Control of reflex reversal in stick insect walking: effects of intersegmental signals, changes in direction, and optomotor-induced turning

Katja Hellekes, Eric Blincow, Julia Hoffmann, and Ansgar Büschges
Department of Animal Physiology, Zoological Institute, Biocenter Cologne, University of Cologne, Cologne, Germany

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Hellekes K, Blincow E, Hoffmann J, Büschges A. Control of reflex reversal in stick insect walking: effects of intersegmental signals, changes in direction, and optomotor-induced turning. J Neurophysiol 107: 239–249, 2012. First published October 12, 2011; doi:10.1152/jn.00718.2011.—In many animals, the effects of sensory feedback on motor output change during locomotion. These changes can occur as reflex reversals in which sense organs that activate muscles to counter perturbations in posture control instead reinforce movements in walking. The mechanisms underlying these changes are only partially understood. As such, it is unclear whether reflex reversals are modulated when locomotion is adapted, such as during changes in walking direction or in turning movements. We investigated these questions in the stick insect Carausius morosus, where sensory signals from the femoral chordotonal organ are known to produce resistance reflexes at rest but assistive movements during walking. We studied how intersegmental signals from neighboring legs affect the generation of reflex reversals in a semi-intact preparation that allows free leg movement during walking. We found that reflex reversal was enhanced by stepping activity of the ipsilateral neighboring rostral leg, whereas stepping of contralateral legs had no effect. Furthermore, we found that the occurrence of reflex reversals was task-specific: in the front legs of animals with five legs walking, reflex reversal was generated only during forward and not backward walking. Similarly, during optomotor-induced curved walking, reflex reversal occurred only in the middle leg on the inside of the turn and not in the contralateral leg on the outside of the turn. Thus our results show for the first time that the nervous system modulates reflexes in individual legs in the adaptation of walking to specific tasks.

motor control; behavioral task; locomotion; proprioception

FEEDBACK FROM LEG SENSORY organs that signal position, movement, and force plays an essential role in the generation and adaptation of locomotion (summaries in Bässler 1983; Büschges 2005; Grillner 1981; Orlovsky et al. 1999; Pearson 2008). In many systems, the effects of sensory inputs during walking differ from those seen in postural control, and these changes can occur as reflex reversals (Duyens et al. 2000). For example, the Golgi tendon organs of vertebrates elicit reflexes in posture that act to decrease muscle tension and the resultant forces (Prochazka 1996). During walking, these receptors instead amplify muscle tension in the stance phase (Pearson and Collins 1993). Similar reflex reversals are found in other receptors of both invertebrates (crayfish: DiCaprio and Clarac 1981; Sillar and Skorupski 1986; insects: locust, Theophilidis and Burns 1990; Zill 1985; stick insect, Bässler 1976, 1986, 1988) and vertebrates (cat, Forsberg et al. 1975; rat, Fouad and Pearson 1997; human, Duysens et al. 1990). The occurrence of reflex reversals can depend on the phase activity in rhythmic movements (e.g., Fouad and Pearson 1997; Pearson and Collins 1993; Skorupski and Sillar 1986) or, more generally, on the behavioral state of the animal (e.g., Bässler 1988; Zill 1985; for review, see, e.g., Büschges and El Manira 1998; Clarac et al. 2000; Pearson 1993).

In most systems, the specific mechanisms underlying the generation and modulation of reflex reversals have not been determined. It is also unclear how reflex reversals are integrated with other processes controlling and directing walking. For example, it is not known whether regulation of reflex reversals occurs as part of the processes mediating interleg coordination in walking. In addition, walking patterns can be extensively adapted in changing the direction of progression and during visually guided stepping (Pang and Yang 2002). It has not been determined how such changes affect the occurrence of reflex reversals, although their regulation must be part of the adaptation of walking patterns (Pang and Yang 2002; Pearson 1993).

We have studied this problem by utilizing the reflex effects of the stick insect femoral chordotonal organ (fCO). Signals from the fCO produce resistance reflexes (RR) in quiescent animals at rest that could function in postural load compensation (Bässler 1974; for summary, see Bässler 1993). These reflexes have been shown to change when the animal generates active leg movements (Bässler 1976, 1988). Afferents of the chordotonal organ that signal joint flexion then inhibit extensor firing and assist the generation of flexor activity. These changes represent a reflex reversal and occur as the first part of a sequence of muscle activities termed the “active reaction” (AR; Bässler 1988). Several studies have examined the underlying neural elements within the local premotor network that mediate both the resistance and assistance reflexes of the fCO in a distributed fashion (e.g., Bässler 1988; Bässler and Büschges 1990; Driesang and Büschges 1996; for review, see Bässler and Büschges 1998).

However, no information is currently available on how the reflex reversal is initiated and regulated in vivo in a walking animal. Thus, at present, it is not known which upstream neuronal processes of the femur-tibia (FTi) control network underlie the behavioral state-dependent alterations in the processing of fCO signals (see Büschges and El Manira 1998). Several studies have demonstrated effects of descending inputs on local reflexes in insects (Knop et al. 2001; Mu and Ritzmann 2008a; Ridgel et al. 2007), but these experiments were not performed in animals that were walking. To address this issue, we have 1) studied the role of intersegmental signals for the local generation of reflex reversal, and 2) tested whether the generation of the local reflex reversal depends on the specific motor behavior of the animal. To study the effects of leg...
coordination, we investigated the influence of leg stepping in ipsi- and contralateral neighboring legs on the generation of the reflex reversal in the middle leg, using a semi-intact preparation in which the remaining legs were walking on a slippery surface (Graham 1981; Gruhn et al. 2006). To examine the behavioral specificity of reflex reversal, we elicited reflexes by chordotonal organ stimulation during forward and backward walking and in optomotor-induced turning during stepping sequences.

MATERIALS AND METHODS

Experiments were performed on adult female stick insects (Carausius morosus Br.) at room temperature (20 –22°C) under reduced light conditions. Animals were obtained from a breeding colony maintained at the University of Cologne. The experimental procedures reported in the study comply with the German National and State Regulations for Animal Welfare and Animal Experiments.

Positioning of the experimental animal for walking. The experimental animals were positioned above a slippery surface at a distance of about 8–12 mm so that the resting angle of the FTi-joint in the middle and hindlegs was ~90° (Fig. 1; Epstein and Graham 1983; Graham 1981; Gruhn et al. 2006).

The stick insect was fixed dorsal side up to a foam-covered metal rod by means of dental cement (Protemp II; 3M ESPE, St. Paul, MN) placed on the pro-, meso-, and metathorax (Fig. 1). The investigated leg was glued to an extension of the rod. The coxa and femur of that leg were immobilized, but the tibia hung over the edge of the rod extension with a FTi-joint angle of 110°.

Preparation for fCO stimulation and recording of motor activity. A small window was cut dorsally into the femoral cuticle, allowing for mechanical stimulation of the fCO and for extracellular recordings from tibial muscles and motor nerves (Fig. 1; Büschges 1989). The leg cavity was filled with saline (Weidler and Diecke 1969), and the apodeme of the fCO was attached to a moveable clamp controlled by a linear motor. Mechanical displacements of the apodeme were produced by applying voltages to the motor by means of a stimulus generator (electronics workshop; Zoological Institute, Cologne, Germany). The fCO was stimulated with ramp-and-hold stimuli that produce displacements of 300 and 400 μm from the starting position. These displacements induce FTi-joint angles between 110 and 50 or 30°, respectively (Weiland et al. 1986). The activity of the extensor tibiae motoneurons was recorded extracellularly from the extensor nerve F2 via hook electrode (modified after Schmitz et al. 1988). In the majority of experiments, the activity of the flexor tibiae muscles was monitored via an electromyogram (EMG; Fig. 1; e.g., Rosenbaum et al. 2010). Walking activities were monitored by myographic recordings of the flexor tibiae (front legs) and/or the retractor coxae muscles of the middle leg, depending on the experiment. Based on the particular experiment, either the front or middle leg was stimulated and recorded from in the manner described above. Other legs were either amputated or allowed to freely walk, depending on the specific experimental condition (see below).

Experiments with stepping neighboring legs. The middle leg was fixed perpendicular to the body axis, and the ipsilateral hindleg was removed (modifications of leg numbers between the different experimental conditions are not shown in Fig. 1). Three different experimental conditions were used to investigate the influence of intersegmental signals on the middle leg: 1) the ipsilateral front leg and all three contralateral legs were present and stepping; 2) only the contralateral legs were present and stepping; and 3) only the ipsilateral front leg was present and stepping.

Experiments on forward and backward walking. For the experiments on forward and backward walking, the left front leg was fixed at a position of 45° anteriorly. All other legs were free to move.

Experiments on curve walking. The left middle leg was immobilized while all other legs were free to move (Fig. 1). Walking movements were induced by tactile stimulation of the animal’s abdomen or head with a small paintbrush (Fig. 1), depending on desired walking direction (Graham and Epstein 1985). Once stepping was initiated, tactile stimulation ceased. An animal was considered to be walking forward or backward when the unrestrained legs exhibited a clear forward or backward stepping pattern. Curve walking was induced by optomotor stimulation. To accomplish this, we placed two screens laterally in front of the stick insect and projected a vertical black and white stripe pattern by means of an optomotor projector (Scharstein 1989). To elicit curve walking in the stick insects, the stripes on both screens moved to either the right or left, which elicited the respective walking direction (Fig. 1; Gruhn et al. 2011).

Physiological signals were amplified and filtered (e.g., von Uckermann and Büschges 2009) and recorded using a Micro 1401 A/D converter (sampling rate, 12.5 kHz) and Spike2 data acquisition/analysis software (version 5.04; Cambridge Electronic Design).

Statistical analysis was done with the modified Student’s t-test (Dixon and Massey 1996). Data were considered to differ significantly at P < 0.01. To determine the reliability of the frequency of occurrence of AR, we defined the 95% confidence intervals for the different experimental situations (Hayes 1988). If the confidence intervals of the mean values do not overlap, the influences are statistically significant. We performed an additional control to test whether the activity of tibial motoneurons during fCO stimulation in the front leg during forward and backward walking differs relative to the activity without fCO stimulation. Therefore, we recorded responses to fCO stimulation and compared them with sequences in which the voltages were generated and recorded but the fCO was not fixed into the stimulator clamp and hence not displaced. We then determined the chance level for a motoneuronal pattern similar to the AR. Evaluation of the data and plotting of the graphs were performed with OriginPro 8.5 and CorelDRAW X4. In the text and figures, “N” gives the number of animals, and “n” gives the sample size.

RESULTS

Processing of fCO signals depends on the ipsilateral stepping front leg. In the first set of experiments, we investigated whether the generation of the reflex reversal in the FTi-joint in a given segment depended on locomotor activity in neighboring legs. To do so, we stimulated the fCO of the middle leg while recording tibial extensor nerve and in some experiments also flexor muscle activity in preparations that differed in the number of neighboring legs stepping. Specifically, we used three experimental conditions.
In the first condition, the ipsilateral front leg as well as all contralateral legs were free to move (Fig. 2A). The fCO of the left middle leg was stimulated with ramp-and-hold stimuli while the activity of tibial extensor motoneurons was recorded from nerve F2. On tactile stimulation of the abdomen with a paintbrush, the experimental animals started forward stepping movements with their free legs (gray shaded area in Fig. 2B). At rest, fCO elongation induced the typical RR activation in tibial motoneurons (Fig. 2B, 1st fCO stimulus and inset) with slow (SETi) and fast (FETi) extensor tibiae motoneurons being activated on elongation of the fCO (for details, see Bässler 1993). However, during stepping sequences, a reflex reversal was seen to occur, and fCO elongation produced inhibition of firing of tibial extensor motoneurons and activation of flexor motoneurons (Fig. 2B, e.g., 4th stimulus and inset). Extensor activity resumed toward the end of the elongation phase of the stimulus. This motor activity on elongation signals from the fCO is characteristic of the AR (Bässler 1976, 1988). No systematic modification was detected on fCO relaxation (for a detailed analysis of the time course in motoneuron activity during the AR, see Bässler 1986, 1988 and Nothof and Bässler 1990]. Figure 2C shows the same situation together with electromyographic recordings of motor activity of the stepping neighboring legs. The muscles monitored are active in the stance phase, i.e., the flexor tibiae for the two front legs and the retractor coxae in the contralateral middle leg. The difference in the influence of fCO elongation on tibial motoneuron activity between the resting animal and the animal stepping with the other legs is apparent in average peristimulus time (PST) histograms of the extensor activity (Fig. 2, D and E) for both situations. During fCO elongation in the inactive animal, extensor activity increases drastically as FETi and SETi are activated, and during the hold phase of the stimuli, only SETi shows a higher frequency (Fig. 2D). In the stepping animal, fCO elongation leads to a cessation in extensor activity. Extensor activity resumes toward the end of fCO elongation, which is known as part II of the AR (for details, see Bässler 1986, 1988; Fig. 2E). The results were the same in all six animals tested with the probability of occurrence of the reflex reversal in the middle leg being on average 61.5% (N = 6, n = 397; see summary in Fig. 4A) and reaching up to 94% for individual animals. Thus we have observed that stepping in neighboring legs promotes the occurrence of reflex reversal. Interestingly, virtually the same results were gathered from the second condition in which only the left ipsilateral front leg was left intact and free to step, whereas all other legs except the

![Image of the experimental setup.](https://example.com/image.png)

Fig. 2. Reflex reversals in the middle leg. A: stick figure of the experimental setup. The ipsilateral hindleg was removed in all experiments to prevent contact with the apparatus. B: resistance reflex (RR) in a resting animal and reflex reversal during walking. In this sequence, 1st the animal was standing, and displacement of the apodeme of the chordotonal organ (fCO stim) mimicking joint flexion induces RR activation firing in tibial extensor motoneurons (tib ext F2). Walking was then elicited by tactile stimulation producing bouts of activity in flexor muscles and extensor motoneurons of the middle leg (gray shaded area). Elongation stimuli of the mesothoracic fCO no longer generate a RR but can induce a reflex reversal [active reaction (AR)], i.e., inhibiting activity of extensor motoneurons and activating the flexor muscle (reflex reversal) during fCO elongation. The 2 insets give enlarged presentations of the motor activity during the RR and the AR (see text for details). C: original recording from a similar experiment with additional presentation of electromyographic recordings of flexor motor activity in the front leg muscles and retractor coxae muscles in the contralateral middle legs to monitor the stepping situation of neighboring legs. D and E: peristimulus time (PST) histograms (1 animal) of extensor firing frequency during RR (D) and reflex reversals (E) in walking (data from 1 animal). FlxTi, flexor tibiae muscle; RetCx, retractor coxae motor nerve; fCO stim, mechanical displacement of the apodeme of fCO signaling FtI-joint flexion (up on traces); pro, prothoracic (front leg); meso, mesothoracic (middle leg); c, contralateral; i, ipsilateral; avg., average; n, number of stimulations; *tactile stimulation; p, prothorax; m, meso-, metathorax.
stimated left middle leg were removed ($N = 5$; Fig. 3A). During stepping sequences of the ipsilateral front leg, fCO signals indicating joint flexion promoted the generation of reflex reversal in tibial motoneurons (Fig. 3A). During front leg stepping, the probability for generating the AR reached 93% for individual animals, being on average 57.6% for the five animals tested ($n = 116$; Fig. 4B). The difference between the influence of fCO elongation on tibial extensor motoneuron activity is also evident in PST histograms of the average extensor motoneuron activity for the resting (Fig. 3C, top) and the stepping animal (Fig. 3C, bottom; only stepping stimuli with AR, inset: all stepping stimuli). Therefore, stepping in the ipsilateral front leg is sufficient to produce an increased frequency of reflex reversal in the middle leg.

These results raise the question of whether stepping activity of contralateral legs affects processing of fCO signals on the insect’s ipsilateral side. To test this, we stimulated the fCO in the left middle leg of experimental animals, in which all contralateral legs were left intact and free to move while the ipsilateral left front and hindlegs were removed (Fig. 3B). Intriguingly, under this condition, we rarely observed reflex reversal in tibial motoneurons of the left middle leg on fCO elongation. The lack of inhibitory influence of fCO elongation on extensor activity is again clear in PST histograms of the extensor activity, as shown in Fig. 3D. Both at rest and during stepping elongation signals, from the fCO elicited an increase in extensor activity, i.e., motor activity representing a RR. Quantification of the probability of occurrence of AR during walking revealed that only in 3.9% of the elongation stimuli ARs were generated ($n = 934$; $N = 7$; Fig. 4C).

In summary, our results show that intersegmental signals from ipsilateral front legs significantly influence the processing of local sensory information of the fCO in mesothoracic tibial motoneurons, whereas contralateral stepping legs showed no such influence (see Fig. 4, A–D).

**Processing of fCO signals depends on walking direction.** In the next set of experiments, we tested whether the generation of reflex reversal depends on the specific walking direction. We used an experimental setup that readily allowed us to induce animals to walk forward or backward by applying mechanical stimuli to the abdomen or antennae, respectively (Rosenbaum et al. 2010). We chose the front leg for fCO stimulation, since front leg movement shows marked differences between forward and backward walking (which is not the
compare the differences in fCO processing in the front leg of walking stick insects during both walking directions, we determined the frequency of occurrence of reflex reversals during forward and backward walking. Reflex reversals occurred in front legs of forward walking animals in 67% of trials (N = 7, n = 99; Fig. 6A) compared with 14% during backward walking (N = 7; n = 101; Fig. 6B). To test the effect of fCO displacement on the likelihood of reflex reversal during walking, we compared tests in which the stimulus displaced the chordotonal ligament to similar intervals in which the stimulus waveforms were generated but did not produce fCO displacement. We determined the chance level for a motoneuronal activity pattern similar to the AR in the same stimulus intervals, which was 14.9% for forward walking and 11.4% for backward walking. Thus the average likelihood of AR occurrence during forward walking (67%) clearly exceeded the chance level of flexor activity (N = 3, n = 67; for test and evaluation, see MATERIALS AND METHODS), which was, however, not true for the likelihood of AR generation during backward walking (14%) with the chance level being 11.4% (N = 3, n = 35).

**Reflex reversals in curve walking.** Each leg of the tethered insect participating in curve walking can be seen as walking on either the inside or outside of the curve. Both patterns produce substantially different movements (Gruhn et al. 2006, 2009). During the stance phase, movement of a middle leg on the inner side of the curve is directed toward the body of the animal, as if pulling the animal via flexion of the FTi-joint toward the side of the turn. In contrast, a middle leg on the outer side pushes backward along the length axis of the animal (Gruhn et al. 2009, 2011). We studied whether the occurrence of reflex reversals differed in the two middle legs during optomotor stimulation, which induces turning in direction similar to walking in a curve (Gruhn et al. 2006). The experimental animal was tethered on a wooden stick on a slippery surface, and all legs except the left middle leg were free to move. The mesothoracic fCO was stimulated while recording the activity of tibial motoneurons from nerve F2 extracelularly and the activity of the flexor tibiae muscle by means of EMGs. Motor activity of the contralateral middle leg was monitored by an EMG of the flexor tibiae (Fig. 7A). The stimulated middle leg was walking on either the inside or outside of the curve (Fig. 7, A and B, respectively) depending on the direction of the optomotor stimulus. The activity observed in the tibial extensor motoneurons changed systematically during turning. The tibial extensor activity was generally higher when the leg walked on the outside of the curve compared with when it walked on the inside. The activity of the flexor tibiae muscle showed the reverse pattern and was increased on the inner side of the curve to pull the animal in that direction (cf. extensor and flexor tibiae activity in Fig. 7, A and B, as well as extensor activity in Fig. 7, C and D). The effects of mechanical stimulation of the chordotonal organ during turning differed according to the behavioral use of the leg. Stimuli indicating joint flexion induced reflex reversals in 56.9% of trials when the leg was walking on the inner side of the curve (N = 9; n = 226; Fig. 8A). In contrast, when the leg was walking on the outside of the curve, the same stimulus produced reflex reversal only in 7.3% of trials (N = 8; n = 246; Fig. 8B). This difference is apparent in Fig. 7, C and D, which shows the average extensor tibiae motoneuron activity plotted as a PST histogram for both conditions. Remarkably, the pooled data showed no consistent
influence of fCO stimulation on tibial extensor activity to be detected for legs walking on the outside of a curve (Fig. 7 D). The data further support the hypothesis that the generation of the reflex reversal depends on not only the behavioral task, but also the specific function and use of the individual segmental leg.

DISCUSSION

The present study has demonstrated that the occurrence of fCO-mediated reflex reversals in stick insects is modulated in response to the insect’s current behavioral task. Our results showed that reflex reversal is influenced by intersegmental signals from neighboring ipsilateral (rostral) legs, as reversals in the middle leg are more frequent during stepping of the ipsilateral front leg but not the contralateral legs. Our experiments also demonstrated that the frequency of reflex reversal shows a strong dependence on the direction of walking, as reflex reversals were generated in forward walking but rarely in backward walking. Finally, during optomotor-induced curve walking, increases in reflex reversals occur in the middle leg walking on the inside of the curve but not in the leg walking on the outside of the curve. These results support the idea that the processing of movement-related feedback from the fCO can be specifically modulated in a single segment when the direction of walking of an individual leg is changed.

Reversal of reflex effects of the chordotonal organ during walking. fCO-mediated reflexes were among the first reflexes to show reversals induced by changes in behavioral state (Bässler 1976). Similar effects have now been demonstrated in a number of vertebrate and invertebrate locomotor systems (for summaries, see Büschges and El Manira 1998; Clarac et al. 2000). In quiescent animals, elongation of the chordotonal ligament that signals flexion of the FTi-joint has been demonstrated to produce RR that activate the tibial extensor motoneurons and inhibit tibial flexor motoneurons.

Bässler first demonstrated that, in animals that were active (i.e., showing motoneuron firing associated with leg movements), displacements of the chordotonal ligament, which corresponds to joint flexion, produces excitation in tibial flexor motoneurons and inhibits tibial flexor motoneurons (Bässler 1988). Sensory signals indicating a change in joint angle, therefore, do not elicit motor activity to resist the apparent joint movement but instead “assisted” and reinforced activity in the muscle that would produce the joint movement.

Fig. 5. Reflex reversals when walking forward and backward. The fCO was displaced (top traces) in the front leg while monitoring the tibial extensor (tib ext F2) and flexor activities (bottom trace) in that leg. A: in this sequence, the animal was 1st standing, then walked forward, and then stopped briefly and walked backward. Resistance responses were elicited during standing and walking backward, whereas reflex reversals occurred during forward walking. B and C: expanded traces of responses during forward (B) and backward (C) walking. D and E: PST histograms of firing frequency of the front leg tibial extensor motoneurons during fCO stimulation in forward (D) and backward (E) walking (D: n = 30; E: n = 30; data from 1 animal). CI, common interval; SETi and FETi, slow and fast extensor tibiae, respectively, nAR, no active reaction.

Fig. 6. Reflex reversals depend on walking direction. Bar histograms show the probability of occurrence of reflex reversals in the front leg tibial muscles during displacement of the front leg fCO in animals that were walking forward and backward. Each histogram shows 2 exemplary experiments (dark bars) and the mean values of all experiments (light bars). Reflex reversals occurred during forward but not backward walking. Arrows indicate walking direction.
Bässler called this reflex reversal the AR because it was associated with a change from a resting to an active locomotory state. The reversed reflexes were postulated to fulfill several functions during walking. Flexor motoneurons are active in walking during the stance phase, and therefore assistance reflexes could serve to amplify flexor muscle tensions and aid in generating support and propulsion. The signals from the fCO could also be important in phase transitions, such as initiation and termination of the stance phase. This implied that sensory signals from the chordotonal organ could access and influence pattern-generating neurons, an idea that has been supported by studies of sensory processing in local circuit nonspiking interneurons (Driesang and Büschges 1996). However, little was known about which signals contribute to the generation of the “active locomotor state” in the segmental ganglia of a walking animal. The active state was typically elicited in earlier studies by prolonged and repetitive touching of the experimental animal with a paintbrush on the abdomen, head, or antennae. As a result, slow and fast motor neurons innervating the leg muscles were activated and generated sequences of bursts of alternating activity with their antagonists. In addition, movement was observed in the neighboring legs or leg stumps, which were free to move, as well as in the antennae (Bässler 1974, 1986, 1988). It is important to note that many of the previous investigations examining reflex reversal were performed in preparations that were largely restrained because legs were either immobilized or removed (e.g., Akay and Büschges 2006; Bässler 1976, 1986, 1988; Bässler and Büschges 1990; Driesang and Büschges 1993) except for one study that directly demonstrated that the AR was associated with movements at the FTi-joint in the same leg (Weiland et al. 1986; compare with Bässler 1974).

Our experiments utilized preparations in which other legs performed walking movements during chordotonal stimulation. Tactile stimuli were used only to initiate walking in the
 experimental animals, and there was no ongoing stimulation of sensory neurons in the cuticle, e.g., tactile hairs, of the experimental animal during the stepping movements in the neighboring legs. This provides further support for Bässler’s contention that the active locomotor state induced by tactile stimulation is effectively analogous to the locomotor state of the freely walking insect (Bässler 1993).

To gain an understanding of the mechanisms controlling and modulating reflex reversals, we measured the frequency of occurrence of reflex reversals in behaving animals. The occurrence of a reflex reversal may on one hand be affected as part of the general mechanisms that regulate stepping in single legs, such as those controlling the rate of walking. However, it appears more likely that reflex reversals are contributing mechanisms that are active in the generation of functional leg kinematics for specific walking patterns, such as changes in direction or turning trajectories. In the following section, we will discuss how the modulation of reflex reversals in behaving animals may reflect the functional needs of the neural system for control and adaptation of walking.

The role of stepping neighboring legs and intersegmental signals. Our data show that stepping movements of the ipsilateral front leg promote the generation of the reflex reversal in the FTi-joint control network in the mesothoracic ganglion. These findings are consistent with previous studies that have shown that the prothoracic segment and the front leg play an important role in determining the locomotor state of the caudal segmental ganglia. Ludwar et al. (2005a,b) demonstrated that stepping of the front leg produces activation of motoneuron pools in the deafferented, ipsilateral middle leg. The motoneuronal activity in the mesothoracic motoneurons was found to be modulated in phase with stepping of the front leg. In a succeeding study, Borgmann et al. (2009) showed that front leg stepping activates and entrains the central pattern generator (CPG) driving the thoracocoxal motoneurons in the ipsilateral (half of) mesothoracic ganglion. Front leg stepping was, however, only accompanied by a general tonic activation of leg motoneurons in all other segmental ganglia of the walking system. It should be noted that the situation appears to be different for the prothoracic segment carrying the front leg, as in forward walking AR is consistently observed (see Fig. 2). Although the stance activity during curve walking showed for the outside leg that AR was only generated when the stimulated leg walked on the inside of the curve and not when the stimulated leg walked on the outside of the curve. From these results, it is clear that local information processing of fCO signals depends on the actual movement to be generated in the specific motor behavioral task, thereby only being generated for the leg that actually needs to generate marked FTi-joint flexion to generate stance. Interestingly, the quantitative evaluation of tibial motoneuron activity during curve walking showed for the outside leg that flexion signals from the fCO did not modify tibial motoneuron activity at all, not even expressing the time course of activation resembling RR in the FTi-joint on flexion signals at rest. This clearly differs from the opposite inside leg, but it also differs from the situation in straight walking, where the generation of AR is consistently observed (see Fig. 2). Although the stance kinematics of the middle leg do not drastically differ between walking straight or walking on the outside of a curve (cf. Gruhn et al. 2009), sensorimotor processing of movement signals from the FTi-joint appears to be different in either situation.

Intersegmental and descending control of local processing of proprioceptive signals. We have shown that the effects of signals indicating FTi-joint flexion are modulated by neural signals arising from stepping in the ipsilateral rostral leg. Specific modulation also occurs during changes in the direction of walking (forward vs. backward) and in optomotor-induced curve walking. At present, we do not know which specific neural signals transmitted to the mesothoracic segment underlie the modification in processing of local fCO signals.

Several previous studies have shown that descending signals from the brain can modulate local reflexes. In locusts, Knop et
al. (2001) demonstrated that lesioning axons of descending neurons in the connective strongly affected the reflex effects of ipsilateral fCO signals on mesothoracic tibial motoneurons. Mu and Ritzmann (2008a) found that lesions of the rostral connections in cockroaches produce marked changes in the reflex motor activity induced by propriocceptive signals in the mesothoracic leg. These studies suggest that descending signals from the brain (supraspinal and/or the suboesophageal ganglion) can play a decisive role in determining the locomotor state and processing of sensory inputs in the thoracic ganglia. According to these findings, it is on one hand conceivable that the modifications observed are mediated via individual descending pathways to the mesothoracic segment specifically modulating local sensorimotor processing underlying the generation of the motor output for a specific motor behavior. On the other hand, they could be the result of a few “strategic” task-specific modifications in sensorimotor processing changing the state of local neural processing elicited by descending signals that modify the sequence of sensorimotor interactions. Both possibilities are in accordance with the notions formulated by Mu and Ritzmann (2008b) on the role of cascading reflex changes induced by descending signals from the brain.

Functions of reflex reversals: reinforcement of movement in the control of walking. We have found the generation of reflex reversals in forward walking front legs and in middle legs that walked the inside of a curve. What specific functions do reflex reversals serve during locomotor behaviors? One function that has been postulated for reflex reversal (during ARs) is the enhancement of motor activities (reviews in Büschges and El Manira 1998; Duyssens et al. 2000; Pearson 1993). When the fCO reflexes reverse, signals indicating joint flexion inhibit extensor firing and can activate and reinforce activity in the tibial flexor muscle. During forward walking, this effect would enhance activity in the muscle that provides propulsion. In addition, during curve walking, the flexor muscle acts to pull the animal in the direction of the turn. The increased probability of reflex reversal in the leg walking on the inside of the curve may reflect enhancements of the mechanisms that generate force during the turn. Thus, in both of these situations, the modulation of chordotonal reflexes may reflect the utilization of sensory inputs to amplify muscle contractions and movements. The mechanisms underlying the modulation of fCO reflexes have not yet been determined. However, a number of nonspiking interneurons that have been identified in the mesothoracic ganglion contribute to the generation of both resistance and assistance reflexes, i.e., reflex reversals (Büssches 1990; Driesang and Büschges 1993, 1996). In addition, recent studies (von Uckermann and Büschges 2009) have documented how rhythmic changes in membrane potential of these interneurons during walking contribute to tibial motoneuron activity.

Previous accounts have indicated that reinforcement of movement is a significant factor in terrestrial locomotion (for summary, see Clarac et al. 2000; e.g., insects, Bässler 1976, 1988, 1992; crustaceans, El Manira et al. 1991; Leibrock et al. 1996; Skorupski and Sillar 1986; cats, Gossard et al. 1994; McCrea et al. 1995; Pearson and Collins 1993; humans, Grey et al. 2007; for reviews, see Bässler and Büschges 1998; Cattaert and Le Ray 2001; Pearson 1995, 2008; Yang and Gorassini 2006). These studies demonstrated that reinforcement of movement by sensory feedback is state dependent and only occurs when the locomotor system is active (e.g., Bässler 1988; Skorupski and Sillar 1986). In some cases movement reinforcement is also phase dependent and linked to the activity of CPGs that generate rhythmic locomotor actions (e.g., El Manira et al. 1991; McCrea et al. 1995; Skorupski 1992). Studies on the cat and the crayfish provided evidence for alterations in the activity of pathways processing sensory information underlying the generation of reinforcement of movement during walking (reviews: crayfish, Cattaert and Le Ray 2001; cats, Pearson 1995, 2008). In the cat hindleg, movement and force feedback reinforce stance motor output in a phase-dependent manner when the central neural networks, i.e., the hindleg CPGs, are active (e.g., Pearson 1995, 2008). In crayfish, the mechanisms underlying reflex reversal and reinforcement of movement have been documented in preparations expressing rhythmic motor activity (“active locomotion”, e.g., Chrchri and Clarac 1990; Skorupski and Sillar 1986). In cats, rhythmic activity was initiated and maintained by perineum stimulation, pharmacological treatment, or electrical stimulation of the mesencephalic locomotor region (e.g., McCrea et al. 1995; Pearson and Collins 1993). In the present study, walking in stick insects was experimentally initiated but maintained by the animal. In both preparations in the crayfish and cat, the pattern of motor activity generated was considered to represent forward walking. However, it is still unclear whether the generation of reinforcement of movement is also affected by varying and adapting the specific motor behavior (cf. Pearson 2008).

The present study provides evidence that processing of propriocceptive information depends on not only the behavioral state, but also the specific locomotor task, i.e., the control of the movement of an inside curve walking leg compared with an outside curve walking leg. Subsequent studies will examine the neural mechanisms underlying the modification of local processing of sensory inputs in a situation when the animal adjusts the direction of walking movements in making a left or right turn. Similar changes may occur in other locomotory systems and provide insight into the mechanisms that coordinate and adapt walking patterns.

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Present address of E. Bünelow: Dept. of Biology, Univ. of Saskatchewan, 112 Science Pl., Saskatoon, SK S7N 5E2, Canada.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: K.H. and A.B. conception and design of research; K.H., E.B., and J.H. performed experiments; K.H., E.B., and J.H. analyzed data; K.H., E.B., J.H., and A.B. interpreted results of experiments; K.H. and...
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