Activity Patterns and Timing of Muscle Activity in the Forward Walking and Backward Walking Stick Insect *Carausius morosus*

Philipp Rosenbaum,* Anne Wosnitza,* Ansgar Büschges, and Matthias Gruhn

Department of Animal Physiology, Zoological Institute, University of Cologne, Cologne, Germany

Submitted 19 April 2010; accepted in final form 23 July 2010

Rosenbaum P, Wosnitza A, Büschges A, Gruhn M. Activity patterns and timing of muscle activity in the forward walking and backward walking stick insect *Carausius morosus*. J Neurophysiol 104: 1681–1695, 2010. First published July 28, 2010; doi:10.1152/jn.00362.2010. Understanding how animals control locomotion in different behaviors requires understanding both the kinematics of leg movements and the neural activity underlying these movements. Stick insect leg kinematics differ in forward and backward walking. Describing leg muscle activity in these behaviors is a first step toward understanding the neuronal basis for these differences. We report here the phasing of EMG activities and latencies of first spikes relative to precise electrical measurements of middle leg tarsus touchdown and liftoff of three pairs (protractor/retractor coxae, levator/depressor trochanteris, extensor/flexor tibiae) of stick insect middle leg antagonistic muscles that play central roles in generating leg movements during forward and backward straight walking. Forward walking stance phase muscle (depressor, flexor, and retractor) activities were tightly coupled to touchdown, beginning on average 93 ms prior to and 29 and 35 ms after touchdown, respectively. Forward walking swing phase muscle (levator, extensor, and protractor) activities were less tightly coupled to liftoff, beginning on average 100, 67, and 37 ms before liftoff, respectively. In backward walking the protractor/retractor muscles reversed their phasing compared with forward walking, with the retractor being active during swing and the protractor during stance. Comparison of intact animal and reduced two- and one-middle-leg preparations during forward straight walking showed only small alterations in overall EMG activity but changes in first spike latencies in most muscles. Changing body height, most likely due to changes in leg joint loading, altered the intensity, but not the timing, of depressor muscle activity.

I N T R O D U C T I O N

Freely behaving animals often display much more complex locomotor outputs than those observed in reduced preparations because of the needs to respond to environmental contingencies and to produce goal-directed movements. Despite considerable work devoted to understanding how this behavioral plasticity arises (humans: Lamb and Yang 2000; van Deursen et al. 1998; salamander: Ashley-Ross and Lauder 1997; fish: Orger et al. 2008; lamprey: Islam et al. 2006; fruit fly: Frye and Dickinson 2004a,b; cockroach: Watson et al. 2002a,b; stick insect: Dürr and Ebeling 2005; Gruhn et al. 2009a,b), we are still only beginning to understand the underlying mechanisms on the neuronal level (Akay et al. 2007; Pick and Strauss 2005; Ridgel and Ritzmann 2005; Ridgel et al. 2007; Schaefer and Ritzmann 2001).

For the stick insect *Carausius morosus* substantial knowledge exists about leg kinematics during adaptive locomotor behaviors such as different walking directions, turning, and gap climbing (Blaesing and Cruse 2004; Cruse 1976a; Cruse et al. 2009; Dürr and Ebeling 2005; Gruhn et al. 2009b; Jander 1985). Substantial information also exists about the central neural mechanisms that generate the locomotor output (for review, see Bässler and Büschges 1998; Büschges 2005; Büschges and Gruhn 2008), although very little is known about the timing of leg muscle activity during walking (Epstein and Graham 1983; Graham and Epstein 1985). This information is important because 1) the exact timing of muscle activities during swing-to-stance transitions is needed to assess how sensory input induces them (Büschges and Gruhn 2008) and 2) detailed knowledge of straight walking muscle activity is required to correctly interpret the alterations in muscle activity that occur during locomotor output changes such as turns (Cruse et al. 2009; Gruhn et al. 2009a; Mu and Ritzmann 2005; Ridgel et al. 2007), changes in walking speed (Gruhn et al. 2009b), and switches between tunneling and climbing (Harley et al. 2009) and forward and backward walking (Akay et al. 2007).

In the present study we set out to bridge this gap in knowledge by recording stick insect leg muscle activity during forward and backward walking. Studying neuronal or muscular activity in behaviors such as different walking directions, turning, and gap climbing (Blaesing and Cruse 2004; Cruse 1976a; Cruse et al. 2009; Dürr and Ebeling 2005; Gruhn et al. 2009b; Jander 1985) the exact timing of muscle activities during swing-to-stance transitions is needed to assess how sensory input induces them (Büschges and Gruhn 2008) and 2) detailed knowledge of straight walking muscle activity is required to correctly interpret the alterations in muscle activity that occur during locomotor output changes such as turns (Cruse et al. 2009; Gruhn et al. 2009a; Mu and Ritzmann 2005; Ridgel et al. 2007), changes in walking speed (Gruhn et al. 2009b), and switches between tunneling and climbing (Harley et al. 2009) and forward and backward walking (Akay et al. 2007).

For the stick insect *Carausius morosus* substantial knowledge exists about leg kinematics during adaptive locomotor behaviors such as different walking directions, turning, and gap climbing (Blaesing and Cruse 2004; Cruse 1976a; Cruse et al. 2009; Dürr and Ebeling 2005; Gruhn et al. 2009b; Jander 1985). Substantial information also exists about the central neural mechanisms that generate the locomotor output (for review, see Bässler and Büschges 1998; Büschges 2005; Büschges and Gruhn 2008), although very little is known about the timing of leg muscle activity during walking (Epstein and Graham 1983; Graham and Epstein 1985). This information is important because 1) the exact timing of muscle activities during swing-to-stance transitions is needed to assess how sensory input induces them (Büschges and Gruhn 2008) and 2) detailed knowledge of straight walking muscle activity is required to correctly interpret the alterations in muscle activity that occur during locomotor output changes such as turns (Cruse et al. 2009; Gruhn et al. 2009a; Mu and Ritzmann 2005; Ridgel et al. 2007), changes in walking speed (Gruhn et al. 2009b), and switches between tunneling and climbing (Harley et al. 2009) and forward and backward walking (Akay et al. 2007).
because the respective interleg interactions through the surface are not present and the moving legs therefore exert no force on the body.

We have used the slippery surface setup to investigate the activity of the three major muscle pairs of the stick insect middle leg with respect to touchdown and liftoff during forward and backward straight walking as a first form of adaptive behavior.

**METHODS**

**Animals**

All experiments were performed on adult female stick insects (*Carausius morosus*). Animals were reared in the animal facility of the institute in a 12-h/12-h light/dark cycle at 20–22°C and were fed with blackberry leaves (*Rubus fructicosus*) without restriction.

**Experimental setup**

In all experiments, animals walked on a 13.5 × 13.5 cm polished nickel-coated brass plate divided into two halves. To allow unimpeded walking under tethered conditions and remove mechanical coupling between the legs, the plate was covered with a lubricant composed of 95% glycerin, 5% saturated NaCl, and a small amount of electrode cream (Marquette Hellige, Freiburg, Germany). This created a slippery surface and also allowed recording of tarsal contact by electric current flow during ground contact (Gruhn et al. 2006). The animal was glued ventral side down on an 80 × 3 mm (length × width) balsa rod using dental cement (ProTempII, ESPE, Seefeld, Germany) so the legs and head protruded from the rod and all joints were unrestrained. Animal height above the substrate was adjustable, but was typically 10 mm. Experiments were performed in a darkened Faraday cage at room temperature.

Walking was elicited by projecting a progressive striped pattern (pattern wave length 21°) onto two 13.5-cm-diameter round glass screens (Scharstein 1989) placed at right angles to each other and at a 45° angle to the walking surface, about 6–7 cm away from the eyes of the animal. Reflections on the polished brass plate further increased the field of view. Alternatively, a single white stripe on dark background (toward which the animals orient with straight walking sequences) was placed in front of the animal. If the animal did not begin locomotion spontaneously, walking was elicited by light brush strokes to the abdomen. Backward walking was elicited by gentle pulls on the antenna (Graham and Epstein 1985).

**Electrophysiology**

Muscle activity (electromyogram [EMG]) was recorded using two twisted, coated copper wires (OD: 57 or 49 µm) placed in each muscle about 1 mm apart and held in place with dental cement (ProTempII, ESPE) or tissue adhesive (Vetbond; 3M, St. Paul, MN). Figure 1A shows the approximate sites for the EMG wire placement in the cuticle of the leg and thorax. All recordings were differentially amplified. The EMG signal was preamplified 100-fold (electronics workshop, Zoological Institute, Cologne, Germany); band-pass filtered (100 to 2,000 Hz), when necessary further amplified 10- to 1,000-fold, and imported into Spike2 (version 5.05, CED, Cambridge, UK) through an AD converter (Micro 1401k II; CED). A reference electrode was placed in the abdomen of the stick insect.

In most experiments, two antagonistic joint muscles were recorded simultaneously. **Protractor coxae** and **retractor coxae** EMGs were recorded in the thorax, **depressor trochanteris** and **levator trochanteris** in the coxa, and **extensor tibiae** and **flexor tibiae** in the femur. In two experiments three muscles, in three experiments four muscles, and in one all six muscles were recorded from simultaneously. These experiments gave the same results as the others.

**Recording tarsal contact**

To determine the exact moment of the switch between stance and swing we used middle leg tarsal contact as a switch to open and close an electric circuit (Gruhn et al. 2006). Briefly, we used a 2- to 4-mV amplitude square-wave signal generated with a pulse generator (Model MS501, electronics workshop; Zoological Institute) and applied to one half of the slippery surface and a lock-in amplifier to the other half. The resistance between the plate through tarsus and tibia into the copper wire, but during swing, when the leg was in the air, the circuit was disconnected. Amplifier output was fed into a CED AD converter and digitized using Spike2.

Due to the low-pass filter properties of the lock-in amplifier and the gradual liftoff/touchdown of the tarsus, the signal was not exactly square. We therefore used thresholds set close to the transition point to define the timing of tarsal contact and manually checked each event. Touchdowns could be determined at a resolution of <1 ms.
Liftoff transitions were less steep and more delayed because of delayed tearing of the lubricant from the tarsus due to a capillary action and occasional upward movements of the leg during stance without complete liftoff. To have comparable liftoff times in all experiments we therefore always defined liftoff as the time point with the steepest ascending slope.

**Optical recording and digital analysis of leg movements**

Optical recordings of forward and backward walking were performed and analyzed as in Gruhn et al. (2009a). In brief, we recorded walking sequences with a high-speed video camera (Marlin F-033C; Allied Visions Technologies, Stadtroda, Germany) that was externally triggered at 100 fps. Insect head, thorax, and legs were marked with fluorescent pigments (Dr. Kremer Farbmühle, Aichtstetten, Germany) mixed with dental cement. During the recording of walking sequences, the animal was illuminated with blue light-emitting diode arrays (12 V AC/DC; Conrad Electronic, Berlin). The video files were analyzed using motion-tracking software (WINanalyze 1.9; Mikromak Service, Berlin). AEP describes the anterior extreme position of the leg at touchdown, whereas PEP is the posterior extreme position at liftoff. In forward stepping AEP in stance is anterior to PEP, whereas in backward stepping AEP in stance is posterior to PEP. AEP and PEP values are always given in millimeters in the form $x\_\text{FW}$, $x\_\text{BW}$, $y\_\text{FW}$, $y\_\text{BW}$. Positive $x$-values indicate points anterior and posterior to the coxa, respectively; $y$-values are given with respect to the axis perpendicular to the length of the animal. Larger $y$-values denote more distant points, smaller values more central points. Figure 1B shows a schematic drawing of the stick insect with the tracked reference points for the analysis of leg kinematics marked as gray dots. As an example for the step length vector determination and its direction, the right middle leg is drawn at two fictive positions, one anterior (ML–AEP) and one posterior (ML–PEP). The vectors for all steps connecting the two positions, normalized to the origin in the AEP, gave direction in degrees and step length in millimeters. The 0°–180° axis was always parallel to the body axis and crossed the AEP; 90° always points inside perpendicularly. The simultaneous recordings of the EMG trace and the camera trigger and tarsal contact signals allowed frame-by-frame correlation of filmed movement and EMG and tarsal contact traces. In calculating middle leg movement vectors all steps were transposed to reflect walking as a right leg regardless of which leg was being recorded from.

**Data analysis and figure preparation**

Leg positions were measured with their $x$ and $y$ coordinates. Care was taken to choose animals of the same size and leg lengths. The number of animals used for a given condition ($N$) and the number of steps evaluated ($n$) are given in the figures. The sample size for the kinematics analysis of straight forward walks was $N = 5$ ($n = 125$), for backward walks $N = 3$ ($n = 83$). Cycle period was calculated from touchdown to touchdown, as determined from the tarsal contact trace. For comparisons of EMG activity of the six different muscles between intact forward and backward walking and between intact and reduced forward stepping preparations, EMG traces were rectified and smoothed ($\tau = 50$ ms) and each single data point of each step was exported in Excel (Microsoft, Redmond, WA) to allow averaging. In each step the minimum muscle activity was set to zero and the maximum to one. In several cases, weak cross talk from the antagonist muscle was removed mathematically using the EMG trace from the antagonist: the activity of the EMG in the antagonist was triggered to the same point in time as that of the EMG in the agonist (i.e., liftoff or touchdown of the tarsal contact trace, common for both EMGs) and exported in the same way as before. Then its minimum activity was set to 0, but its maximum to an arbitrary value of 0.5, due to the smaller size of the antagonist signal in the agonist EMG. The normalized activity of the antagonistic muscle was then subtracted from the corresponding value of the muscle under investigation (see Supplemental Fig. S1).1

First spike latencies with respect to liftoff or touchdown were calculated relative to the tarsal contact signal (see preceding text). The absolute latency was then normalized with respect to the corresponding step cycle and averaged for the plot in Fig. 1C. Average swing/stance phase duration was calculated from each evaluated step from liftoff to touchdown for swing and from touchdown to liftoff for stance.

All angles were analyzed using the Watson–Williams test, the circular analogue of the two-sample $t$-test (Matlab, circular statistics toolbox; Berens 2009). Circular variance of vector angles was tested using the variance test in the same toolbox (Matlab, circular statistics toolbox; Berens 2009). For all other statistical analyses, a nonparametric Wilcoxon $U$ test (Matlab, Statistics toolbox; The MathWorks, Natick, MA) was used, except for the comparison of integrals of depressor activity, where a standard Student’s $t$-test was used. Statistical significance was assumed at values of $P < 0.01$. Figures were prepared in Origin 6.1 (OriginLab, Northampton, MA) and Photoshop 6.0 (Adobe Systems, San Jose, CA).

**RESULTS**

Understanding how animals adapt their motor behavior to changing environmental conditions requires measuring limb kinematics and muscle activity in different behaviors. We have shown elsewhere that stick insect leg kinematics differ in straight and curve walking and examined the effect of reducing leg number on these changes (Gruhn et al. 2009a). Here we compare middle leg kinematics during forward and backward walking in intact animals and then examine muscle activity in these two behaviors in the intact and reduced preparations.

**Kinematics of straight forward versus backward walking in the middle leg**

Figure 2A shows a schematic drawing of the stick insect with marked anterior and posterior extreme positions (AEP and PEP plus SD) of the right middle leg in forward and backward straight walking. The data for forward walking (gray) were taken from Gruhn et al. (2009a). AEP is defined as tarsus position at touchdown and PEP as tarsus position at liftoff, always with respect to the direction in which the animal moves. During forward walking (FW), the leg is moved anteriorly during swing and posteriorly during stance. This order of leg movements is reversed during backward walking (BW). In backward walking each step’s AEP is therefore more caudal along the long axis of the animal than the PEP. Forward steps were significantly longer (mean step length FW: 16.2 ± 5.4 mm; BW 9.9 ± 4.7 mm, $P < 0.0001$) and their movement direction was on average more parallel to the body length axis than were backward steps (Fig. 1A). To compare movement vector angles, we mirror-imaged the forward step movement angles in Fig. 2B along the horizontal axis. The resulting mean angles of forward (8.8 ± 17.3°, gray) and backward (36.1 ± 20.3°, black) steps are shown in Fig. 2D and differed significantly ($P < 0.0001$) from each other. The variability between the movement vector angles of single steps is similar in both directions and spans angles over a range of 83° during FW and 88.5° during BW between the respective extremes. Mean touchdown position along the transverse axis was significantly

1 The online version of this article contains supplemental data.
closer to the midline in FW versus BW (y-positions: AEPFW 16.9 ± 3.3 mm; AEPBW 19.6 ± 2.9 mm, P < 0.0001), but mean liftoff position was not significantly different (y-positions: PEPFW 14.4 ± 2.9 mm; PEPBW 13.8 ± 2.6 mm, P = 0.32). However, because during backward walking the movement is more inward directed in each step, the PEP is generally reached after a shorter step length (Fig. 2, B–D). Taken together, these data show that in intact animals middle leg backward stepping is not simply reversed forward walking, but is instead altered to having shorter and more inward directed steps, albeit with a similar degree of variability as seen for forward stepping.

Stance duration alone determines cycle period

In stick insects walking on nonslippery surfaces, in which the different legs are coupled mechanically through the ground on which the animal walks, step cycle period depends on stance duration (Graham 1972, 1985; Wendler 1964). We tested whether this relationship is also present in slippery surface forward and backward walking and, to test for interleg interactions, in animals with reduced leg numbers (only the two middle legs or only one single middle leg). In all these cases, the cycle period varied linearly with stance duration but did not depend on swing duration, which was essentially constant at all cycle periods (Fig. 3).

Phasing of leg muscle activity

EMG recordings of various leg muscles during walking have been made (Fischer et al. 2001; Graham and Epstein 1985), but with few exceptions, only the activities of single muscle pairs were recorded (Bässler 1993; Cruse and Pflüger 1981; Epstein and Graham 1983). In addition, the timing reference for the beginning or end of muscle activity relative to step cycle, if present at all, was not precise. To remedy this lack we made comprehensive paired EMG recordings of all three major muscle pairs controlling leg movements: the protractor/retractor coxae, levator/depressor trochanteris, and the extensor/flexor tibiae muscles at a time during both forward and backward walking.

Figure 4 shows the activity of the muscles of the most proximal leg joint, the thorax–coxa joint, the protractor and retractor coxae muscles, which serve to protract and retract the leg, respectively. The traces in Fig. 4A show raw EMG activity, those in Fig. 4B rectified and smoothed (τ = 50 ms) activity, and those in Fig. 4C mean rectified activity from one stepping sequence (gray trace) and from five animals (black trace). In forward walking protractor activity began before the liftoff of the leg, reached its main activity during swing, and then decreased toward the end of swing. In backward walking the protractor was barely active during swing but began at the transition between swing and stance and reached peak activity

FIG. 2. Kinematics of a forward and backward walking stick insect middle leg on a slippery surface. A: schematic drawing of a stick insect with the mean anterior extreme position (AEP) and posterior extreme position (PEP) values (and SD error bars) of the right middle leg for forward (gray) and backward (black) walking. Note that for the backward walking animal the AEP is posterior to the PEP; the gray line marks the X₀-value for the middle leg. B and C: step-to-step variability in angle and length of stance movement of forward (B) and backward (C) steps normalized to touchdown position (AEP); the average stepping vector is drawn in black in both cases. D: average stepping vectors for forward (gray) and backward (black) walking from B and C; the average vector for forward walking from B was mirrored across the horizontal plane for easier comparison. N = animal number, n = step number.

about 100 ms into stance. This activity pattern was the same for the retractor muscle except that it showed stance activity during forward walking and swing activity during backward walking.

Figure 5 shows the activity of the muscles of the next most distal leg joint, the coxa–trochanter joint, the depressor trochanteris and levator trochanteris muscles, which serve to depress and lift the leg, respectively. The traces in Fig. 5A again show raw EMG activity, those in Fig. 5B rectified and smoothed (τ = 50 ms) activity, and those in Fig. 5C mean rectified muscle activity from one stepping sequence (gray trace) and from five animals (black trace). Depressor activity

![Figure 4](http://jn.physiology.org/)  
**FIG. 4.** Right middle leg protractor and retractor EMG recordings during forward (left column) and backward (right column) walking on a slippery surface. Gray boxes mark swing phase. A: raw EMG recordings. B: rectified and smoothed traces of EMGs in A. C: mean rectified and smoothed traces of recordings (gray) from one and from 5 animals (black). Gray boxes mark the average swing duration; shaded area shows swing duration SD. Double asterisks mark where cross talk from the retractor was removed mathematically from the protractor traces. N = animal number, n = step number.

![Figure 5](http://jn.physiology.org/)  
**FIG. 5.** Right middle leg levator and depressor activity during forward (left column) and backward (right column) walking on a slippery surface. Gray boxes mark swing phase. A: raw EMG recordings. B: rectified and smoothed traces of EMGs in A. C: mean rectified and smoothed traces of recordings from one (gray) and from 5 animals (black). Gray boxes mark the average swing duration; shaded area shows swing duration SD. N = animal number, n = step number.
began very shortly after swing beginning, was active throughout swing, and declined shortly after stance beginning. Provided the animal was maintained at a constant height about the substrate (see following text), depressor activity was the same in forward and backward walking. Moderate levator muscle activity was present at the beginning and middle of stance, with a substantial peak of activity occurring just before the stance to swing transition. Levator activity decreased and reached a minimum shortly after swing beginning. As with the depressor, levator activity was the same in forward and backward walking.

The last muscles analyzed (Fig. 6) were the extensor tibiae and flexor tibiae muscles, which move the femur–tibia joint and extend and flex the tibia, respectively. The traces in Fig. 6A again show raw EMG activity, those in Fig. 6B rectified and smoothed ($\tau = 50$ ms) activity, and those in Fig. 6C mean rectified muscle activity from one stepping sequence (gray trace) and from five animals (black trace). Peak extensor activity occurred around liftoff in forward and in backward walking, whereas flexor activity peaked during the first half of stance in forward and backward walking.

Latency of muscle timing during forward and backward walking

Reliably comparing muscle activity in different walking directions and across preparations requires determining the exact timing of muscle activity within the step cycle. Swing to stance and stance to swing transitions are two such points. Figures 7 and 8 show first spike latencies relative to these points for all six muscles in forward and backward walking, respectively, from five animals each. The gray areas mark mean swing duration averaged across all steps and animals.

The protractor, levator, and extensor muscles move the middle leg forward, up, and extend the femur–tibia joint. During forward walking these movements occur during swing. We therefore measured the first spikes in these muscles relative to liftoff (Fig. 7, A, C, and E). Activity occurred earliest in the levator (mean first muscle potential 99.9 ± 64.2 ms before liftoff), followed by the extensor (66.9 ± 47.3 ms) and then the protractor (36.5 ± 36.3 ms). The retractor, depressor, and flexor muscles move the leg backward, down, and flex the femur–tibia joint. During forward walking these movements occur during stance. We therefore measured the first spikes in these muscles relative to touchdown (Fig. 7, B, D, and F). Activity occurred earliest in the depressor with the mean first muscle potential 93.1 ± 33.9 ms after touchdown, 22% into the swing phase. The first flexor activity occurred next with mean first muscle potential 9.0 ± 13.3 ms after touchdown. Single first spikes occurred just before touchdown, confirming previous findings for the timing of this muscle (Gruhn et al. 2006). First retractor activity was more variable, with mean first muscle potential 34.6 ± 33.6 ms after touchdown and first activity occurring ≤50 ms before touchdown. The joint activation sequence in swing is thus the same as that for stance, i.e., first coxa–trochanter, then femur–tibia, and finally thorax–coxa. The high SD values result from the high variability in the stepping of the stick insect on the slippery surface. Walking sequences with many consecutive straight forward steps do not occur often and every step has a slightly different direction and stance duration.

As was shown earlier in the kinematics and EMG data, in backward walking protractor and retractor timing is the reverse of that in forward walking. To continue to compare the timing of functional swing and stance muscles in the two walking
directions, in backward walking sequences we therefore referenced retractor activity to liftoff and protractor activity to touchdown, but continued to reference the activity of the other muscles as before (Fig. 8, A–F). Sequence of levator and extensor activation as well as the latencies for the first muscle potential (100.2 ± 60.5 ms, Fig. 8C; 56.8 ± 48.0 ms, Fig. 8E, respectively) were the same as in forward walking (\( P_{\text{Lev}} = 0.98; P_{\text{Ext}} = 0.31 \)). During backward walking the retractor activated 18.5 ± 36.5 ms before liftoff (Fig. 8A), dramatically different from this muscle’s activation in forward walking (Fig. 7B), but barely not significantly different from the timing of the functionally analogous protractor during forward walking (\( P = 0.012 \)) (Fig. 7A).

Except for the difference mentioned earlier that the protractor is a stance phase muscle in backward walks, the timing and activation sequence of the functional stance phase muscles were also similar in forward and backward walking. The depressor again activated first (Fig. 8D), although only halfway through swing at 64.9 ± 25.1 ms before touchdown, significantly later than that in forward walking (\( P < 0.0001 \)). The protractor and flexor activated next at almost the same time: 10.9 ± 34.4 and 5.3 ± 28.6 ms after touchdown (Fig. 8, B and F). Flexor timing did not differ significantly from that in forward walking (\( P = 0.12 \)). Despite their large SDs, protractor timing in backward walking (10.9 ± 34.4 ms) and retractor timing in forward walking (34.6 ± 33.6 ms) did differ significantly (\( P < 0.0001 \)).

In summary, these data show that 1) only the muscles controlling the thorax–coxa joint showed large changes when walking direction changed, and 2) with respect to liftoff and touchdown, the timing of functionally analogous muscles in swing and stance is almost the same in both directions.

Muscle activity in reduced preparations

Many studies on stick insect walking have been conducted in preparations with reduced leg number (e.g., Akay et al. 2001, 2004; Fischer et al. 2001; Gabriel and Büschges 2007; Gabriel et al. 2003; von Uckermann and Büschges 2009). Because these preparations lack interleg sensory interactions, it is important to test whether data from such experiments are applicable to intact animals. Leg kinematics in straight forward
walking and turning change only little in reduced preparations (Gruhn et al. 2009a), but muscle activity in reduced preparations has not been measured. We therefore next compared forward walking muscle activity in intact and two-legged (2L) and one-legged (1L) animals.

Figure 9 shows mean rectified and smoothed extensor and flexor EMGs from 112 to 125 steps from five different animals for each leg number condition. Extensor activity began about 100 ms before the stance–swing phase transition, peaked between the stance–swing transition and the first third of swing, and lasted throughout swing. Flexor activity was also similar in all leg number conditions. It started at the beginning of stance and the greatest activity occurred during the first 100 ms of stance. Similar data were found for the levator/depressor and protractor/retractor. In no case were major differences in EMG activity of the three antagonistic muscle pairs found between the intact, 2L, or 1L preparations (data not shown).

Removal of four or five legs to produce 2- or 1-middle legged preparations, however, did alter the timing of first muscle activity in all three muscle pairs, at least under some reduced leg number conditions. The first swing phase muscle to be activated, the levator, was on average activated 92.8 ± 99.4 ms before liftoff in the 2L preparation and 88.4 ± 37 ms before liftoff in the 1L preparation. For both preparations this time was not significantly ($P_{2L} = 0.15$, $P_{1L} = 0.61$) later than that in intact animals; neither were the values for the 1L and 2L preparations significantly different from each other ($P = 0.38$). The second muscle to be activated in swing, the extensor, activated significantly later in both reduced preparations than that in intact animals, with first activity occurring on average 29.7 ± 38.9 ms (2L) and 33.2 ± 27.9 ms (1L) before liftoff ($P < 0.0001$). The timing of the first extensor spike in these two reduced preparations, on the other hand, did not differ significantly from each other ($P = 0.47$). The third muscle activated in swing, the protractor, activated 19.9 ± 32.5 ms before liftoff in the 2L preparation, significantly later than that in intact animals ($P = 0.0005$) (1L preparations were not investigated in this muscle).

All stance muscles showed small changes in activation timing. The depressor activated slightly but significantly earlier than that in intact animals in both the 2L (99.6 ± 24 ms, $P < 0.0001$) and 1L (123.7 ± 32.5 ms, $P < 0.0001$) preparations.
The flexor activated at statistically equivalent times in both reduced preparations (2L, 16 ± 12.5 ms; 1L, 15.9 ± 9.5 ms, P = 0.9), with both preparations also differing significantly from intact animals (P < 0.0001). In the 2L preparation the retractor showed a large and significant change in the timing of first activity (10.2 ± 34.5 ms after touchdown, P < 0.0001) compared with that in intact animals (1L preparations were not investigated in this muscle).

The data show that middle leg muscle activity changes only slightly in reduced preparations. Nonetheless, the presence of clear changes in the latencies of most muscles indicates that interleg sensory input does contribute to the timing of middle leg swing and stance muscle activation.

Depressor muscle activity depends on animal height

Depressor activity is strongly influenced by movement, strain, and load-related inputs from the trochanteral hair plate (Cruse et al. 1993; Schmitz 1986a,b), campaniform sensilla (Borgmann et al. 2005), and from the femoral chordotonal organ (Hess and Büschges 1997, 1999). In our experimental setup the animals were attached to a small wooden dowel held at a fixed height above the slippery surface. The animals therefore could not regulate their height and thus did not experience the changes in leg loading that would occur in completely free walking. We therefore tested the effect of decreased and increased load by lifting or lowering the tethered animal during walking sequences and comparing the depressor activity under these conditions. Figure 10, A–C shows middle leg depressor trochanteris and levator trochanteris recordings from a single stick insect while the animal walked at 10 (the physiological walking height and height of all other experiments shown here), 13, and 7 mm above the slippery surface. The normalized rectified and smoothed depressor activities from all recorded steps of all animals under the different conditions are shown in Fig. 10D. Increasing walking height from 10 to 13 mm (Fig. 10B) had little effect on the depressor activity (Fig. 10A). At both heights the depressor was mainly active during the second half of swing, although in three of six animals, as in this example, slightly fewer depressor spikes occurred in stance at a height of 13 mm. Levator activity showed no detectable changes in activity. At a height of 7 mm the depressor was active not only in the second half of swing.
but also throughout two thirds of stance, continuing until leverator activity began. Since the depressor activity was very similar during swing, we compared the integrals under the rectified and smoothed EMG traces after touchdown from 178 to 221 steps from six animals at all three heights. Four of six animals showed higher mean depressor activity during stance at 7 mm compared with the other two conditions. Even with all animals at this condition pooled together, average depressor activity was significantly greater at 7 mm than that in the other two situations ($P < 0.0001$), whereas the activity was the same in the animals at 10 and 13 mm height ($P = 0.49$). These differences in depressor activity at different animal heights made it very important to measure depressor activity in freely walking animals that could control their own body height. We therefore dismounted one stick insect from the wood dowel after first recording depressor and leverator activity under tethered conditions at different heights. The glycerin was then wiped from the slippery surface and the still completely wired animal was allowed to walk freely on the surface while we continued to record stepping tarsal contact (Fig. 10E). Under these conditions, depressor EMG activity did not start at a different time from the values at all heights seen in the tethered animal. The stippled trace of rectified and smoothed average EMG activity in this animal (Fig. 10D) shows the similarity in mean rectified activity for the freely walking animal and the averaged six animals fixed at 7 mm. The time course of depressor activity pattern, however, was very similar to that seen in tethered walking at 7 mm height (Fig. 10C), i.e., depressor activity lasted long into stance. This suggests that, when the animal has to control its own height during walking, the depressor acts not only to lower the leg to the ground (swing activity), but also acts during stance to help carry the animal’s weight and keep it at a specific height above the ground.

DISCUSSION

Kinematics/cycle period

Multiple studies have investigated insect forward and curve walking (walking and turns on solid substrate: Cruse 1976b; Cruse et al. 2009; Jindrich and Full 1999; Ridgel et al. 2007; Rosano and Webb 2007; Stauber and Heisenberg 1990; Wendler 1966; Zollikofer 1994a; Zolotov et al. 1975; air-cushioned Styrofoam ball: D¨urr and Ebeling 2005; Frantsevich and Mokrshov 1980; Jander 1982; slippery surface: Camhi and Nolen 1981; Gruhn et al. 2009a; Mu and Ritzmann 2005; Ridgel et al. 2007). Backward walking has been investigated in crustacea (Ayers and Davis 1977a; Chasserat and Clarac 1980), scorpions and spiders (Bowerman 1981), and stick insects (Graham and Epstein 1985). However, this quite early stick insect work was only qualitative and did not have precise measurements of stance–swing transitions.

The data presented here confirm observations by Graham and Epstein (1985) that stick insects can perform coordinated backward walks on a slippery surface. Our data show that forward and backward steps are equally variable but backward steps are significantly shorter and therefore more inward directed than forward steps. Touchdown positions were only slightly different in the transverse axis to the animal (y), and liftoff positions were unchanged, in forward and backward walking. This observation is consistent with the activity of the femur–tibia joint muscles, especially the flexor, being very similar in forward and backward walking because it is flexor activity that determines y-position at stance end. It should be noted, though, that backward stepping was elicited by a continuous pull on the antennae, whereas forward walking was undisturbed. This may be an additional reason for some of the observed changes in vector length and direction.

Cycle period depended only on stance duration in both forward and backward slippery surface walking. This has been long known for stick insects walking forward on nonslippery substrates (Graham 1972, 1985; Wendler 1964), although it was unclear whether this relationship held for forward walks on a slippery surface or for backward walks. Our findings confirm previous results (B¨uschges et al. 1995; Gabriel and B¨uschges 2007; Gruhn et al. 2009b) showing that in pharmacologically activated neuron preparations only stance motor neuron firing duration, and in single leg preparations only leg stance duration, vary with cycle period. We have not tested whether the time of onset of muscle bursting changes with the rate of walking. Such dependence has been shown in a previous study of muscle activities in cockroach (e.g., Delcomyn 1989), where the phase of the onset of bursts in a leg stance phase muscle shifted with respect to the neighboring leg when walking speed increased and bursting was initiated earlier during rapid walking. However, on the level of the single leg, such a shift has been shown to occur in flexor motor neurons in the stick insect middle leg, albeit not with respect to a neighboring leg (Gabriel and B¨uschges 2007). It is therefore not unlikely that shifts in the muscle activity onset of stance-related muscles in the stick insect exist with respect to neighboring legs.

Muscle activity and latencies in forward versus backward walking

Recent work on stick insect muscles (Guschlbauer et al. 2007; Hooper et al. 2007, 2009) highlights the slow responses of these muscles to neural input and thus the importance of direct measurement of muscle activation in describing how neural activity generates behavior in this system. Our EMG recordings of the six main middle leg muscles showed that only the muscles controlling the thorax–coxa joint (protractor, retractor) had large changes in activity when stick insects reversed walking direction (Fig. 11). Figure 11A shows the average onsets of muscle activities of the functional swing phase muscles, timed to liftoff, and Fig. 11B the average onsets of activities of the functional stance phase muscles timed to touchdown, in both walking directions with their respective SDs. The levator and extensor muscles are always functional swing muscles and the depressor and flexor always stance muscles. The protractor and retractor muscles, alternatively, switched from being (respectively) functional swing/stance muscles to being stance/swing muscles when walking direction reversed (Fig. 11C; see also Fig. 11A, which shows, in addition to swing onsets, the average end of retractor activity during stance in forward walking and the average end of protractor activity during stance in backward walking). The activity of these muscles is thus determined by each muscle’s function in the behavior that is being produced. Provided this switch is noted, the activation sequence of functional swing and stance
A general finding was that first activity timing was relatively imprecise for most muscles with SDs ranging from 13.3 (flexor in forward walking) to 64.2 ms (levator in forward walking). One reason for high lever and extensor variability could be that these muscles are timed to liftoff and measurement of the liftoff signal can be less precise than that of the touchdown signal. However, pro- and retractor muscle timing variability was similar in forward and backward walking despite different reference points being used in the two walking directions. Furthermore, the flexor shows much higher variability in backward (28.6.4 ms) (reference point touchdown) than that in forward (13.3 ms) walking and the opposite is the case for the depressor. These results suggest that variability differences do not result from a lack of precision in determining liftoff or touchdown times, but are instead true differences in motor patterning.

Another interesting observation in this context was the early activation of swing muscles before the actual kinematically observed onset of the swing movement and the activation of stance muscles before or around touchdown and therefore before the kinematically observed stance movement. This finding can be explained with the muscle properties reported for stick insects and smaller animals in general (Guschlbauer et al. 2007; Hooper et al. 2007, 2009). With decreasing diameter of a given muscle, the forces needed to overcome the passive forces of its antagonist become so large that muscle activity has to start well before an observed movement that is caused by the muscle contraction (Hooper et al. 2009). In this case, as opposed to that in large animals such as cat or human, the role of gravity in moving a limb becomes negligible and thus the early onset of muscle activity in levator and depressor is needed to counteract the respective antagonist before movement can begin.

Previous work has shown that signals from movement and load sensors are important for inducing stance–swing and swing–stance transitions during walking (Büschges and Gruhn 2008; Büschges et al. 2008; Cruse et al. 2004). In most of this work, however, motor activity timing was inferred from leg kinematics, which means that the measurement of sensory input timing with respect to motor output was imprecise. It is therefore useful to compare our precisely measured muscle activities and these previous data.

1 Tibia extension signals arising from the femur–tibia joint have been identified as a trigger for depressor activity in swing (Bucher et al. 2003; Hess and Büschges 1999). The depressor activation times we measure, well into swing (93 ms prior to touchdown), and at a time when the middle leg tibia is known to be well extended (e.g., von Uckermann and Büschges 2009), are consistent with this conclusion (Fig. 11, A and B).

2 Leg loading, as occurs after touchdown, has been reported to initiate retractor activity (in forward walking), as a result of signals from the trochanteral campaniform sensilla (Akay et al. 2004, 2007), and flexor activity, as a result of signals from the femoral campaniform sensilla (Akay et al. 2001). Although the mean activation times we measure for these muscles agree with this hypothesis, first activation of both muscles was either prior to touchdown or so shortly after that it is difficult to imagine load signals from trochanteral or femoral campaniform sensilla alone to activate either muscle at stance onset.

3 Load signals arising from the trochanteral campaniform sensilla (Akay et al. 2007) have been reported to support ongoing depressor and retractor activity during stance (Bässler 1967, 1972; Schmitz 1986a). This matches the finding that depressor activity was increased and prolonged when the animal was lowered to the surface or walked freely on the slippery surface.

4 Our data show that during swing the levator is activated first, then the extensor, and finally the protractor (Fig. 11A). This sequence matches the predicted effects of known sensory influences: leg unloading signaled by the femoral and trochanteral campaniform sensilla activates extensor (Akay et al.
process. Understanding the dependence of cycle period on stance duration (Gabriel and Büschges 2007). It is thus possible that the durations of individual joint movements were measured and that our experiments did not test for the effects of independently altering the durations of individual joint movements. Flexor activity does indeed play an important role in determining cycle period (Gabriel and Büschges 2007). It is thus possible that the retractor–protractor switch does not pose a difficulty for understanding the dependence of cycle period on stance duration because thorax–coxa joint activity is simply not a part of this process.

Implications of pro- and retractor switch in forward and backward walking

The finding that the timing of the pro- and the retractor muscles, which control the thorax–coxa joint, switched independently of that of the muscles for the other joints, and that it depended on the functional role of the muscle rather than on the muscle itself, raises questions about the neuronal control of forward and backward walking. How does the nervous system alter the control for the joint network to reverse the motor pattern and how is the similar timing of muscle activity achieved? This includes the question on how cycle period continues to depend on stance duration in backward walking, even though under this condition the stance phase muscle at the thorax–coxa joint, and only at this joint, switches.

One explanation for the retractor–protractor switch is that the phase coupling between the thorax–coxa joint pattern generator and the pattern generators of the other joints, the coxa–trochanter and femur–tibia joints, is altered centrally so that the thorax–coxa central pattern generator’s “protractor motor neuron driving interneurons” fire during stance in backward walking. An input with this effect has not been identified, nor would this mechanism explain why cycle period continues to depend on stance duration in backward walking. That is, if in forward walking the thorax–coxa central pattern generator’s cycle period depends on “retractor interneuron” burst duration, it is unclear why switching the pattern generator’s phase relative to the coxa–trochanter and femur–tibia central pattern generators would change the dependence of the pattern generator on retractor interneuron burst duration. This could be explained if this dependence is not associated with retractor (forward walking) and protractor (backward walking) activity duration, but only with flexor and depressor durations (note that in Fig. 3 whole leg stance vs. phase durations, not individual joint movement durations, were measured and that our experiments did not test for the effects of independently altering the durations of individual joint movements). Flexor activity does indeed play an important role in determining cycle period (Gabriel and Büschges 2007). It is thus possible that the retractor–protractor switch does not pose a difficulty for understanding the dependence of cycle period on stance duration because thorax–coxa joint activity is simply not a part of this process.

Interleg influences on muscle activity in the forward walking animal

Reducing leg number caused only relatively small alterations in overall leg muscle activity but shifted the average latency of the first muscle spikes in the pro–retractor, in the extensor and the flexor muscles, and the depressor, whereas no effect was seen on levator activity. These data are consistent with kinematics analyses of straight walking and turning stick insects showing that single legs produced leg movements with changes in the precise leg positioning when the number of legs was reduced (Gruhn et al. 2009a). These changes are also in line with considerable evidence suggesting that interleg influences could play a prominent role in shaping leg motor output (Borgmann et al. 2007, 2009; Ludwar et al. 2005). In our data the greatest changes in forward walking between intact and reduced animals were in the extensor and retractor muscles, where the onset of activity shifted by about 25–30 ms toward liftoff and thus activation started later than that in the intact animal. In the case of the flexor, the effect was a shift of 5–10 ms, yet this muscle was also activated significantly later in the reduced preparations. Interestingly, the effect of the reduction was not always a delay in the activation, but in the case of the retractor and the depressor muscle a significantly earlier activation, demonstrating that the sensory intersegmental effects influence the timing in both directions and, in the case of the depressor, even the ablation of the contralateral leg and the lack of its sensory input may have an effect on timing. The activity of the above-cited muscles may be especially influenced by interleg influences because they largely determine the leg’s anterior and posterior extreme positions and stance and swing phase duration, all extremely important components of interleg coordination (Cruse 1990; Cruse et al. 1998). The origins of the sensory input from the neighboring legs exerting these influences is unclear, although Ludwar et al. (2005) demonstrated that flexion signals from the front leg femoral chordotonal organ can facilitate middle leg retractor activity and also contralateral influences have been...
shown to exist, although not for the depressor muscle (Stein et al. 2006).

**Local influence on depressor muscle activity**

When the animals were tethered $\pm 1$ cm above the slippery surface, depressor activity strongly decreased very early in stance, but in animals tethered at a lower height, depressor activity continued throughout the greater part of stance. Increased depressor duration was also seen in freely walking animals. These data suggest that sensory input in freely walking animals prolongs depressor activity so that, in addition to lowering the leg at stance end, the muscle also helps to support the animal during stance. Work in stick insect and other insects suggests that the sense organs most likely responsible for this effect are, again, the campaniform sensilla, the same organs involved in switching protractor to retractor activity at touchdown (see preceding text and Akay et al. 2004, 2007). However, the role of campaniform sensilla in the magnitude control of motor neuron activity in stick insects is much less understood. Cruse et al. (1993) previously demonstrated in double-treadwheel experiments that stick insects walking with small distances between body and wheel push the wheel away from the body, resulting in increased depressor activity. In cockroach different subgroups of tibial campaniform sensilla react to increases or decreases in body load (Noah et al. 2004; Zill et al. 2009) and fire prolonged spike trains when legs actively support the body. Increased load also increases cockroach trochanteral extensor motoneuron firing, the functional analog of stick insect depressor motoneurons (Quimby et al. 2006). These and our data suggest that local mechanisms controlling depressor activity are a major component of the support of body load and maintenance of body height.

**Conclusions**

We have described the activity and timing of all major middle leg muscles during forward and backward walking in the tethered stick insect. As the animal switched from forward to backward walking the major observed change was that the functional stance muscle of the thorax–coxa joint switched from retractor to protractor, with both muscles showing the same activity times when serving as stance muscles. These findings demonstrate again the modular structure of the neuronal networks driving leg movement. They also suggest potential ways in which the CNS controls adaptive walking behaviors and how sensory input may be differentially modulated, depending on behavioral task. With these data at hand it will now be possible to study the effect of selective manipulation of single-sense organs on these now well-defined behaviors.

**Acknowledgments**

We thank H.-P. Bollhagen, J. Sydow, and M. Dübbert for excellent technical support and Drs. U. Bässler, S. Gruhn, S. L. Hooper, and J. Schmidt for comments on earlier versions of the manuscript and support with the statistical analysis.

**Grants**

This study was supported by Deutsche Forschungsgemeinschaft Grant Bu 857/810 to A. Büschges.

**Disclosure**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**References**


