THE TREADING-ON-TARSUS REFLEX IN STICK INSECTS: PHASE-DEPENDENCE AND MODIFICATIONS OF THE MOTOR OUTPUT DURING WALKING

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Summary

In stick insects walking forwards or backwards, a treading-on-tarsus (TOT) reflex of the middle leg could be elicited by mechanical stimulation of the ipsilateral front leg tarsus. The probability of eliciting a TOT reflex in the middle leg depended on the phase of the stimulus within the middle leg's step cycle and on the position of the middle leg relative to its normal anterior and posterior extreme positions. During a TOT reflex the functional swing muscle (protractor coxae in forward walks and retractor coxae in backward walks) was inhibited while the functional stance muscle was activated. This suggests that the step generator of the middle leg contains two hierarchical levels: the higher level would determine the timing of swing and stance independent of walking direction; the lower one would drive the appropriate muscle groups for swing and stance depending on which programme – forward or backward walking – was running. The stimulus information from the front leg necessary to elicit a TOT reflex was observed only in the ipsilateral connective. In terms of the model, this information would have to be processed according to the actual state of the higher level of the step generator. Movement and position of the front leg and tarsal contact of the middle leg were not relevant for the TOT reflex of the middle leg. Steps with a TOT reflex were significantly prolonged, and compensation for this disturbance of walking coordination was made within the next step.

Introduction

The crucial influence of peripheral sense organs on the motor programme during walking in stick insects has been well demonstrated recently and gives some interesting insights into the generation of the motor pattern (see Bässler, 1983). One example is an intersegmental reflex that results in 'targeting movement' of a hind leg (Cruse, 1979). During the swing part of the step cycle – when the leg is moved forward above the ground – the hind leg aims for a close approach to the actual position of the ipsilateral middle leg. The sources of this reflex are numerous sense organs at the leg joints of the middle leg which affect the motor

output of the muscles moving the hind leg (Cruse *et al.* 1984; Dean, 1984). The functional advantage of this reflex is that the legs of the stick insect can expect to find support near the position at which the ipsilateral anterior leg stands. If the leg steps into space, where there is no support, the walk is at least slowed down and often stopped while the hind leg makes searching movements until it finds support. If the hind leg steps onto the tarsus of the middle leg, the hind leg often lifts off again and is replaced slightly posteriorly without an interruption of walking.

This treading-on-tarsus or TOT reflex was first found in decerebrated animals walking at speeds of less than $2\,steps\,s^{-1}$ (Graham, 1979). If, in faster walks, the hind leg steps onto the middle leg no TOT reflex is observed, because the middle leg is pulled out from under the hind leg tarsus. Graham showed that a TOT reflex in the hind leg can also be elicited by touching the middle leg tarsus with a fine brush. If the stimulus is applied in the early hind leg stance of the step cycle – when the leg is moved to the rear in contact with the ground – after the hind leg has been properly placed the leg is lifted off and replaced again as described above. If the tarsal stimulus on the middle leg is applied during the early swing of the hind leg this swing is prematurely terminated. These results show that the hind leg response strongly depends on the phase of the leg within the step cycle. Therefore, the sensory information from the middle leg has to be processed with state information from the motor programme of the hind leg.

Phase-dependent reflex modulations in locomotory systems are also well known in vertebrates. Investigations of the stumbling corrective reaction in the walking intact cat (Forssberg, 1979) and the walking spinal cat (Forssberg *et al.* 1977), and of reflexes evoked by cutaneous stimuli in other vertebrates (e.g. turtles, Lennard, 1985), have shown that intralimb and interlimb reflexes are gated or modulated at the spinal level by the currently active locomotor programme.

The intersegmental TOT reflex of the stick insect has been described in free-walking decerebrated animals only. The present investigation was designed to give a more detailed description of the phase-dependence of the TOT reflex, and of the resulting modifications of the motor output, in walking intact animals. Our study is focused on the front–middle leg pair of a tethered walking stick insect and shows that the reflex can reproducibly be elicited in preparations suitable for electrophysiology. This reflex might, therefore, be a valuable tool in investigations of processing of intersegmental information during a motor programme.

Materials and methods

All experiments were performed on female imagines of the stick insect, *Carausius morosus*, raised in laboratory culture. The insects were glued with their ventral side to a small holder so that head, antennae and all legs were freely movable. The animals were positioned above a treadwheel with the coxae about 8 mm above the surface of the wheel. The wheel consisted of a styrofoam disc, 410 mm in diameter and 19 mm thick, mounted on a ball-bearing. The weight of the wheel forced the animals to walk slowly. The static friction – measured at th

outer margin of the wheel – was about 10 Ncm and the moment of inertia was about $6.5\,kg\,cm^2$. Despite the large inertia and high friction the animals walked with a properly coordinated step pattern.

For the experiments with fixed front legs, the tibia of the right front leg was fixed with Plasticene to a rim of balsa wood placed beside the wheel. The tarsus was allowed to grip the rim. The stimulus at the front leg tarsus was applied with a fine brush, either by hand or with an electromechanical device (a loudspeaker with a brush attached to the membrane *via* a lever).

For nerve recordings the animal was opened by a dorsal cut along the midline from just behind the prothorax to the posterior edge of the metathoracic segment. Four small insect pins were inserted into the pleurae to spread the tergal halves. The gut was then removed to expose the mesothoracic muscles and the central nervous system in this region. Fatty tissue around lateral nerves nl2, nl3 and nl5 was carefully removed leaving the tracheal supply of the muscles and the ganglion intact. Activity of the motoneurones in the nerves was recorded with tungsten hook electrodes, using silicon grease as insulating material (Schmitz *et al.* 1988). The signals were conventionally filtered, amplified and stored on a four-channel magnetic tape recorder (Teac A3440). Normally only two nerve recordings were taken simultaneously and the on–off signal of the loudspeaker was stored to monitor the stimulus. To evaluate the data an electrostatic pen-recorder (Gould ES 1000) was used. The accuracy was normally ±10 ms but could be increased to ±1 ms if necessary. The movement of the loudspeaker–brush system lagged behind the electrical signal by 15 ms. This dead-time was taken into account in the evaluation.

In the experiments in which movement of the middle leg was monitored, an optoelectrical device was used, as described elsewhere (von Helversen & Elsner, 1977). In brief, a light beam was reflected by a small piece of metal foil (Scotch lite) glued to the distal femur of the middle leg and focused on a photosensitive diode (TSE PXY-10, TS Optoelectronic). After appropriate electronic compensation of scattered light, the electrical signal was FM-modulated and stored on tape. The position data and nerve recordings were evaluated using a high-resolution digital storage oscilloscope (Natic-System/Apple IIe) with the possibility of cursor read-out.

Results

Intact animals, fixed on a holder, were allowed to walk with all six legs on the high-inertia treadwheel, which forced the animals to walk at a rate of about $0.5-1\,step\,s^{-1}$. The tarsus of the right front leg was stimulated by hand with a fine brush. The stimulus was applied depending on the middle leg's movement as judged by eye. In most cases where the stimulus was applied in the early stance, the middle leg was then lifted off again and replaced a little behind the position it reached before. The lifting of the leg was performed by flexion of the coxa–tro-chanter joint and was often accompanied by a short extension followed by flexion

of the femur–tibia joint. The strength of this reflex varied within a walk and among individuals. Sometimes the middle leg moved back by as much as half the normal step amplitude; in other cases the TOT reflex was observed as a brief twitch of the leg which seemed to be replaced at the same position it reached before.

In some cases the stimulus to the front leg was applied just before the middle leg was set down. In these cases the downward movement of the middle leg was stopped and the middle leg was lifted again by flexion in the coxa–trochanter joint before it was set down. This additional movement of the leg prolonged the current swing of the middle leg. These experiments show that it was not necessary for the tarsus of the middle leg to have made contact with the surface. This means that the sense organs of the middle leg which measured contact with the substratum were not essential for this behaviour.

It was not necessary for the front leg to perform steps. In all cases in which the front leg was held in a prolonged stance (Wendler, 1964) by placing it on a holder beside the wheel, the TOT reflex of the middle leg could be elicited by stereotypically touching the front leg tarsus at appropriate times with a brush moved by an electromechanical device. The reflex was also independent of front leg position. The TOT reflex could be elicited from various positions, even those corresponding to the anterior extreme position (AEP) of the front leg in normal steps.

The relevant information from the stimulated front leg to the reacting middle leg flowed only within the ipsilateral connective: after cutting one connective between the pro- and mesothoracic ganglia the TOT reflex on the operated side was abolished in all cases, whereas the contralateral leg pair on the intact side reacted as usual.

Phase-dependence of the TOT reflex

The normal activity of motoneurones in the nl2 nerve to the protractor coxae muscle of the middle leg in a walking animal with a fixed front leg is shown in the first part of Fig. 1. The main activity of the neurones with the larger spikes was concentrated in a burst with a rather sharp onset at about the beginning of the swing. This burst outlasted the swing and reached into the early stance and sometimes halfway through the latter, as previously described (Graham & Wendler, 1981). The motoneurones with the smaller spikes were often active throughout the whole step with increasing activity starting near the end of the stance. Although the animals were prepared for electrophysiological recordings, they walked in a properly coordinated way with swing durations of about 400 ms and step durations between 1200 and 1800 ms.

· To investigate the phase-dependence of the TOT reflex, the moment of stimulation of the front leg tarsus was determined as a fraction of the step cycle in the middle leg. Because changes in the duration of the period in the ongoing step were expected, owing to stimulation, the phase of the stimulus was calculated within the step cycle of the last undisturbed step immediately before the stimulus was applied. Therefore, the time elapsed from the onset of the nl2 burst to the
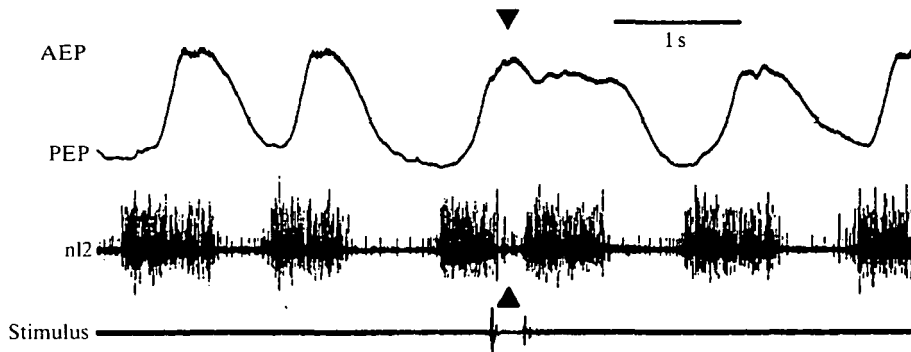
Fig. 1. Motor pattern in mesothoracic nerve nl2 during tethered forward walking. The movement of the right middle leg is monitored in the upper trace. AEP and PEP are anterior and posterior extreme positions, respectively. Upward deflection implies forward movement (swing) of the leg. The middle trace shows activity of the protractor coxae motoneurones. In the lower trace the on–off signal of the stimulus device is shown.
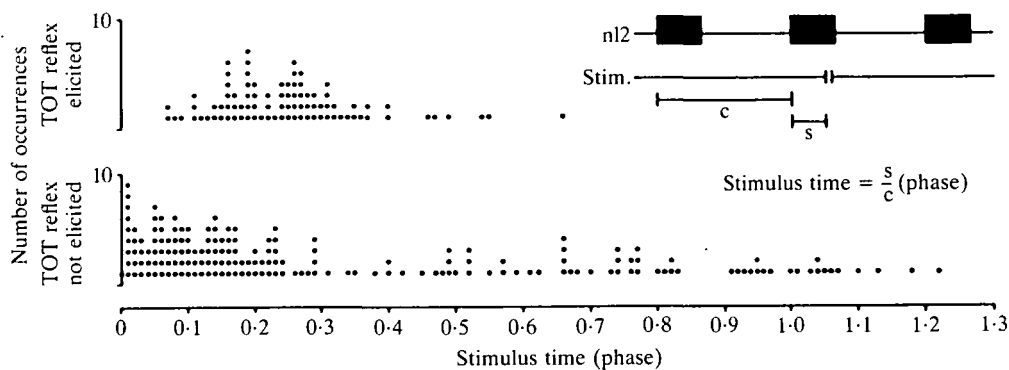

Fig. 2. Dependence of the TOT reflex on step phase. The abscissa shows the phase of a stimulus to the front leg tarsus in the step cycle of the middle leg calculated as shown in the inset. The upper band of points represents stimuli eliciting a TOT reflex. The lower band of points represents stimuli which were not successful. Number of animals, 11; number of stimuli, 265.

stimulus was measured and divided by the time between the last two onsets of nl2 bursts (see inset of Fig. 2). A stimulus was classified as successful if it led to a visible TOT-reflex movement of the middle leg as described above. The results are shown in Fig. 2: each dot represents a stimulus and is shown on the abscissa according to its phase. The resolution of 0·01 corresponds to 10 ms in a 1000 ms step. A few phase values greater than 1 were observed from steps which, at the time of the stimulus, were somewhat longer than their previous ones. The ordinate ▶ divided to give the frequency of occurrence of unsuccessful (lower band of
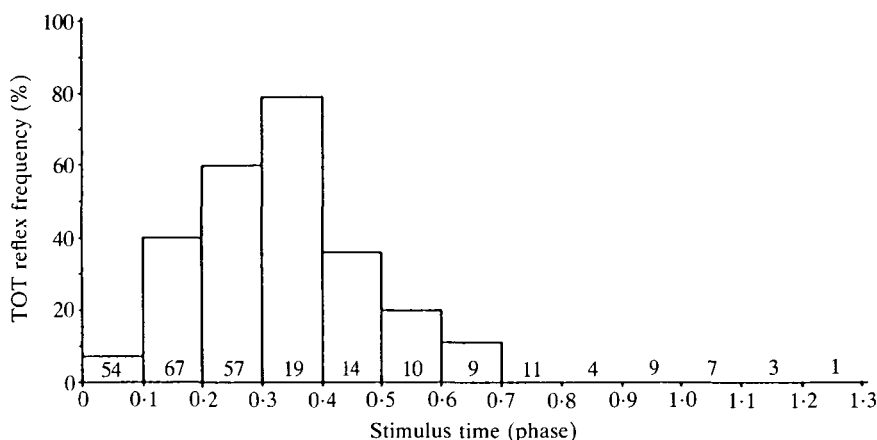
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Fig. 3. Probability of eliciting a TOT reflex against step phase. The probabilities are calculated within classes of 0·1 from the points shown in Fig. 2. The total number of stimuli per class is given in each column. Same abscissa as in Fig. 2.

points) and successful (upper band of points) stimuli for each class according to the criterion stated above.

265 stimulus responses obtained from 11 animals were evaluated quantitatively. An attempt was made to collect data randomly distributed over the step period. Nevertheless, most of the data were grouped between phases 0 and 0·5. Fewer data are shown for other phase values because stimuli within the range 0·5–1·0 very often led to a cessation of walking. Although these stimuli never elicited a TOT reflex, only continuous walks were evaluated to investigate effects on the timing of the step. Also not shown in Fig. 2 are data from nine other animals in which efforts were made to elicit TOT reflexes and where only the successful stimuli were evaluated. The 101 successful stimuli in the latter series were also exclusively grouped in the range between 0·1 and 0·6.

Fig. 2 shows that during the very early swing (0·0–0·07) and during the second half of the stance (above 0·7) no TOT reflex was elicited. Between 0·07 and 0·7 it was possible to elicit TOT reflexes with different probabilities depending on phase. This is shown more clearly in Fig. 3 where the probability, rather than the absolute occurrence, is shown in phase classes of 0·1. The probability reached a maximum of about 80 % between 0·3 and 0·4. This phase interval corresponds approximately to the time of the transition from swing to stance in the walks investigated here.

Although the position of a leg varies with the phase in the step cycle, one cannot take position information from Figs 2 and 3 because the absolute position – i.e. AEP and posterior extreme position (PEP) – varied considerably among the animals. Therefore, another evaluation was made using the signal of the position diode. Fig. 4 shows the probability of eliciting a TOT reflex for varying leg positions, normalized for each animal to the mean AEP of the undisturbed steps immediately before stimuli were applied. As can be seen, the probability of a TO
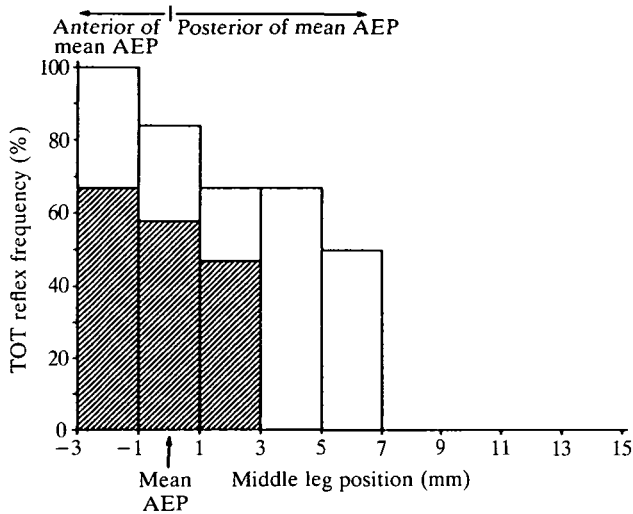
Fig. 4. Probability of eliciting a TOT reflex against middle-leg position. The horizontal axis shows the position of the middle leg at the moment of stimulation of the front-leg tarsus. The axis reads in millimetres from the mean AEP (which corresponds to zero) to which the actual position of the leg was normalized. The open columns show the probabilities of eliciting TOT reflexes during the swing, whereas the hatched columns give the probabilities of eliciting TOT reflexes during the stance of the middle leg.

reflex increased towards the mean AEP position and was maximal for steps in which, at the time of the stimulus, the position of the leg was more anterior than the mean AEP of this animal. However, during the swing of the step (open columns) the probability for a given position was always higher than during the stance (cross-hatched columns). This shows that, in addition to the actual position of the middle leg, the state of this leg – swing or stance – played an important role in the initiation of the TOT reflex.

Modifications of the motor output

As stated above, the normal motor pattern to the main leg muscles producing swing (protractor coxae *via* nerve nl2) and stance (retractor coxae *via* nerve nl5) was an alternating rhythmical activity. During a step with a TOT reflex this regular pattern was modified (Figs 1, 5, 9B). In all cases in which a visible TOT reflex movement was observed there was a distinct pause in the burst activity of the nl2 motoneurones after a certain latency. This pause affected at least the large and medium-sized spikes, although very often the motoneurones with the smaller spikes were also inhibited. In 14 animals the latency from the time of stimulation to the start of the pause was evaluated quantitatively (Fig. 6A). The mean latency was 68 ± 22 ms ($\bar{x} \pm$ s.d., $N = 147$). The pause duration was on average 213 ± 69 ms and, in spite of considerable variation, significantly correlated with the moment of stimulation ($r = 0.35$, $P < 0.01$, $N = 138$). This means that the later the
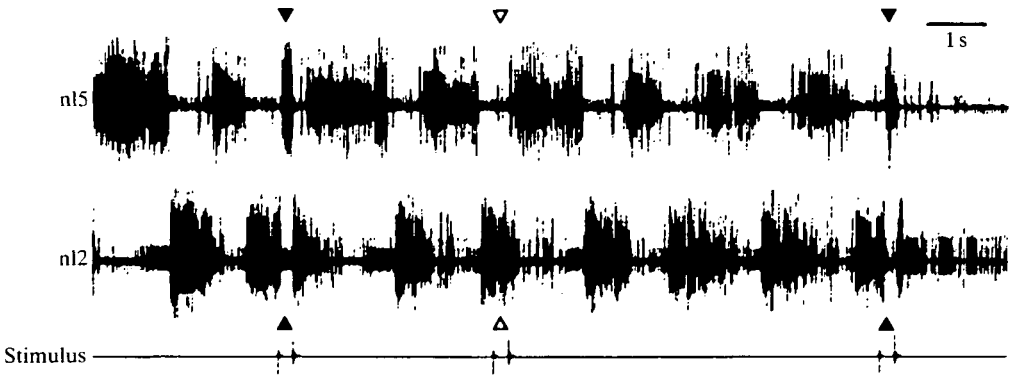
Fig. 5. Recordings of nerves nl5 and nl2 during tethered forward walking. Nerve recordings from motoneurones to the retractor coxae (upper trace) and protractor coxae (middle trace) of a right middle leg showing alternating activities in undisturbed steps. The lower trace shows the on–off signals of the stimulus device. In the first and third trial the stimulus was properly timed and each elicited a TOT reflex (filled arrowheads), the second stimulus was applied too early in the swing and did not result in a TOT reflex (open arrowheads).
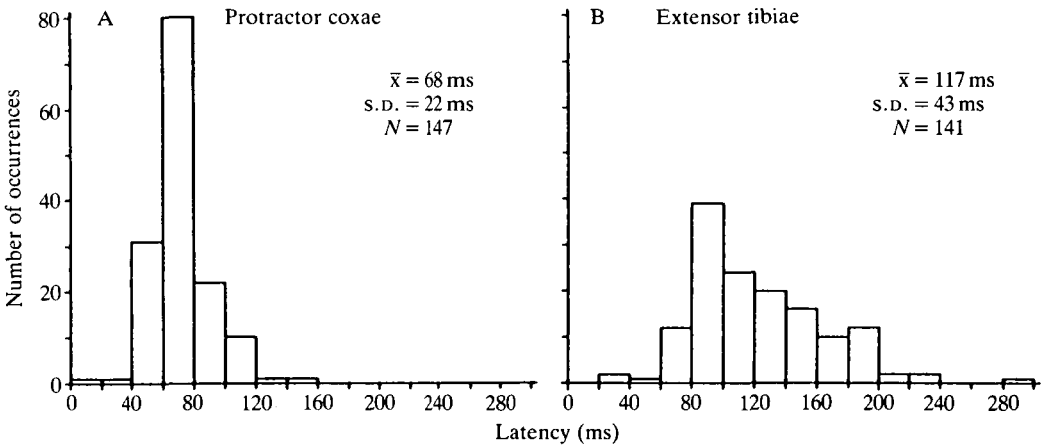

Fig. 6. Latencies to activity changes in nerves nl2 and nl3. (A) Histogram of the latency from the moment of the stimulus at the front leg tarsus to the beginning of the pause in the protractor activity of the middle leg. (B) Histogram of the latency for the additional activation of the FETi motoneurone. The bin width is 20 ms.

stimulus was applied after the onset of the nl2 burst the longer was the pause duration.

The modification of the motor pattern in the motoneurones of the retractor muscle was evaluated only qualitatively. During the TOT reflex there was a distinct activation of motoneurones which were normally not active in this phase of a step (Figs 5, 9B). This activation could only be seen in those cases in which the
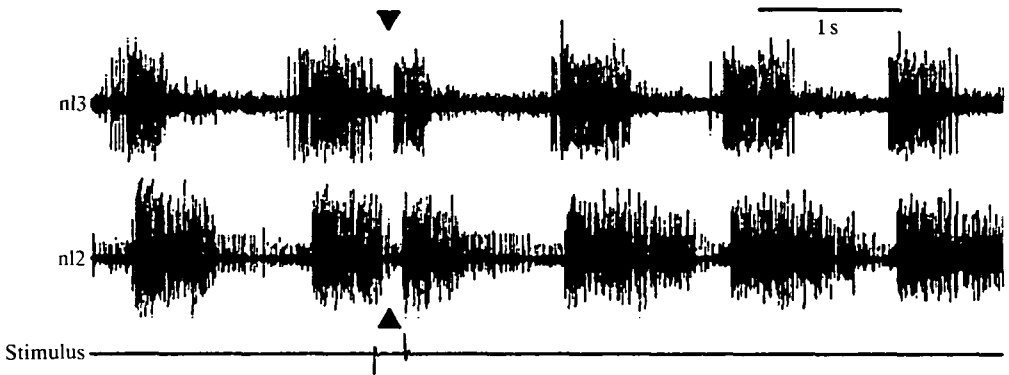
Fig. 7. Recordings of nerves nl3 and nl2 during tethered forward walking. The regular motor pattern in both nerves is typically disturbed during a TOT reflex (filled arrowheads). The spikes with the large amplitude in nerve nl3 (upper trace) belong to the FETi motoneurone.

stimulus also led to a pause in the nl2 activity. The additional activity appeared as a short burst of spikes with large amplitudes. The latency for this activation was some 10 ms longer than that for the nl2 inhibition, and the duration of the additional nl5 burst was somewhat shorter than the pause in the nl2 moto-neurones.

The fast extensor tibiae (FETi) motoneurone in nerve nl3 showed one burst of activity in an undisturbed forward step, starting within the last 10 % of the stance and ending at approximately the middle of the swing (Fig. 7). After a stimulus which elicited a TOT reflex, the FETi clearly showed an additional burst of activity with a mean duration of 169 ± 60 ms ($N = 141$). The latency for its activation was somewhat longer and more variable than that for the nl2 inhibition (Fig. 6B). The slow extensor tibiae (SETi) motoneurone showed a distribution of activity similar to that of the FETi, but possessed some continuous activity throughout the whole step cycle, increasing towards the end of the stance. In steps with a TOT reflex, the SETi was also reflexly activated in parallel with the FETi. However, the smaller spikes of the SETi were often masked by the larger FETi spikes, so quantitative evaluation was difficult.

Influence on timing/coordination

The animals often stopped walking when stimulated during the stance of the middle leg, especially when the stimulus occurred during the second half of the stance, as stated above. If the animals continued walking after a stimulus without eliciting a TOT reflex, the only observed effect was that the step was prolonged for about 100 ms (Wilcoxon-test, $0 \cdot 01 < P < 0 \cdot 05$, $N = 177$).

Steps in which a TOT reflex occurred were significantly longer than the undisturbed steps immediately before ($P < 0 \cdot 01$, $N = 138$). This significant pro-longation of about 410 ms was more than twice the mean pause in the nl2 activity

or the duration of the additional FETi and nl5 bursts. The duration of the next step immediately after the step with a TOT reflex showed no changes compared with the last step before the TOT reflex ($P > 0 \cdot 1$, $N = 100$).

Backward walking

Pulling gently on the antennae caused the animals to walk backwards for several steps. During the stance, the leg was moved from rear to front and *vice versa* for the swing. The functional swing muscle was now the retractor coxae and the functional stance muscle was the protractor coxae. The steps were not as regular as in forward-walking animals and the legs often grasped the wheel a second time (Graham & Epstein, 1985).

In backward-walking animals it was also possible to elicit TOT reflexes in the middle leg by stimulation of the front leg tarsus. As described above for the forward-walking animal, a successful stimulus led to an additional lifting and replacing of the leg. The lifting was produced by flexion in the coxa–trochanter joint, and was often accompanied by a short extension followed by flexion in the femur–tibia joint. The highest probability of eliciting TOT reflexes occurred when stimulating the front leg tarsus at about the time of the transition from swing to stance of the middle leg, as in forward-walking animals. However, in backward walking the middle leg was now near its posterior extreme position, far away from the front leg. In this case the TOT reflex had no obvious functional relevance. It was not possible to elicit TOT reflexes in the middle leg by stimulation of the hind leg tarsus.

The motor patterns of the retractor and protractor coxae motoneurones in backward-walking animals (Fig. 8A) were not as regular as during forward walks, which makes quantitative evaluation difficult. In addition, to ensure that the grasping described above was not wrongly interpreted as a TOT reflex, only 37 sequences from six animals were evaluated in which the animals showed relatively regular backward walks. In all cases in which a visible TOT-reflex movement was elicited, there was a reduction in the activity of retractor motoneurones and an activation of protractor motoneurones (Fig. 8). The latencies to these changes in activity were similar to those measured in forward-walking animals. This shows that the reflex affected the functional swing and stance muscles in qualitatively the same manner as in forward walking. This is even more obvious in averaged post-stimulus-time histograms showing stimulus responses of the same animal for forward (Fig. 9A,B) and backward walking (Fig. 9C,D).

Activity in the ipsilateral connective during standing and walking

The ipsilateral connective between pro- and mesothoracic ganglia was found to carry the information necessary to elicit a TOT reflex in the middle leg. Recordings from this connective in standing animals showed an increased activity in interneurones (Fig. 10) 40–50 ms after a stimulus at the front leg tarsus. Several interneurones which were rarely active in the undisturbed standing animal were activated after stimulation. However, in most cases the activities of the meso
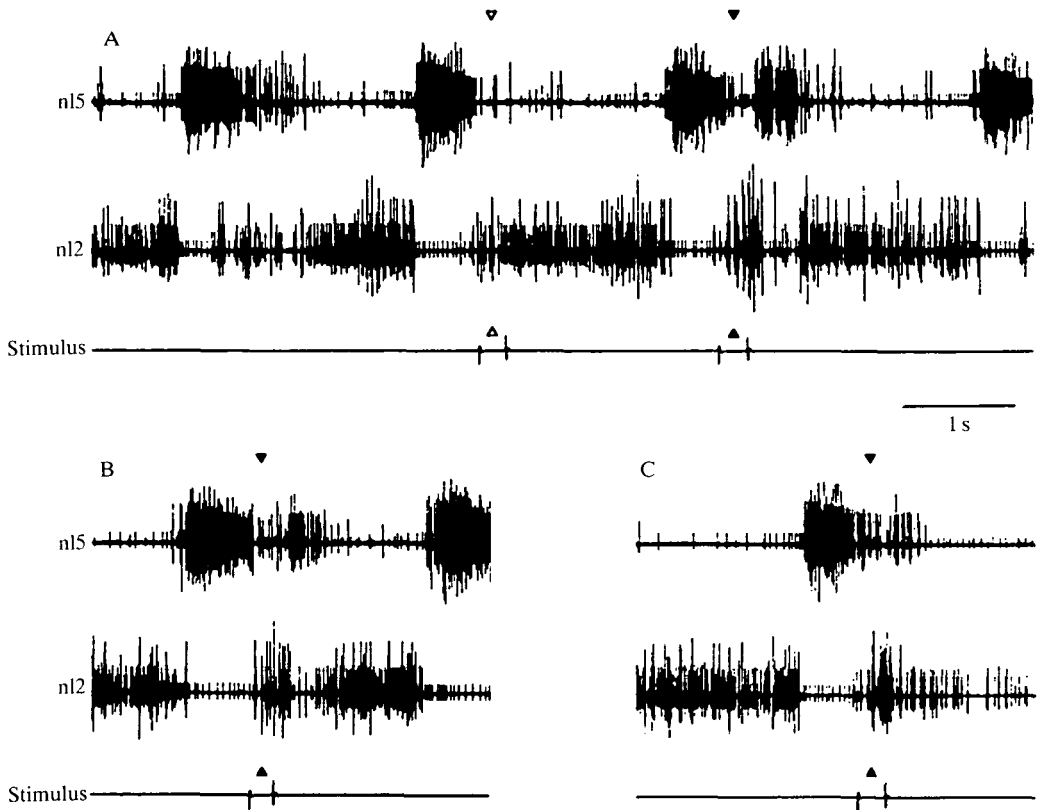
Fig. 8. Recordings of nerves nl5 and nl2 during tethered backward walking. The first stimulus in A was not successful because it was applied too late within the stance of the middle leg (open arrowheads). The second stimulus in A was properly timed and elicited a TOT reflex (filled arrowheads) with a reduction of retractor activity and an increase in protractor activity. This is also shown for two further examples in B and C.

thoracic protractor and retractor motoneurones were not obviously affected by stimulation of the front leg tarsus. In the few cases in which effects could be observed, the responses of the motoneurones varied, i.e. the motoneurones could show increased or decreased activity.

Recordings from the connective in walking animals showed activity in so many interneurones that it was not possible to evaluate single interneurones. However, averaging the activity of all interneurones with larger spike amplitudes that were triggered by the stimulus showed an increased activity after 40–50 ms (Fig. 11). To prevent artificial summation of the modulated activity during the step cycles, in this case the stimuli were applied with an even distribution within the middle leg's step cycle. The histogram includes some stimuli which did not lead to a TOT reflex. The latency to peak activation shown in this histogram is the same as in histograms derived only from stimuli which elicited TOT reflexes.
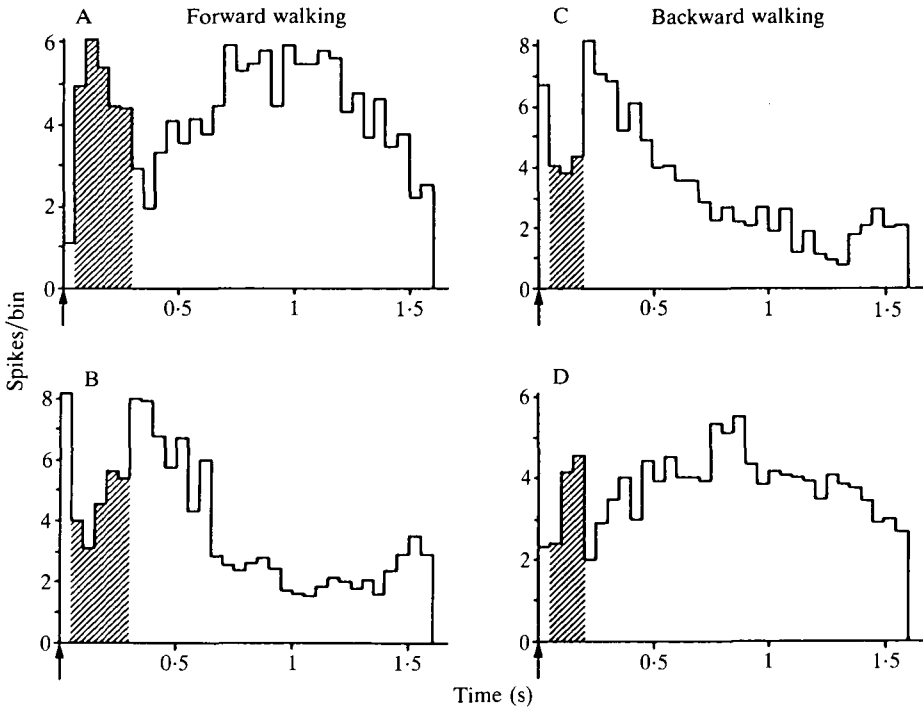
Fig. 9. Post-stimulus-time histograms of the activities of retractor (A,C) and protractor (B,D) coxae motoneurones during TOT reflexes in forward- (A,B) and backward-walking animals (C,D). The activities of the motoneurones were averaged after being triggered at the onset of stimulation of the front leg tarsus (arrows). All stimuli were applied at about the transition from swing to stance. For one animal 13 TOT reflexes were averaged during forward walks and 12 TOT reflexes during backward walks. Bin width is 50 ms. The decrease in the motoneurones' activities to the functional swing muscle, protractor in forward walks (B) and retractor in backward walks (C), started for both situations within 50–100 ms after stimulation. The duration of decreased activity is represented by the cross-hatched classes for each situation. Within this time interval the functional stance muscle, retractor in forward (A) and protractor in backward walks (D), is activated. After the interval of decreased activity in the protractor motoneurones in forward walks (B) there is an additional increase in activity which is also true for the retractor motoneurones in backward walks (C). Because the stimuli were applied at about the end of the swing, the appropriate motoneurones' activities of the functional swing muscles (B and C, respectively) can only be seen in the first 50 ms class. The following stance is characterized by the slow decrease of the activities in the motoneurones of the functional swing muscle (B and C, respectively) and the increase in the motoneurones' activities of the functional stance muscle (A and D, respectively).

Discussion

The TOT reflex between adjacent ipsilateral leg pairs was first described by Graham (1979) in decerebrated free-walking animals. Our results show that this reflex also occurs in tethered walking intact animals, permitting electrophysiolog

Fig. 10. Recording from the right connective of a standing animal between the pro- and mesothoracic ganglia. Beside many small units in the standing animal, middle- and large-sized units are also active. After a stimulus to the right front leg tarsus (lower trace), both small units and large units, which are normally rarely active, show an increased activity in the ipsilateral connective.
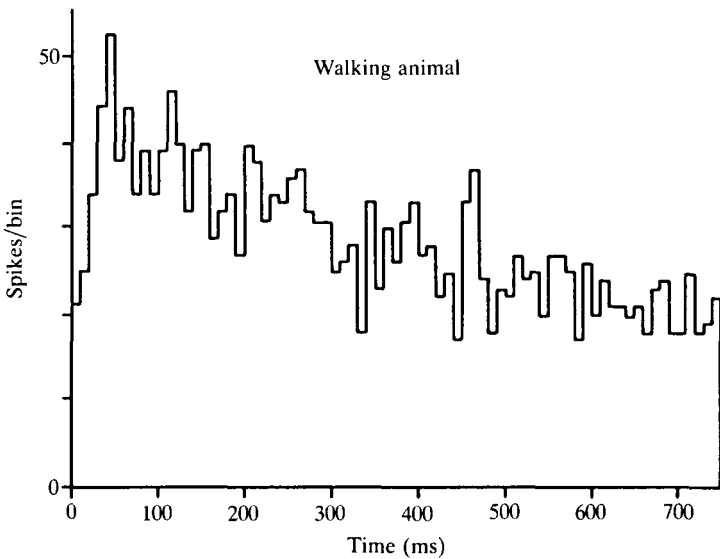
Fig. 11. Post-stimulus-time histogram for all large units in the right connective between the pro- and mesothorax. The activities of all large units were averaged after being triggered at the onset of stimulation of the front leg tarsus ($N = 20$). The moment of stimulation was evenly distributed within the step cycle of the middle leg to avoid artificial summation of increased activity at given phases. The maximum of increased activity is reached between 40 and 50 ms after the stimulus. Bin width is 10 ms.

cal investigation. This study was performed on the TOT reflex between front and middle legs. The fixed-front-leg experiment shows that the front leg does not necessarily have to perform walking movements. This finding facilitated a detailed examination of phase-dependence. The experiments with backward-walking

animals show that the control mechanism is independent of the walking direction and that the information from the front leg is processed with information on the state (swing or stance) of the middle leg.

This processing probably occurs in the mesothoracic ganglion. The fixed-front-leg experiment shows that position information from the front leg is not taken into account for triggering the TOT reflex. However, the experiment does not imply that the front leg is in a standing mode, but rather that it is in a so-called 'prolonged stance phase' (Wendler, 1964; Bässler, 1979). In the latter case it has been shown by Cruse & Saxler (1980) that a 'standing leg of a walking stick insect' produces force oscillations in the same rhythm as the walking legs. This observation, as well as investigations of feedback loops (Schmitz, 1985), indicates that the fixed front leg is in a walking mode. So, in principle it might be possible that the TOT reflex is controlled within the front leg's ganglion.

However, it is proposed that the mesothoracic ganglion controls the expression of the middle leg's TOT reflex for the following reasons. (1) Bässler et al. (1985) showed that the step generators – the neuronal systems which produce the motor output to perform a step – are situated within the ganglion of each leg. Such a step generator is not necessarily restricted to a cluster of central interneurones but might include the periphery and even motoneurones. (2) A comparison of the recordings from connectives in standing and walking animals showed no principle differences. This suggests that the information flow along the connective is independent of the animal's mode (standing or walking). However, the TOT reflex is only effective in the walking mode, whereas in the standing mode the animal shows another reflex, the compensatory leg-placing reflex (Chr. Walter, cited in Bässler, 1983; Fricke & Schmitz, 1988). (3) The experiments with backward-walking animals show that the reflex depends on the state (swing or stance) of the middle leg.

The latter finding strongly indicates that the step generator contains two levels. One level, higher in hierarchy, determines the timing of swing and stance. The other one steers the appropriate muscle groups for swing and stance depending on which programme – forward or backward walking – is running. This is supported by results of Bässler et al. (1985) and Cruse (1985).

The TOT reflex in either walking direction produces an additional activation of the extensor tibiae motoneurones and (as indicated by the leg movement) an activation of the levator trochanteris motoneurones, while the retractor unguis motoneurones are apparently inhibited. The motoneurones of the 'swing muscle' are inhibited and those of the 'stance muscle' are excited. The swing muscle is the protractor coxae and the stance muscle is the retractor coxae for forward walking, and vice versa for backward walking. This shows that the stimulus information from the front leg is gated by the actual state of the higher level of the step generator which determines swing and stance.

Three different hypotheses, which might explain the dependence of the TOT reflex within the step cycle, are examined in the present paper. First, one could propose an absolute time window within the step cycle. Second, one could assume

that it is the relative time (phase) rather than the absolute time. The third possibility is that the effect depends on position within the middle leg's movement.

The first hypothesis can be rejected, because plotting the occurrences of a TOT reflex against absolute time of the stimulus within a step cycle shows larger scattering than that shown in Fig. 2, where the occurrence is shown over relative time (phase). The results show that the control depends on more than one parameter. The position of the middle leg relative to its transition points (AEP or PEP) certainly plays an important role, as Fig. 4 shows. Beside the parameter 'position', however, the state of the leg within the step (swing or stance) is also important because the probability for a given position is higher during the swing than during the stance.

The sense organs in the middle leg measuring tarsal contact or load on the leg must play a minor role, as the TOT reflex could be elicited before termination of the leg's swing. This seems to be sensible because the TOT reflex might be designed not only to react to stepping onto the front leg tarsus but also to prevent the middle leg landing too near to a disturbed front leg. The same effect is shown by the premature termination of the swing after stimulation of the adjacent anterior leg tarsus during the very early swing of the hind leg (Graham, 1979). This effect was not observed for the front–middle leg pair in the present study. The reason may be that premature swing terminations only occur in decerebrated animals or that it is a special feature of the middle–hind leg pair.

On the basis of the current data it is only possible to speculate upon features of the neuronal mechanism. In the forward-walking animal the most constant parameter is the latency of the beginning of the protractor motoneurones' inhibition. This inhibition is also the earliest effect in the motoneurones recorded in the present study. Although no levator trochanteris motoneurones were recorded, the data measured by Graham (1979) allow an estimation of the levator activation. The fastest effect in the leg movement (levation of the leg) was observed after 105 ± 20 ms. If one takes into account a delay of some 10 ms for the electromechanical transduction within the muscle, the activation of the levator motoneurones is not faster than the inhibition of the protractor motoneurones. The latter seems to be the fastest and most invariant parameter and may possibly be performed by only one interneurone which is controlled by the leg's position and state as discussed above. It seems unlikely that intersegmental interneurones directly inhibit the 'swing motoneurones', because the latter are different for forward and backward walks. In addition, some effects should be expected in the experiments with standing animals, but no clear effect was observed. The other effects (i.e. extensor and retractor activation, pause duration, amplitude of the movement etc.) are much more variable and depend on currently undefined parameters.

It is shown here that steps with a TOT reflex are significantly longer than the step immediately before. This is probably not a direct effect of the TOT reflex, but results from coordinating influences from the other legs which play an important role in the timing of the steps. The parameters of the following step are not

significantly changed compared with undisturbed steps, as expected from experiments with other disturbed walks in the stick insect (Cruse & Schwarze, 1988).

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