

Straight walking and turning on a slippery surface

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SUMMARY

In stick insects, walking is the result of the co-action of different pattern generators for the single legs and coordinating inter-leg influences. We have used a slippery surface setup to understand the role the local neuronal processing in the thoracic ganglia plays in the ability of the animal to show turning movements. To achieve this, we removed the influence of mechanical coupling through the ground by using the slippery surface and removed sensory input by the successive amputation of neighboring legs. We analyzed the walking pattern of the front, middle and hind legs of tethered animals mounted above the surface and compared the kinematics of the straight walking legs with those of the curve walking inside and outside legs. The walking pattern was monitored both electrically through tarsal contact measurement and optically by using synchronized high-speed video. The vectors of leg movement are presented for the intact and a reduced preparation. Animals showed the ability to walk in a coordinated fashion on the slippery surface. Upon change from straight to curve walking, the stride length for the inside legs shortens and the vector of movement of the inner legs changes to pull the animal into the curve, while the outer legs act to pull and push it into the turn. In the reduced two-leg and in the single-leg preparation the behavior of the legs remained largely unchanged in the behavioral contexts of straight walking or turning with only small changes in the extreme positions. This suggests that the single stepping legs perform given motor programs on the slippery surface in a fashion that is highly independent not only of mechanical coupling between but also of the presence of the other legs.

Key words: walking, leg coordination, kinematics, stick insect.

INTRODUCTION

Walking in all kinds of organisms shows a high degree of variability in the stepping kinematics despite its often stereotypical appearance. This form of locomotion assures survival through great adaptability and the possibility of coping with unpredictable terrain. This is also the case during walking in the stick insect, *Carausius morosus*, which has been a very useful organism for gaining insight into basic principles of joint and leg coordination (Bässler and Büschges, 1998; Büschges, 2005; Büschges and Gruhn, 2008). The types of leg movements observed in this six-legged animal share major similarities with those performed by vertebrates such as the cat (Pearson, 2004; Büschges, 2005).

However, currently, we know only little about the neural basis for more complex adaptive behaviors such as turning in the intact animal. Previous studies that have investigated turning in insects have mostly looked at body trajectories and stepping patterns, either in freely moving animals [bee (Zolotov et al., 1975); fruit fly (Strauss and Heisenberg, 1990); ants (Zollikofer, 1994a; Zollikofer, 1994b; Zollikofer, 1994c); cockroach (Franklin et al., 1981; Jindrich and Full, 1999); stick insect (Rosano and Webb, 2007)] or animals under tethered conditions [e.g. stick insect (Jander, 1982; Jander, 1985; Dürr, 2005; Dürr and Ebeling, 2005); cockroach (Camhi and Nolen, 1981; Mu and Ritzman, 2005)], and they have mostly focused on the behavior as a task for the whole motor system. During free turning in the stick insect, the anterior part of the body is moved into the curve by the inner pro- and mesothoracic legs with pulling-like inward movement of the femur, while the outer pro- and mesothoracic legs support the body displacement with a pushing-like movement through extended retraction of the leg. At the same time, the metathoracic legs both push slightly against the turning direction with the extension of the femur during

stance. Jander described the changes in phase relationships of the single legs in detail (Jander, 1982). The insect achieves the necessary differences of moving speeds between the inner and outer legs through variation of the step length and also, in sharp turns, through different stepping frequencies (Jander, 1982; Dürr, 2005; Dürr and Ebeling, 2005).

The important questions that arise from these studies are: what the mechanisms are that control changes in the coordination of different joints as they are used in varying behavioral contexts; how the animal configures its neuronal output for a given limb in order to be able to complete the necessary adaptations in joint coordination; and what potential role sensory feedback has in generating the related leg movements?

We have a fairly good idea about the organization and actions of those networks that control and coordinate the muscle activity in the different leg joints during simple stepping movements at the level of the single leg (for a review, see Büschges, 2005; Büschges and Gruhn, 2008). At the level of simple walking tasks such as straight forward and backward walking, information about the underlying networks is just emerging (Akay et al., 2007). However, changes from a stereotypical walking pattern are required of any insect that moves in variable environments. Thus, when trying to answer the above questions, a fundamental problem lies in the uncertainty about the contributions of various local and inter-leg influences to such changes. These can be direct sensory feedback in a given leg, the actions of existing inter-leg coupling between central neural networks and finally, coupling between all legs with ground contact through the substrate on which the animal moves (for a review, see Grillner, 1981; Bässler and Büschges, 1998; Büschges and Gruhn, 2008). For example, one could have the notion from the known 'coordination rules' that control the coordination

of the legs among each other (Cruse, 1990; Dürr et al., 2004; Dürr, 2005) that the touch down and lift off positions of each leg were influenced by their neighbors during turning. Therefore, the kinematics of the single leg could depend on the presence of the neighboring legs. However, the degree to which the movement of the single leg and its coordination with the other five legs is controlled by the three factors above is yet unknown.

A useful way to separate inter-leg influences from local influences has been the single walking leg preparation in the stick insect (e.g. Karg et al., 1991; Bässler, 1993), where all legs but the one under investigation are amputated at the level of the mid-coxa. Studies under these conditions have demonstrated that single legs can produce inter-leg sensory influence on their neighbors as postulated (Cruse et al., 2004; Ludwar et al., 2005; Borgmann et al., 2007). Yet, although this preparation allows good electrophysiological access to the neuronal networks (Schmidt et al., 2001; Gabriel and Büschges, 2007; Akay et al., 2007), it is also restricted in one plane and the possibility to answer questions on single leg stepping during adaptive walking patterns such as turning is limited. Therefore, it remains unclear whether the nervous system can generate the appropriate context-dependent leg movements in a single leg in the absence of neighboring legs or whether, or to what extent, the kinematics are influenced by mechanical coupling through the ground and/or sensory feedback from the neighbors.

We have, therefore, used the slippery surface setup as introduced by Gruhn et al. (Gruhn et al., 2006) to elicit straight walking and turning in tethered stick insects where the legs were not restricted in their movements. The slippery surface for the intact tethered preparation allows the reduction of mechanical coupling between stepping legs and has been used successfully to study walking and turning in the cockroach and the stick insect (e.g. Camhi and Nolen, 1981; Cruse and Epstein, 1982; Epstein and Graham, 1983; Tryba and Ritzmann, 2000a; Tryba and Ritzmann, 2000b; Mu and Ritzman, 2005). On such a greased surface, the animal lacks inter-leg sensory feedback through being tethered and through the lack of substrate coupling. Yet, in the above cited studies, the problem remains that sensory input to all legs may have had an impact on the motor activity in all the other legs even when substrate coupling was absent. Therefore, we have combined the slippery surface preparation with the single leg approach to resolve this problem.

In the present study, we analyzed the movement patterns of the front, middle and hind legs during straight walking and turning in the intact animal, and compared the findings with the reduced preparations in order to determine whether the single leg is able to generate the kinematics associated with turning, and how dependent these leg movements in each leg are on inter-leg mechanical coupling and sensory information through the presence of neighboring legs. If the single leg is indeed capable of producing proper turning movements, then one can postulate that the main information for context-dependent leg motor control resides in the respective hemiganglion and that inter-leg sensory information may only have modulatory influence.

MATERIALS AND METHODS

Animals

Experiments were performed on adult female stick insects (*Carausius morosus* Br.) with a length of 7.5 cm and leg lengths that were the same between animals. The animals were reared in the animal facility of our institute at 20–22°C under a 12h:12h light:dark cycle and fed blackberry leaves *ad libitum*.

The slippery surface setup

The surface on which the animals walk and the electrical measurement of tarsal contact that was used to verify touch down and lift off positions for single legs as determined by high-speed video analysis has been described in detail in Gruhn et al. (Gruhn et al., 2006). Briefly, the plate consists of two nickel coated brass halves insulated against each other. Current was applied to the plates separately through two plugs at the base of each plate. Slipperiness and simultaneous conductivity was conveyed through a glycerin/saturated NaCl-solution mix at a ratio of 95:5 [viscosity approx. 435.8 cStokes as determined through use of a table in Römpf (Römpf, 1966)], which was applied with a soft cloth to ensure an almost even distribution of a very thin film. Small artifacts at contact of each leg also allowed to us to monitor the legs that were not directly connected to the two lock-in amplifiers. A very small signal voltage (2–4 mV) and an amplifier with high input resistance (1 M Ω) were chosen in order to avoid affecting the walking behavior of the animal. This allowed us to keep the current passing through the tarsus and tibia between 2 and 4 nA.

Optical stimulation

Walking episodes were elicited as optomotor responses as described previously (Gruhn et al., 2006). Briefly, moving stripes were projected onto two glass screens (diameter 130 mm; Marata screens, Linos Photonics, Göttingen, Germany) in front of the animal, positioned left and right of the head at right angles to each other, and at a distance of 70 mm from the eyes. The wavelength of the striped pattern was kept constant at $\lambda=21$ deg. The contrast frequency of the moving stimuli was varied between 0.35, 0.72, 1.07 and 1.49 Hz. λ was not varied throughout the experiments. Forward walking was induced by a progressive pattern on both screens with stripes moving outward whereas curve walking was induced by moving stripes in the same direction on both screens. Luminance of the striped pattern was adjusted by the voltage of the halogen lamps in the projectors. The experiments were set up in a darkened Faraday cage and performed in a darkened room at 22–24°C.

Preparation and experimental procedure

The animals were glued (two-component glue, ProTempII, ESPE, Seefeld, Germany) ventral side down onto a balsa stick that was thinner than the width of the insect (3×5×100 mm, W×H×L). The head and legs protruded from the front and side of the stick to allow their free movement. The area of the coxae of all legs as well as the major part of the abdomen was left free of glue. The balsa stick was inserted into a brass tube that was connected to a micromanipulator. This permitted us to adjust the position of the animal at approximately 8–15 mm above the slippery surface, which corresponds to the height of the insect during free walking. The velocity of the striped pattern was set, and moving pattern and video recording were started simultaneously. If the animal did not start locomotion spontaneously, it was either stimulated with a brush at the abdomen (Bässler and Wegener, 1983) or with a puff of air to the antennae. The striped pattern was kept moving until the animal stopped walking or until after 3 min of continuous recordings. For experiments with two-legged (2L) and one-legged (1L) animals, we induced autotomy of the pro- and metathoracic legs with a pair of forceps (Schmidt and Grund, 2003) or cut the legs at the level of the coxae after recording from the intact animal. After that we allowed a minimum of 30 min for recovery.

Optical recording and digital analysis of leg movements

We recorded walking sequences from above with a high-speed video camera (Marlin F-033C, Allied Vision Technologies, Stadtroda, Germany) at 100 frames s^{-1} . The camera was externally triggered and pictures were fed into a PC through a FireWire interface and then assembled into a video (*.avi) file ('fire-package'-software, Allied Vision Technologies, Stadtroda, Germany). The legs were marked at the distal end of the femur and the tibia. We used orange and yellow fluorescent pigments as markers (gold-orange, catalogue #56200 and yellow, catalogue #56150, Dr Georg Kremer Farbmühle, Aichstetten, Germany), which were dissolved in two-component glue (ProTempII, ESPE, see above). Additional markers, pigments dissolved in a shellac/alcohol solution, were set at the center of the thorax between the pro-, meso- and metathoracic legs, as well as at the end of the prothoracic segment and in the middle of the head. During the recording of walking sequences, the animal was illuminated with blue LED arrays (12 V AC/DC, Conrad Electronic, Germany). In addition, we used a yellow filter in front of the camera lens for suppression of the short wavelength of the activation light to have a high contrast for the video recordings. The video files were analyzed using motion tracking software (WINalyze, v. 1.9, Mikromak service, Berlin, Germany). AEP describes the anterior extreme position of the leg at touch down whereas PEP is the posterior extreme position at lift off. Most of the time, the AEP position for a given step and, thus, its stance phase is anterior to its PEP. However, in sideward stepping of the inside legs, the lift off position can be anterior to the touch down position. In these cases, the labeling of AEP and PEP remains the same, that is, marking the touch down and lift off positions, respectively. The AEP and PEP values are always given in millimeters in the form $xx.x; yy.y$ (s.d._x;s.d._y). x -values are given with respect to the length of the animal and for each leg, a virtual 0 line was drawn across the animal at the level of the coxa. Thus, positive x -values reflect points anterior of the coxa of the respective leg, negative values reflect those posterior to the coxa. y -values are given with respect to the axis along the length of the animal and are always absolute values. Larger y -values denote more distal points, smaller values denote more central points (Fig. 1B). The distance between tarsus and leg joint was not analyzed in this paper. For the calculation of the movement vectors of each leg, all steps were transposed to reflect walking as a left leg, irrespective of the actual position. Then all inside, outside and straight steps were averaged in their respective groups and evaluated. The mean step length between AEP and PEP for the front, middle and hind legs with respect to the body axis was calculated from the x - and y -positions of the single vectors. For the calculation of the movement vector angles between AEP and PEP, the body axis served as 0 deg. mark against which the angle of a leg was calculated (Fig. 1B). Thus, 90 deg. marks an angle at which the leg is moved perpendicular to the body axis. Figures were prepared with Origin (v. 6.1, Origin Lab Corporation, Northampton, MA, USA) and Photoshop software (v. 6.0, Adobe Systems, San Jose, CA, USA).

Data analysis

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 Tables 1–3. The sample size for the straight walks was left at an N of 3 because the criterion for straight walks was a clearly straight head posture throughout the walking sequence and because straight walks were further identifiable through the symmetrical leg movements on either side

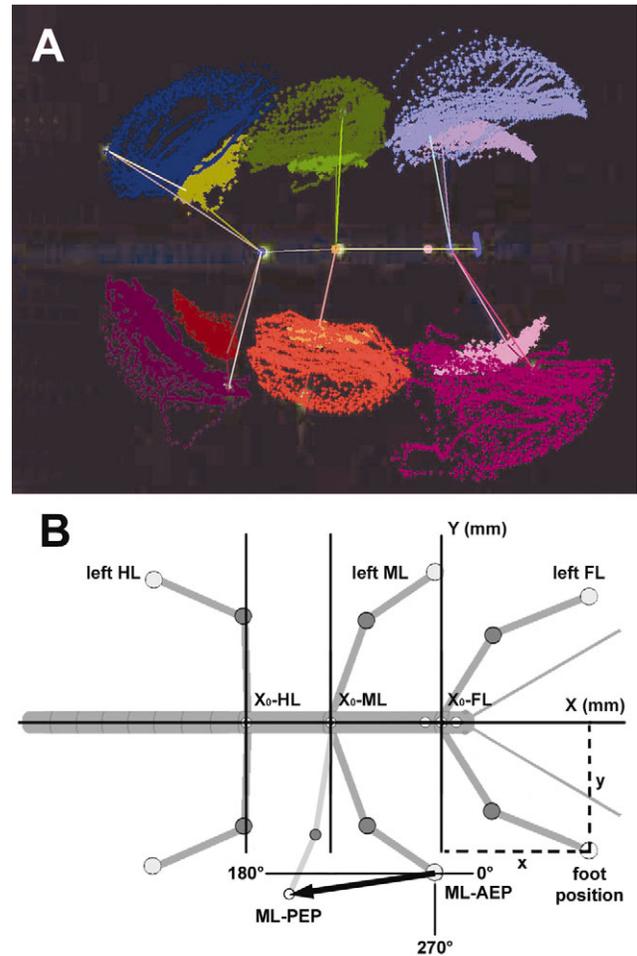


Fig. 1. (A) Overlay of all tracked frames from a single walking sequence. Tracked dots mark the distal ends of the femora and the tibiae of all legs, the head is pointing to the right. The animal was performing a left turn in the depicted sequence. (B) Schematic drawing of the stick insect with the points tracked. x -values always denote points along the axis of the animal whereas y -values mark points perpendicular to the animal. The x_0 -value was always set at the level of the coxae for each leg to give a clear reference point. As an example for the determination of the vector for step length and direction, the right middle leg is drawn in two arbitrary positions, one anterior extreme position (ML-AEP) and one posterior extreme position (ML-PEP). The vectors for all steps connecting the two positions, normalized to the origin in the AEP, gave direction in deg. and step length in mm. The 0–180 deg. axis was always parallel to the body axis and crossed the AEP, 270 deg. always points away perpendicularly. ML, middle leg; FL, front leg; HL, hind leg.

of the animal. For turning in the intact animal, we recorded steps from $N=8$ animals to account for the variability in turning behavior as the animals showed a range of wide to narrow turns (see also Results). For the reduced preparations of two-legged and single-leg animals, we used an N of 4 and 3 animals, respectively, where we chose head movement only to determine the direction of the turn. In using head posture alone and not having five more legs to judge turning behavior from, we may have introduced a bias towards more easily identifiable narrow turns in our sample of the two-leg or the single-leg preparations. However, this has no consequence for the conclusions drawn.

We used linear statistics to describe significance levels between distributions of AEP and PEP- x - and y -values, and tested

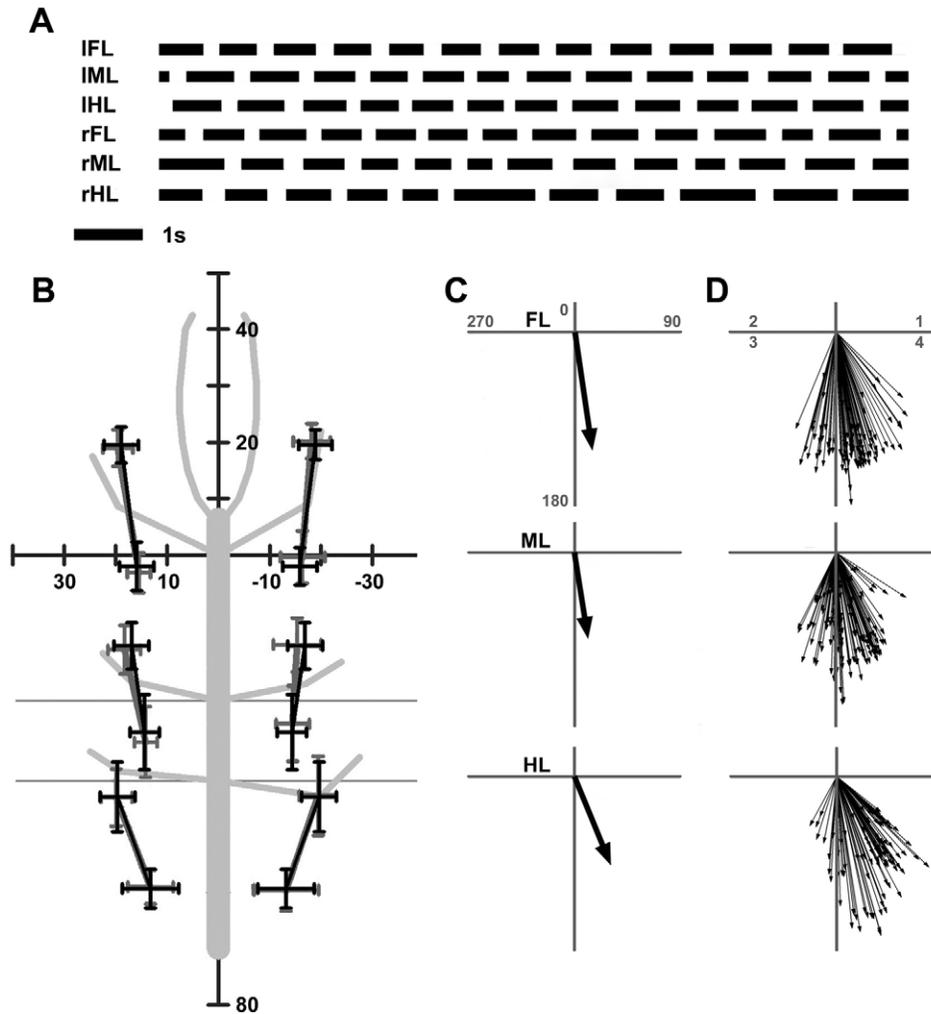


Fig. 2. Straight walking of the intact stick insect on the slippery surface. (A) Pattern of straight walking stepping sequence on the slippery surface. The black bars mark the stance, the white gaps mark the swing phases for the six legs. From top to bottom; left front leg (IFL), left middle leg (IML), left hind leg (IHL) and right front leg (rFL), right middle leg (rML) and right hind leg (rHL). (B) Schematic drawing of a stick insect with the mean anterior extreme position (AEP) and posterior extreme position (PEP) values (and s.d.) for the straight walking animal. Dark gray marks the values for the averaged steps from the left and right side plotted separately, black marks the values for all pooled, averaged and mirror imaged data points. The light gray lines mark the respective x_0 -value for the middle and hind legs. (C) Mean vectors of stance phase movement drawn to the mean step length as calculated between AEP and PEP. (D) Step-to-step variability in angle and length of all stance phase movement vectors for the front, middle and hind legs. All vectors of the right side legs were mirror imaged to show only left side-stepping.

separately for significance using the Mann–Whitney test (*U*-test) because we could not assume normal distribution and because the positions are determined by the activity of two muscle systems. The same applies to the step lengths that were defined as the length of the vector between mean AEP and PEP. For the significance level, we chose $P < 0.05$. The angles of the stance phase movement vector for each leg were determined as the angles between AEP and PEP values to give the mean direction of the step and not the mean movement vector. Therefore, also in this case, we used the *U*-test for the comparison. Again, significance levels were $P < 0.05$. To exclude the effect of individuals, AEP and PEP values, step lengths and angles of stance phase movement between inside and outside legs were tested for each individual animal and under all three experimental conditions. Within each animal and between all animals of each experimental group, i.e. intact, 2L- and 1L-preparation, all inside and outside stepping sequences during turns were significantly different from each other with respect to angle or step length or both whereas left and right legs in the straight walking animals showed no significant differences ($P < 0.05$). Therefore, inside, outside and straight steps of all animals under one experimental condition were pooled and compared. As a test for variance, the *F*-test was applied, with a significance level of 0.05. All values are given as means \pm s.d.

RESULTS

Prior work has shown that stick insects can walk in a coordinated fashion on slippery surfaces, such as mercury or greased glass or metal (Graham and Cruse, 1981; Cruse and Epstein, 1982; Epstein and Graham, 1983; Cruse and Schwarze, 1988; Cruse and Knauth, 1989; Gruhn et al., 2006). However, in these previous studies, neither the leg kinematics were measured nor were the movements of the single legs in straight walking compared with the ones during curve walking, and also whether the resulting movement pattern is influenced by the presence of neighboring legs.

Straight walking on the slippery surface in the six-legged animal

In order to analyze the stepping pattern of stick insects on the slippery surface, stick insects tethered above the walking platform were induced to walk straight with a progressive optomotor stimulus. As shown previously by Gruhn et al. (Gruhn et al., 2006) and in Fig. 2A, stick insects are capable of walking in a coordinated fashion on a slippery surface. We first compared the vectors of the stance phase movement between touch down and lift off between the front, middle and hind legs of a straight walking animal. The marks for the coordinates of the mean AEP at touch down and the coordinates of the mean PEP at lift off are shown in Fig. 2B. The positions are shown with s.d. and connected to show the general direction of stance phase movement for each

Table 1. Mean x - and y -values of the AEP and PEP in the left ('left') and right ('right') straight walking front, middle and hind legs and the pooled values ('straight') with their respective s.d. for the intact animal

	Direction	N	n	Mean (x)	s.d. (x)	Mean (y)	s.d. (y)
Front leg							
AEP	Left	3	52	19.0	3.2	19.5	2.9
	Straight		99	19.5	3.2	18.9	3.3
	Right		47	20.2	3.1	18.2	3.6
PEP	Left	3	52	-3.2	3.6	15.4	2.3
	Straight		99	-2.0	4.3	15.9	3.4
	Right		47	-0.4	4.6	16.4	4.3
Middle leg							
AEP	Left	3	56	9.0	4.3	18.3	3.2
	Straight		125	9.8	4.1	16.9	3.3
	Right		69	9.9	4.8	15.4	4.6
PEP	Left	3	56	-7.4	6.3	14.2	2.2
	Straight		125	-5.6	6.7	14.4	2.9
	Right		69	-4.1	6.7	14.5	3.3
Hind leg							
AEP	Left	3	45	-3.0	5.1	19.9	3.1
	Straight		99	-2.8	6.2	19.6	3.4
	Right		54	-2.7	7.1	19.4	3.7
PEP	Left	3	45	-18.8	3.0	13.2	4.4
	Straight		99	-19.1	3.4	13.2	5.5
	Right		54	-19.3	3.7	13.2	6.3

x -values are given with respect to the coxa of the given leg in mm; negative values represent positions posterior of the coxa. y -positions are given in mm from the midline of the animal; larger values represent more distal points. N =number of animals; n =number of steps evaluated.

leg. The steps of the left and right legs were plotted separately (in gray) and then pooled and plotted again (in black) for comparison of the two sides with the averaged pooled data. The x - and y -mean values of all left and right legs and the pooled values with their respective s.d. for the straight walking intact animals are given in Table 1. The AEP of all tarsi are more distal from the body axis than the lift off PEP creating an average movement vector towards the center and the rear. Between the leg pairs, the middle legs are always placed slightly more centrally than the front and hind legs. The stance phase movement of the straight walking front legs takes place almost entirely anterior of the prothoracic coxae. At the same time, the middle legs operate in a range anteriorly and posteriorly around the mesothoracic coxae, while the hind legs touch down and lift off on average posteriorly of the metathoracic coxae. For the subsequent comparison with the stepping pattern during turns, left and right legs of the straight walking sequences were pooled into a single group.

We chose to describe the direction of stance phase movement through the mean vector angles for the straight walking front, middle and hind legs. These mean vectors are plotted in Fig. 2C as for the left legs only, meaning that the right legs were mirror imaged. Their angles were: FL, 171.7 ± 13.0 deg.; ML, 171.2 ± 17.24 deg.; and HL, 157.9 ± 17.83 deg., which means that all legs perform a slight inward movement during straight walking stance phases. The mean step length between AEP and PEP for the front, middle and hind legs with respect to the body axis as calculated from the x - and y -positions of the single vectors was 22.6 ± 3.8 mm, 16.2 ± 5.4 mm and 18.2 ± 6.1 mm, respectively (see Fig. 2C; Fig. 4A). In all legs, there is considerable step-to-step variability in the direction of their stance phase movements and step lengths during walking episodes. This is shown in Fig. 2D, with the movement vectors of all steps tracked and normalized to the AEP ($N=3$, $n_{FL}=99$, $n_{ML}=125$, $n_{HL}=99$). To visualize left and right leg vectors in one plot, the vectors of the right legs were again mirror imaged.

Turning in the six-legged animal

We then compared the leg movement patterns of the straight walking stick insect with those of the turning animal. Left or right turning was elicited at random by an optomotor stimulus with black stripes moving in the respective direction. Again, we recorded AEP and PEP coordinates of each stance phase for the three leg pairs. The steps of all legs, marked as arrows between AEP and PEP of a typical straight walking sequence (Fig. 3Ai) and two curve walking sequences (Fig. 3Aii,Aiii) are shown in Fig. 3A. They show that nearly all steps of all legs in a straight walking sequence are outside-in and front-to-back movements, highly symmetrical between the contralateral sides (Fig. 3Ai). However, turning leads to a great asymmetry between inside and outside legs clearly visible in the two extreme examples (Fig. 3Aii,Aiii). The mean AEP and PEP for all pooled steps from left and right turns projected as if all turns were left turns are shown with s.d. in Fig. 3B after testing inside vs outside leg in each animal to exclude the effect of individuals (see Materials and methods). Red marks the pooled values for the inside legs, dark yellow for the outside legs. For better comparison, we also show the mean positions of the straight walking animal (black). The higher standard deviation in the AEP and PEP values for the inside and outside legs reflects an increased variability in stepping pattern during the turns ($P < 0.05$). The x and y values and their respective s.d. are summarized in Table 2.

The mean AEP for the inside front leg ($N=8$, $n=315$) was significantly more posterior and more lateral ($P < 0.001$) whereas the mean PEP was significantly more anterior and more central when compared with the straight walking front leg ($P < 0.001$). However, the outside front leg had a significantly more centrally and anteriorly located AEP ($P < 0.001$) whereas the PEP was significantly more anterior and more lateral than in the straight walking animal ($P < 0.001$). Interestingly, the PEP x -positions of the inside and outside front legs were not significantly different from each other ($P=0.93$). The changes in the touch down and lift off positions and movement patterns of the middle leg are equally striking. The AEP

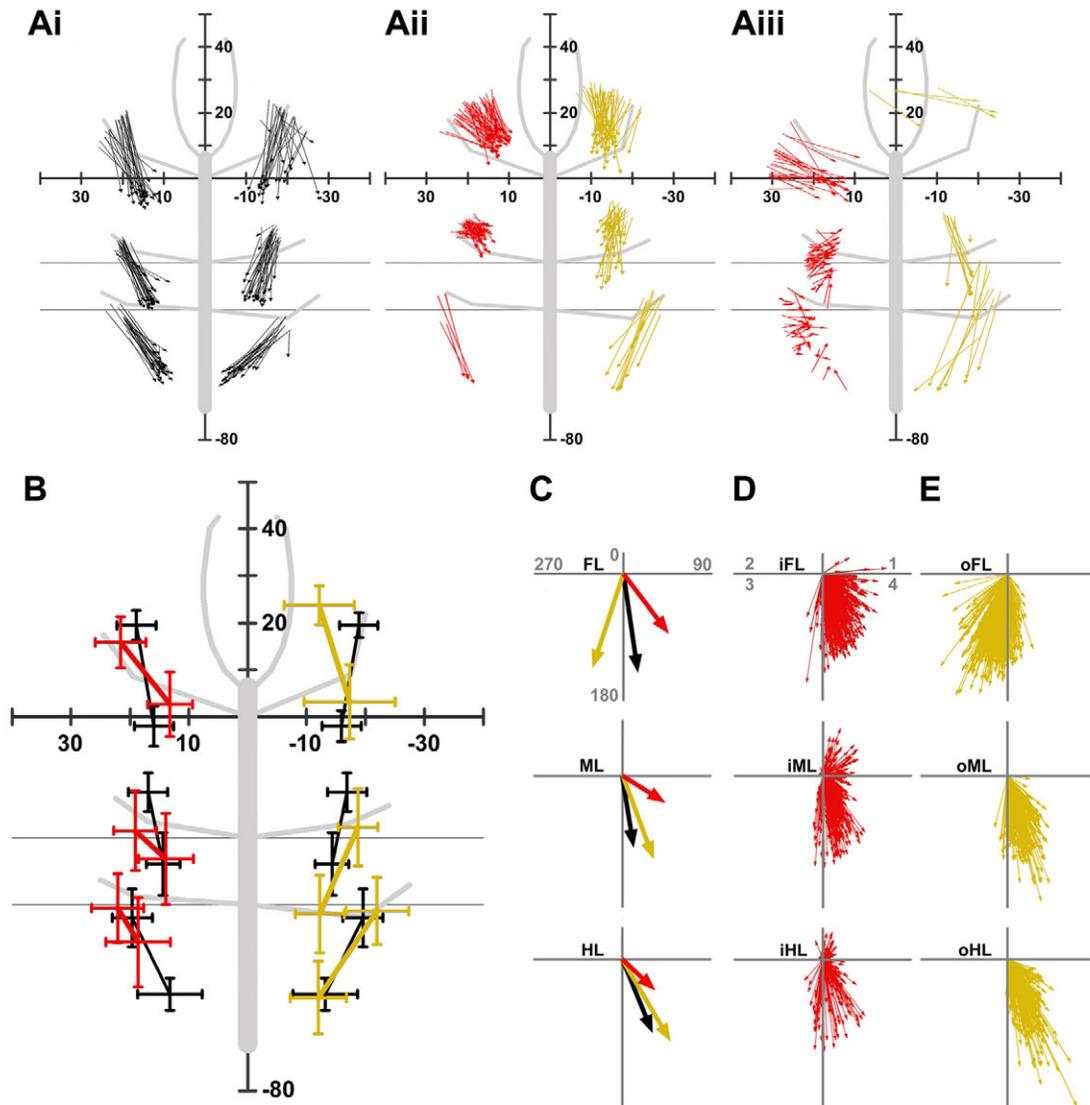


Fig. 3. Turning in the intact stick insect on the slippery surface. (Ai-iii) Three typical walking sequences with the anterior extreme position (AEP) and posterior extreme position (PEP) connected by arrows for one straight walking sequence (Ai, black), and two curve walking sequences (Aii and Aiii) of differing steepness. Red marks inside legs, yellow marks outside legs. (B) Schematic drawing of a stick insect with the mean AEP and PEP values (and s.d.) for the straight walking and turning animal. Red marks the values for the averaged steps for the inside leg, dark yellow for the outside legs and black for the straight stepping legs (from Fig. 2). The light gray lines mark the respective x_0 -value for the middle (ML) and hind (HL) legs. (C) Mean vectors of stance phase movement of the inside (red), outside (dark yellow) and straight stepping (black) left legs, drawn to the mean step length as calculated between AEP and PEP. (D,E) Step-to-step variability in angle and length of all stance phase movement vectors for the inside (D, red) and outside (E, yellow) front, middle and hind legs (iFL, inside front legs; iML, inside middle legs; oFL, outside front legs; oML, outside middle legs). All vectors of the right side legs were again mirror imaged to show only left side stepping; coloring is the same as in A.

of the inside leg is now significantly more caudally and laterally located than that of the straight walking leg ($P < 0.001$) whereas the mean PEP does not change. At the same time, the outside middle leg has a significantly more posterior and lateral AEP ($P < 0.001$) and a significantly more caudal and central PEP than the straight walking leg ($P < 0.001$). In this case, the AEP between the inside and outside legs was the same. The hind legs show a major change in movement pattern only for the inside leg of a turn. Although touching down close to but still more laterally than at the AEP of the straight walking leg ($P_x = 0.008$; $P_y < 0.001$), the inside leg often moves very little during a stance phase and has a significantly more anterior mean PEP ($P < 0.001$) that is relatively close to the AEP. The outside hind leg, however, shows a more moderate change in

movement pattern resulting in an only slightly (yet significantly) more lateral ($P_y = 0.001$) but otherwise unchanged AEP. During stance, the leg is moved to a PEP that is significantly more posterior ($P < 0.001$) but has the same distance to the body axis as in the straight walking animal. Again, the AEP of the inside and outside legs were the same.

The resulting vectors from averaging the vector lengths and the direction of all inside (red) and outside (dark yellow) stance phase vectors between AEP and PEP are plotted in Fig. 3C. The respective values with s.d. are plotted in Fig. 4 and given in Table 3. For comparison, the mean vector for the respective straight walking legs is added in black. In comparison with straight walking, all inside legs show a significantly shortened step ($P < 0.001$) and significantly

Table 2. Mean x - and y -values of the AEP and PEP in the inside ('in'), straight ('straight') and outside ('out') stepping intact animal and the reduced two-leg and single-leg preparations

	Direction	N	n	Mean (x)	s.d. (x)	Mean (y)	s.d. (y)
Intact animal							
Front leg							
AEP	In	8	315	15.9	5.4	21.6	4.3
	Straight	3	99	19.5	3.2	18.9	3.3
	Out	8	277	23.8	4.1	12.2	5.9
PEP	In	8	315	2.6	6.9	13.2	3.8
	Straight	3	99	-2.0	4.3	15.9	3.4
	Out	8	277	3.2	7.9	17.4	7.8
Middle leg							
AEP	In	8	264	1.5	8.5	19.1	3.7
	Straight	3	125	9.8	4.1	16.9	3.3
	Out	8	178	2.3	8.3	18.8	3.4
PEP	In	8	264	-4.5	9.8	13.9	4.7
	Straight	3	125	-5.6	6.7	14.4	2.9
	Out	8	178	-16.3	8.3	12.3	4.2
Hind leg							
AEP	In	5	192	-0.8	7.4	22.1	4.4
	Straight	3	99	-2.8	6.2	19.6	3.4
	Out	5	163	-1.4	7.2	22	5.3
PEP	In	5	192	-8.0	9.5	18.6	5.5
	Straight	3	99	-19.1	3.4	13.2	5.5
	Out	5	163	-19.8	7.7	12.0	4.8
2L-preparation							
Front leg							
AEP	In	4	192	18.9	4.6	18.0	4.3
	Straight	3	63	24.0	1.8	12.5	2.6
	Out	4	166	24.5	2.3	10.2	3.9
PEP	In	4	192	8.4	6.8	9.9	2.2
	Straight	3	63	6.8	6.7	12.7	2.6
	Out	4	166	5.2	5.0	16.8	4.7
Middle leg							
AEP	In	4	105	11.1	5.0	16.8	3.2
	Straight	3	103	16.6	3.6	12.3	3.8
	Out	4	80	17.3	2.3	11.2	2.7
PEP	In	4	105	8.8	4.0	9.1	3.0
	Straight	3	103	6.5	4.2	11.0	2.6
	Out	4	80	1.7	6.7	13.3	2.7
1L-preparation							
Front leg							
AEP	In	3	85	13.5	8.6	20.8	4.7
	Out	3	98	25.5	1.8	5.1	6.1
PEP	In	3	85	5.7	4.2	9.6	3.2
	Out	3	98	10.5	5.8	10.0	4.9
Middle leg							
AEP	In	4	78	3.8	7.5	18.8	2.6
	Out	3	52	14.2	4.3	13.1	4.0
PEP	In	4	78	4.9	4.4	9.8	2.9
	Out	3	52	1.5	6.1	13.9	3.2

Straight stepping values are the pooled values from Table 1. x -values are given with respect to the coxa of the given leg in mm; negative values represent positions posterior of the coxa. y -positions are given in mm from the midline of the animal; larger values represent more distal points. N =number of animals; n =number of steps evaluated. All values are given with s.d.

smaller angles ($P<0.001$), reflecting the pulling-like movements of the inside legs. For the outside legs of a turn, a significant increase in the angle of the mean vector is only found in the front leg ($P<0.001$), while the step length in this leg remains unchanged. In the middle and hind outside legs, the opposite can be observed: the angle of the stance phase movement is slightly but significantly smaller ($P_{ML}<0.001$; $P_{HL}<0.01$) but the step length is significantly increased ($P<0.001$).

An additional change between straight walking and turning is that the movement direction of the stance phase vectors of the inside steps in all legs is more variable than in the straight walking animal ($P<0.05$). The combined vectors of all steps recorded are given in

Fig. 3D,E and are plotted as left leg movements, both for the inside and outside legs. They are again normalized to the AEP to show the variability of length and direction of the movements observed. The majority of vectors between AEP and PEP for the front, middle and hind inside legs (red), plotted in Fig. 3D, are in quadrant 4 showing a front-to-back and outside-in movement of all legs. For the inside middle and hind legs, an additional second group of vectors is in quadrant 1, marking steps in the back-to-front, outside-in direction. Note that the depiction of vectors in this graph does not account for the additional variability in the location of touch down and lift off. By contrast, the outside leg vectors in Fig. 3E (dark yellow) show a similar variability than in the straight walking

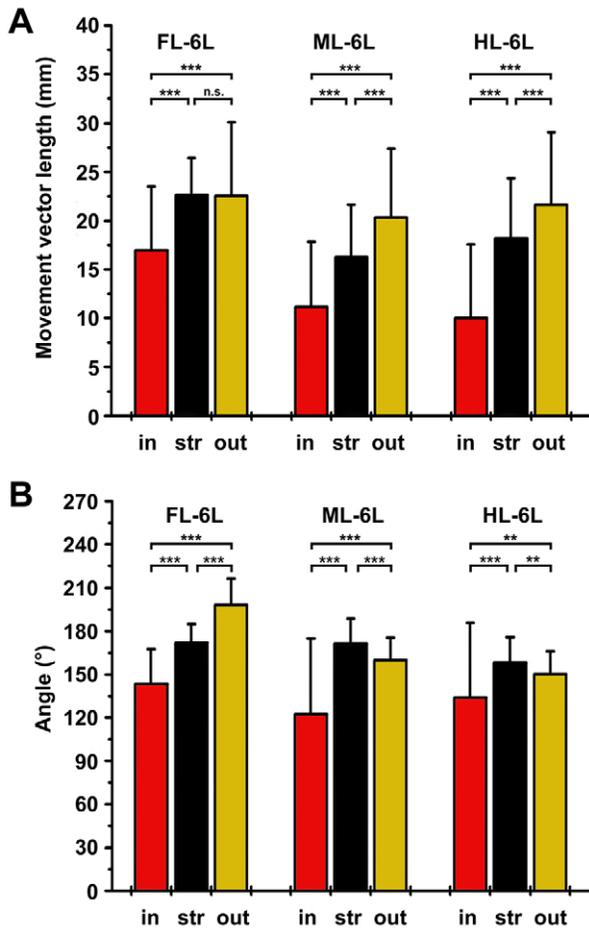


Fig. 4. Turning vs straight stepping in the intact stick insect. (A) Mean step length of front, middle and hind legs during inside turning (red, in), straight stepping (black, str) and outside leg stepping (dark yellow, out) in mm. (B) Mean angles of the stance phase movement vector between AEP and PEP in front, middle and hind legs under the three behavioral conditions, given in deg. Labeling is the same as in A. A 90 deg. angle means a movement perpendicular to and towards the body, 180 deg. a front-to-back movement parallel to the body. Significance levels are: ** $P < 0.01$; *** $P < 0.001$; n.s., not significant. FL, front legs; ML, middle legs; HL, hind legs.

animals. Here, only the direction is shifted so that the majority of vectors for the front leg are in quadrant 3, and show an inside-out and front-to-backwards shift described earlier. The middle and hind leg vectors are in quadrant 4 and show, a slight but significantly reduced variability in their front-to-backwards but outside-in movement of the legs, compared with the straight walking animal ($P < 0.05$).

Thus, turning behavior of the intact stick insect on the slippery surface involves changes in the combination of at least two factors. On the inside of the turn, the step length between AEP and PEP is shortened significantly while the tarsi are placed more laterally in relation to the PEP. This produces a movement in the inside front and middle legs as if to pull the body into the curve, while the inside hind leg can either act as if to pull, or simply work as a pivot around which the animal rotates. In contrast, the outside front legs act as if to pull, the middle and hind legs as if to push the body on a radius around the curve. This is done with no or relatively small changes in the step length between AEP and PEP. Instead, the outside leg

placement changes. The front leg touches down more anteriorly and centrally, creating an inside-out movement of the leg and a marked change in the angle of the movement vector during stance. At the same time the tarsi of the middle and hind outside legs are placed more laterally and move backwards and inside towards the body during each stance phase.

Straight walking and turning in the two-leg preparation

Does the presence of neighboring anterior or posterior legs affect the movement pattern of a single leg and the coordination of its joints during straight forward stepping or turning on the slippery surface? We first removed the middle and hind legs to yield a two-leg–front leg preparation (2L–FL), which we subsequently reduced to a single-leg–front leg preparation (1L–FL) to investigate such a potential influence.

During straight walking, the AEP and PEP of the front legs in the two-legged animal are both positioned significantly further anteriorly and centrally in comparison with the intact animal ($P < 0.001$). Their AEP and PEP in the straight walking and turning 2L–FL-preparations are summarized in Fig. 5Ai. For better comparison, Fig. 5A also shows the data for the intact straight walking animal as gray connections between AEP and PEP. The respective x - and y -values of AEP and PEP (in mm) for the front and middle legs and the s.d. are given in Table 2. In addition to the changes in AEP and PEP, the step length is significantly reduced to 17.6 ± 6.6 mm ($P < 0.001$) and the angle of stance phase movement is slightly but significantly bigger (178.3 ± 12.5 deg.; $P = 0.001$) than that measured for the straight walking front legs in the intact animal (Fig. 5B,E,F).

The AEP and PEP of the front legs in the turning 2L-preparation are shown in red (inside legs) and dark yellow (outside legs) in Fig. 5A. Turning was determined by head position pointing in the direction of the turn, as described in the Materials and methods section. The connections between AEP and PEP of the intact turning animal are shown in lighter shades of their respective colors for better comparison. Generally, the leg positions are in a similar orientation to the body compared with those in the intact animal. Yet, the inside 2L–front leg on average touches down and lifts off significantly more anteriorly and centrally than the inside 6L–front leg ($P < 0.001$). The outside 2L–FL, however, only has a significantly more central AEP ($P_x = 0.251$; $P_y < 0.001$) and a significantly more anterior and slightly but significantly more central PEP ($P_x < 0.001$; $P_y < 0.05$) (see Table 2). Despite of the changes in AEP and PEP, the mean stepping movement angles for the inside and for the outside legs of the turning 2L–FL-preparation remain the same as those of the turning intact animal ($P_{in} = 0.086$; $P_{out} = 0.588$) (Table 3). By contrast, the step length of both legs was reduced significantly to 14.1 mm (inside leg, s.d.=5.7; $P < 0.001$) and 21.1 mm (outside leg, s.d.=5.8; $P = 0.002$) (Fig. 5E,F; Table 3). Interestingly, the x -positions of the AEP and PEP in the straight stepping and the outside 2L–FL-preparation are the same. No change is seen in that the vast majorities of the inside (Fig. 5C) and outside (Fig. 5D) leg vectors, normalized to the touch down position, are in quadrant 4 and 3, respectively. Yet, the inside legs show a decreased, the straight stepping legs an increased and the outside front legs an unchanged variability of stance phase vectors after the reduction of the preparation to 2L–FL ($P < 0.05$).

In analogy to the front leg experiments, we also removed the front and hind legs to yield a two-leg–middle leg preparation (2L–ML). We refrained from studying the hind legs as they are known to perform only backward walking when they are the only remaining legs (Bässler et al., 1985). The mean AEP and PEP

Table 3. Mean stride length as determined by the mean stepping vector length and mean angle of stance phase movement for the inside ('in'), straight ('straight') and outside ('out') stepping intact animal for the front, middle and hind legs. For the reduced two-leg- and single-leg preparations only the values for the front and middle legs are given

Direction		Length l (mm)						Angle α (deg.)					
		In		Straight		Out		In		Straight		Out	
Leg	Preparation	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean (α)	s.d. (α)	Mean (α)	s.d. (α)	Mean (α)	s.d. (α)
Front leg	Intact animal	17.0	6.6	22.6	3.8	22.6	7.6	143.3	24.0	171.7	13.0	198.0	18.1
	2L-preparation	14.1	5.7	17.6	6.6	21.1	5.8	140.8	20.2	178.3	12.5	197.1	15.3
	1L-preparation	16.5	3.8	–	–	16.2	5.1	121.5	34.5	–	–	198.1	14.4
Middle leg	Intact animal	11.1	6.7	16.2	5.4	20.3	7.1	122.3	52.5	171.2	17.2	159.6	15.2
	2L-preparation	10.5	3.7	10.8	3.2	16.1	5.6	112.5	40.0	171.9	21.0	188.8	13.2
	1L-preparation	12.1	4.4	–	–	13.5	6.0	89.6	42.2	–	–	180.2	19.8
Hind leg	Intact animal	10.0	7.6	18.2	6.1	21.7	7.4	133.7	51.9	157.9	17.8	149.9	16.0
	2L-preparation	–	–	–	–	–	–	–	–	–	–	–	–
	1L-preparation	–	–	–	–	–	–	–	–	–	–	–	–

For the 1L–ML-preparation, only inside and outside stepping was analyzed. N =number of animals; n =number of steps evaluated. All values are given with s.d.

positions in straight walking and turning 2L–ML-preparations are shown in Fig. 5Aii, again, with the connections between AEP and PEP from the intact animal given in lighter shades of the respective colors. The resulting mean vectors of all stance phase movements are given in Fig. 5B.

During straight walking, the 2L–ML-preparation has mean AEP and PEP that are both positioned significantly more anteriorly and centrally than in the intact animal ($P<0.001$) (see Table 2 for the summary of x - and y -values). In addition, as seen in the 2L–FL-preparation, the step length of the straight walking 2L–ML-preparation is significantly reduced (to 10.8 ± 3.2 mm; $P<0.001$), while the angle of the mean stance phase movement vector remains unchanged ($P=0.588$) (Fig. 5B).

In the turning 2L–ML-preparation, inside steps are performed significantly ($P<0.001$) more anteriorly and centrally to the mesothoracic coxae and almost at a right angle to the body axis. Still, the angle of the mean inside stance phase vector in the 2L–ML-preparation is slightly but barely not significantly smaller than that of the inside middle leg in the intact animal ($P=0.05$) and the inside step length is also not significantly reduced ($P=0.656$) (Fig. 5E,F). The outside middle legs, however, show a much more distinct change in movement pattern, when compared with the intact animal, than all other legs investigated. They touch down significantly more anteriorly and centrally than the outside legs of the intact turning animal ($P<0.001$) but in almost the same location than the 2L–ML straight stepping legs ($P_x=0.889$; $P_y=0.02$). Their PEP is significantly anterior to the outside middle leg PEP of the intact animal ($P<0.001$), yet significantly posterior to the straight stepping 2L–ML ($P<0.001$). The resulting mean angle of stance phase movement is significantly bigger than that of the outside leg in the intact animal (188.8 ± 13.2 deg.) and that of the straight stepping leg in the 2L–ML-preparation ($P<0.001$). The outside step length is now significantly shorter than in the intact preparation (16.1 ± 5.6 mm; $P<0.001$) (Fig. 5E,F) but still significantly longer than in the straight stepping 2L–ML ($P<0.001$). The movement pattern now resembles more that of the outside front leg. The graphs with all stance phase movement vectors normalized to their AEP in Fig. 5C,D show that the range of possible vectors is similar for both, inside (Fig. 5C) and outside (Fig. 5D) steps. It is, however, noteworthy that the number of long steps is generally reduced and that the direction of outside leg vectors appears to have shifted over to quadrant 3 compared with the intact animal. As a result, the overall variability for the stepping angle in the inside and outside

middle legs is significantly reduced ($P<0.05$), while the variability for the straight walking legs remains the same as in the intact animal.

Altogether, the movements of the inside and outside front and middle legs in the reduced 2L-preparation can still be readily distinguished from those of the straight walking animal and they are generally similar to the turning movements of the legs in the intact animal. Interestingly, two-legged animals seem to express more narrow turns, which is reflected in the reduction in the numbers of long steps more or less parallel to the body and the resulting changes in the mean angles of the stance phase movement vectors between AEP and PEP. This will be discussed later.

Turning in the single-leg preparation

Is the single leg still capable of performing the stepping movements of a turning animal during an optomotor response or does the removal of the contralateral leg change these capabilities? To test this, we further reduced the two-legged animals to single-leg preparations to study context-dependent single leg stepping. As single-leg preparations usually step only for relatively short periods of time and straight stepping is not easy to determine in single-leg animals, we only analyzed stepping sequences in which the animal was clearly turning as determined by a continuously bent head position, which leads the direction of turning (Gruhn et al., 2006). Fig. 6A shows the mean AEP and PEP of the turning single-front leg and single-middle leg preparations. For better comparison, both movement types are combined in one figure and also show the data for the 2L-preparation and the intact turning animal as connections between AEP and PEP in progressively lighter shades of the respective colors (front leg: Fig. 6Ai,Aii; middle leg: Fig. 6Aiii,Aiv). The mean stance phase vectors of all single leg steps, normalized to one origin, are shown in Fig. 6B, and all AEP and PEP values for the 1L-preparation are given in Table 2.

The single front leg performs clear inside-leg-like movements upon the appropriate turning stimulus (red trace, Fig. 6Ai). In comparison with the intact animal (light pink) and to the 2L–FL-preparation (light red), however, there are differences in the touch down and lift off positions. The AEP of the inside front leg is on average significantly further caudal and lateral than in the 2L–FL-preparation ($P<0.001$) but not different from the intact animal ($P_x=0.11$; $P_y=0.59$). The mean PEP of the 1L-inside front leg is more caudally located than in the 2L–FL-animal but not as far as in the intact animal. Compared with the 2L-preparation its y -position remains unchanged ($P=0.14$). The resulting mean angle of the vector

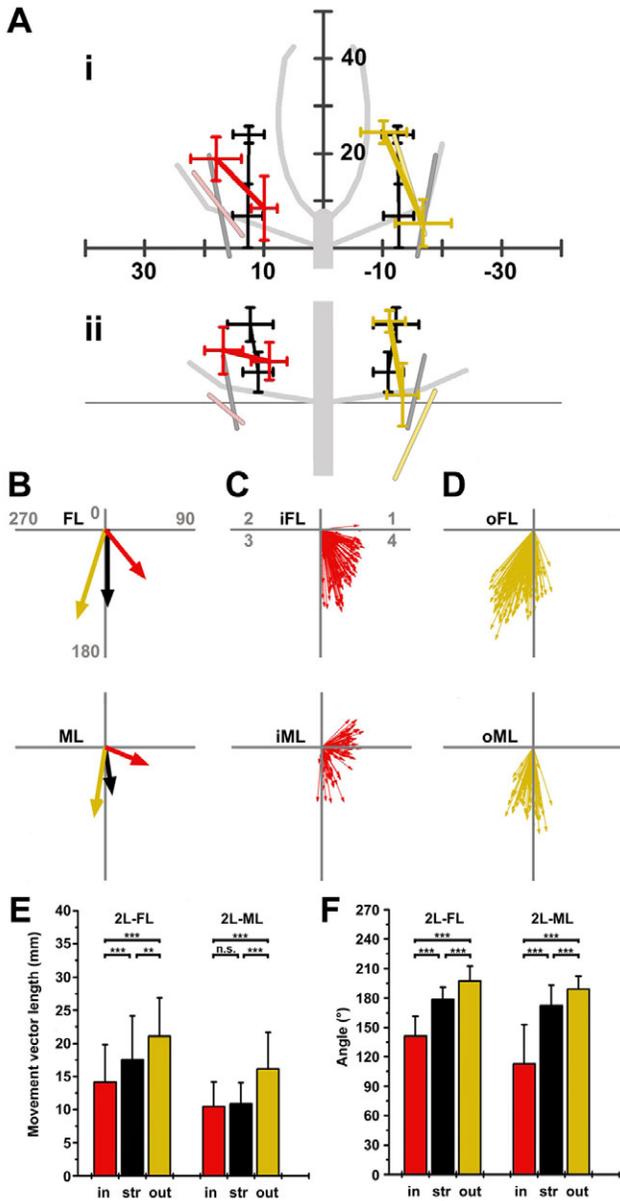


Fig. 5. Turning and straight stepping in the two-leg preparation (A) Schematic drawing of a stick insect with the mean anterior extreme position (AEP) and posterior extreme position (PEP) values (and s.d.) for the straight walking and turning two-legged-preparation (2L). Red marks the values for the averaged steps for the inside leg, dark yellow for the outside legs and black for the straight stepping legs. Pink (inside), light yellow (outside) and gray (straight walking) mark the connections between AEP and PEP in the respective legs of the intact animal. (Ai) 2L–front leg preparation (2L–FL). (Aii) 2L–middle leg preparation (2L–ML). (B) Mean vectors of stance phase movement of the inside (red), outside (dark yellow) and straight stepping (black) left legs, drawn to the mean step length as calculated between AEP and PEP for the front and middle legs. (C,D) Step-to-step variability in angle and length of all stance phase movement vectors for the inside (C, red) and outside (D, dark yellow) front and middle legs. All vectors of the right side legs were again mirror imaged to show only left side stepping; coloring is the same as in Figs 3 and 4. (E) Mean step length of front and middle leg 2L-preparation during inside turning (red, in), straight stepping (black, str) and outside leg stepping (dark yellow, out) in mm. (F) Mean angles of the stance phase movement vector between AEP and PEP in the front and middle legs under the three behavioral conditions, given in deg. Coloring is the same as in E. Significance levels in E and F are: ** $P < 0.01$; *** $P < 0.001$; n.s., not significant. iFL, inside front legs; iML, inside middle legs; oFL, outside front legs; oML, outside middle legs.

between AEP and PEP is 121.5 ± 34.5 deg. and thus significantly smaller than that in the 2L–FL-preparation and in the intact animal ($P < 0.001$) (Fig. 6B). Therefore, the direction of movement for the single inside front legs describes a more extreme pull-like movement into the direction of turning. The mean step length of the inside front leg is significantly longer than in the 2L-preparation ($P < 0.01$) but not significantly different from the intact preparation ($P = 0.269$) (see Table 3).

On the outside of the turn, the single front leg also produces clear outside-leg-like stepping movements but touches down significantly more anteriorly and centrally than in the other two preparations ($N = 3$, $n = 98$; $P < 0.001$). The mean step length is also significantly shorter (16.2 ± 5.1 mm; $P < 0.001$), leading to a PEP that is significantly more anterior and more central than in the two other outside leg preparations ($P < 0.001$). The mean angle of the outside leg stance phase movement vector, however, remains at 198.1 ± 14.4 deg., virtually unchanged from the other two preparations (Fig. 6B,E,F; Table 3). Interestingly, the step lengths of the inside and outside 1L–FL-preparations are not significantly different from each other ($P = 0.28$).

As found for the single-front leg preparation, the single middle leg also produces distinctly different movements depending on its function as an inside or outside leg during turns. For the inside of the turn, the AEP and PEP are shown in Fig. 6Aiii. The leg has a mean touch down position that is slightly but significantly more anterior than the AEP of the inside middle leg in the intact animal ($N = 4$, $n = 74$; $P_x = 0.047$) (Fig. 6Aiii). However, the PEP of the 1L–ML inside leg is actually anterior to its AEP and is significantly more anterior and central than the PEP of the intact inside leg ($P < 0.001$). Compared with the 2L–IL, the 1L–ML step occurs significantly more posteriorly and tarsal touch down occurs more laterally ($P < 0.001$). The resulting mean angle of the stance phase movement in the 1L–ML inside leg is again significantly reduced to 89.6 ± 42.2 deg. ($P < 0.001$), and is now perpendicular to the body axis. At the same time, the step length is 12.1 ± 4.4 mm slightly, yet significantly longer than in the intact preparation or in the 2L–ML inside leg ($P < 0.05$) (Fig. 6B,E,F).

The outside middle leg stepping movements are again clearly distinct from those of the inside leg but, just as in the 2L-preparation, also deviate significantly from the movements observed in the outside middle legs of the intact animal. Both AEP and PEP are again significantly more anterior and the AEP is significantly more central than that of the outside middle leg of the intact animal ($P < 0.001$). This creates a much straighter stepping movement vector as compared with the intact outside middle leg. However, the movement of the outside 1L–ML is relatively similar to the stepping movements of the outside 2L–ML. Its AEP is significantly more posterior and more lateral than the 2L–ML outside AEP ($P_x < 0.001$; $P_y < 0.01$) whereas the PEP only differs slightly in the y -position ($P_x = 0.92$; $P_y = 0.032$). Due to these shifts in AEP and PEP, the mean angle of the stance phase movement is significantly reduced to 180.1 ± 19.8 deg. compared with the angle in the 2L outside middle leg ($P = 0.011$). Yet, it is significantly larger than the angle observed in the intact animal ($P < 0.001$) (Fig. 6B). At the same time, the step length of the outside 1L–ML is once again further and significantly reduced to 13.5 ± 6.0 mm ($P < 0.01$). As for the front legs, the step lengths of the inside and outside 1L–ML-preparations are not significantly different from each other ($P = 0.545$).

The vectors for all inside and outside steps of the 1L-preparation, normalized to the AEP are given Fig. 6C,D, with inside steps in 6C and outside steps in 6D. Changes in the distribution of movement vectors in the front leg can be observed on the inside, which now

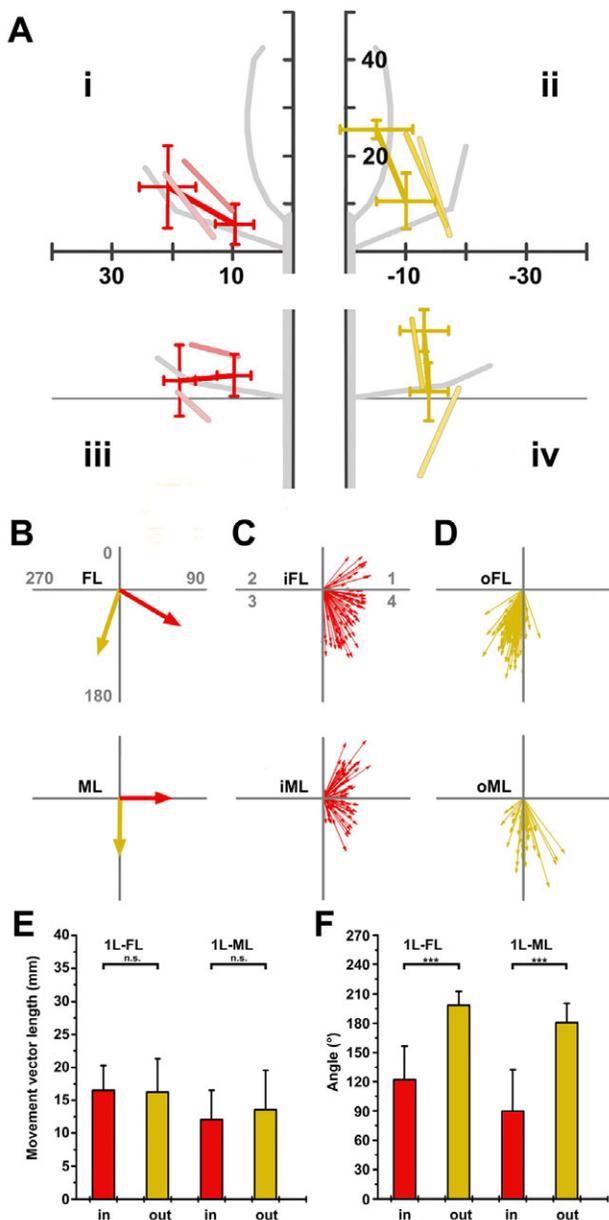


Fig. 6. Turning in the single-leg preparation. (A) Schematic drawings of a stick insect segment with the mean anterior extreme position (AEP) and posterior extreme position (PEP) values (and s.d.) for the turning 1L-preparation in the inside (Ai) and outside (Aii) front leg, and the inside (Aiii) and outside (Aiv) middle leg. The darkly colored traces with error bars mark the single-leg preparations, the lighter shaded red and yellow bars mark the connection between mean AEP and PEP in the 2L (red) and the intact preparations (light red or yellow) for comparison (B) Mean vectors of stance phase movement of the inside (red) and outside (yellow) stepping left legs, drawn to the mean step length as calculated between AEP and PEP for the single front and middle legs. (C,D) Step-to-step variability in angle and length of all stance phase movement vectors for the inside (C, red) and outside (D, dark yellow) front and middle legs (iFL, inside front legs; iML, inside middle legs; oFL, outside front legs; oML, outside middle legs). All vectors of the right side legs were again mirror imaged to show only left side stepping; coloring is the same as in Figs 3, 4 and 5. (E) Mean step length of front and middle leg 1L-preparation during inside turning (red, in), and outside leg stepping (dark yellow, out) in mm. (F) Mean angles of the stance phase movement vector between AEP and PEP in the front and middle legs under the two behavioral conditions, given in deg. Coloring is the same as in E. Significance levels in E and F are: *** $P < 0.001$; n.s., not significant.

also shows occasional back-to-front movements during stance phase and an increased variability, compared with the 2L-preparation ($P < 0.05$) (Fig. 6C). At the same time the outside front leg stepping vector distribution remains unchanged. The range of possible movement vectors in the inside middle leg is significantly increased again but still smaller than in the intact animal ($P < 0.05$). Meanwhile, the variability of outside middle leg vectors is now even significantly more variable than the ones of the intact animals ($P < 0.05$) (Fig. 6D).

In summary, with the exception of the single middle outside legs, we could observe the same general movement patterns during turns in the 1L-preparation that can also be found in the intact animal or the two-legged preparation. For the single outside middle leg, there is a similarity to the outside stepping movements in the 2L-ML-preparation. These stepping movements, however, share similarities with straight stepping in the 2L-ML-preparation with respect to the mean step length and the angle of the stance phase movement vector. For all legs in the 1L-preparation, we noted an increased tendency to perform leg movements that are on the extreme end of the spectrum of what is found in the intact animal. This will be discussed later.

Inside middle leg step-to-step variability

The intact stick insect can perform a range of turns from wide to narrow. For the middle leg stance phase during a tight turn, this can lead to a reversal in stepping direction to a back-to-front movement (Jander, 1985; Dürr, 2005; Dürr and Ebeling, 2005). For the stick insect turning on the slippery surface, we have observed a similarly great range of movement patterns for the inside middle leg, which generally did not change with increasing reduction of the preparation. This is also reflected in the movement vector variability in Fig. 3C,D, Fig. 5C,D, Fig. 6C,D. For the inside middle leg we essentially observed a continuum between three types of movement: (1) a front-to-back, outside-in movement; (2) a strictly outside-in movement that can take place either anteriorly, posteriorly or at the same level as the coxa; and (3) a back-to-front outside-in movement. The range of variability in the movement vectors, however, does not give the information on the distribution of AEP and PEP among preparations. We, therefore, plotted the x - vs y -values of the AEP and PEP in the inside middle legs of the three preparations and then the frequencies with which they occur to see if this frequency changes with increasing reduction to a single-leg preparation (Fig. 7A-I).

Let us first compare the x -value distributions among preparations. X -values with a positive sign mark positions anterior to the coxa, those with negative signs mark positions posterior to the coxa. In the intact animal, the tarsus was seen to be placed anywhere between +17 mm and -25 mm along the axis (Fig. 7A,B). However, there appear to be two main areas of preferred placement, one around +10 mm and the other around -10 mm. The PEP x -position also appear to be widely distributed between +15 mm and -24 mm, with a majority of lift offs occurring between +8 mm and -10 mm and around -18 mm (Fig. 7A,B). The wide distribution of touch down positions anteriorly and posteriorly of the coxa reflects the great variability in stepping patterns seen in the intact animals. It results from mostly front-to-back directed steps, which also explain the posteriorly located second concentration of touch down and lift off positions.

In both reduced middle leg preparations, we observed more sideways-directed movements on the slippery surface. This causes a more narrow distribution among the touch down and lift off positions for the inside leg, which are, however, largely within the range of positions observed in the intact animal. Fig. 7D shows the distributions of touch down and lift off positions of the inside middle

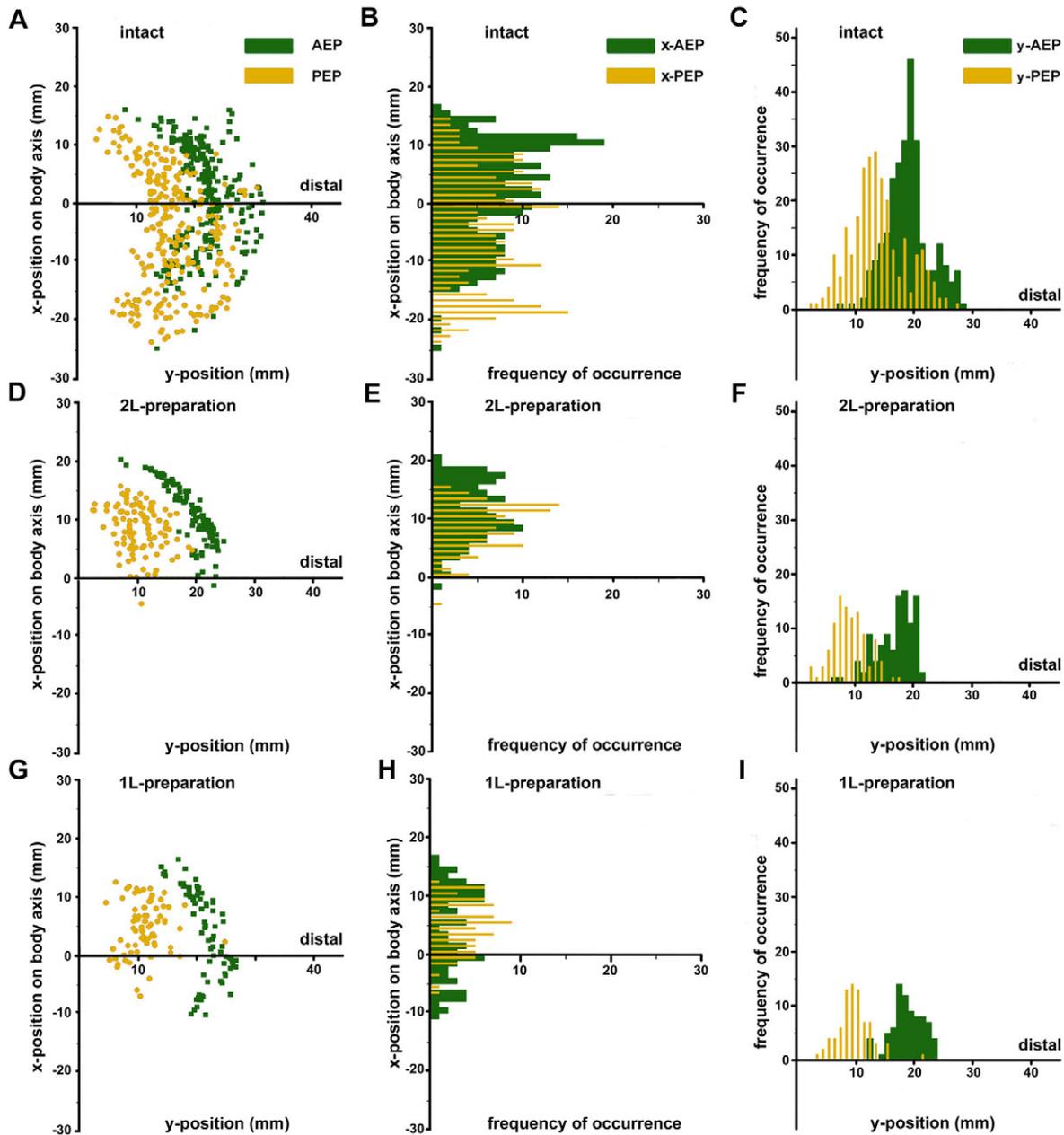


Fig. 7. Variability of the anterior extreme position (AEP) and posterior extreme position (PEP) during inside stepping in the middle leg. (A–C) Intact animal, (D–F) 2L-preparation and (G–I) 1L-preparation. A, D, G: Scatter plots of the AEP and PEP of the middle inside leg in the intact, the 2L- and the 1L-preparation. B, C, E, F, H, I: Histograms of the frequency of the occurrence of AEP and PEP x - (B, E, H) and y -positions (C, F, I) in the inside middle leg of the intact animal, the 2L-preparation and the 1L-preparation. AEP in green; PEP in orange. All positions are given in mm.

leg in the 2L-preparations. The frequency of occurrence of AEP and PEP x -positions is shown in Fig. 7E. The AEP is now more anterior, and quite uniformly distributed between +21 mm and –2 mm around the coxa. The PEP x -position is on average slightly more posterior than that of the AEP but also ranges only from +16 mm to –4 mm (Fig. 7E). Overall, the variability of the AEP x -positions is significantly reduced ($P < 0.05$) over that of the inside steps of the middle leg in the intact animal. The distribution of AEP and PEP in the single-leg preparation is plotted in Fig. 7G and the frequency of occurrence of the AEP and PEP x -positions for the respective steps is plotted in Fig. 7H. Compared with the 2L-preparation and as mentioned earlier, the single-leg preparation has

the same if not even an increased tendency to produce more sideways movements than the inside leg in the intact turning animal. This is reflected by an increased variability in AEP and PEP x -positions ($P < 0.05$) with a relatively wide distribution between +17 mm and –11 mm at touch down (AEP), and a more narrow distribution of PEP closer to the coxa, which ranges from +13 mm to –7 mm (Fig. 7H).

When it comes to the AEP and PEP y -positions in the intact animal, they are both more uniformly distributed than the x -positions. The AEP ranges from 28 mm to 6 mm from the center of the mesothorax, with the majority of steps touching down around 19 mm. Lift offs occur within 27–3 mm from the mesothorax but

the majority of PEP occurs around 13 mm (Fig. 7A,C). The uniform distribution of AEP and PEP y -values reflects the mostly sideways outside in stepping independent of the front-to-back or back-to-front direction, together with the occurrence of occasional longer front-to-back steps (compare Fig. 3D, red iML).

With the increasing reduction of the preparation to 2L–ML and 1L–ML, this occurrence of touch down away from the body and lift off closer to the body is conserved, although the variability is, with the exception of the 6L– vs 1L–AEP y -position significantly reduced ($P < 0.05$). The majority of touch down y -positions in both, the 2L– and the 1L–ML-preparations remains at 19 mm distance from the center of the mesothorax and, thus, at the same location as in the intact animal. Only the majority of PEP y -positions in the two preparations is shifted towards the body and is now at 9 mm distance (Fig. 7F,I). The separation between the touch down and lift off y -positions increases with increasing reduction, again reflecting the increasing abundance of clear sideward steps and simultaneous lack of long front-to-back steps in inside middle leg stepping.

Taken together, one can summarize that the distribution of x -positions for the touch down and lift off points shifts toward the axis through the middle leg coxa and the variability in stepping patterns narrows with the increasing reduction of the preparation. At the same time, the pattern of AEP and PEP y -positions remains similar but also decreases in variability. This may have implications for the context-dependent modulation of single leg stepping through intersegmental sensory influence but may also be a consequence of the definition of a turning animal as stated in the methods. Both shall be discussed later.

DISCUSSION

From kinematics and electrophysiological studies, it is yet unknown how much of the generation of turning movements in an animal depends on descending inputs from higher brain centers, how much on the flow of information between the networks of the legs, and how much on the specific action of local networks controlling the movement of the single leg itself. Earlier studies either did not separate active from passive components (Jander, 1982; Jander, 1985; Dürr, 2005; Dürr and Ebeling, 2005) or did not study adaptive behavior (Graham and Cruse, 1981; Epstein and Graham, 1983; Ludwar et al., 2005).

To eliminate passive displacement of the legs through ground coupling as a factor within the walking system that could shape leg kinematics, we therefore used a preparation in which the tethered insect walks on the so-called 'slippery surface' (Gruhn et al., 2006). We analyzed the stepping pattern in the straight walking animal and compared the kinematics with those of the inside and outside legs of the turning animal. Then, we sequentially reduced the number of legs to dissect inter-leg from local influences in the animal to test how much single leg stepping kinematics is dependent on the presence of neighboring legs. When we selectively removed all but the front or middle legs, and subsequently even reduced the animal to a single-leg preparation, it was still possible to distinguish between inside and outside leg movements. With the exception of the middle outside leg, the legs performed stepping behavior similar to their respective legs in the intact turning animal.

Straight walking and turning in the intact animal

We have found that the stepping patterns of the intact straight walking stick insect on the slippery surface are qualitatively very similar to those observed for freely walking stick insects (Cruse, 1976; Rosano and Webb, 2007) or tethered stick insects walking on a sphere (Dürr, 2005; Dürr and Ebeling, 2005). Similar movement

patterns have also been reported for stick insects walking on an oiled glass surface and on a mercury surface (Graham and Cruse, 1981; Cruse and Epstein, 1982; Epstein and Graham, 1983).

When the intact animal on the slippery surface changes from straight walking to turning, the stepping pattern is clearly altered in all legs. On the inside of a turn during the stance phase, a reduction in movement vector angle and stride length in all legs act together as if to pull the body into the curve. In addition, the inner hind leg can also stay almost stationary and as if it were a pivot around which the animal rotates, similar to what has been described by Dürr and Ebeling for the stick insect tethered over a rotating ball (Dürr and Ebeling, 2005). On the outside of the turn, however, the direction of the leg movement and the stride length are altered independently. In the front legs, the stride length remains constant while it increases in the middle and hind legs. The increase in movement vector angle then causes the outside front leg to perform a pulling-like movement into the turn, while the small decrease in angle in the middle and hind legs performs pushing-like movements on an imaginary radius around the curve. As with the straight walking animal, these leg movements are qualitatively similar to turning shown in stick insects on a sphere and even to cockroaches turning freely or crayfish turning on a treadmill (Jander, 1982; Cruse and Saavedra, 1996; Jindrich and Full, 1999; Dürr and Ebeling, 2005).

Dürr and Ebeling noted previously that the front legs in the stick insect take the leading role in the initial phase of turning behavior (Dürr and Ebeling, 2005). From this finding, one could have expected that the animal, being suspended over the slippery surface is not capable of changing its single leg kinematics without the passive displacement of the body and the legs, caused by front leg turning activity. Instead, the comparison of leg movements between straight walking and turning in the intact stick insect on the slippery surface demonstrates that passive leg displacement during insect stepping, when coupling through the ground is present (Jander, 1985; Jindrich and Full, 1999), is not necessary to produce turning-like kinematics in all legs. This implies that changes in the leg movement patterns occur actively. The fact that there are such active changes in the placement and movement of all legs during turning on the slippery surface also suggests that there is an active reconfiguration of the motor activity for each leg. This active reconfiguration appears to occur in a coordinated fashion in the intact animal, as the PEPs in the front legs and the AEPs in the middle and hind legs of the turning animal have the same position along the body axis. A quantitative comparison between AEP and PEP values obtained in the intact stick insect turning on the slippery surface with the data from the stick insect turning on an air cushioned ball (Dürr and Ebeling, 2005) should yield the effect that passive leg displacement due to ground coupling has on the leg kinematics during turns. It should also be noted that leg kinematics alone cannot predict the dynamics of turning behavior under these conditions; however, this study intended to exclude mechanical coupling between the legs as a decisive factor, and to focus on the neuronal coupling between the legs.

The location and topology of the networks that control the change in stepping kinematics for straight walking and turning in the single stick insect legs are unknown. As lesion experiments in the stick insect and the cockroach suggest (Graham, 1979a; Graham, 1979b; Schaefer and Ritzmann, 2001), local thoracic networks could be capable of producing the necessary kinematics without descending information. By contrast, as in the fruit fly or the cockroach, the central body complex (CBC) in the cerebral ganglion is highly likely to participate in generating descending signals from the brain to produce correct turning behavior (Strauss and Heisenberg, 1993;

Ridgel et al., 2007; Mu and Ritzman, 2008a; Mu and Ritzman, 2008b). As of now, there are no electrophysiological results in the stick insect elucidating the mechanism underlying turning. However, behavioral analysis has suggested that the front legs take a leading role in the organized execution of turns (Dürr and Ebeling, 2005) and a body trajectory analysis by Rosano and Webb (Rosano and Webb, 2007) supports this finding but suggested the additional contribution to turning by the posterior legs.

Our finding, that turning involves a change in the angle of stance phase movement, a change in the stance phase duration as reflected by the step length, or both in all legs, suggests that these two parameters are independently modulated locally, depending on the leg in question and its behavioral function. Moreover, step length in the outside legs must be controlled through pattern generators controlling the action of the pro-/retractor coxae system whereas inside leg step length is largely determined by the activation of the flexor/extensor tibiae system.

Straight walking and turning in the reduced preparation

From the so called 'coordination rules', which are known to control coordinated stepping in insects and crustaceans (Cruse, 1990; Dürr et al., 2004), one could imagine that the observed kinematic changes of each leg during turning were influenced by and therefore depended on the presence of the neighboring legs and, in fact, it is known that stepping patterns of insects change as a result of amputation (Wendler, 1965; Pearson and Iles, 1973; Graham, 1977; Delcomyn, 1991a; Delcomyn, 1991b).

For the specific case of turning behavior, however, our results from the reduced preparations support the notion that each leg is driven by a specific motor program that depends on the turning direction, and that these motor programs create kinematics that are indeed quite robust. In both, the two-leg and the single-leg preparation, front and middle legs produced the movement patterns expected for the respective leg function. This suggests that the basic information as to where to place the foot during a given motor program, such as inside curve stepping, resides in the local circuitry of the single leg, and that it is not only highly independent of passive leg displacement but also independent of the presence of coordinating sensory information from the other legs.

Four differences in comparison with the intact animal, however, should be noted that point to inter-leg influences: (1) in both cases of reduction, there was an anteriorly directed shift in AEP and PEP in the front and the middle legs. This was independent of the function of the leg as either inside or outside leg and was also observed in the 2L straight walking animals. (2) Outside leg stepping in 2L-ML and the 1L-ML preparation, as determined by head movement, became similar to but was yet significantly different from straight stepping in the two-leg preparation. (3) Step lengths in the inside and outside front legs, and the inside and outside middle legs of the 1L-preparations as determined by the movement vector length were not significantly different from each other. (4) We observed that the animals showed a tendency to perform more extreme turning movements leading to less variability in stepping pattern and the distribution of AEP and PEP along the body axis for the inside middle leg.

All four alterations compared with the intact animal imply that, even though the presence of neighboring legs may not be necessary to produce basic context dependent leg movements, it still influences the motor output in a given leg. There are indications that such input may shape the extreme touch down and lift off positions in the form of targeting information (Graham, 1979b; Cruse, 1985; Schmitz and Hassfeld, 1989). The reason for the more anterior placement of the

tarsi, observed in all reduced preparations could be a lack of inter-leg sensory information or a reduced general neuronal activity allowing the legs to reach their PEP threshold earlier. This could also be the reason behind the reduction in the range of possible touch down and lift off positions in the middle inside leg. In the present example, however, the modulating sensory input seems to come from both, anteriorly and posteriorly located legs because the shift in AEP/PEP was seen in the front and in the middle legs of the reduced preparations.

The similarity between outside and straight stepping in the reduced middle leg preparations indicates that there may in fact only be two basic stepping patterns present in the middle leg: an inside stepping pattern and a straight/outside pattern. These two basic patterns could be largely fixed but the straight/outside pattern may then be shaped into either straight or outside leg stepping kinematics through inter-leg sensory influence, when neighboring ipsilateral legs and their position information are present. One could also imagine an alternative explanation: one can see similarities in the stepping pattern between the 2L-ML-preparation and the front legs in the intact animal. It is, therefore, also conceivable that the lack of sensory information from the front legs causes the middle legs to assume a front-leg-like role and the corresponding kinematics. In this case and in the case of the reduced variability in the inside leg stepping pattern, it is not clear in which way this shaping effect of the motor output by sensory signals may occur. The notion, however, that shaping of one general motor pattern such as the straight/outside pattern into two more refined ones may be mediated by the action of descending signals is supported by recent findings in the cockroach where it was shown that a reflex response that is involved in the execution of searching/inside leg turning is altered after removal of descending input from the brain (Mu and Ritzmann, 2008a). One word of caution should be added about the interpretation of the reduced variability in the stepping kinematics of the inside middle leg. It is appealing to think that a lack of sensory input from neighboring legs would cause this less variable stepping pattern. However, it cannot be excluded that more shallow turns of the 2L- and the 1L-preparation were missed in the data acquisition because they are harder to identify than in an intact animal with six legs turning. This could also have led to a more narrow distribution of the data. One next step will be to understand the basis for the observed variability in stepping movements.

The fact that the step lengths in the inside and outside front or middle legs becomes the same in our 1L-preparations stresses again the point that different joint control networks primarily contribute to a given movement pattern. This selective contribution depends on the function of the leg as inside or outside leg of a turn, as on the inside the flexor activity largely determines stance phase, while on the outside, this is determined by retractor activity. This drive that determines the stride width of the leg appears to undergo additional modulation depending on the presence of neighboring legs.

Potential sensory information involved in turning kinematics

From the present study, it becomes clear that two factors contribute to turning kinematics in the single leg. One is a motor program for turning that resides in a given leg, and the other is that this motor program is shaped by the presence of neighboring legs. But what type of information from the other legs is used to shape the motor output? In the stick insect, it has been found that there is a weak inter-leg influence from the femoral chordotonal organ (fCO), which measures the movement of the tibia (e.g. Ludwar et al., 2005; Stein et al., 2006; Borgmann et al., 2007) (for a review, see Büschges

and Gruhn, 2008). Whether sensory information from the fCO plays a role in coordinating leg movements during turns, however, remains unknown. Another potential candidate to send modulating input to a neighboring leg and its joint central pattern generators (CPGs) may be the campaniform sensilla (CS) located at the base of each leg. Despite the fact that the animals in this study were suspended over the slippery surface, the CS measure the increase in load created by the touching down or lifting of the leg. This signal is reported to the local motor network and CS input to the CNS has been shown to be differentially processed in the stick insect, depending on whether the animal is walking forwards or backwards (Akay et al., 2004; Akay et al., 2007) [summary in Zill et al. (Zill et al., 2004)]. This way, they may also influence the switch in pro- and retractor activity that can occur from one inside step to another and back within two cycles, and they could also influence the motor networks in neighboring legs. The important difference between backward walking and turning, however, is that during turns such a reversal in stepping pattern occurs independently of the contralateral leg. This is in agreement with the findings by Dürr that contralateral coupling during turning is generally weak (Dürr, 2005). The mechanisms that underlie such a contralateral uncoupling still need to be elucidated.

A similar switch in the processing of sensory input is also likely to happen in the cockroach, where sensory input has long been known to have a large impact on inter-leg coordination. Here, a reversal in muscle activation from joint extension during stance to extension during swing has been observed for the inside leg during turning (Pearson and Iles, 1973; Mu and Ritzmann, 2005). Similarly, in cockroach climbing, changes in the activation of the thorax-coxa joint has been shown to generate changes in the sensory response to the increased strain in the cuticle, thereby increasing muscle activity in the legs to accomplish the new postural tasks (Watson et al., 2002).

Conclusions

Altogether, the goal of our study was to understand the importance of local neuronal processing in the thoracic ganglia for the ability of the stick insect to show turning movements. Our findings demonstrate that each stick insect leg performs movement patterns during turns that are characteristic for its function as an inside or outside leg. Compared with earlier studies on turning in intact insects, we have expanded the investigation to the analysis of leg movements also in an increasing state of reduction in the number of legs stepping. We could demonstrate that these movement patterns are at the same time independent of the presence of other legs or of the connection between the tarsi through the ground on which the animal moves and thus due to local CNS activity. In addition, it becomes clear that the presence of other legs influences these locally generated and context-dependent movement patterns of the single legs by shaping them into the patterns observed in the intact animals. We are now able to investigate specific sensory mechanisms that underlie the neuronal activities relevant for each leg during and between straight walking and turning. This should help us to understand the physiological mechanisms behind the generation of adaptive locomotor movements.

LIST OF ABBREVIATIONS

AEP	anterior extreme position
CBC	central body complex
CPG	central pattern generator
CS	campaniform sensilla
fCO	femoral chordotonal organ

HL	hind leg
IL	inside leg
ML	middle leg
PEP	posterior extreme position

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