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A MOVEMENT GENERATED IN THE PERIPHERAL NERVOUS SYSTEM: RHYTHMIC FLEXION BY AUTOTOMIZED LEGS OF THE STICK INSECT *CUNICULINA IMPIGRA*

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SUMMARY

Autotomized legs of the stick insect *Cuniculina impigra* bend rapidly and rhythmically at the femur-tibia joint. These flexions occur at a frequency of 1–6 Hz immediately after autotomy and decrease in frequency and amplitude with time. Each flexion is produced by a burst of 1–14 action potentials in a single motor axon of the flexor tibiae muscle (bursting axon). These rhythmic discharges are generated in a very restricted part of the crural nerve, which contains the bursting axon, close to the autotomy point and appear whenever the nerve is cut in the immediate vicinity of this generator region. Rhythmic flexion can also be elicited by electrical stimulation of the crural nerve.

The bursting axon is of small diameter. It innervates all or most of the flexor tibiae muscle in which it produces relatively large EPSPs. Each EPSP elicits one muscle twitch. These fuse into a brief tetanus, whose amplitude is proportional to the number of spikes in a burst. Each tetanus produces one flexion.

This behaviour does not occur in the autotomized legs of several related species.

INTRODUCTION

Many animals discard, or autotomize, certain parts of their body when attacked or pursued by an enemy. In some cases the autotomized body part carries out vigorous movements, which divert the attention of the pursuer. When the discarded body part contains components of the central nervous system (CNS), e.g. the autotomized tails of lizards, the generation of the motor neurone activity underlying the movements need not differ from the generation of normal motor neurone activity. However, some autotomized legs of arthropods contain no parts of the CNS yet still perform rhythmic bending movements (e.g. the Opiliones and some spiders). In the Opiliones the movements of the femoro-patellar and the tibia-basitarsal joints are generated by neurogenic pacemakers in the proximal half of the femur. Each bending movement is caused by a burst of motor impulses (see Miller, 1977). Miller called these movements

Key words: Stick insect, autotomy, burst-generation, flexor tibiae muscle.

twitches. However, since each bending movement is based on a brief tetanus, I use the word flexion instead of twitch.

Stick insects have long legs, which can be easily grasped by a predator. Probably for this reason all stick insects have a well-developed autotomy mechanism (Beier, 1968) that separates the leg with the help of a special autotomy muscle at the boundary between the trochanter and the femur (a morphologically preformed breakpoint) (Schindler, 1979). In most stick insect species the femur-tibia joint flexes briefly right after autotomy, then opens to a 90° angle where it remains. However, in one of the species raised in our laboratory, *Cuniculina impigra* Redtenbacher (syn. *Baculum impigrum* Brunner) the autotomized leg flexes rhythmically at the femur-tibia joint for a period of time. This large insect (the body of the female is about 9 cm long with forelegs about 8 cm long) presented an excellent opportunity for a neuronal analysis of this behaviour.

MATERIAL AND METHODS

Experiments were conducted on females of *Cuniculina impigra* from colonies at the University of Kaiserslautern, maintained at 26–28 °C and a relative humidity of 50–70 % under a 12: 12 light to dark cycle.

Although autotomy is most easily induced by twisting the femur around its longitudinal axis (Schindler, 1979), for most of the experiments this method could not be used because the leg had to be immobilized before autotomy. Usually the leg was amputated with a razor blade at the autotomy boundary. The results using this method were identical to those obtained after the occasional spontaneous autotomy.

The force of the movements was measured with a force transducer (Swema SG-4-25), in combination with a balanced bridge (Hellige TF 19) and a pen recorder (Hellige He 16). Extracellular recordings were made using conventional suction electrodes (for the nerve recordings) or 50-μm insulated copper wires (for the myograms) and a Grass P-15 amplifier. Intracellular recordings were made using glass microelectrodes filled with 3 mmol l^{-1} KCl (20–40 MΩ), an amplifier (WPI M701 or one built in the workshop) and magnetic tape recorder (Racal Store 4). These records were played back on a storage oscilloscope or on a pen recorder (Siemens Mingograf EEG 8 or Hellige He 18).

To measure the velocity of propagation, recordings were made using a special double electrode arrangement (U. Koch, personal communication). Each of the two electrodes consisted of a narrow chamber that was insulated from the surrounding bath and filled with insect saline in which a silver electrode with a large surface area was immersed. The electrodes were separated by 3 mm.

The technique used for preparation is described separately for each individual experiment. The animals were not anaesthetized. The experiments were carried out at a room temperature of 20–23 °C.

RESULTS

Description of the behaviour

Not all legs moved after spontaneous autotomy or amputation at the autotomy boundary. Most of them, however, performed a series of very short, vigorous flexion-

At first, the frequency of these movements was high (always greater than 1 Hz) so th[illegible] single flexions often overlapped and the leg appeared to quiver with its femur-tibia joint almost completely flexed. The frequency of flexions decreased with time. Between two non-overlapping flexions the angle formed by the femur and the tibia was usually less than 90°. At first the leg was almost completely flexed by each movement, but this amplitude quickly decreased. Many legs stopped moving after a few minutes; others continued for up to 30 min.

To quantify the behaviour, the femur of the intact animal was fixed with Scutan (dental adhesive), and a force transducer was attached to the tibia. Fig. 1 shows a typical, but relatively short, recording beginning with the autotomy. Of the 45 legs tested, 12 did not move after separation. The frequency of the flexions for the remaining 33 legs was 1·2–5·5 Hz during the first 6 s (the usual frequency was between 2 and 3·5 Hz). The amplitude varied, especially at the beginning, often from one flexion to the next. Mean amplitude and frequency decreased with time.

The behaviour appeared only if the leg was separated within 0·5 mm of the autotomy boundary (the length of the middle leg femur is about 22 mm and that of the hindleg femur, about 30 mm). Amputation at the coxa or at the proximal region of the femur did not elicit any movements. If the leg was first cut off at the coxa (no movements), a second cut at the autotomy boundary usually elicited flexions. A third cut about 0·5 mm further down the femur immediately abolished movement. Hence, there appears to be a special structure very near the autotomy boundary that generates the rhythmic pattern. It is referred to below as the generator region.

Neural basis of the behaviour

The femur was opened on its dorsal side over most of its length (only 1–2 mm at each end were left intact). The extensor tibiae muscle, both main tracheae, and all nerves and tendons in the dorsal third of the femur were removed to expose the flexor tibiae muscle and the crural nerve, which innervates it. The crural nerve was severed about 5 mm from the femur-tibia joint, and its proximal stump was inserted into a suction electrode. Intracellular recordings of a muscle fibre were made simultaneously with a microelectrode (see Fig. 2).

Each single flexion was produced by a burst of 1–14 small action potentials in the crural nerve. For the 25 legs tested the mode for the first 30 s after separation of the leg was 4–7 spikes per burst. The number of spikes per burst decreased over time and often changed abruptly from one burst to the next (Fig. 2). In the first minutes after autotomy other neurones were sometimes active, but these fired with a constant frequency and never in bursts. Activity could still be recorded in the nerve after muscle contractions were no longer discernible (often for more than half an hour afterwards).

In a few preparations extracellular recordings were also made from F2, the nerve that innervates the extensor tibiae muscle. After separation of the leg the slow extensor tibiae motor neurone (SETi) was sometimes active for a while, but its discharge never took the form of bursts.

Every extracellularly-recorded action potential in the nerve was accompanied by a large EPSP in the intracellular muscle recording. The amplitude of the first EPSP of a burst was 5–20 mV. This is smaller than the fast EPSPs in the extensor tibiae musc[illegible]

Fig. 2. Simultaneous extracellular recording of the crural nerve (top trace) and intracellular recording of a muscle fibre in the flexor tibiae muscle of an autotomized leg (lower trace).

Bässler & Storrer, 1980) and in the retractor coxae muscle (Graham & Wendler, 1981) of *Carausius*, but larger than the slow EPSPs of the same muscles. The half-time of rise for EPSPs of this amplitude was long, lying between 3 and 10 ms. Every muscle fibre that was penetrated showed the same kind of responses.

Determination of the amount of force

The force was measured at the tibia, 16 mm from the femur-tibia joint as in Fig. 1, and at the same time a myogram of the flexor tibiae muscle was recorded mid-femur with two 50-μm insulated copper wires. Fig. 3 shows that each flexion is elicited by a brief tetanus which is composed of as many twitches as there are spikes in the myogram. The maximum force generated during a flexion was plotted as a function of spikes per burst for each of the four legs (including the legs represented by Figs 3 and 4) that had the most variation in the number of spikes per burst from a total of ten legs. The representative plot in Fig. 4 shows that the maximum force was proportional to the number of spikes per burst. All four legs behaved the same in this respect, but their proportionality factors differed (see also Fig. 3). The maximum force generated by a particular number of spikes per burst declined in all legs over the course of time (the decline was slowest in the leg shown in Fig. 4). For this reason the number of spikes per burst and the maximally generated force appear well-correlated only if one does not plot too many successive flexions.

Neurogenic origin of the behaviour (isolated nerve preparations)

In four legs the crural nerve was severed at the level of the subcoxal joint and at the middle of the femur and stripped from the leg. The distal (femoral) end was inserted into a suction electrode. No activity could be registered initially. The nerve was then shortened bit by bit from its coxal end until rhythmic bursts appeared, usually when the last cut was at the level of the coxa-trochanter joint. The bursts were exactly like those seen after autotomy. After 26–44 min the rhythmic discharges ceased. Then the crural nerve was shortened further. This caused in all cases the reappearance of a rhythmic firing. A further shortening of the nerve beyond the trochanter-femur boundary abolished the rhythmic activity.

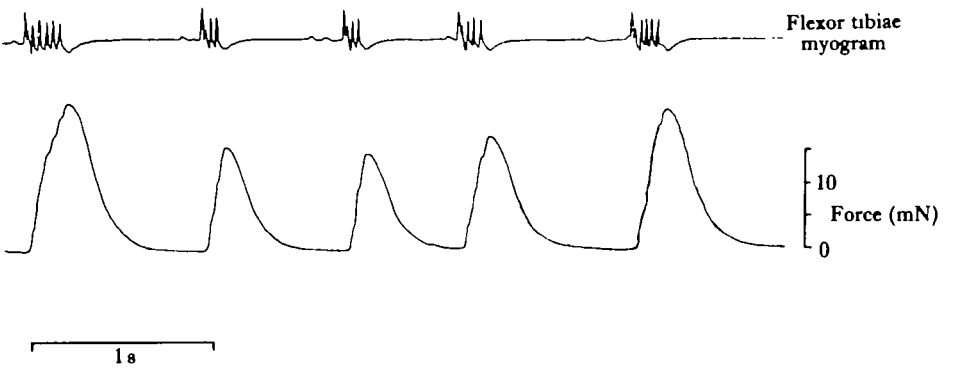

Fig. 3. Simultaneously recorded flexor tibiae myogram and force of flexion in an autotomized hind-leg. The intervals between spikes within a burst and the variation of the number of spikes per burst are relatively large.

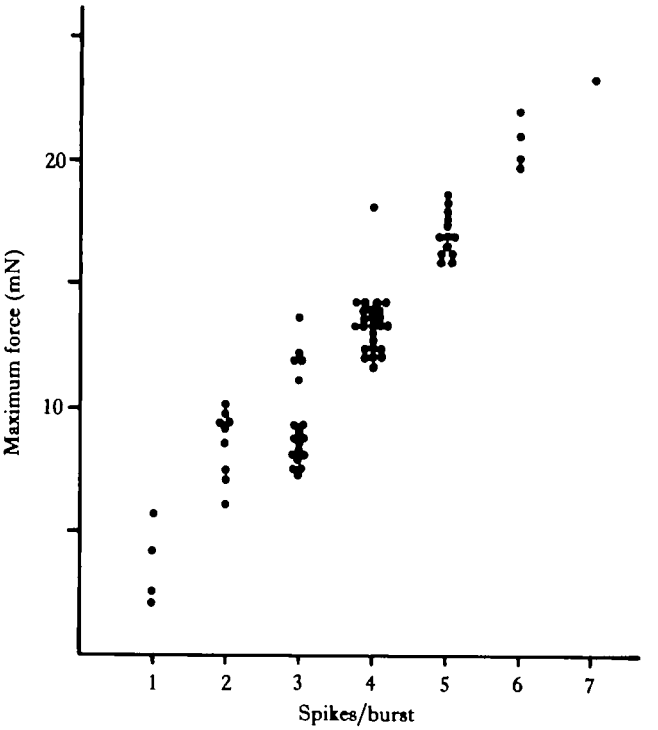

Fig. 4. Maximum force generated during a flexion as a function of spikes per burst from 75 successive flexions of an autotomized hindleg that exhibited an especially high variation of spike number per burst and an especially small decline in force amplitude over time.

In three other legs the nerve was isolated in the same way as before, but the proximal (coxal) end was inserted into a suction electrode. The nerve was then shortened from its femoral end. A cut at about 0·5 mm distal to the trochanter-femur boundary produced the typical bursts of action potentials which now travelled from the generator region in the proximal direction. Cutting the nerve at the level of the trochanter-femur boundary abolished these discharges completely.

Electrical stimulation

The crural nerve was exposed in the coxa, the trochanter, the first few millimetres of the femur, and in the distal third of the femur (10 legs). Its connections to the flexor tibiae muscle remained intact. Recordings were made with a suction electrode in the distal third of the femur from the cut end of this nerve. After a stimulation suction electrode had been placed on the nerve at about the level of the coxa-trochanter joint, the crural nerve was severed at the level of the subcoxal joint. This was necessary because in the intact crural nerve the activity after electrical stimulation was so high (probably due to reflex activation) that the small action potentials were masked.

A d.c.-stimulus of 1–3 V (with stimulating electrode positive) immediately produced steady activity from many fibres. This activity usually declined rapidly, after which fairly regular bursts from a single unit could be seen for a long time. Its single spikes and bursts possessed all the characteristics of the discharges that occur after autotomy (as a control the leg was cut off at the end of each experiment). Th

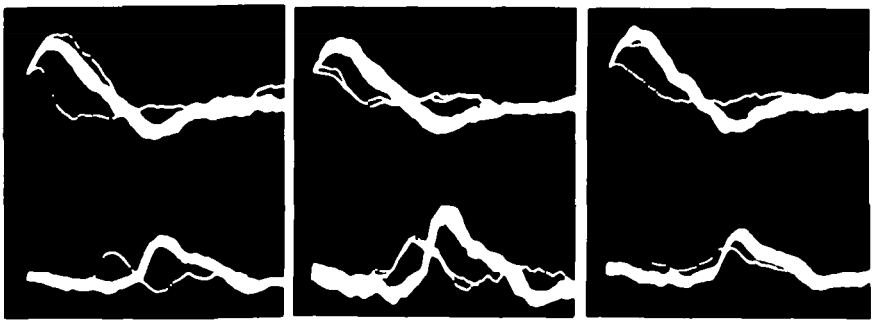

Fig. 5. Three double-electrode recordings of crural nerve responses to d.c.-stimulation in the trochanter. The traces of all impulses in a burst as well as one or two efferent action potentials of other units have been superimposed by a trigger mechanism. Upper traces are from the proximal electrode; lower traces from the distal electrode. The distance between electrodes is 3 mm. In the middle recording the amplification of the lower trace is higher.

discharges produced clearly visible rhythmic contractions in the muscle. The higher the stimulus voltage, the more spikes per burst and the stronger the muscle contractions. Usually the interburst interval decreased also. The rhythmic discharges could be elicited for several hours without any noteworthy decrement of spike amplitude.

Velocity of spike propagation and the spike shape

The spikes were recorded mid-femur with two electrodes 3 mm apart. The crural nerve was stimulated electrically in the trochanter. As described above this elicited rhythmic bursting from the bursting axon as well as steady activity in a few other neurones (8 legs). In Fig. 5 the traces of all the impulses (4–7) in each of three bursts have been superimposed. Conduction velocities between 1 and $1{\cdot}5\,m\,s^{-1}$ were measured for the bursting axon. They suggest a quite small axon diameter which is consistent with the small amplitude of the measured spikes. Fig. 5 also shows that the shape of the action potential of the bursting axon does not differ from that of other neurones of similar amplitude and velocity of propagation.

Behaviour of the autotomized legs of other phasmids

Spontaneous autotomy or amputation of the leg at the trochanter-femur boundary does not lead to rhythmic flexions in the other species of our phasmid colonies, *Acrophylla wülfingi, Carausius morosus, Ctenomorphodes briareus, Extatosoma tiaratum* and *Eurycantha calcarata. Acrophylla* and *Ctenomorphodes* belong to the same subfamily (Phasmatinae) as *Cuniculina* (Beier, 1968) and were also investigated electrophysiologically (three legs each). In none of these legs did the crural nerve show rhythmic activity after autotomy. Directly after the leg was separated from the body, there were high frequency discharges, but these quickly disappeared in all cases, and no further action potentials followed.

DISCUSSION

The rhythmic flexions of autotomized legs were produced exclusively by rhythmic contractions of the flexor tibiae muscle, with the elasticity of the extensor tibiae muscle serving as the 'antagonist'. Although the extensor motor neurones were usually silent, they could occasionally produce a constant tension in the extensor muscle (continuous activity of the SETi) and thereby increase its rigidity.

Each flexor contraction was elicited by a burst of action potentials from a single motor axon, the 'bursting axon'. The bursting axon is apparently very thin as its conduction velocity was low and its extracellularly recorded action potentials were small. As shown in Fig. 5, this low conduction velocity was responsible for the relatively long duration of the extracellularly-recorded action potentials.

The bursting axon resembled a typical slow fibre in terms of its conduction velocity, amplitude of extracellularly-recorded potentials and the rise time of the EPSPs it produced in the muscle fibre. The EPSP amplitude was, however, more similar to that of a fast fibre of *Carausius*. The axon appeared to innervate all or almost all of the flexor tibiae muscle. It is not known whether this fibre is used in the normal life of the stick insect. Phasmids have at least 12 excitatory flexor motor neurones, which

have not yet been characterized individually (B. Debrodt, personal communication). It is, therefore, not possible to homologize the bursting axon with a flexor motor neurone in other phasmids and any speculations on the evolution of this mechanism would be premature.

The muscle twitch elicited by a single action potential of the bursting axon decays relatively slowly so that the individual twitches from a burst summate to a brief tetanus, whose amplitude is proportional to the number of spikes per burst.

The spike generating mechanism seems to be activated by injury to the nerve, probably to the bursting axon itself. Perhaps the bursting axon is especially sensitive to the ions released from the other cut axons. The generator region is located at the proximal end of the femur and is only a fraction of a millimetre long. If the injury occurs proximal to this generator region (as in autotomy), the action potentials are conducted distally. If the injury is distal to the generator region, they are conducted proximally. The smallness of the axon fibre in the relatively large nerve precluded any search for the morphological correlate of the generator region.

The number of spikes per burst decreased and the interburst interval increased with time. These processes were apparently not due to fatigue of the generator region, since they were not evident in the responses to electrical stimulation. Probably the effect of the injury declines gradually and the generator mechanism can thus be reactivated by a new injury. The decline in the force of a flexion is a consequence of the decreasing number of spikes per burst and of the decrease in the force generated by a particular number of spikes with time. The latter is probably mainly due to lack of oxygen since the tracheae have also been cut.

The flexions of the autotomized *Cuniculina* leg differ from the myogenic muscle contractions of isolated locust legs (Hoyle & O'Shea, 1974) but are similar to those of the autotomized Opiliones leg (Miller, 1977). In Opiliones the rhythmic movements are also neurogenic. The number of active units is not known, but probably only one unit is involved per muscle. The duration of the behaviour is similar to *Cuniculina*. In both cases a flexion is produced by a burst of 1–15 spikes, and the frequency of bursts and the number of spikes per burst decrease with time. The generator regions are both in the proximal region of the femur and are activated by injury. Neither rhythm can be influenced by sensory input. This was not tested directly for *Cuniculina*. However, since the known sensory fibres from the femur do not join the crural nerve until the trochanter or the coxa, no connection between the femoral sense organs and the crural nerve exists in the autotomized leg.

There are, however, a few differences between the Opiliones and *Cuniculina*. In the Opiliones several joints flex independently of each other. Also, the generator region appears to be more extensive since an injury to the cephalothorax can also trigger the behaviour.

Most probably the behaviour developed independently in *Cuniculina* and in the Opiliones. It may be possible to find among the species related to *Cuniculina impigra* one whose autotomized leg exhibits a behaviour intermediate between that of *Cuniculina* and of normal phasmids.

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