RESEARCH ARTICLE

Segment-specific and state-dependent targeting accuracy of the stick insect

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SUMMARY

In its natural habitat, *Carausius morosus* climbs on the branches of bushes and trees. Previous work suggested that stick insects perform targeting movements with their hindlegs to find support more easily. It has been assumed that the animals use position information from the anterior legs to control the touchdown position of the ipsilateral posterior legs. Here we addressed the question of whether not only the hindleg but also the middle leg performs targeting, and whether targeting is still present in a walking animal when influences of mechanical coupling through the ground are removed. If this were the case, it would emphasize the role of underlying neuronal mechanisms. We studied whether targeting occurred in both legs, when the rostral neighboring leg, i.e. either the middle or the front leg, was placed at defined positions relative to the body, and analyzed targeting precision for dependency on the targeted position. Under these conditions, the touchdown positions of the hindlegs show correlation to the position of the middle leg parallel and perpendicular to the body axis, while only weak correlation exists between the middle and front legs, and only in parallel to the body axis. In continuously walking tethered animals, targeting accuracy of the hindlegs and middle legs parallel to the body axis barely differed. However, targeting became significantly more accurate perpendicular to the body axis. Our results suggest that a neural mechanism exists for controlling the touchdown position of the posterior leg but that the strength of this mechanism is segment specific and dependent on the behavioral context in which it is used.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/22/4172/DC1

Key words: walking, motor control, sensory feedback, intersegmental coordination.

Received 10 June 2013; Accepted 31 July 2013

INTRODUCTION

If terrestrial animals want to walk through any kind of environment, they need to know how to move their legs to reliably find a foothold. This information becomes particularly relevant when navigating through an unknown or irregular terrain. For cats (McVea and Pearson, 2007; McVea et al., 2009; Wilkinson and Sherk, 2005) and humans (Mohagheghi et al., 2004; Patla and Vickers, 2003) it is known that targeting of leg movements is primarily mediated by visual information that is captured on average two steps ahead. Likewise Niven and colleagues showed that locusts visually target their front legs towards the position of a ladder rung and information about the position of the rung is acquired before leg swing is initiated (Niven et al., 2010). But how do animals find an appropriate foothold when visual information is not available? In the same study, Niven and colleagues also observed that placement of the middle leg in locusts was not visually guided (Niven et al., 2010). For this purpose, the control system has to have information not only about the environment but also about the actual positions of the aiming and the targeted leg. This information can be provided by several kinds of sense organs. Cats, for example, use information from muscle receptors and cutaneous receptors in the skin to match sensory information from different joints and reliably represent the position of the limb relative to the body in the dorsal root ganglia (Stein et al., 2004). This information is also transferred to area 5 in the posterior parietal cortex where it is integrated with memorized visual information in order to perform appropriate leg movements (McVea

et al., 2009), which are in turn generated in the local networks of the spinal cord (for review, see Grillner and Jessell, 2009; Kiehn et al., 2010). Similarly, it is known from work on stick insects that proprioceptive inputs of several sensory structures in the leg influence the protraction end point of all legs (Wendler, 1964; Bässler, 1977; Dean and Wendler, 1983; Cruse et al., 1984).

In their natural habitat, stick insects (*Carausius morosus*) live in a complex three-dimensional maze of twigs and leaves to which they have to constantly adapt their locomotor behavior. As nocturnal animals they primarily rely on mechanosensory information from the antennae, and do not use vision to guide their front legs towards an appropriate foothold (Dürr, 2001; Blaesing and Cruse, 2004; Schütz and Dürr, 2011). How the stick insect guides its hindlegs towards an appropriate foothold has also been the focus of several earlier investigations (e.g. Cruse, 1979; Cruse et al., 1984; Dean, 1984; Dean, 1989; Dean and Wendler, 1983), in which it was shown that the touchdown position of the hindleg depends on the position of the standing middle leg (Cruse, 1979). The sense organs that appear to be primarily responsible for targeting parallel to the body axis are hair rows and hair fields on the coxa (Cruse et al., 1984; Dean and Wendler, 1983), while targeting information perpendicular to the body axis seems to originate primarily from the femoral chordotonal organ (fCO) (Cruse et al., 1984). Information about the posture of the middle leg is transmitted *via* the ipsilateral connective (Dean, 1989); Brunn and Dean (Brunn and Dean, 1994) described three interneurons, each signaling the angle of one leg joint, that

together could encode the tarsus position. This has led to the inclusion of targeting into coordination rules, which assume targeting of all legs during walking in the stick insect (Cruse, 1990; Cruse et al., 1995).

However, it is not known whether stick insects guide their middle legs towards an appropriate foothold equally well, and whether they also use position information from the front legs. In addition, it is still unclear how information from sense organs that detect angular positions and velocities of joints is incorporated into a reference frame for motor control. In recent years, more and more evidence has suggested that the behavioral state of an animal is important for the effectiveness of sensory processing on to the motoneurons (Clarac et al., 2000; Duysens et al., 2000; Pearson, 1993; Akay et al., 2007; Hellekes et al., 2012) (for review, see Büschges and El Manira, 1998), but it is not known to what extent movement of the anterior leg, limb joint constraints or effects of mechanical coupling through the ground influence the targeting accuracy of the middle leg or hindleg, and at which time point the information used for targeting is sampled.

We have therefore investigated the placement of middle leg and hindleg towards their anterior neighbor in the stick insect *C. morosus* to study spatial coordination of the legs and foot placement without visual guidance under two behavioral conditions: either the first step after standing or during continuous walking. We measured the targeting accuracy of the two legs and compared their performance with each other, and under the two behavioral conditions, to find out whether there were segment-specific and state-dependent differences. By tethering the animal above a slippery surface we could reliably remove mechanical coupling of leg movements through the ground (Gruhn et al., 2006). Targeting in the continuously walking animal under these conditions would emphasize the role of neuronal control mechanisms underlying this behavior.

MATERIALS AND METHODS
Animals
All experiments were performed on adult female stick insects (*C. morosus*, Sinety 1901). Animals were reared in the animal facility of the institute on a 12h:12h light:dark cycle at 23–25°C and were fed with blackberry leaves (*Rubus fructiosus*) *ad libitum*.

Experimental setup
The general setup is an adaptation of the setup described in detail previously (Gruhn et al., 2006). In all experiments, animals were tethered above a 13.5×13.5cm polished nickel-coated brass plate. To allow unimpeded stationary stepping or walking under tethered conditions and to minimize mechanical coupling between the legs, the plate was covered with a lubricant composed of 95% glycerin and 5% saturated NaCl. The animals were glued ventral side down on a 3×5×100mm (width×height×length) balsa rod using three droplets of dental cement (ProTempII, ESPE, Seefeld, Germany) along the length of the thorax such that the legs and head protruded from the rod and all joints were unrestrained. Animal height above the substrate was adjustable, but was typically set to 10mm, measured from the coxae of the front leg and hindleg on both sides. Experiments were performed in a darkened Faraday cage at room temperature.

In the continuous walking sequences, walking was elicited by projecting a progressive striped pattern (pattern wavelength 21deg) onto two 13.5cm diameter round glass screens placed at right angles to each other and at a 45deg angle to the walking surface, about 6–7cm from the animal's eyes (Scharstein, 1989) [for a detailed
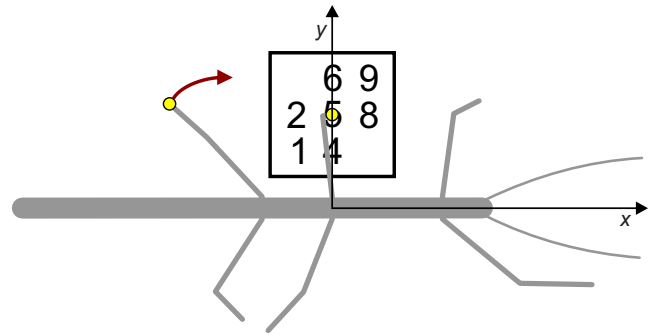

Fig. 1. Schematic drawing of the stationary stick insect with the coordinate system of the labeled standing positions of the middle leg. The yellow dots mark the tracked positions on the animal. The red arrow marks the movement direction of the posterior leg, in this case the hindleg. Experiments with the front leg as the target leg were carried out accordingly (see also schematic diagrams in Figs 2 and 4).

description see Gruhn et al. (Gruhn et al., 2006)]. Reflections on the polished plate further increased the field of view. Alternatively, a single white stripe on a 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. In all sequences with the previously positioned, standing anterior leg, stepping of the posterior leg was also elicited by light brush strokes to the abdomen.

To analyze the precision of the first steps, we carefully placed the tarsus of the anterior leg on a small 5×10mm cardboard platform with a particularly rough surface. This small platform was attached to a brass tube, which was connected to a micromanipulator (see Cruse, 1979). Exact positioning of the anterior leg was achieved by moving the platform to one of seven aiming positions. In all experiments the tarsus was not artificially fixed to the platform. The location of these positions was defined by the central position (no. 5) directly underneath the femur–tibia joint when the tibia was perpendicular to the surface, and the femur was perpendicular to the body (see Fig. 1). The other six tested positions were arranged around position 5 as follows: positions 1 and 2 were 5mm posterior, while positions 8 and 9 were 5mm anterior. Positions 1 and 4 were 5mm central, while positions 6 and 9 were 5mm distal of position 5. Positions 3 and 7 could be taken up by the anterior leg, but very often caused the animal to re-position the anterior leg. Therefore, we focused our analysis on the remaining positions. The standing position of the anterior leg was randomly changed after each step of the posterior leg. Fig. 1 gives a schematic representation of the stationary stick insect with the seven aiming positions relative to the body, shown for the stepping hindleg and stationary middle leg. The same general setup was used for the front leg. A smaller version of this representation is also given as insets in Figs 2 and 4. In supplementary materials Figs S1 and S2, the tarsus was glued to the platform with dental cement (see above), and successively moved between positions 1–9.

Optical recording and digital analysis of leg movements
Optical recordings of the steps were performed and analyzed as previously (Gruhn et al., 2009a). In brief, walking sequences were recorded with a high-speed video camera (Marlin F-033C; Allied Visions Technologies, Stadtroda, Germany) that was externally triggered at 100 frames s^{-1}. Insect head, thorax and legs were marked
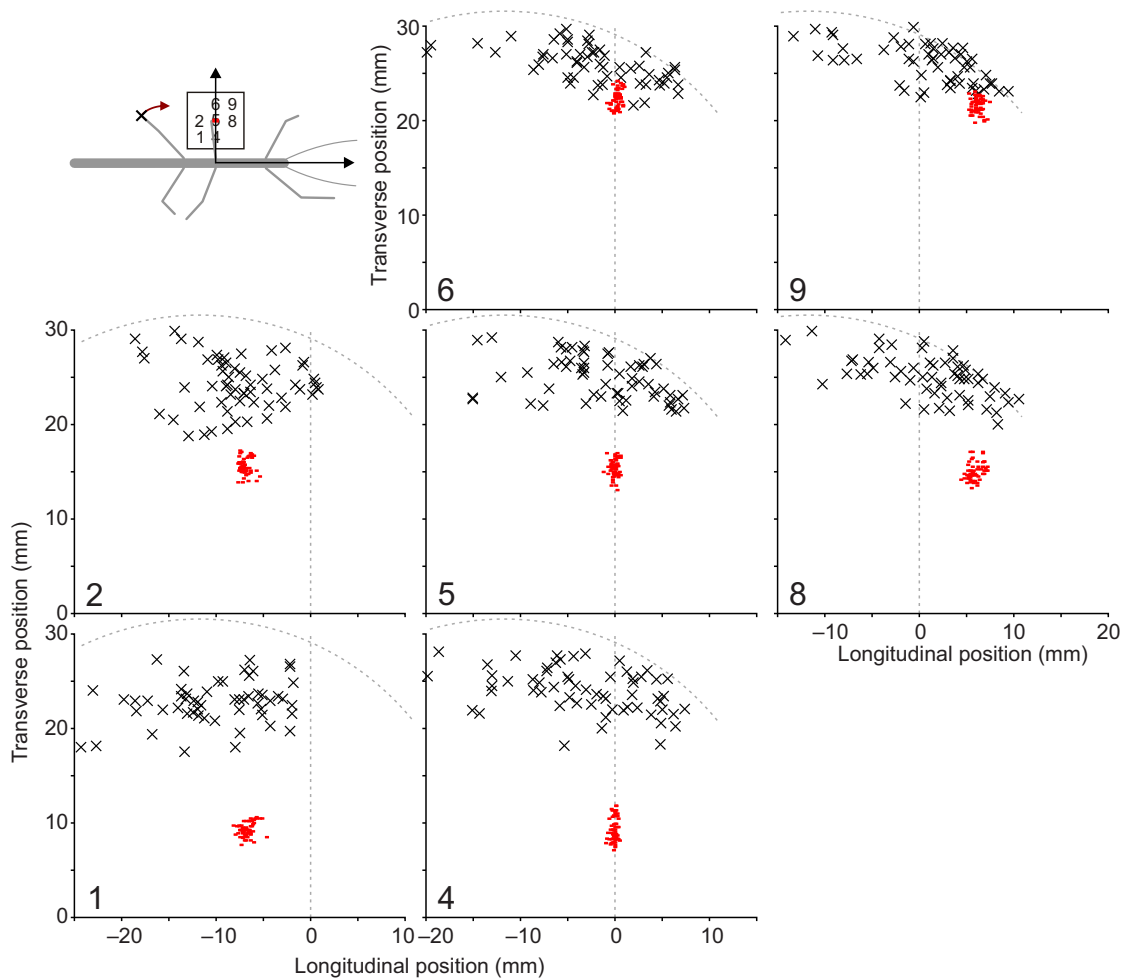
Fig. 2. Standing positions of the middle leg (red dots) and touchdown positions of the stepping hindleg (black crosses) on the slippery surface. Each plot shows data from one of the seven standing positions of the middle leg. The vertical dotted line marks the position of the middle leg coxa, which is located at zero on the *x*-axis. The dotted semi-circle depicts the calculated average maximum range of the fully stretched hindlegs. The inset gives a schematic overview of the standing positions of the middle leg. *n*=60 for each position.

with fluorescent pigments (Dr Kremer Farbmühle, Aichstetten, 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, Germany). The video files were analyzed using motion-tracking software (WINanalyze 1.9; Mikromak Service, Berlin, Germany). Position values are always given in millimeters in the form *xx.x*; *yy.y* (s.d.*x*; s.d.*y*). A virtual 0 line was drawn across the animal at the level of the coxa of the anterior leg (Fig. 1). Positive and negative *x*-values indicate points anterior and posterior to this coxa, respectively; *y*-values are given with respect to the axis perpendicular to the length of the animal. Larger *y*-values denote more distal points, smaller values more central ones. Fig. 1 shows a schematic drawing of the stick insect with the tracked reference points for the analysis of leg kinematics marked as yellow dots and the standing positions of the anterior leg. All steps were transposed to reflect walking by a left leg regardless of which leg was being recorded.

Data analysis and figure preparation

Leg positions were measured with their *x* and *y* coordinates in millimeters. Care was taken to choose intact animals of the same size (mean ± s.d. animal length: 77.2±2.8 mm). The number of

animals used (*N*) for the continuous walks was eight; for the standing middle leg or front leg it was six. The number of steps evaluated (*n*) under the respective conditions is given in the figures or figure legends.

For statistical analyses, Mann–Whitney *U*-test, Hotellings T^2-test and Pearson's correlation test were used (Matlab, Statistics toolbox; The MathWorks, Natick, MA, USA). Statistical significance was assumed at values of *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

RESULTS
Targeting accuracy of the hindleg towards the middle leg

First, we analyzed whether the hindlegs of *C. morosus* target the position of the ipsilateral middle leg during the first steps. We tethered the animals above a slippery surface and placed one middle leg onto one of seven pre-defined standing position markers on a cardboard platform. Each position was used 10 times in a randomized order. The touchdown position of the first step by the hindleg was recorded, after initiating walking of the animal through a brush stroke to the abdomen. Sequences in which the middle leg moved before the hindleg had finished its swing phase were not evaluated.

A plot with the position of the standing middle leg and the respective touchdown position of the stepping ipsilateral hindleg
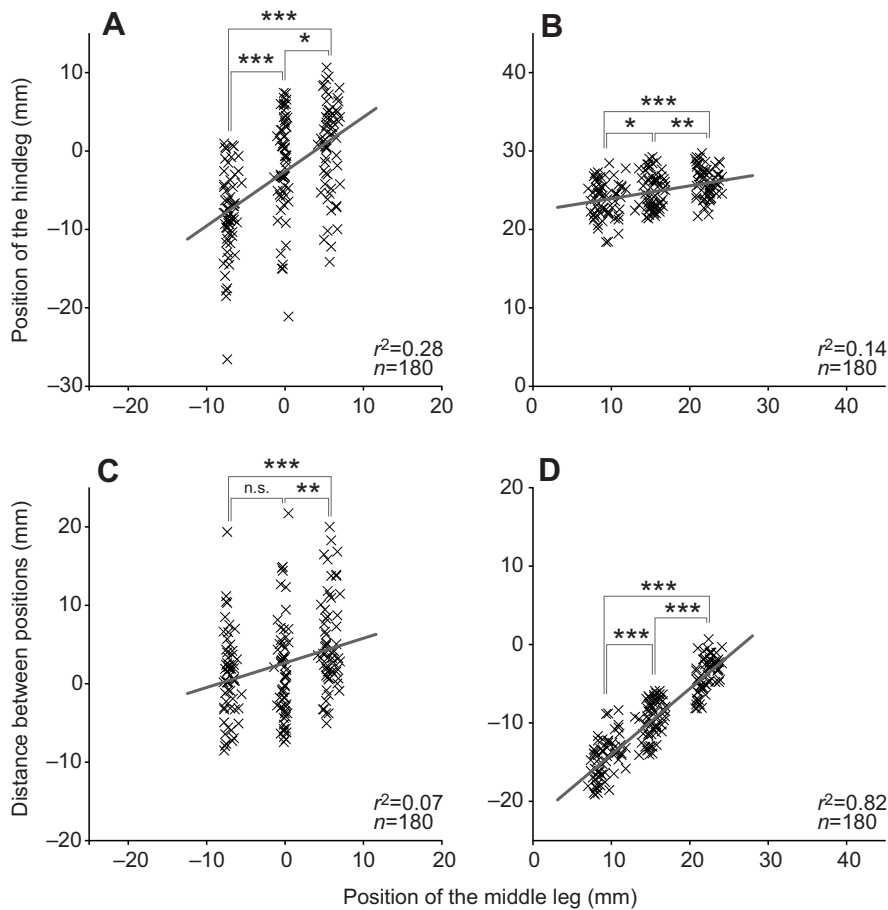
Fig. 3. Scatter plot of the middle leg standing positions against the touchdown positions of (A,B) and distances from (C,D) the ipsilateral hindleg. Data are separated into the components parallel (A,C) and perpendicular (B,D) to the body axis. Each panel includes linear correlation and a test for significant differences between the groups of data using the Mann–Whitney U-test. The pairs of data plotted belong to middle leg standing positions that only differ in the considered coordinate. In A and C, these are positions 2, 5 and 8. In B and D, these are positions 4, 5 and 6. n is the number of steps; $*P<0.05$, $**P<0.01$ and $***P<0.001$.

(Fig. 2) shows that all seven investigated positions of the middle leg were within reach of the hindleg (dotted semi-circle in Fig. 2). The touchdown position of the hindleg was often anterior to the position of the middle leg coxa (vertical dotted line in Fig. 2). Only when the middle leg was standing at positions 1 or 2 did the hindleg rarely touch the ground anterior to the middle leg coxa. Taking the position of the middle leg coxa as a reference, it becomes apparent that the touchdown positions of the hindleg were more anterior for farther anterior standing positions of the middle leg. Similarly, for more lateral standing positions of the middle leg, the touchdown positions of the hindleg were on average also more lateral.

We quantified these qualitative observations by testing whether the three groups of touchdown (hindleg) positions, either parallel (Fig. 3A) or perpendicular (Fig. 3B) to the body axis were significantly different from one another. Significant differences between the three groups of data are a prerequisite for linear correlation. We then looked for linear correlation between the data groups. For 180 pairs of positions each, we performed a pair-wise analysis of data from the middle leg standing positions that only differed along one of the two axes.

On average, the x-coordinate of the touchdown position of the hindleg increased with the increasing x-coordinate of the standing middle leg (Table 1A). Although the variability of the hindleg touchdown positions for the three middle leg positions along the body axis (2, 5 and 8) was relatively large, the groups of touchdown positions were nevertheless all significantly different from one another (Fig. 3A, Table 1B). We used these data pairs to identify a linear correlation parallel to the body axis. With a coefficient of determination of $r_x^2=0.28$, such a correlation can indeed be assumed.

To test for a possible correlation perpendicular to the body axis, we used middle leg positions 4, 5 and 6 (Fig. 3B). Although the mean values of these three data groups did not apparently differ much

Table 1. Touchdown positions and distances of the posterior leg with respect to the targeted position of the anterior leg (A) and P-values for significant differences between the data groups (B)

	Hindleg–middle leg		Middle leg–front leg	
	Position	Distance	Position	Distance
A. Touchdown position/distance				
x: 2	−8.0±5.3	0.9±5.3	−10.1±4.8	3.7±4.6
x: 5	−1.8±6.3	1.6±6.3	−7.0±5.0	7.3±5.0
x: 8	0.7±5.8	5.0±5.9	−5.5±5.8	13.0±5.8
y: 4	23.8±2.3	−14.5±2.6	19.8±2.9	−8.6±2.7
y: 5	24.9±2.2	−9.5±2.4	20.6±3.2	−2.8±2.9
y: 6	26.0±1.9	−3.8±2.3	21.3±2.6	3.1±2.6
B. P-values				
2–5	<0.0001	0.6612	0.0002	0.0001
5–8	0.0255	0.0024	0.0280	<0.0001
2–8	<0.0001	0.0002	<0.0001	<0.0001
4–5	0.0154	<0.0001	0.1333	<0.0001
5–6	0.0058	<0.0001	0.1825	<0.0001
4–6	<0.0001	<0.0001	0.0022	<0.0001

(A) Position and distance data are means ± s.d. (in mm). For targeting along the long axis of the animal (x), the values are given with respect to anterior leg positions 2, 5 and 8; for positions perpendicular to the long axis of the animal (y), values are given with respect to anterior leg positions 4, 5 and 6.
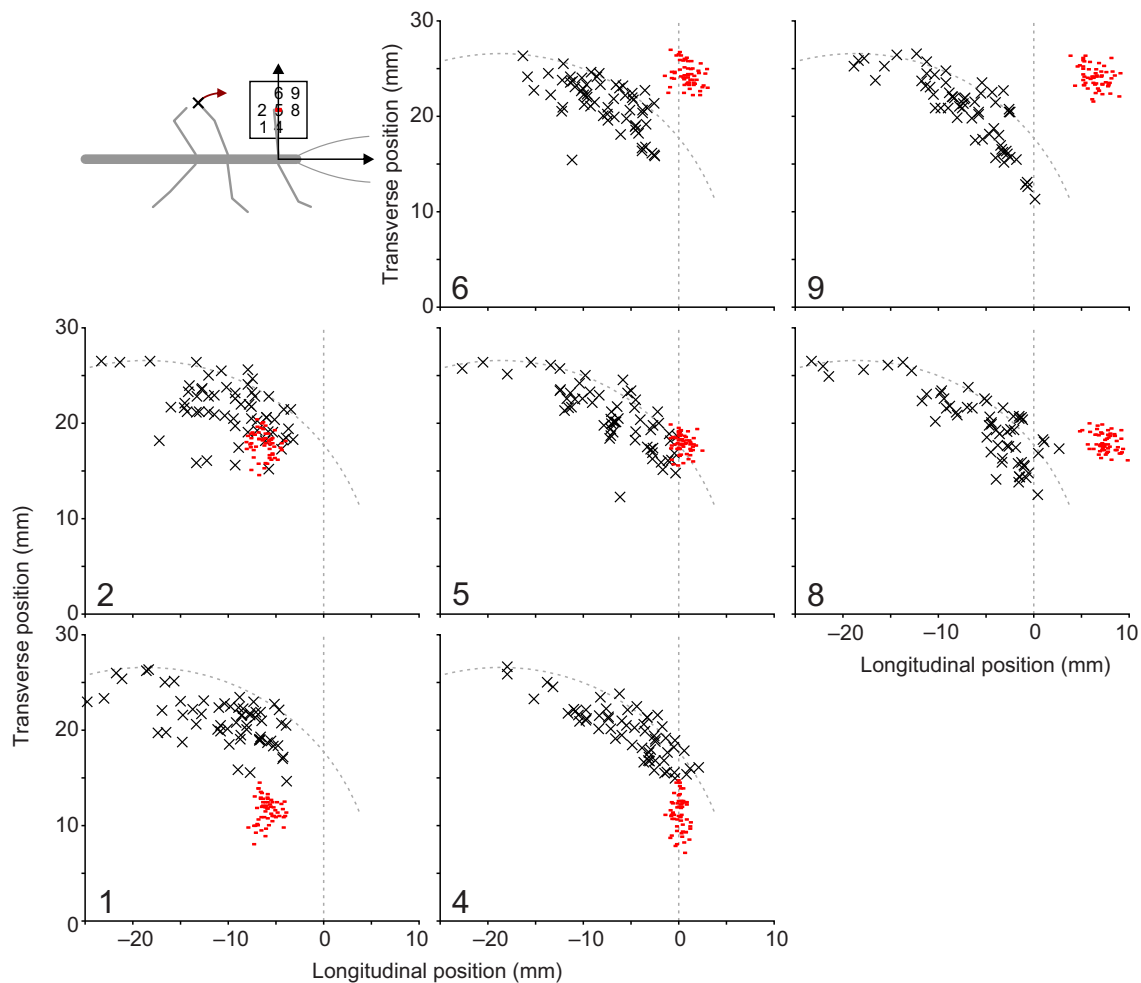(B) P-values were obtained from the Mann–Whitney U-test.

Fig. 4. Standing positions of the front leg (red dots) and touchdown positions of the middle leg (black crosses) on the slippery surface. Each plot shows data from one of the seven positions of the standing front leg. The vertical dotted line marks the level of the front leg coxa, which is located at zero on the *x*-axis. The dotted semi-circle depicts the calculated average maximum reach of the fully stretched middle legs. The inset gives a schematic overview of the standing positions of the front leg. *n*=60 for each position.

(Table 1A) they were still significantly different from each other (Table 1B) as a result of their small variability. The linear correlation along this axis was smaller but still present (r_y^2=0.14).

We also calculated the distance between the standing position of the middle leg and the touchdown position of the hindleg parallel (Fig. 3C) and perpendicular to the body axis (Fig. 3D). These values were plotted against the standing position of the middle leg. We calculated their mean values, and tested for significant differences between the groups and for linear correlation. This comparison helps to estimate the targeting accuracy of the hindleg. No significant difference in the distances between the middle leg and hindleg for the differing middle leg standing positions would suggest targeting by the hindleg. A systematic increase in the distance between the two positions with a more anteriorly or distally standing middle leg would instead indicate weak or no targeting by the hindleg. On average, the distance parallel to the body axis between middle leg standing positions 2 and 5 and the respective hindleg touchdown positions did not increase significantly, while the distance at position 8 was significantly bigger than those at positions 2 and 5 (Table 1). There was no correlation between the standing positions of the middle leg and the distance to the touchdown position of the hindleg along the body axis (r_x^2=0.07), again supporting targeting

of the hindleg towards the standing middle leg parallel to the body axis. In contrast, the average distance between hindleg touchdown and the standing middle leg at the three positions perpendicular to the body axis increased significantly from one standing position to the next by about 5 mm each (Fig. 3D, Table 1). Because of the small variability within the groups, and the big systematic increase of the mean values, the linear correlation between these standing positions and the distances was strong (r_y^2=0.82), suggesting no or only minor targeting of the hindleg towards the standing position of the middle leg perpendicular to the body axis. For easier comparison of all coefficients of determination, the r^2-values of all evaluations are also listed in Table 2. We repeated the series of experiments with the middle legs of the same animals glued to the standing platform. This did not change the distribution of touchdown positions, and the targeting accuracy in both directions was largely unchanged (data not shown, see supplementary material Fig. S1).

Targeting accuracy of the middle leg towards the standing front leg

To our knowledge, targeting of the middle leg towards the front leg has not been studied quantitatively. To test the targeting accuracy of the middle leg, we therefore performed the same experiments as
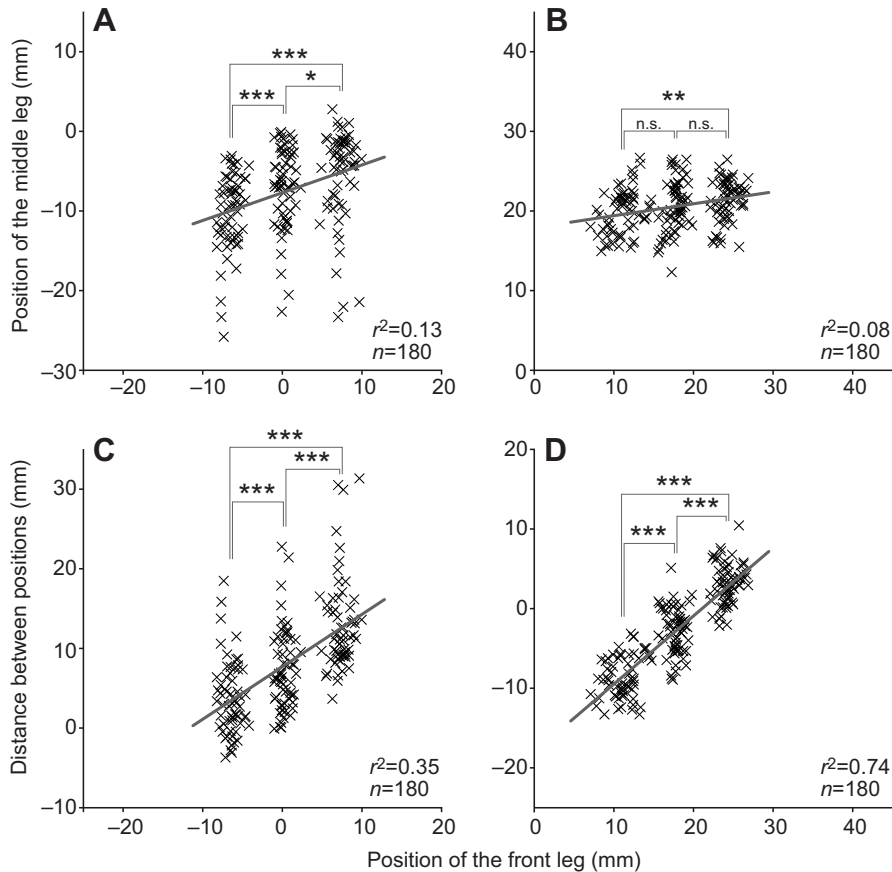
Fig. 5. Scatter plot of the front leg standing positions against the touchdown positions of (A,B) and distances from (C,D) the ipsilateral middle leg. Data are separated into the components parallel (A,C) and perpendicular (B,D) to the body axis. Each panel also shows linear correlation and a test for significant differences between the groups of data using the Mann–Whitney U-test. The pairs of data plotted belong to front leg standing positions that only differ in the considered axis. In A and C, these are positions 2, 5 and 8. In B and D, these are positions 4, 5 and 6.

above with the standing front and stepping middle leg. A plot of the seven different standing positions of the front leg, and the respective touchdown positions of the stepping ipsilateral middle leg (Fig. 4), shows that the touchdown of the middle leg usually occurred close to its maximum reach (dotted semi-circle in Fig. 4). The middle leg only rarely had its touchdown anterior to the front leg coxa (vertical dotted line in Fig. 4). The front leg positions 6, 8 and 9 were actually out of reach for the middle leg. To identify a potential systematic dependence between the touchdown position of the middle leg and the standing position of the front leg, we plotted these two positions against each other and tested for linear correlation parallel (Fig. 5A) and perpendicular to the body axis (Fig. 5B). Again, we used 180 pairs of data from front leg positions that only differed along one of the two axes.

To identify a potential correlation parallel to the body axis we used positions 2, 5 and 8 of the standing front leg (Fig. 5A). Although the scatter of touchdown positions along the body axis was relatively large, the average x-coordinate of the touchdown position increased significantly parallel to the body axis with increasing x-coordinate of the standing front leg (Table 1), but they were only weakly correlated ($r_x^2=0.13$). To test for a correlation perpendicular to the body axis, we used positions 4, 5 and 6 (Fig. 5B). Here, the mean values of the three data groups did not change significantly (Table 1). Consequently, no linear correlation along this axis was detected ($r_y^2=0.08$).

We then calculated the distances between the position of the standing front leg and the touchdown position of the middle leg parallel (Fig. 5C) and perpendicular to the body axis (Fig. 5D). Although the touchdown positions of the middle leg were on average more anterior when the front leg was standing in a more anterior position (Fig. 5A), the distance between the middle leg and front

leg tarsus parallel to the body axis also increased significantly from positions 2–8 (Table 1). With values of 7.3 and 13.0 mm, the difference between the distances at positions 5 and 8, respectively, is particularly big. This might be caused by the fact that the middle leg was still anatomically able to reach position 5, while this was not possible for position 8. We found a linear correlation between the position of the standing front leg and the distance to the middle leg touchdown parallel to the body axis ($r_x^2=0.35$), which is again indicative of only weak targeting of the middle leg towards the standing position of the front leg in this direction. Perpendicular to the body axis, the average distance increased from one standing position to the next significantly by about 5 mm (Fig. 5D, Table 1), resulting in a strong linear correlation between the standing position of the front leg and the distance to the middle leg touchdown position ($r_y^2=0.74$). This again means no or only weak targeting of the middle leg towards the front leg in this axis. We again repeated the series of experiments with the front legs of three of the animals glued to the platform. This had only minor effects on the distribution of touchdown positions or the targeting accuracy in both directions (data not shown, see supplementary material Fig. S2).

Targeting accuracy in the tethered walking animal
The experimental condition of a standing anterior leg corresponds to a situation where the animal starts locomotion after standing still, but this is a special case that may have limited relevance for the freely locomoting animal. Therefore, we also analyzed the targeting precision of the hindlegs and middle legs on to their anterior neighbor during tethered stationary walking. The animal was tethered above the slippery surface as before, but this time the middle or front legs were not placed in one of the defined positions but moved freely. This approach differs from that of Dean and Wendler, who looked

Table 2. Coefficients of determination of the linear regressions parallel (r_x^2) and perpendicular (r_y^2) to the body axis and size of the evaluated data groups

	Hindleg–middle leg			Middle leg–front leg		
	r_x^2	r_y^2	n	r_x^2	r_y^2	n
Position *versus* standing anterior leg	0.28	0.14	180	0.13	0.08	180
Distance *versus* standing anterior leg	0.07	0.82	180	0.35	0.74	180
Touchdown of the posterior leg	0.15	0.30	216	0.19	0.15	494
Last lift-off of the posterior leg	0.30	0.51	356	0.27	0.18	501
Next lift-off of the anterior leg	0.07	0.22	216	0.20	0.06	494
Random variables	−0.04	−0.07	356	−0.01	0.01	501
Distances *versus* lift-off of posterior leg	0.09	0.15	356	0.64	0.72	501

r^2-values are given for the touchdown position and the distance of the posterior leg against the standing position of the anterior leg. Additionally, r^2-values are given for the touchdown position of the posterior leg against the position of the anterior leg at three different time points, and against a set of random variables. Finally, the distance of the posterior leg against the positions of the anterior leg at the time of the last lift-off of the posterior leg is shown.

All linear regressions of the real data are significantly different from zero ($P<0.001$). The linear regressions with the random variables are not significantly different from zero ($P>0.05$).

n is the number of steps.

at targeting in stick insects walking on a treadwheel (Dean and Wendler, 1983), and was chosen to remove mechanical influences between the legs and investigate especially the neuronal basis of targeting. The position of the posterior leg used for the analysis was again its touchdown position. However, as it is not known at what time during the step cycle of the posterior leg its touchdown position is determined, we tested whether we could see a correlation of this touchdown position with the position of the anterior leg at three different time points during its step cycle: (1) the position of the anterior leg at the time when the posterior leg finished its swing phase and touched the ground (comparable to the control with a standing anterior leg, only without pre-defined positions); (2) the position of the anterior leg at the time when the posterior leg was lifted off the ground and began its swing phase; and (3) the next posterior extreme position that the anterior leg takes up after lift-off in the posterior leg (this point can be identical to that in 1, but need not be so).

We calculated the coefficients of determination for each of these three combinations, and, to ensure that the results were not caused by noise, we also calculated the coefficients of determination between the touchdown positions of the posterior leg and a set of random variables. The random variables had the same distribution as the real data (front leg: x between −10.2 and 28.7 mm; y between 0.5 and 31.2 mm; middle leg: x between −11.2 and 15.4 mm; y between 2.0 and 28.3 mm). Table 2 lists the number of data pairs and the corresponding r^2-values of the linear regressions. All linear regressions of the real data are significantly different from zero ($P<0.001$), while the linear regressions with the random variables are not ($P>0.05$). In addition, all coefficients of determination of the real data are bigger than the values for the used random variables. The strongest linear correlation in both directions for the middle leg and hindleg as posterior legs was found between the touchdown position of the posterior leg and the position of the anterior leg at the time of lift-off of the posterior leg (scenario 2). For all further evaluations of targeting during walking, we therefore used this position. We determined all lift-off and touchdown events of the posterior leg, and identified the position of the anterior leg for all lift-off events of the posterior leg. If the anterior leg was performing a swing phase at that time point, the corresponding touchdown position of the posterior leg was removed from the data set.

All data pairs from the hindleg and middle leg are plotted in Fig. 6. Most of the time, the touchdown positions of the hindleg were

posterior to the middle leg coxa (dotted vertical line in Fig. 6), but occasional stepping to more anteriorly located positions occurred. The mean values and the overall scatter of the touchdown positions of the hindleg perpendicular to the body axis were similar to those of the hindleg touchdown positions in all experiments with predefined middle leg standing positions (Fig. 6; mean ± s.d. y=20.0±4.3 mm; see for comparison Fig. 2), but were slightly shifted caudally (x=−13.1±6.4 mm; see Fig. 2). As the reference positions of the middle leg were taken at the time of lift-off of the hindleg, the middle leg had not completed its stance phase and thus had not reached its lift-off position. Therefore, the middle leg positions are comparably far rostral, and distances to the hindleg touchdown positions were larger than those for the standing middle leg. Under tethered walking conditions, the touchdown positions of the hindlegs were on average 16.1±5.7 mm posterior (x-distance) of the middle leg positions, while the lateral distribution of the two data groups was similar (y-distance=−4.4±3.3 mm). Most of the middle leg positions were within the reach of the hindleg. We tested for linear correlation of the hindleg and middle leg positions and distances
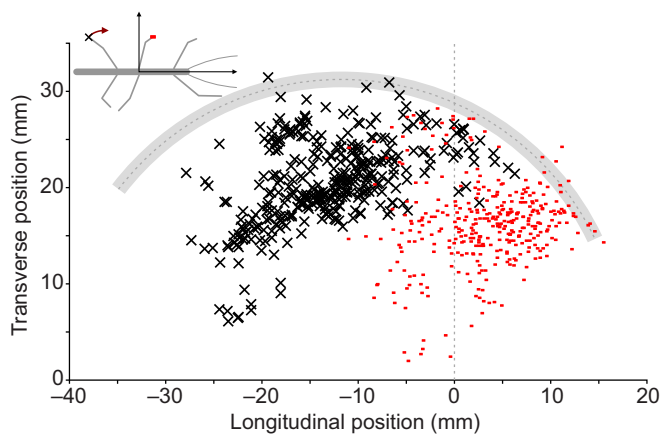

Fig. 6. Scatter plot of middle leg and hindleg positions during walks on the slippery surface. The red dots represent the position of the middle leg at the time of lift-off of the hindleg. The black crosses show the subsequent touchdown position of the hindleg. The vertical dotted line marks zero on the x-axis and also the position of the coxa of the middle leg. The dotted semi-circle depicts the calculated average maximum range and standard deviation (gray area) of the fully stretched hindlegs. n=356.
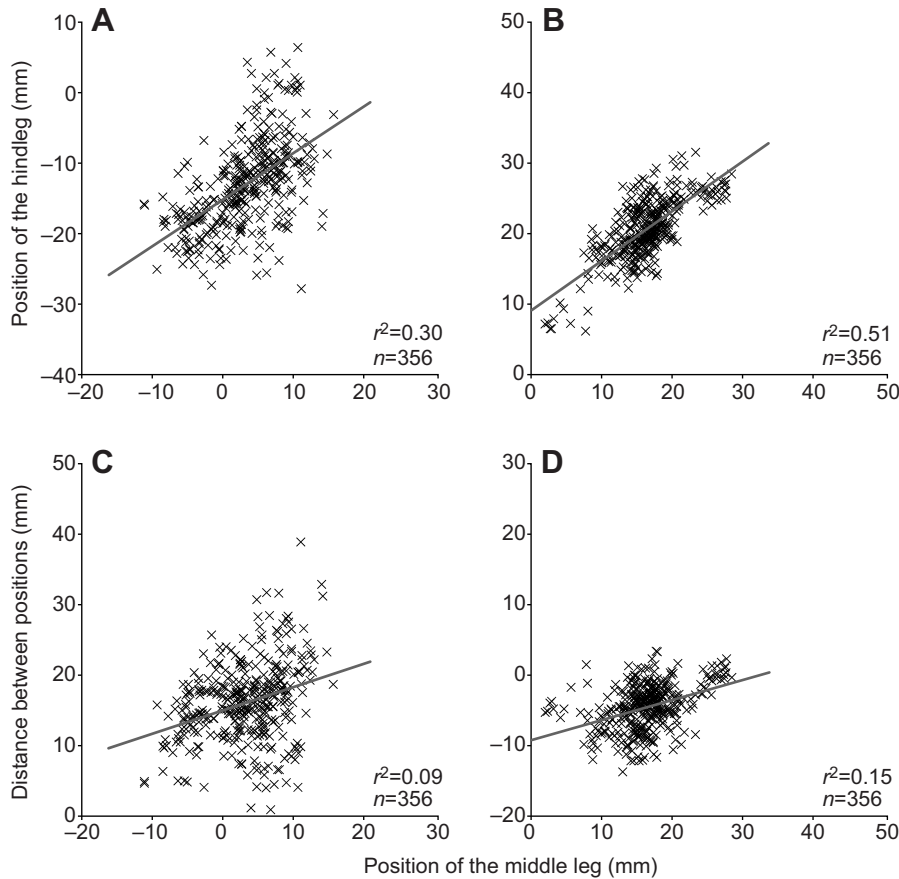
Fig. 7. Scatter plot with a test for linear correlation of the position of the middle leg at the time of lift-off of the hindleg against the subsequent touchdown position of the hindleg (A,B) and against the distance between the middle leg and hindleg (C,D). The plots are separated into components parallel (A,C) and perpendicular (B,D) to the body axis.

parallel and perpendicular to the body axis (Fig. 7). The coefficient of determination for positions parallel to the body axis (Fig. 7A) was similar to the results with standing middle leg and targeting hindleg ($r_x^2=0.30$, cf. Fig. 3A). A much stronger linear correlation was found for the positions perpendicular to the body axis (Fig. 7B; $r_y^2=0.51$). Distances between the two positions either parallel (Fig. 7C; $r_x^2=0.09$) or perpendicular (Fig. 7D; $r_y^2=0.15$) to the body axis showed only very weak linear correlations. Altogether it appears that the state of activity of the middle leg positively influences the targeting accuracy of the hindleg perpendicular to the body axis when the animal locomotes steadily, while no additional improvement was found for the aiming precision along the body axis.

To find out whether there were also state-dependent changes in the aiming precision of the middle on to the front leg, we repeated this analysis for these two legs under tethered walking conditions. We again determined all lift-off and touchdown events of the middle leg and their positions, and also identified the position of the front leg for all lift-off events of the middle leg. If the front leg was performing a swing phase at the time, the corresponding touchdown position of the middle leg was again not included in the analysis. The majority of touchdown positions of the middle leg were close to the leg's maximum reach, with no touchdown positions anterior of the coxa of the front leg (Fig. 8, vertical dotted line). The overall distribution (mean ± s.d. values: $x=-9.3\pm4.4$ mm; $y=18.2\pm2.5$ mm) was similar to that of the touchdown positions with standing front leg (cf. Fig. 4). Interestingly, the spread among touchdown positions of the middle leg was much smaller than that among the touchdown positions of the hindleg (cf. Fig. 6). Similar to the middle leg and hindleg, the reference positions of the front leg were taken at the

time of lift-off of the middle leg. As a result, the front leg positions are all relatively far anterior and in most cases even out of reach for the middle leg (dotted semi-circle in Fig. 8). There was only a very small overlap in the spread of the middle and front leg positions parallel to the body axis. On average, the touchdown positions of the middle leg were 22.7±6.3 mm (x-distance) posterior to the front leg positions while the lateral distribution of the two data groups was similar (y-distance=-1.4±4.3 mm). Despite the large distance, with $r_x^2=0.27$, one can assume linear correlation between the positions of the middle and front leg along the body axis (Fig. 9A). This value was in the same range as that for the walking middle and targeting hindleg (cf. Fig. 7A, Table 2) and about twice as high as the coefficient of determination of the standing front and targeting middle leg (cf. Fig. 5A, Table 2). Perpendicular to the body axis, there was only a slight linear correlation between the positions of the middle and front leg (Fig. 9B; $r_y^2=0.18$), but this was still more than twice as large as that between the standing front and targeting middle leg (cf. Fig. 5B, Table 2). The distance between the touchdown position of the middle leg and the position of the front leg at middle leg lift-off also showed a strong linear correlation parallel (Fig. 9C; $r_x^2=0.64$) as well as perpendicular to the body axis (Fig. 9D; $r_y^2=0.72$). Overall these results indicate targeting of the middle leg to the position of the moving front leg along the body axis and at least a slight targeting perpendicular to the body axis. Similar to the findings for targeting of the hindleg to the middle leg, the targeting accuracy of the middle leg to the front leg appears to improve in a state-dependent manner, once the animal locomotes steadily.

In summary, markedly extending earlier assumptions, the middle leg is less precise than the hindleg in finding its anterior neighbor
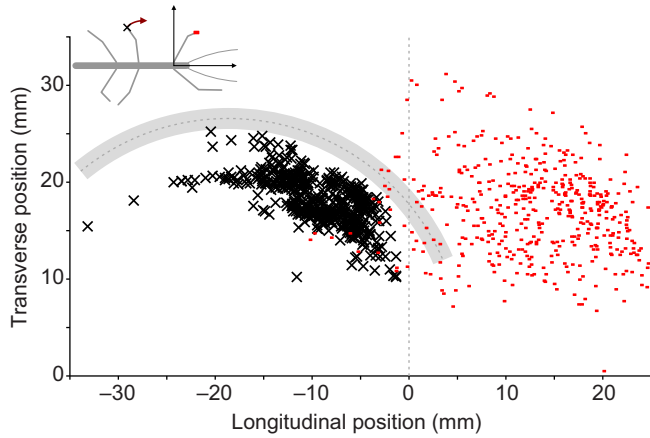
Fig. 8. Scatter plot of the positions of the front leg and middle leg during walks on the slippery surface. The red dots represent the position of the front leg at the time of lift-off of the middle leg. The black crosses show the subsequent touchdown position of the middle leg. The vertical dotted line marks zero on the *x*-axis, and also the position of the coxa of the front leg. The dotted semi-circle depicts the calculated average maximum range and standard deviation (gray area) of the fully stretched middle legs. *n*=50.

in the standing and the walking animal. In addition, we found that movement of the respective anterior leg seems to be of importance for targeting accuracy, suggesting not only a segment-specific but also a state-dependent effect.

DISCUSSION

We have investigated the aiming accuracy of the middle legs and hindlegs of stick insects on a slippery surface. With our analyses we demonstrated that targeted leg movements towards their rostral

neighboring leg can occur under certain conditions, even without mechanical coupling through the ground, but that the strength of this ability is not equal between the hindlegs and middle legs, or between standing and walking animals.

Targeted leg movements without mechanical coupling

In earlier investigations it was shown that stick insects can perform targeted movements with their hindlegs and that the touchdown position of the hindleg depends on the position of the middle leg, which was standing on a separate platform when the rest of the legs were walking on the same treadwheel (Cruse, 1979). This constitutes a setup where the first step of the hindleg is virtually mechanically uncoupled from the standing middle leg. A different approach to study the neuronal control of stepping used animals tethered above a slippery surface, and showed that stick insects are able to perform normal walking movements under this condition (Graham and Cruse, 1981; Cruse and Epstein, 1982; Epstein and Graham, 1983; Graham and Epstein, 1985; Gruhn et al., 2006; Gruhn et al., 2009a). However, information about targeting movements of the legs on the slippery surface is relatively scarce and inconclusive. While Graham, Cruse and colleagues (Graham and Cruse, 1981; Cruse et al., 1995) reported targeting of the legs based on the distribution of the touchdown and lift-off positions of ipsilaterally neighboring legs, Epstein and Graham claimed that they could not observe targeting behavior during their experiments with walking stick insects (Epstein and Graham, 1983). By specifically analyzing the linear correlation of corresponding pairs of leg positions of stick insects tethered above a slippery surface setup, we can confirm that stick insects actually perform targeted leg movements towards their anteriorly neighboring leg even in the absence of mechanical coupling through the ground. However, targeting precision is different between thoracic segments. This targeting in the absence of mechanical coupling provides
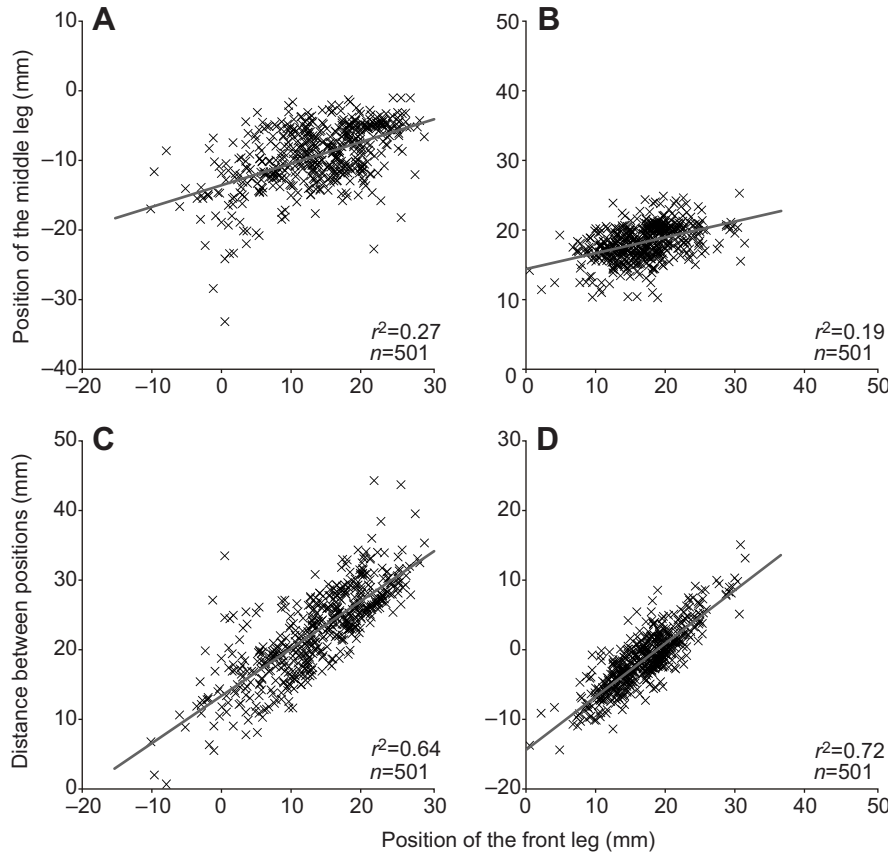

Fig. 9. Scatter plot with a test for linear correlation of the position of the front leg at the time of lift-off of the middle leg against the subsequent touchdown position of the middle leg (A,B) and against the distance between the front and middle leg (C,D). The plots are separated into components parallel (A,C) and perpendicular (B,D) to the body axis.

evidence for a neuronal mechanism that must be involved in spatial coordination of leg movements.

Targeting accuracy of hindlegs and middle legs is different

By comparing the targeting accuracy of the hindlegs towards the middle legs with the targeting accuracy of the middle legs towards the front legs, we showed that the precision has a segment-specific quality, and that targeting of the hindleg was distinctly more accurate than targeting of the middle leg. In fact, when the front leg is standing and the middle leg performs its first step of the walking sequence, this step forwards can hardly be called targeted at all (see Results). This is a novel result because none of the previous studies investigating the targeting behavior of stick insects (e.g. Cruse, 1979; Cruse et al., 1984; Dean, 1984; Dean and Wendler, 1983) measured the accuracy of the middle leg foot placement towards its ipsilateral front leg to compare it with the targeting accuracy of the hindleg, although middle leg targeting was reported by Cruse et al. as unpublished observations (Cruse et al., 1995). In earlier studies, it was assumed from comparing distances between average touchdown and lift-off positions of neighboring legs (Cruse, 1976) that the hindlegs showed better targeting than the middle legs (Cruse, 1979). With our results we can now confirm this to be the case. It is, however, interesting that targeting perpendicular to the body axis in both legs was virtually non-existent in our study, unlike in earlier studies. However, in these previous studies, the targeting hindleg was either standing (Cruse, 1979; Cruse et al., 1984) or moving (Dean and Wendler, 1983; Dean, 1984) along a treadwheel. The possibility cannot be excluded that under these conditions the treadwheel may have a predefining influence on leg movement perpendicular to the body axis. In addition, the position analyses were performed between the touchdown position of the hindleg and the position of the middle leg at the same time, which, as will be discussed below, may not be the best choice for the moving animal.

It remains unclear why the targeting of the hindleg is more accurate than that of the middle leg. The induction of the first step by a light touch to the abdomen was the same when activating either leg and thus seems unlikely to be the reason for the difference. One explanation for the distinctly better targeting accuracy of the hindlegs compared with the middle legs could be based on simple anatomical constraints for the middle legs. The middle leg is the shortest leg of the stick insect (Cruse, 1976) and is anatomically not capable of reaching all posterior extreme positions of the front leg, while the distinctly longer hindleg (Cruse, 1976) is anatomically capable of reaching almost every posterior position of the middle leg. This could also lead to better targeting accuracy of the hindleg by simply bumping into the middle leg. Such an effect, however, may only be relevant at the beginning of a movement when the body is not simultaneously displaced forwards by the movement of several legs at the same time.

The reason for the better targeting performance by the hindlegs may be that the center of mass of the stick insect is located close to and posterior to the coxae of the hindlegs (Cruse, 1976). It might therefore be of greater importance for the stability of the animal to reliably find foothold with the hindlegs than with the middle legs. As a consequence, processing of sensory information on the target leg's location in the resting animal may be different between the mesothoracic and the metathoracic segment. So far, no direct evidence exists to support this hypothesis in the case of targeting. However, Hellekes and colleagues (Hellekes et al., 2012) have shown that there is segment specificity in the processing of sensory information from the fCO, which signals the femur–tibia joint angle, and which could also be integrated with other known sensory signals

to yield distance information to a neighboring leg. Further implications of this differential processing will be discussed below.

Targeting accuracy changes between standing and moving target leg

Interestingly, targeting performance improved when the animal was moving as compared with when the animal was stationary. We found this to be true for the middle leg targeting the front leg parallel to the body axis, as well as for hindleg and middle leg targeting perpendicular to the body axis. This suggests that targeting precision is in fact dependent on the state of the animal, i.e. the movement of the legs.

It is currently unknown at what time or at what position of the target leg the targeting information is read out in order to produce aimed movements by the targeting leg. For exact targeting, the animal would have to know the position of the target leg at the point at which it touches down, which, during walking, is not trivial because the target position has to be obtained and extrapolated while the target leg is still moving towards this position. However, the time at which the information is acquired can be assumed to be within a time frame that allows the nervous system to process the information and for the targeting leg to actually produce a targeted movement that is not made obsolete by the forward movement of the animal.

One can get a rough estimate for the minimal time span necessary for this information transfer by calculating conduction times. First, the position information from the sense organs of the targeted leg has to be transmitted to the local thoracic ganglion. Spikes take 12 ms to travel from the stick insect tarsus to the ganglion, and from data from the stick insect and locust it can be assumed that it takes about 2 ms for the first spikes to travel from coxal sense organs to interneurons within the same hemiganglion (Fisch, 2007; Höltje and Hustert, 2003). The information then has to travel to the neighboring segment. Hardly any direct connections from sensory neurons into neighboring segments have so far been demonstrated (Hustert, 1978), but with connective lengths averaging about 17 mm between the prothorax and mesothorax and 10 mm between the mesothorax and metathorax (Cruse, 1976), and with conductance velocities within the connective of about 2–2.8 mm s^{-1} (Brunner et al., 1990), one can assume at least another 4–9 ms until the first spikes reach the neighboring ganglion. Depending on how far distal in the leg the innervated muscle is, it takes an additional 1–5 ms for the motoneuron spikes to travel to the neuromuscular end plate (Höltje and Hustert, 2003). Finally, the muscle needs a minimum of 20–40 ms to build up the muscle tension needed for movement of the leg (Guschlbauer et al., 2007; Hooper et al., 2009; Blümel et al., 2012). It is unclear how many synapses and interneurons have to be crossed before the information reaches the motoneurons of the targeting leg, but both intersegmental and local interneurons have been described that could take part in the targeting process (Brunn and Dean, 1994). Altogether, in the most conservative estimate, and without considering synaptic transmission, it would take at least 27 ms to process and target a measured leg position, most likely more. This estimate seems to match the finding by Schütz and Dürr (Schütz and Dürr, 2011) that re-targeting of an ongoing swing movement by the front leg occurs with a delay of about 40 ms after antennal contact with an object. Therefore, the position information has to be collected and read out during the swing phase of the targeting leg.

Taking the above considerations into account, the position of the target leg at the time at which it finishes its swing phase and touches the ground, or even the posterior extreme position of the target leg,

does not leave enough time for processing. They could only have a good correlation with the touchdown position if one assumes a perfect prediction of this position by the animal. Indeed, the r^2-values were very small (see Table 2). As we did not know the exact point in time that is used by the animal, we chose the position of the target leg at the time when the targeting leg lifted off the ground and began its swing phase. This is well above the range reported by Schütz and Dürr (Schütz and Dürr, 2011), and hence leaves enough time (on average 141±57 ms; A.W., unpublished) for the neuromuscular system to transmit and process the information. However, we cannot exclude the possibility that the time point at which the placement of the foot is actually decided is later, similar to Schütz and Dürr (Schütz and Dürr, 2011), or even earlier, as has been reported for vertebrates that use visual and mechanosensory information to guide leg trajectories during walking [cat (McVea and Pearson, 2007; McVea et al., 2009; Wilkinson and Sherk, 2005), human (Mohagheghi et al., 2004; Patla and Vickers, 2003)]. In the case of humans wanting to place their foot at a specific target position, it has been reported that they fixate on this position on average two steps ahead, and at least 800–1000 ms before the limb is placed on the target area (Patla and Vickers, 2003).

The questions that arise now are why targeting of the hindleg and the middle leg generally improved during walking, why this was not the case for the hindleg in parallel to the body axis, and what the underlying neuronal mechanisms could be. It is known that sensory information signaling leg angles is integrated by intersegmental and local interneurons and could therefore also be used to provide the targeting information for the hindleg (Brunn and Dean, 1994). The fCO is primarily responsible for targeting accuracy perpendicular to the body axis, by measuring the angle between the femur and tibia (Bässler, 1977; Cruse et al., 1984). The processing of fCO activity changes between standing and walking animals (Bässler, 1974; Bässler, 1976; Bässler, 1988; Stein et al., 2006; Hellekes et al., 2012). In addition, it is known that fCO signals from an anterior leg in the actively stepping animal affect the next posterior leg (Ludwar et al., 2005; Stein et al., 2006). So far, however, no interneurons have been described that solely receive position information from the fCO. Most of the interneurons receive a combination of movement velocity and acceleration information from the femoral chordotonal organ (Büschges, 1989; Brunn and Dean, 1994). Altogether, these findings make it very plausible that fCO signals from the anterior leg may help to target the posterior leg to its anterior neighbor perpendicular to the body axis, but that they are only processed to do so in a state-dependent manner; that is, if the animal is actually walking.

Targeting of the hindleg in parallel to the body axis seems to be primarily controlled by coxal hair rows and hair fields, which measure the position of the coxa, and protraction and retraction movements of the leg (Bässler, 1977; Dean and Wendler, 1983; Cruse et al., 1984). So far, no data exist on state-dependent or thoracic segment-dependent processing of this type of sensory information; however, it is again known that signals from the fCO are processed differently in the different thoracic segments (Hellekes et al., 2012). Therefore, in addition to the state dependence of sensory processing, a different segment-specific processing in the metathorax may be responsible for the lack of improvement in hindleg to middle leg targeting when the animal switches from standing to walking. In other words, as the hindlegs could be more important for the animal's stability, their targeting parallel to the body axis is already almost at its best in the standing animal.

Interestingly, this state-dependent influence of sensory input on the spatial coordination between the legs also matches the description of movement-induced temporal coordination in the stepping stick insect (Borgmann et al., 2009) and its improvement with acceleration (Gruhn et al., 2009b). It also bears similarities with the changes in the coordinating influences between straight and curve walking (Dürr, 2005). The fact that these influences may not be equally strong between different thoracic segments also matches earlier descriptions of stick insect walking, in which the front legs have been described to act as 'feelers' (Cruse, 1976), and is also in accordance with the finding that temporal coupling between middle legs and hindlegs during walking is much stronger than that of either leg to the front legs (Dürr, 2005; Grabowska et al., 2012). The functional significance of this could be that the front legs may, in addition to their function in locomotion, also be used for exploratory purposes, while the middle legs and hindlegs serve mostly as an entity for locomotion. In this context, it will be interesting to see whether targeting accuracy changes with ground properties such as solid planar ground or even irregular profiles such as stair-like structures or even grids, which more closely resemble the natural habitat of a stick insect.

In conclusion, our data, together with the findings of previous studies, support a notion in which stick insect middle legs and hindlegs can aim at their anterior neighbor either when performing a first step or during steady walking. However, the correlations are not always very strong, especially for the first step in the standing animal. This suggests that processing of the relevant sensory information is differently achieved in middle legs and hindlegs as the hindleg is more accurate than the middle leg in finding its anterior neighbor under both conditions. The fact that movement of the animal strongly improves targeting accuracy suggests that processing of information on leg position to produce spatial coordination in the stick insect is not only segment specific but also state dependent and supports previous findings of state-dependent and segment-specific processing of sensory information for temporal coordination.

AUTHOR CONTRIBUTIONS

A.W. and M.G. conceived and designed the experiments; J.E., A.W. and M.G. collected the data; A.W. and J.E. analyzed the data; and A.W. and M.G. wrote the paper.

COMPETING INTERESTS

No competing interests declared.

REFERENCES

Akay, T., Ludwar, B. C., Göritz, M. L., Schmitz, J. and Büschges, A. (2007). Segment specificity of load signal processing depends on walking direction in the stick insect leg muscle control system. *J. Neurosci.* **27**, 3285-3294.

Bässler, U. (1974). Vom femoralen chordotonalorgan gesteuerte reaktionen bei der stabheuschrecke *Carausius morosus*: messung der von der tibia erzeugten kraft im aktiven und inaktiven tier. *Kybernetik* **16**, 213-226.

Bässler, U. (1976). Reversal of a reflex to a single motoneuron in the stick insect *Carausius morosus. Biol. Cybern.* **24**, 47-49.

Bässler, U. (1977). Sensory control of leg movement in the stick insect *Carausius morosus. Biol. Cybern.* **25**, 61-72.

Bässler, U. (1988). Functional principles of pattern generation for walking movements of stick insect forelegs: the role of the femoral chordotonal organ afferences. *J. Exp. Biol.* **136**, 125-147.

Blaesing, B. and Cruse, H. (2004). Stick insect locomotion in a complex environment: climbing over large gaps. *J. Exp. Biol.* **207**, 1273-1286.

Blümel, M., Hooper, S. L., Guschlbauerc, C., White, W. E. and Büschges, A. (2012). Determining all parameters necessary to build Hill-type muscle models from experiments on single muscles. *Biol. Cybern.* **106**, 543-558.

Borgmann, A., Hooper, S. L. and Büschges, A. (2009). Sensory feedback induced by front-leg stepping entrains the activity of central pattern generators in caudal segments of the stick insect walking system. *J. Neurosci.* **29**, 2972-2983.

Brunn, D. E. and Dean, J. (1994). Intersegmental and local interneurons in the metathorax of the stick insect *Carausius morosus* that monitor middle leg position. *J. Neurophysiol.* **72**, 1208-1219.

Brunner, M., Karg, G. and Koch, U. T. (1990). An improved system for single unit isolation from multiunit nerve recordings by velocity analysis. *J. Neurosci. Methods* **33**, 1-9.

Büschges, A. (1989). Processing of sensory input from the femoral chordotonal organ by spiking interneurones of stick insects. *J. Exp. Biol.* **144**, 81-111.

Büschges, A. and El Manira, A. (1998). Sensory pathways and their modulation in the control of locomotion. *Curr. Opin. Neurobiol.* **8**, 733-739.

Clarac, F., Cattaert, D. and Le Ray, D. (2000). Central control components of a 'simple' stretch reflex. *Trends Neurosci.* **23**, 199-208.

Cruse, H. (1976). The function of the legs in the free walking stick insect, *Carausius morosus*. *J. Comp. Physiol.* **112**, 235-262.

Cruse, H. (1979). The control of the anterior extreme position of the hindleg of a walking insect, *Carausius morosus*. *Physiol. Entomol.* **4**, 121-124.

Cruse, H. (1990). What mechanisms coordinate leg movement in walking arthropods? *Trends Neurosci.* **13**, 15-21.

Cruse, H. and Epstein, S. (1982). Peripheral influences on the movement of the legs in a walking insect *Carausius morosus*. *J. Exp. Biol.* **101**, 161-170.

Cruse, H., Dean, J. and Suilmann, M. (1984). The contributions of diverse sense organs to the control of leg movement by a walking insect. *J. Comp. Physiol. A* **154**, 695-705.

Cruse, H., Bartling, C., Dreifert, M., Schmitz, J., Brunn, D. E., Dean, J. and Kindermann, T. (1995). Walking: a complex behavior controlled by simple networks. *Adapt. Behav.* **3**, 385-418.

Dean, J. (1984). Control of leg protraction in the stick insect: a targeted movement showing compensation for applied forces. *J. Comp. Physiol. A* **155**, 771-781.

Dean, J. (1989). Leg coordination in the stick insect carausius morosus: effects of cutting thoracic connectives. *J. Exp. Biol.* **145**, 103-131.

Dean, J. and Wendler, G. (1983). Stick insect locomotion on a walking wheel: interleg coordination of leg position. *J. Exp. Biol.* **103**, 75-94.

Dürr, V. (2001). Stereotypic leg searching movements in the stick insect: kinematic analysis, behavioural context and simulation. *J. Exp. Biol.* **204**, 1589-1604.

Dürr, V. (2005). Context-dependent changes in strength and efficacy of leg coordination mechanisms. *J. Exp. Biol.* **208**, 2253-2267.

Duysens, J., Clarac, F. and Cruse, H. (2000). Load-regulating mechanisms in gait and posture: comparative aspects. *Physiol. Rev.* **80**, 83-133.

Epstein, S. and Graham, D. (1983). Behavior and motor output of stick insects walking on a slippery surface. *J. Exp. Biol.* **105**, 215-229.

Fisch, K. (2007). Untersuchungen zur Rolle und Funktion tarsaler sensorischer Signale bei der Laufmustergenerierung im Mittelbein der Stabheuschrecke *Carausius morosus*. MSc thesis, University of Cologne.

Grabowska, M., Godlewska, E., Schmidt, J. and Daun-Gruhn, S. (2012). Quadrupedal gaits in hexapod animals – inter-leg coordination in free-walking adult stick insects. *J. Exp. Biol.* **215**, 4255-4266.

Graham, D. and Cruse, H. (1981). Coordinated walking of stick insects on a mercury surface. *J. Exp. Biol.* **92**, 229-241.

Graham, D. and Epstein, S. (1985). Behavior and motor output for an insect walking on a slippery surface. *J. Exp. Biol.* **118**, 287-296.

Grillner, S. and Jessell, T. M. (2009). Measured motion: searching for simplicity in spinal locomotor networks. *Curr. Opin. Neurobiol.* **19**, 572-586.

Gruhn, M., Hoffmann, O., Dübbert, M., Scharstein, H. and Büschges, A. (2006). Tethered stick insect walking: a modified slippery surface setup with optomotor stimulation and electrical monitoring of tarsal contact. *J. Neurosci. Methods* **158**, 195-206.

Gruhn, M., Zehl, L. and Büschges, A. (2009a). Straight walking and turning on a slippery surface. *J. Exp. Biol.* **212**, 194-209.

Gruhn, M., von Uckermann, G., Westmark, S., Wosnitza, A., Büschges, A. and Borgmann, A. (2009b). Control of stepping velocity in the stick insect *Carausius morosus*. *J. Neurophysiol.* **102**, 1180-1192.

Guschlbauer, C., Scharstein, H. and Büschges, A. (2007). The extensor tibiae muscle of the stick insect: biomechanical properties of an insect walking leg muscle. *J. Exp. Biol.* **210**, 1092-1108.

Hellekes, K., Blincow, E., Hoffmann, J. and Büschges, A. (2012). Control of reflex reversal in stick insect walking: effects of intersegmental signals, changes in direction, and optomotor-induced turning. *J. Neurophysiol.* **107**, 239-249.

Höltje, M. and Hustert, R. (2003). Rapid mechano-sensory pathways code leg impact and elicit very rapid reflexes in insects. *J. Exp. Biol.* **206**, 2715-2724.

Hooper, S. L., Guschlbauer, C., Blümel, M., Rosenbaum, P., Gruhn, M., Akay, T. and Büschges, A. (2009). Neural control of unloaded leg posture and of leg swing in stick insect, cockroach, and mouse differs from that in larger animals. *J. Neurosci.* **29**, 4109-4119.

Hustert, R. (1978). Segmental and interganglionic projections from primary fibres of insect mechanoreceptors. *Cell Tissue Res.* **194**, 337-351.

Kiehn, O., Dougherty, K. J., Hägglund, M., Borgius, L., Talpalar, A. and Restrepo, C. E. (2010). Probing spinal circuits controlling walking in mammals. *Biochem. Biophys. Res. Commun.* **396**, 11-18.

Ludwar, B. C., Göritz, M. L. and Schmidt, J. (2005). Intersegmental coordination of walking movements in stick insects. *J. Neurophysiol.* **93**, 1255-1265.

McVea, D. A. and Pearson, K. G. (2007). Stepping of the forelegs over obstacles establishes long-lasting memories in cats. *Curr. Biol.* **17**, R621-R623.

McVea, D. A., Taylor, J. L. and Pearson, K. G. (2009). Long-lasting working memories of obstacles established by foreleg stepping in walking cats require area 5 of the posterior parietal cortex. *J. Neurosci.* **29**, 9396-9404.

Mohagheghi, A. A., Moraes, R. and Patla, A. E. (2004). The effects of distant and on-line visual information on the control of approach phase and step over an obstacle during locomotion. *Exp. Brain Res.* **155**, 459-468.

Niven, J. E., Buckingham, C. J., Lumley, S., Cuttle, M. F. and Laughlin, S. B. (2010). Visual targeting of forelimbs in ladder-walking locusts. *Curr. Biol.* **20**, 86-91.

Patla, A. E. and Vickers, J. N. (2003). How far ahead do we look when required to step on specific locations in the travel path during locomotion? *Exp. Brain Res.* **148**, 133-138.

Pearson, K. G. (1993). Common principles of motor control in vertebrates and invertebrates. *Annu. Rev. Neurosci.* **16**, 265-297.

Scharstein, H. (1989). A universal projector for optomotor stimulation. In *Dynamics and Plasticity in Neuronal Systems* (ed. N. Elsner and W. Singer), pp. 116. New York, NY: Thieme.

Schütz, C. and Dürr, V. (2011). Active tactile exploration for adaptive locomotion in the stick insect. *Philos. Trans. R. Soc. B* **366**, 2996-3005.

Stein, R. B., Weber, D. J., Aoyagi, Y., Prochazka, A., Wagenaar, J. B., Shoham, S. and Normann, R. A. (2004). Coding of position by simultaneously recorded sensory neurones in the cat dorsal root ganglion. *J. Physiol.* **560**, 883-896.

Stein, W., Büschges, A. and Bässler, U. (2006). Intersegmental transfer of sensory signals in the stick insect leg muscle control system. *J. Neurobiol.* **66**, 1253-1269.

Wendler, G. (1964). Laufen und Stehen der Stabheuschrecke *Carausius morosus*: Sinnesborstenfelder in den Beingelenken als Glieder von Regelkreisen. *Z. Vgl. Physiol.* **48**, 198-250.

Wilkinson, E. J. and Sherk, H. A. (2005). The use of visual information for planning accurate steps in a cluttered environment. *Behav. Brain Res.* **164**, 270-274.