J. exp. Biol. **105**, 127–145 (1983)
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MOTOR OUTPUT OF THE DENERVATED THORACIC VENTRAL NERVE CORD IN THE STICK INSECT *CARAUSIUS MOROSUS*

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(*Received 5 July 1982—Accepted 31 January 1983*)

SUMMARY

The denervated thoracic ventral nerve cord produces a motor output which is similar to that observed in the intact animal during irregular leg movements (seeking movements) or rocking, but not walking.

When the nerves to some legs are left intact, and the animal walks on a wheel, the motor output in the protractor and retractor motor neurones of the denervated legs is modulated by the stepping frequency of the walking legs. The modulation is similar to that observed in the motor output to a not actually stepping leg of an intact walking animal.

When only the crural nerve of one leg is left intact, stimulation of the trochanteral campaniform sensilli induces protractor and retractor motor output to that leg and the leg behind it. In this case the motor output to the ipsilateral leg is in phase. Stimulation of the femoral chordotonal organ influences activity in motor neurones of the extensor tibiae (FETi and SETi) but not those of the protractor and retractor coxae muscles.

In a restrained leg of an intact animal stretching of the femoral chordotonal organ excites FETi and SETi as long as the other legs walk (as in a walking leg) and inhibits FETi and SETi (as in a seeking leg) when the other legs are unable to walk.

INTRODUCTION

The denervated thoracic ganglion of the cockroach produces an alternating rhythmic activity in the motor neurones to the mesothoracic and metathoracic levator and depressor muscles of the femur. The activities of the ipsilateral middle and hind leg muscles are coordinated in a similar manner to those in an intact walking animal (Pearson, 1972; Pearson & Iles, 1970, 1973). These experiments have usually been interpreted as indicating that walking movements in insects are primarily driven endogenously (e.g. Delcomyn, 1980; see Selverston, 1980). However, only one pair of antagonists was studied. So it is possible that the recorded activity could be a characteristic of a movement other than walking in the intact animal, e.g. a seeking movement (Pearson & Iles, 1970, 1973; Ayers in Selverston, 1980).

To investigate the neuronal control of leg movements in the stick insect *Carausius*

Key words: Denervation, walking, seeking, rocking.

morosus, we have first characterized the motor output during the rhythmic movements, walking and rocking, and the irregular, seeking movements. Walking and rocking movements are known to be modulated by afference signals (Bässler, 1977*b*; Pflüger, 1977). We have then been able to determine the nature of the output from the denervated thoracic ganglia, and examine the effect of afferences in the partially denervated ganglion.

METHODS

The animals were fixed, by means of pins, ventral side down on a cork plate. In the experiments with only partially denervated thoracic ganglia or intact animals this plate was 5 mm wide, 70 mm long and 2 mm thick. It was glued onto a 1 × 5 mm steel rod clamped above a walking wheel like that of Graham (1981) but with a fixed axle. The cork plate had cutouts at the leg bases so that movements of the coxae were unimpeded. The vertical distance between the ventral surface of the body and the wheel rim was 5 mm.

To denervate thoracic ganglia the whole thorax was opened from the dorsal side. The body-wall was pinned open by insect pins and the gut moved outside the body. The body cavity was filled with saline (Bässler, 1977*a*). The liquid remained inside the body. Animals treated in this way show a motor output similar to that found in free-walking animals (D. Graham & D. Godden, in preparation). To record from a denervated half-ganglion the nerves na, nl_4, np and the unpaired nerve (Marquardt, 1940) were cut not far from the ganglion (see Fig. 1). All branches of nl_2 which do not go to the protractor coxae muscle were cut close to the main branch, the other ones close to the muscle. Nerve nl_5 was severed from the retractor coxae muscle and then exposed to its origin from the crural nerve. The crural nerve was cut distal to the origin of nl_5. During this procedure nerve nl_3 was also exposed. It was cut either close to the ganglion (no nl_3 recording) or close to its entry into the coxa. All tissue which connected the ganglion to its surroundings was removed. This side of the ganglion was now connected to the rest of the animal only by the connectives and the tracheae.

To limit reduction in oxygen supply, only those tracheae which travel to the gut and dorsal body wall were cut. In no case did these tracheae fill with saline so far that tracheae travelling to the ganglion were blocked. Also, no spiracles were blocked, although some may have closed as a result of denervation.

We recorded from 2–4 nerve stumps at the same time using suction electrodes. The signals were amplified by Grass P15 amplifiers and recorded on tape. From the tape all spikes above a certain threshold of one nerve were counted automatically every 100 ms and plotted (as spikes/s = Hz) on a Hellige He 18 pen-recorder (e.g. Fig. 2). These recordings were used to evaluate regular bursting sequences. At the same time the original tape recordings were shown on a storage oscilloscope for comparison and photographed if required. The common inhibitor (CI) could be recognized if its spikes in both nerves were seen. Because these spikes were very small, their frequency could only be measured when no strong excitatory activity occurred in at least one of the nerves. The threshold of the spike-counting system was chosen so that the CI-spikes were not counted.

To activate the denervated animals they were stimulated vigorously at th

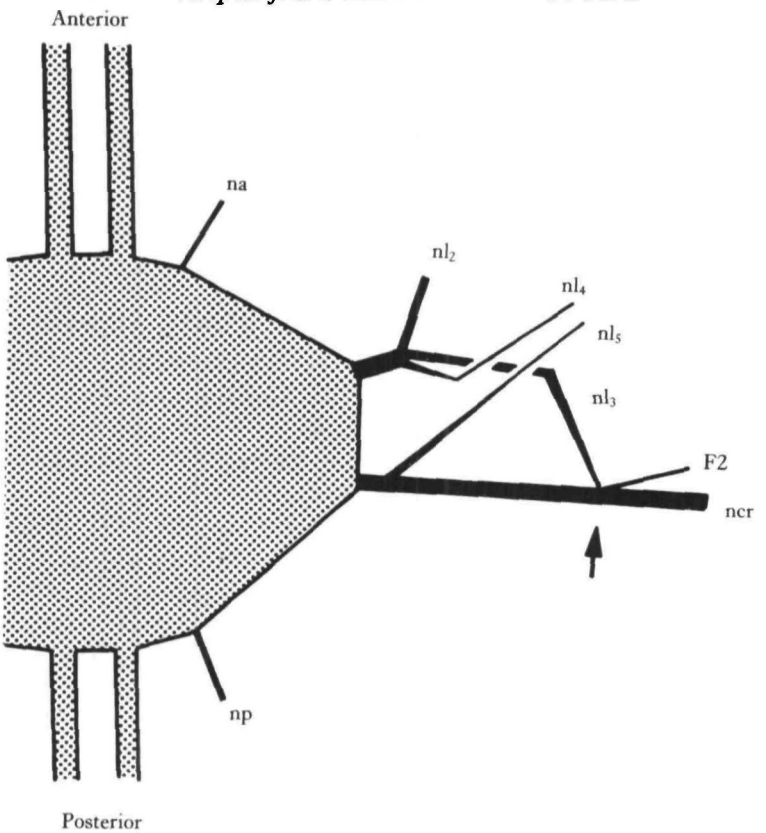

Fig. 1. Schematic representation of the stick insect meso- or metathoracic ganglion and the nerves leaving it. The arrow indicates the border between trochanter and femur. Nerves are named as in the text.

abdomen. Stimulation was by hand, sometimes by one of us, sometimes by the other. There were no significant differences between results obtained by each of us.

The experiments were restricted to meso- and metathoracic ganglia. The following types of experiments were performed:

(1) Records from nl_2 and nl_5 of a denervated half-ganglion. From the other thoracic half-ganglia at least one was not denervated. The legs of the intact half-segments walked on the wheel (recordings from four meta- and four mesothoracic ganglia).

(2) Records from one half-ganglion (nl_2 and nl_5, in some cases additionally nl_3 of the same or nl_2 of an adjacent ganglion) which was denervated with the exception of the crural nerve (ncr). All other half-ganglia were denervated. Stimulation of sense organs in the leg with intact crural nerve (for number of experiments see Results).

(3) Thoracic ventral nerve cord totally denervated. Records from nl_2 and nl_5 of one half-ganglion. (13 meso-, 18 metathoracic ganglia.)

(4) Thoracic ventral nerve cord totally denervated. Records from nl_5 of two adjacent half-ganglia. (Two right meso- and right metathoracic ganglia, two left meso- and right mesothoracic ganglia and two left meta- and right metathoracic ganglia.)

(5) Thoracic ventral nerve cord totally denervated. Records from nl_2 and nl_5 of both a right meso- and a right metathoracic ganglion. (Fourteen animals.)

(6) Thoracic ventral nerve cord totally denervated. Records from nl_2, nl_3 and nl_5 of a right mesothoracic ganglion. (Five animals.)

(7) Intact animals. Records from nerve F2 (extensor nerve) of the right hind leg. Stimulation of the right metathoracic femoral chordotonal organ. Right middle leg autotomized. The other legs walked on the wheel (see Fig. 13).

RESULTS

Neural characterization of leg movements in intact animals

In daylight a stick insect is normally inactive. When it is disturbed it may rock or walk or perform irregular (seeking) movements. Seeking movements are made by the active animal when the tarsi find no contact, and by the restrained animal when trying to escape or when a walking wheel is stopped (Bässler, 1973, 1979; Cruse & Saxler, 1980).

In extracellular recordings from the leg nerves of a denervated *Carausius* thoracic ganglion the activities of the motor neurones of the extensor tibiae, the retractor coxae and the protractor coxae can be identified. All three muscles are innervated by a common inhibitor (CI). The extensor tibiae possesses a fast (FETi) and a slow (SETi) neurone, which both leave the ganglion *via* nerve nl_3 (Marquardt, 1940; Bässler & Storrer, 1980). In extracellular recordings from this nerve FETi produces the largest spikes. SETi has significantly smaller spikes which in some cases did not stand out against the other activities. The retractor coxae is innervated by four (Graham & Wendler, 1981*a*) or five (Igelmund, 1980) excitatory axons. They travel in nerve nl_5. The last branch of this nerve running along the muscle contains only retractor coxae motor neurones. In the denervated preparation they cannot always be labelled individually. All protractor motor neurones travel in nerve nl_2 and produce the largest spikes. Sometimes respiratory activity with small spikes can also be seen in nl_2. The motor output during different leg movements is described qualitatively (Table 1). The description of the inactive animal is based on Bässler & Storrer (1980); that of walking on Graham & Wendler (1981*b*), Graham & Bässler (1981), Cruse & Pflüger (1981), von Buddenbrock (1921); that of rocking on Pflüger (1977) and that of seeking movements on Bässler (1973, 1976). All these experiments were briefly repeated and could be verified.

Activity of protractor and retractor motor neurones of a denervated half-ganglion during walking of the other legs

In these experiments the animals were fixed over a walking wheel. In a first experiment the metathoracic ganglion was only denervated on the left side. The legs of all the other half-ganglia walked on the wheel. As in intact animals walking had to be started by gently touching the abdomen. During walking the activity in nl_2 (protractor neurones) and nl_5 (retractor neurones) of the denervated half-ganglion showed regular alternating modulation (Fig. 2). The protractions of the ipsilateral middle leg were watched by eye and marked by hand on a third channel. The modulation frequency corresponded exactly with the walking frequency of the other legs. Then the metathoracic ganglion was also denervated on the right side. The activity of

Table 1. *Characterization of leg movements in intact animals*

	General	Coordination between different muscle groups of the same leg	Coordination between adjacent legs	Common inhibitor	Occurrence after cutting of the neck connectives	Influence of stretching of the femoral chordotonal organ on extensor motor neurones
Inactive (no movement)	Only some of the slow units active, not modulated	no	no	inactive	+	excitation
Walking	Most motor neurones active, antagonists alternate	constant	in antiphase	regularly modulated	−	excitation
Seeking	Most motor neurones active, antagonists alternate	no	no	no regular modulation	+	brief inhibition or no influence
Rocking	Only smaller units active, very regular alternation	constant	ipsilateral in phase; contralateral in antiphase	only rarely active	−	excitation

For references see text.

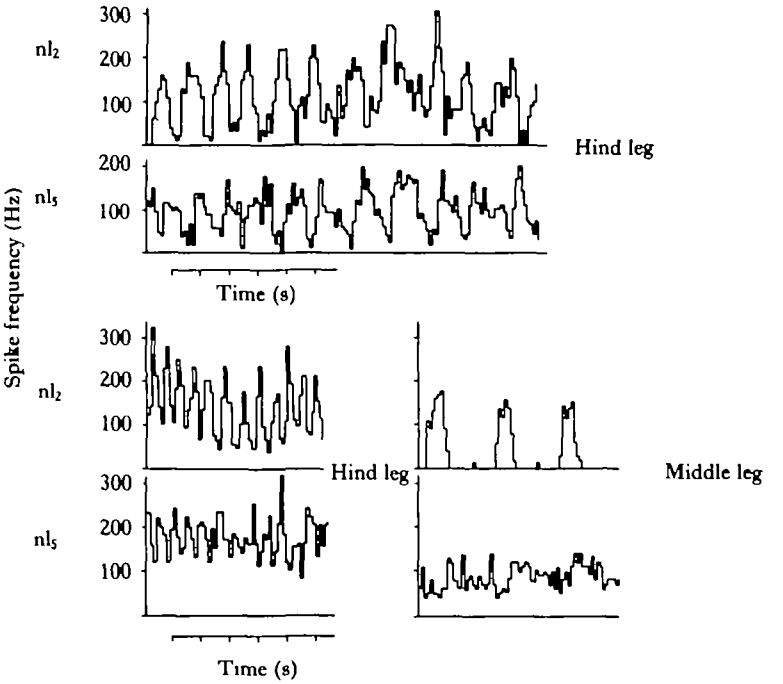

Fig. 2. Spike frequency in records from nl_2 (protractor neurones) and nl_5 (retractor neurones) of one side of a denervated ganglion with the rest of the ventral nerve cord intact. The spikes of all excitatory motor neurones are counted automatically every 100 ms and plotted as spikes/s. Intact legs walking on the wheel. The modulation frequency in these typical examples corresponds exactly with the stepping frequency of the walking legs.

denervated left side of the metathoracic ganglion did not change. Thus, the ganglion to be recorded from on one side was always denervated in a single operation in all the following experiments.

Fig. 2 shows three examples of motor output in different animals. Recordings from meso- or metathoracic ganglia did not differ significantly. The summed spike-frequency of the excitatory units was not zero during minimum activity in at least one of the nerves. CI was active but the frequency was difficult to measure, because the small spikes were often masked by the larger excitatory spikes. In some parts of the recordings CI could be observed for several seconds. Its frequency was 4–30 Hz and was only weakly modulated. The maximum frequency corresponded with the maximum frequency in the excitatory units. In most cases the largest excitatory units (probably fast or semifast units) were not active.

After successive denervation of the other half-ganglia the motor output remained the same as long as one leg remained intact (in all cases a front leg) and walked (three meta- and one mesothoracic record). The modulation of protractor and retractor activity remained coordinated with the walking movements of the walking leg. When the walking frequency of the remaining leg was slowed down, by braking the wheel by hand, the modulation was still coordinated with the walking movements. The rhythm disappeared when the walking wheel was stopped by hand. The motor output then became irregular like that described below.

The front legs are not the only ones which can walk alone. Cruse & Saxler (1980)

showed that one middle leg as well as both hind legs of an intact animal can walk on a wheel while the other legs are standing on a platform.

The motor output changed dramatically (see below) when the last half-ganglion was denervated or when the animal autotomized the last intact leg.

Totally denervated thoracic ventral nerve cord

Activity of protractor and retractor motor neurones in one half-ganglion

Simultaneous recordings from nl_2 (protractor motor neurones) and nl_5 (retractor motor neurones) of a meso- or metathoracic ganglion in the totally denervated thoracic ventral nerve cord were made in type (3), (5) and (6) experiments. The rest of the nervous system (including all connectives) was intact. A single unit with small spikes was normally active in one or in both nerves when the animal was not stimulated. Stronger spontaneous activity in protractor or retractor motor neurones never occurred.

To obtain stronger activity of protractor or retractor motor neurones the animals had to be mechanically stimulated vigorously at the abdomen. After the end of stimulation the motor neurones normally became quiet within a few seconds. The long lasting activity, which was normal in the experiments above only very rarely occurred. The stimulation required in these experiments was greater than in the walking preparation. After some time the animals habituated to the stimulus. Then activity could only be produced after a rest period of 10–20 min. Two different kinds of activity were obtained: vigorous activity and weak activity.

Vigorous activity

Altogether vigorous activity was obtained for 48 min in mesothoracic and for 53 min in metathoracic ganglia. The results were similar for both ganglia and are therefore combined. Figs 3 and 4 show typical results. In most cases only one muscle of a pair was active at a given moment. The excitatory neurones of the antagonist were silent or only weakly active. Activity normally occurred in isolated bursts. Within such a burst the activity was usually continuous, the largest units were active and the spike-frequency was high. The bursts overlapped only slightly or not at all. The firing rate was occasionally regularly modulated, at 0·8–3 Hz, during a long burst (Fig. 3, last record).

The duration of the bursts, when it could be determined unequivocally, was measured in all the records with longer lasting activity. Protractor (nl_2) bursts were nearly always clearly marked, but retractor (nl_5) bursts sometimes died away without any well-defined end point especially towards the end of an active sequence. Thus, there are more measurements for protractor (nl_2) burst duration. The protractor bursts were shorter on average and varied less in duration than the retractor bursts (Figs 5, 6). This was also found in each individual animal.

Protractor and retractor activity normally alternated very irregularly. Sometimes 'regular' alternation occurred. In the following, alternation is termed regular, when at least two full burst cycles were present and the durations of the corresponding bursts were similar (the longer burst of two successive bursts of the same motor neurones was not longer than three times that of the shorter one). Of the 101 min of strong activity, 15 min can be called regularly alternating (7 min in the meso- and

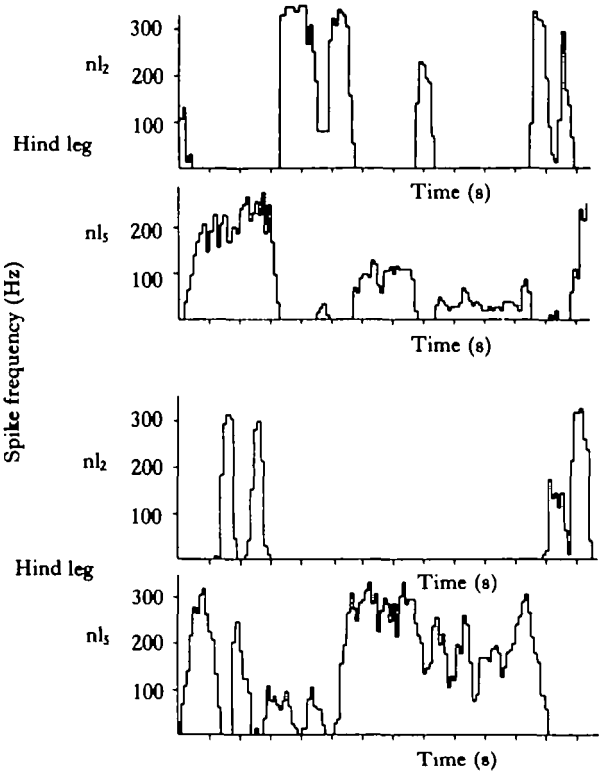

Fig. 3. Spike frequency in records from nl_2 (protractor neurones) and nl_5 (retractor neurones) of one half-ganglion in the denervated thoracic ventral nerve cord (counting as in Fig. 2). Vigorous motor output when the animal was mechanically stimulated at the abdomen. A regular sequence occurs at the beginning of the lower record.

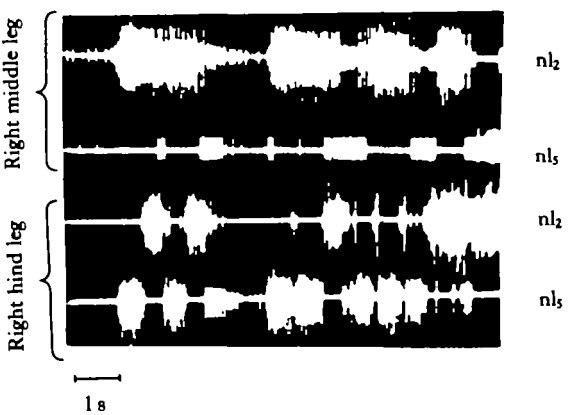

Fig. 4. Records from nl_2 (protractor) and nl_5 (retractor) of a right middle and hind leg from the denervated thoracic ventral nerve cord. The animal was mechanically stimulated at the abdomen. Vigorous motor output. Regular sequences in the second half of the middle leg records and at the beginning and in the second half of the hind leg records. This is the most regular sequence of all four-channel records.

Fig. 5. Histograms of the durations of protractor (nl_2) and retractor (nl_5) bursts in middle leg records in the denervated thoracic ventral nerve cord. Vigorous motor output.

Fig. 6. Histograms of the durations of protractor (nl_2) and retractor (nl_5) bursts in hind leg records in the denervated thoracic ventral nerve cord. Vigorous motor output.

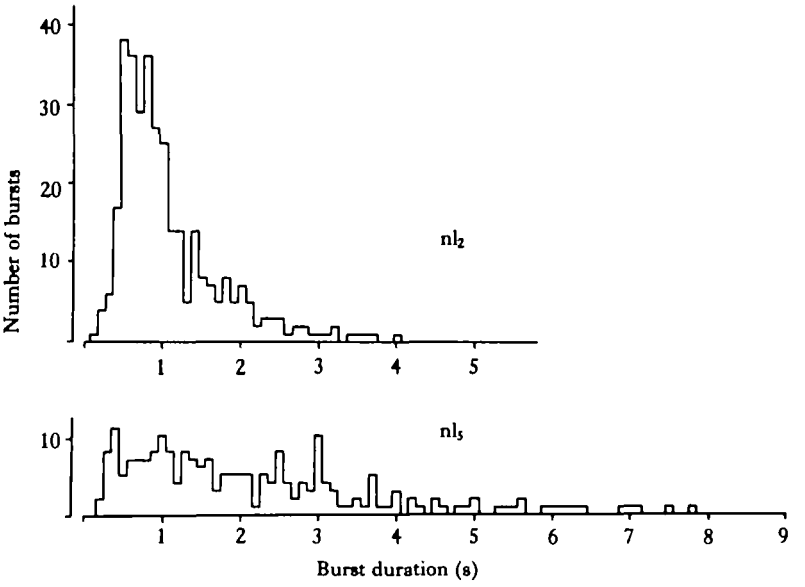
Number of bursts
40
30
20
10
nl2
1
2
3
4
5
10
nl5
1
2
3
4
5
6
7
8
9
Burst duration (s)

Fig. 5

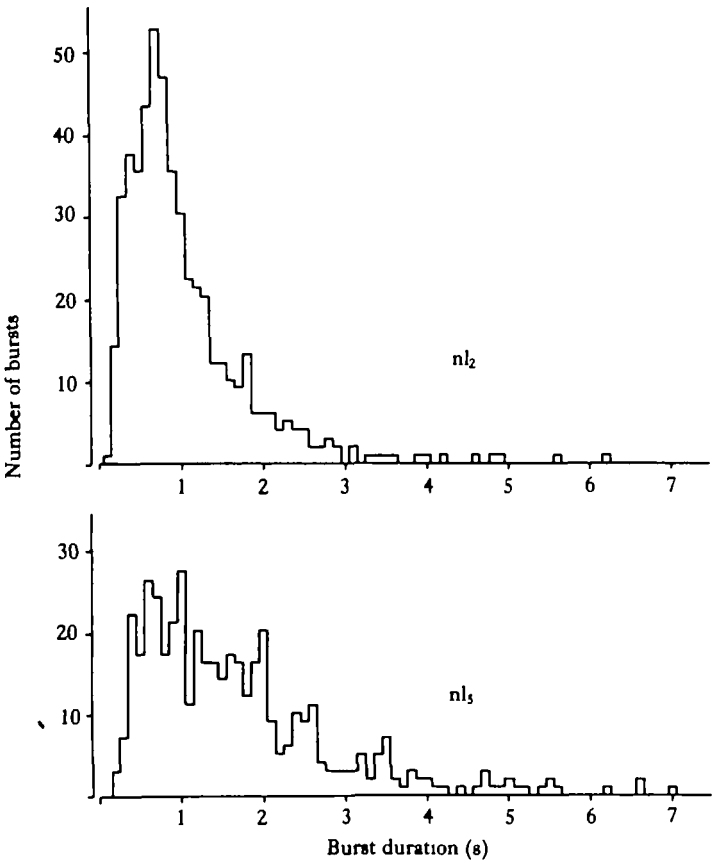
Number of bursts
50
40
30
20
10
nl2
1
2
3
4
5
6
7
30
20
10
nl5
1
2
3
4
5
6
7
Burst duration (s)

Fig. 6

8 min in the metathoracic ganglion). The percentage of regular alternation was different in different animals and was also different in the two ganglia of the same animal. The extreme values for one ganglion were nearly 30 % and zero.

The CI neurone could only be identified in about one half of the records. The activity of this neurone was correlated with the activity of the excitatory units of protractor or retractor: when the activity of the excitatory motor neurones of one of the two muscles was high the CI activity was also high. During long bursts the CI frequency decreased towards the end. A clear increase in firing rate at the change from retractor to protractor activity or *vice versa* as in walking animals (Graham & Wendler, 1981*b*) never occurred.

Weak activity

Sometimes, mostly at the end of a longer period of activity, when the motor output was relatively weak, the protractor and retractor activity alternated very regularly (Fig. 7). This rhythmical alternation could last for 20 s. The frequency of alternation was between 1 and 5 Hz. Only units with small spikes were active. CI only rarely fired. These characteristics correspond most clearly to rocking. Altogether approximately 8 min (approximately 2 min in the mesothoracic and approximately 6 min in the metathoracic ganglion) of this type of motor output were obtained (not included in the 101 min of vigorous activity).

In five animals [belonging to type (3) experiments] the neck connectives were cut towards the end of the experiments. After stimulation these animals showed the same kind of vigorous motor output as before. Regular sequences occurred (Fig. 8). Regular sequences of low intensity corresponding to rocking were never seen.

Coordination of the protractor and retractor neurones with other motor neurones in the denervated thoracic ventral nerve cord

At first the regular alternating sequences (defined above) in the type (4) and type (5) experiments were selected. During vigorous activity no significant coordination between meso- and ipsilateral metathoracic ganglion could be found. During regular alternations of nl_2 and nl_5 activity in one half-ganglion the alternation in the other half-ganglion was often irregular (Fig. 4, first half) or the frequency of regular alternation was different in the two half-ganglia (Fig. 4, second half).

Fig. 7. Records from nl_2 (protractor) and nl_5 (retractor) of a right metathoracic ganglion in the denervated thoracic ventral nerve cord. First half shows weak, alternating output.

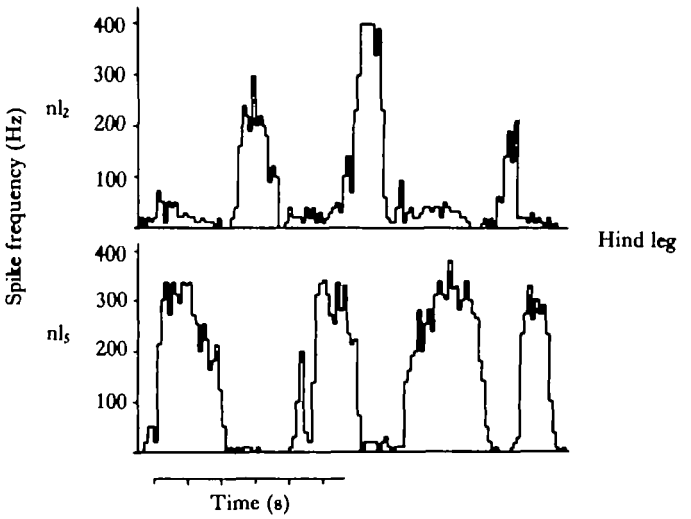

Fig. 8. Spike frequency in records from nl_2 (protractor neurones) and nl_5 (retractor neurones) of a right metathoracic ganglion in the denervated thoracic ventral nerve cord with neck connectives cut (spike counting as in Fig. 2). The animal was mechanically stimulated at the abdomen.

The regular alternations of weak activity, which may correspond to rocking, were coordinated in adjacent ipsilateral and contralateral half-ganglia (Figs 9, 10). The activities of the corresponding motor neurones of the ipsilateral meso- and metathoracic ganglia were in phase and those of the two half-ganglia of one segment were in antiphase.

In the records of type (6) experiments (nl_2, nl_3 and nl_5 of a mesothoracic ganglion) the sequences of regular alternation were selected too. FETi, and when detectable SETi, were either continuously active or their modulation frequency was different from that of the protractor and retractor motor neurones (Fig. 11). A regular alternation of weak activity was not observed in any of these animals.

In summary: during vigorous regular alternation of the protractor and retractor motor neurones there was no obvious coordination between motor neurones of the same leg or of the ipsilateral adjacent leg. The weak regular alternation was coordinated with the motor neurones of adjacent half-ganglia.

Stimulation of the femoral chordotonal organ

The whole ventral nerve cord other than the crural nerve of a middle or hind leg was denervated. The leg with the intact crural nerve was fixed by Scutan. The receptor apodeme of the femoral chordotonal organ was held by a clamp which could be moved by a pen-motor (details of the stimulation technique: Bässler, 1972). The crural nerve was cut in the middle of the femur as were the nerves F121, F122 and F2 (Bässler, 1977*a*). The following structures were still innervated: the muscles inside the coxa, the proximal parts of the extensor and flexor tibiae, the femoral part of the retractor unguis and all sense organs of the coxa, the trochanter and the proximal part of the femur. The femoral chordotonal organ was stimulated with ramp-and-hold functions with an amplitude of 300 μm and a rise time of 300 ms. The activity of nl_2, nl_3 and nl_5 of the stimulated leg was recorded (three middle and three

Fig. 9. Records from the right nl_5 (retractor) of a meso- and a metathoracic ganglion in the denervated thoracic ventral nerve cord. Weak, regularly modulated motor output.

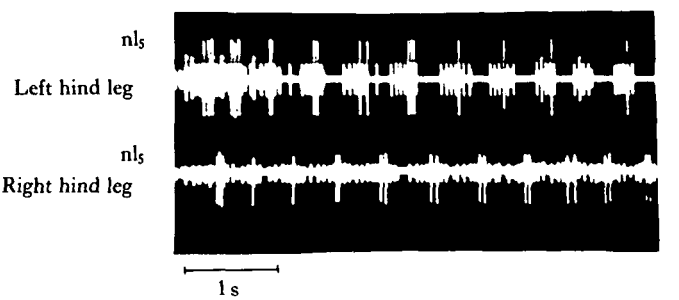

Fig. 10. Records from the right and left nl_5 (retractor) of a metathoracic ganglion in the denervated thoracic ventral nerve cord. Weak, regularly-modulated motor output.

Fig. 11. Records from nl_2 (protractor), nl_3 (extensor) and nl_5 (retractor) of a right mesothoracic ganglion in the denervated thoracic ventral nerve cord. The animal was mechanically stimulated at the abdomen.

hind legs). No obvious differences between middle and hind legs could be found.

In the resting or weakly active animal stretching of the femoral chordotonal organ produced an activation of FETi and SETi as previously observed (Bässler & Storrer, 1980). There was no significant influence on the motor neurones of the protractor and retractor coxae.

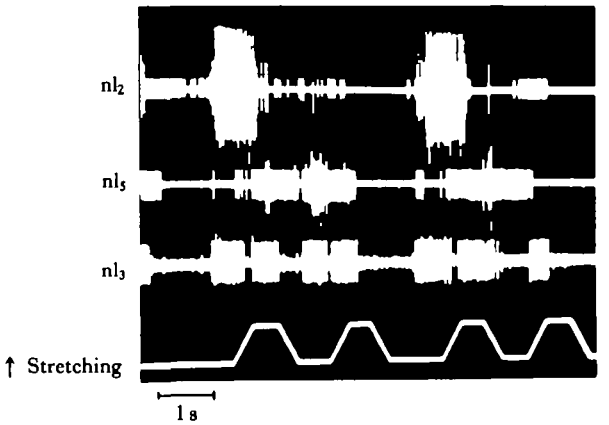

Fig. 12. Records from nl_2 (protractor), nl_3 (extensor) and nl_5 (retractor) of a right metathoracic ganglion in the thoracic ventral nerve cord, denervated except for the crural nerve of the leg under consideration. Stimulation of the corresponding femoral chordotonal organ during vigorous motor output.

During strong activity of the animal the motor output in nl_2, nl_3 and nl_5 was similar to that in the totally denervated preparation (see above). Stretching of the femoral chordotonal organ in those animals produced either no reaction or a brief inhibition of FETi and SETi (Fig. 12). This is typical for restrained active animals performing seeking movements (Bässler, 1973, 1976). Again, no significant reaction of the protractor and retractor motor neurones could be seen.

In an additional experiment (three animals) the prothoracic ganglion was left intact and the front legs could walk on a wheel as above. The mesothoracic ganglion was totally denervated and the metathoracic ganglion was denervated with the exception of the right crural nerve. The right metathoracic femoral chordotonal organ was stimulated. When the front legs walked, the motor outputs in protractor and retractor motor neurones were similar to those described above. Stretching of the femoral chordotonal organ excited FETi and SETi and releasing the chordotonal organ inhibited FETi and SETi. The response was similar to that described below.

Stimulation of the femoral chordotonal organ in intact animals

The response to stimulation of the femoral chordotonal organ was used to test whether a particular leg in an intact animal was in the walking or in the seeking state.

Intact animals were fixed over a wheel (Fig. 13). The right hind leg was restrained by Scutan perpendicularly to the long axis of the body. It was surrounded by a basin filled with saline. The dorsal side of the femur was removed together with the extensor tibiae muscle, exposing the receptor apodeme of the femoral chordotonal organ and the nerve F2 (the extensor nerve). Records from this nerve were obtained using a suction electrode. The femoral chordotonal organ was ramp-wise stimulated as described above (amplitude, 300 μm; rise time, 500 ms). The total numbers of FETi and SETi spikes per 100 ms were counted automatically. The right middle leg was removed because its movements would have been hindered by the basin. All the other legs could walk on the wheel (five animals).

Resting animals responded as described by Bässler & Storrer (1980). When the

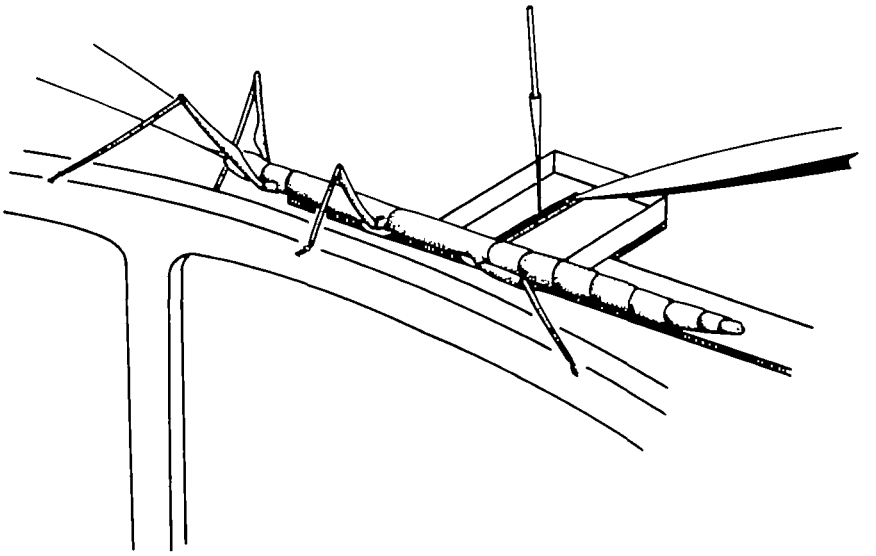

Fig. 13. Experimental set-up for recording the electrical activity of the extensor motor neurones during stimulation of the femoral chordotonal organ in the restrained hind leg of a walking animal. For details see text.

animals walked, stretching of the femoral chordotonal organ produced an increase in firing rate of FETi and SETi (Fig. 14, top) and releasing it caused a decrease in firing rate. The restrained hind leg apparently behaved like a walking leg. Then the wheel was stopped by hand. As long as the animal was active (indicated by firing of FETi) stretching of the femoral chordotonal organ produced a brief inhibition of FETi and SETi (Fig. 14, bottom, original record as by Bässler, 1976) and releasing it had no detectable effect. Thus, when no other leg was able to walk the restrained hind leg behaved like a leg performing seeking movements. All five animals showed the same kind of responses.

Stimulation of the trochanteral campaniform sensilli

The whole ventral nerve cord other than the crural nerve of a middle leg was denervated. This middle leg was cut at the middle of the femur. The attachments of the levator trochanteris and the depressor trochanteris muscles at the trochanter were cut as well as all muscles inside the body which end at the coxa. This prevented these muscles from applying forces to the cuticle and thus from stimulating the campaniform sensilli. The operated leg was free. The campaniform sensilli (description: Tatar, 1976) were stimulated by rhythmically pressing the base of the femur with tweezers. The stimulus was recorded by voice. The separation of stimuli varied between 0·5 and 3 s. Records were made from nl_2 and nl_5 of the stimulated middle leg and from nl_2 of the ipsilateral hind leg (five animals).

Strongly active animals showed a motor output similar to that above, when the campaniform sensilli were not stimulated. Stimulation produced firing of the retractor motor neurones. The end of stimulation excited the protractor motor neurones of the same leg. These two groups of motor neurones could always be driven by rhythmical stimulation of the campaniform sensilli as long as the animal had a certain level

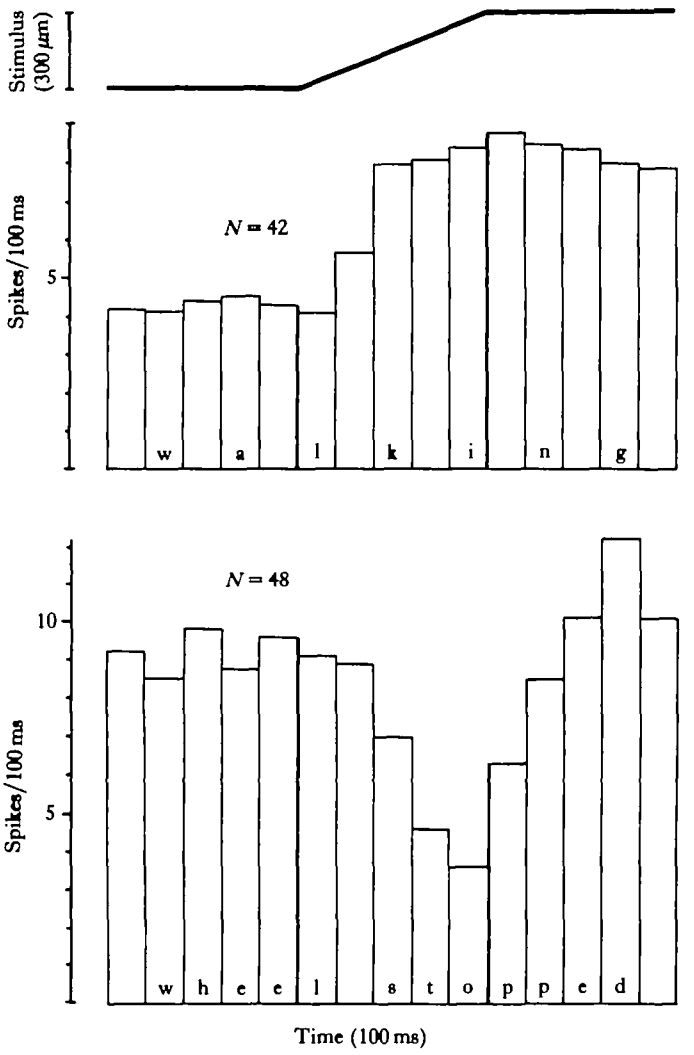

Fig. 14. Summed reaction of FETi and SETi to ramp-wise stretching of the femoral chordotonal organ (top trace) in a hind leg exposed as in Fig. 13. The upper plot gives the mean of 42 stimuli of one animal in spikes/100 ms during walking, the bottom plot the reaction of the same animal when the wheel was stopped by hand (mean of 48 stimuli).

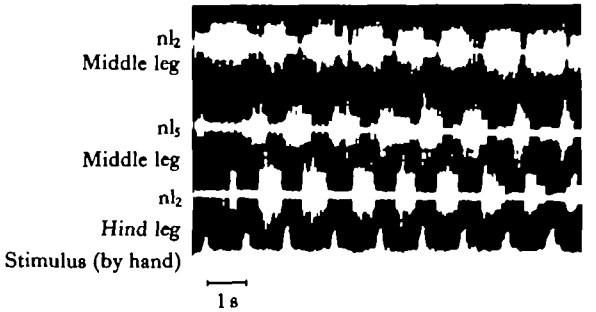

Fig. 15. Records from nl_2 (protractor) and nl_5 (retractor) of a right mesothoracic and nl_2 of a right metathoracic ganglion. Animal operated as described in the text. Stimulation of the campaniform sensilli.

of activity. Rapid alterations in stimulation frequency caused corresponding alterations in burst frequency. In most cases the protractor motor neurones of the ipsilateral hind leg could also be driven. They fired roughly at the same time as the protractor motor neurones of the stimulated leg (Fig. 15).

DISCUSSION

In stick insects with a denervated thoracic ventral nerve cord, which are stimulated at the abdomen, at least two different types of motor output can be distinguished in protractor and retractor motor neurones: (1) a vigorous output which 85 % of the time alternates irregularly and (2) a relatively weak, regularly-alternating output.

The relatively weak, regularly-alternating motor output shows many characteristics of the output during rocking in the intact animal (Table 1): the CI is only rarely active, the muscles of the different legs are coordinated in the same way as in rocking, the oscillation can be superimposed onto other movements (Fig. 3) and it disappears after removal of the head ganglia. This shows directly that the basic rocking rhythm can be produced without sensory input from the legs. This was also shown indirectly by Pflüger (1977).

The irregular sequences of vigorous output are similar to those observed during seeking movements. The regular alternating sequences (defined in Results) of this kind of motor output can be characterized as follows: the muscles of one leg and of different legs are not coordinated; CI has no maximum firing rate at the transitions from protractor to retractor activity and *vice versa* as in walking animals; the behaviour is not altered by cutting the neck connectives; SETi and FETi either do not respond to stretching of the femoral chordotonal organ or they are briefly inhibited. All these criteria correspond to seeking and not to walking (see Table 1). Apparently, the denervated thoracic ventral nerve cord was no longer able to produce a 'walking' motor output.

The motor output of a denervated half-ganglion during walking of other legs can be characterized as follows: it is regularly modulated and coordinated with the walking legs. Stretching of the femoral chordotonal organ during such oscillations excites FETi and SETi (resistance reflex). These characteristics are also seen in intact walking animals (Table 1). Some other characteristics are different from those of a walking motor output: the difference between maximum and minimum activity is less than in a walking leg, where during minimum activity the motor neurones are silent (Graham & Wendler, 1981*b*) and hyperpolarized (D. Graham & D. Godden, in preparation). CI is not modulated. This motor output apparently only partly fits walking. But it is likely to fit the motor output of a leg which unmoveably stands on a fixed platform while the other legs walk on a wheel. Such a leg exerts a backward directed force on the platform which is rhythmically modulated by the stepping of the other legs. The modulation is coordinated with the stepping legs (Cruse & Saxler, 1980). Although electrophysiological records have not been made in such animals, it is obvious from the force records that the retractor motor neurones are not normally silent during minimum force. Studies on first instar larvae of *Extatosoma* (Bässler, 1979) have shown that the leg on the platform is actually in the walking state but remains continuously in the stance phase. The modulations in motor output are supposed to b

produced by a rhythmically oscillating component within the CNS. Stimulation of the femoral chordotonal organ produces resistance reflexes (Cruse & Schmitz, 1983). There is only one difference between the motor output of a leg on a platform and that of a denervated leg: in the leg on a platform it is always the retractor which does not go back to zero, whereas in a denervated leg both muscles may behave in this way. In summary: the motor output of a denervated half-ganglion during walking of other legs is likely to correspond to the motor output of a standing leg of a walking animal, but without the bias of this leg towards retraction.

The stump of an autotomized leg moves with small amplitude. The movements are coordinated with the movements of the walking legs (Wendler, 1964). D. Graham (personal communication) has recorded the electrical activity of the retractor muscle of an autotomized leg and found a weak rhythmical firing. Legs remaining in a prolonged swing phase ('saluting' legs produced by crossing the receptor apodeme of the femoral chordotonal organ; Graham & Bässler, 1981) also show a weak rhythmical modulation of the motor output which is coordinated with the walking legs. A leg which is in the walking state but is unable actually to step thus receives a motor output which is weakly modulated in the stepping frequency of the walking legs. All these rhythmically modulated motor outputs of non-stepping legs in the walking state are apparently similar and are called here walking motor output type 2. The motor output of a normally walking leg is called type 1. The type 2 motor output may occur in an intact as well as in a denervated ganglion. It must therefore be timed by phasic sensory input from walking legs and not by rhythmical stimulation of the leg's own sense organs.

The type 2 motor output can be regarded as the expression of a rhythmically oscillating component within the CNS. The interaction of this oscillating component and the peripheral influences from the same intact leg are discussed by Bässler (1979). He showed that this interaction is able to coordinate the activities of several legs.

In our experiments a denervated ganglion produced a type 2 motor output only if at least one other leg was able to walk. A fixed leg of an intact animal showed a walking-like reflex only if the other legs walked (see Results). Therefore, under our experimental conditions, the ventral nerve cord adopted the walking state only if sense organs of at least one leg signalled that the leg actually stepped. A mesothoracic or metathoracic half-ganglion produced the type 1 motor output if these afferences came from the corresponding leg; in all experiments so far described it produced the type 2 motor output if the afferences came from other walking legs. The modulation frequency of both types of motor output could be modified by external influences (e.g. decelerating the wheel). The following conclusions may be drawn from these results:

(1) The thoracic ventral nerve cord as a whole apparently adopts the walking state even if not all the legs are able to walk.

(2) Type 1 motor output is only produced if sense organs in the corresponding leg signal that a step is occurring. Apparently the final structure of this type of motor output is patterned under the influence of phasic sensory input.

(3) Both types of motor output are timed by phasic sensory input. Results (above) suggest that the trochanteral campaniform sensilli are such sense organs. D. Graham (personal communication) also found that position receptors on the subcoxal joint were able to influence the walking motor output.

(4) The walking motor output of different legs can adopt different types. Therefore, each leg seems to have its own pattern generator.

Under our experimental conditions a denervated thoracic ventral nerve cord produced rocking and seeking movements unless some phasic sensory input from a leg was present. This shows the existence of a central oscillator for rocking. A central oscillator (definition: Bässler, 1983) for walking may exist, but no sort of stepping motor output in the denervated nerve cord has yet been demonstrated in the absence of phasic sensory input from the limb.

This work was supported by the Deutsche Forschungsgemeinschaft Ba/578.

REFERENCES

BÄSSLER, U. (1972). Der 'Kniesehnenreflex' bei *Carausius morosus*: Übergangsfunktion und Frequenzgang. *Kybernetik* **11**, 32–50.

BÄSSLER, U. (1973). Zur Steuerung aktiver Bewegungen des Femur-Tibia-Gelenkes der Stabheuschrecke *Carausius morosus*. *Kybernetik* **13**, 38–53.

BÄSSLER, U. (1976). Reversal of a reflex to a single motoneuron in the stick insect *Carausius morosus*. *Biol. Cybernetics* **24**, 47–49.

BÄSSLER, U. (1977*a*). Sense organs in the femur of the stick insect and their relevance to the control of position of the femur-tibia-joint. *J. comp. Physiol.* **121**, 99–113.

BÄSSLER, U. (1977*b*). Sensory control of leg movement in the stick insect *Carausius morosus*. *Biol. Cybernetics* **25**, 61–72.

BÄSSLER, U. (1979). Interaction of central and peripheral mechanisms during walking in first instar stick insects, *Extatosoma tiaratum*. *Physiol. Entomol.* **4**, 193–199.

BÄSSLER, U. (1983). *Neural Basis of Elementary Behavior in Stick Insects*. Heidelberg: Springer.

BÄSSLER, U. & STORRER, J. (1980). The neural basis of the femur-tibia-control-system in the stick insect *Carausius morosus*. I. Motoneurons of the extensor tibiae muscle. *Biol. Cybernetics* **38**, 107–114.

BUDDENBROCK, W. VON (1921). Der Rhythmus der Schreitbewegungen der Stabheuschrecke *Dixippus*. *Biol. Zbl.* **41**, 41–48.

CRUSE, H. & PFLÜGER, H.-J. (1981). Is the position of the femur-tibia-joint under feedback control in the walking stick insect? II. Electrophysiological records. *J. exp. Biol.* **92**, 97–107.

CRUSE, H. & SAXLER, G. (1980). Oscillations of force in the standing legs of a walking insect (*Carausius morosus*). *Biol. Cybernetics* **36**, 159–163.

CRUSE, H. & SCHMITZ, J. (1983). The control system of the femur-tibia joint in the standing leg of a walking stick insect *Carausius morosus*. *J. exp. Biol.* **102**, 175–185.

DELCOMYN, F. (1980). Neural basis of rhythmic behavior in animals. *Science, N.Y.* **210**, 492–498.

GRAHAM, D. (1981). Walking kinetics of the stick insect using a low-inertia counter-balanced pair of independent treadwheels. *Biol. Cybernetics* **40**, 49–57.

GRAHAM, D. & BÄSSLER, U. (1981). Effects of afference sign reversal on motor activity in walking stick insects (*Carausius morosus*). *J. exp. Biol.* **91**, 179–193.

GRAHAM, D. & WENDLER, G. (1981*a*). The reflex behaviour and innervation of the tergo-coxal retractor muscles of the stick insect *Carausius morosus*. *J. comp. Physiol.* **143**, 81–91.

GRAHAM, D. & WENDLER, G. (1981*b*): Motor output to the protractor and retractor coxae muscles in stick insects walking on a tread wheel. *Physiol. Entomol.* **6**, 161–174.

IGELMUND, P. (1980). Untersuchungen zur Stellungs- und Bewegungsregelung der Beine der Stabheuschrecke *Carausius morosus*: Neuronale Grundlagen der Pro- und Retraktion der Coxa. Diplomarbeit, Köln.

MARQUARDT, F. (1940). Beiträge zur Anatomie der Muskulatur und der peripheren Nerven von *Carausius* (*Dixippus*) *morosus*. *Zool. Jb. (Anat.)* **66**, 63–128.

PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–193.

PEARSON, K. G. & ILES, J. F. (1970). Discharge patterns of coxal levator and depressor motoneurones of the cockroach *Periplaneta americana*. *J. exp. Biol.* **52**, 139–165.

PEARSON, K. G. & ILES, J. F. (1973). Nervous mechanisms underlying intersegmental coordination of leg movements during walking in the cockroach. *J. exp. Biol.* **58**, 725–744.

PFLÜGER, H.-J. (1977). The control of the rocking movements of the phasmid *Carausius morosus* Br. *J. comp. Physiol.* **120**, 181–202.

SELVERSTON, A. I. (1980). Are central pattern generators understandable? *Behavl. Brain Sci.* **3**, 535–571.

[redacted]ATAR, G. (1976). Mechanische Sinnesorgane an den Beinen der Stabheuschrecke *Carausius morosus*. Diplomarbeit, Köln.

WENDLER, G. (1964). Laufen und Stehen der Stabheuschrecke *Carausius morosus*: Sinnesborstenfelder in den Beingelenken als Glieder von Regelkreisen. *Z. vergl. Physiol.* **48**, 198–250.