

THE CONTROL SYSTEM OF THE FEMUR-TIBIA JOINT IN THE STANDING LEG OF A WALKING STICK INSECT *CARAUSIUS MOROSUS*

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SUMMARY

The control system of the femur-tibia joint of the stick insect (*Carausius morosus*) shows different properties depending on whether the animal is standing or walking. The properties of the system were examined when the animal was walking on a treadmill and when the examined leg rested on a platform fixed beside the wheel. The results show that the properties of the system in the standing leg of a walking animal are similar to those of a walking animal rather than those of the standing animal. This indicates that the state of the leg (standing or walking) does not seem to be controlled by the neural subsystem of the leg itself but by a more generalized system, which differs in its properties depending upon whether the whole animal is standing or walking. Furthermore the results show how the behaviour of the system changes for the two alternative states.

INTRODUCTION

A feedback system controls the position of the femur-tibia joint in the leg of the stick insect, *Carausius morosus*. This control system has been shown to be active with negative feedback in the fixed animal (see the review of Bässler, 1982), the free standing animal and the free walking animal (Cruse, 1981; Cruse & Pflüger, 1981). However, there are quantitative differences in the reflex response depending on whether the animal is passive (fixed or standing) or walking. The duration of the reflex response and its gain are smaller in the latter case than in the former. In the case of the struggling movements, even the sign of the response can be changed (Bässler, 1976). It is of interest to know at what level within the central nervous system such reflex changes are made. The question can be investigated using this reflex, since it is possible to place the animal in a situation where one leg is standing while the remaining legs are walking. This can be done by holding the animal so that its legs walk on a treadmill and one leg stands on a platform beside the treadmill. This leg is therefore stationary relative to the body. When the position of the platform is not too far to the rear, within the normal range of movement of this leg, it will stand on the platform whilst the animal is walking with the other five legs (Wendler, 1964). In this way, one can arrange to have 'the standing leg of a walking animal'. It is now

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In the middle of the femur. To record from the extensor nerve the electrodes were inserted more proximally. The potentials were amplified conventionally (Grass P-15/AC preamplifier) and recorded on a four-channel tape recorder. The values from the position transducer were amplified (Hellige TF 19) and after frequency modulation recorded on the same tape. For further details see Schmitz (1980).

The results were filmed with an oscilloscope camera (Recordine) with a speed of 250 mm/s and evaluated by hand. Because of the higher film speed and the lower noise ($\leq 50 \mu\text{V}$ in the extensor recording, $\leq 80 \mu\text{V}$ in the flexor recording) the influence of the masking effect of large spikes was clearly decreased. In the recording from the extensor nerve F2 the potentials of the fast extensor tibiae (FETi) motoneurone and of the slow extensor tibiae (SETi) motoneurone can be distinguished (Fig. 2A). The third known unit, the common inhibitor was present but has not been evaluated here.

In the EMG recording from the flexor muscle, no reliable discrimination is possible as this muscle is controlled by more than six excitatory motoneurones (Debrodt, 1980). Therefore as in Cruse & Pflüger (1981) the muscle potentials were divided into three classes related to the amplitudes of the fast rising part of the potential. The class F1Ti(1) contained all muscle potentials with an amplitude between $100 \mu\text{V}$ and $200 \mu\text{V}$, the class F1Ti(2) contained all potentials with an amplitude between $200 \mu\text{V}$ and $400 \mu\text{V}$, and the class F1Ti(3) all potentials with an amplitude larger than $400 \mu\text{V}$. Muscle potentials with an amplitude smaller than $100 \mu\text{V}$ could not be counted (Fig. 2B). For a discussion of the reliability of this classification see Cruse & Pflüger (1981). The potentials were counted within time intervals of 100 ms and then calculated as spikes/s.

The frequency values for the time interval before the beginning of the stimulus and the immediately following time interval (first part of the ramp) were tested for significant differences (χ^2 -test). This was repeated for the values of both time intervals during the ramp part of the stimulus. A significant difference with $P \leq 5\%$ in the figures is shown by one asterisk, for $P \leq 1\%$ by two asterisks and for $P \leq 0.1\%$ by three asterisks. The two time intervals during the ramp part of the stimulus are normalized to 100 ms each. In Figs 5, 6 and 9 the corresponding frequency values are drawn as striped columns. As the beginning of the ramp can only be determined

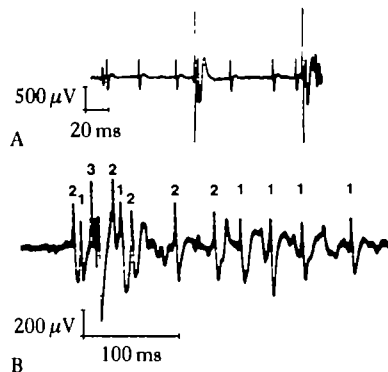


Fig. 2. Examples of recordings: (A) from the extensor nerve and (B) from the flexor tibiae muscle. In (A) the small spikes from the SETi motoneurone and the large spikes from the FETi motoneurone can be seen. Each spike is followed by a muscle potential. The first spike belongs to the common inhibitor neurone. In (B) the classification of the muscle spikes related to their amplitude is shown.

within a range of 20 ms, the interval before the ramp may also show a small change in the frequency value as a response to the very beginning of the stimulus.

The experiments were started at least 1.5 h after implantation of the electrodes. The longest recording was maintained for 3 days. Flexion and extension of the femur-tibia joint was performed in four intact standing animals. The time between flexion and extension was at least 30 s. In walking animals the time between two consecutive stimuli of flexion or extension was at least 10 s. These experiments were performed with three intact animals. In two control experiments the receptor apodeme (RA) of the femoral chordotonal organ was cut as described earlier (see Bässler, 1982). A total of 211 responses in standing animals and 217 in walking animals were recorded, and 81 (standing) and 97 (walking) were evaluated quantitatively. The other responses showed no qualitative differences.

As a measure of correlation either the product-moment correlation coefficient r was used or, when a normal distribution could not be assumed, Pawlik's correlation equivalent CP (Lienert, 1973).

RESULTS

Fig. 3 shows recordings from animals walking on the treadmill without stimulation. Fig. 3A shows a record from an animal walking on the treadmill with all six legs. The beginning of the flexor burst marks the beginning of a protraction movement. No obvious change occurred when the receptor apodeme of the femoral chordotonal organ was cut. Fig. 3B shows a record of the right hind leg standing on the force transducer while the intact animal walks with the other five legs. The extensor

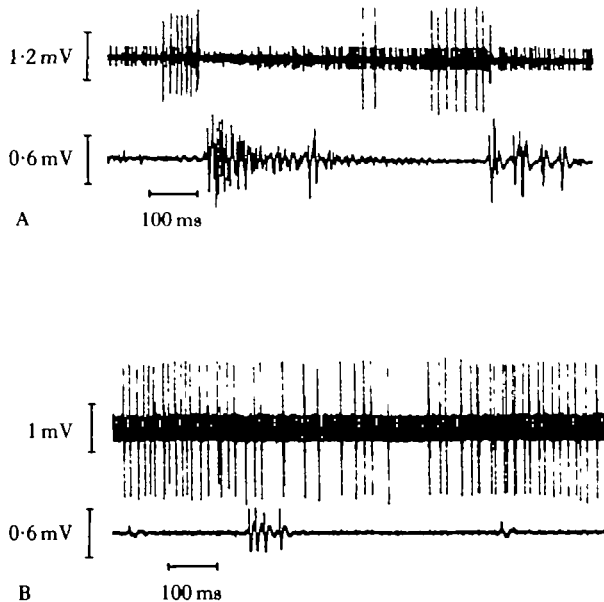


Fig. 3. Upper trace: recording from extensor nerve. Lower trace: recording from flexor muscle. (A) Recording of a hind leg of an animal walking on the treadmill with all six legs. (B) Recording of a hind leg standing on a platform while the intact animal walks on the treadmill with the other five legs. For further explanations see text.

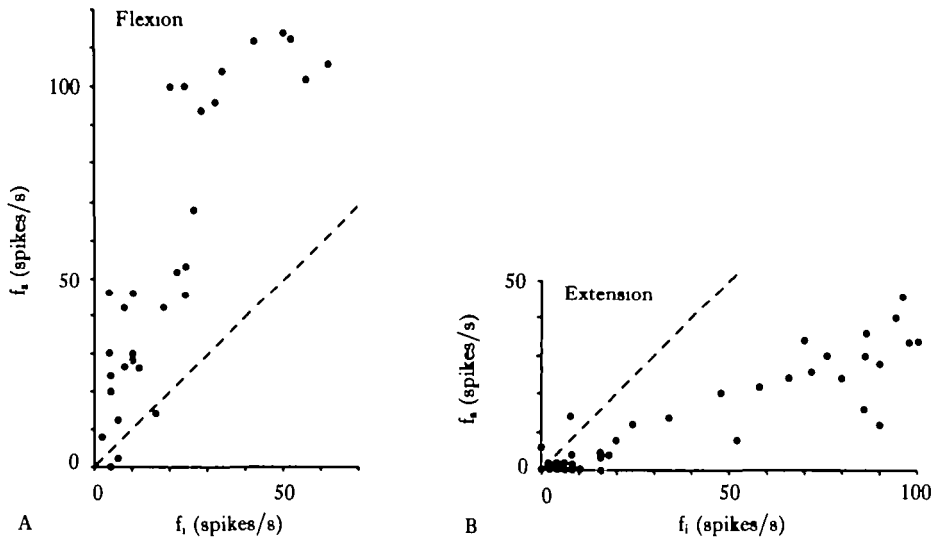


Fig. 4. Response of the SETi motoneurone to flexion of the femur-tibia joint, (A) responses to flexion, (B) responses to extension. f_i : mean initial frequency during the 500 ms before the beginning of the ramp. f_a : mean frequency calculated from the five time intervals between 0.3 s and 0.8 s after the end of the ramp. For further explanation see text.

motoneurons show long bursts, with a mean frequency of about 100 Hz in the SETi motoneurone and of about 30 Hz in the FETi motoneurone. The duration of these bursts is very variable and can continue for several seconds. The pauses between bursts vary between 50 ms and 1 s.

Standing leg — standing animal

As the experimental arrangement differs in some respects from that described by Cruse & Pflüger (1981) (here the animal is fixed dorsally over a wheel, hind legs are used instead of middle legs, and the evaluation of EMG data was somewhat different), the control experiments with standing animals had to be repeated. When stimulating the standing leg by flexion or extension of the femur-tibia joint, the force measurements showed no significant differences from the earlier measurements for the middle legs of free-standing animals (Cruse, 1981).

However, in electro-physiological measurements the individual responses were found to vary considerably. Several response parameters were correlated with the level of SETi activity ('initial frequency' f_i) in the 500 ms before the stimulus. In flexion experiments, the maximum frequency of the FETi motoneurone ($r = 0.69$; $P \leq 0.1\%$), and in extension experiments, the maximum frequency of the FITi(2) class ($r = 0.59$; $P \leq 0.1\%$) and the FITi(3) class ($CP = 0.68$; $P \leq 0.1\%$), were correlated with f_i . The SETi motoneurone, like other motoneurons of this system, showed a response to the ramp and hold stimulus which was predominantly phasic. The duration of its recovery phase was also correlated with the initial frequency f_i . This was shown by measuring the mean frequency f_a in the 500 ms between 0.3 s and 0.8 s after the end of the ramp (Fig. 4). Small differences $|f_a - f_i|$ indicate a short recovery phase.

By this method, possible masking of SETi activity by FETi spikes or flexor muscle

cross talk can be excluded. The results show that there is a correlation between mean initial frequency and the speed of recovery measured by the value $|f_a - f_i|$. This is not due to differences between individuals, as this correlation can also be observed for an individual animal.

As the results obtained from standing legs of walking animals show that there is a high resting frequency in the SETi motoneurone, it appears to be most sensible to compare these results with those from standing animals which also show high initial frequency. However, as the responses of standing legs of walking animals show a fast decay in the recovery phase, the more critical test is to compare these results with those from standing animals that have a low initial frequency, since these also show a relatively fast decay. If one finds differences in this case, the effect must be even more obvious in all other cases. Thus in Fig. 5A the mean values of all those responses to flexion are shown which had a mean initial frequency of SETi motoneurone smaller than 18 s^{-1} . Fig. 5B shows the mean values of responses to extension with a mean initial frequency smaller than 50 s^{-1} . Thus, force measurements and electrophysiological measurements in the standing animal show no important differences from the results obtained by Cruse & Pflüger (1981). However, electrophysiological results are described in more detail, which allows a closer comparison with the results obtained in walking animals.

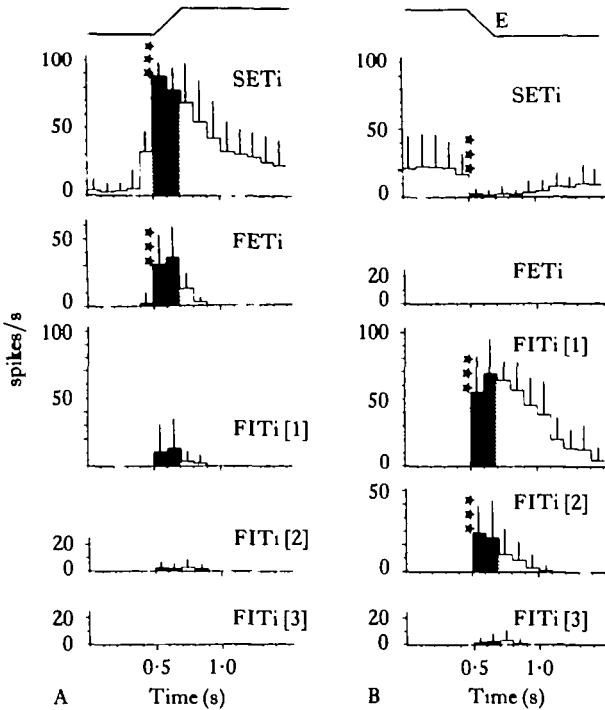


Fig. 5. Flexion (A) and extension (B) of the femur-tibia joint in the standing animal. The upper trace shows the position of the platform of the force transducer. The frequency values obtained during the ramp part of the stimulus are shown by striped columns. The bars show the values of the standard deviation. To make the figure as clear as possible the bars are drawn in one direction only. The asterisks indicate significant differences between values of consecutive intervals (see Methods).

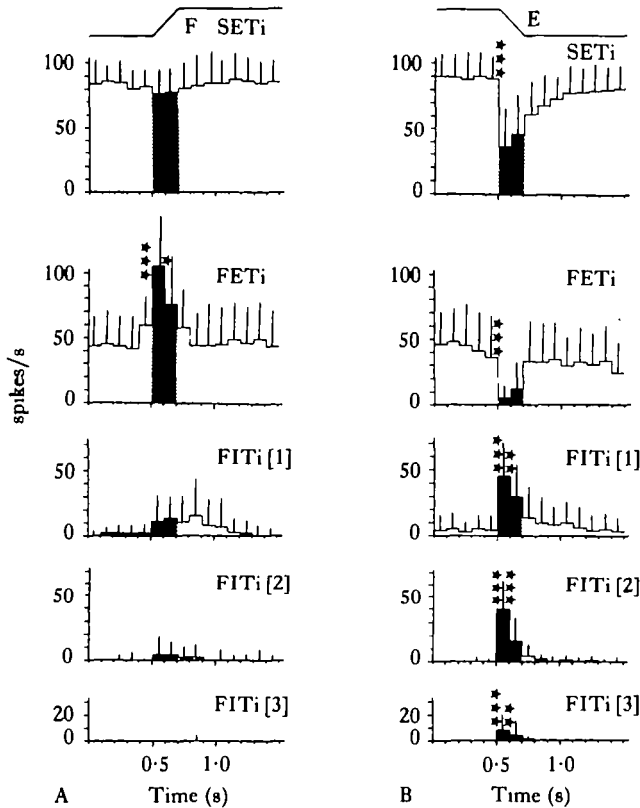


Fig. 6. Standing leg of the walking animal. Electrophysiological recordings during (A) flexion and (B) extension of the femur-tibia joint. Mean values of motoneurone responses to (A) 34 flexions, F, and (B) to 39 extensions, E, of the femur-tibia joint. For further explanations see Fig. 5.

In experiments with the standing leg of the walking insect, no correlation between initial frequency and amplitude or decay time of reflex response was found (Fig. 6). The SETi motoneurone seems to be at its upper saturation level. Therefore, during flexion no response to the stimulus can be seen. Qualitatively the same results are obtained as in the standing animal, but there are quantitative differences. Even during the ramp part of the stimulus, in flexion experiments, the FETi, and in extension experiments all three flexor classes, show significant decrease in the response. This has not been observed in the standing animals. To show this difference in more detail, the response of FETi motoneurones to flexion is shown in Fig. 7A for the standing animal and in Fig. 7B for the standing leg of the walking animal, but with a higher time resolution compared to the earlier figures. Here the individual spike intervals were measured and then the mean spike frequency for intervals of 25 ms duration were calculated. The results show that in the walking animal the phasic response is added onto a resting frequency of about 60 Hz and it ceases abruptly when the ramp ends. In the standing animal the response continues after the end of the ramp, decaying with a half time value of about 150 ms. Analog computer simulation showed that these responses can be described by a high pass filter with a time constant of about 200 ms for the standing animal and a time constant of ≤ 10 ms for the walking animal.

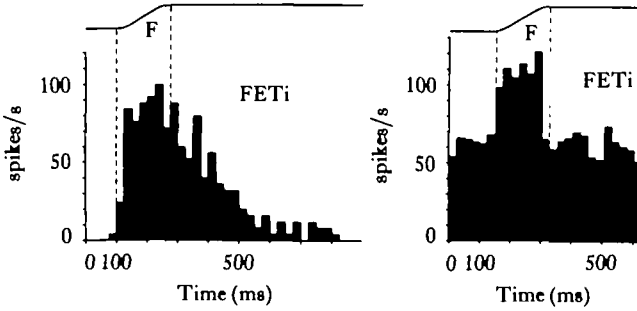


Fig. 7. Mean values of responses at the FETi motoneurone to 10 stimuli. The upper trace shows the position of the platform. Beginning and end of the ramp are demonstrated by interrupted vertical lines. (A) Responses for the standing animal and (B) for the standing leg of the walking animal.

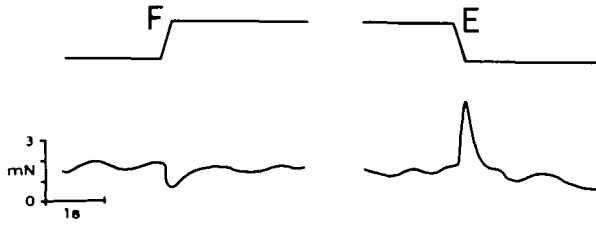


Fig. 8. Recordings from the standing leg of the walking insect. Upper trace: Position of the force transducer platform. Lower trace: force measurement of the responses to flexion (F) and extension (E) of the femur-tibia joint.

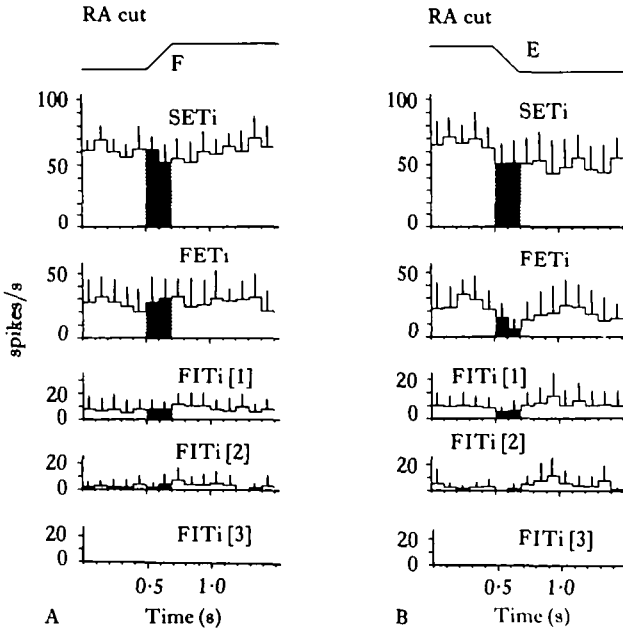


Fig. 9. Standing leg of the walking animal. The same experiments as described in Fig. 6 but with the receptor apodeme of the femoral chordotonal organ cut. Mean values of responses to (A) flexion stimuli and to (B) extension stimuli. For further explanations see Fig. 5.

The small excitation of flexor units during flexion (Figs 5, 6) might be due to a general stimulation of the animals, as cocontraction of extensor and flexor units was regularly observed when the animal was touched slightly on any part of the body.

Fig. 8 shows two examples of force measurements in the standing leg of the walking animal. The system responds with negative feedback. The mean values of the force changes during the ramp are given in Table 1 and show that the gain of the reflex is reduced to about 30% of that found in the standing animals. The half-time values of the decay of force in walking animals are 0.26 ± 0.14 s ($N = 25$) after flexion and 0.28 ± 0.09 s ($N = 31$) after extension (mean value \pm s.d.). These values are much smaller than those measured in the standing animals which may average 4.5 s after flexion and 1.5 s after extension. The walking values, however, are similar to those of free-walking animals, which were roughly estimated by Cruse (1981) to be around 0.1 s.

Our experimental stimulus moved not only the femur-tibia joint, but to some degree also the other joints of the leg. Therefore to control for the possibility that the response measured in the walking animals might derive from other sense organs of the leg, the experiments were repeated after cutting the receptor apodeme of the femoral chordotonal organ. The results of these control experiments (Fig. 9) show that stimulation of the chordotonal organ is essential for a significant response. As in earlier experiments (Cruse & Pflüger, 1981) the resting frequency of the SETi motoneurone is decreased after this operation.

DISCUSSION

Both electrophysiological and force measurements show that in the standing hind-leg of a walking stick insect the control system of the femur-tibia joint is active. However, comparing these results with those obtained in the standing animal the half-time values are significantly shorter in the walking animal. In the FETi motoneurone and in all three flexor classes the amplitude of the reaction decreases even during the ramp part of the stimulus, whereas in the standing animal no significant changes can be seen within the two time intervals during the ramp. This is even more obvious when taking into account the results obtained using higher initial frequencies in the standing animal.

It is not possible to evaluate the gain of the control system from the electrophysiological recordings for the following reason. Looking at individual motoneurons (extensor) or classes of motoneurons (flexor) no quantitative statements can be given since it is not yet known how the different units quantitatively act together to produce the muscle force. However, the force measurements show that the gain of the reflex loop is significantly smaller in the walking animals than in the standing animal (Table 1).

Force measurements and electrophysiological recordings show that the behaviour of the feedback system in the standing leg of the walking insect is very different from that of an animal standing on a wheel or standing free. These results agree with those obtained in free-walking animals: (a) the dynamic properties of the reflex responses mainly occur during the ramp in both situations and, (b) in both situations the gain (obtained from force measurements) is decreased compared to that in standing

Table 1. *Maximum amplitude and standard deviation of force changes.*

All values were obtained from the same three animals.

| | Flexion | Extension |
|--------------------------------|---------------------------------|---------------------------------|
| Standing animal | -5.0 (± 2.5) mN N = 35 | 11.0 (± 2.6) mN N = 30 |
| Standing leg of walking animal | -1.8 (± 0.6) mN N = 24 | 2.8 (± 1.8) mN N = 29 |
| Percent decrease | 64 % | 74 % |

animals. Some minor quantitative differences might be produced by differences in the classification of the flexor amplitudes, or possibly by individual differences between the animals, or perhaps by differences between the middle leg and hind leg. The qualitative agreement means that the control system of the femur-tibia joint behaves as if it were in the walking state although the leg is in fact standing. Thus the state of the leg (standing or walking) is not controlled by the neural subsystem belonging to the leg itself but by the behaviour of the organism as a whole, which is either in a standing or a walking state. In addition, the results support the assumption that a leg standing on a platform is in the walking mode (prolonged retraction). This point is important for the interpretation of experiments concerning the coordination between the legs (Cruse & Epstein, 1982).

The results show that in agreement with earlier findings, cited in the introduction, the system has high-pass filter properties which means that it shows a phasic response. For quantitative considerations, however, in every channel [SETi, FETi, FITi(1)–(3)] different nonlinearities have to be taken into account. The most important ones are saturation level in SETi and different threshold values for SETi, FETi and for the flexor classes, probably as a result of the size principle (Davis, 1971). When the animal changes from the standing to the walking state, the time constant of this high-pass filter is diminished in every channel.

There are many possible mechanisms that could produce this change in the dynamic properties, including, for example, the selection of other sensory units of the chordotonal organ or neuronal systems with recurrent self inhibition, the gain of which is increased in order to obtain shorter time constants. A very simple non-linear mechanism should also be mentioned which can describe the decrease of both properties, i.e. time constant and gain. One can assume that the input signal has to exceed a threshold level before any output is produced. If a value controlled from the CNS is subtracted from the input signal before it reaches the threshold calculation, the system has the following properties. With the increase of this value the gain is decreased and also the 'effective' time constant, as only a smaller part of the upper peak of this reflex response appears above the threshold.

There are numerous reports of reflex modulations in other animals. Therefore only a few selected examples illustrating different mechanisms will be mentioned here. Pure gain changes as found for the M-C-joint of the cheliped of the crayfish (Evoy, 1977) might simply be the result of change of the reference value of a position servo loop. Other authors have demonstrated clear qualitative changes depending upon the

behavioural context (stick insect: Bässler, 1974, 1976; rock lobster: DiCaprio & Clarac, 1981; cat: Forssberg, Grillner, Rossignol & Wallén, 1976). Particularly interesting are the results of Wieneke & Dernier van der Gon (1974), who investigated the reflexes controlling joint position in man. Here the reflex gain is diminished during active movements. This decrease is interpreted as a means of avoiding the damping effect of the feedback loop in order to allow faster limb movements. In the stick insect this effect should be increased by the additional shortening of the reflex response (see also Cruse, 1981, for discussion).

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