tangle wires with the other legs or bite through them, thereby breaking the wires. Thus we were only able to obtain clear EMG data for two animals (of more than 50 trials). Again, these data were sufficient to make some qualitative comparisons.

T1 WALKING KINEMATICS. As in the cricket, the cockroach protracts the T1 leg during swing phase. Reaching forward during swing involves a reduction of the body-coxa (BC) joint angle (points A-B in Fig. 9*A*; $n = 12$ steps, $n = 2$ animals). To accomplish foot set-down, the FT joint is extended at midswing phase followed by extension of the CTr joint that depresses the leg onto the substrate (point B in Fig. 9*A*). These actions are in contrast to those of the T2 and T3 legs during walking where both FT and CTr joints extend during stance with CTr joint extension preceding that of the FT joint (Watson and Ritzmann 1998a). Subsequent to foot set-down (point B Fig. 9*A*), the CTr joint flexes and then extends again, while the FT joint either flexes throughout stance (Fig. 9*A*) or is held at a relatively constant angle after a brief flexion (Fig. 9*B*). During the stance phase, the coxa is rotated posteriorly at the body-coxa (BC) joint (Fig. 9*A*). The second extension of the CTr joint during stance coupled with the rotation of the BC joint thrusts the T1 leg away from the anterior of the body (Fig. 9*A*).

We observed more variability in the T1 coordination than was found for T2 and T3 legs. However, the variants appeared to fall into two basic walking strategies. These strategies differed in joint kinematics after foot set-down. One strategy simply involved further extension of the CTr joint. We refer to this simpler movement as *strategy 1* (Fig. 9*B*). The other movements, which we refer to as *strategy 2,* involved the biphasic extension of the CTr joint during stance that is described in the previous paragraph. (Fig. 9*A*; $n = 9$ steps, $n = 2$ animals).

T1 TREADMILL WALKING EMGs AND KINEMATICS. The two animals from which we successfully recorded EMGs during treadmill walking used *strategy 1* kinematics (Fig. 9*B*; $n = 10$ steps). The use of *strategy 1* kinematics by these animals was not a consequence of wiring the animals for EMGs as *strategy 1* kinematics were also observed during tethered walking from an animal not wired for EMGs (data not shown). The motor activity was consistent with the unique kinematics of T1 legs and is in contrast to T2 and T3 legs during walking. That is, the SETi burst onset occurred about 40 ms prior to that of Ds (Fig. 9*B*). Also, the SETi and Ds activity began prior to foot touch down during the swing phase (Fig. 9*B*). During protraction of the T1 leg, where the animal brought the leg forward, SETi bursts at an initial high frequency and was occasionally coupled with recruitment of the fast extensor tibia motor neuron (FETi; Fig. 9*B*). After the FT joint extended, SETi activity declined and Ds activity began. The early high-frequency SETi was followed by much lower frequency secondary activity

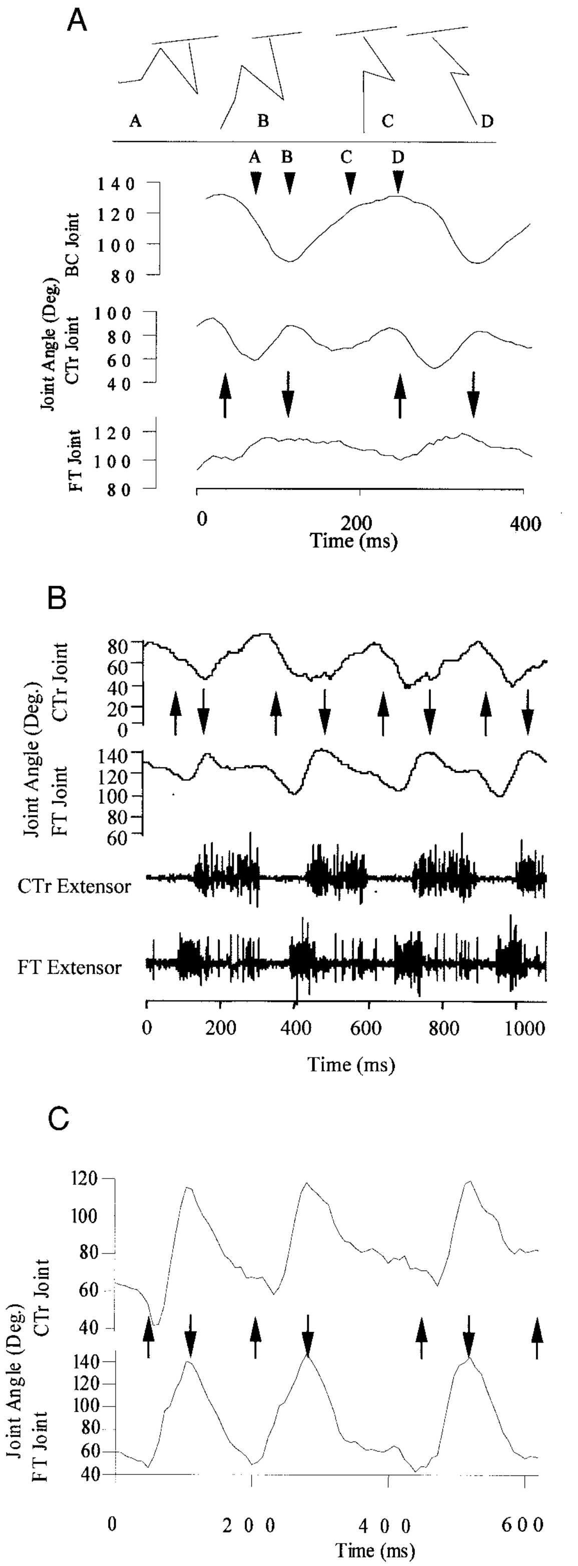

FIG. 9. *A*: CTr and FT joint-angle data from prothoracic leg kinematics during treadmill walking. Joint kinematic record as in Fig. 2*A*, but includes the BC joint position (see also Fig. 1). Stick figures (*top*) show select prothoracic leg joint positions during the step cycle. A–D and arrowheads mark where on the kinematic record the stick figure data were taken from during the step cycle (arrows as in Fig. 2). *B*: prothoracic leg CTr and FT leg joint motion records synchronized with CTr and FT extensor EMG records during treadmill walking at about 4.4 Hz (arrows as in Fig. 2*B*). *C*: prothoracic leg CTr and FT leg joint kinematic records during tethered searching for a foothold (arrows as in Fig. 2).

after foot set-down and resulted in SETi having a distinct biphasic pattern of activity (Fig. 9*B*).

Both the joint kinematics and EMG activity of the T1 leg are consistent with data reported from front legs of other insects (Burns and Usherwood 1979). Moreover, aspects of them are qualitatively similar to the data we report on the T2 leg searching behavior. In particular, the extension of the FT joint prior to that of the CTr during swing and the fact that SETi fires a high-frequency burst prior to the onset of Ds activity are reminiscent of T2 searching. However, although extension of the T1 CTr joint during walking began during leg protraction (Fig. 9, *A* and *B*), there was also extension during stance (Fig. 9, *A* and *B*). Thus the CTr joint movement is more complex and variable during T1 walking than T2 searching, but T2 searching is more similar to T1 walking than it is to T2 walking (Fig. 9*A*).

T1 SEARCHING KINEMATICS. We also briefly looked at joint kinematics during T1 searching movements (Fig. 9*C*; $n = 2$ animals, $n = 10$ cycles). There were some differences in the coordination of T1 CTr and FT joint kinematics during searching versus walking. Searching involves larger joint excursions than walking (Fig. 9*C*). This finding is not unexpected as we induced searching movements by raising the tether, pitching the animals' anterior end away from the substrate. In contrast to T1 walking, searching involved CTr extension primarily during leg protraction (Fig. 9*C*). Thus T1 searching kinematics (Fig. 9*C*) resemble those of T2 searching (Fig. 7*A*) as both involve extension of the FT joint prior to the CTr joint and both joints extend during leg protraction.

DISCUSSION

A variety of experiments using deafferented cockroaches suggested that coordination of muscles acting on a joint can be centrally patterned (Pearson 1972; Pearson and Iles 1970). However, it is unclear whether the fictive coordination produced by these restrained preparations is the same as that underlying actual walking, struggling or rocking behaviors (Reingold and Camhi 1977). Further, it is known that feedback from leg sensory structures is important in coordinating leg joint movements in a normal way during walking (cat: Duysens and Pearson 1980; Pearson et al. 1992; stick insect: Bassler 1983; locust: Burns 1979; cockroach: Delcomyn and Usherwood 1973; Krauthamer and Fourtner 1978; Spirito and Mushrush 1979) and searching for a foothold (stick insect: Karg et al. 1991).

It is clear from these studies that understanding the neural basis of behaviors such as walking and searching requires clearly defining the behavior examined and studying the behavior under circumstances where essentially normal movements and sensory feedback are produced. To define the behaviors examined, we characterized the relationship between motor neuron activity and kinematics of some of the joints involved. To ensure that the behaviors studied involved essentially normal movements, we compared the behavior of our tethered preparation to similar data sets from freely walking animals on a treadmill. We found that many of the joint kinematic and motor parameters measured during tethered walking are similar to those during treadmill walking. Our subsequent analysis focused on those aspects of the tethered movements that are similar to treadmill walking. Where differences in the kinematics and neural control were observed, we will speculate on the possible role of sensory feedback in causing these differences.

Our data further showed that T2 CTr and FT joint kinematics during searching were markedly different from those observed for walking. Preliminary observations suggest that there are also some differences of CTr and FT joint coordination in the T1 legs during walking versus searching. While the T1 and T2 searching joint kinematics were similar, the T1 walking joint movements also shared some similarities with T2 searching during initial leg protraction. Our data suggest several possibilities for neural control of two leg joints during two different behaviors. For example, compared with walking, there is a delay in onset of T2 CTr extension during searching. This delay may be the result of direct inhibition of Ds, reduced excitation, delayed excitation or, more likely, a combination of these mechanisms. We begin to address these hypotheses in the companion paper that follows (Tryba and Ritzmann 2000).

Comparison of tethered and treadmill T2 walking

While many of the T2 joint kinematics and EMG parameters for tethered and treadmill walking were similar, there were some differences. For example, although we found a linear relationship between mean EMG activity and mean joint velocity, the slope of the line was lower for tethered data than had been previously shown for treadmill walking.

There are several possible explanations for this difference. First, it is possible that the insertion of EMG wires into the legs somehow influenced the rate of leg extension during tethered walking. This was not the case in treadmill walking but could be when the animal is tethered. While we did not test this hypothesis directly, FT joint angular velocity during extension was actually lower in tethered animals that are not wired for EMGs than in animals during treadmill walking (Table 1). These data suggest that electrode implantation alone cannot account for the decline in joint velocity during tethered walking. Second, the tethered animals are not experiencing the normal whole body inertia of a freely moving animal, and that could account for some of the decrease in joint velocity relative to EMG frequency. Third, the decline in mean joint velocity may also result from the relative degree of slippage or drag that the foot experiences during tethered walking. For example, it may be that with application of different amounts of oils to the glass plate, or using oils of different viscosity, the tethered joint velocity during walking would more closely resemble that during treadmill walking. One would expect a lower joint velocity if the resistance to foot movement on the glass plate is higher than during walking on a treadmill.

There is some evidence to suggest that the tethered animals experience an increase in resistance to foot movement during stance, and this results in a decline in extension velocity at a given muscle activation rate. Compared with freely walking cockroaches, the relationship between Ds frequency and step period is lower for animals that have an increase in resistance to extension when they drag a weight (Pearson 1972). Along these lines, animals that were climbing over obstacles had a lower slope in this relationship than animals walking on a treadmill (Watson et al. 1998). In that case, the forward inertia of climbing animals would presumably be less and extension resistance would increase as the animal climbed against gravity. Additionally, during walking in locust, there is a decrease in swing duration when extension resistance is increased (Newland and Emptage 1996). Thus if there is an increase in resistance to extension during tethered cockroach walking, one would a expect a decline in flexion duration at a given step period for both the CTr and FT joints, and this was shown to be the case (Table 1).

We also noted that the mean extension velocity during tethered walking (at a particular rate) shown in Table 1 was higher than would be expected to be achieved by increasing slow motor neuron activity alone (i.e., extrapolation of regression lines in Fig. 6, *A* and *B*). Although we did not record EMGs during tethered walking from animals represented in Table 1, we suspect these animals achieved higher extension velocities and faster walking rates (than animals in Fig. 6, *A* and *B*) via the recruitment of fast motor neurons.

Tethered T2 leg and freely behaving searching

The tethered preparation provided several technical advantages to studying searching. While freely behaving cockroaches produce leg searching movements following loss of a foothold as they walk over uneven terrain (Franklin and Pearson 1984), it would be difficult to define leg searching kinematics and motor coordination under these circumstances. True joint-angle calculations in three dimensions require a lateral and ventral view. Obtaining an appropriate ventral view would not be tractable under conditions involving terrain complex enough to reliably evoke searching leg movements (see Franklin and Pearson 1984). It would also be problematic in freely moving animals to collect enough data at similar joint cycle rates to make comparisons between data sets. Ideally, the true joint angles should be measured so there can be consistency among different investigators studying the same behaviors.

There is qualitative data from locusts to support the notion that the tethered searching described here shares several similarities with searching movements of freely behaving insects (Franklin and Pearson 1984). These similarities include the following: the searching leg undergoes rapid elevation and depression, there is marked extension at distal joints during the aerial phase, the searching pattern continues for several cycles, the behavior is terminated when the animal's legs stopped cycling, and the behavior ceases if the leg engaged in searching encounters an object and finds a foothold.

Tethered searching and T2 motor neuron activity

Our data are also consistent with and provide further support for the hypothesis proposed by Delcomyn (1987) that searching and walking are distinct behaviors. Delcomyn (1987) examined searching Ds activity of cockroaches tethered above a glass plate. He found that for T2/T3 legs there was a decrease in Ds frequency and inter-burst interval during searching compared with walking. We also found that there was a lower frequency of Ds activity and a higher SETi frequency during searching. These findings may in part account for the smaller CTr and larger FT joint excursions during searching versus walking. Further, we extended the initial observations of Delcomyn (1987) because we included the neural control and kinematics of the T2 CTr and FT joints and we quantitatively compared many aspects of searching joint kinematics to those observed for walking.

We can now also define the searching behavior by extension of the FT and CTr joints during leg protraction. In all cases of searching examined, the onset of FT extension preceded that of the CTr joint. Further, the onset of the extension cycle of searching included a characteristic high-frequency SETi activity that was accompanied by little or no Ds activity (Fig. 7*B*). Thus searching included characteristic patterns of motor activity and the consequent joint kinematics. As both the T2 CTr and FT joint kinematics and motor activity of searching are markedly different from walking, either of these parameters can be used to identify ongoing searching (Figs. 2, 3, and 7). In contrast to our data, Delcomyn (1987) actually recorded two different patterns of Ds motor activity, and it is not clear whether both result from variability in searching or are different behaviors. Whether one pattern or the other was observed depended on whether some or none of the legs were supported by substrate (Delcomyn 1987). There is evidence that different behaviors may be expressed depending on degree of substrate contact. For example, it is known that the flight motor activity is evoked following substrate removal and loss of tarsal contact (Kramer and Markl 1978). To control for this possibility, searching in this study was always examined under conditions where the tarsi of all legs could contact the substrate. Furthermore, characterization of multiple joints ensured that we were examining the same behavior in each preparation.

T1 walking and searching

The CTr and FT joint movements vary among the three sets of legs. T1 legs showed distinct differences from T2 legs during walking while T1 leg data included fewer differences between searching and walking than T2 legs did. For example, the onset of FT extension that is followed by CTr extension during initial protraction of the leg is not markedly different from T1 walking. Thus there may be similar neural architecture underlying the coordination of the principal leg extensors during onset of T1 searching and walking (Fig. 9, *A*–*C*). During T2 searching (that bears kinematic resemblance to T1 searching) and T1 walking, the onset of the extension cycle included high-frequency SETi activity at a time when there was cessation of Ds activity (Figs. 7*B* and 9*B*). There does appear to be some consistency in the searching behavior of T1 and T2 legs. Both T1 and T2 searching involves extension of the FT joint prior to CTr extension and continued extension throughout protraction (Figs. 9*C* and 7*B*). This observation suggests there may be similar Ds and SETi motor neuron activity during these behaviors. Since activity of Ds and SETi and the resultant CTr and FT joint movements are clearly different for T1 versus T2 and T3 legs during walking (T1: Fig. 9*B*; T2: Fig. 2*B*; T3: Watson and Ritzmann 1998a), it appears that walking involves different neural mechanisms coordinating the FT and CTr joints in the T1 legs than in T2 (or T3) legs. The unique coordination of the CTr and FT joints for each of the legs during walking may reflect the roles these legs play during walking. For example, the fact that the T1 legs extend the principal leg joints (primarily the FT joint) during the protraction phase of walking allows the animal to use the front legs to investigate a larger area ahead of it for footholds than if T2 or T3 walking kinematics were used.

Coordination of CTr and FT joints during walking and searching

Several reports have suggested that movement at multiple joints in a single leg is generated by the activity of separate joint oscillators (Bassler 1993; Grillner 1981; Nothof and Bassler 1990). The appropriate coordination of the joint oscillators to produce walking or searching movements is thought to be orchestrated by sensory feedback (Bassler 1993; Bassler et al. 1991; Braunig and Hustert 1983; Hess and Buschges 1999; Pearson et al. 1976; Zill et al. 1981). Our data suggest that distinct mechanisms couple the movements of the CTr and FT joints during the onset of the extension cycle when the animal searches versus when it walks. The fact that there is more variability in Ds onset times during searching suggests that the CTr and FT joint coordination during walking is more tightly coupled (Fig. 3*A*), and this result would not necessarily be expected if the same mechanisms were responsible.

Different mechanisms are also necessary to account for the coordination of multiple legs during searching and tripod walking. For example, walking does not involve simultaneous extension of any segmental pair of legs (Pearson 1976). However, the in-phase extension of segmental leg pairs can be observed for T1-T2 legs during tethered searching (see also Franklin and Pearson 1984). The 0.5 phase relationship between legs on opposite sides of the same segment during walking has suggested the possibility that reciprocal inhibition of the CTr joint oscillators coordinates the phase relationship between contralateral leg pairs (Pearson 1976; Pearson and Fourtner 1975). This coordination does not appear to be the case during searching.

Role of afferent information in the behavior of legs during searching and walking

Leg loading and joint angle information may play a role in modulation of CTr and FT joint coordination (see also, Bassler 1993; Bassler et al. 1991). Several lines of evidence suggest that some of the differences in T1–T3 Ds and SETi coordination and joint kinematics may be due in part to differences in loading. For example, in both insects and cats, transition from stance to swing phase requires unloading of extensors and leg extension (Cruse 1976; Cruse and Saxler 1980; Pearson 1976; Whelan et al. 1995). In our study, induction of tethered T1 and T2 leg searching resulted in the T3 legs extending and remaining in stance phase until the tilted substrate was rolled. Presumably rolling the pitched plate unloaded T3 legs allowed them to engaged in searching. As is the case for walking, a reduction in load supported by a leg probably permits the transition from stance to swing during searching. We suspect that unloading the legs alone allows leg protraction to occur but did not induce the searching motor pattern. Instead, unloading the legs at a particular joint angle may permit expression of the searching motor pattern. The fact that induction of tethered searching occurs over a relatively small range of glass plate angles, regardless of walking rate or rate of pitching the glass plate, suggests that there may be specific joint angle requirements met under these circumstances for induction of searching.

Conclusions

Our data show that tethered walking T1 and T2 leg CTr and FT joint coordination closely resembles that during treadmill walking. This conclusion is based both on joint kinematic data and principal leg extensor motor neuron activity. Additionally, the kinematics of walking were distinct from searching. Accordingly, we found a very different CTr and FT extensor EMG pattern during T2 searching versus walking.

In the companion paper, we take advantage of our tethered preparation to investigate differences in control of the T2 CTr and FT joints during walking and searching. In addition to the properties examined here, we are also able to record intracellularly from the thoracic ganglia during either behavior. We use this technique to examine the hypothesis that inhibition patterns extensor motor neuron activity during walking as was suggested for stick insects (Buschges 1998; Godden and Graham 1984) and Ds during searching (this study).

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