DACTYL SENSORY INFLUENCES ON ROCK LOBSTER LOCOMOTION

II. ROLE IN INTERLEG COORDINATION

BY UWE MÜLLER* AND FRANÇOIS CLARAC†

Laboratoire de Neurobiologie et Physiologie Comparées, Place Peyneau, F-33120 Arcachon, France

Summary

1. The effects of cyclic electrical stimulation of the dactyl sensory nerve (DN) on the walking pattern of rock lobsters were examined at the two crucial points within the step cycle: the anterior extreme position (AEP) and the posterior extreme position (PEP).

2. Stimulation during the occurrence of the PEP affected neither the movement pattern of the stimulated leg itself nor that of the ipsilateral adjacent legs.

3. Stimulation of the same intensity during the occurrence of the AEP interrupted the oscillation of the stimulated leg and affected the phase relationships of the ipsilateral adjacent legs.

4. The possibility that indirect influences were mediated by coupling to the substratum can be excluded. Neuronal connections may therefore exist between the funnel canal organs (FCO) of a single leg and the motor output of the adjacent legs. The discussion deals with whether the described channels alone are able to fulfil the requirements of a 'coordinating mechanism' as described in the literature.

Introduction

Of major interest in the analysis of walking behaviour is the identification of the parameters responsible for the production of proper coordinated movement output. In undisturbed walking, the effects of these so-called 'coordinating mechanisms' cannot be specifically resolved, because their function is to produce and maintain the requisite stable state. Information can, however, be obtained by applying disturbances in specific phase intervals of the oscillatory pattern. Cruse and Müller (1986) have described one such technique for analysing the coupling mechanisms between ipsilateral legs of crayfish. In this case the oscillation of a

*Present address: Fakultät für Biologie der Universität, Postfach 8640, D-4800 Bielefeld 1, FRG.
†Present address: LNF 2, 31 chemin J. Aiguier, BP 71, 13402 Marseille, France.

single leg was experimentally prolonged by blocking the power stroke mechanically for a short time. When the leg resumed oscillation, its phase relationship to the adjacent legs was different from that in the stable state. The adjacent legs then reacted to the disturbance, demonstrating the existence of coordinating mechanisms. As this study on crayfish was exclusively a behavioural analysis, the question of which physiological mechanisms (including sense organs) are involved in these mechanisms still remains open.

The previous paper described intrasegmental and intersegmental leg reflexes in the rock lobster *Jasus lalandii* which could be obtained by electrical stimulation of the dactyl sensory nerve of a single leg. It was shown that these reflexes can reset the whole walking pattern, including the responses of adjacent ipsilateral legs. There are various possible explanations for the responses of the adjacent legs. (a) The most obvious one is that these responses are only an indirect effect resulting from the mechanical coupling of the legs: lifting a leg from the substratum increases the load on the adjacent legs. (b) It is also possible that the responses are mediated by other, proximal sense organs (which would also be an indirect effect) or (c) the reflex might be caused directly by the stimulation of the dactyl sensilla. In the latter case, the dactyl sensilla would be an integral part of the coordinating mechanisms mentioned above. To resolve this problem we investigated whether the effects observed were also apparent when the stimulated leg was not itself involved in the walking pattern.

The analysis of the phase–response curves (PRCs, Müller and Clarac, 1990) showed that the reaction of a stimulated leg and that of the neighbouring legs were strongly dependent on the state of the leg when the stimulus was applied. A stimulation delivered at the transition from return to power stroke (the occurrence of the anterior extreme position; AEP) had the strongest effect on the duration of the step cycle. By contrast, a stimulation at the transition from power to return stroke (the occurrence of the posterior extreme position; PEP) had no visible effect. In the experiments described below all the stimulations were triggered by the leg movement of the animal itself, when switching from power stroke (PS) to return stroke (RS) or *vice versa*. This second part of the study demonstrates that phasic afferent input applied to the dactyl nerve at these two crucial points in the step cycle is able to disturb, maintain and even modulate a complete walking pattern.

Materials and methods

The experimental arrangement was designed to study the influence of repetitive stimulation of the dactyl nerve in fixed phase situations during a walking sequence. A block diagram of the experimental apparatus is shown in Fig. 1. The position signal of the movement transducer was connected to a peak discriminator which produced two trigger signals on separate channels, just when the motion of the leg switched from return stroke to power stroke (AEP, 1) or from power stroke to return stroke (PEP, 2). Either of the two trigger signals could be selected by means
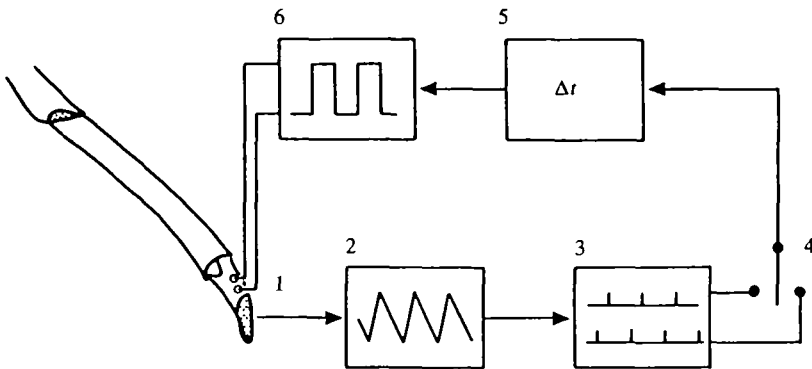
Fig. 1. Method for position-dependent electrical stimulation of the dactyl nerve (DN). (1) Position electrode, located at the tip of the dactyl. (2) Movement transducer. (3) Peak discriminator. (4) Switch, used to select triggering when leg motion switched from return stroke to power stroke or power stroke to return stroke. (5) Delay. (6) Pulse generator. The movement of the stimulated leg or that of an adjacent leg could be used for triggering.

of a switch. The signal could be delayed and was linked to the trigger input of a stimulator applying a stimulus to the dactyl nerve. All other stimulation parameters and recording techniques were the same as those described in the previous paper. Phase relationships were calculated using circular statistics (Batchelet, 1981). The concentration parameter is a measure of the distribution of phase. It can vary between 0 for a random distribution and 1 for a locked phase between the measured events. The mean phase angles were also calculated as fractions between 0 and 1, which correspond to 0 and 360°, respectively.

Results

Repetitive stimulation at two opposite phase intervals

Stimulation at the PEP

When an animal was walking on a driven belt at a constant speed ($8\,\mathrm{cm\,s^{-1}}$), a repetitive stimulation applied at the occurrence of the PEP had no visible effect on the walking pattern, as shown in Fig. 2A with a stimulation of the DN of leg 4. This is tested quantitatively in Fig. 2B: the phase relationships of the AEP of leg 5 in the period of leg 3 (defined between one AEP and the subsequent AEP, see top inset) during normal walking (open histogram) and during stimulation (hatched histogram) were measured. The histograms show a concentration parameter of 0.94 and a mean phase angle of 0.8 for normal walking, not significantly different from the corresponding values of 0.96 and 0.82 during stimulation. Qualitatively similar results were obtained when leg 5 was stimulated in the same way as described here for leg 4.
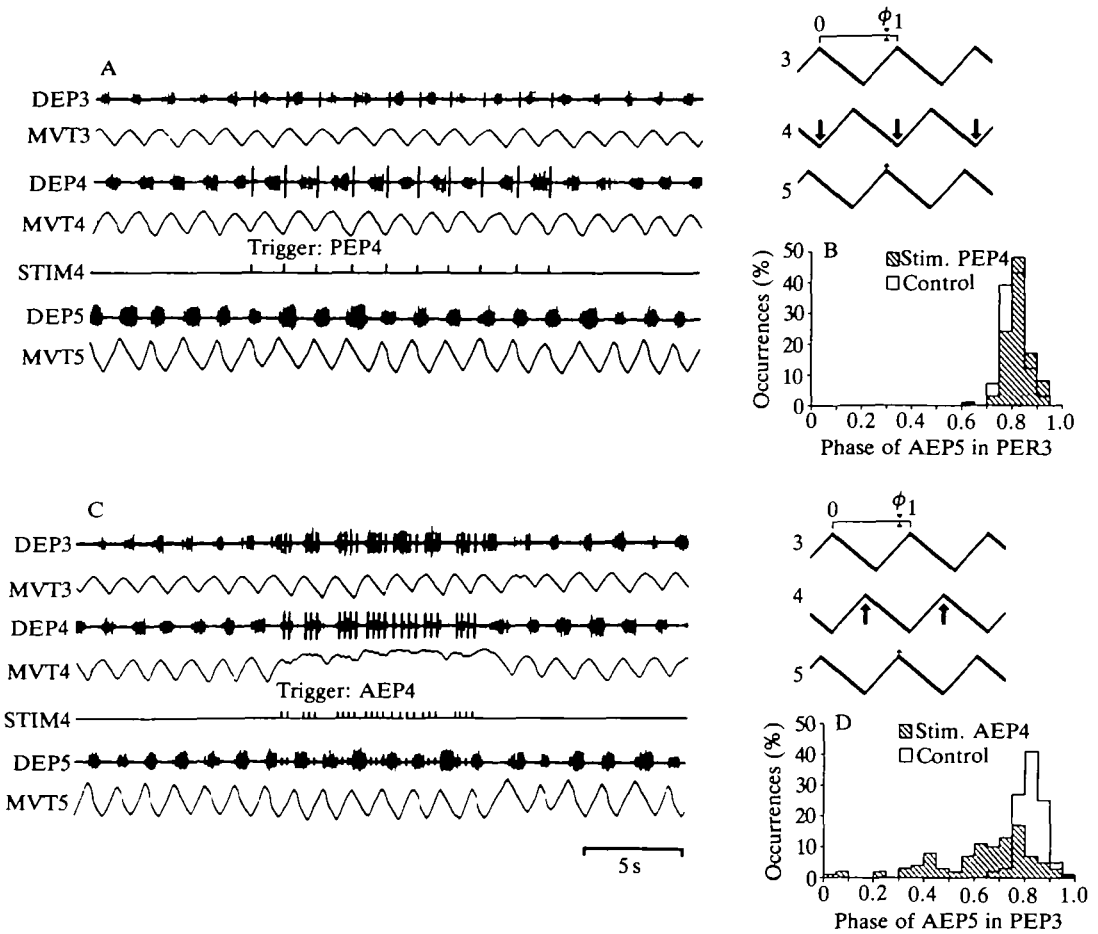
Fig. 2. Position-dependent electrical stimulation of the DN of leg 4. (A) Stimulation of leg 4 at the occurrence of its PEP (see arrows in the inset). The EMGs of the depressor muscles (DEP) and the movement of legs 3–5 (MVT) are shown. Downward deflection in the movement traces indicates a power stroke, upward deflection a return stroke. Trace 5 is the stimulus mark. The vertical bars between the depressor bursts are stimulus artefacts. The time is given on the abscissa. (B) Phase histogram of leg 5 measured in leg 3. The phase (ϕ) is defined as the relative time interval of the occurrence of the AEP of leg 5 within the period (PER, measured from AEP to AEP) of leg 3 (see the dashed line in the inset). Open histogram: during normal walking. Hatched histogram: same parameters when stimulating leg 4 at the occurrence of its PEP. (C) Stimulation of leg 4 at the occurrence of its AEP (see arrows in the inset). All other parameters were the same as those given for A. (D) Phase histogram of the occurrence of the AEP of leg 5 measured in the cycle of leg 3 during normal walking (open histogram) and when leg 4 was stimulated at the occurrence of its AEP (hatched histogram). All other parameters were the same as those given for B.

Stimulation at the AEP

In contrast to these results, Fig. 2C shows the situation where the stimulus arrived at the opposite phase interval (AEP, see top inset). The stimulation inhibited the movement of leg 4 as well as the activity of its depressor muscle. [The same effect could also be obtained by continuous but low-frequency (25 Hz) stimulation of the dactyl nerve over a long time interval (about 5 s, data not shown) but the response fatigued rapidly with this strong stimulation. We therefore chose a 'smooth' stimulation with short pulses, triggered by definite parts of the step cycle.] Measurements of the phase relationships between legs 5 and 3 in Fig. 2D (measured in the same way as in Fig. 2B) show that in the situation during stimulation (hatched histogram) the concentration parameter (0.53) was significantly smaller than in the control (0.96, open histogram). Furthermore, a shift of the mean phase angle was observed from 0.80 in the unstimulated case to 0.68 in the stimulated one.

Stimulation of leg 4 at the occurrence of AEP, unlike stimulation at the occurrence of PEP, therefore had strong influences on the whole walking pattern of the legs investigated.

When legs 3 and 5 were stimulated as described above, different results were obtained: stimulation of leg 3 at its AEP (see Fig. 3A, top inset) led to a complete inhibition of the depressor activity and movement of the leg, but no strong influences on the other legs were visible. In contrast to Fig. 3A, Fig. 3B shows that stimulation of leg 5 at its AEP (see top inset) strongly affected the front neighbouring legs. The movement of leg 5 shifted in the rostral direction. In no case was it possible to inhibit completely the leg movement itself (as was possible with legs 4 and 3), but an inhibition of the depressor of leg 5 and an excitation of the depressor of leg 4 were observed. The movement traces show that the stepping frequency of both anterior neighbouring legs 3 and 4 increased significantly.

Modulation of the walking pattern by legs that are not involved in walking

As mentioned in the Introduction, the responses of the adjacent legs might also be thought to result from indirect influences, mediated by coupling to the substratum or by other sense organs. To test this possibility, in a series of experiments we fixed the meropodite of the stimulated leg at the carapace, using a rubber band. All leg movements along the thoraco-coxopodite (T-C) and coxo-basipodite (C-B) joint were thus completely inhibited, so the leg was unable to reach the substratum and was therefore not involved in walking. Nevertheless, the leg could be stimulated in a phase-dependent way by triggering the stimulator with the switchpoints of the adjacent legs.

When leg 4 was fixed, the depressor muscle of leg 4 discharged tonically. This can be called a resistance reflex, as the leg was continuously lifted. Stimulation of the dactyl nerve of leg 4 at the AEP of leg 5 (which is approximately at the virtual phase of the PEP of leg 4, if it could perform walking movements, see Fig. 4A and top insets) modulated the tonic activity of the depressor of leg 4 in antiphase with
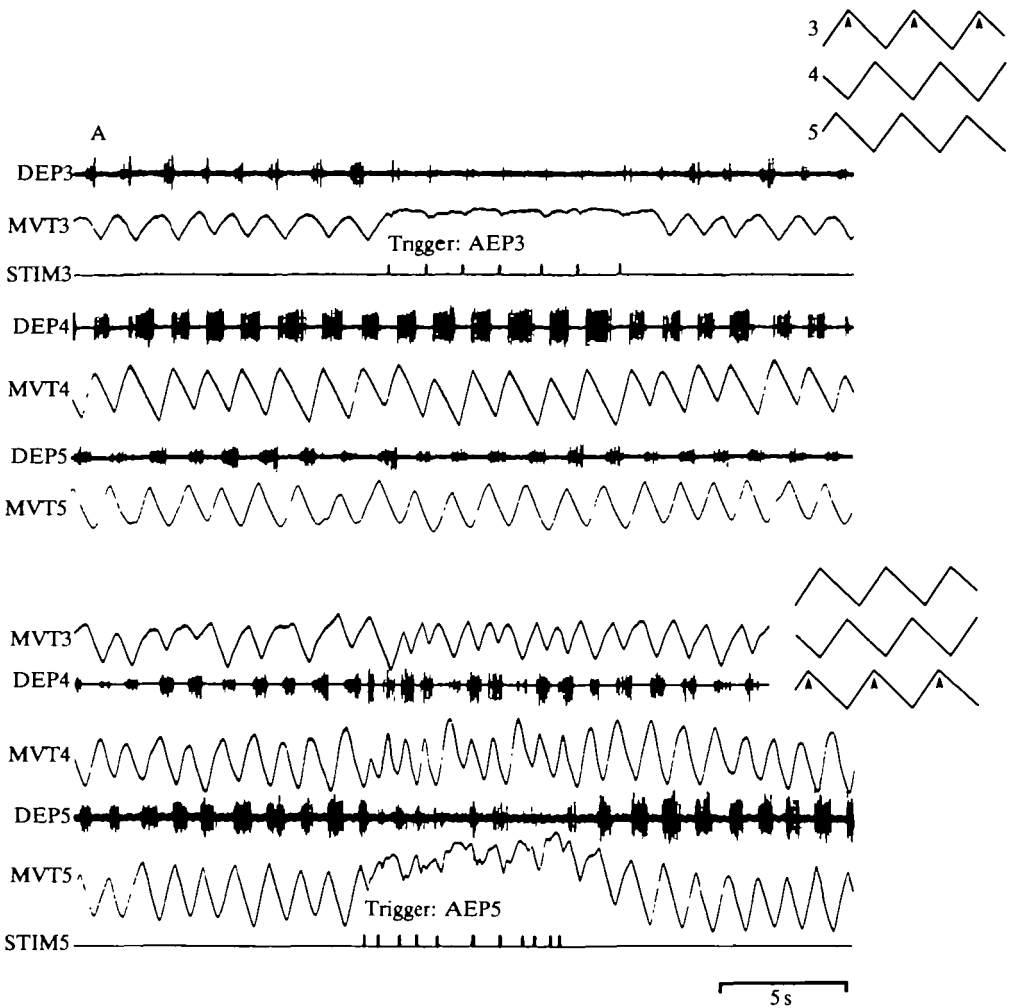
Fig. 3. Position-dependent electrical stimulation of the DN of leg 3 (A) and leg 5 (B) at the AEP. The EMGs of the depressor muscles (DEP) and the movement of legs 3–5 (MVT) are shown. The time is given on the abscissa. (A) The stimulus mark is trace 3. The arrowheads in the inset top right show the times when a stimulus was applied to leg 3. This was at the occurrence of the AEP of leg 3 during the step cycle. (B) The stimulus mark is trace 6. The arrowheads in the inset show the times when a stimulus was applied to leg 5. This was at the occurrence of the AEP of leg 5 during the step cycle.

the depressor activity of leg 5. The pattern of the EMG discharge then became similar to that obtained in normal walking (see, for example, Fig. 2A). Once the stimulation of leg 4 was discontinued the tonic discharge started again.

On stimulation of an active leg 5 at a phase angle of 0.1–0.2, a decrease in the period of leg 4 occurred (Müller and Clarac, 1990). When leg 5 was fixed and not able to walk, as shown in Fig. 5A,B, a repetitive stimulation of leg 5, triggered
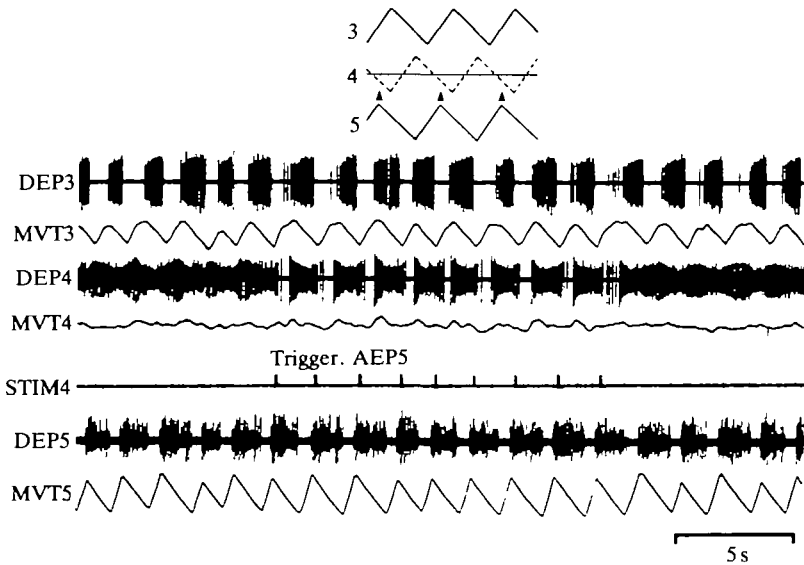
Fig. 4. Position-dependent electrical stimulation of the DN of leg 4 at the occurrence of the AEP of leg 5 with leg 4 fixed. The EMGs of the depressor muscles (DEP) and the movement of legs 3–5 (MVT) are shown. The time is given on the abscissa. The stimulus mark is trace 5. The arrowheads in the inset show the times when a stimulus was applied to leg 4: this was at the occurrence of the AEP of leg 5 during the step cycle of leg 5. The dashed movement trace in the inset shows the expected movement of leg 4 if it had been involved in walking.

with the PEP of leg 4 (which normally occurs with an approximate phase angle of 0.2 during the step cycle of leg 5, see inset, Fig. 5) was also able to increase the step frequency of leg 4 (second trace). In Fig. 5A, the levator muscle of leg 5 was recorded in addition (third trace) to show its strong excitation during stimulation. The depressor muscle of leg 4 (upper trace) also showed an additional excitation, compared with the unstimulated case. Fig. 5B shows a similar experiment, where the movement of leg 3 (upper trace), which also increased in frequency, was additionally recorded. The quantitative analysis of this effect in Fig. 5C shows that the strongest effects of decreasing the step period occurred during the first part of the stimulation, reaching values slightly above 50 % of the normal step duration, followed by a slow adaptation. Later during the stimulation the step period remained at values of approximately 75 % (see legend for description).

When leg 5 was stimulated at a phase angle of approximately 0.8 of its cycle, an increase in the period of leg 4 occurred (Müller and Clarac, 1990). In the experiments described above (Fig. 5) we were able to use the PEP of leg 4 for triggering the stimulation of leg 5. As there is no corresponding triggerpoint in the cycle of leg 4, we took the AEP of leg 4 as the triggerpoint and delayed the stimulation electronically by 250 ms. This stimulation appears approximately at a point in the virtual cycle of leg 5 just before the AEP (inset, Fig. 6). Stimulation of leg 5 in this case led to a decrease in the stepping frequency of leg 4, as shown in
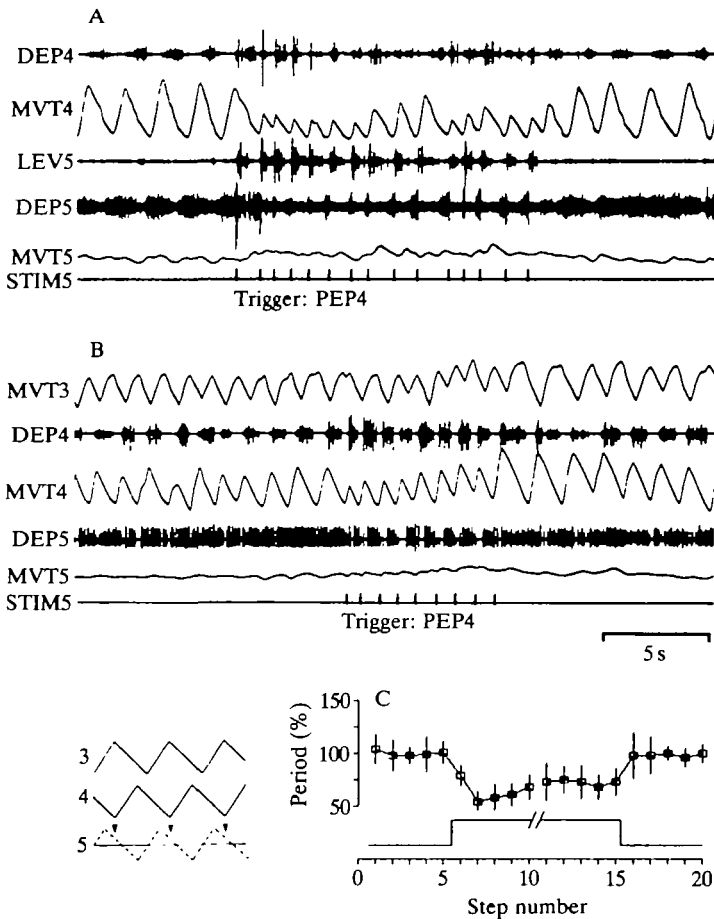
Fig. 5. Position-dependent electrical stimulation of the DN of leg 5 at the occurrence of the PEP of leg 4 with leg 5 fixed. Examples from two different animals are shown in A and B. The time is given on the abscissa. (A) Traces 1 and 2 show the EMG of the depressor muscle (DEP) and the movement of leg 4 (MVT). Traces 3–5 show the EMGs of the levator and depressor muscles and the movement trace of leg 5. Trace 6 is the stimulus mark. The inset shows the times when a stimulus was applied to leg 5. This was at the occurrence of the PEP of leg 4 during the step cycle of leg 4. (B) Trace 1 shows the movement of leg 3. Traces 2–5 show the EMGs of the depressor muscles and the movement traces of legs 4 and 5. All the other parameters correspond to those given for A. (C) Changes in step duration of leg 4 during sequential stimulation of leg 5 as described for A and B. Ordinate: changes in relative step duration (as a percentage of the mean period in the unstimulated case). Abscissa: sequential numbering of the five steps before, the first five steps during, the last five steps during and the first five steps after stimulation. Each measurement is the average of 11 measurements. Bars indicate the standard deviation.
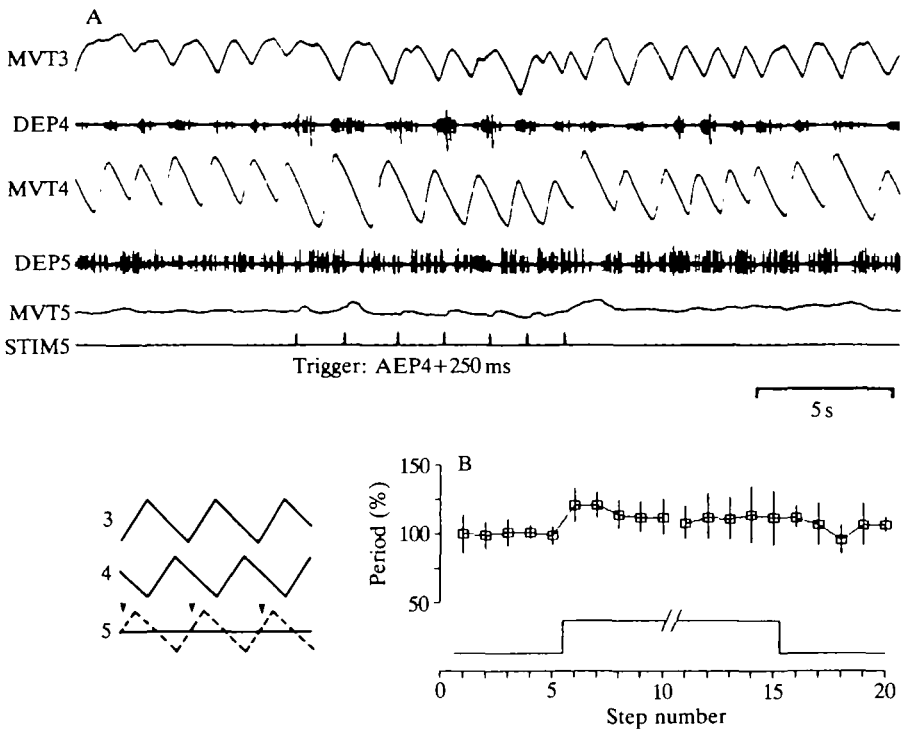
Fig. 6. Electrical stimulation of the DN of leg 5, 250 ms after the occurrence of the AEP of leg 4 with leg 5 fixed. (A) The time is given on the abscissa. Trace 1 shows the movement of leg 3 (MVT). Traces 2–5 show the EMGs of the depressor muscles (DEP) and the movement traces of leg 4 and 5. Trace 6 is the stimulus mark. The arrowheads in the inset shows the times when a stimulus was applied to leg 5. The stimulating pulses in this case were delayed 250 ms from the time of occurrence of the trigger pulses indicating the AEP of leg 4. (B) Changes in step duration of leg 4 during a sequential stimulation of leg five as described for Fig. 6A. The abscissa corresponds to the sequential number of the five steps before, the first five steps during, the last five steps during and the first five steps after stimulation. The changes in step duration compared with the unstimulated mean step duration are plotted on the ordinate. The parameters of the plot correspond to Fig. 5C. Each measurement is the average of seven single measurements.

Fig. 6A. Fig. 6B gives a quantitative analysis of this effect. The strongest effects of increasing the step period were observed during the first part of stimulation, here reaching values of 120 % of the normal step duration, again followed by a slow adaptation. Later during stimulation the resulting period remained at values of aproximately 110 %. In the first steps after stimulation a slight overcompensation was observed in some cases.

When stimulating the fixed leg 5 at a point close to the PEP of its virtual cycle, it was possible to modulate the tonic discharge periodically (Fig. 7; as was demonstrated with leg 4 in Fig. 4). Slow irregularities in the movement pattern
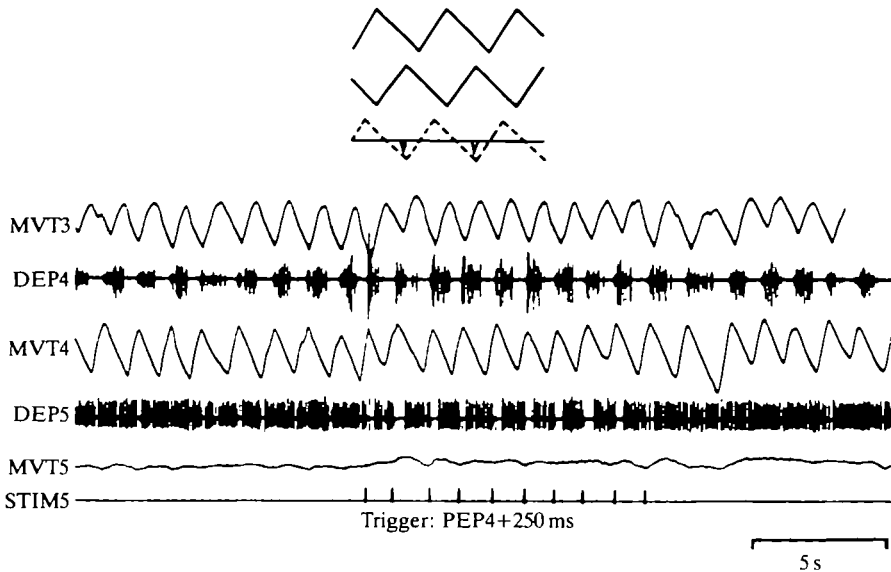
Fig. 7. Electrical stimulation of the DN of leg 5, 250 ms after the occurrence of the PEP of leg 4 with leg 5 fixed. The arrowheads in the insets show the times when a stimulus was applied to leg 5. The stimulating pulses in this case were delayed 250 ms from the time of occurrence of the trigger pulses indicating the PEP of leg 4. This corresponds approximately to the virtual PEP of leg 5. All other parameters were the same as those given for Fig. 6A.

and also an additional excitation of the depressor of leg 4 were observed. This is not surprising, as the adjustment of the 'correct' phase angle for stimulation in this experiment is a very rough approximation based on former observations of the mean phase angles between legs 4 and 5. Nevertheless, it is obvious that the movements of the anterior neighbour were not affected to any great extent during this stimulation.

The influence of DN stimulation of leg 5 on the behaviour of leg 4, shown in the last three experiments, can be summarized as follows (see Fig. 8). A DN stimulation of leg 5 leads either to a decrease in the step duration of leg 4 [71 %, standard deviation (s.d.) 17 %; squares] – correlated with a shift of the AEP in the posterior direction, whereas the PEP was almost unaffected (5 %, s.d. 14 %) – or to an increase in the step duration (115 %, s.d. 15 %; triangles) – which was correlated with a shift of the PEP in the posterior direction (18 %, s.d. 20 %), and the AEP was almost unaffected (−5 %, s.d. 22 %). The control (circles) shows the results given in Fig. 7. In this case the measurements were in the range of normal deviations during undisturbed walking.

Discussion

The results of this paper demonstrate the importance of two crucial points within the step cycle, the switch from RS to PS, which corresponds to
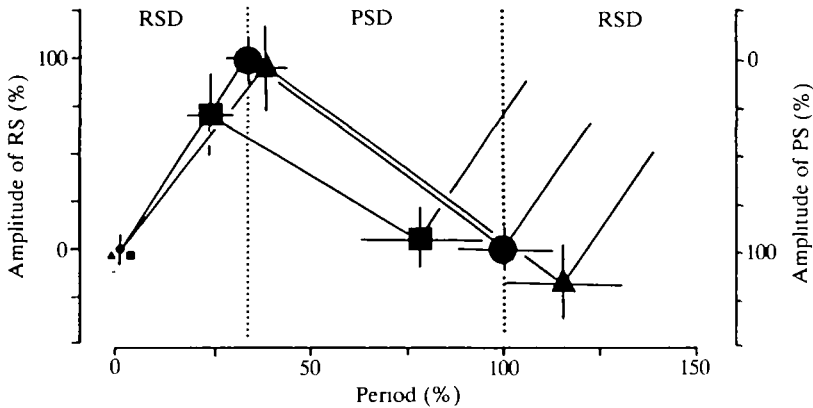
Fig. 8. Summary of the experiments described in Figs 5–7. Abscissa: average step duration of leg 4 as a percentage of the mean period of undisturbed walking. Ordinate: left side, amplitude of the average relative stride length during the return stroke (RS); right side: same parameters during the power stroke (PS). The symbols indicate the phase of occurrence (abscissa) and the relative position of AEP or PEP (ordinates). Each symbol shows the mean of 80–174 single steps. Vertical bars indicate the standard deviation of the spatial (position) parameters, horizontal bars the standard deviation of temporal parameters (step duration). Squares, Fig. 5A,B; triangles, Fig. 6A; circles, Fig. 7. RSD, return stroke duration; PSD, power stroke duration.

the occurrence of the AEP, and the switch from PS to RS, which corresponds to the occurrence of the PEP. As shown in Fig. 2A,B, a repetitive stimulation of the dactyl nerve of leg 4 at the occurrence of PEP did not affect the step cycle of the leg itself or that of its neighbouring legs. This does not necessarily mean that the leg did not respond to a stimulation at its PEP, as at this phase interval the leg performs the switch from PS to RS, even without receiving additional stimulation from the dactyl nerve. Therefore, any reaction of the leg to the stimulation may have been masked by its own inherent motion. Nevertheless, the data show that the applied electrical stimulation was within the physiological range, as in this situation no disturbances of the walking pattern (such as startle responses) or other behaviour patterns (such as escape reflexes) were observable.

In contrast, stimulation at the opposite phase interval, the AEP of leg 4 (Fig. 2C,D), inhibited the leg movement. As leg 4 at its AEP switches from the return stroke to the power stroke, its response to the stimulus was the opposite of its normal motion. The effect of repetitive stimulation was a pause in the leg motion at the level of the AEP. The response of the neighbouring legs 3 and 5 to this interruption was a disturbance in the phase relationship between their oscillations. The main reason for this disturbance is that legs 3 and 5 had a slight phase shift in their oscillations (see Fig. 2D, control) and therefore their reactions to the stimulation of leg 4 were different.

In the analysis of the PRCs in the previous paper (Müller and Clarac, 1990) differences in the intensity of the influences were observed among the three legs

examined. This indicates that some sort of hierarchy exists concerning the return of the legs to their normal walking pattern after a disturbance. Fig. 3A,B illustrates this hierarchy: although a stimulation of leg 3 at the AEP had no dramatic effects on the posterior legs, stimulation of leg 5 was able to increase the frequency of the whole walking pattern. During stimulation of leg 3, leg 5 remained motionless at the AEP, whereas stimulation of leg 5 was unable to inhibit its motion completely. We interpreted this behaviour as a strong inherent excitation of the leg 5 oscillator, which contrasts with the situation observed in leg 3. Again, this interpretation is consistent with the finding that, unlike leg 3, leg 5 is always active during undisturbed walking.

Though the previous results (Müller and Clarac, 1990) clearly show interseg-mental influences of DN stimulation, the question remains whether the observed effects were due to an interleg coordinating mechanism, or whether they were secondary effects, resulting from interference with the normal movement of the legs. Preventing a stimulated leg from participating in the walking pattern seems to be an appropriate method of excluding the effects of mechanical coupling of the legs.

As the effects of leg 5 on the more anterior neighbouring legs were the most prominent, we chose this leg for further experimentation. When the stimulated leg itself is fixed, differences in loading among the unstimulated legs cannot occur. Thus, any reactions of unstimulated adjacent legs point to the existence of coordinating mechanisms activated by the dactyl sensilla and directed to the movement output of the adjacent legs. The method of analysing the PRCs of the neighbouring legs during these experiments would doubtless have been the most precise method of analysis, but was not adopted for the following reason: inhibition of one of the most important walking legs leads in many cases to strong irregularities in the whole walking pattern. The effects of single stimuli can therefore be masked by these irregularities. This problem can be overcome by applying stimulation in the parts of the cycle where the strongest effects are to be expected.

The resulting effects, summarized in Fig. 8, are within the ranges expected on the basis of the PRCs given in the previous paper (Müller and Clarac, 1990). The quantitative analysis in Fig. 5C shows that the strength of the induced effect decreased slightly with repetition of the stimulus. The induced values shifted from 50% to 70% of the normal period, indicating a limited adaptation to electrical stimulation. This can also be seen from the prolongation of the step duration as well as from the overcompensating effects observed at the end of stimulation in Fig. 6B. Fig. 7 shows stimulation of leg 5 during a phase interval which corresponded approximately to the PEP of leg 5 in the undisturbed walk. This has no conspicuous effect on the posterior neighbouring legs. On the contrary, in the leg itself the stimulation had the effect of periodically inhibiting the tonic depressor muscle discharge. Thus the pattern becomes similar to that observed when the leg is involved in walking.

The funnel canal organs are able to modulate the complete walking pattern,

depending on the phase in which a stimulus is applied. In the stimulated leg itself, it is possible to influence the switch from PS to RS by shifting the PEP in an anterior direction (for example Fig. 3B). In an anteriorly positioned leg, it is possible to influence both turning points: the switch from PS to RS, shifting the PEP in a posterior direction, or the switch from RS to PS, shifting the AEP in a posterior direction (see Fig. 8). As mechanical coupling can be overruled, the basic prerequisites for the ability to play a functional role in the control of interleg coupling would be fulfilled.

Nevertheless, the question has to be discussed whether the results demonstrate a direct intersegmental pathway from the dactyl sensillae of one leg to the motor output of adjacent legs, or whether an interaction of different reflex loops, including sense organs other than the stimulated ones, would be more probable. In each part of the step cycle numerous receptors located in proximal leg regions were stimulated (see Clarac, 1977, 1985; Evoy and Ayers, 1982; Bush and Laverack, 1982; Klärner and Barnes, 1986). The thoraco-coxal muscle receptor organ (TCMRO) monitors the leg movements along the thoraco-coxopodite (T-C) joint. The merocarpopodite (M-C) joint is controlled by two chordotonal organs $(MC_{1,2})$ and also by the myochordotonal organ (MCO). The cuticular stress detectors $(CSD_{1,2})$, located in the basi-ischiopodite region, are proprioceptors that are activated during the power stroke of a leg.

The possible influences on some of the important sense organs under the experimental conditions described can be roughly summarized in the following way. (1) If a leg is completely elevated (as was the case in our experiments) any cycle-dependent effects of loading or unloading the leg as well as effects of joint movements are absent. Therefore, only tonic influences can act on the chordotonal organs and on CSD_2. The experiments of Klärner and Barnes (1986) on crayfish, however, have demonstrated that chronic stimulation of CSD_2 produces only very weak effects. (2) The TCMRO could certainly be excited without any joint movement. Experiments by Sillar *et al.* (1987) have shown that phasic input on the TCMRO facilitates an in-phase pattern in the motor discharge of adjacent ganglia. In our experiments, however, stimulation of the dactyl nerve promoted an antiphase pattern in adjacent legs.

Considering the high degree of redundancy among the existing sense organs, it is clear that we cannot completely rule out the possibility that sense organs other than the stimulated ones participate in the mediation of the observed reflexes. Although it has been shown that the funnel canal organs can influence and modulate a walking pattern, it is not yet certain that the information provided by these receptors is the same as that which coordinates a normal undisturbed walk. The effects described here might play a role only under extreme loading situations (as were found in the stick insect, Cruse, 1985). Nevertheless, the ability of specific sense organs to play a major role in leg coordination has been demonstrated.

Comparison with other results

There is now considerable evidence that the reflexes described here may be

common to all crustaceans, as their existence has also been demonstrated in crabs (Libersat *et al.* 1987*a*,*b*), *Homarus* (D. Cattaert, unpublished results) and *Astacus* (U. Müller, unpublished results). In isolated thoracic ganglia preparations of the crayfish, stimulation of the dactyl nerve leads to a response in the levator and depressor motoneurones, which is quite similar to that obtained in the muscles of an intact animal (A. Chrachri, personal communication).

The experiments in Figs 5A–C and 6A,B demonstrate the existence of an anteriorly directed influence from leg 5 onto leg 4. This influence in all cases inhibits the return stroke and induces a power stroke in leg 4. Chasserat and Clarac (1983) have analysed a phase-dependent influence of leg 5 on the period duration of leg 4 in rock lobsters, which supports the data obtained in our experiments.

Other experiments on rock lobsters (Clarac, 1978, 1982, 1985; confirmed by a model calculation by Cruse, 1983) have demonstrated that the load on each leg (monitored by specific sensory afferents) is responsible for the antagonism between adjacent legs. Autotomy of a single leg makes its stump move in phase with its anterior neighbouring leg, whereas the posterior leg stays in opposition. Within the stump, the two remaining sense organs, TCMRO and the C-B chordotonal organ, are slightly stimulated, whereas other afferents of that leg are strongly reduced.

Cruse *et al.* (1983) have also demonstrated the dependence of leg 4's force on the power stroke of leg 5 in rock lobsters, by placing leg 4 on a force transducer while leg 5 continues walking. Under these experimental conditions leg 4 does not step, but exerts a rhythmically modulated force which reaches a maximum during the return stroke and a minimum during the power stroke of leg 5. This finding also suggests that inhibitory influences are exerted on the return stroke of leg 4 during the power stroke of leg 5.

In a statistical analysis of walking parameters, Clarac and Chasserat (1986) and Chasserat and Clarac (1986) measured the time interval between the occurrence of the PEP of leg 4 and the AEP of leg 5, called the '5on–4off interval'. Correspondingly, the time interval between the occurrence of the AEP of leg 4 and the PEP of leg 5 was labelled the '4on–5off interval'. As the 5on–4off interval seemed to be more stable, always showing positive values, the authors hypothesized some underlying neuronal mechanism which might operate like a 'synchronizing delay' between the power stroke of the driving leg 5 and the return stroke of the anterior driven leg 4. In contrast, our results seem to show control of the 4on–5off interval. Therefore, other receptors (such as the CSD or chordotonal organs) may be responsible for the previous findings.

In their analysis of the influences of CSD_2 on leg coordination in crayfish, Klärner and Barnes (1986) measured the '4on–3off' interval during normal walking and when CSD_2 was stimulated tonically. When a wire depressed the compliant cuticle of the CSD_2 in leg 4, the duration of this interval varied significantly more. It is, however, possible that CSD_2 could also have a much stronger phase-dependent influence on leg coordination than that which could be demonstrated with this chronic stimulation procedure.

In insects, the existence of forward-directed influences which inhibit the start of the return stroke of the forward leg as long as the controlling leg performs a return stroke has been demonstrated in the grasshopper by Graham (1978) and in the stick insect by various authors (Dean and Wendler, 1982; Cruse and Epstein, 1982; Foth and Graham, 1983). These results are not quite compatible with our data, as we have observed a reinforced excitation of the power stroke, rather than an inhibition of the return stroke, of the forward leg. This is not very surprising, however, as recent investigations by Cruse and Schwarze (1988) have shown that the interleg coordinating mechanisms of crustaceans and insects generally differ.

References

BATCHELET, E. (1981). *Circular Statistics in Biology.* London, New York: Academic Press.

BUSH, B. M. H. AND LAVERACK, M. S. (1982). Mechanoreception. In *The Biology of Crustacea,* vol. 3, *Neurobiology, Structure and Function* (ed. H. L. Atwood and D. C. Sandeman), pp. 399–467. New York: Academic Press.

CHASSERAT, C. AND CLARAC, F. (1983). Quantitative analysis of walking in a decapod crustacean, the rock lobster *Jasus lalandii.* II. Spatial and temporal regulation of stepping in driven walking. *J. exp. Biol.* **107**, 219–243.

CHASSERAT, C. AND CLARAC, F. (1986). Basic processes of locomotor coordination in the rock lobster. II. Simulation of leg coupling. *Biol. Cybernetics* **55**, 171–185.

CLARAC, F. (1977). Motor coordination in crustacean limbs. In *Identified Neurons and Behaviour in Arthropods* (ed. G. Hoyle), pp. 167–187. Plenum Press: New York.

CLARAC, F. (1978). Locomotory programs in basal leg muscles after limb autotomy in the Crustacea. *Brain Res.* **145**, 401–405.

CLARAC, F. (1982). Decapod crustacean leg coordination during walking. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid and C. Fourtner), pp. 31–37. New York, London: Plenum Press.

CLARAC, F. (1985). Stepping reflexes and the sensory control of walking in Crustacea. In *Feedback and Motor Control in Invertebrates and Vertebrates* (ed. W. J. P. Barnes and M. H. Gladden), pp. 379–400. London: Croom Helm.

CLARAC, F. AND CHASSERAT, C. (1986). Basic processes of locomotor coordination in the rock lobster. I. Statistical analysis of walking parameters. *Biol. Cybernetics* **55**, 159–170.

CRUSE, H. (1983). The influence of load and leg amputation upon coordination in walking crustaceans: A model calculation. *Biol. Cybernetics* **49**, 119–125.

CRUSE, H. (1985). Coactivating influences between neighbouring legs in walking insects. *J. exp. Biol.* **114**, 513–519.

CRUSE, H., CLARAC, F. AND CHASSERAT, C. (1983). The control of walking movement in the leg of rock lobster. *Biol. Cybernetics* **47**, 87–94.

CRUSE, H. AND EPSTEIN, S. (1982). Peripheral influences on the movement of the legs in a walking insect *Carausius morosus. J. exp. Biol.* **101**, 161–170.

CRUSE, H. AND MÜLLER, U. (1986). Two coupling mechanisms which determine the coordination of ipsilateral legs in the walking crayfish. *J. exp. Biol.* **121**, 349–369.

CRUSE, H. AND SCHWARZE, W. (1988). Mechanisms of coupling between the ipsilateral legs of a walking insect *Carausius morosus. J. exp. Biol.* **138**, 455–469.

DEAN, J. AND WENDLER, G. (1982). Stick insects walking on a wheel: perturbations induced by obstruction of leg protraction. *J. comp. Physiol.* **148**, 195–207.

EVOY, W. H. AND AYERS, J. L. (1982). Locomotion and control of limb movements. In *The Biology of Crustacea,* vol. 4, *Neural Integration and Behaviour* (ed. D. C. Sandeman and H. L. Atwood), pp. 61–105. New York: Academic Press.

Foth, E. and Graham, D. (1983). Influences of loading parallel to the body axis on the walking coordination of an insect. I. Ipsilateral effects. *Biol. Cybernetics* **47**, 17–23.

Graham, D. (1978). Unusual step patterns in the free walking grasshopper, *Neoconocephalus robustus*. II. A critical test of legs interaction underlying different models of hexapod coordination. *J. exp. Biol.* **73**, 159–172.

Klärner, D. and Barnes, W. J. P. (1986). Crayfish cuticular stress detector CSD_2. II. Activity during walking and influences on leg coordination. *J. exp. Biol.* **122**, 161–175.

Libersat, F., Clarac, F. and Zill, S. (1987a). Force-sensitive mechanoreceptors of the dactyl of the crab: Single-unit responses during walking and evaluation of function. *J. Neurophysiol.* **57**, 1618–1637.

Libersat, F., Zill, S. and Clarac, F. (1987b). Single-unit responses and reflex effects of force-sensitive mechanoreceptors of the dactyl of the crab. *J. Neurophysiol.* **57**, 1601–1617.

Müller, U. and Clarac, F. (1990). Dactyl sensory influences on rock lobster locomotion. I. Intrasegmental and intersegmental leg reflexes during standing and walking. *J. exp. Biol.* **148**, 89–112.

Sillar, K. T., Clarac, F. and Bush, B. M. H. (1987). Intersegmental coordination of central neural oscillators for rhythmic movements of the walking legs in crayfish, *Pacifastacus leniusculus*. *J. exp. Biol.* **131**, 245–264.