

## Perturbation of leg protraction causes context-dependent modulation of inter-leg coordination, but not of avoidance reflexes

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### Summary

All animals capable of legged locomotion execute fast, adaptive compensatory movements in response to perturbation of a step cycle. In terms of motor control, such adaptive behaviour typically involves changes in the kinematics of the perturbed limb as well as changes in coordination between legs. Moreover, the unpredictable variety of real life situations implies that compensatory responses should be sensitive to the behavioural context of the animal. We have investigated the extent to which the compensatory response of a walking stick insect (*Carausius morosus*) adapts in parallel to strong context-dependent adaptation of step kinematics and inter-leg coordination. The behavioural contexts we chose were straight walking and visually induced curve walking, for both of which the steady state limb kinematics and inter-leg coupling strengths were known. In case of curve walking, we further distinguished contexts according to whether the inner or the outer leg was perturbed. The three contexts differed strongly with respect to the set of joint actions before perturbation. Upon mechanical perturbation of front leg protraction, we studied context-dependent differences in a local avoidance reflex of the perturbed leg, as well as in coordination mechanisms that couple the step cycles of the perturbed leg to its unperturbed neighbours.

In all three walking contexts, obstacle contact caused an avoidance movement of the front leg that deviated from the unperturbed swing trajectory. Swing duration was increased while step distance was decreased; however, both effects vanished in the subsequent unperturbed step. The prevailing immediate reaction of the three leg joints

were retraction of the coxa (>76%), leivation of the femur (>80%), and flexion of the tibia (>80%), regardless of the behavioural context and, therefore, joint action prior to perturbation. Moreover, activation of each one of these joint actions was shown to be independent of the other two. Thus, local avoidance reflexes are not modulated by the descending visual information that causes transition from straight to curve walking, but are composed of context-independent joint actions.

Perturbation of the front leg also caused significant shifts of the touch-down position of the perturbed leg and of its unperturbed neighbours. If the inner front leg was perturbed, this shift could persist until the subsequent step. Perturbation affected both the spatial location and the timing of touch-down and lift-off transitions in unperturbed neighbouring legs. These effects on inter-leg coordination were context-dependent. For example, time delay to lift-off of the contralateral neighbour was shortened in inner and straight walking legs, but not in outer legs. Finally, a targeting mechanism that determines foot placement in stick insects was shown to be affected by perturbation in a context-dependent manner.

We conclude that the immediate compensatory response of the perturbed leg is not adapted to the behavioural context in spite of strongly differing step kinematics, whereas the compensatory effect on inter-limb coupling is context-dependent.

Key words: stick insect, *Carausius morosus*, curve walking, avoidance reflex, leg coordination, context-dependency.

### Introduction

The central requirement for legged locomotion is the simultaneous control of propulsion, clearance above ground and balance of the body frame. Apart from solving this multiple control problem, the neural controller needs to be adaptive in order to account for varying physical conditions of

the environment, such as slope and compliance of the substrate, but also for changes in behavioural context of the animal. For example: whereas control of a light, running insect with low clearance may well entirely rely on centrally generated rhythms and inherent viscoelastic properties of the musculoskeletal system known as ‘preflexes’ (Jindrich and

Full, 2002), the mere reduction of speed by the same animal increases the relevance of neurally mediated leg coordination and reflexes (e.g. Watson et al., 2002; Zill et al., 2004). Much as in vertebrates, slow walking in arthropods is neurally controlled by an interaction of central pattern generator networks (Bässler and Büschges, 1998; Delcomyn, 1999), local proprioceptive feedback (Graham, 1985; Pearson, 1995), and inter-leg coordination mechanisms (Cruse, 1990) (for a review, see Büschges, 2005). The relative contribution of the latter two is the basis for fast compensatory reactions to unexpected changes within the environment, or to any perturbation of the step cycle. Thus, understanding adaptive properties of fast compensatory reactions may lead to greater understanding of adaptive neural control of legged locomotion in general.

The objective of the present study was to reveal the extent to which fast compensatory responses to mechanical perturbations are context-sensitive in walking stick insects (*Carausius morosus*), a model system of multi-legged locomotion. In particular, we studied the context sensitivity of fast avoidance reflexes and of associated changes in inter-leg coordination in response to mechanical perturbation of front leg protraction. Straight walking stick insects were known to respond to such perturbation with an avoidance reflex that is characterised by prolonged swing duration, followed by irregular steps of the perturbed leg (Cruse and Epstein, 1982; Dean and Wendler, 1982). The latter studies further showed that perturbation also affected inter-leg coordination, for example, by a rearward shift of the lift-off position of the anterior neighbour leg. Here, we compared the course of such compensatory responses in three walking situations that strongly differ in limb kinematics (Dürri and Ebeling, 2005) and coupling strength between adjacent legs (Dürri, 2005). Moreover, we studied the effect of the perturbation on a spatial coupling mechanism that causes a protracting leg to touch down near the footprint of the anterior leg (Cruse, 1979).

Our analysis focused on the control of front leg movements and on coupling between the perturbed front leg and its unperturbed neighbouring legs. Generally, insect front legs are frequently used in different behavioural contexts, e.g. grooming in crickets (Honegger et al., 1979), catching prey in praying mantids, or digging in cicada larvae. In walking cockroaches, front legs have more degrees of freedom than the other legs (Watson et al., 2002) and are influenced more strongly by descending signals than are posterior legs (Schaefer and Ritzmann, 2001). In stick insects, antennal tactile cues can trigger fast re-targeting of an ongoing front leg swing movement (Dürri and Krause, 2001), lack of appropriate ground contact information can induce the smooth transition from a swing movement into cyclic searching movements, e.g. in *C. morosus* (Dürri, 2001) and *Aretaon asperrimus* (Bläsing and Cruse, 2004), and visual motion cues can trigger the kinematic transition from straight to curve walking (Dürri and Ebeling, 2005). In the latter case, front leg motor networks can be considered a primary target of descending visual

interneurons because of the magnitude and speed of the front leg response.

A common observation that was crucial to the design of our experiments is that the kinematics of front leg movements often undergo dramatic changes as the animal enters a new behavioural context. Here, we experimentally brought about such kinematic changes by causing the animal to enter a steady curve walk by means of a large-field visual motion stimulus. Thus, by altering the behavioural context from straight walking into a left or right turn, we forced the animal to change its front leg kinematics (Dürri and Ebeling, 2005) such that the activation pattern of the three leg joints differed considerably. This then allowed us to test whether perturbation caused a consistent, i.e. context-insensitive, activation pattern of all leg joints during all compensatory responses, or whether compensatory responses differed between contexts. Since we always disturbed right front legs, the prevalent activation pattern of the perturbed leg corresponded to three distinct contexts: straight walking leg perturbed, inner curve walking leg perturbed, and outer curve walking leg perturbed.

Concerning the avoidance reflex, the following alternative hypotheses were tested. (1) Different avoidance reflex movements would indicate that the nature of the reflex depended on the current activation pattern of the contributing muscle groups. (2) Identical avoidance reflex movements would indicate recruitment of the same set of muscles irrespective of the current movement context of the leg. In the first case, the avoidance reflex could be the result of the muscle activation pattern having switched to the antagonistic pattern, i.e. by each joint reversing its action. Context-dependent reflex activity has been described in a number of arthropod species. It can be a result of selective activation of distinct motor patterns depending on which set of mechanoreceptors are stimulated (Siegler and Burrows, 1986), or due to gain modulation (Belanger et al., 2000), sign reversal (Bässler and Büschges, 1998), gating (Staudacher and Schildberger, 1998) or likelihood modulation (Gras and Bartels, 1998), depending on the current behavioural state.

Concerning compensatory modulation of inter-joint coupling, we tested whether or not perturbation caused significant differences in spatial footfall patterns, temporal delays between stance–swing transitions between adjacent legs, and in spatial coordination according to the targeting mechanism described by Cruse (Cruse, 1979). Following the notion that each coupling mechanism is characterised by information flow from a sender leg to a receiver leg (Dürri, 2005), we expected that perturbation of the sender leg would affect the step cycle of the receiver leg.

Our results show that obstacle contact leads to an avoidance movement that, in spite of drastically different kinematics at the time of perturbation, remains the same in all three walking contexts. Moreover, we present evidence that each one of the three leg joints is affected independently of the others. In contrast, we found that compensatory leg coordination differed in a context-dependent manner.

## Materials and methods

### Experimental animals and set-up

In all experiments, we used adult female stick insects *Carausius morosus* Brunner 1907, which were bred in a predominantly parthenogenetic colony at the University of Bielefeld, Germany. We selected seven intact animals that showed spontaneous locomotion. Segment lengths of the right front leg coxa, femur and tibia were measured with an accuracy of 0.1 mm using a calliper. To allow for better visibility of the leg joints in video analysis, the thorax–coxa (ThC), coxa–trochanter (CTr), and femur–tibia (FTi) joints (Fig. 1A)

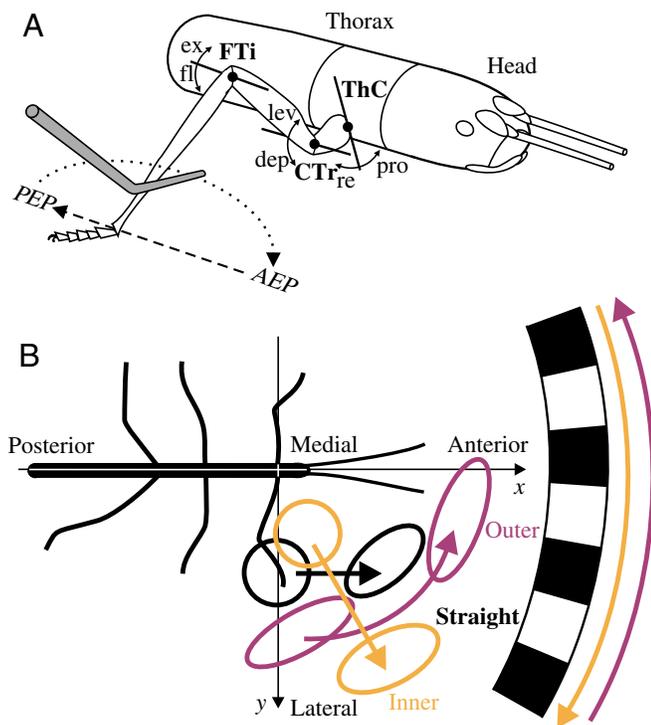


Fig. 1. Schematic drawings of the experimental setup and behavioural contexts. (A) Right front leg of a stick insect with thorax–coxa joint (ThC), coxa–trochanter joint (CTr) and femur–tibia joint (FTi) indicated. Each joint is responsible for different motion components of the leg: ThC, protraction/retraction (pro/re); CTr, levation/depression (lev/dep); FTi, extension/flexion (ex/fl). During swing movements (curved dotted arrow), the leg moves through the air from the posterior extreme position (PEP) to the anterior extreme position (AEP). During stance movements (straight broken arrow), the leg pushes the body forward, moving from AEP to PEP in body coordinates. An obstacle (grey probe) was held into the swing trajectory to examine effects of perturbation. (B) Schematic trajectories of typical swing movements of the right front leg (arrows) and approximate areas of PEPs and AEPs. In straight walking (black trajectory), front leg movements are symmetrical and approximately parallel to the body long axis, whereas in curve walking, leg movements differ between inner (orange trajectory) and outer (purple trajectory) front legs. The coordinate system used for analyses has its origin between the front leg coxae. Curve walking was elicited by means of a large-field visual motion stimulus of a rotating stripe pattern. It was always the right front leg that was perturbed.

were marked by black dots near the distal ends of each segment.

A detailed description of the experimental design has been given in a previous study (Dürr and Ebeling, 2005) and will be briefly reiterated here. Stick insects walked on a light Styrofoam ball (diameter 19.7 cm, mass 11.6 g, moment of inertia  $7.5 \times 10^{-2} \text{ g m}^2$ ) over which they were tethered in a fixed position. The support was counterbalanced in order to make the animals carry their own weight only. Tethering did not constrain head or leg movements. The optomotor turning response to a large-field visual motion stimulus was exploited to elicit steady curve walking behaviour. This motion stimulus was a rotating paper cylinder (height 32 cm, diameter 27.4 cm) with a vertical black-and-white stripe pattern ( $\lambda=24^\circ$ , temporal frequency=1.6 Hz, mean luminance  $\approx 60 \text{ cd m}^{-2}$ , brightness contrast  $\approx 90\%$ , angular velocity  $=38.1 \text{ deg s}^{-1}$ ). Visual motion caused a turning response in the same direction. Since we only perturbed right front legs, curve-walking trials with clockwise turning direction meant that the front leg on the inner side of the curve was perturbed, whereas trials with counter-clockwise turning direction meant that the front leg on the outer side of the curve was perturbed. Therefore, clockwise trials are termed ‘inner leg’ trials, whereas counter clockwise trials are termed ‘outer leg’ trials throughout this study. A lack of stimulus motion led to straight walking, which was considered the reference behavioural context. Trial duration was 21 s. There was no rotation of the stripe cylinder during the first 7 s, then the stimulus began and continued for the following 14 s. Direction of rotation was randomised. Between trials, the insects were left in the set-up for at least 1 min with neither illumination nor rotation of the stripe cylinder. From time to time, drops of water were offered by means of a paintbrush. We selected trials that showed consistent walking behaviour before and after obstacle contact and an obvious turning reaction after stimulus onset. Out of seven stick insects used, five individuals contributed to each sample per walking context with at least four trials. Each experimental context had different sample sizes: inner leg, 51; straight walking, 64; and outer leg, 58.

As animals walked, they rotated the ball underneath them; its movement was recorded optically. The data collected by the optical tracking system were saved on a PC that also controlled the set-up by means of an AD/DA converter. Walking behaviour was monitored and recorded from above using a video camera (1.4 m above the set-up; Fricke GmbH, CCD-7250, Lübbecke, Germany) with a frame rate of 50 Hz and an optical resolution of 0.395 mm per pixel. Video sequences were stored on tape (Panasonic NV-F70) and captured as AVI files for further processing on a PC (MiroVideo 30plus, Pinnacle Systems GmbH, Braunschweig, Germany). AVI files were analysed frame-by-frame using software that was custom-written by Jure Zakotnik (Dept. Biological Cybernetics, University of Bielefeld, Germany). The video system and PC were synchronised by TTL-trigger pulses of the video camera. A frame code generator (Magnasonic VTG 200, Spitzer-Mileger, Basel, Switzerland) was used to match single video frames to the data of the tracking system.

*Perturbation of swing movements*

Only one swing movement of the right front leg was perturbed per trial. The obstacle that we used to perturb front leg movements was constructed from metal wire (diameter 2 mm) that was bent so that it could be handled from outside the striped cylinder. It was painted grey to approximately match mean luminance of the visual stimulus and, therefore, minimise visual contrast. Its angular size with regard to its distance from the animal's eye was approx. 1.14°, which is well below the inter-ommatidial angle of the compound eye. Owing to its size and contrast, but also to lack of any observed orientation response toward or away from the obstacle, we assume it was invisible to the stick insect. Shortly before perturbation, the obstacle was moved from the right side and behind the animal and inserted into the workspace of the leg. Only swing movements were obstructed (Fig. 1A). In many cases, even quick withdrawal of the probe after the initial contact could not avoid multiple contacts between the obstacle and the front leg. In the selected trials shown in Fig. 2A, there

is one short obstacle contact each. In other trials, obstacle contact could take place up to a maximum of four times before either the leg successfully moved around the obstacle or the obstacle was withdrawn. Single obstacle contacts of longer duration than in the examples shown in Fig. 2 were also common. The range of obstacle contact duration was 20–280 ms with a median of 60 ms. The avoidance reaction was an obligatory criterion, i.e. we did not select trials in which the leg touched down in response to obstacle contact or grasped hold of the obstacle.

Since our analysis of reflex behaviour was based on the video frames immediately before and after the first instance of obstacle contact, variability of contact number and duration did not affect the presented results. Contact between the obstacle and the leg occurred either at the front leg tibia (93%) or tarsus (7%). There was no systematic effect of contact location (proximal tibia, distal tibia or tarsus) in any of the three walking contexts. For the analysis of the movement components before and after perturbation, we chose a time

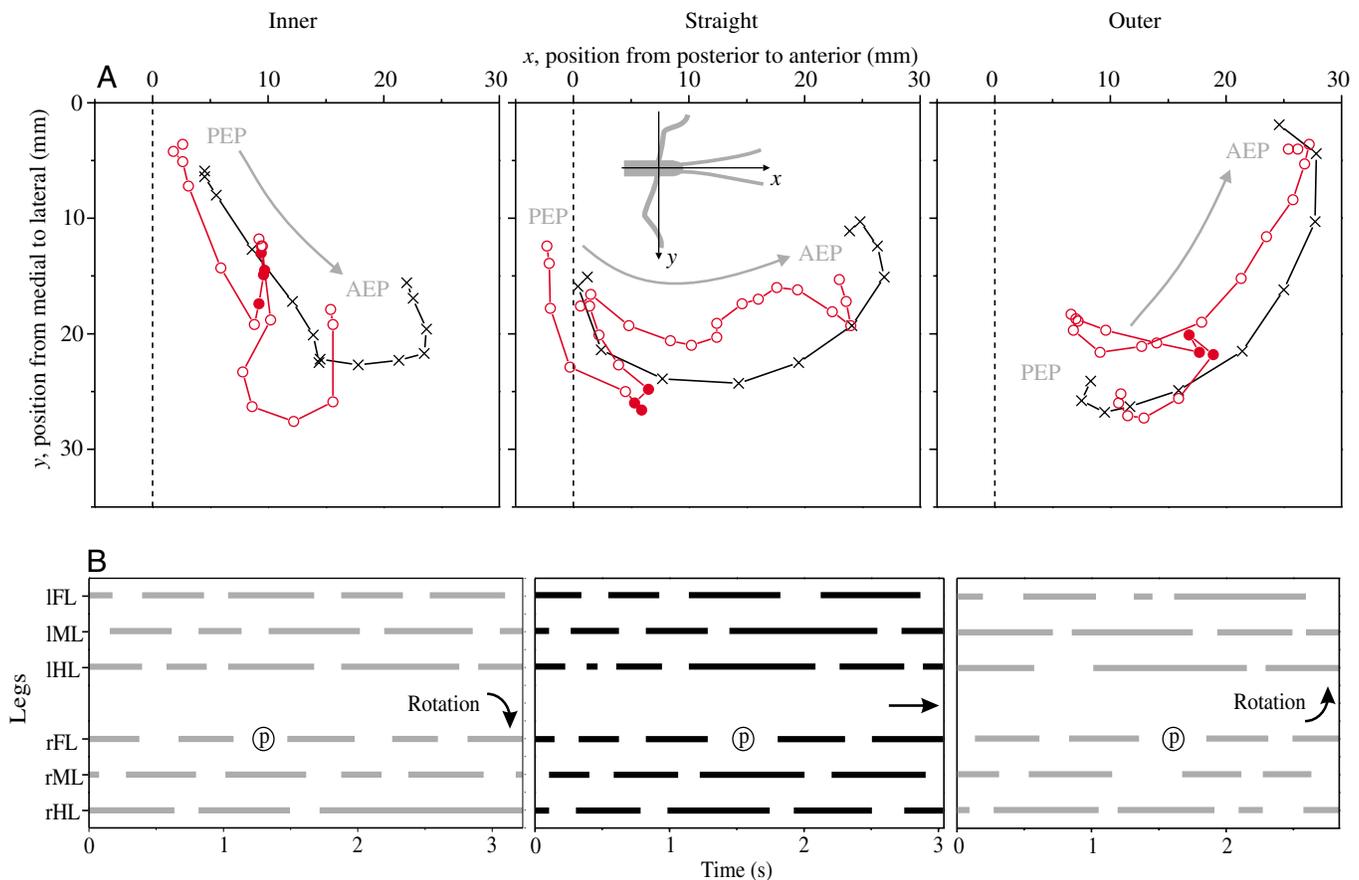


Fig. 2. Representative swing trajectories and step patterns. (A) Top-view of front leg swing trajectories of two subsequent steps: unperturbed (black crosses) and perturbed (red circles) for inner front leg in curve walking (left), straight walking (centre), and for outer leg in curve walking (right). Data points were recorded at a frame rate of 50 Hz. Obstacle contact (filled circles) could last for a variable time span (here: 60–80 ms), eliciting an avoidance reflex that resulted in a deviating swing trajectory until touch-down (AEP). (B) Step patterns of all six legs in straight and curve walking. Traces from top to bottom: IFL, left front; IML, middle; IHL, hind leg; rFL, right front; rML, middle; rHL, hind leg. Arrows indicate direction of visual pattern motion and turn direction. The data belong to the same trials shown in A. The perturbation ('p') was applied during a swing movement of the right front leg. Perturbation causes only small effects in the stepping rhythm of the six legs.

window of 20 ms, i.e. the distance covered between two video frames. This time window was long enough to resolve the displacement caused by the movement, but was short enough to detect the immediate reaction to perturbation.

Each perturbation response was analysed as a sequence of three subsequent swing movements: unperturbed reference, perturbed and unperturbed control. Ideally, perturbation responses were elicited twice per trial: first during the straight walking period (first 7 s) and later during one of the two curve-walking situations (after at least 14 s). In curve walking trials, perturbation responses were analysed only after at least four step cycles had been completed following the onset of cylinder rotation to ensure the transition was long enough to monitor steady curve walking behaviour (Dürr and Ebeling, 2005). Swing trajectories were analysed from video sequences following frame-by-frame digitisation of five points on the body. A point between the hind leg coxae and another between the front leg coxae together defined the body long axis of the animal and the body-fixed coordinate system. Three points near the coxa-trochanter joint (CTr), femur-tibia joint (FTi) and tibia-tarsus joint (TiTa), respectively, defined the leg plane and joint angles. A custom-written computer programme calculated all coordinates in a body-fixed Cartesian coordinate system with the long axis being the x-axis and the origin between the front leg coxae. Segment lengths were used to estimate the height of the FTi and TiTa joints above the walking surface and thus to calculate the CTr and FTi joint angles, as well as the protraction and pronation of the leg-plane. The latter are equivalent to the two degrees of freedom of the ThC joint. Only the protraction component was analysed in this study. A sixth marker was used to label contact position with the obstacle or extreme positions, i.e. the frames in which a swing-stance or stance-swing transition occurred.

#### *Statistical analysis and error estimation*

We analysed our data using non-parametric tests in SPSS software (Statistics Package for the Social Sciences, SPSS Inc., Chicago, IL, USA): the Wilcoxon test for pair-wise comparison (dependent samples) and the Mann-Whitney *U*-test (independent samples). Circular statistics were applied to the distributions of extreme positions. This analysis was based on shift vectors between pairs of subsequent touch-down or lift-off positions of each trial, determining direction and spatial extent of the shift in extreme positions induced by perturbation. The mean vector of the entire sample was then tested for a significant direction [Rayleigh test (see Fischer, 1993)]. Orientation and length of the mean vector were also used as a measure of shift due to coordination influences. We considered test results as statistically significant if  $P=0.05$ . Asterisks indicate significance levels: \*\*\*,  $P<0.001$ ; \*\*,  $P<0.01$ ; \*,  $P<0.05$ .

Because of our experimental arrangement (see above), video recordings could only be analysed in two dimensions. The *z*-component of the three-dimensional (3D) coordinates, necessary for the calculation of joint angles in Fig. 5, was computed from the difference between the real segment length

and the apparent segment length measured from the video projection, considering the inclination of the leg segment. Since identical projection lengths can refer to ambiguous postures in 3D, we thoroughly examined those video sequences in question and confirmed that the FTi joint never moved below the CTr joint and that the tarsus never moved above the FTi joint.

Sometimes, apparent projection lengths slightly exceeded the real segment lengths because of discretisation of real length into pixel units. In these cases, the *z*-coordinate of the segment endpoint would have been undefined. To avoid such singularities, the true segment length was increased by 1 mm (i.e. the femur was increased by approx. 6.1% and the tibia by approx. 6.5%) before calculating *z*-coordinates and joint angles. Although this method introduced a systematic error to the absolute values of all joint angles (mean error  $\pm$  s.d. was  $3.96\pm 2.17^\circ$  in the CTr joint and  $-8.89\pm 4.22^\circ$  in the FTi joint), the relative change of joint angles between two subsequent frames, i.e. angular velocity, was affected only very little (overestimation by  $1.49\pm 0.15\%$  in the CTr joint, and  $1.42\pm 0.38\%$  in the FTi joint). The sign of angular velocity, i.e. direction of movement, was not affected at all. As joint angular velocities were only used in the figure and table that determine the prevalent direction of the perturbation-induced change, the introduced error did not affect the discussed results.

## **Results**

### *General observations*

Front leg swing movements were perturbed in different behavioural contexts that strongly differed in the course of the foot trajectory (see schematic in Fig. 1B and representative trials in Fig. 2A). These marked differences in trajectory translate into differences of the current set of joint actions because the three joints of the stick insect leg can be considered hinge joints with only one rotational degree of freedom (Fig. 1A). In straight walking, the foot trajectory of the front leg is nearly parallel to the body long axis (Fig. 1B). Therefore, straight walking requires the contribution of all three leg joints: the ThC joint accounts for protraction/retraction; the FTi joint is essentially responsible for the width of the support, i.e. extension/flexion, and the CTr joint controls the height above ground during stance movements (levation/depression) and governs lift-off and touch-down of the leg (Fig. 1A). In curve walking, the inner front leg tarsus is directed along an approximately diagonal line from medial to anterior lateral, with strong contribution of the FTi joint and little or no contribution of the ThC joint. In contrast, the swing movement of the outer leg follows a semi-circular arc from lateral to anterior-medial, with a strong contribution of the ThC joint and little or no contribution of the FTi joint. Therefore, prior to perturbation, each one of the three walking contexts were characterised by a distinct activation pattern of the front leg joints.

Obstacle contact led to a disruption of the swing movement. Three typical avoidance movements are shown in Fig. 2A,

which are representative examples of perturbed swing trajectories. Fig. 2B provides the corresponding step patterns of the same trials as in Fig. 2A, illustrating the impact of perturbation on walking sequences. Perturbation of a front leg swing movement affected its trajectory and touch-down position in all behavioural contexts (Fig. 2A). After the initial obstacle contact, the leg remained in contact with the obstacle for 60–80 ms. Then, the leg withdrew from the obstacle, followed a caudal and medial loop, and returned to a trajectory

similar to the unperturbed movement. Since this avoidance movement took longer than a normal swing movement, the stance movements of all unperturbed legs temporally prolonged in straight walking, whereas in both curve walking contexts, only the contralateral legs prolonged their stance phases (Fig. 2B). An extremely prolonged stance phase occurs in the inner hind leg (Fig. 2B, left step pattern), revealing only very weak coupling to the stepping rhythm of the other legs. This is a common feature of the step pattern during curve walking (Dürr and Ebeling, 2005; Jander, 1985) and may be unrelated to the perturbation.

#### *Impact on step cycle and the avoidance reflex of the perturbed leg*

Perturbation affected both spatial (Fig. 3A: swing distance) and temporal step parameters (Fig. 3B,C: swing duration and duty cycle). Perturbed swing movements were of decreased distance (88–93%), longer duration (132–139%) and lower duty cycle (67–74%).

In straight walking, swing distance of the perturbed and subsequent step decreased to about 93%, whereas in the outer leg, only the perturbed step was affected by a drop to about 88% (Fig. 3A). Swing distance of the inner leg was not affected. Avoidance movements had a significantly prolonged swing duration (132–139%; Fig. 3B), but there was no effect on the subsequent unperturbed step. Accordingly, the duty cycle dropped from 80–87% to approx. 60% in all three behavioural contexts, which shows that the prolonged swing movement is not followed by a prolonged stance movement (Fig. 3C). Stance duration depends on the overall walking velocity and varies considerably among trials (data not shown). However, stance duration of the perturbed step significantly decreased in straight walking to 83% ( $P < 0.01$ ) and in the inner leg to 78% ( $P < 0.001$ ).

Step parameters and the effect of perturbation were context-dependent, in that swing movements of the inner front leg were 23% shorter and took 8% longer than in straight walking, and 26% shorter and 13% prolonged in the outer leg. This indicates lower swing velocity in the inner front leg. In parallel, the duty cycle in inner front legs was 5–8% lower. Stance duration is lowest in the outer leg and differs by 41 and 46% from straight walking and the inner front leg, respectively.

Among trials of one behavioural context, the characteristics of obstacle contact differed in terms of timing and stimulus site. For example, perturbation could occur from soon after lift-off until just before touch-down and at any point along the tibia or tarsus. Since timing of the perturbation might have affected the avoidance reflex, statistical tests were calculated to take into account possible effects of swing velocity or limb posture. Trials were binned according to three time intervals of equal duration, equivalent to thirds of the average unperturbed swing duration. There was no systematic effect of perturbation time on any of the tested movement variables. Similarly, statistical controls were calculated by binning the trial according to three contact regions along the leg (proximal tibia, distal tibia, and tarsus). Again, no systematic effect on reflex action was found

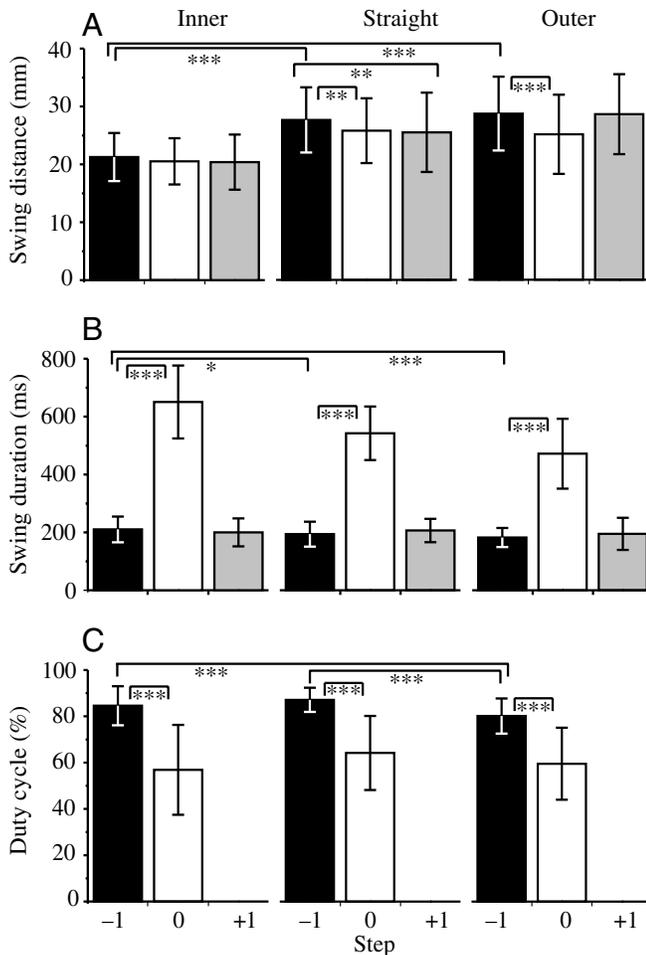


Fig. 3. Spatial and temporal effects of perturbation. The impact of perturbation on the perturbed leg itself was investigated over a walking sequence of three subsequent steps (-1, 0, +1), step 0 being the perturbed step (white). Bars indicate mean values of step parameters  $\pm$  s.d. (Inner,  $N=51$ ; Straight,  $N=64$ ; Outer,  $N=58$ ). Perturbation affected swing distance (A), swing duration (B), and duty cycle, i.e. the fraction of the step cycle spent in stance (C). Data of the perturbed step 0 and the subsequent unperturbed step +1 (grey) were tested with reference to step -1 (black), prior to perturbation (Wilcoxon test). Perturbation caused a reduction of swing distance and duty cycle and a prolongation of the swing duration, the only exception being swing distance of inner legs, which remained unchanged (A, left). Step parameters in unperturbed walking depend on the behavioural context and differ between straight and curve walking (Mann-Whitney  $U$ -test). Significance levels: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

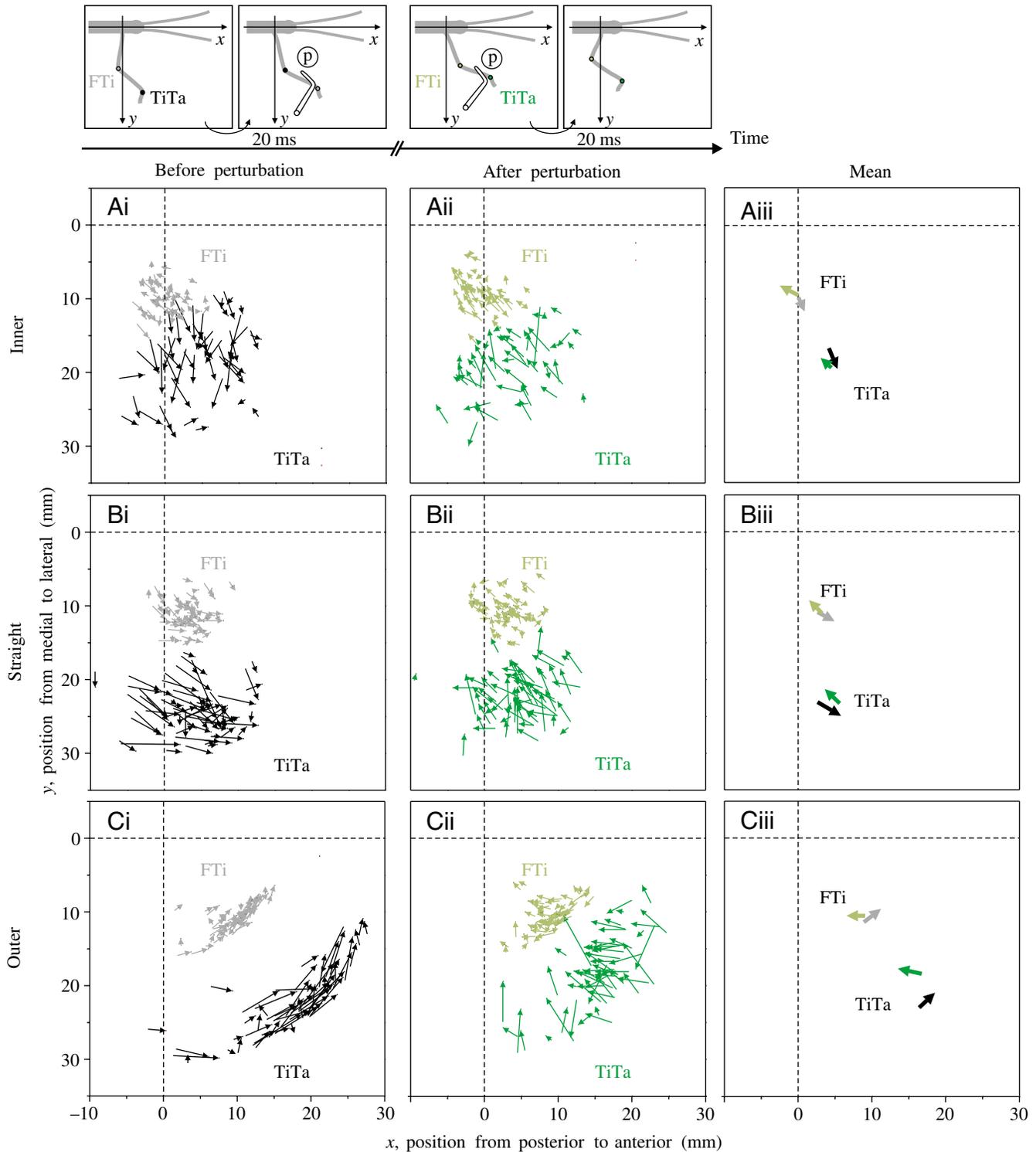


Fig. 4. Immediate reaction of the perturbed leg to obstacle contact. (Top) Sketch of the experimental sequence. (A–C) Top view of leg movements before and after perturbation in the three behavioural contexts: inner leg (A), straight walking (B) and outer leg (C). Vectors indicate the displacement of the femur–tibia joint (FTi; grey in i, light green in ii) and tibia–tarsus joint (TiTa; black in i, dark green in ii) within 20 ms immediately before (i) and after (ii) obstacle contact. (iii) The mean vectors. Note that vectors do not indicate the action of these joints but rather their displacement caused by the movement of the joints proximal to them. The direction of the FTi vectors reflects the contribution of the ThC and CTr joints and, therefore, two motion components only (protraction/retraction, levation/depression). The direction of the TiTa vectors additionally includes a contribution of the FTi joint (extension/flexion). Vector length indicates swing velocity. Before perturbation, leg movements exhibit prominent differences between behavioural contexts, indicating that different muscle groups were active at the moment of obstacle contact. In contrast, the patterns of movement after obstacle contact were all very similar, seemingly converging to a common position.

(data not shown). Thus, all data could be pooled for further analysis of reflex behaviour.

Qualitatively, all immediate avoidance movements of the perturbed leg appeared to lift the foot up and move it closer towards the base of the leg. Three representative single tarsus trajectories are shown in Fig. 2A. To illustrate the common trend of all avoidance movements, displacement vectors in Fig. 4 illustrate the knee and foot trajectories immediately prior to obstacle contact (Fig. 4Ai,Bi,Ci) and immediately upon release from the obstacle (Fig. 4Aii,Bii,Cii). Each arrow indicates the instantaneous direction and velocity of the displacement the FTi joint, i.e. the ‘knee’, and of the TiTa joint, i.e. the base of the foot. Right panels show the mean displacement vector of all movements. Prior to perturbation, the TiTa joint (black arrows in Fig. 4Ai,Bi,Ci) is displaced laterally in inner legs (Fig. 4Ai), anteriorly in straight walking legs (Fig. 4Bi), and anterior-medially in outer legs (Fig. 4Ci). Displacement of the FTi joint (grey arrows) follows this pattern with lower velocity. Immediately after release of the obstacle, the TiTa joint (TiTa; dark green arrows in Fig. 4Aii,Bii,Cii) most often is displaced posterior-medially in each one of the three contexts. Again, displacement of the FTi joint (light green arrows) largely follows this pattern with lower velocity, yet with posterior displacement being more pronounced in the outer legs. The average displacement vectors in Fig. 4Aiii,Biii,Ciii illustrate that foot trajectories (TiTa) are directed into very similar directions after obstacle contact, although their direction prior to contact was very different.

Next, we analysed the relative contribution of the three leg joints. Qualitatively, this can be extracted from Fig. 4 because, owing to the degrees of freedom of the two basal leg joints, the tangential and radial components of the FTi joint displacement illustrate the action of the ThC joint and CTr joint, respectively. In contrast, the displacement of the TiTa joint reflects the combined action of all three leg joints. Note that, owing to a leverage effect, small changes of the ThC or CTr joint angles produce a much larger TiTa displacement than equal changes of the FTi joint. Fig. 5 takes a closer look at what happens at the individual joint by showing joint angle velocities prior to and after obstacle contact. Lines that cross the abscissa indicate a switch in action of the joint, i.e. an activation of the antagonistic muscles upon perturbation. The slope of each line indicates the magnitude of the change. Median joint angle velocities in Fig. 5 (arrowheads) confirm that the joint actions were context-dependent before perturbation, but reveal a default activation pattern during the avoidance movement. In straight walking, median values for each joint differ significantly from zero (Mann–Whitney *U*-test against zero,  $P < 0.01$ ), showing that all three leg joints contribute to the movement. In both curve walking contexts, the median value of only one joint significantly deviated from zero (Mann–Whitney *U*-test against zero,  $P < 0.001$ ), showing that a single joint dominated the movement before perturbation: the FTi joint causes extension in inner legs, whereas the ThC joint governs protraction in outer legs.

After perturbation, avoidance movements involved

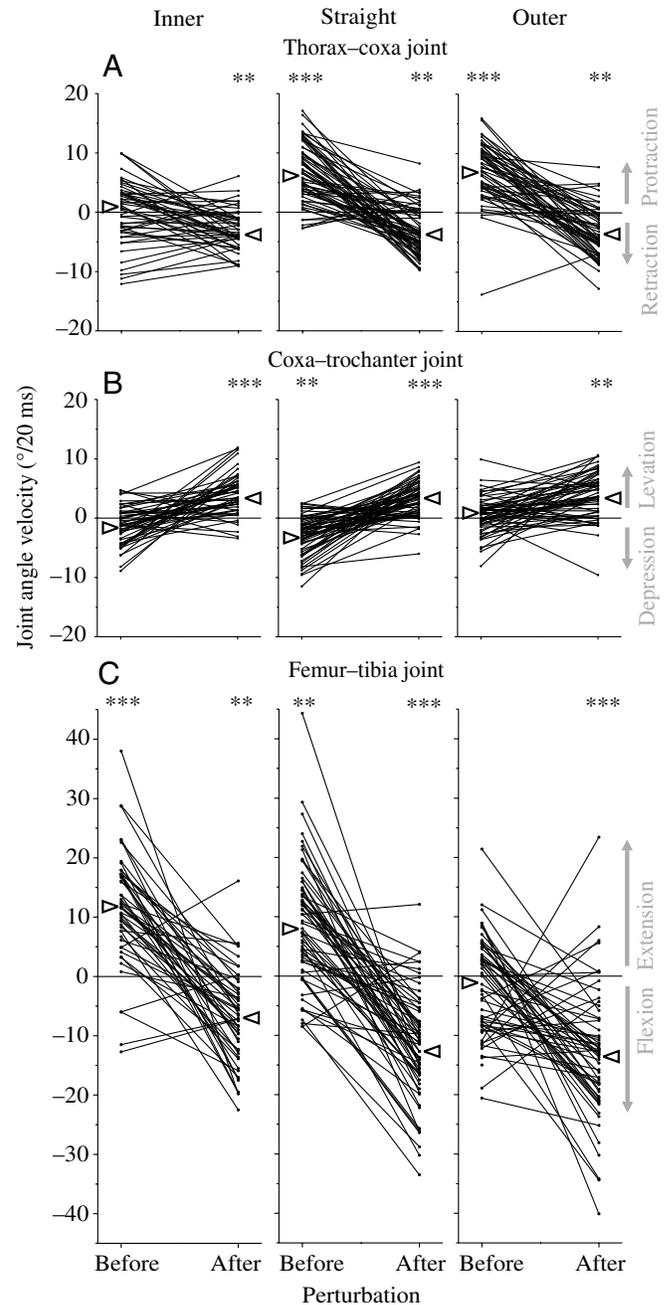


Fig. 5. Motion components before and after perturbation. Joint angle velocities and motion components during the 20 ms immediately before and after obstacle contact in the three behavioural contexts. (A) Positive thorax-coxa (ThC) joint velocity causes protraction of the leg. (B) Positive coxa-trochanter (CTr) joint velocities result in levation of the femur. (C) Positive femur-tibia (FTi) joint velocities are due to extension of the tibia. Median velocities are indicated by arrowheads. Before perturbation, the combination of motion components is context-dependent. The swing phase of straight walking is characterised by protraction, depression and extension (centre), whereas in curve walking, only one motion component predominates: FTi extension in the inner leg (left) and ThC protraction in the outer leg (right). In contrast, the avoidance reflex consists of the same motion components in all three behavioural contexts: retraction, levation and flexion (Mann–Whitney *U*-test of median velocities against zero). Significance levels: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

Table 1. Joint action in the transition from unperturbed walking to avoidance movements

Action	ThC joint			CTr joint			FTi joint		
	Inner	Straight	Outer	Inner	Straight	Outer	Inner	Straight	Outer
Switch	54	81.3	77.2	62.7	79.7	43.8	76.5	71.9	56.2
Reinforce	18	1.6	5.3	29.4	9.4	43.9	5.9	20.3	33.4
Reduce	28	17.2	17.6	7.9	11.0	12.3	17.6	7.9	10.6

Motion component	Retraction			Levation			Flexion		
	82	76.6	79.0	86.3	89.1	80.7	80.4	89.1	87.7

Percentage of joint action of thorax–coxa (ThC) joint, coxa–trochanter (CTr) joint and femur–tibia (FTi) joint in three behavioural contexts: inner leg, straight walking and outer leg. Joint angle velocities were analysed before and after perturbation on a single-trial basis. All values indicate percentages of trials.

Of the three types of joint action defined, switching between two motion components occurred most frequently regardless of behavioural context and leg joint. Whereas switching clearly dominated in straight walking, it is less consistent in both curve-walking contexts. The avoidance movement context-independently comprised the motion components retraction, levation and flexion, each one in 77–89% of all trials.

retraction, levation and flexion, irrespective of the behavioural context (Fig. 5). As can be seen by the fact that lines in Fig. 5 often cross the abscissa, and may do so in both directions, the joint action often reversed sign in response to perturbation. This might indicate that the avoidance reflex switches to antagonistic muscles, which would necessarily result in movement away from the contact surface. Alternatively, the avoidance reflex could be caused by a default activation pattern of retraction, levation, and flexion, with a non-significant number of trials deviating from this rule. To assess whether switching to antagonistic muscles provided a better explanation of the reflex action than a default activation pattern does, we calculated the relative frequencies of three reaction types on a single-trial basis. The results are shown in Table 1. The reaction types we distinguished were: *switch*, i.e. a sign reversal in joint angle velocity; *reinforce*, i.e. same sign, but with greater magnitude than before perturbation; *reduce*, i.e. same sign, but with decreased magnitude.

In response to perturbation, joint action switched in the ThC and CTr joint in about 80% of all trials in straight walking, whereas joint action of the FTi joint switched only in 71.9% of trials (Table 1). In curve walking, switching was the most frequent reaction type, too, but occurred less frequently and less consistently than in straight walking (between 43.8 and 77.2%, depending on joint and context). Given that the predominant joint actions in unperturbed straight walking were protraction, depression and extension, reversal of the current motor action in all three leg joints would have resulted in retraction, levation and flexion. Across all behavioural contexts, these putative default motion components occurred with frequencies between 76.6 and 89.1%. Indeed, response type ‘activation of a default pattern’ is more probable than switching in eight out of nine cases. This indicates that the avoidance reflex is not a result of switching to antagonistic muscles, but should be regarded as a default movement pattern of a leg, regardless of its previous movement.

Concerning the neural implementation of this default avoidance movement, it is important to note that the frequencies of concurrent retraction, levation, and flexion are nearly equal to the expected frequencies in case of their independent action, i.e. without any coupling. Accordingly, the products of  $P_{\text{retraction}} \times P_{\text{levation}} \times P_{\text{flexion}}$  listed in Table 2 (numbers in parentheses) hardly differ from the real frequencies in all three behavioural contexts. Whereas each one of the default motion components, retraction, levation, and flexion, was observed in about 80% of all cases (Table 1), the combination of the three of them occurred only in about 60% of all trials (Table 2). Thus, we conclude that the three leg joints are not coupled to each other during initiation of the avoidance reflex. In summary, the generation of a particular motion component during an avoidance movement is context-independent. Upon obstacle contact, joint action did not switch sign or reduce strength consistently. Instead, the default combination of retraction, levation, and flexion occurred most frequently in each of the behavioural contexts leading to the same reflex action by independent action of the leg joints.

Table 2. Context-independent composition of the avoidance reflex

Reflex	Inner	Straight	Outer
re+lev+fl	58.8 (56.9)	60.9 (60.8)	59.7 (55.9)

Percentage of trials in the three behavioural contexts with a reflex composition of retraction (re), levation (lev) and flexion (fl). Numbers in parentheses are the expected percentage in case of completely independent (i.e. uncoupled) action of each joint. The combined reflex action occurs with approx. 60% frequency in all behavioural contexts of the perturbed front leg and very closely matches the expected frequencies in independent joints.

*Impact of perturbation on leg coordination*

Having analysed the response of the perturbed leg itself, we investigated whether the impact of the altered step cycle in the perturbed leg caused a change in coordination with neighbouring legs. As the front legs are known to send coordinating information to the contralateral front leg and to the ipsilateral middle leg (Cruse, 1990; Dürr, 2005), we focused on these two neighbours. Both spatial effects on the location of extreme positions and temporal effects such as the

delay of lift-off in the receiver leg were analysed for context-dependency.

First, we assessed perturbation-related shifts of the posterior extreme position (PEP, lift-off) and the anterior extreme position (AEP, touch-down) of the two receiver legs. In general, extreme positions varied considerably, as shown in Fig. 6 for all straight walking trials (black crosses). The PEPs of each of the three legs were distributed in broad oval areas, whereas the front leg AEPs dispersed along the swing

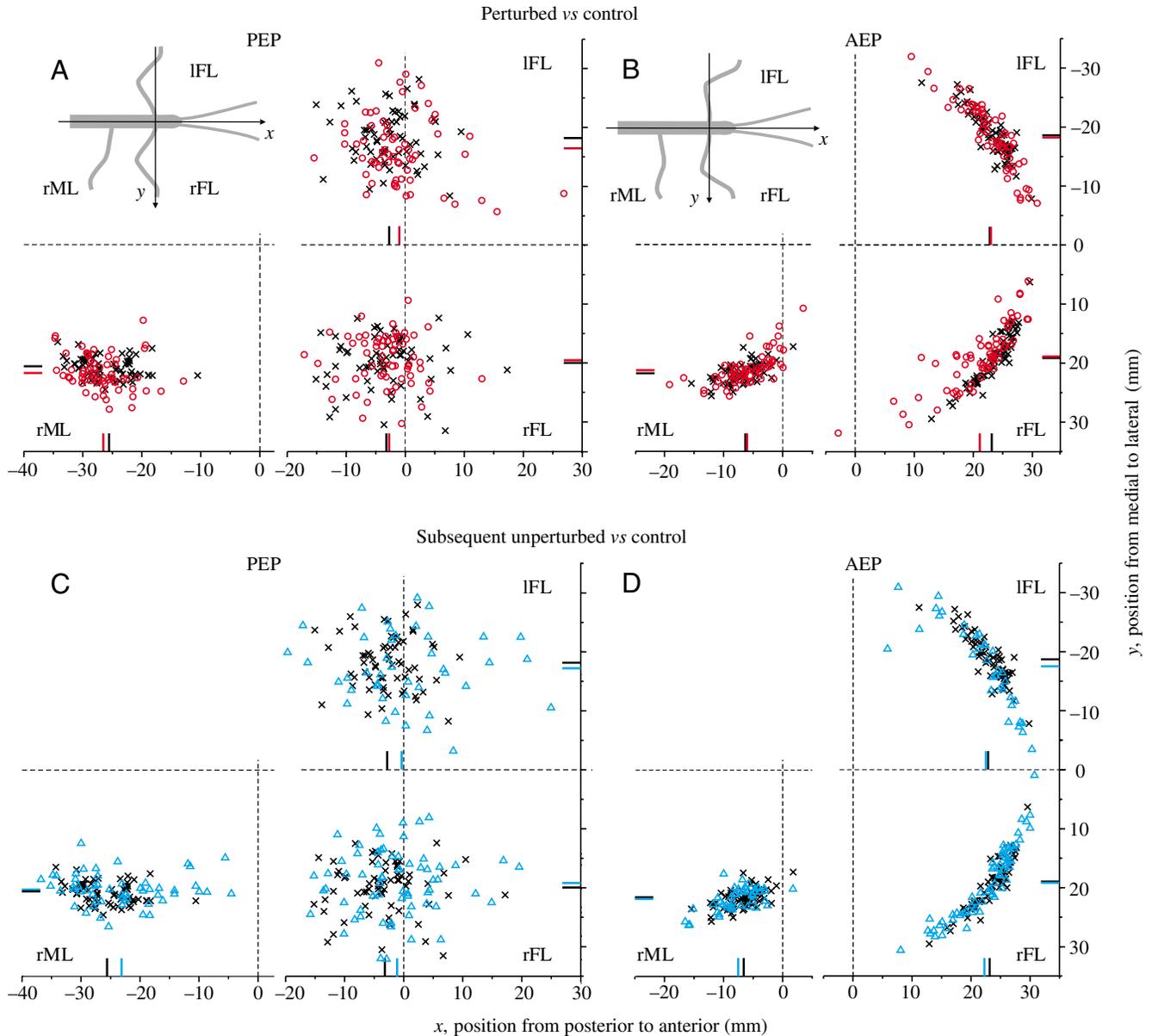


Fig. 6. Impact of perturbation on extreme positions of swing movements. Top view of posterior extreme position (PEP) and anterior extreme positions (AEP) in unperturbed and perturbed straight walking of the right front leg (rFL), the contralateral left front leg (IFL), and the ipsilateral right middle leg (rML). (A) PEPs and (B) AEPs of unperturbed swing movements (black crosses) and their subsequent perturbed swing movements (red circles). Red, blue and black bars indicate mean values. Both in perturbed and unperturbed steps, PEPs dispersed more widely than AEPs. Perturbation caused a posterior-lateral shift of PEPs of the right middle leg and a posterior shift of AEPs of the right front leg. (C) PEPs and (D) AEPs of unperturbed reference (black crosses) and the subsequent unperturbed swing movements after the perturbed step (blue triangles) of the same walking sequence as in A and B. Most effects of perturbation vanished in the subsequent step (for statistics, see Fig. 7 and text).

trajectories. Significant shifts often appeared rather subtle because of the scattered distribution of the extreme positions, but were fairly consistent throughout their distribution. In response to mechanical perturbation (red circles in Fig. 6A,B), the AEPs of the perturbed front leg (rFL) were more dispersed and lay systematically posterior to the unperturbed reference AEPs (Fig. 6B). Middle leg PEPs shifted to posterior-lateral (Fig. 6A). In the subsequent unperturbed step (blue triangles in Fig. 6C,D), there were no shifts of extreme positions. Note that, according to the definition of a step cycle beginning and ending with a PEP, the PEPs of the perturbed steps preceded perturbation in the right front leg, but lagged perturbation in neighbouring legs. Therefore, in the right front leg, PEPs were virtually unaffected by perturbation and did not differ from PEPs of unperturbed steps. In contrast, the distribution of PEPs of both neighbouring legs is shifted after perturbation.

Statistical significance of these effects was tested for by means of circular statistics on the shift vectors with respect to the unperturbed extreme position. Shifts were considered significant if the mean direction of these shift vectors deviated from zero [Rayleigh test (see Fischer, 1993)], which is equivalent to a consistent and systematic shift in most trials. The result is presented in Fig. 7 for the three behavioural contexts with mean shift vectors indicating the direction and the spatial extent of the shift. Strongest effects occurred if the inner front leg was perturbed (Fig. 7A) in which case perturbation even affected the neighbouring unperturbed legs and the subsequent step. In straight walking, AEPs of the perturbed leg were shifted rearward in the perturbed and subsequent step, whereas PEPs and AEPs of the ipsilateral middle leg and PEPs of the contralateral front leg were only affected in the perturbed step (Fig. 7B). Perturbation of the outer front leg caused shifts of the AEPs of the perturbed leg and of the ipsilateral middle leg. Moreover, PEPs of the contralateral front leg shifted, but subsequent steps remained unaffected (Fig. 7C).

Arcs in Fig. 7 illustrate the contribution of the front leg and middle leg ThC joints to shifts in tangential direction.

Although the ThC joint lacks contribution to unperturbed steps of the inner front leg, shifts of its extreme positions due to perturbation clearly contain tangential components. In straight walking, perturbation caused only small shifts with both radial and tangential components, revealing the contribution of all leg joints (Fig. 7B). Perturbation of the outer front leg led to a rearward shift of the AEP in tangential and radial direction in the perturbed leg and to a radial outward shift of the PEP in

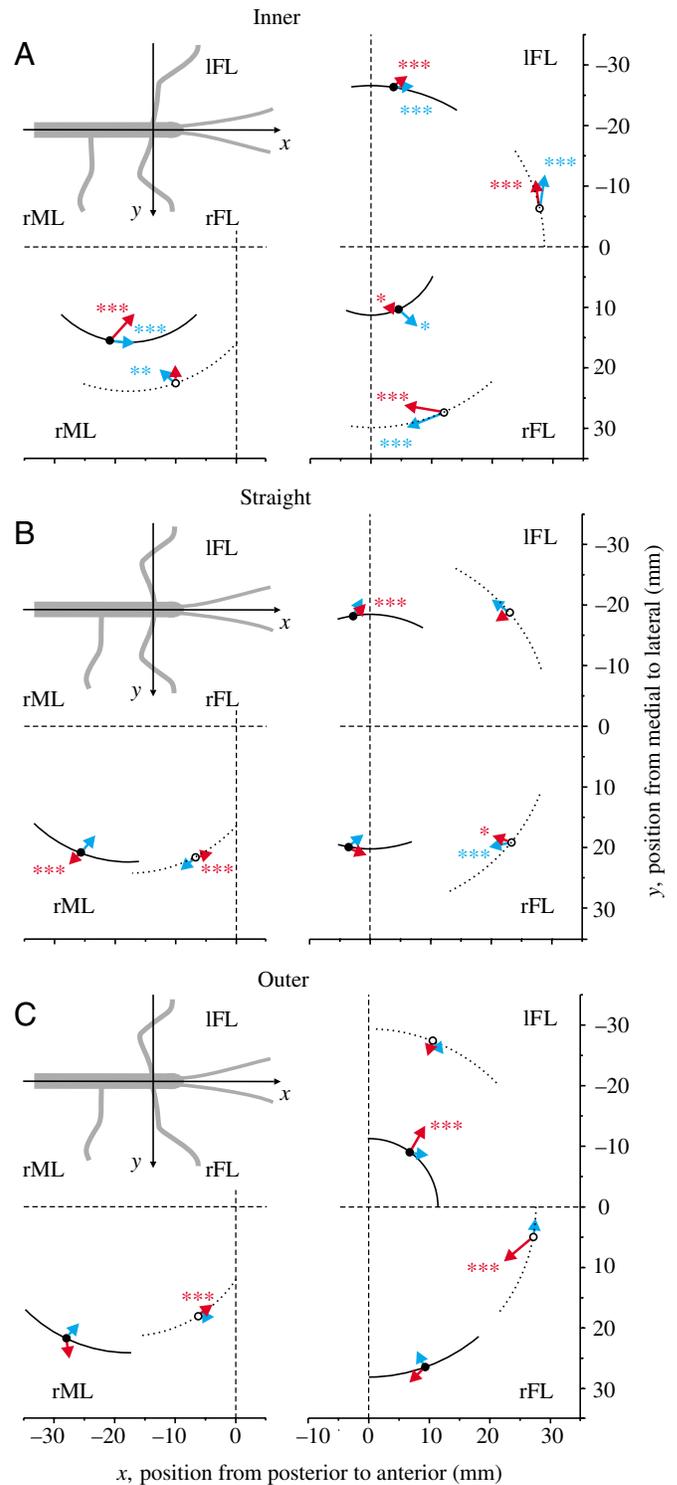


Fig. 7. Shifts of extreme positions due to perturbation. Top view of shift vectors, indicating the mean shift of extreme positions of the perturbed right front leg (rFL), the contralateral left front leg (IFL), and the ipsilateral right middle leg (rML). As reference, mean unperturbed PEPs (filled circles) and AEPs (open circles) are shown for the three legs. Different walking contexts are shown: (A), inner leg; (B), straight walking; (C), outer leg. Arrow length indicates the spatial extent of the shift. Statistical significance was tested for mean direction of the shift. Significance levels: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . Arcs (solid lines, PEP; dotted lines, AEP) illustrate tangential components of the shift, i.e. the contribution of the front leg and middle leg ThC joints. The impact of perturbation was strongest when the inner front leg was perturbed (A) with significant shifts in all three legs in the perturbed (red arrows) and subsequent step (blue arrows). Shifts were less pronounced if perturbation occurred in straight walking (B) or the outer front leg (C). The mean distance between the prothoracic and mesothoracic coxae was taken from Cruse (Cruse, 1976).

the contralateral front leg (Fig. 7C). The latter must be due to the FTi joint. The differences between the three panels in Fig. 7 illustrate that the perturbation caused context-dependent changes on leg coordination, as extreme positions of both the perturbed sender leg and also of neighbouring receiver legs were affected to different extents.

Apart from the impact of perturbation on spatial leg coordination, we also found significant effects on temporal

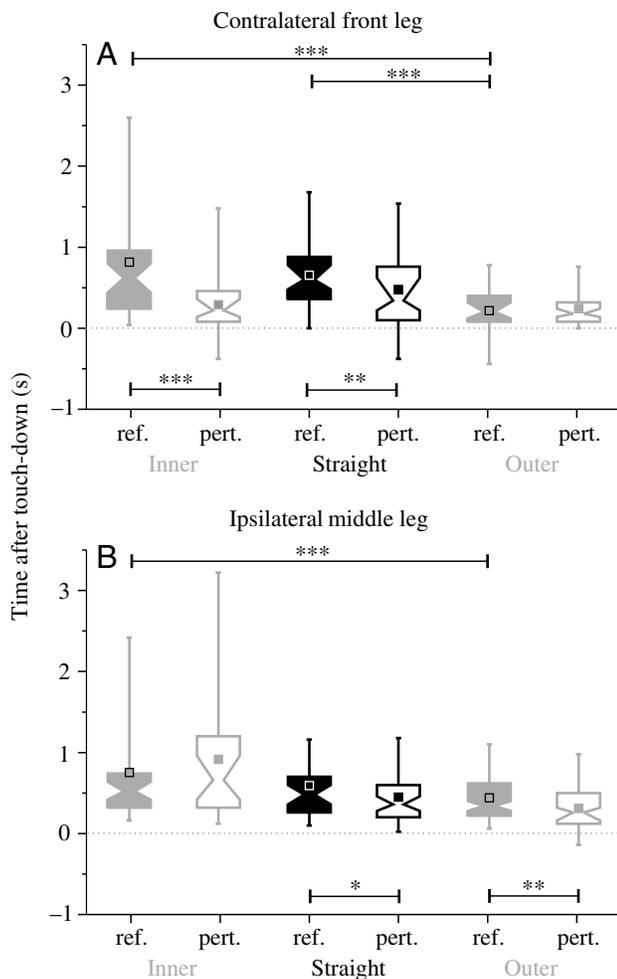


Fig. 8. Temporal coordination between contralateral (A) and ipsilateral (B) legs. Time delays of lift-off of two receiver legs relative to touch-down of the perturbed sender leg. (A) Delay between AEP of the perturbed front leg and PEP of its contralateral neighbour. In unperturbed reference steps (ref., filled symbols), the delay was significantly shorter if the sender leg was an outer leg (grey, right; Mann–Whitney *U*-test). After perturbation (pert., open symbols), delays significantly decreased if the sender leg was in the straight walking (black, middle) or inner leg context (grey, left; Wilcoxon test). (B) Delay between AEP of the perturbed front leg and PEP of its ipsilateral middle leg. In unperturbed steps, delays significantly differed between outer and inner leg contexts. In the perturbed steps, the middle leg lifts off earlier in straight walking and in outer legs. Boxes, 25–75% of the data; notch, 95% confidence interval of the median (centre of notch); whiskers, 5% and 95% percentiles. Squares, mean. Significance levels: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

coordination. Fig. 8 illustrates the delay of lift-off, i.e. stance–swing transition, in the contralateral and ipsilateral receiver legs relative to the time of touch-down in the perturbed sender leg. According to coordination rules 1 and 2 (*sensu* Cruse et al., 1995), a sender leg inhibits lift-off in a neighbouring receiver leg until touch-down (rule 1), but excites lift-off soon after touch-down (rule 2). As both of these coordination rules couple the perturbed front leg to its contralateral neighbour (Dürr, 2005), we assessed the time delay between the AEP of the perturbed front leg (touch-down in sender leg) and the PEP of the neighbouring leg (lift-off of receiver leg). The results are shown in Fig. 8A. Values below zero, which indicate violations of rule 1, occurred in unperturbed trials only if the sender leg was an outer leg and in perturbed trials if the sender leg was in the straight walking or inner leg context. In each of these situations, violations occurred in almost 25% of trials. Thus, coupling according to rule 1 appears to be weakened in the straight walking and inner leg context, but strengthened in the outer leg context.

As for rule 2, median delays in the unperturbed reference situations were 600 or 620 ms if the sender leg was in the straight walking or inner leg context, respectively, but only 200 ms if the sender leg was an outer leg. Temporal coupling according to rule 2 was context-dependent in that perturbation caused a significant decrease in delay only if the sender leg was in the straight walking or inner leg context.

The ipsilateral middle leg receives coordinating information from the perturbed front leg according to two further coupling mechanisms, affecting its timing of stance–swing transition (rule 3 *sensu* Cruse et al., 1995) and the spatial targeting of its touch-down position (rule 4 *sensu* Cruse et al., 1995; Cruse, 1979). According to rule 3, likelihood of lift-off in the receiver leg continuously increases during retraction of the sender leg, i.e. increases with time after touch-down of the sender leg. Therefore, Fig. 8B shows the time delay between front leg AEP and PEP of the middle leg. As expected from measurements on coupling strength of rule 3 in unperturbed walking (Dürr, 2005), middle legs rarely lifted off the ground before touch-down of the front leg, i.e. only very few values lay below zero and none in unperturbed situations. Delays of unperturbed trials differed significantly only between outer (median: 340 ms) and inner legs (median: 510 ms), possibly as a result of larger scatter in the inner leg context. In perturbed steps, lift-off occurred significantly earlier in the straight walking and outer leg context (differences in median: straight walking 100 ms, outer leg 90 ms), but not in the inner leg context. Thus, the effect of perturbation on coupling according to rule 3 is context-dependent.

Finally, we analysed context-dependency and sensitivity to perturbation of the targeting mechanism that is known to cause the middle leg (receiver leg) to touch down near the PEP of the ipsilateral front (sender leg). In unperturbed straight walking (Fig. 9B, black symbols), the middle leg AEPs (filled squares) lay close to front leg PEPs (filled circles) with strongly overlapping standard deviations (s.d.). In curve walking, middle leg AEPs and front leg PEPs revealed a much

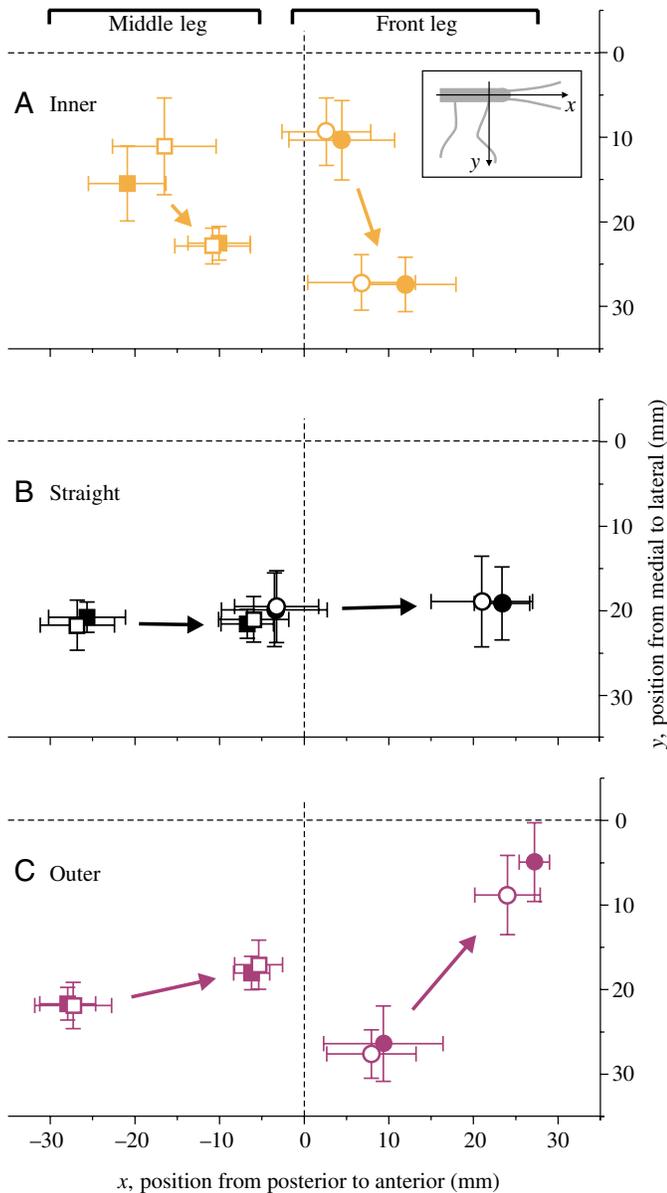


Fig. 9. Context-dependent targeting of middle leg AEP. Targeting of the right middle leg towards the ipsilateral front leg is illustrated by the vicinity of the middle leg AEP and the front leg PEP in straight walking. Values are means  $\pm$  s.d. (Inner,  $N=51$ ; Straight,  $N=64$ ; Outer,  $N=58$ ). (A) Inner curve-walking leg perturbed (orange symbols). (B) Straight walking leg perturbed (black symbols). (C) Outer curve-walking leg perturbed (purple symbols). Locations of front leg extreme positions (circles) and swing directions (arrows) reflect context-dependent kinematics. Squares show extreme positions of middle legs. Filled symbols, unperturbed extreme positions; open symbols illustrate the effect of perturbation. In straight walking (B), middle leg AEP was regulated to lie close to front leg PEP. In curve walking, middle leg swing direction shifted as the animals adapted to the behavioural context of curve walking. Touch-down location remained regulated, as indicated by the small s.d. (A, inner legs; C, outer legs), although not towards the front leg PEP. Perturbation of the front leg does not affect the targeting mechanism in a context-dependent manner (see text).

greater distance, with front leg PEPs and middle leg AEPs having shifted into opposite directions (Fig. 9A,C), thus confirming our previous results (Dürr and Ebeling, 2005). Nevertheless, the s.d. of middle leg AEPs remained as small as during straight walking, indicating that the AEP remained regulated during curve walking. In response to perturbation (open symbols), middle leg AEPs shifted significantly more rostrally, i.e. closer towards the front leg PEP in the straight walking context (Fig. 9B, black symbols) and in the outer leg context (Fig. 9C, purple symbols). For statistics see Fig. 7 and corresponding text. Perturbation caused no significant shift in PEP of straight walking and outer legs, but resulted in significantly caudal shifts of the inner leg PEP. Thus the context-dependent shift of extreme positions was even more pronounced after perturbation, because middle leg AEPs tended to shift in walking direction whereas front leg PEPs tended to shift posteriorly. We conclude that spatial coupling due to Cruse's targeting mechanism is context-dependent with the effect being emphasised after perturbation.

### Discussion

The experiments analysed context-dependency of local leg reflexes and associated changes in leg coordination due to perturbations of front leg swing movements in three different behavioural contexts. These contexts were controlled by means of a visual motion stimulus that caused characteristic, context-specific differences in leg kinematics and coordination. In particular, the movement of the leg to be perturbed differed such that the three leg joints contributed to different extents to the swing movement (Figs 1, 2, 5). The results gained in the unperturbed reference situations were perfectly consistent with previous experiments in the same setting (Dürr and Ebeling, 2005; Dürr, 2005), including the profound changes of step pattern and leg coordination during curve walking. Mechanical perturbation of a swing movement typically caused an avoidance movement which deviated from the normal front leg swing trajectory in that it consisted of a caudal and medial loop (Fig. 2A). Movement trajectories of the avoidance responses in straight walking stick insects were very similar to those measured by Schmitz et al. (Schmitz et al., 2001). Behavioural responses other than an avoidance reflex were possible, consistent with the observation of Cruse et al. that the relative frequency of avoidance reflex decreases during late protraction whereas the relative frequency of a grasping reaction increases proportionally (Cruse et al., 1998). However, these responses were excluded from the analysis. Irrespective of the behavioural context, the overall step pattern appeared to be hardly affected by the perturbation which resembles earlier findings on perturbation in straight walking stick insects on a tread-wheel (Cruse and Epstein, 1982; Dean and Wendler, 1982). In contrast to all previous studies, we were able to measure the extent to which perturbation-related changes in leg kinematics and coordination are context-sensitive, particularly resulting from modulation by descending visual information. Immediate changes on the step parameters of the perturbed leg

were context-dependent (Fig. 3), but the kinematics of movement trajectories (Fig. 4) and individual leg joints (Fig. 5) were not. Perturbation affected leg coordination both spatially (Figs 6, 7) and temporally (Fig. 8) in a context-dependent way.

#### *Context-independent avoidance reflex*

Given the marked kinematic differences between behavioural contexts, one could expect differences in reflex action due to factors such as swing velocity, combination of moving joints, timing of perturbation, or location of stimulus site. To dissociate such factors from context-dependent modulation of reflex activity, we tested for systematic differences within each behavioural context. Although obstacle-contact sites (proximal tibia, distal tibia and tarsus), swing velocity and current leg position varied (Fig. 4), there were no significant differences in joint angle velocities after perturbation. Similarly, avoidance movements elicited by chemical stimulation of a locust hind leg were found to be stereotypic, irrespective of the stimulus site (Newland, 1998). In contrast, tactile stimulation of the same leg tibia and femur depends on stimulus site, and evokes different avoidance movements, each of which draws the leg most effectively away from each stimulus (Siegler and Burrows, 1986). Timing of perturbation within the swing movement had no systematic effect on reflex joint action either, although it was correlated with the progress of protraction and, therefore, the current position of the leg could have influenced the output of the motor system and the reflex gain. Leg position (Field and Coles, 1993) and gain changes due to previous motion (Büschges and Wolf, 1996) were shown to have a systematic influence on the resistance reflex in the FTi joint of the locust.

We conclude from our analysis that all differences of avoidance reflexes would have resulted from context-dependency on a higher level because none of the low-level influences such as leg position and velocity had an effect. However, since reflexes turned out to follow a default pattern with independent activation of all three leg joints, we found no evidence for adaptation to the behavioural context. This is reminiscent of the treading-on-tarsus reflex that does not adapt to forward or backward walking in the stick insect (Schmitz and Haßfeld, 1989).

One can imagine two alternative mechanisms for the joint action in response to perturbation. Firstly, avoidance movements could affect the motor action of active muscles only and perturbation could cause switching to antagonistic muscle groups. In this case, we should have found context-dependent differences in the composition of motion components after perturbation due to different patterns of muscle activities prior to perturbation (Fig. 5). This, however, was not the case. Rather, we found a highly similar reflex composition in the three behavioural contexts analysed. Secondly, there could be a context-independent activation of a set of muscle groups that generates a default avoidance movement, regardless of joint action prior to perturbation. In straight walking, both alternative mechanisms would cause retraction, levation and flexion of the leg, but the second

mechanism also holds true for the two curve walking contexts (Table 1). Each behavioural context reveals about the same percentage of each motion component of the avoidance movement: retraction, levation and flexion (approx. 80%). The combination of these three motion components is very similar, too (approx. 60%), suggesting independent activation of muscle groups of different joints (Table 2). This is somewhat in contrast to the interpretation that the composition of stereotypic avoidance movements might be due to strong inter-joint coupling during reflex action. For example, in the stick insect, simulated flexion of the tibia by elongation of the femoral chordotonal organ depolarises trochanteral levator motoneurons in the active animal (Hess and Büschges, 1997). Inter-joint coupling was also found in the coxo-basipodite chordotonal organ of the crayfish (LeRay and Cattaert, 1997), indicating a common principle in arthropods. Our results suggest that this coupling may not always be obligatory because the likelihood of the effect is considerably less than 100%. It is possible that the sensory input that elicits the avoidance reflex does not cause a common drive to motoneurone pools of different joints, but rather acts independently on each one of them. This independent action could be either mediated by separate afferents and interneurons or it could be due to a sub-optimal common drive to levator, flexor and retractor motoneurone pools which then independently reach supra-threshold activation levels, each one with a likelihood of 80%. Compared to earlier work on insects, the high likelihood of 77–82% of retraction being part of avoidance movements suggests coupling of all three joints, not just between the FTi and CTr joints (Table 1). Finally, the good match of predicted and measured likelihood of the combined action of all three joints (Table 2) strongly suggests coupling *via* a parallel feed-forward pathway from the reflex-triggering sensor to the motoneuron pools, rather than coupling *via* joint angle sensors.

#### *Context-dependent impact on spatial leg coordination*

Obstacle contact led to an avoidance movement that temporarily deviated from the normal swing trajectory. Associated with this deviation, touch-down positions (AEPs) appeared more broadly scattered than in the unperturbed step (Fig. 6B). In all behavioural contexts, AEPs were significantly shifted rearward (Fig. 7), resulting in a shortened step length (Fig. 3A). This was more pronounced in curve walking (Fig. 7A,C) than in straight walking (Fig. 7B). Whereas middle and hind leg AEPs are known to depend on the position of the anterior leg (e.g. Cruse, 1979) (see below), front legs are thought to aim at a set AEP. After perturbation, however, they stay short of the AEPs in the reference trials. Rather than assuming a higher control level to trigger a step of precaution after obstacle contact, we favour the hypothesis of a context-dependent position control mechanism, most probably a change in the set AEP, to cause the observed deviations. Regulation of the AEP is further supported by the finding of Dean that targeting of hind leg swing movements is robust against external forces (Dean, 1984). However, the observation

that the scatter of front leg AEPs is considerably larger than that of the position-controlled middle leg AEPs (Fig. 6B,C) may also be an indication of a relatively weak gain of the control loop. If so, the deviation might not reflect a change in set point, but rather the different start condition after termination of the avoidance reflex.

In both curve walking contexts, the deviation of the AEP after the avoidance reflex is more severe than in straight walking (Fig. 7), particularly in the tangential direction, which is due to significantly less protraction in the ThC joint. Although a context-dependent modulation of the set AEP by descending visual motion information could explain the strong difference in swing direction between unperturbed outer and inner legs, it can hardly account for the shifts observed after perturbation in these contexts. An additional shift of the set AEP due to perturbation could give rise to this effect.

Concerning spatial coordination of front and middle legs, Fig. 9 shows that perturbation affected targeting of middle leg AEPs towards front leg PEPs. Ipsilateral leg pairs are known to be coupled *via* a so-called targeting mechanism (Cruse, 1979; Dean and Wendler, 1983). As a putative neural substrate of this mechanism, target positions have been shown to be coded in space by appropriate mapping of proprioceptive information coming from the joint angle sensors of the anterior leg (Brunn and Dean, 1994). Our results suggest that middle leg targeting is subject to context-dependent modulation. Methodologically, it is worth noting that we analysed the extreme positions of both legs, unlike Dean and Wendler, who related the front leg posture at the time of middle leg touch-down (Dean and Wendler, 1982). In case of straight walking, the small variance of middle leg AEP and its close proximity to front leg PEP (Fig. 9B) confirm findings of previous studies. In response to perturbation, the middle leg AEP is shifted even slightly closer towards the front leg PEP.

In curve walking, middle leg AEPs still vary remarkably little, indicating that the direction of swing movement remains regulated during turning. If so, touch-down is no longer targeted towards the front leg PEPs, but towards a modulated target position (Fig. 9A,C, squares). This position is shifted in the direction of heading, i.e. laterally in inner legs and medially in outer legs. In contrast, front leg PEP always shifts in the counter direction of heading. Since the AEP tends to shift in the opposite direction of the front leg PEP, perturbation leads to an increased distance between middle leg AEPs and front leg PEPs. Therefore, we suggest that the touch-down location of Cruse's targeting mechanism is modulated in different contexts. The effect of perturbation may be explained by an increase of this modulation, in which case it would be independent of behavioural context.

In contrast to previous perturbation experiments (Cruse and Epstein, 1982; Dean and Wendler, 1982), we examined only immediate effects of perturbation on leg coordination, not long-term adaptations to an altered load situation or the like. Fig. 7 reveals the asymmetric effects of perturbation on the next steps in the perturbed leg and in the two receiver legs to which the perturbed leg is coupled. Apart from the AEP of the

perturbed leg, all other extreme positions underwent considerably stronger shifts in the inner leg context than in the outer leg context, with intermediate effects in the straight walking context. Theoretically, this could have been due to asymmetric strength of coupling mechanisms. Coupling strength is known to be much stronger between ipsilateral leg pairs than between contralateral leg pairs (Dürr, 2005). Moreover, when compared to straight walking, ipsilateral coupling strength is increased in outer legs and decreased in inner legs. Therefore, in the present experiments, compensatory responses of the ipsilateral and contralateral receiver legs were likely to depend on the context of the perturbed sender leg.

#### *Context-dependent impact on temporal leg coordination*

In Fig. 8, we analysed the temporal coupling of the perturbed sender leg and its contralateral (Fig. 8A) and ipsilateral (Fig. 8B) receiver legs. In the contralateral receiver leg, coupling mechanisms associated with Cruse's rules 1 and 2 (Cruse et al., 1995) should have inhibited lift-off until the swing movement of the perturbed leg was completed (rule 1) and enhanced lift-off soon after (rule 2) (Cruse and Knauth, 1989). Our results show significant effects of perturbation in the straight walking and inner leg contexts only (Fig. 8A), i.e. in situations when the receiver leg was an outer leg or straight walking leg. The weaker coupling of rule 1, as shown by its higher percentage of violation, could be due to the receiver leg having been more strongly coupled to the step rhythm of its unperturbed ipsilateral neighbour than to its perturbed contralateral neighbour. In other words, an outer or straight walking receiver front leg would not have 'waited' until termination of the prolonged swing phase of its obstructed contralateral neighbour, but had lifted off in the step rhythm of its ipsilateral neighbours. Conversely, weak ipsilateral coupling would have favoured that an inner receiver front leg 'obeyed' the contralateral rule 1, which is what we found. However, this asymmetric ipsilateral coupling cannot explain the strengthening of rule 2-type coupling after perturbation.

In the ipsilateral receiver middle leg, the coupling mechanism associated with Cruse's rule 3 should have increased the likelihood of lift-off with increasing time after touch down of the perturbed leg (Cruse and Schwarze, 1988). The results in Fig. 8B revealed significant effects only in the straight walking and outer leg context. In both cases, the delay was shortened, indicating that the receiver leg lifted off earlier. As in the contralateral case, context-dependent strengthening of coupling between outer legs could explain the difference between unperturbed outer and inner legs in Fig. 8B, but cannot explain the context-dependent effect of perturbation.

Thus, since temporal coupling was affected by perturbation, we conclude that the induced context-dependent differences in leg coordination were not simply due to asymmetric coupling strengths. Rather, at least part of the effects must have been due to context-dependent responses related to perturbation. In

summary, this shows that perturbation of swing movement causes compensatory reactions in immediate limb kinematics and in inter-leg coordination. Whereas several effects on coordination depend on the current behavioural context, the kinematics of the immediate avoidance reflex is context-insensitive.

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