The Role of Leg Touchdown for the Control of Locomotor Activity in the Walking Stick Insect

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Abstract

Much is known on how select sensory feedback contributes to the activation of different motor neuron pools in the locomotor control system of stick insects. However, even though activation of the stance phase muscles *depressor trochanteris*, *retractor unguis*, *flexor tibiae* and *retractor coxae* is correlated with the touchdown of the leg, the potential sensory basis of this correlation or its connection to burst intensity remain unknown. In our experiments, we are using a trap door setup to investigate how ground contact contributes to stance phase muscle activation and burst intensity in different stick insects species, and which afferent input is involved in the respective changes.

While the magnitude of activation is changed in all of the above stance phase muscles, only the timing of the *flexor tibiae* muscle is changed if the animal unexpectedly steps into a hole. Individual and combined ablation of different force sensors on the leg demonstrated influence from femoral campaniform sensilla (feCS) on flexor muscle timing, causing a significant increase in the latencies during control and air steps. Our results show that specific load feedback signals determine the timing of *flexor tibiae* activation at the swing-to-stance transition in stepping stick insects, but that additional feedback may also be involved in flexor muscle activation during stick insect locomotion. With respect to timing all other investigated stance phase muscles appear to be under sensory control other than that elicited through touchdown.
Introduction

Leg movement during steps in vertebrates and invertebrates alike is the joint result of central rhythmic activity combined with sensory feedback, which are ultimately also responsible for the coordinated movement of the limbs and body (e.g. insects, Büschges et al. 1995; Büschges2005; turtle, Robertson et al. 1985; mammals, Brown1911; for review see Kiehn and Kjaerulff1998; Pearson2008). The relative importance of central output vs. sensory feedback for the timing of the stance and swing muscles has been discussed since the early days of neuroscience (Brown1911; Prochazka et al. 2000; Sherrington1910). Since then, proprioceptive sensory feedback from leg sense organs has been known to be integrated with central pattern generator activity of the individual leg joints to control the magnitude of activation and the timing of phase transitions between muscle antagonists (for detailed reviews see: Büschges2005; Büschges and Gruhn2008; Duysens et al. 2000; Ekeberg et al. 2004; Orlovsky et al. 1999). The transition between the stance and swing phases of a step cycle has been investigated particularly well, and is known to be affected both by position and load signals from the same as well as from neighboring legs (Bässler1967; Bässler1993; Conway et al. 1987; Cruse1985; Cruse1990; Duysens and Pearson1980; Gorassini et al. 1994; Hiebert and Pearson1999; Wendler1964; Zill et al. 2009). In vertebrates, the relative contributions of central vs. peripheral influences during step cycle transitions were clarified through trap door experiments with cats. These experiments, in which a cat stepped into a suddenly appearing hole in the ground, have shown that the first phase of leg extensor activity is centrally driven up until at least 30ms after touchdown (Gorassini et al. 1994; Hiebert et al. 1994) and sensory feedback appears to take effect only thereafter. In this case, loading of the leg only influences the magnitude of muscle activation. For invertebrates, namely the stick insect, several sensory inputs are known to provide a sensory input that facilitates transitions between antagonist muscle pairs (for review see (Büschges2005). Position and load feedback, as well as coordinating influences from the neighboring legs, for example, are known to affect the stance to swing transition (Bässler1967; Bässler1977; Cruse1985; Cruse1990; Wendler1964). Similarly, in the swing to stance transition, flexor muscle activation has been described to be tightly coupled with the touchdown of the leg (Bässler et al. 1991; Berendes et al. 2013; Cruse1985; Gruhn et al. 2006; Rosenbaum et al. 2010). Yet, in this latter case it is unresolved to what extent the
coupling is dependent on sensory feedback or on central drive. Recently, a laser-assisted trap door setup has been developed to help studying of the role of local sensory input for the activation of select stick insect leg muscles (Berendes et al. 2013). With this approach, similar to that used in the cat (Gorassini et al. 1994), it was shown that flexor activation was drastically delayed or even absent, when ground contact was unexpectedly lowered during swing, and it was suggested that missing load feedback might be the cause of this delayed flexor activation (Berendes et al. 2013). Load sensing, which in vertebrates can be provided by tendon organs (Prochazka et al. 1997), in insects is provided by campaniform sensilla (CS) (Büsches and Gruhn 2008; Zill et al. 2004). In the stick insect and the cockroach, femoral and trochanteral campaniform sensilla (feCS or trCS), are capable of signaling tarsal ground contact, and have been reported to take part in the initiation and maintenance of stance (Akay et al. 2001; Akay et al. 2004; Rosenbaum et al. 2010; Zill et al. 2004; Zill et al. 2009). Their feedback can influence the magnitude and timing of motoneuron activity. A load increase has been shown to initiate and increase depressor activation (Borgmann et al. 2012; Cruse et al. 1993; Rosenbaum et al. 2010; Watson et al. 2002; Zill et al. 2004; Zill et al. 2009), as well as its the duration (Pearson and Bradley 1972; Rosenbaum et al. 2010; Watson and Ritzmann 1998a; Watson and Ritzmann 1998b; Zill et al. 1999), and ablation of feCS was shown to reduce flexor tibiae muscle EMG magnitude (Akay et al. 2001). However, so far no direct demonstration exists for the sensory control of stance muscle activation through the ground contact signal in general, and the influence of CS or other sense organs in this context in particular (for review see Büsches 2005).

We have used the trap door approach to allow the systematic investigation of the influence that lack of ground support has on the timing of activation of the major stance phase muscles of the stick insect. The muscles investigated were the retractor coxae in forward and protractor coxae in backward walking, and the flexor tibiae, the depressor coxae and the retractor unguis. Furthermore, we have analyzed the sensory influences from the leg onto flexor muscle activation. For this purpose we selectively ablated either single or multiple sense organs, amputated parts of the leg and compared latencies of the initial activation of the flexor muscle between normal steps on ground and steps into the hole. Finally we compared the response in the stick insect Carausius morosus to that of the two other phasmid species Cuniculina impigra and Aretaon asperrimus, which are often used
interchangeably in motor control research to determine if the findings are transferrable between species.

Material and Methods

Experimental animals

The experiments were carried out on adult female stick insects of the species *Carausius morosus, Cuniculina impigra* and *Aretaon asperrimus*. The animals were taken from a colony at the Biocenter of the University of Cologne, kept at 20-22°C under a 12h light / 12h dark cycle. The stick insects were fed blackberry leaves (*Rubus fructiosus*) *ad libitum*.

Experimental Setup

The setup used has been described in detail in Berendes et al. 2013. In brief, animals were tethered (see below) above a slippery surface which had a separate platform for one leg (49 mm x 34 mm, stainless steel surface) integrated into it at the same height. This platform could be lowered pneumatically (SLS-6-25-P-A; FESTO mini slide, Esslingen, Germany) to levels in 2mm intervals below the original walking surface level. Ground contact of the leg was detected electrically (Gruhn et al. 2006) by means of a lock-in-amplifier (electronics workshop, Zoological Institute, Cologne) that amplifies the voltage at the animal during application of a specific small current to the slippery surface at any platform level. In addition, a sheet of laser light (LG series, 1mW, 660nm, Lasertechs, Aschaffenburg, Germany) was used to detect virtual ground contact after lowering the platform (photodetector SLCD-61N4, Silonex, Montreal, Canada). Directly following manual initialization, the drop of the lowerable platform occurred after the next tarsal liftoff, which in turn was detected by the ground contact detection circuit. The activation of the laser light sheet together with the drop of the platform allowed signaling the next passing of the tarsus through the former ground level. The surface was brought back to its original position pneumatically at any time thereafter chosen by the experimenter. Tarsal ground and virtual ground detection signals were fed into an AD converter (Micro1401, CED, Cambridge, UK), and recorded using Spike2 software (Version 7, CED, Cambridge, UK). The period of maximum amplitude in the tarsal contact trace marked the swing phase (tarsus lifted off) while that of minimum amplitude marked stance (tarsus in contact with surface). The onset
of the digital pulse produced by the laser signal marked the time when the tarsus crossed the virtual ground level for the first time. Any additional deflection in the laser contact trace could be either caused by a passing of the leg through the laser signal, or the misreading of the laser signal by the detector, caused by positional changes of the leg in the light path. High-speed video (AOS S-PRI, AOS Technology AG, Baden Daettwil, Switzerland, resolution: 400 x 1024 Pixel, frame rate: 500 fps, shutter speed: 2000 µs) during some of the experiments was used to ensure the accuracy of the electrical and the laser-sheet measurements.

**Preparation**

The animal was glued ventral side down (two component glue, ProTemp II, ESPE, Seefeld, Germany) onto a balsa stick as described in Berendes et al., 2013. The animal was induced to autotomize the hind and front legs as described in (Schmidt and Grund 2003). If autotomy could not be induced, legs were removed by cutting them leg off with a pair of scissors at the level of the coxa. Forward walking was elicited by brush strokes to the abdomen, backward walking by gently pulling on the antennae. Electromyograms (EMG) of the stance phase muscles *retractor coxae* in forward and *protractor coxae* in backward walking, the *flexor tibiae*, the *depressor coxae*, and the tibial branch of the *retractor unguis* (RUI2) were performed using two twisted copper wires (51 µm outer Ø) (Rosenbaum et al. 2010). The location of the EMG recording sites was according to described locations (Radnikow and Bässler 1991; Rosenbaum et al. 2010). The freshly cut-off tips of the wires were inserted through small holes in the cuticle into these muscles and held in place by ProTemp II glue. The signal was preamplified 100-fold with an isolated preamplifier (MA101, electronics workshop, Zoological Institute, Cologne), and further amplified 10-fold with a main amplifier/signal conditioner (MA102, electronics workshop, Zoological Institute, Cologne). For all experiments, the signal was band pass-filtered 100 Hz- 1000 Hz. Crosstalk from the *extensor tibiae* muscle was present in most of the flexor EMGs recorded. Flexor activity, however, was easily distinguished from extensor potentials, based on its larger amplitude and lower frequency. The *flexor tibiae* muscle is multiply innervated and shows different innervation patterns over the length of the muscle (Debrodt and Bässler 1989; Goldammer et al. 2012). As it has been previously reported that the latencies of activation in this muscle can depend on the site of the recording proximally or distally within the femur (Berendes et al. 2013), care was taken to place the EMG wires always at the end of the proximal third of
A threshold line was placed above the level of the extensor potentials to determine the time of the first large flexor potential unit of every stance phase.

All experiments involving ablation and amputation were carried out 24hrs after surgery on animals which had not been used for control experiments previously, because of the deterioration of the EMG signal over this prolonged period. However, care was taken to have equal numbers of recordings from control animals without ablation. Campaniform sensilla (CS) were ablated as described previously (Zill et al. 2011) by destroying the caps and subsequent insertion of a fine minuten pin into the destroyed cap. Ablated CS included: tibial campaniform sensilla (tiCS, group 6A and B, Zill et al. 2011) and two additional groups anterior and posterior from these; femoral campaniform sensilla (feCS, Akay et al. 2001) and trochanteral campaniform sensilla (trCS, group 1- 4, Bässler1977; Zill et al. 2012). In addition to the CS, the retractor unguis tendon was cut. For this purpose, a window was cut into the cuticle of the tibia. The tendon was lifted with an insect pin, while care was taken not to damage nerves or muscles in the leg, and then cut with a pair of scissors. Afterwards, the cuticle was closed. For tarsus ablation, the tibia was cut just above the tibia-tarsus-joint. Different sense organs or the tendon were either destroyed alone or in combinations thereof as described in the results section.

Evaluation of muscle latencies and magnitude of muscle activity

Stance phase muscle activation latencies were calculated by using the first EMG spike of the stance phase muscle in question that was visible above noise level, with respect touchdown, as measured by the tarsal contact signal or the virtual touchdown signal at the pass through the laser light sheet. For comparison of EMG activities between steps on ground and steps into the hole, original recordings were rectified and smoothed (time constant 20ms). The integral of muscle activity was compared between the first 200ms after touch-down and 200ms after the virtual touch-down, unless the antagonist was activated earlier and a second step was initiated within the time window. In that case, the steps were not used for analysis. The time window of 200ms was chosen after a comparison of the stance phase lengths to represent an average length before the start of swing phase muscle activity. In addition to the total value of the integral, the maximum value of the integrals in the 200 ms interval after TD and vTD, respectively, was also measured, and all values exported to Origin (vers. 8.5, Origin Lab Corporation, Northhampton, MA, USA).
The number of animals used for each experiment were as follows: 3 each for the *retractor coxae*, *protractor coxae*, and *depressor trochanteris*, 9 for the *flexor tibiae*, and 4 for the *retractor unguis* for the stance phase muscle activity analysis in *C. morosus*. For the comparative analysis of flexor muscle activity, we used data from 9 animals of *C. morosus*, 5 of *Cuniculina impigra* and 4 of *Aretaon asperrimus*. For ablation experiments, the femoral campaniform sensilla were ablated in 3, the tibial or trochanteral campaniform sensilla were ablated in 5 animals. In 3 animals, the femoral *and* trochanteral campaniform sensilla were ablated together. In 5 animals the tarsus was cut off, *and* the tibial, trochanteral, *and* femoral campaniform sensilla were ablated. Finally, the *retractor unguis* tendon was cut in 7 animals. The number of animals used for a specific condition (*N*) and the number of steps evaluated (*n*) for a specific experiment are also given in the figures.

**Statistics**

All statistics were performed using Origin (Vers. 8.5, Origin Lab Corporation, Northhampton, MA, USA). The data sets acquired in this study were tested for normal distribution using the Shapiro-Wilk test. According to this test, none of the data presented in this study were distributed normally. For this reason, all tests for statistical significance were performed using nonparametric statistical tests. For data sets dependent on each other, the Wilcoxon signed rank test was used. For this purpose, latencies, the integral or the amplitude measured during three control steps in one walking sequence were pooled. The Mann-Whitney-U-test was used to compare independent data sets. Significance was marked in the figures with the following symbols: * for a significance level of $p < 0.05$; ** for a significance of $p < 0.01$ and *** for $p < 0.001$. *N* denotes the number of animals, while *n* represents the number of steps.
During walking, the different stance phase muscles are activated in a specific sequence that is influenced by sensory input (Büschges 2005; Rosenbaum et al. 2010). In this context, ground contact has been demonstrated to be of great importance for the activation of flexor tibiae muscle (Berendes et al. 2013). However, it remains unclear to what extent the activity of other stance phase muscles (protractor and retractor coxae, depressor trochanteris, retractor unguis) may also be modified by sensory feedback elicited by ground contact of the tarsus. In addition, it also remains unclear what sensory signals are mediating the coupling between ground contact and stance muscle activation. Here we use a trap door setup described by Berendes et al. (2013) to investigate the influence of ground contact on all stance phase muscles and pin down potential sensory signals that could mediate this relationship.

**Influence of ground contact on stance muscle activation latency**

In the first set of experiments we implanted EMG wires into the stance phase muscles retractor coxae (during forward walking), its antagonist the protractor coxae (during backward walking), and depressor trochanteris, flexor tibiae and retractor unguis, which act as stance muscles in both walking directions. Figure 1 shows example recordings from each of these muscles with EMG, rectified and smoothed EMG, and with the respective tarsal contact trace for one step on ground and one subsequent step into the hole (Fig.1 A,C-G) and the recording sites (Fig.1B).

The retractor coxae moves the coxa posteriorly during stance in forward steps. It was activated around the time of touchdown, and no difference in muscle activation was discernible between steps on ground and steps into the hole (Fig.1C). Retractor activation latency in three animals during steps on ground (SG) started on average -34.0 ms before touchdown in control steps (SD=32.1; N=3, n=27), and not significantly different from the -33.5ms for the steps into the hole (SD=21.0; N=3, n=9). During backward walking, the protractor coxae takes over the role of the retractor, but moves the leg forward during the stance phase. An example of the activity is shown in Fig. 1A and, at larger time resolution, in Fig.1D. The average latency of protractor coxae activation for the control steps of three animals was -19.4 ms (SD=24.7; N=3, n=27), not significantly different from the -34.1 ms for
the steps into the hole (SD=34.3; N=3, n=9). The third stance phase muscle investigated, the
depressor trochanteris is located in the coxa and is responsible for lowering the leg. Its
activation both, during steps on ground and steps into the hole occurred well before
touchdown (Fig 1E). In the animals tested, depressor activity began on average -93.5 ms
before touchdown in control steps (SD 35.6; N=3, n=27), which was not significantly different
from the latency of -105.5 ms before steps into the hole (SD 39.9; N=3, n=9). The correlation
of flexor tibiae activation to the time touchdown has been shown previously (Berendes et al.
2013; Gruhn et al. 2006; Rosenbaum et al. 2010). Figure 1F shows an example of flexor
activity during a ground supported step and after stepping into the hole. Similar to earlier
results by Berendes et al., upon steps into the hole, the latency of activation for the flexor
was shifted significantly (p<0.001) to a later point in time (mean=36.3ms,SD=19.1; N=9,
n=57) as compared to regular steps on ground (mean=10.2 ms, SD=3.0; N=9, n=171). The last
leg muscle tested was the 2nd part of the retractor unguis, located in the proximal tibia,
which is responsible for lowering and flexing the claw (Fig. 1G). The retractor unguis was
activated around the time of lift off, on average -130.3ms before touchdown for the control
steps (SD=63.3; N=4, n=36), and -142.1 ms, for the steps into the hole (SD=74.5; N=4, n=12),
which was again not significantly different from one another. The results are summarized in
figure 2. Of all stance muscles investigated, stepping into a hole only affected the latency of
activation of the flexor tibiae significantly, confirming earlier results on this particular muscle
(Berendes et al. 2013). All other muscles are activated before touchdown and do not show a
change in latency. So, with respect to timing, this suggests that ground contact affects only
in the flexor muscle.

Influence of ground contact on stance muscle activation strength

Despite the lack of influence of ground contact signaling on the activation latency of the
stance phase muscles other than the flexor, there was still the possibility that ground
contact alters the activity in an ongoing stance phase after touchdown, as has been
described for the flexor and depressor muscles (Gabriel and Büschges2007; Rosenbaum et
al. 2010). To explore this, we compared the strength of EMG activity within a time window
of 200ms either after real touchdown or after the passing through the laser sheet. As a
measure, we took the ratio of either the maximal amplitudes during steps with and without
ground contact, or that of the total area of the rectified and smoothed integrals of the EMG
activity of the different stance phase muscles during steps with and without ground contact. The maximum amplitude gives an idea about the maximum intensity of muscle activation, whereas the total area can be an additional measure of the activity within the predefined time window.

We first compared the maximum of the integral amplitudes during steps into the hole to those during steps on ground. If the ratio was smaller than 1, then the maximum activity of the muscle during steps into the hole was smaller, and if it was greater than 1, then activity during steps into the hole had increased. No significant differences between the maximum amplitudes under the two situations were seen in the retractor coxae \( (A_{\text{max-ratio}}^{\text{ret coxae}}=1.04, \ SD=0.23) \). The maximum depressor activity was significantly greater after steps into the hole \( (A_{\text{max-ratio}}^{\text{depr}}=1.51, \ SD=0.5, \ p<0.01) \), whereas protractor coxae, flexor and retractor unguis maximum activity were significantly greater during steps on ground \( (A_{\text{max-ratio}}^{\text{pro coxae}}=0.68, \ SD=0.22, \ p<0.5; \ A_{\text{max-ratio}}^{\text{flex tibiae}}=0.9, \ SD=0.25, \ p<0.01; \ A_{\text{max-ratio}}^{\text{ret unguis}}=0.86, \ SD=0.16, \ p<0.05) \) (Fig 3A).

The analysis of the total area under the integral of the rectified EMG activity within a 200ms time window after either the real or the fictive touchdown showed that the SIH to SG activity ratios in the depressor was clearly increased after stepping into the hole (mean ratio\(_{\text{depr}}\)=1.75, SD=0.63, \( p<0.05 \)), whereas retractor and protractor coxae, flexor tibiae and retractor unguis activity was clearly decreased (mean ratio\(_{\text{ret coxae}}\)=0.73, SD=0.19, \( p<0.05 \); mean ratio\(_{\text{pro coxae}}\)=0.42, SD=0.13, \( p<0.01 \); mean ratio\(_{\text{flex tibiae}}\)=0.67, SD=0.38, \( p<0.001 \); mean ratio\(_{\text{ret unguis}}\)=0.8, SD=0.22, \( p<0.01 \)) (Fig 3B). All values including median and IRQ range are summarized in table 1.

This demonstrates that, despite the fact that the timing of muscle activation is only affected in the flexor, ground contact, nevertheless, does influence the magnitude of activity in all other stance muscles. This influence, however, only affects already ongoing muscle activity and the sign of the influence can either be positive or negative.

**Influence of ground feedback on flexor tibiae in different phasmid species**

Two additional phasmid species are often used in locomotor research in addition to the well known species *Carausius morosus*, which have been used above. These are *Cuniculina impigra*, which is similar in its body proportions to *Carausius morosus* but of bigger size, and *Aretaon asperrimus*, which is similar in size as *Carausius morosus*, but has shorter legs and
shows more frequent walking activity. Since each preparation offers particular advantages to
the experimenter (Berg et al. 2013; Jeck and Cruse 2007), and especially Cuniculina and
Carausius are often used interchangeably, we wanted to verify that the observed effect is a
general phenomenon in phasmids.

We focused the subsequent comparative analysis on the activity of the flexor tibiae muscle
in the three species, since it is affected by ground contact both in the timing and the
magnitude of the response. During normal steps on ground, the latency of the first EMG
spike in the flexor muscle is shortest in Carausius (mean=10.2ms, SD=3.0; N=9, n=171),
followed by that in Aretaon (mean=14.9ms, SD=6.4; N=4, n=63), and that in Cuniculina
(mean= 21.4ms, SD=17.9; N=5, n=75). Between the Carausius and the other two species this
difference was significant (p<0.01 and p<0.001, resp.), while the difference was not
significant between Aretaon and Cuniculina. Activation of the flexor muscle after steps into
the hole occurred in all three species significantly later than after their respective steps on
ground (C.m.: mean=36.3ms, SD=19.1; N=9, n=57; A.a.: mean=190.5ms, SD=90.3; N=4, n=21;
C.i.: mean=50.0ms, SD=28.0; N=5, n=25; for all species p<0.001), and was also significantly
different from one another in all species (Fig4A). Similar to C.morosus, the maximum
amplitude of the rectified flexor EMG was also lower in Aretaon (93.3 %, SD=71.1) and
Cuniculina (86.4 %,SD=29.1), but these changes were not significant over the values from the
steps on ground (Fig4B). However, as for C.morosus (see above), the overall flexor tibiae
activity for a time window of 200ms after touchdown was also significantly lower for the
other two species during steps into a hole compared to steps with ground contact (p<0.001).
While flexor activity in C.morosus dropped to 67% of its original value (SD=38.6),
A.asperrimus flexor activity dropped to 50.5 % (SD=38.5), and C.impigra flexor activity
dropped to 62.8 % (SD=28.4) (Fig4C).

In summary, while there are differences between the three phasmid species, all of them
show the same dependency of both timing and magnitude of flexor activation on the
presence of a ground contact signal. Aretaon shows a particularly dramatic dependence of
flexor activation on the touchdown signal. Thus, all three species lend themselves to
investigating the relationship between sensory feedback caused by ground contact and the
activation of the flexor tibiae muscle.

Potential sources of touch-down feedback for flexor tibiae activation
We next investigated potential sources for the sensory feedback that influences flexor latency in *Carausius morosus*. Candidate sense organs that could influence either the timing or magnitude of the *flexor tibiae* activity by measuring the moment of ground contact, are the trochanteral (trCS, group 1-4, Bässler 1977; Zill et al. 2012) and femoral campaniform sensilla (feCS, Akay et al. 2001), as well as tibial campaniform sensilla (tiCS, group 6A and B, Zill et al. 2011), including two additional groups anterior and posterior of tiCS 6A and 6B. In addition, in a subset of experiments, we cut the *retractor unguis* tendon to remove all potential input associated with activity in this muscle. A multipolar receptor connected to the *retractor unguis* tendon similar to ones found for more proximal muscles (Bässler 1977; Bräunig et al. 1981; Matheson and Field 1995) would be another potential candidate for the detection of the ground contact. Removal of sense organs or of the tarsus were either performed individually or in combinations thereof and recordings made 24hrs following the procedure. Examples of a FlxTi recording during SG and SIH in a control animal and in a different animal 24hrs after ablation of the feCS are shown in figure 5A and B, respectively. Ablation of either all trochanteral (trCS) or tibial CS (tiCS) did not have an influence on the latency of *flexor tibiae* activation during regular steps on ground (trCS abl: SG mean=12.9ms; SD=6.2; N=5, n=15) (tiCS abl: SG mean=9.9ms, SD=3.2; N=5, n=90)(Fig. 5C). However, the flexor latency was significantly prolonged (p<0.05) if the femoral campaniform sensilla (feCS) were ablated (feCS abl. SG mean=15.9ms, SD=9.2 ms; N=3, n=27). Interestingly, if the feCS were ablated together with the trCS, the latency of flexor tibiae activation after touchdown was not significantly prolonged compared to control animals (feCStrCS abl. SG mean=11.8ms, SD=5.6; N=3, n=54). Cutting the RetUng tendon at the level of the mid-tibia prevented the flexion of the tarsus before touchdown and a passive stretch of the tendon at touchdown, effectively removing proper feedback from such a hypothesized stretch receptor. This procedure produced a large increase in the variability of flexor latencies after touchdown (Var_tendon int: 15.5 ms; Var_tendon cut: 240.9 ms), but the mean values were not significantly different from controls (SG mean=21.4 ms, SD=43.5; N=7, n=93)(Fig5C).

For all these experiments, we also measured the latencies for the steps into the hole with respect to the virtual touchdown. In all cases, the SIH latencies of flexor muscle activation increased significantly (Fig5D). In all three cases where single CS groups were ablated, this procedure also increase the latency of the flexor response to stepping into the hole significantly over control steps into the hole (trCS abl: SIH mean=57.0 ms; SD: 27.0; N=5, n=15,
p<0.01; tiCSabl SIH mean=60.1ms, SD=28.3; N=5, n=30, p<0.001; feCSabl: SIH mean=73.2ms, SD=39.4; N=3, n=9, p<0.01). Interestingly, when feCS and trCS were ablated together (feCStrCSabl SIH mean=25.7ms, SD=14.9; N=3, n=18), no significant difference in SIH latency over controls existed. Cutting the retractor unguis tendon had the same effect on SIH latencies as on latencies during steps on ground, i.e. the variability was greatly increased (Var tendon int: SIH=94.1 ms; Var tendon cut: SIH=326.8 ms), but the mean latency was unchanged (tendon cut SIH mean=52.2ms, SD=70.3; N=7; n=31). To test if redundancy from a potential interplay of different sense organs was responsible for the absence of effects during steps on the ground in most ablation experiments, we then ablated all the above CS groups and additionally removed the tarsus. Animals without these CS and without tarsus showed a great reluctance to perform continuous stepping with that leg and the procedure greatly increased the variability of latencies recorded (VarSG=243.9 ms; VarSIH=254.2). In addition, mean latency for flexor activation during steps on ground and steps into the hole was delayed significantly (SG mean=40.4ms, SD=61.0, p<0.05; SIH mean=61.7ms, SD=52.8, p<0.05; N=5, nSG=69, nSIH=23; Fig 5C,D).

Finally, we wanted to know if ablation of any of the sense organs or the tendon, on top of the effect on the latency of flexor activation, also affected the magnitude of activation of the flexor muscle. In intact animals, flexor activity during steps into the hole was significantly reduced, both in maximum amplitude and total activity as measured through the integral of the rectified and smoothed EMG (see above and Fig 5E,F). In all ablation situations but one, the maximum integral amplitude and total flexor activity were significantly reduced during steps into the hole over steps on ground (for a summary of all values, incl. the median and IRQ range see table 2), but never significantly different from the reduction in the intact animal (Fig.5E,F). The maximum amplitudes were reduced to between 82-90% of the SG value, while the total integral area was reduced to between 61-78% of the SG value. The observed changes, however, are not a result of qualitative changes in the flexor EMG compared to the control animals, such as through changes in the occurrence of large or small units or delayed recruitment single units, in any of our single or double ablation protocols (compare figures 1 F and 5A to figure 5B). The only case with a result significantly different from control animals was the experiment where all the above CS fields and the tarsus were ablated. These animals showed a great reluctance to perform steps with the leg
that had undergone the ablations. Here again, the variability was greatly increased, but neither the mean maximum amplitude nor the mean total activity were significantly different between steps on ground and steps into the hole. Instead, these animals often lacked large flexor units (data not shown), and both values were significantly different from the control animals without any surgery.

In summary, the only single CS group with a clear effect on flexor activation latency during regular ground steps is that of the femoral CS. However, feCS ablation and all other single CS ablations did also affect the flexor activation latency during steps into the hole. Cutting the *retractor unguis* tendon only affected the variability of the response, while ablation of all of the above CS groups and the tarsus (incl. the tendon) together affected the latencies during steps on ground and into the hole in addition to increasing the variability. Only this latter procedure affected the change in magnitude of the flexor response seen between SG and SIH, which, was absent after this particular procedure only.
We have used a recently developed foot-in-the-hole setup to study systematically which leg muscles depend on the touchdown of the leg they are moving for their activation during walking, and to what extent. We have compared data from three different phasmid species and singled out the flexor tibiae (FlxTi) muscle as the only muscle that is dependent on touchdown for its activation. We performed a series of ablation experiments to determine the source of the sensory feedback that contributes to this dependence. Only ablation of the local femoral campaniform sensilla directly affected flexor activation. However, a greatly increased variability in the latency of activation after lesioning the tendon of the tarsal flexor muscle retractor unguis suggests the involvement of additional sensory signals in flexor muscle activation at touchdown.

Dependence of stance muscle latency on touchdown

The influences of sensory feedback about touchdown on the timing of the stance muscles in the stick insect are only partly known (Berendes et al. 2013). We therefore first analyzed the latencies of these muscles with respect to the electrically determined touchdown in a 2-middle-leg-preparation during regular steps on a slippery surface. We found that the flexor and the depressor latencies matched values reported in earlier studies for the intact, six-legged animal (Gruhn et al. 2006; Rosenbaum et al. 2010). Similarly, the RetUng muscle was activated during swing as reported earlier (Fischer et al. 2001). Only the latencies for the activation of RetCx (forward) and ProCx (backward) were shifted forward from the mean latencies which had been reported for the 6-legged stick insect by Rosenbaum et al. (2010). However, Rosenbaum and colleagues also reported a significant forward shift for RetCx activation in the 2-leg preparation, and the data reported show that activity very often began well before touchdown for stance in the respective directions for both muscles (Rosenbaum et al. 2010). Part of the discrepancy to the data from the 6-legged animal could be the result of a generally large variability in the latencies of this muscle. The latency shift observed by Rosenbaum and colleagues, and our latencies, however, also suggest a timing influence caused by the activity of adjacent legs for this particular muscle. Previous studies have already shown that front leg stepping influences activation of the middle leg RetCx motor neurons during forward walking (Borgmann et al. 2007; Borgmann et al. 2009), but
the source or sources for such an inter-leg sensory influence are as yet not clear. One of
them could be the front leg femoral chordotonal organ (fCO)(Ludwar et al. 2005).

**Effects of lack of ground support on stance muscles**

Stepping into the hole (SIH) should reveal if ground contact affects the activation of a given
muscle through a shift in its activation latency. A similar approach in cat, in which the animal
stepped into a suddenly appearing hole in the ground showed that ground contact
influences the leg stance muscle *lateral gastrocnemius* only in the magnitude of its
activation, and not until 30ms after touchdown (Gorassini et al. 1994; Hiebert et al. 1994).
The SIH scenario in the stick insect affected activation strength and latency of the different
stance muscles to a different extent, and only in select muscles. The latency of activation
was only affected in the FlxTi muscle, where it was significantly greater for SIH than for steps
on the ground. This matches and confirms earlier reports by Berendes and colleagues
(2013), and shows that the touchdown signal appears to be only necessary for the timing of
this muscle. In contrast, the intensity, or magnitude of muscle activity was influenced in all
muscles albeit in different directions. RetCx, ProCx, FlxTi and RetUng showed a significant
decrease, the DepTr a significant increase in activity. This corroborates the importance of
local feedback for the magnitude control.

For all of these muscles, at least single local sources for sensory influences are known.
Activation of the RetCx during forward walking is facilitated by activation of the fCO through
extension of the femur-tibia joint (Bässler1986). In addition, the ventral coxal hair plate
vcxHP is known to report the position of the coxa and its stimulation initiates and sustains
RetCx activation (Bässler1977; Cruse et al. 1984). ProCx activation, on the other hand, is
influenced by stimulation of coxal hair rows (Cruse et al. 1984), and it does not seem unlikely
that their stimulation also helps in initiating protraction during stance in backward walking.
Local load signals are also known to influence both, RetCx and ProCx activation and activity.
Feedback from the trochanteral CS supports termination of ProCx and initiation of RetCx
(Akay et al. 2004; Akay et al. 2007) as well as an increase in RetCx activity (Schmitz1993)
during forward, and *vice versa* for ProCx activity during backward walking (Akay et al. 2007).
In addition, stimulation of the trCS is able to sustain stance (Bässler1977). It is therefore
interesting that the latency in the RetCx in forward, or ProCx in backward walking is
unchanged during SIH. This suggests that the local touchdown signal is of relatively little
importance for their timing when they are used as stance muscles in the two-leg preparation. The lack of change in DepTr activation latency upon SIH supports earlier studies (Bässler1967; Bucher et al. 2003; Graham and Bässler1981; Hess and Büschges1999), in which it was shown that the extension of the tibia, as measured by the fCO is highly influential for depressor activation. It is also known, that sustained load such as that caused by ground support and signalled by trCS increases depressor activity, but only during ongoing stance (Rosenbaum et al. 2010; Zill et al. 2012; Zill et al. 2013). Interestingly, we found that also lack of ground support leads to an increase in DepTr activity. This is likely caused by a second mechanism at work in this case, providing the positive feedback. Its source is probably the trochanteral hair plate (trHP) that is located at the base of the trochanter and signals the position of the joint (Schmitz1986). The ablation of this hair plate causes a strong reduction in downward directed force, in turn suggesting that its activation during the lowering of the leg in SIH increases DepTr activation (Schmitz1986). The RetUng displayed no change in activation latency during SIH but showed a marked decrease in activity following the passing through the fictive ground level. Up to recently, very little information was available on sources of sensory feedback for this muscle in the stick insect. Several sense organs on the tarsi of the locust have been reported to feed back onto RetUng motor neurons (Laurent and Hustert1988) and recent studies in stick insects have shown that RetUng activity, and the forces generated by the resulting tarsal flexion are reported back and further enhance RetUng activity with an average delay of 35ms (Zill et al. 2010; Zill et al. 2014). This finding explains the reduction of mean activity when the animal steps into a hole. Finally, for the FlxTi muscle, we found both a drastic increase in latency and a reduction in overall activity, confirming earlier results by (Berendes et al. 2013). Clearly, the initial ground contact has a strong influence on both timing and magnitude of this muscle. This effect was seen in all three phasmid species investigated, albeit to different degrees. While the morphologically more similar species Carausius morosus and Cuniculina impigra showed similar changes in FlxTi latencies during SIH, Aretaon asperrimus expressed a particularly strong dependence of FlxTi activation on touch down. In any case, however, all three species lend themselves to the further investigation of this phenomenon. All magnitude changes most likely represent underestimations of the effect that loading in an intact freely walking animal would have (Schmitz and Dallmann2014), owed to the fact
that the animals were tethered above a slippery surface and did not have to carry their own weight. Furthermore, the effect of the step into the hole on the magnitude in all but the DepTr muscle parallels the reduction in EMG amplitude in cat lateral gastrocnemius and vastus lateralis muscles, the ankle and knee extensors (Gorassini et al. 1994). The increase in DepTr activity could be part of a corrective response elicited by the above mentioned stimulation of the trochanteral HP (Schmitz 1986).

Interestingly, the studies on the EMG response to missing ground support in cat have demonstrated that the magnitude control also strongly depended on the posture and state of the contralateral leg, which we did not monitor (Gorassini et al. 1994). In the cat, extensor muscle activity was increased compared to control during steps into the hole when the contralateral leg was in swing (Gorassini et al. 1994). In the stick insect, we never observed a reduction in the response to stepping into the hole, suggesting that the response is quite stereotypical and the state of the contralateral leg is of relatively little importance in this context. Nevertheless it cannot be excluded that the state of the ipsilateral front or hind legs might have been able to influence the response had they not been removed for the experiment.

Sensory influences on flexor tibiae activation

The tight correlation of touchdown and FlxTi activation seen in our and in previous studies suggests that this particular leg muscle is strongly influenced by the transient loading of the leg at touchdown signaled through campaniform sensilla (Berendes et al. 2013; Gruhn et al. 2006). Since, the swing to stance transition in a step cycle provides an excellent opportunity to investigate the relative importance of central and peripheral influences during such a phase transition, we directed our attention to ablating potential candidate sources for this feedback. This excluded ablating the tarsus which had previously been shown not to be directly involved in FlxTi timing (Berendes et al. 2013). The FlxTi is known to receive sensory input from movement and load sensors. Movement signals from the fCO are known to support and enhance FlxTi activity at the beginning of and during stance (for review see Büschges et al. 2008). However, this feedback is most likely not instrumental for the initial activation of the muscle. Furthermore, load feedback from various leg campaniform sensilla has been shown to influence FlxTi motor neuron activity (Akay et al. 2001; Akay et al. 2004; Zill et al. 2014). Yet no direct demonstration exists that stimulation of these or other CS is
causal for FlxTi timing at the beginning of stance (for review see Büschges2005). When ablating single groups of sense organs, we found that only ablation of the feCS caused a significant increase in FlxTi latency during normal ground steps, the response one would expect for sensory feedback that is a major contributor to the activation of the muscle. The only other single ablation to also cause an increase in latency was that of the trCS. This change, however, was not significant.

This suggests that the main timing information for the activation of the FlxTi through touchdown in the two-leg preparation is provided by the local feCS. These particular CS had previously only been shown to provide important feedback for the magnitude control of FlxTi activity (Akay et al. 2001; Akay et al. 2004). No previous evidence existed that trCS affected flexor activity at all (Büschges and Gruhn2008), and future intracellular recordings will have to verify this influence. Cutting the retractor unguis tendon in the tibia only affected the variability of the response, suggesting that any multipolar receptor associated with the tendon is not directly involved in FlxTi activation.

**FlxTi activation during steps into the hole**

Despite of a further increase in activation latency and a marked decrease in FlxTi activity, stepping into the hole nevertheless results in FlxTi activation in 81% of the cases (Berendes et al. 2013). This could suggest a weak centrally controlled activation of the muscle, not completely unlike that in the cat (Gorassini et al. 1994). The presence of networks able to generate such rhythmicity in the stick insects is known from pharmacological experiments which have used the muscarinic agonist pilocarpine in deafferented preparations (Büschges et al. 1995; Büschges1995). The fact, however, that the flexor tibiae fails to become active in 19% of the SIH shows that this central influence is not very strong in the non-deafferented animal. In addition to feedback on touchdown through the feCS, it is therefore likely that additional sensory feedback adds to eliciting flexor activation during SIH in the two-leg preparation we used.

One possible sensory mechanism compensating for the lack of input from the feCS could be passive flexion in the femur tibia joint through passive forces at the end of extensor activity. This could elicit enough flexion movement (Hooper et al. 2009) to assist in activating FlxTi MNs during SIH through stimulating the femoral chordotonal organ which has long been known to be involved in reinforcement of flexion movement (Bässler1973; Bässler1977).
During normal steps, the time course of this passive flexion is too slow, as extensor tibiae MN activity often lasts just up to the beginning of FlxTi MN activity (Hooper et al. 2009; Rosenbaum et al. 2010). However, during SIH this movement could help in the belated FlxTi activation. Compared to the intact leg, the latency of FlxTi activation during SIH was increased even further in all single CS ablation experiments, while no single ablation changed the SIH effect on the magnitude of FlxTi activity. This implies that not only the trCS, or the feCS, together with the taCS (Zill et al. 2014), but also the tiCS have an influence on the flexor MNs. Such an influence from the latter has not been shown yet. Two reasons for these effects of CS ablation on FlxTi timing during steps into the hole are conceivable. For one, ablation of any given CS could reduce inherent tonic activity (Zill et al. 2012; Zill et al. 2013; Zill et al. 2014) to zero and thereby decrease the likelihood of flexor activation. On the other hand, it is known that CS do not only report transient or continuous load that is externally applied, but also load that is self-generated by cuticular strain due to muscle contractions (Zill et al. 2012; Zill et al. 2014). This means that strain developed by muscles such as the DepTr and the RetUng, which are activated independently of touchdown, theoretically could in turn stimulate trCS and tarsal CS (taCS) and thereby activate the flexor muscle. So far, however, such an indirect effect has not been shown, and CS activation through muscle contractions has been shown in resisted movements only (Zill et al. 2011; Zill et al. 2012; Zill et al. 2014). Both mechanisms could serve as explanations for the finding that all single CS ablations further increased the FlxTi activation latency during SIH compared to the increase observed in the intact leg. Finally, it cannot be excluded that additional inter-leg sensory influences that we eliminated through tethering a reduced preparation may play a role in the freely walking animal, especially on a non-slippery ground. Such sensory feedback might add up to help activating the FlxTi in combination with local feedback in the intact animal.

The effect of the ablation of all CS in conjunction with removing the tarsus on the flexor muscle activation and why this procedure prevented the reduction in maximum amplitude that is seen in the intact animal, while still increasing the FlxTi activation latency remains unclear.

In summary, we have shown that, while ground contact influences all stance phase muscles of the stick insect middle leg, only the timing of the flexor tibiae is largely controlled by loading feedback upon touchdown. This is different from the current assumption that also
the timing of retractor coxae MNs is dependent on the onset of load signaling (Ekeberg et al. 2004). The source for the activation signal for this latter muscle remains unknown. The sensory feedback controlling flexor muscle timing, on the other hand, originates mostly from the local femoral campaniform sensilla, which had previously only been implied in FlxTi magnitude control. In the absence of ground support, lack of phasic campaniform sensilla activity contributes to a belated flexor tibiae activation. This could assist in the following searching movements for renewed ground support.
Table 1: ratios of the burst integrals during steps into the hole (SIH) vs. steps on ground (SG) for the stick insect stance muscles. FlxTi: flexor tibiae; RetCx: retractor coxae; ProCx: protractor coxae; DepTr: depressor trochanteris; RetUng: retractor unguis.

<table>
<thead>
<tr>
<th>SIH to SG burst integral ratio</th>
<th>mean</th>
<th>SD</th>
<th>median</th>
<th>IRQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FlxTi total area</td>
<td>0.67</td>
<td>0.38</td>
<td>0.62</td>
<td>0.57</td>
</tr>
<tr>
<td>FlxTi maximum burst amplitude</td>
<td>0.9</td>
<td>0.25</td>
<td>0.93</td>
<td>0.37</td>
</tr>
<tr>
<td>RetCx total area</td>
<td>0.73</td>
<td>0.19</td>
<td>0.79</td>
<td>0.23</td>
</tr>
<tr>
<td>RetCx maximum burst amplitude</td>
<td>1.04</td>
<td>0.23</td>
<td>0.97</td>
<td>0.41</td>
</tr>
<tr>
<td>ProCx total area</td>
<td>0.42</td>
<td>0.13</td>
<td>0.42</td>
<td>0.25</td>
</tr>
<tr>
<td>ProCx maximum burst amplitude</td>
<td>0.68</td>
<td>0.22</td>
<td>0.69</td>
<td>0.31</td>
</tr>
<tr>
<td>DepTr total area</td>
<td>1.75</td>
<td>0.63</td>
<td>1.7</td>
<td>0.48</td>
</tr>
<tr>
<td>DepTr maximum burst amplitude</td>
<td>1.51</td>
<td>0.5</td>
<td>1.34</td>
<td>0.45</td>
</tr>
<tr>
<td>RetUng total area</td>
<td>0.8</td>
<td>0.22</td>
<td>0.83</td>
<td>0.32</td>
</tr>
<tr>
<td>RetUng maximum burst amplitude</td>
<td>0.86</td>
<td>0.16</td>
<td>0.84</td>
<td>0.3</td>
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</table>
Table 2: Burst integral percentage and maximum burst percentage of *flexor tibiae* (FlxTi) muscle during steps into the hole (SIH) in comparison to that during steps on ground (SG) for the intact leg and for different ablation scenarios. trCS: trochanteral campaniform sensilla; RU tendon: retractor unguis tendon; fCS: femoral campaniform sensilla; tiCS: tibial campaniform sensilla.

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<tr>
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<th>median</th>
<th>IRQ</th>
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<tbody>
<tr>
<td><strong>Burst Integral % of FlxTi activity in SIH compared to SG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66.513</td>
<td>38.359</td>
<td>61.582</td>
<td>57.03</td>
</tr>
<tr>
<td>trCS abl.</td>
<td>72.163</td>
<td>27.531</td>
<td>73.731</td>
<td>39.347</td>
</tr>
<tr>
<td>RU tendon cut</td>
<td>78.739</td>
<td>41.844</td>
<td>72.729</td>
<td>60.608</td>
</tr>
<tr>
<td>fCS abl.</td>
<td>67.147</td>
<td>22.96</td>
<td>60.186</td>
<td>29.023</td>
</tr>
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<td>fCS, trCS abl.</td>
<td>60.967</td>
<td>32.096</td>
<td>56.497</td>
<td>37.74</td>
</tr>
<tr>
<td>tiCS abl.</td>
<td>61.981</td>
<td>33.512</td>
<td>59.022</td>
<td>57.247</td>
</tr>
<tr>
<td>Tarsus, tiCS, fCS, trCS abl.</td>
<td>118.767</td>
<td>65.525</td>
<td>97.796</td>
<td>97.754</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th></th>
<th>mean</th>
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<th>median</th>
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<tbody>
<tr>
<td><strong>Maximum Burst Amplitude % of FlxTi SIH compared to SG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90.209</td>
<td>25.275</td>
<td>92.557</td>
<td>36.607</td>
</tr>
<tr>
<td>trCS abl.</td>
<td>82.17</td>
<td>24.943</td>
<td>76.424</td>
<td>37.807</td>
</tr>
<tr>
<td>RU tendon cut</td>
<td>85.144</td>
<td>27.115</td>
<td>87.457</td>
<td>37.787</td>
</tr>
<tr>
<td>fCS abl.</td>
<td>84.246</td>
<td>19.082</td>
<td>82.846</td>
<td>8.113</td>
</tr>
<tr>
<td>fCS, trCS abl.</td>
<td>87.246</td>
<td>22.483</td>
<td>87.721</td>
<td>23.658</td>
</tr>
<tr>
<td>tiCS abl.</td>
<td>90.072</td>
<td>22.778</td>
<td>90.918</td>
<td>18.317</td>
</tr>
<tr>
<td>Tarsus, tiCS, fCS, trCS abl.</td>
<td>106.828</td>
<td>37.488</td>
<td>107.608</td>
<td>29.781</td>
</tr>
</tbody>
</table>
**Figure 1:** A. Example trace for a recording of three consecutive steps of the *protractor coxae* muscle during backward walking on the ground (after touchdown, TD) and one step into the hole (after virtual touchdown, vTD); top trace marks laser signal, 2nd trace from top is the electrical tarsal contact trace, followed by the rectified and smoothed EMG and the original EMG recording. Grey shaded area under the rectified trace marks 200ms time window for analysis. B. Schematic drawing of the right middle leg, with marked EMG recording sites and scheme for current flow during touchdown, lift off and the virtual touchdown. C-G. Example EMGs of all stance phase muscles during one step on the ground (after TD) and one step into the hole (after vTD); traces are the same as in A. C. Retractor coxae (RetCx) during forward walking. D. *Protractor coxae* (ProCx) during backward walking. E. *Depressor trochanteris* (DepTr). F. *Flexor tibiae* (FlxTi). G. Retractor unguis (RetUng). Solid vertical lines mark TD or vTD, dashed vertical lines mark begin of muscle activity in EMG trace, asterisks mark platform drop. The additional deflections in the laser signal in C and E are movement artifacts caused by misreading of the detector.

**Figure 2:** Schematic comparing activation latencies for the different stance muscles during steps on ground (SG, open boxes) and steps into the hole (SIH, filled boxes). RetCx: *retractor coxae*, N=3, nSG=27, nSH=9; ProCx: *protractor coxae*, N=3, nSG=27, nSH=9; DepTr: *depressor trochanteris*, N=3, nSG=27, nSH=9; FlxTi: *flexor tibiae*, N=9, nSG=171, nSH=57; RetUng: *retractor unguis*, N=4, nSG=36, nSH=12. N=number of animals, n=number of steps; the grey shaded area marks stance.

**Figure 3:** Ratio of the maximal burst amplitude (A) and total integral of rectified and smoothed burst activity normalized to SG burst activity (B) of the stance phase muscle during the 200 ms period after touch down between steps into the hole (SIH) and steps on ground (SG). RetCx: *retractor coxae*, N=3, nSG=27, nSH=9; ProCx: *protractor coxae*, N=3, nSG=24, nSH=8; DepTr: *depressor trochanteris*, N=3, nSG=27, nSH=9; FlxTi: *flexor tibiae*, N=9, nSG=171, nSH=57; RetUng: *retractor unguis*, N=4, nSG=36, nSH=12. N=number of animals, n=number of steps.

**Figure 4:** Comparison of latency of *flexor tibiae* (FlxTi) activation and burst properties for the three species *Carausius morosus*, *Cuniculina impigra* and *Aretaon asperrimus* during steps on the ground and steps into a hole. A. latency of FlxTi activation. *Carausius morosus*: N=9, nSG=171, nSH=57; *Aretaon asperrimus*: N=4, nSG=63, nSH=21; *Cuniculina impigra*: N=5, nSG=75, nSH=25. N=number of animals B,C. Ratio of the maximal FlxTi burst amplitude (B) and total integral of rectified and smoothed FlxTi burst activity normalized to SG burst activity (C) during the 200 ms period after touch down between steps into the hole (SIH) and steps on ground (SG). *Carausius morosus*: N=9, nSG=171, nSH=57; *Aretaon asperrimus*: N=4, nSG=63, nSH=21; *Cuniculina impigra*: N=3, nSG=39, nSH=13. N=number of animals, n=number of steps.

**Figure 5:** Effects of ablation experiments. A. example of *Flexor tibiae* (FlxTi) activation during steps on ground (SG) and steps into the hole (SIH) in an intact animal. B. example of the FlxTi activation during SG and SIH C. Latencies of FlxTi activation after ablation of different sense organs during steps on the ground (SG). D. Scatter plot with the values of FlxTi activation latencies for comparison between SG and steps into a hole (SIH) after ablation of different sense organs; the median and IRQ of the SG values are shown in C and therefore omitted for the sake of clarity. E,F. Ratio of the maximal FlxTi burst amplitude (E) and total integral of rectified and smoothed FlxTi burst activity normalized to SG burst activity (F) during the 200
ms period after touch down between steps into the hole (SIH) and steps on ground (SG) after ablation of different sense organs. Control: N=9, n_{SG}=171, n_{SIH}=57; tibial campaniform sensilla, tiCS ablated: N=5, n_{SG}=90, n_{SIH}=30; femoral campaniform sensilla, feCS ablated: N=3, n_{SG}=27, n_{SIH}=9; femoral and trochanteral campaniform sensilla, feCS and trCS ablated: N=3, n_{SG}=54, n_{SIH}=18; trCS ablated: N=5, n_{SG}=45, n_{SIH}=15; retractor unguis, RetUng tendon cut: N=7, n_{SG}=93, n_{SIH}=31; tarsus, ti-, fe-, trCS ablated: N=5, n_{SG}=69, n_{SIH}=23. N=number of animals, n=number of steps. Solid vertical lines mark touchdown (TD) or virtual TD (vTD), dashed vertical lines mark begin of muscle activity in EMG trace, asterisks mark crosstalk from the extensor tibiae muscle, the number sign marks the time of the platform drop.
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