FUNCTIONAL PRINCIPLES OF PATTERN GENERATION FOR WALKING MOVEMENTS OF STICK INSECT FORELEGS: THE ROLE OF THE FEMORAL CHORDOTONAL ORGAN AFFERENCES

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Summary

A rampwise stretch of the femoral chordotonal organ is known often to elicit a response in the active decerebrate stick insect that is termed an 'active reaction', and which can be considered to represent part of the step cycle. During the first part of the response, the flexor motor neurones are excited and the excitatory extensor motor neurones are inhibited, forming a positive feedback loop. When the chordotonal organ reaches a position corresponding to a flexed femur-tibia joint, the flexor motor neurones are inhibited and the extensor motor neurones are excited.

In this study, extracellular and intracellular recordings showed that, during an active reaction, the excitation of the retractor unguis motor neurones usually paralleled that of the flexor motor neurones, whereas the protractor coxae motor neurones were less strongly coupled to this system.

The first part of the active reaction occurred only at low stimulus velocities. At high stimulus velocities negative feedback was present. The first part therefore represents some kind of velocity-control-system for active flexions.

Electrical stimulation of the nerve containing the axons of trochanteral campaniform sensilla and of the hairfield trHP decreased the likelihood that concurrent chordotonal organ stimulation would elicit an active reaction. Furthermore, most of the active reactions that occurred under these stimulus conditions involved only the flexor tibiae muscle.

The results indicate that: the walking pattern generator is composed of subunits that are only loosely coupled centrally; it probably does not include a central pattern generator; and generation of an active reaction is a two-step process.

Introduction

Each of the stick insect's legs can be considered to possess its own pattern generator for walking movements (von Buddenbrock, 1921; Foth & Bässler, 1988).

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1985). It is the interaction of these six pattern generators that seems to coordinate the movements of the legs (for a summary see Bässler, 1983).

In this paper the concept of pattern generator is understood to encompass all the sensory and central nervous structures responsible for timing the motor output (for a distinction from the concept of central pattern generator, see Bässler, 1986c). Many other details of the fine structure or shape of the motor output besides its timing are determined by the pattern generator for the walking movements of a single leg of the stick insect. The pattern generator includes, in addition to its central nervous parts, sense organs that register leg position, and campaniform sensilla that measure leg load (for a summary see Bässler, 1987). It is likely to be a relaxation oscillator with two states, stance phase and swing phase.

The transition from stance to swing phase is triggered when position-sense organs signal that the leg has reached its posterior extreme position, when campaniform sensilla report a decrease in leg load, and when certain coordinating influences from the other legs are present (for a summary see Bässler, 1987; Cruse, 1985c). An important position-sense organ is the femoral chordotonal organ, which has units sensitive to position, velocity or acceleration, as well as units that respond to a combination of these parameters (Hofmann, Koch & Bässler, 1985; Hofmann & Koch, 1985).

In intact stick insects the active and the inactive states are well defined and easy to distinguish. Stretching the femoral chordotonal organ elicits a resistance reflex only in the inactive state. In daylight the animal adopts the active state only when it is disturbed. The transition from the inactive to the active state and vice versa occurs very rapidly (Bässler, 1983). Decerebrate stick insects differ in several respects: spontaneous active movements may occur in daylight and the transition from the active to the inactive state takes some time, often many minutes (Graham, 1979; Bässler, 1973). During this transition the decerebrate animal shows no spontaneous movements but the resistance reflex is switched off, as in the active intact animal. Additionally, if the animal is fastened above a motor-driven treadband, walking movements can be elicited by moving the band, although no spontaneous movements occur before or after the band is moved by the motor (Bässler, 1986a). Apparently, decerebrate animals are often in a state in which they do not actively move but can respond to sensory stimulation in a way corresponding to that in the intact active animal. This state regularly occurs for some time at the transition from the active to the totally inactive state, but sometimes also spontaneously (Bässler, 1986a). It is now termed the intermediate state. In this paper dealing with decerebrate animals, ‘inactive’ means that the animal did not show spontaneous changes in the motor output for several minutes, and ‘active’ that spontaneous fluctuations in the motor output were present just before stimulus onset.

In restrained forelegs of decerebrate stick insects, Cuniculina impigra, either in the active or in the intermediate state, a stretch of the femoral chordotonal organ (in the intact leg this is equivalent to flexing the femur–tibia joint) often leads initially to an activation of the flexor tibiae and retractor unguis muscles. These
muscles contract during the stance phase of a normal foreleg step. For the femur–tibia joint this is a reflex reversal (positive feedback) compared with the strong resistance reflex in the intact inactive animal (for a summary see Bässler, 1983) although the resistance reflex includes some minor assisting components (Bässler, Hofmann & Schuch, 1986).

As soon as the chordotonal organ senses an almost fully flexed femur–tibia joint in an animal which is in the active or in the intermediate state, the excitation of these muscles declines rapidly. At the same time the extensor tibiae, which contracts during a normal foreleg swing phase, is excited. Hence, when the femoral chordotonal organ senses a flexed joint, i.e. the position of this joint towards the end of the stance phase, these muscles show the same behaviour as during the transition from stance to swing phase. Other findings also suggest that this reaction corresponds to a component of the motor output for walking. (i) When this reaction occurs in the extensor and flexor, the protractor motor neurones (which fire during a normal swing phase) tend to be active together with the extensor motor neurones, whereas the retractor motor neurones (which fire during a normal stance phase) tend to be active together with the flexor motor neurones (Bässler, 1986b). (ii) The reaction can also be produced in hindlegs (Bässler, 1973). When the forelegs are intact, the retractor unguis motor neurones preferentially fire together with the extensor motor neurones as in a stance phase of forward walking. When forelegs and middle legs have been removed, the retractor unguis motor neurones preferentially fire together with the flexor motor neurones as in a stance phase of backward walking (U. Nothof, unpublished results). When the forelegs are intact, the hindlegs walk forwards and when the forelegs are lacking, they walk backwards (Bässler, Foth & Breutel, 1985).

An earlier paper, Bässler (1986b) distinguished between the reaction in an active decerebrate animal (reaction type 1) and that in a decerebrate animal which did not spontaneously move (reaction type 2). The distinction between these two types was based on the behaviour of the contralateral leg. As this leg had to be restrained or partly removed here, both reaction types had to be included in one term, which is now called the active reaction.

The present study attempts to elucidate the functional principles underlying the processing of chordotonal organ afferences during the generation of the active reaction of Cuniculina forelegs. Intracellular and extracellular records have been used to indicate how the chordotonal organ information is distributed, what kinds of chordotonal organ afferences are involved and whether the active reaction consists of a fixed action pattern. Concurrent stimulation of other afferences has also been made, to investigate whether they too are involved.

**Materials and methods**

All experiments were carried out on adult female stick insects, Cuniculina impigra Redtenbacher (syn. Baculum impigrum Brunner) that had been decerebrated by aspiration of the supraoesophageal ganglion (see Bässler, 1973). They
were touched now and then on the abdomen to keep them in the active or intermediate state (for definition see Introduction).

Intracellular recordings from the prothoracic ganglion were performed as described previously (Bassler, 1986b). The animals were restrained ventral side up with the left foreleg at a subcoxal angle of about 60°; the prothorax and femur were opened ventrally; most of the flexor tibiae muscle was removed, and the femoral chordotonal organ was mechanically stimulated. During every experiment extracellular recordings were made from F2, the nerve containing the axons of the extensor motor neurones (for anatomical terminology see Bassler, 1983).

Intracellular records were made from the cell bodies of several motor neurones. The extensor neurones were identified by comparison with the extracellular F2 recording (one-for-one correlation of intracellular and extracellular impulses), and the protractor neurones were identified by comparison with recordings from the n12 nerve near its entry into the ganglion. Since this nerve also projects to muscles other than the protractor coxae muscle, the part of the nerve near the ganglion (which was the only region accessible to recording) contains other neurones as well. However, it is known from intracellular muscle recordings that the larger extracellular spikes are all from the protractor motor neurones (M. Strantz, unpublished results). For this reason, intracellular recordings were analysed only from those neurones that showed large extracellular spikes in the n12 nerve. The retractor unguis motor neurones were identified by comparison with extracellular recordings from the entire femoral part of this muscle, which was inserted into a large-tipped suction electrode. As the flexor tibiae muscle had been removed, the flexor motor neurones had to be identified by their response to stimulation of the femoral chordotonal organ. Experiments on preparations with the flexor tibiae muscle intact have shown that flexor motor neurones own all of the cell bodies in the anterior region lateral to the connective which, during longer lasting inactivity, are reproducibly hyperpolarized by a stretch of the femoral chordotonal organ and depolarized by its relaxation (B. Debrodt & U. Bassler, in preparation). Flexor motor neurones that respond differently (e.g. Siegler, 1981) are not encompassed by this method of identification. The locations of all neuronal somata in the prothoracic ganglion of Cuniculina correspond approximately to those of cells in the mesothoracic and metathoracic ganglia of Carausius (Storrer, Bassler & Mayer, 1986).

Figures showing simultaneous extracellular recordings were photographed from the screen of a storage oscilloscope; figures showing only intracellular recordings are from a chart recorder (Hellige He18, upper corner frequency 100 Hz). Extracellular recordings from nerve F2 were made using a paraffin-oil hook electrode (Bassler, 1977) and from nerve n12 using a suction electrode.

The femoral chordotonal organ was mechanically stimulated as described previously (Bassler, 1986b). The stimulus used here was a ramp-and-hold stretch. The stimulus is considered to be only the movement itself and not the constant state of stretch before or after the movement. Unstimulated means that there was no change in the degree of extension of the chordotonal organ. The minimum
stretch of the chordotonal organ corresponded to a joint angle of about 110°. Since
the stimulus amplitude was 600 μm, the maximum stretch corresponded to a joint
position of about 40° (for details see Bässler, 1986b).

Nerve Tr1 supplies the campaniform sensilla fields 2, 3 and 4 on the anterior side
of the trochanter and the hairfield BF1, labelled trHP by later authors (Hofmann
& Bässler, 1982). To stimulate this nerve electrically, it was first cut as distally as
possible and then the end was taken up into a suction electrode. The nerve was
stimulated with 1.5 ms rectangular stimuli at a frequency of 120 Hz. This high
stimulus frequency was chosen because during natural stimulation (with rather low
amplitude) the impulse frequency of the campaniform sensilla approaches this
range (Hofmann & Bässler, 1986). The stimulus amplitude, which ranged from 0.6
to 2.0 V for all the experimental animals, was increased from zero until there was a
distinct increase in flexor force. The amplitude used in the subsequent experiment
was somewhat higher. The Tr2 nerve supplying the campaniform sensilla on the
posterior side of the trochanter was cut in most cases.

To measure the force of the extensor tibiae muscle, the muscle tendon was cut
where it joined the tibia and mounted in a clip connected to a force transducer
(Swema). The force generated by the flexor tibiae muscle was measured indirectly
by attaching a force transducer to the tibia about 2 cm from the femur–tibia joint
(femur–tibia angle 90–100°, extensor tendon cut). The forces measured in this
way are shown in the figures. Since the extensor force was measured directly and
the flexor force indirectly, the amplitudes shown in the recordings are not directly
comparable.

Results

Intracellular recording from motor neurones

The active reaction seems to be a complicated reaction of the motor neurones of
several muscles. Therefore, it was necessary to test whether all these motor
neurones are rigidly coupled during an active reaction (i.e. whether there is an
underlying fixed action pattern) or whether the motor neurones are only loosely
coupled. This was investigated by comparing intracellular records from several
motor neurones with the active reaction expressed in extracellular records from
the extensor motor neurones. The shape of the recorded reaction additionally
gave insight into some functional principles of the generation of the active
reaction.

The femoral chordotonal organ of the left foreleg was stimulated mechanically
with ramp-and-hold stimuli. Ramp rise times ranged from 0.5 to 3.0 s. In all
experiments extracellular recordings were made from the F2 nerve, containing the
axons of the extensor tibiae motor neurones, to identify the active reaction. An
active reaction was scored when during a stretch stimulus there were no spikes of
the excitatory extensor motor neurones until the stimulus reached half-amplitude
(first part of the active reaction). During the second half of the stimulus or
immediately after the end of the stimulus these neurones resumed activity (second
Fig. 1. Comparison of FETi's response to stretch of the chordotonal organ (ChO) during an active reaction in the decerebrate active animal (top) and during a resistance reflex in the inactive animal (bottom). Two independent intracellular records. Stimulus trace valid for both records.

part of the active reaction). The following analysis of intracellular responses includes only responses that occurred during an active reaction as defined above.

The percentage of active reactions differed from animal to animal. On rare occasions it was nearly 100% and in some animals no active reactions occurred. Normally between 20 and 40% of the stimuli elicited an active reaction provided the animal could be activated by touching the abdomen. The time the animal remained in the active or intermediate state was normally not very long in these highly dissected preparations.

Six intracellular recordings were made from the fast extensor tibiae motor neurone (FETi). These recordings showed that whenever an active reaction was evident in the F2 extracellular recording (42 times), the FETi neurone was hyperpolarized at the onset of the stimulus ramp and strongly depolarized towards the end of the ramp (compare Bässler, 1986b). Fig. 1 compares a typical FETi response during an active reaction with the response in an inactive animal. Twenty-two active reactions showed a steep hyperpolarization at stimulus onset (as in Fig. 1), but in the other 20 active reactions the hyperpolarization started more gradually. In the reactions with steep hyperpolarization the latency between stimulus onset and the beginning of hyperpolarization was between 15 and 30 ms. When the same animals were inactive for a longer period, the latency between stimulus onset and the beginning of depolarization was between 8 and 15 ms. Apparently, the latency of the active reaction is longer than that of the resistance reflex in the inactive animal.

Three recordings were made from a slow extensor tibiae motor neurone (SETi). When the extracellular recording showed an active reaction (22 times), the response of SETi was identical to that of FETi: it was first hyperpolarized and then depolarized (Fig. 2A). The responses of FETi and SETi during active reactions contrasted sharply with their behaviour in the inactive animal (Fig. 1).
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Recordings were made from nine flexor motor neurones in six animals. It was not possible to characterize individual flexor neurones (for identification see Materials and methods) because no myograms were recorded in parallel. Fig. 2B compares a typical response during an active reaction in the F2 recording with the response in an inactive animal. Each flexor motor neurone showed this resistance reflex during the inactive state but in most cases the amplitude was lower. During every active reaction (56 times) the flexor motor neurones were first depolarized and then strongly hyperpolarized (reflex reversal compared with the inactive state). If, before stimulus onset, the flexor motor neurone was only weakly excited, the initial depolarization was greater (Fig. 3C) than when the neurone was already strongly depolarized (Figs 2B, 3A). The hyperpolarization always coincided with the beginning of firing of FETi and SETi, and although it could show varying steepness (compare Fig. 3A and C from the same cell), it was always so pronounced that no flexor motor neurone action potentials were recorded during FETi and SETi activity (Fig. 3A). In active, unstimulated animals FETi and SETi usually spiked at different times from the flexor soma. However, occasionally both would spike simultaneously (see end of Fig. 3B).

Four recordings were made from a retractor unguis motor neurone (two from a fast and two from a slow motor neurone – the method of characterization of these motor neurones is unpublished). These motor neurones were difficult to identify because the soma spikes were very small. The retractor unguis neurone always behaved like a flexor motor neurone during an active reaction (26 times, Fig. 3D), but showed no clear response to chordotonal organ stretch in the inactive animal. In the active, unstimulated animal these neurones were usually inhibited when FETi and SETi were strongly excited, but simultaneous excitation was observed occasionally (for example, see beginning of Fig. 3D).

Five recordings were made from a protractor coxae motor neurone (for identification see Materials and methods). When the extracellular recordings
showed an active reaction of the extensor motor neurones, the response of the protractor neurone sometimes resembled that of the FETi and SETi: it was hyperpolarized during the first part of the stimulus ramp and strongly depolarized towards the end of the ramp (Fig. 4A). Often the initial hyperpolarization was slight or totally absent. These two variations were sometimes difficult to distinguish and were observed 52 times. In most cases the transition from hyperpolarization to depolarization in the protractor neurone did not coincide with the onset of extensor action potentials, but it was always after the middle of the stimulus. Sometimes maximum depolarization was reached considerably later (Fig. 4B). For 12 other active reactions the protractor neurone exhibited a different behaviour: there was no initial hyperpolarization, and depolarization either occurred before the middle of the stimulus ramp (Fig. 4C) or not at all. In a

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Fig. 3. (A–C) Intracellular recordings from flexor motor neurones (middle traces) and simultaneous extracellular recordings from the F2 nerve (extensor motor neurones). (A,C) Active reactions; (B) example of coactivation of FETi (large spikes in top trace) and flexor motor neurone at the end of the record. Chordotonal organ not stimulated; (D) simultaneous recordings from the F2 nerve (top trace) and a retractor unguis motor neurone (intracellular) during an active reaction.
Fig. 4. Simultaneous recordings from the F2 nerve (extensor motor neurones) (top traces) and a protractor motor neurone (intracellular) (middle traces) during active reactions produced by stretching of the chordotonal organ (bottom traces, upwards). During the two active reactions in A the neurone behaves like FETi and SETi (compare with Figs 1, 2A). In B and C the neurone behaves differently.

few cases there was no clear relationship between stimulus and response even though the extensor motor neurones showed an unambiguous active reaction. Inactive animals gave no clear response to the stimulus, and in active unstimulated animals there was no apparent correlation between the excitation level of the protractor coxae motor neurone and that of the extensor motor neurones.

**Extracellular recording from the motor neurones of the extensor tibiae, retractor unguis and protractor coxae muscles**

In general, chordotonal organ stimulation was less likely to elicit an active reaction during intracellular recording than during extracellular recording. As this difference was probably attributable to the preparation procedure, which might
also have changed the coupling between the motor neurones of different muscles, additional extracellular recordings were made. Decerebrate animals were immobilized as for intracellular recordings. In contrast to the experimental set-up used previously (Bässler, 1986b), most of the flexor tibiae muscle was removed so that the campaniform sensilla probably received very little stimulation from muscle contractions. The chordotonal organ was stimulated as in the preceding section.

Extracellular recordings were made from the F2 nerve, containing FETi, SETi and CI1, in every experiment. Additional recordings were made from the tip of the femoral retractor unguis muscle, using a wide-tipped suction electrode, or from nerve n12, containing the protractor motor neurones, or both.

In 63 of the 72 recorded active reactions in the extensor motor neurones, the excitation in the retractor unguis motor neurones increased during the first part of the stimulus and decreased towards its end (Fig. 5). The decline in retractor unguis firing rate always coincided with the onset of FETi and SETi discharge, but sometimes did not reach zero (Fig. 5A). In the remaining nine cases there were practically no retractor unguis impulses during the stimulus ramp. Earlier experiments (Bässler, 1986b) have shown that the force generated by the retractor unguis muscle is always strictly correlated with flexor force, i.e. it increases during the first part of an active reaction and declines to zero in the second half. The instances found here in which retractor unguis activity did not decline completely to zero could have resulted from the fact that the campaniform sensilla were not stimulated by contraction of the flexor tibiae muscle as was possible in the earlier experiments.

In active, unstimulated animals the discharges of the extensor motor neurones and the retractor unguis motor neurones usually alternated. However, coactivation was sometimes observed (see Fig. 5C, beginning of trace).
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Fig. 6. Simultaneous extracellular recordings from the F2 (extensor motor neurones) and the n12 (protractor motor neurones) nerves during active reactions (referred to by letter in the text) produced by stretching of the chordotonal organ (bottom traces, upwards). In B and C the responses of the protractor motor neurones parallel that of FETi and SETi; in A and E they behave differently.

The protractor motor neurones also gave an active response during 138 of the 176 active reactions scored for the extensor motor neurones (Fig. 6B,C), but the onset of firing in extensor and protractor motor neurones only rarely coincided (see also Bässler, 1986b). During 38 active reactions in F2, the discharges of protractor and extensor motor neurones were not coupled (Fig. 6A,E).

Apparently, there is only a weak coupling between the extensor-flexor system and the protractor motor neurones, and a stronger, but not absolute, coupling with the retractor unguis motor neurones. Therefore, the following experiments were restricted to the extensor-flexor system.

Velocity-dependence of the active reaction

Active reactions occur only if the stimulus velocity is relatively low. They are rare for stimulus durations of less than 300 ms (Bässler, 1986b), but it has not been investigated whether another reaction type occurs instead. To answer this question the earlier recordings were reanalysed, and new experiments were carried out on five decerebrate animals as follows. Animals were immobilized dorsal side up and all legs except for the right foreleg were amputated. This leg was fixed at an angle of about 60° to the animal's longitudinal axis, and the extensor tibiae muscle was removed to expose the F2 nerve for extracellular recordings. Myograms were recorded simultaneously from the distal half of the flexor tibiae muscle, and the chordotonal organ was stimulated in the usual manner. Animals were evaluated only if they showed at least 70 % of active reactions with slow ramp speeds.

At ramp rise times of less than 100 ms the extensor motor neurones were always excited during the first half of the stimulus. The animals thus responded with a resistance reflex to high-velocity stimuli. For stimulus durations between 100 and 300 ms there could be either a resistance reflex or an active reaction (onset of extensor discharge after the middle of the stimulus). Often there was no distinct response. In this velocity range the same animal could respond with either a resistance reflex or an active reaction. At stimulus durations longer than
300–500 ms active reactions predominated (see examples in Fig. 7, compare with Fig. 10A).

Thus, the response to stimulation of the chordotonal organ depended on how fast it was stretched. The extensor motor neurones were excited during the first half of the stimulus by higher ramp velocities, and the flexor motor neurones by lower velocities. A further decrease in ramp velocity did not affect the transition from flexor to extensor activity (Bässler, 1986b).

**Stimulation of the Tr1 nerve and the femoral chordotonal organ**

Stimulation of the trochanteral campaniform sensilla appears to inhibit the transition from stance to swing phase in the walking animal (Bässler, 1977; Cruse,

![Graph showing simultaneous extracellular recordings from the F2 nerve (upper traces) and a flexor myogram (middle traces) during different velocities of chordotonal organ (ChO) stimulation. First and second line from one animal, third line from another one. Typical active reactions only occur with slow ramp velocities.](image)
1985a,c; Pearson, Fourtner & Wong, 1973), whereas a sudden cessation of stimulation appears to trigger the transition from stance to swing phase (Bässler & Wegner, 1983; Cruse, 1985c; Bässler, 1986b). In none of these experiments was the stimulus strictly defined.

In the present experiments the Tr1 nerve was stimulated electrically. This excites axons coming from the campaniform sensilla fields 2, 3 and 4 and from the hairfield trHP. The campaniform sensilla can respond either tonically or phasotonically to frontward or rearward loading of the coxa-trochanter joint. Units that respond to rearward loading are more common (Hofmann & Bässler, 1986). Although an electrical stimulus is physically reproducible and precisely defined, physiologically it is, if anything, less precise than the stimuli used in the studies cited above, because it probably generates a combination of afferences that does not normally occur. For example, nerve stimulation may correspond to signals from the campaniform sensilla signalling that the femur was bent simultaneously towards the front and towards the back. Furthermore, signals from the hairfield trHP could be indicating a raised femur.

At first the forces generated by the flexor and extensor tibiae muscles were measured during stimulation of the Tr1 nerve at a frequency of 120 Hz in a preliminary experiment on five animals. The extensor force was measured directly at the cut tendon, and the flexor force was measured indirectly at the tibia. Tr1 stimulation caused the force generated by the flexor to rise and the force generated by the extensor to disappear. These results were obtained at the beginning of the experiments from all five animals, but only one animal continued to show this response after prolonged repetition of the stimulus. The responses of the other four became weaker and had longer latencies during the course of the experiment.

The results seem to be similar to those obtained with mechanical stimulation of the campaniform sensilla. As both kinds of stimuli were rather unspecific and the reactions were somewhat variable, it is not possible to state that the described responses are unequivocally attributable to a stimulation of campaniform sensilla axons. Tr1 nerve stimulation can therefore only be used as a stimulus changing the responsiveness of motor neurones to chordotonal organ stimulation. This gives insight only into the way the chordotonal organ afferences are processed but makes no deductions possible about the processing of campaniform sensilla afferences.

In seven more animals a rampwise stretch of the chordotonal organ with a rise time of 0.5 s was presented either alone or in conjunction with longer-lasting stimulation of the Tr1 nerve at a stimulus frequency of 120 Hz. Before the start of a series of experiments the animals were activated by touching their abdomens. Five of the seven animals gave an active reaction to over 50% of the chordotonal organ stimuli, presented alone, and only these five were used for further analysis. The other two animals became inactive relatively quickly.

Fig. 8 illustrates the essential findings of this experimental series. (i) In the absence of Tr1 nerve stimulation a stretch of the femoral chordotonal organ usually elicited an active reaction, i.e. first the flexor force increased, then after a
certain position had been reached, flexor force declined and extensor force increased simultaneously. Sometimes the transition from flexor to extensor activity did not take place until shortly after the end of the stimulus, e.g. as in the animal shown in Fig. 8. (ii) The onset of Tr1 nerve stimulation raised the flexor force (or lowered the extensor force), and its offset lowered the flexor force. (iii) Stimulation of the Tr1 nerve lessened the likelihood that a stretch of the femoral chordotonal organ would trigger an active reaction. (iv) Chordotonal organ stimulation elicited fewer active reactions than normal in response to the first stimulus after the end of Tr1 nerve stimulation (Fig. 8F).

To quantify the above observations, the responses to chordotonal organ stretch were classified under seven categories. (1) Complete active reaction as described above. (2) Active reaction with gap, in which the flexor force started to decline more than 200 ms before extensor force started to rise. (3) Active reaction only from the extensor. During the stimulus the flexor force was zero or, more rarely, at low constant value. The extensor force was either already at zero or, less commonly, declined at stimulus onset. Towards the end of the stimulus it increased sharply. (4) Active reaction only from the flexor. The flexor behaved normally; no increase in extensor force was detectable towards the end of the stimulus (see examples in Fig. 9). (5) The transition from flexor to extensor...
activity occurred during the first half of the stimulus, in most cases right at the beginning of the stimulus. Because this corresponds to the behaviour of the inactive animal (resistance reflex), the response type was called the 'inactive reaction'. (6) No reaction. Flexor and extensor forces did not change noticeably during or immediately after the stimulus. (7) No classification. Although flexor and extensor forces changed during the stimulus, the change could not be classified under any of the reaction types 1–5.

As the transition from one reaction type to the next was gradual, assigning a response to a particular category was sometimes difficult. For example, response D in Fig. 8, which was classified as a 'complete' active reaction, differs only slightly from the responses shown in Fig. 9, which were all classified as 'only flexor' responses. The latter are not very different from responses E and C in Fig. 8, which were classified as 'no reaction'.

Fig. 10 shows the relative frequency distribution of the above response types for two situations. The first was without Tr1 nerve stimulation (Fig. 10A). After a Tr1 stimulus the response to the first chordotonal organ stimulus was never included (see above). The second was with Tr1 nerve stimulation (Fig. 10B). Stimulation of the Tr1 nerve had two noteworthy effects: it decreased the relative frequency of active reactions, and within the active reaction group it increased the relative frequency of only flexor reactions.

A closer look at the only flexor reactions revealed that without Tr1 nerve stimulation the flexor forces for this response type were often slight. Forces over 0.5 mN were measured for only 12 responses, and in all these cases the flexor force fell to zero at the end of the stimulus. There is some probability that in many of these weak responses the extensor force was too low to be detected, i.e. they were complete, but weak active reactions. Tr1 nerve stimulation not only increased the relative frequency of the response type, it also changed the shape of the response. Now, large flexor forces occurred more often. In 18 of 25 responses with maximum flexor forces higher than 1 mN, flexor force fell to zero at the end of stimulation.
Fig. 10. Relative frequency distribution of the different reactions to a stretch of the femoral chordotonal organ. For definitions of the categories and details of the stimulation see text. (A) Tr1 nerve unstimulated \( (N = 265) \). (B) Tr1 nerve stimulated at a stimulus frequency of 120 Hz \( (N = 187) \).

(see Fig. 9A). However, in the other seven cases, although the forces declined, they remained well above zero (see Fig. 9C).

Only 23 of the 57 responses to chordotonal organ stimulation directly after Tr1 nerve stimulation could be classified as active reactions, and most of these were weak or incomplete.

**Discussion**

*The active reaction is the result of chordotonal organ stimulation*

Several lines of evidence suggest that indirect influences from other sense organs are not involved in the generation of an active reaction in the way that the chordotonal organ induces reflex muscle contractions that in turn stimulate other sense organs and induce other reflexes. (i) The latency of the first part of the active reaction in the FETi is only 15–30 ms but the latency for force increase in the flexor or extensor tibiae muscles is approximately 30 ms (Storrer & Cruse, 1977). (ii) The active reaction could be released when either the flexor tibiae muscle or the extensor tibiae muscle (see also Bässler, 1986b) has been removed or when the nerves from the trochanteral campaniform sensilla have been cut.
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The active reaction may be a component of the walking programme

This has already been postulated on the basis of earlier experimental findings (Bässler, 1986b). The results presented here lend further support to the hypothesis that the first part of the active reaction corresponds to the stance phase and the second part to the swing phase: during constant stimulation of the campaniform sensilla on the trochanter, free-walking animals can terminate the stance phase, but are unable to begin the swing phase (Bässler, 1977). This corresponds to the increase in only flexor responses after Tr1 nerve stimulation. For further evidence see below.

The walking pattern generator may consist of subunits, which are only loosely coupled centrally

The results presented above show that the motor neurones of different muscles are only loosely coupled during an active reaction. The coupling strength varies markedly. In every case in which an active reaction occurred in the extensor motor neurones, the responses of FETi and SETi were similar. The excitation of Cl1 (Hale & Burrows, 1985) also increased slightly towards the end of the stimulus (Bässler, 1986b). The response of Cl1 in the inactive animal corresponds to that during an active reaction, but baseline activity is lower (Bässler et al. 1986). Hence, in contrast to FETi and SETi, the response of Cl1 is not changed when the animal goes from the inactive to the active or intermediate state. During all active reactions in nerve F2, the flexor motor neurones also showed an active response, i.e. they were excited during the first part of the active reaction and inhibited during the second part. The retractor unguis motor neurones usually also showed an initial increase in activity and then a decline in activity at the end of the stimulus, but sometimes their activity did not fall completely to zero or did not appear during the first part of the active reaction. The protractor motor neurones responded only in part like the extensor motor neurones. Thus, during an active reaction the FETi, SETi and flexor motor neurones appear to be strongly coordinated with each other. The retractor unguis motor neurones are not completely coupled to these neurones and the coupling of the protractor motor neurones is even weaker. In earlier experiments (Bässler, 1986b) this coupling was also not complete, although it was stronger than reported here. Possibly this is because in the earlier experiments contractions of the flexor tibiae muscle stimulated the trochanteral campaniform sensilla, which could in turn inhibit the protractor motor neurones (see Bässler & Wegner, 1983).

Thus the neural generator for the active reaction is apparently composed of subunits that are only loosely coupled with each other. This would make sense for a component of the walking pattern generator. The stick insect foreleg is able to walk forwards, backwards and sideways (during turning), and the coordination of the movements of individual joints must be different for each direction. Grillner (1981) proposed that the walking pattern generator is composed of subunits that have a great deal of independence from each other. In the walking leg these
subunits appear to be coupled partly \textit{via} the sensory systems and partly \textit{via} the central nervous system.

Evidently, the subunit responsible for the femur–tibia joint is used not only for walking but also for other active movements. The following section demonstrates that the findings presented here also describe the active movements of restrained, non-walking animals.

\textit{The first part of an active reaction is likely to control the velocity of a flexion movement}\n
At low stimulus velocities, the first part of the stimulus ramp excites the flexor motor neurones. As the stimulus signals a flexion of the femur–tibia joint, this is a positive feedback. It would speed up the flexion in the closed-loop system. High ramp velocities excite the extensor motor neurones, slowing down the flexion in the closed system.

Flexions of the femur–tibia joints of restrained animals do, in fact, appear to be under velocity control (Weiland & Koch, 1987). The switch from acceleration to deceleration usually occurred here with ramp rise times between 100 and 300 ms. Since the amplitude of the ramp stimulus was equivalent to a joint movement of about 70°, the switch occurred at joint velocities between 230 and 700°s\(^{-1}\). Most values for velocity of flexion movements measured in intact, restrained animals lie in this range (G. Weiland, personal communication).

There also appears to be velocity control in free-walking animals during the stance phase (Cruse, 1985b) and the swing phase (Dean, 1984). Hence, all findings indicate that the first part of the active reaction is responsible for the control of the velocity of flexion movements during walking as well as during active movements of restrained legs.

\textit{The velocity-sensitive and position-sensitive units of the chordotonal organ contribute to the active reaction}\n
The first part of the active reaction is velocity-dependent, but the transition to the second part is position-dependent (Bässler, 1986b) so that both position- and velocity-sensitive units must contribute to the response. A velocity signal of low value apparently excites flexor and inhibits extensor motor neurones whereas a position signal indicating joint flexion excites extensor and inhibits flexor motor neurones. The simplest kind of interaction of these two signals would be a superposition of velocity and position effects. But then the depolarization of flexor motor neurones and the hyperpolarization of extensor motor neurones should decline steadily during the whole stimulus. Instead, the decline was initially very small or zero and increased markedly immediately before or after stimulus offset. This is the behaviour of a threshold effect (relaxation oscillator): when a certain parameter reaches the threshold the flexors are inhibited and the extensors are excited. Apparently the signals for velocity and position contribute to this parameter. But the parameter is not formed by a simple superposition of the two signals, because the transition from flexor to extensor activity is independent of
velocity (Bässler, 1986b) and high velocities excite the extensor motor neurones. Apparently, the active reaction is formed by a complicated neuronal relaxation oscillator which receives input from the position and the velocity signal of the chordotonal organ.

In the crayfish, *Pacifastacus leniusculus*, a backwards movement of the leg at the thorax–coxa joint is registered by the TCMRO, a muscle receptor organ in that joint. Stimulation of the TCMRO leads first to an excitation of the remotor motor neurones. This positive feedback is produced by the velocity-sensitive cell of the TCMRO (Skorupski & Sillar, 1986). When the position-sensitive cell of the TCMRO signals that a leg has reached its posterior extreme position, the remotor motor neurones are inhibited and the promotor motor neurones are excited (Sillar, Skorupski, Elson & Bush, 1986). The great similarity between this reaction and the active reaction of *Cuniculina* suggests that this mechanism also plays a role in the control of walking in other animals.

**Interaction of Tr1 nerve afferences and chordotonal organ afferences**

Electrical stimulation of the Tr1 nerve excited many of the axons leading from the campaniform sensilla of fields 2, 3 and 4 on the anterior surface of the trochanter and from the hairfield trHP (Hofmann & Bässler, 1982). The individual axons of the campaniform sensilla signal load in different directions; some react tonically and some phasotonically to a constant load (Hofmann & Bässler, 1986). Electrical stimulation generates constant excitation in receptor axons that would not be simultaneously active under natural conditions.

Electrical stimulation of the Tr1 nerve had two effects on the formation of the active reaction: it decreased the number of active reactions and usually only the flexor tibiae muscle was excited in the active reactions that did occur. What can be deduced from these facts? The extensor motor neurones are excited by the joint reaching a moderately flexed position, as well as by the end of Tr1 nerve stimulation. There are two possible hypotheses for the interaction of these two sensory inputs (given that the active reaction is formed by a relaxation oscillator).

1. The afferences of the Tr1 nerve contribute directly to the formation of the active reaction. For example, the signal from the campaniform sensilla is added to the velocity and position signal from the chordotonal organ to form the parameter which is responsible (by reaching a threshold) for the release of the transition from flexor to extensor activity. (2) The chordotonal organ afferences (signals for velocity and position) are first processed separately to produce the active reaction in the way described above. The signal from the Tr1 nerve afferences is introduced on a second level and its effect is simply superimposed on the active reaction.

A system according to hypothesis (1) would (in the case of Tr1 nerve stimulation) (i) decrease the number of active reactions and (ii) delay the transition from flexor to extensor activity in the active reactions that do occur because the parameter would reach the threshold not at all or later. Additionally (iii), the active reactions that do occur during Tr1 nerve stimulation should be normal (except for the delayed transition); there is no reason for only flexor
reactions which do not decrease to zero. Although i was observed, there was no indication of ii and iii.

A system according to hypothesis 2 would not delay the transition from flexor to extensor activity under Tr1 stimulation and it would bias the whole active reaction towards flexion so that all kinds of only flexor reactions would be possible. But, it should not decrease the frequency of active reactions. These predictions as well are only partly observed. Apparently, only a combination of both hypotheses is able to explain all the experimental data. The following hypothesis explains all the results: the chordotonal organ afferences are processed in a first step to form the active reaction. The result of this first step resembles the motor output without Tr1 nerve stimulation. The reactions generated by Tr1 nerve stimulation are incorporated in a second step, shifting the active reaction in the direction of flexor activity. The decrease in frequency of active reactions as a result of Tr1 nerve stimulation indicates that these afferences must also affect the formation or, more probably, the expression of the active reaction, but not in the manner suggested by the first hypothesis. Perhaps the afferences decrease the amplitude of the response to chordotonal organ stimulation so that, although the active reaction is still formed, its amplitude is below measurable limits. This would explain why responses classified as no reaction during Tr1 nerve stimulation often included very weak only flexor responses (e.g. Fig. 8B,E).

According to these considerations the afferences from the chordotonal organ are processed in two steps. In the first step, signals from the velocity- and position-sensitive units produce an excitation pattern that closely corresponds to the excitation pattern of the motor neurones during an active reaction. Afferences from the Tr1 nerve decrease, for unknown reasons, the probability of the occurrence of an active reaction. In the second step, a constant excitation, such as is generated by Tr1 nerve stimulation, can be added to this excitation pattern.

The latency of hyperpolarization of FETi during an active reaction is longer than that of depolarization during a resistance reflex. This indicates that the pathway of the active reaction is longer than that of the resistance reflex. As during active reactions an initial depolarization of FETi was not present, one has to conclude that the fast pathway of the resistance reflex is switched off in the active animal.

The indirect conclusions reached here do not contradict the present understanding of how typical thoracic reflexes are organized in other Orthoptera (for summaries see Siegler, 1984; Burrows, 1987). There are two types of local interneurones: spiking ones and nonspiking ones. The longest pathway is from afferents to spiking and then nonspiking interneurones and finally motor neurones. Shorter reflex pathways are: afferent to spiking interneurone to motor neurone, afferent to nonspiking interneurone to motor neurone and monosynaptic connections between afferents and motor neurones. In the locust, afferences from the femoral chordotonal organ can synapse directly upon a motor neurone and a spiking local interneurone (Burrows, 1987). The direct connections between afferences and motor neurones (and perhaps also the bisynaptic pathways) might
be the basis for the resistance reflex. It remains an open question how these reflex pathways are switched off during the active reaction. The active reaction might use the longest pathway. The active reaction would then be produced by the spiking interneurones. Indeed, Burrows (1985, fig. 6D) has described the response of a spiking local interneurone that resembles an active reaction. Nonspiking interneurones would be the basis for amplitude reduction and the superposition of the effects of Tr1 nerve stimulation. As the nonspiking interneurones also project onto premotor nonspiking interneurones of other joints, they would also provide the basis for the weak central coupling between individual joints.

The concept proposed here contradicts two important other concepts: the hierarchical command structure (for a summary see Kupfermann & Weiss, 1978) and the central pattern generator (CPG, e.g. Delcomyn, 1980). However, it is similar to the half-centre model (for a summary see Lundberg, 1981).

The proposed concept does not require a CPG that can generate a rhythmic output without afferences. This agrees with other results on stick insects (Bässler & Wegner, 1983) and cockroaches (Pearson, 1985; Zill, 1986).

Under natural conditions stimulation of the campaniform sensilla is not independent of the stimulation of the chordotonal organ. The flexor tibiae is the most powerful muscle in the prothorax and therefore the main source of load on the trochanter. When the femoral chordotonal organ triggers the transition from the first to the second part of the active reaction, flexor force declines, decreasing the load on the trochanter and hence the excitation of the campaniform sensilla. Decreased excitation of the campaniform sensilla seems to lessen flexor excitation, thus reinforcing the effect of the chordotonal organ afferences. It can be assumed that during free walking the transition from the stance to the swing phase always occurs when the force of the flexor tibiae (and other stance phase muscles) falls below a certain threshold.

According to this concept, the decision to switch from stance to swing phase is made at the lowest level, i.e. the pattern generator of a single leg. Higher authorities (e.g. coordinating influences) that ‘want’ to modify this decision must be incorporated in this decision process, i.e. they must raise or lower the flexor force. Coordinating influences have, indeed, been shown to change the strength of the motor output of the other legs (Cruse & Saxler, 1980; Bässler & Wegner, 1983; Bässler, Dübner & Fahrig, 1987). Thus, according to the proposed concept, a higher authority would not be able to command the transition from stance to swing phase. It would only be able to submit a ‘recommendation’ to the ‘democratic decision-making process’ at the lowest level.

The first draft of this article was translated by Camilla Strausfeld.

References


Walking in stick insects


