Cooperative Mechanisms Between Leg Joints of *Carausius morosus* II. Motor Neuron Activity and Influence of Conditional Bursting Interneuron

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Brunn, Dennis E. and Antje Heuer. Cooperative mechanisms between leg joints of *Carausius morosus*. II. Motor neuron activity and influence of conditional bursting interneuron. J. Neurophysiol. 79: 2977–2985, 1998. The activity of the motor neuron pools of the protractor coxae muscle and of the thoracic part of the depressor trochanteris muscle during forward walking in the stick insect was investigated, and a spiking local interneuron, able to produce "endogenous bursting" and innervating both motor neuron pools, was identified. Extracellular recordings of the motor neurons innervating the protractor and the thoracic depressor of front, middle, and rear legs, respectively, were made with oil-hook electrodes from the peripheral nerves n2c and n4a while the animals were walking on a styrofoam treadwheel. The corresponding leg movements were registered and phase histograms were created with the software Spike2. Intracellular recordings were made in the neuropile of the metathoracic ganglion with glass electrodes filled with the dye Lucifer yellow. In all three legs measured (front, middle, and rear), both motor neuron pools increased their activity during the swing movement. The increase in the activity of the protractor motor neurons started at the end of the stance ~100 ms before reaching the posterior extreme position (PEP), and the activity of the large-sized depressor motor neurons increased as soon as the tarsus was lifted at the PEP. A local spiking interneuron was identified that excited both motor neuron pools. In 4 of 23 recordings the interneuron started to burst in synchrony with protractor and thoracic depressor motor neurons. During bursting a depolarizing stimulus reinforcer and a hyperpolarizing stimulus inhibited the activity of both motor neuron pools. Thus we conclude that the thoracic part of the depressor trochanteris muscle might be a component of the neuromuscular system that shapes the swing movement. The two proximal joints, subcoxal and coxa-trochanter, connected mechanically via the thoracic part of the depressor trochanteris muscle, are also connected neurally by segmental and intersegmental spiking interneurons (this paper) and by nonspiking local interneurons (see companion paper).

INTRODUCTION

Insect locomotion always requires the use of many muscles and joints tuned well to each other. Speed and walking direction may be changed quickly because of changes in the environment, and all muscles contributing to leg movements are coordinated in slightly different patterns depending on the walking condition itself (Duch and Pflüger 1995). However, during undisturbed forward walking the same sets of muscles are activated repeatedly, thus leading to a quasi-rhythmic motor output. Although stick insect walking is strongly influenced by sensory inputs (Bässler 1977; Cruse et al. 1984; Graham 1985), steady walking, like other rhythmic motor behaviors, requires a pattern-generating neural network. A prime example of a pattern-generating network and an object of extensive investigation on all levels is the stomatogastric system of crustaceans (Elson and Selverston 1992; Harris-Warrick 1988; Selverston and Moulins 1985). Leech swimming (Friesen 1989), locust flight (Robertson and Pearson 1985), and lamprey locomotion (Grillner et al. 1991) are other well-investigated rhythmically active systems.

In all described pattern-generating networks, rhythmically active interneurons are known to be an essential element. In several systems these rhythmically active neurons are conditional; i.e., their ability to produce rhythmic behavior depends on modulatory inputs either centrally or peripherally (Bal et al. 1988).

The muscarinic agonist pilocarpine activates the pattern-generating network underlying locomotion in arthropods, thus leading to a rhythmic activity in motor neurons and in some interneurons (Büsches et al. 1995; Chrachri and Clarac 1990; Ryckebusch and Laurent 1994). In some species the activated pattern can be very similar to the activity pattern during walking and is therefore called "fictive locomotion" (Chrachri and Clarac 1990). In locusts, spiking local interneurons have been described that were rhythmically active during both fictive locomotion (induced by pilocarpine) and during walking on a treadwheel (Wolf and Laurent 1994).

In contrast to some well-known central pattern generators in the crustacean stomatogastric system, little is known about the interneurons involved in the generation and maintenance of rhythmic walking behavior in stick insects. Pilocarpine-activated preparations revealed nonspiking interneurons whose membrane potential was modulated in synchrony with the rhythmic activity of motor neurons of one or more joints of the leg (Brunn 1993; Büsches 1995); but so far no spiking interneuron has been described affecting the motor neurons of one or more joints and producing an endogenous oscillating activity.

This paper reports first on the cooperation of the two proximal joints, the subcoxal and the coxa-trochanter, of all three legs (front, middle, and rear) of the stick insect *Carausius morosus* during walking, and then on an identified conditional bursting interneuron that influences the activity of both joints of the rear leg.
METHODS

Preparation

Adult female stick insects (C. morosus Br.) raised in our laboratory colony were used in all experiments.

To investigate the phase relationship between the subcoxal and coxa-trochanter joint of walking stick insects, animals were attached dorsal side up to a balsawood stick (pattex adhesive) mounted on top of a metal holder. Carvings in the balsawood corresponding to the six coxae sites made sure that the animals could perform undisturbed walking. Head with antenna and the abdomen could also move freely.

The holder was positioned above a 1.9-cm-wide lightweight styrofoam wheel of 40-cm diam mounted vertically. The distance between the insect’s coxa and the wheel’s surface was 7 mm (the moment of inertia of the wheel was 1.8 \times 10^{-5} \text{ kg m}^2). Walking on such a lightweight treadwheel proved very similar to free walking (Cruse and Bartling 1995; Graham 1981).

Dissection

For both extra- and intracellular recordings the animal was opened dorsally along the midline above the pro-, meso-, or meta-thoracic ganglion. The gut was removed to expose the ganglia and the peripheral nerves. To slightly spread the tergal halves, pins were inserted into the pleurae and the so-formed cavity was filled with saline (modified after Graham and Wendler 1981). If necessary, fatty tissue was removed from around nerves nl2c and nl4a.

Extracellular activities were recorded by oil-hook electrodes (Schmitz et al. 1988) from the nerves nl2c and nl4a. These nerves comprise the motor neurons of the protractor muscle (nl2c) and the thoracic part of the depressor muscle (nl4a) (Graham 1985; Marquardt 1939). The movements of the legs were monitored by means of a photodiode system (von Helversen and Elsner 1977). Therefore a small piece of light-reflecting foil was attached to the end of the femur of the left front, middle, or rear leg, respectively. The system provides a continuous, analogous signal corresponding to the forward-backward movement of the femur. This signal was stored on a DAT-recorder (Bio-logic) together with the extracellular recordings that were conventionally filtered and amplified. By lightly striking the abdomen with a paint brush, the stick insect was encouraged to walk forward.

To monitor the walking movements of all six legs the animal was recorded on VCR (CCD camera) from above. Only those sequences were used for evaluation where all six legs performed a coordinate walk for at least five successive steps. A total of 1,143 steps were evaluated with step frequency varying from 0.5 to 1 step/s. Data were obtained from a total of 16 animals.

For evaluation all signals were collected into a computer via a CED 1401+ (Cambridge Electronic Design). The upward deflection in the leg position trace corresponds to the swing movement (Fig. 1). Phase histograms were created with the Spike2 software (Cambridge Electronic Design). The start of each swing was marked and served as a trigger point to define the step cycles (Fig. 1; /)

See the companion paper (Brunn 1998) for methods of intracellular recording and staining.

RESULTS

Anatomic situation

The leg of the stick insect has four joints. This paper deals with the two proximal ones, the subcoxal joint between coxa and thorax and the coxa-trochanter joint. The cooperative action of these two joints is mainly required for generating the swing movement, which is not only a movement in a horizontal plane but also in a vertical one. The result is a movement along a curved trajectory.

The up and down movement is performed mainly at the coxa-trochanter joint. The trochantero-femur is moved by two muscles, the levator and the depressor trochanteris. The depressor trochanteris muscle consists of a coxal and a thoracic part (Marquardt 1939). The coxal part is located totally within the coxa, whereas the thoracic part inserts at the thoracic wall and spans the subcoxal joint to attach at the rim of the trochanter (see Brunn 1998, Fig. 1, scheme of leg and muscles).

The thoracic part is innervated by the lateral nerve nl4a (Graham 1985). Only this thoracic part is object of this paper and will be referred to hereafter as the “thoracic depressor.”

The thoracic depressor is innervated by at least nine motor neurons (the maximal number of somata we found by backfill staining). Depending on their spike amplitude, the neurons can be divided into four classes. Combined intra- and extracellular recordings showed that the two classes with the largest and second largest spikes each consist of two motor neurons (Brunn, unpublished results). Intracellular recordings of the thoracic depressor muscle fibers (Heuer, unpublished data) and investigations of the resistance reflex
Protractor motor neuron activity

As can be seen in Figs. 2 and 3 the activity of the protractor motor neurons of all three legs started to increase \( \sim 130 \) ms before the onset of the swing movement. The increase continued during the swing movement. After having reached the maximum, the activity of the motor neurons of the middle leg (Fig. 3A) and the rear leg (Fig. 3B) decreased quite rapidly to a level of low activity that continued during the stance phase. The protractor motor neurons of the front leg (Fig. 2) behaved similarly, although the course was smoother and did not show the sharp peak as in the mesothoracic segment (Fig. 3).

Thoracic depressor motor neuron activity

The activity of the thoracic depressor motor neurons was not uniform. In the front leg two clearly different activity patterns could be distinguished. In three of the seven animals \( (n = 186\) steps) the activity of the thoracic depressor motor neurons increased slightly with the ongoing stance phase (Fig. 2A); in the remaining four animals \( (n = 231\) steps) an increase of thoracic depressor activity took place with the lifting of the leg (Fig. 2B). The difference in these two types of motor output was also observed on the behavioral level. In the first case the front leg movements remained predominantly in a rostral area. After a short stance that rarely exceeded the position of the front leg’s coxa, the swing of the leg ended in an anterior position where it often

Activity pattern during walking

Data were obtained from seven animals (417 steps) in the prothoracic segment, four animals (386 steps) in the mesothoracic segment, and five animals (340 steps) in the metathoracic segment.

Figure 1 shows examples of the activity pattern of the protractor and thoracic depressor motor neurons of the mesothoracic and metathoracic segment. The activity of the protractor motor neurons (nl2c) and the motor neurons of the thoracic depressor (nl4a) are shown together with the corresponding leg position signal.

To show the temporal relation between nl2c and nl4a activity, phase histograms are shown (Figs. 2 and 3). All phase histograms start at the beginning of the swing movement, i.e., 0 corresponds with the posterior extreme position (PEP; see Fig. 1).
remained while performing searching movements. In the second case the leg moved immediately caudad after the swing, thus performing a normal stance movement. At the end of the stance movement the neuronal activity in nL4a decreased almost completely.

In the middle leg the thoracic depressor motor neuron activity increased with the start of the swing movement at the PEP nearly as sharply as the activity of the protractor motor neurons and reached nearly the same level (Fig. 3A). The course of the decrease of both motor neuron pools was approximately identical, but the protractor neurons increased their activity again at the end of the stance movement. The activity of the thoracic depressor motor neurons remained at a rather low level until the start of the next swing movement.

The thoracic depressor motor neurons of the rear leg showed a low but clearly biphasic activity (Fig. 3B). From nearly zero activity at the beginning of the swing, the first peak was reached shortly after the maximal activity of the protractor motor neurons. After a decrease the activity increased again with the ongoing stance movement to drop near zero at the end of the stance.

**Characteristics of a spiking interneuron driving protractor and thoracic depressor motor neurons**

The local spiking interneuron described here was recorded and stained 23 times. In Fig. 4 the morphology of this neuron is shown from dorsal and from sagittal. A typical distinguishing morphological feature was the bifurcations drawn separately in Fig. 4B.

In all preparations with successful staining the morphology of these 23 neurons was so much alike that we consider these neurons to be one single individual interneuron. In all preparations only small depolarizations were observed when the left rear leg or other parts of the animal’s body were stimulated. However, a depolarization of this neuron excited...
several neurons in nerve nl2c and a large unit in nerve nl4a (Fig. 5). A hyperpolarization inhibited an ongoing activity of nl4a neurons (Fig. 6; see also Fig. 8B). At the end of the hyperpolarizing stimulus a postinhibitory rebound excitation occurred in the interneuron and the motor neurons.

The most interesting feature of this interneuron is its ability to produce bursts spontaneously, i.e., without the use of pharmacological agents like pilocarpine. This happened in 4 of the 23 successful recordings. As shown in Fig. 7, the interneuron started to burst rhythmically and in synchrony with bursts of some motor neurons in nl2c and with one of the large units in nl4a.

In another preparation, depolarization of this interneuron reinforced the activity in nl2c and nl4a and delayed the start of the next burst (Fig. 8A). Hyperpolarizations inhibited the activity of both motor neuron pools (Fig. 8B).

Both motor neuron pools are driven nearly simultaneously, not only by local interneurons (for nonspiking interneurons, see companion paper, Brunn 1998) but also by intersegmental interneurons. As shown in Fig. 9 an intersegmental interneuron (morphologically identified) also drives these motor neuron pools, generating a series of bursts that last longer than the stimulus set by the experimenter.

**DISCUSSION**

Stick insect walking has been an object of investigation for over 30 yr (Bässler 1983; Cruse 1990; Wendler 1964). Stick insects as well as cockroaches and locusts are perfectly suited for investigating the mechanisms underlying walking and running. Both the sensory systems and muscle systems were examined thoroughly. The antagonistic muscle systems moving the three proximal leg joints (protractor-retractor coxae, (coxal) depressor-levator trochanteris, and flexor-extensor tibiae) have been of main interest (for review see Bässler 1983; Delcomyn 1985, 1993; Graham 1985). However, in stick insects the thoracic part of the depressor trochanteris muscle innervated by the lateral nerve nl4a was investigated only twice (Cruse et al. 1993; Herrmann and Schmitz 1989). The second dealt with the changes of the...
FIG. 7. Bursting activity. The interneuron spontaneously started to burst rhythmically in synchrony with nl2c and nl4a motor neurons. The activity of the interneuron and the nl4a motor neurons continued for ~3 min; activity of the nl2 motor neurons began to cease after 10 bursts. Current is in the range of 1–6 nA. See note regarding neuron labels in Fig. 1.

contraction force under the influence of proctolin, whereas Cruse et al. (1993) investigated the role of this muscle for the control of body height during walking, focusing on the coupling between the two middle legs.

Our objectives were 1) to investigate the role of this muscle during walking and 2) to assess whether this part of the depressor trochanteris muscle can actually be treated in exactly the same way as the coxal part. Unfortunately, only very few investigations exist that examined the activity of the depressor trochanteris muscle during voluntary activity (see INTRODUCTION). However, most investigators take for granted that the depressor and retractor muscles are active together mainly during the stance phase (Clarac 1978; Epstein and Graham 1983; Graham and Bassler 1981; Wolf 1992). This is certainly true for the coxal part of the thoracic depressor trochanteris (Epstein and Graham 1983).

Our investigation showed for the middle leg that the thoracic depressor motor neurons with large spike amplitudes increase their activity during the swing movement, reaching the peak of activity shortly after that of the protractor motor neurons (Fig. 3A). Cruse et al. (1993) also reported an increase in activity of these nl4a motor neurons during the swing movement.

The same is true for the front leg when performing a regular step (Fig. 2B). The activity of the nl4a motor neurons is not as high as in the middle leg, but the peak of the activity also occurs shortly after that of the protractor motor neurons.

The front legs, having more freedom of movement (Graham 1985), are often used as a second pair of antennae; i.e., in free walking they grope for the ground and do not participate in producing power (Cruse 1976). During our experiments this manifests itself in the second activity pattern (Fig. 2A). While performing searching movements, which the animals do frequently (Cruse 1976), the activity of the nl4a motor neurons is irregular and slightly enhanced when the tarsus contacts the treadwheel.

In the rear leg the peak of the activity coincides with the highest activity of the protractor motor neurons, but the neurons are also active during the stance. Thus in this leg the muscle contributes to both protraction and retraction as suggested by Graham (1985).

The observation that the two motor neuron pools, protractor and thoracic depressor motor neurons, are activated simultaneously during the swing movement deserves detailed discussion. In stick insects the end of the swing movement depends not only on simple termination of activity of those muscles (and motor neuron pools) of the proximal leg joints that generate the swing (levator trochanteris and protractor coxae). As shown by Cruse (1976) and Cruse and Bartling (1995), a considerable ventrad force is generated during the end of the swing phase. Our results imply that this force could be generated by the thoracic part of the depressor trochanteris.

The fact that neurons of these two motor neuron pools are driven nearly simultaneously by a conditional bursting local interneuron is in agreement with this interpretation. However, in contrast to local nonspiking interneurons, local spiking interneurons are thought to have only weak direct influences on motor neurons (for review see Burrows 1992). Their major role is the integration of sensory inputs and the execution of reflexes (Burrows and Siegler 1983; Nagayama and Burrows 1990). Hence the massive influence of the interneuron described here on motor neuron pools innervating muscles involved in walking is an unusual quality.

Bursting neurons are the essential part of rhythmically
FIG. 8. Bursting activity of the neuron in a different preparation. A: during ongoing bursting activity a depolarization of this interneuron reinforced motor neuron activity considerably and prolonged the time before the next burst started. B: a hyperpolarization always inhibited the motor neuron activity. Current is in the range of 1–6 nA. See note regarding neuron labels in Fig. 1.

Graham and Bässler (1981) described oscillating movements of the coxa-trochanter joint during "saluting" behavior and assumed that this joint is under the control of an endogenous oscillator or rhythmic command. The local interneuron described here could be part of this oscillator. Its rhythmic activity might either be suppressed by other neurons (Cazalets et al. 1987; Ramirez and Pearson 1993) or additional activity of rhythm-initiating influences are necessary to induce the bursting property or it may obtain rhythm input from another source. (As shown in Fig. 9 we recorded and stained an intersegmental spiking interneuron that when depolarized caused bursts of one n14a motor neuron and of several n12c motor neurons. The bursts continued after the end of the stimulus.)

The bursting property of the local interneuron was re-
vealed only in the metathoracic ganglion. In the mesothoracic ganglion a local spiking interneuron with a similar morphology as the one described in this paper was recorded. Several tonic depolarizations lasting 6–13 s produced a rhythmic bursting activity (4–8 bursts; burst duration 500–700 ms) of one nl4a motor neuron with a large spike amplitude during the time of the stimulus (Schneider 1996). In these experiments the nl2c was not recorded.

Experiments with pilocarpin-activated stick insects also support the existence of a common drive to both motor neuron pools; if the nl4a motor neurons burst, which they do very rarely, they burst mainly (7 of 10 times) in-phase with the nl2c motor neurons (unpublished data).

As in locust flight the basic rhythmic motor pattern underlying stick insect walking might be generated centrally, but with proprioceptors as important elements for both reporting environmental events as well as serving their purpose as a feedback-control system.

Together with the features discussed here that predestine this interneuron to be part of a rhythm-generating network, we regard this local interneuron as an important part of walking behavior.

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Fig. 9. An intersegmental interneuron initiating bursts in the motor neuron pools of nl4a and nl2c. A section lasting 4 s of the continuous record was removed to show the influence on the nl2 motor neurons when the stimuli are given with a short interval. Current is in the range of 1–6 nA. See note regarding neuron labels in Fig. 1.


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