

IS THE POSITION OF THE FEMUR-TIBIA JOINT UNDER FEEDBACK CONTROL IN THE WALKING STICK INSECT?

II. ELECTROPHYSIOLOGICAL RECORDINGS

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SUMMARY

From earlier force measurements it was assumed that the system controlling the position of the femur-tibia joint in the middle leg of the stick insect is active in walking animals. This hypothesis is confirmed by electrophysiological recordings and is discussed in relation to several earlier findings concerning the function of the control system of the femur-tibia joint.

INTRODUCTION

The leg of the stick insect, *Carausius morosus*, possesses a negative-feedback mechanism that controls the position of the femur-tibia joint. This system is known to function in the absence of active leg movements (Bässler, 1965, 1967, 1972*a, b*; Bässler, Cruse & Pflüger, 1974; Bässler & Storrer, 1980; Godden, 1974; Storrer & Cruse, 1977; Cruse & Storrer, 1977). Force measurements in free-walking animals indicate that the feedback system is also active during the stance phase of the walking middle leg (Cruse, 1981). However, it is possible that the measured reaction could be an active reaction of the feedback mechanism or, alternatively, a passive reaction produced by elastic properties of the muscles, possibly in combination with another reflex acting on a different joint. To resolve this question the present experiments were performed by recording the electrical activity of the muscles concerned.

METHODS

The animal was allowed to walk over a horizontal path, in which a small piece of the margin was cut away. This small platform could be moved by hand (see Cruse, 1981, for details). If by chance the walking animal touched this platform with the middle leg a flexion (F) or extension (E) movement could be superimposed on the normal movement of the femur-tibia joint (Fig. 1). Recordings were made from the flexor tibia muscle (electromyograms, EMG) and from the extensor tibiae nerve (nerve F2) as described elsewhere by (Pflüger, 1977). The flexor myograms, the extensor nerve

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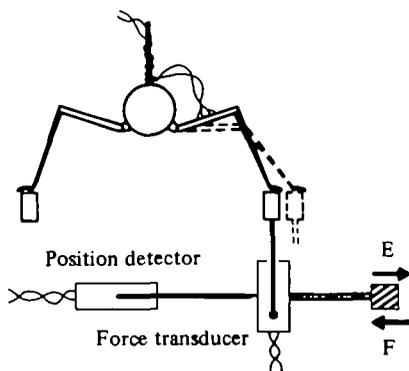


Fig. 1. Schematic cross-section through animal and experimental device when a middle leg is standing on the platform.

potentials and the position of the platform (measured by a Hellige position transducer WL 150) were recorded on FM tape (Philips EL 1020), later filmed by an oscilloscope camera (Recordine) and evaluated by hand.

In our recordings the potentials of the fast extensor tibiae motoneurone (FETi), with a spike amplitude of about 1 mV, and of the slow extensor tibiae motoneurone (SETi), with a spike amplitude of about 300 μ V, could be clearly identified (see Pflüger, 1977; Bässler & Storrer, 1980). Potentials of the inhibitory motoneurone could also be seen. However, these were not seen in all recordings and, as these small potentials could possibly have been mistaken for sensory units, they are not taken into account. In stick insects the extensor nerve contains both motor and sensory fibres (Bässler, 1977*b*). The different units exciting the flexor muscle (at least six, Debrodt, 1980) could not be identified from the EMG. However, from the amplitudes of the different muscle potentials they were divided into three classes of increasing amplitude: FITi (1), FITi (2) and FITi (3). These three classes correspond to amplitudes of < 250 μ V (minimum amplitude 50 μ V) in FITi (1), 250–600 μ V (FITi (2)) and > 600 μ V (FITi (3)). (For further details see Cruse & Schmitz (1981).)

In control experiments the receptor apodeme of the femoral chordotonal organ was cut as previously described (e.g. Bässler, 1965).

RESULTS

The standing animal

Force measurements showed that the reflex response of a standing animal differs from that of a walking one. At first, therefore, experiments were performed with standing animals. The middle leg of the free animal was placed on the movable platform. The platform was then moved, stepwise, outwards or inwards to extend or flex the femur–tibia joint. It is known (Bässler, 1972*b*) that there are large differences in the response of individual animals when the 'state of excitation' is changed in different experiments. These differences are correlated to some extent with the frequency of the SETi motoneurone before stimulation (Cruse & Schmitz, 1981). Therefore, only experiments with values in a defined range were considered (5–4

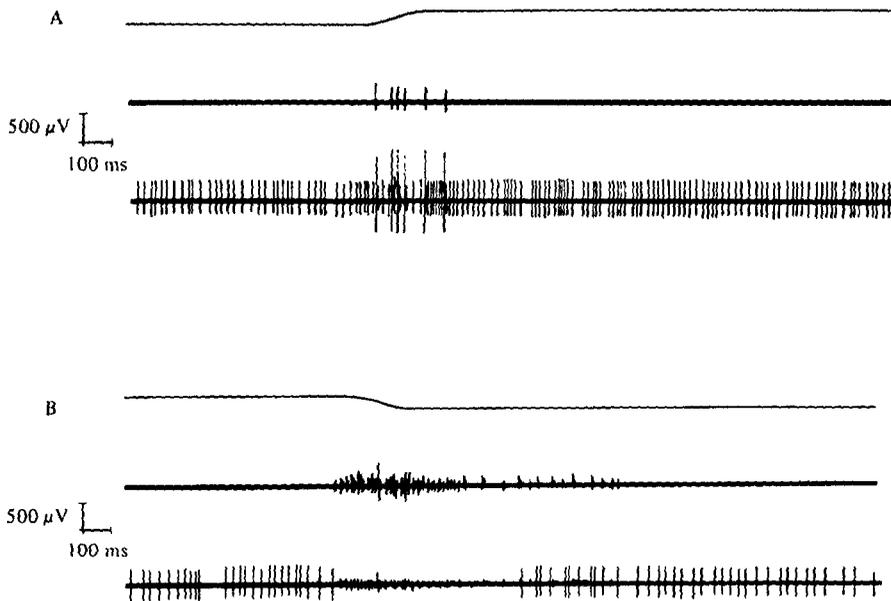


Fig. 2. Standing animal. Two individual responses to flexion (*a*) and extension (*b*). The upper trace shows the position of the platform. The second trace shows the recording from the flexor muscle. The lower trace shows the recording from the extensor nerve. Cross-talk is produced by muscle potentials of the flexor and by those muscle potentials of the extensor elicited by the FETi motoneurone. These are drawings from film prints which because of inadequate fixing showed a too low contrast to print from directly.

spikes s^{-1} for flexion experiments and 30–50 spikes s^{-1} for extension experiments). Two individual results are shown in Fig. 2. The mean values of the responses to 16 flexions are shown in Fig. 3(*a*). The values are expressed as spikes s^{-1} calculated from the number of spikes measured during intervals of 100 ms, 500 ms or during the duration of the stimulus ramp. As the ramp duration was variable, it is symbolized by a 200 ms interval (marked by striped columns). The exact ramp duration is given in the legends. The half time of the falling phase of the SETi activity is several seconds and of the FETi motoneurone is < 100 ms.

The frequency values of the low-amplitude spikes (measured during the time when the spikes with higher amplitudes occur) might be higher than shown, because these spikes might mask the presence of smaller ones.

Fig. 3(*b*) shows the mean values of responses to 17 extensions of the femur-tibia joint of a standing animal. No FETi potentials were observed in these experiments. The half time of the recovery of the SETi frequency is about 1 s and of the falling phase of the flexor group FITi (1) about 500 ms, of the group FITi (2) about 200 ms and of the group FITi (3) < 100 ms. The frequency of the SETi motoneurone before the stimulus is different in flexion and extension experiments (Fig. 3). This is due to the influence of the previous stimulus which had the opposite direction.

Cutting the receptor apodeme of the chordotonal organ reveals that part of the reaction which is not due to stimulation of the chordotonal organ (Fig. 4). After

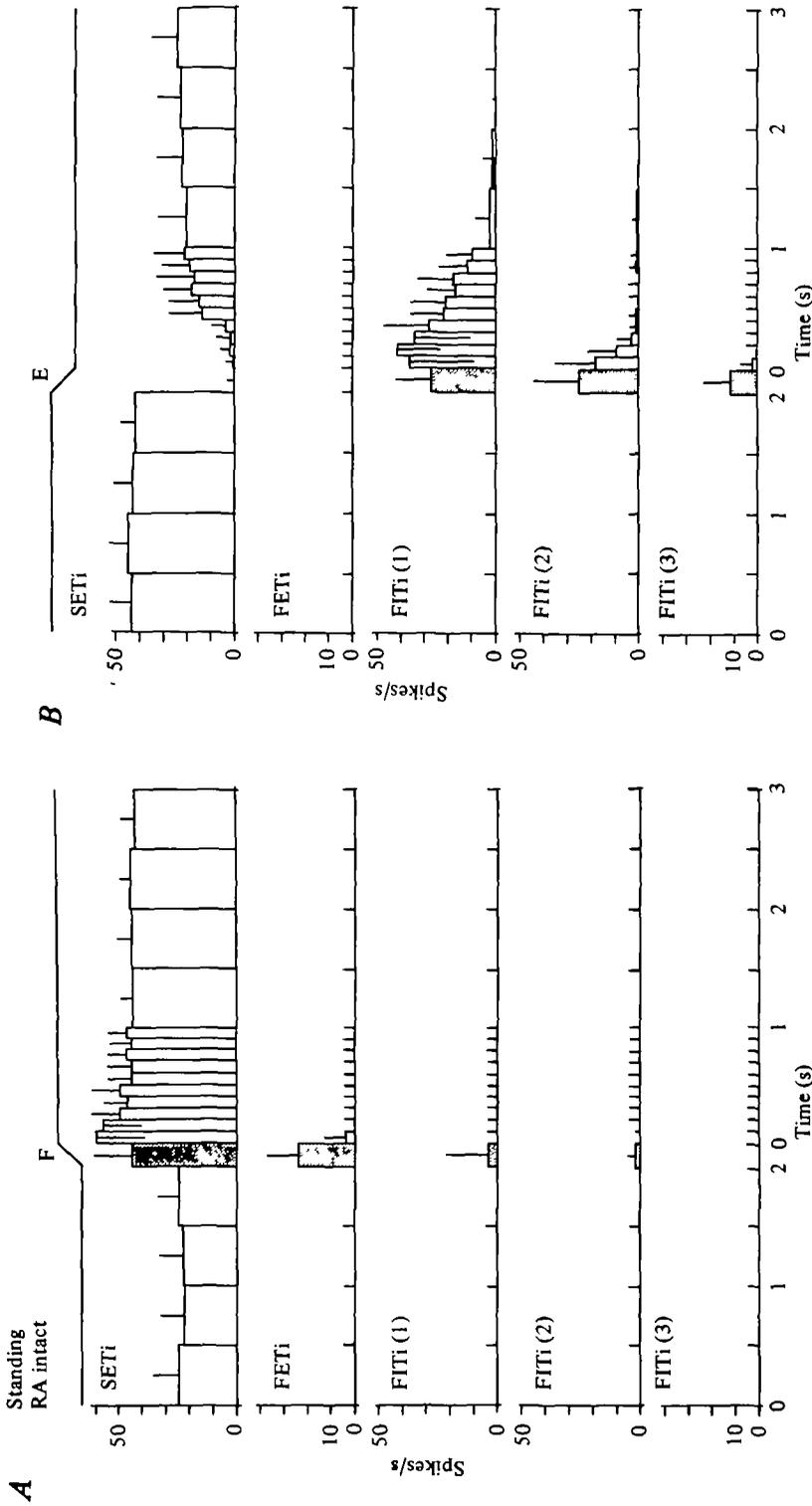


Fig. 3. Standing animal. Mean values of responses to flexion (A) and extension (B) of the femur-tibia joint. The upper trace shows the position of the platform of the force transducer. Mean value of the duration of the ramps are 247 ms for flexion and 267 ms for extension. SETi: slow extensor tibiae motoneuron. FETi: fast extensor tibiae motoneuron. FITi (1-3): three classes of flexor tibiae: muscle potentials. The bars show the values of the standard deviation. To make the figure as clear as possible they are drawn in one direction only. For further explanations see Fig. 1 and the text.

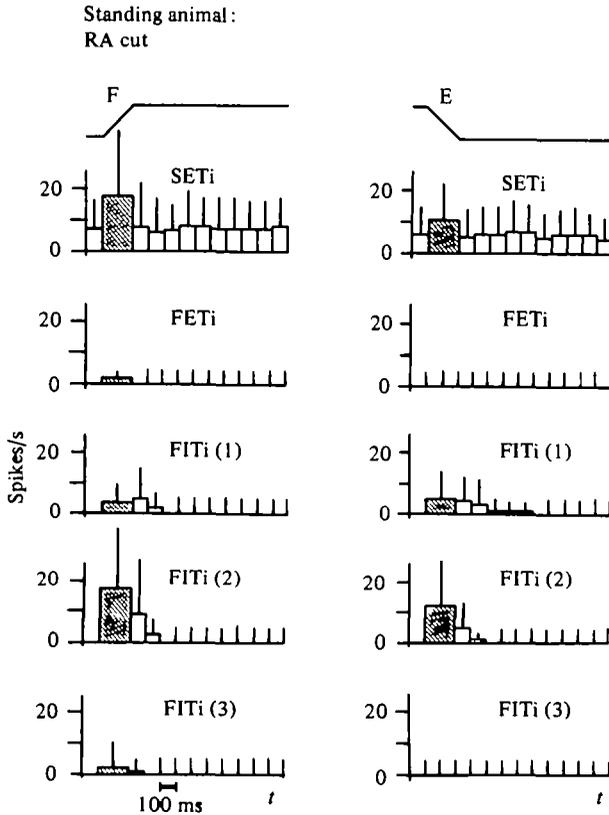


Fig. 4. Standing animal with cut receptor apodeme. Mean values of responses to flexion (F) and extension (E) for 32 flexions and 31 extensions (each from two animals). Mean values of the ramp durations are 252 ms for flexion and 239 ms for extension. For further explanations see Fig. 3 and the text.

cutting the receptor apodeme of the femoral chordotonal organ the responses to flexion and extension are similar. There is no directed reaction, but only a general excitation of both flexor tibiae and extensor tibiae motoneurons, due to the stimulation of other sense organs on the leg (see Cruse & Schmitz, 1981). In addition the spontaneous frequency of the SETi motoneurone is reduced when the receptor apodeme is cut.

The walking animal

Fig. 5(a) shows the mean values for ten normal steps, from eight different animals walking on the horizontal path, using the end of the FETi burst as a reference point. The very distinct burst of the FETi motoneurone coincides with the protraction (swing phase) of the middle leg. However, no detailed comparison between leg movement and timing of the FETi burst was made. The low-frequency output of the SETi motoneurone during the high-frequency FETi burst may be due to a masking effect, as mentioned earlier. During retraction the frequencies of all flexor groups decrease while that of the SETi motoneurone increases. Fig. 5(b) shows the same results obtained from 14 steps of two animals with cut receptor apodemes. There are no

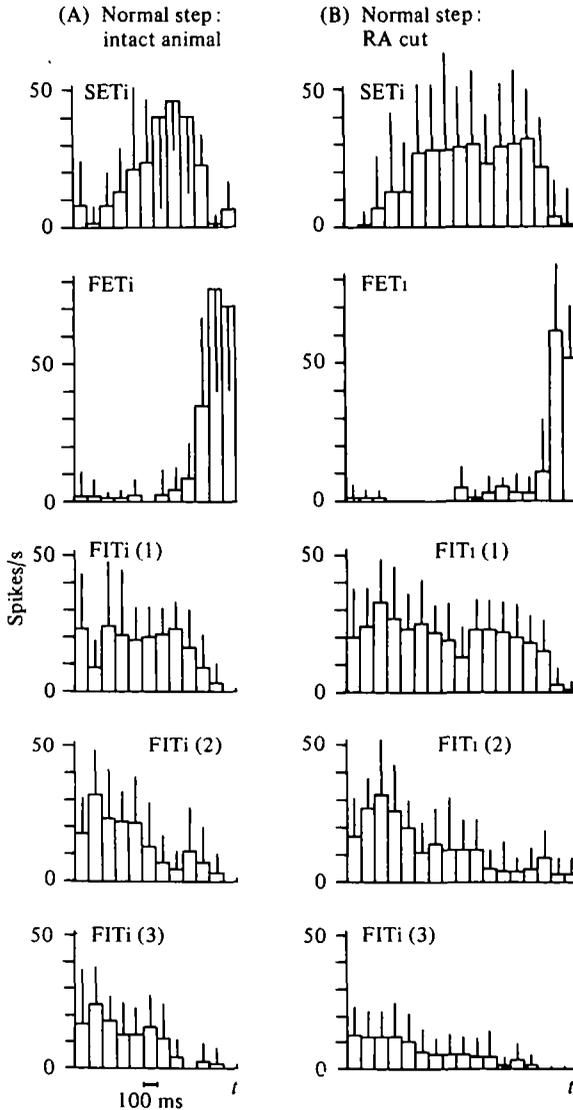


Fig. 5. Normal step. The mean values are calculated after normalizing the step length to the mean value of 1.2 s for the intact animal (A) and of 1.7 s for the animal with receptor apodeme of the investigated middle leg cut (B). For further explanations see Fig. 3 and the text.

differences except for a longer step period (the operated animals walked more slowly) or a slight decreasing activity of the FETi unit and, possibly, of the FITi (3) class.

The effects of stimulation by moving the platform was observed during the retraction (stance phase) of walking animals. Twelve flexion and 12 extension experiments with 3 intact animals were evaluated. Stimuli were applied during the retraction movement of the leg so that they occurred at as many different positions as possible. The position of the beginning of a stimulus within the retraction stroke was measured from the absolute time Δt , between the end of the FETi burst of the last protraction and the beginning of a stimulus. The values lay between 400 and 1300 ms.

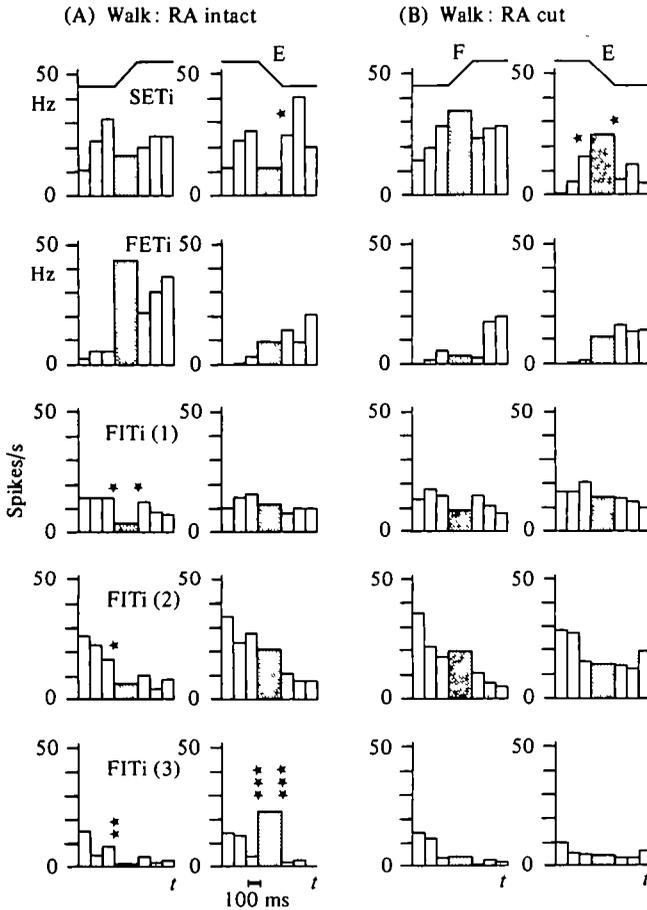


Fig. 6. Walking animal being intact (A) or with receptor apodeme cut (B). Mean values of the responses to flexion (F) and extension (E) of the femur-tibia joint. The mean values are shown for the three time intervals (100 ms each) before the beginning of the stimulus, for the three time intervals after the end of the ramp part of the stimulus and for the time during the ramp part (marked by striped columns). The mean values (\pm s.d.) of the ramp duration is 141 (\pm 31) ms (A, F), 174 (\pm 94) (A, E), 169 (\pm 82) (B, F) and 198 (\pm 66) (B, E). The mean values of two consecutive time intervals were tested for differences (χ^2 test). When the significance level was $P < 5\%$ the two consecutive values are marked by one asterisk, if it was $P < 1\%$ it is marked by two asterisks and by three asterisks when $P < 0.1\%$. For further explanations see Fig. 3 and the text.

For each of the five classes (SETi, FETi, FITi (1), FITi (2), FITi (3)) the frequency before, during and after the ramp of the stimulus was evaluated. We then tested, separately, for each class, whether the change in frequency produced by the stimulus depends upon the value Δt . In all cases, the correlation coefficient was not significantly different from zero ($P > 10\%$). Therefore, all the corresponding values were combined to calculate mean values.

These mean values are shown in Fig. 6. The detailed significance levels for differences between two consecutive values are shown in Table 1. Significant differences occur only between the frequencies during the ramp of the stimulus and the time

Table 1. *The significance levels of the differences between spike frequencies of the consecutive time intervals shown in Fig. 7 (χ^2 -test)*

	Flexion						Extension					
	Intact						RA cut					
SETi	> 20	> 30	> 20	> 30	> 30	> 30	> 20	> 30	= 5.9	= 3.7	> 30	> 30
FETi	> 30	> 30	< 0.1	< 1	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 20
FITi (1)	> 30	> 30	= 4.3	= 4.3	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
FITi (2)	> 30	> 30	= 4.3	> 30	= 7.3	> 30	> 30	> 30	> 30	= 6.8	> 30	> 30
FITi (3)	> 20	> 30	< 0.5	> 30	> 30	> 30	> 30	> 10	> 0.1	< 0.05	> 30	> 30
	RA cut											
SETi	> 20	> 20	> 20	> 10	> 30	> 20	> 30	= 3.7	> 30	= 4.1	> 30	> 30
FETi	> 30	> 30	> 30	> 30	> 10	> 30	> 20	> 30	> 20	> 30	> 30	> 30
FITi (1)	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
FITi (2)	> 10	> 30	> 30	> 20	> 20	> 30	> 30	> 30	> 30	> 30	> 30	> 30
FITi (3)	> 20	> 30	> 30	> 20	> 30	> 30	> 10	> 30	> 30	> 30	> 30	> 30

interval before or after the ramp. In flexion experiments the frequency of the FETi increases and those of the three FITi classes decrease. In extension experiments, the SETi frequency decreases during the ramp (however, only the increase after the ramp is significant) and the FITi (3) frequency increases. In the control experiments (using animals with a cut receptor apodeme), no significant differences between consecutive time intervals are found, with two exceptions (Fig. 6*b*, Table 1). One of these two exceptions occurs before a stimulus and is, therefore, due to a normal change of frequency value during the step. The other occurs at the end of an extension stimulus (SETi), but has the opposite sign of the response of the intact animal. This shows that the frequency change in the intact animal (see Fig. 6*a*) is due to stimulation of the chordotonal organ. The control experiments were made with two animals and 12 walks for either flexion or extension.

DISCUSSION

The results presented show, qualitatively, that a system with negative feedback is active in the femur-tibia joint of the walking stick insect. Measurements of the movement of the leg indicate that this is also true for the coxa-trochanter joint (Wendler, 1964). For crustaceans corresponding results are reported by Barnes, Spirito & Evoy (1972) and Barnes (1975) for the propo-dactylopodit joint of *Cardisoma* and the mero-carpopodit joint of *Astacus* (Barnes, 1977). As discussed by Cruse (1981), these feedback systems can be interpreted as simple servomechanisms which operate in the standing and walking animal. In addition, qualitative differences have been found in the stick insect between the reflex response of standing and of walking individuals. The most obvious difference occurs in the decay of the reflex response. In the standing animal the half-time value of the decay of the SETi amounts to some seconds and of the FITi (1) class to about 500 ms. The duration of the reflex response is much shorter in the walking animal. If a reflex response can be seen (Fig. 6) it seems to occur only during the ramp part of the stimulus in all units in the walking animal.

This agrees with the results obtained by the force measurements (Cruse, 1981). Force measurements alone, of course, cannot distinguish whether these responses are produced by the reflex loop (see Cruse, 1981). However, the agreement with the electrophysiological recordings implies that the main part of the measured force responses is due to the femur-tibia angle control system.

Another difference also exists between walking and standing animals. In the walking animal the largest effects are due to an increase of the FETi activity, during flexion, and of the FITi (3) class, during extension (Fig. 6(a); Table 1). This contrasts with the standing animal, where the strongest responses are found in the SETi motoneurone and the FITi (1) group respectively (Fig. 3). Therefore, in the walking animal the control system excites more strongly those units which show higher amplitude action potentials (extensor) or muscle potentials (flexor). The smaller number of potentials measured in the SETi motoneurone and the FITi (1) class in the walking animal may result from a masking effect by the potentials with high amplitudes. This assumption is supported by results of Cruse & Schmitz (1981). In spite of the slightly higher maximum frequency of the spikes the maximum force value is smaller in walking than in standing animals. This implies that the gain of the reflex response decreases during walking. This is not a contradiction, for it can be explained by the short duration of the reflex response and by taking into account the low-pass filter properties of the muscles. The maximum amplitude of an impulse response of a low-pass filter decreases with decreasing duration of the input impulse.

The results show that the control mechanism of the femur-tibia joint in the middle leg of *Carausius morosus* uses negative feedback in fixed individuals, in free-standing animals with tarsal contact and in the free-walking individual during retraction. As an animal with 'crossed receptor apodeme' (Bässler, 1967) and no tarsal contact shows slow oscillatory movements in the femur-tibia joint, Bässler concluded that the system is also present in the standing animal in the absence of tarsal contact. However, no such movements are observed after the same operation in the walking animal (Bässler, 1967; Graham & Bässler, 1981). This might support the assumption that in a walking animal the negative feedback system is 'switched off', at least during part of the protraction movement. However, another interpretation is possible. The hypotheses of Bässler (1967, 1977a) and Cruse (1980a), concerning the control of movement of the whole leg, postulate that during protraction the reference input to the femur-tibia control system corresponds to a fully stretched joint. The joint indeed has this stretched position, but because of inappropriate sensory input a bent position is first recorded by the chordotonal organ. After adaptation of the control system the recorded position corresponds to a femur-tibia angle of about 90°. Therefore, an error signal is produced by the control system which continuously tries to stretch the joint. Thus no slow oscillations of the kind found in the standing animal would be expected.

In the fixed animal Bässler (1973) found that general stimulation produced a positive feedback response to an extension of the chordotonal organ, corresponding to flexion of the femur-tibia joint (i.e. the force of the flexor muscle was increased). Shortening of the chordotonal organ normally did not have any observable effect. This can be interpreted as a zero gain in the extensor system and a positive gain in the flexor system. As very fast active movements were observed in this situation (much faster than those which occur as reflex responses or during walking) it would support

an interpretation mentioned by both Bässler (1973) and Cruse (1981), namely that the gain of the reflex loop is zero, thus avoiding the damping properties of the feedback mechanism, and is therefore able to produce very fast movements. This effect is further enhanced by positive feedback. This interpretation agrees with the results of Bässler (1973) that the maximum speed of active movements of fixed animals, without tarsal contact, is smaller when the receptor apodeme is cut. Therefore, all the results support the hypothesis of a servomechanism with adaptive control whereby, in the standing and the walking animal, the feedback is negative and, in the fixed animal making active movements, the feedback is zero or positive.

The classification of flexor units concerning the amplitude of the EMG potentials is sensible, for the amplitude value depends only to a small degree upon the position of the electrode in the femur (Debrodt, 1980; Schmitz, 1980). In the extensor muscle the extracellularly recorded EMG potentials excited by the FETi motoneurone are of large amplitude and those excited by the SETi unit of small EMG amplitude. Thus it is possible that in the flexor muscle large EMG potentials are also produced by faster units and small EMG potentials by slower ones. This hypothesis is supported by the similarity of the step responses, for FETi and FITi (3) have short half-time values whereas SETi and FITi (1) have large ones.

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