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The Reflex Behaviour and Innervation of the Tergo-Coxal Retractor Muscles of the Stick Insect *Carausius morosus**

D. Graham** and G. Wendler***
Max-Planck-Institut für Verhaltensphysiologie, Abt. Mittelstaedt, D-8131 Seewiesen, Federal Republic of Germany

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Summary. The retractor coxae muscles of the mesothorax (Fig. 3) were examined by reflex stimulation and simultaneously recording intracellular muscle fiber potentials and the activity in nerve nl_5. Five axons were identified by their action potential amplitudes and reflex characteristics (Figs. 4, 5, 6). The smallest axon 1 is also present in nl_2 and nl_3 and has several of the properties of a common inhibitor (Figs. 6, 9). Four other axons produce depolarisations of the muscle fibers and were classified as slow 2, semi-fast 3, 4 and fast 5 on the basis of the amplitude of the junction potential.

All five axons were active in a resistance reflex produced by rotation of the coxa about the coxo-thoracic axis in the stimulus range 0.01–20 Hz (Figs. 1, 4, 10–12). Each axon showed a different dependence upon the frequency of the stimulus but the strongest responses occurred in the range 0.5–10 Hz.

A similar reflex was present in the metathorax. Axon 2 was often spontaneously active in the rear legs and a weak synchronizing reflex could sometimes be found between right and left hind legs. This reflex could only be detected by the modulation of ongoing activity (Fig. 4). No other interleg reflex pathways were found. Records from nl_2 show that the protractor muscles in both segments have resistance reflexes with higher thresholds (Fig. 4).

Cobalt back filling was used to show the morphology of both groups of neurons (Fig. 9). The soma position and dendrite structure of the retractor neurons are similar to a homologous neuron in locust.

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** Present address: Fachbereich Biologie der Universität, D-6750 Kaiserslautern, Federal Republic of Germany
To whom offprint requests should be sent
*** Present address: Zoologisches Institut der Universität zu Köln, Lehrstuhl Thierphysiologie, Weyertal 119, D-5000 Köln, Federal Republic of Germany

Introduction

When walking at high speed an insect advances by moving a tripod of legs to the rear. As no strong rotation of the body about a vertical axis is observed during the large acceleration produced by each retracting tripod, it would appear that the mesothoracic leg develops approximately the same power output as the combination of front and rear legs on the other side (Hughes 1952; Wendler 1965; Graham 1972; Burns and Usherwood 1979).

The muscles responsible for this strong rearward movement of the middle leg in the stick insect, are a group of three thoracic muscles pulling on a single tendon attached to the posterior rim of the coxa. The nerve supply to these muscles and that innervating the protractor muscle in the mesothoracic segment has been examined by standard physiological techniques to determine the motor output during imposed sinusoidal rotation of the leg about the coxo-thoracic axis at frequencies from 0.01 to 20 Hz.

The stick insect is particularly suitable for such a study because the leg is constrained to rotate about an axis defined by an upper point articulation (the epimeron rib) and the posterior end of a strut (the trochantin) which links the rigid pleuron wall and the ventral rim of the coxa. Furthermore in this insect the body cavity can be opened easily from above exposing the nerves and muscles responsible for movement of the coxa relative to the body.

Methods

Adult female *Carausius morosus* were used exclusively in this investigation. The animals were opened by a cut along the dorsal midline from the prothorax to the posterior edge of the 1st segment of the abdomen. The side walls were then pinned out forming a shallow trough. The gut was lifted clear of the body and either tied off and removed or placed along one side of the body. This simple dissection exposes the thoracic nervous system and the musculature moving the leg coxae. If this dissection is performed on

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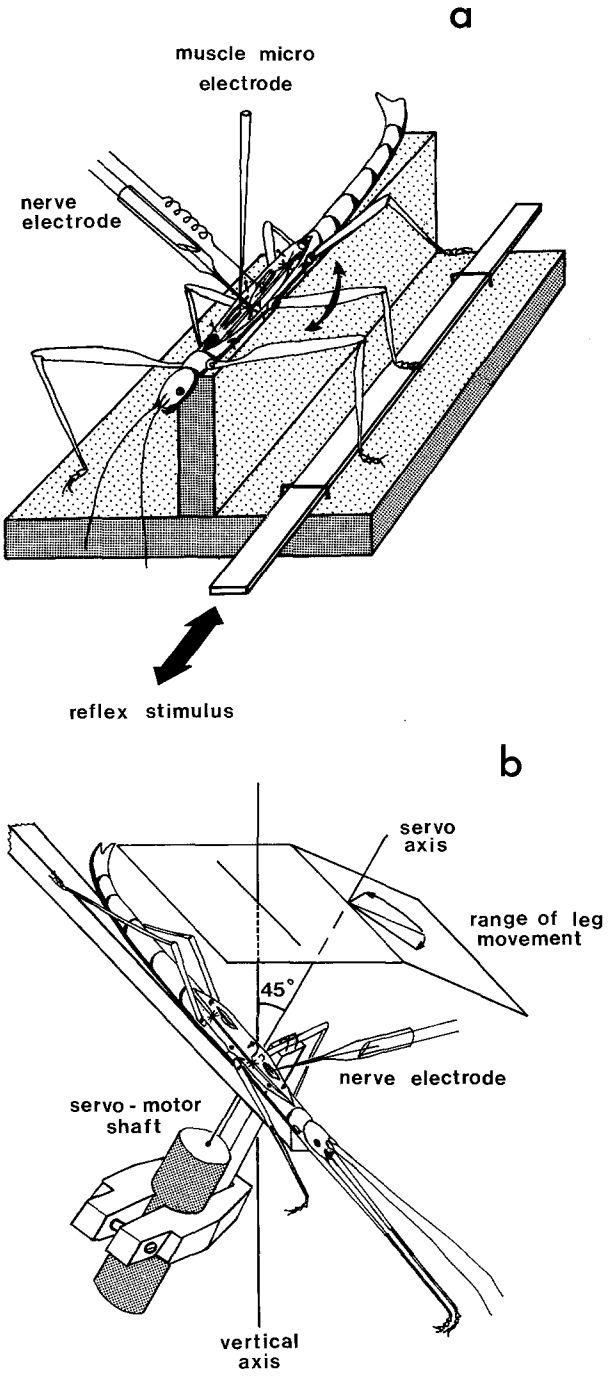

Fig. 1. a Preparation for hand movement of leg and comparison of intracellular muscle and extracellular nerve activity. **b** Preparation for nerve electrode recording of servomovement of the mesothoracic leg. The amplitude of femur movement was 70°

a suitable mounting block the standing legs remain in a normal position and can be manipulated as required (see Fig. 1a). Anaesthesia is not essential as the animal frequently remains in thanatosis (death feigning) during the dissection.

The hemolymph volume is inadequate if the gut is completely removed and either Wood's (1957) sucrose Ringer, or a saline of Weidler and Diecke (1969) was used to fill the body trough

Table 1

	Carausius Ringer[a]	High Na saline[b]	Locust Ringer[c]
Na^+	15 mM/l[d]	180	160
K^+	18	5	8
Mg^{++}	50	25	–
Ca^{++}	7.5	7.5	2
Cl^-	133	250	132
Sucrose	185	–	–
Total	410	466	322
pH	6.6	7.4[e]	7.2

[a] Based upon Wood (1957) hemolymph analysis
[b] Based upon work of Weidler and Diecke (1969)
[c] Hoyle (1953)
[d] 4.5 mM Na_2HPO_4; 6.0 mM NaH_2PO_4
[e] 2 mM of Tris/HCl Buffer

(Table 1). The tracheation system is left intact and the animal respires normally through its spiracles.

Suction electrodes using polyethylene tips or modified Wilkens-Wolfe (1974) hook electrodes were used to record from and stimulate the peripheral nerves.

For the reflex experiments the middle or rear leg was attached to a rotating shaft servo motor driven by a function generator (see Fig. 1 b). The leg was rotated about the coxo-thoracic axis over a range of 60–70° in a smooth sinusoid at frequencies from 0.01–20 Hz. The movement of the Brush (200) pen motor was measured independently. For further details, see Wendler (1974).

Recordings from muscle fibres were made using a conventional glass microelectrode, filled with 3 mol KCl or 1 mol potassium citrate, and various DC amplifiers. Simultaneous recordings of nerve impulses and intracellular potentials were made on an FM tape recorder. The motor neurons were backstained with 0.5 mol cobalt-chloride over distances of 5 mm from the cut ends of the peripheral nerves. A 1.5 V battery was used to provide a current of around 10–20 μA producing heavily stained cells after 12–24 h at 1 °C. Only light staining of the cell body and negligible dendritic filling were achieved after several days without current. A positive potential applied to the cobalt appears to be essential for good filling over large distances in this animal unless intensification methods are used to enhance the cobalt deposit.

Results

Figure 2 shows an external view of the coxo-thoracic leg joint in the mesothorax. The lower end of the axis can be adducted or abducted over an arc of 90° to permit the leg to move out parallel to the body in the thanatotic position. The leg is shown at the end of the retraction stroke in the position used for flat surface walking. Overlap in the soft cuticle between the trochantin and pleuron permits 10° of movement in the anterior-posterior direction but the joint is not capable of large rotations around the long axis of the coxa.

The general anatomy of the mesothorax is shown in Fig. 3. The retractor (tergo-coxal) muscles are the most accessible and are immediately exposed after removal of the gut. The retractor consists of three

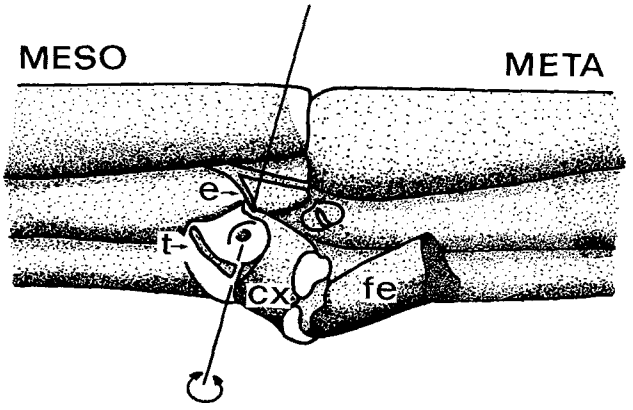

Fig. 2. Side view of the mesothorax showing the coxa (*cx*) of the middle leg, cut at mid-femur (*fe*), the articulation axis, the trochantin strut (*t*) and the epimeron rib (*e*)

separate parts Ra, Rb and Rc. In Marquardt (1939) only two muscles L_1t-cx and $L_{1\alpha}$t-cx are shown with their correct insertion into the tergum. All three retractor muscles are innervated by nerve nl_5 which leaves the nervus cruris before the leg nerve descends into the coxa. Nerve nl_5 passes posteriorly along the body (in most animals) and passes around the pleurosternal muscle L_2p-st before finally coming forward over the inner surface of Ra. The nerve supply for Rb and Rc is provided by a branch of nl_5 which passes under the retractor tendon. This nerve branch also supplies other muscles close to the body wall. The path for nl_5 and the relative positions of the three components of the retractor muscle are similar in the metathorax with the exception that Rc is almost parallel to Ra and Rb over the complete range of coxal movement in this segment and the nerve nl_5 goes directly forward from the nervus cruris.

The protractor of the coxa L_1(p-cx) lies below the retractor muscle and its tendon attaches to the soft cuticle immediately in front of the anterior rim of the coxa. It is innervated by nerve nl_2 which also carries the axons innervating the muscles Lt-p and L_1p-st which hold the dorsal and ventral body-plates together. This nerve shows rhythmic ventilatory activity in several small axons with a period of 5–10 s. Nerve branches containing these axons pass above and below the protractor muscle. Thus recordings from the branches of nl_2 going to the protractor often contain a background activity from these ventilatory motor axons and possibly a contribution from sensory axons.

Nerve Recording

Figure 4a shows the activity in nl_5 (meso) and nl_2 (meso) in response to a sinusoidal forward and rearward rotation of the leg about the coxo-thoracic axis. Four action potentials of different amplitude can be seen in nl_5 at this frequency of movement. The two larger action potentials change slightly in amplitude due to movement of the nerve relative to the electrode and occasionally they summate at the electrode. Two smaller action potentials were also present in the nl_5 record. The larger of the two was most active during forward movement of the leg. The smaller unit fired when the leg was moving towards the end of the retraction stroke. The nl_2 record was made between ventilatory bursts and shows activity in 2 large axons, and 2 smaller axons one of which is active 1:1 with the axon labelled 1 in nl_5. The record also showed some very small potentials which cannot be distinguished in the figure. The synchronous action potentials in nl_2 and nl_5 could be stimulated via nerve nl_3. It was not always possible to detect the potential in nl_2 when stimulating nl_3 or nl_5 but stimulation of either nl_3 or nl_2 always produced a detectable action potential in both distal branches of nl_5. Figure

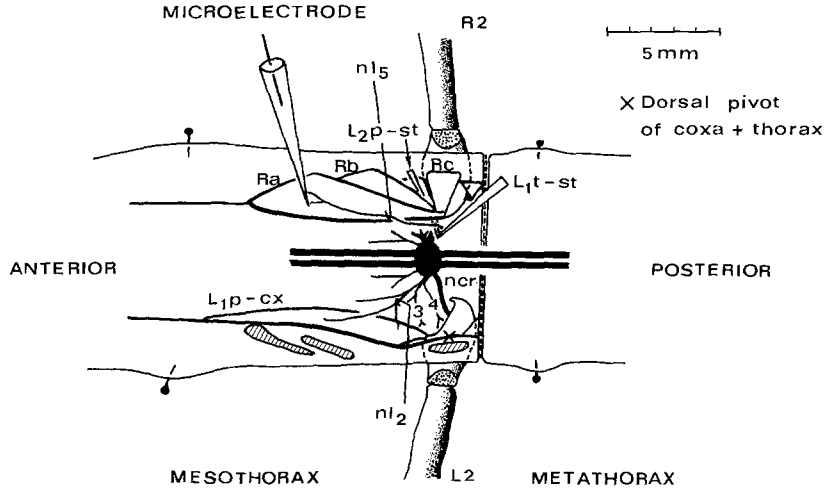

Fig. 3. Anatomy of protractor and retractor musculature (Ra, Rb and Rc) in the mesothoracic segment of an adult stick insect showing nerve recording sites for nl_2 and nl_5. On the left side the retractor muscle has been removed to expose the protractor muscle (L_1p-cx). See also text and Fig. 7

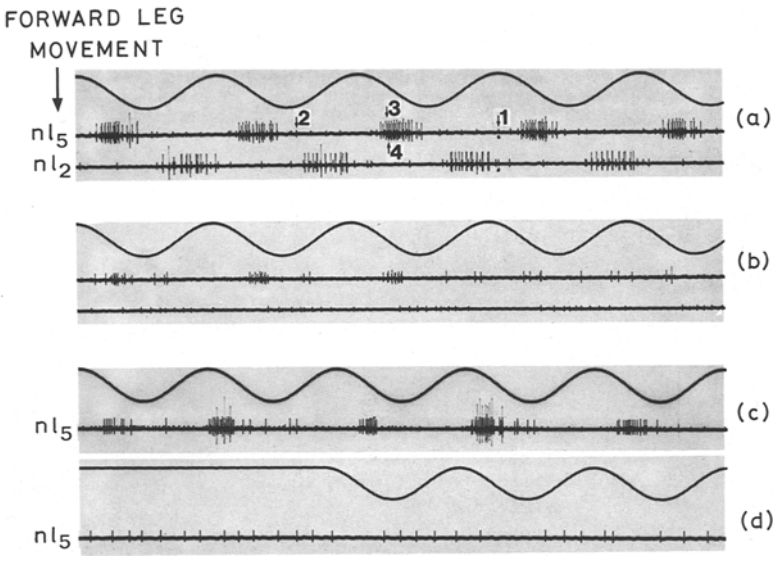

Fig. 4 a–d. Hook electrode records of motor reflexes in response to sinusoidal movement of the leg at 1 Hz in nl_2 and nl_5.
a Resistance reflexes in mesothorax. Four axons identified by numbers (1, 2, 3, 4); see text.
b After cutting both anterior and posterior connectives.
c Resistance reflex in metathorax.
d Interleg reflex (L3) in response to movement of R3

4b shows the responses to movement when the ganglion was isolated by cutting anterior and posterior connectives.The retractor resistance reflex had a lower threshold than that of the protractor and this was often the case in the intact preparation. Figure 4c shows a similar response to hind leg movement in the metathoracic retractor nerve.

The reflex was still present in nl_5 after cutting nl_2, nl_3 and the nervus cruris distal to the exit of nerve branch nl_5. Removal of the coxal hair rows and the hair fields at the articulation between trochantin and coxa produced no change in the reflex. It was also present after cutting the retractor muscle tendon, and cutting away the distal part of the leg at the middle of the coxa. Cutting nl_4, which passes close to the trochantin-coxa hinge and turns down into the leg, abolished the reflex.

One can conclude from these observations that the sense organ responsible for the resistance reflex does not lie in parallel with the muscle fibers or on the tendon, nor does it lie peripheral to the two joints attaching the coxa to the thorax, and that it supplies information to the ganglion through nl_4. A horizontal division of the coxa into upper and lower halves showed that movement of the lower joint was the source of the reflex.

Action Potential Identification

Records from nl_5 in 45 animals show a maximum of five different height spikes (see Figs. 4, 5, and 10). The smallest axon 1 becomes active when the leg is moved beyond its walking range of movement in either direction. This action potential is usually found to occur 1:1 with a small potential in nl_2 and the inhibitor of the extensor tibiae in nl_3 (Bässler and Storrer 1980).

Often in the metathorax and occasionally in the mesothorax one or both of the smaller potentials were present in the standing animal. The spontaneous activity of axon 2 may be increased by a slow forward movement of the leg or decreased by a slow movement to the rear. As the speed of forward movement is increased axon 3 becomes active. This axon produces an action potential approximately 3× the amplitude of that in axon 2. If the leg is moved forward more rapidly, axon 4 fires early in the burst of axons 2 and 3. The fifth, and usually the largest action potential, is from axon 5. This potential can be produced by a sharp forward flick of the leg and is most readily activated during the first movements of a resting animal that has been left quiet for a long period.

The above behavioural tests for identification of the different units can be applied to each new animal. In general axons 1 and 2 have action potentials of ~0.5 mV, 3 and 4 have a larger amplitude (~1.5 mV) and axon 5 may produce either a greater or smaller action potential in the range 1 to 3 mV depending upon the position of the recording electrode relative to the nerve. Activity in axon 1 does not produce a noticeable movement in any of the muscle fibers. Single potentials in axon 2 produce no fiber movement but small bursts produce a slow contraction in some fibers. Single potentials in axons 3 and 4 produce slight twitches in some fibers and stronger ones in others. Single potentials in axon 5 produce strong twitch contractions in approximately half the fibers of Ra and Rb. Forward movement of the leg stretches the retractor muscle and it is concluded that units 2, 3, 4 and 5 which are active at this time are

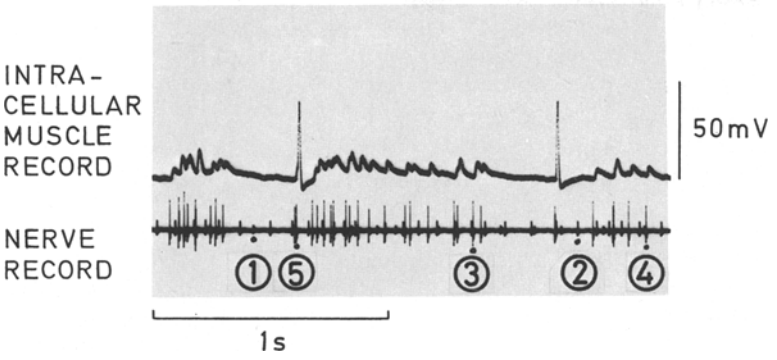

Fig. 5. Simultaneous recording from a fiber of Ra close to the body wall and a suction electrode on nl_5 shows axons 3, 4 and 5 innervating one fiber

excitatory units while unit 1 shows the characteristics possibly associated with an inhibitor.

During active struggling movements considerable summation of spikes occurs but all records analysed so far can be interpreted on the basis of contributions from only five axons. Where only three or four axons appear to be present two units are often found to have a similar height which can be detected by occasional summation in the recording electrode. Alternatively, on rare occasions (2 animals in 45), axons 3 and 4 are found to produce a complex action potential which corresponds to two axons firing with a very short interval between them, as a doublet.

Intracellular Muscle Recording

Intracellular recordings from muscle fibres of Ra and Rb confirm that they are innervated by at least four excitatory axons and one inhibitor. Figure 5 shows a simultaneous record from the nerve nl_5 (suction electrode) and a fiber of Ra close to the insertion of the muscle into the body wall. This record shows the three larger axons 3, 4 and 5 and the corresponding depolarisations produced in the muscle fiber. At first it was difficult to detect the junction potentials from axons 1 and 2. Recent records using a cut tendon preparation showed that both axons produced junction potentials at the tendon end of the muscle fibre which were often difficult to detect close to the body wall. The junction potentials are small (Fig. 6) and the fibers are very long (5–6 mm) in proportion to their diameter (100 μm). The failure to observe junction potentials close to the body wall suggests that innervation may not extend over the whole fiber.

Figure 6 shows examples of the intracellular responses for the 5 axons innervating the retractor muscle. The resting potentials of the fibers ranged from 15–55 mV. Axon 5 produces large overshooting excitatory junction potentials with a time course varying between 20 and several 100 ms. Axons 3 and 4 produced smaller excitatory potentials which rarely exceeded 20 mV in amplitude. Axon 2 produced small

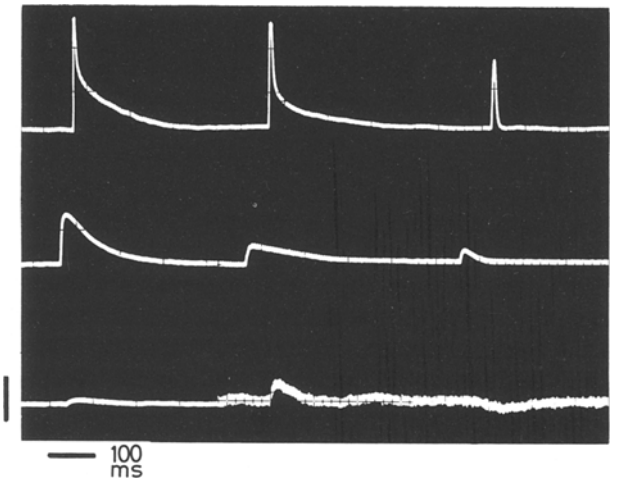

Fig. 6. Junction potentials recorded from muscle fibers in Ra and Rb: upper row, stimulation of axon 5 (scale 15 mV); middle row, stimulation of axons 3 or 4 (15 mV); bottom row left (15 mV) and center (1 mV) axon 2, right (1 mV) axon 1

junction potentials in the range 0.2–3 mV and axon 1 gives small inhibitory junction potentials of less than 0.2 mV with a duration of 150 ms.

Figure 7 shows a tracing of an electron microscope cross section of the nerve nl_5 at various positions relative to the branch point. There are some 20 or more axons in the proximal part of nl_5. Seven of these axons have a diameter equal to or greater than 5 μm and 8 range from 1–3 μm in diameter. The 7 larger axons pass into the lateral branch going under muscles Ra and Rb. At least 8 smaller axons also pass into this branch. In the anterior branch passing over Ra there are 7 axons greater than 4 μm in diameter and several below this size. From the electrophysiological observations only 5 axons give detectable potentials during reflex activation and one must conclude that either reflex stimulation does not activate the extra axons or these profiles are simply the result of branching prior to entry into different fibers of the muscle. Early branching of this kind has been reported by Walther (1980) from a study of serial

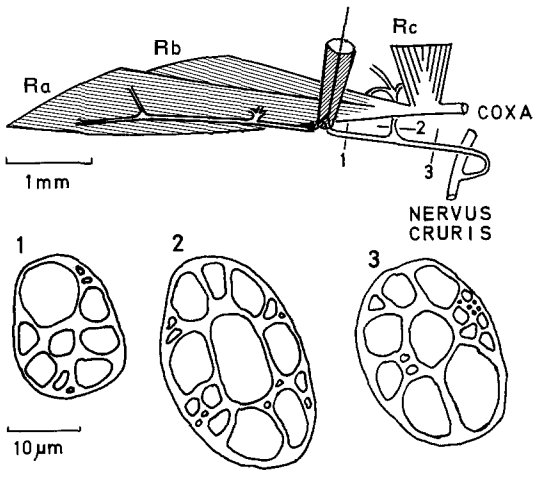

Fig. 7. Tracings of electron microscope sections anterior (1), lateral (2) and posterior or proximal (3) to the primary branch point of the retractor nerve nl_5

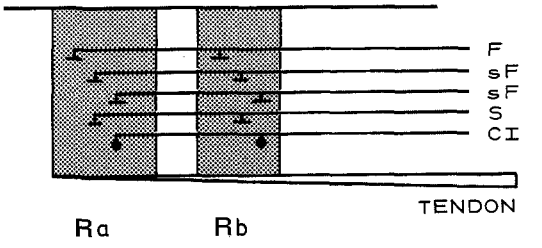

Fig. 8. Innervation of the muscles Ra and Rb

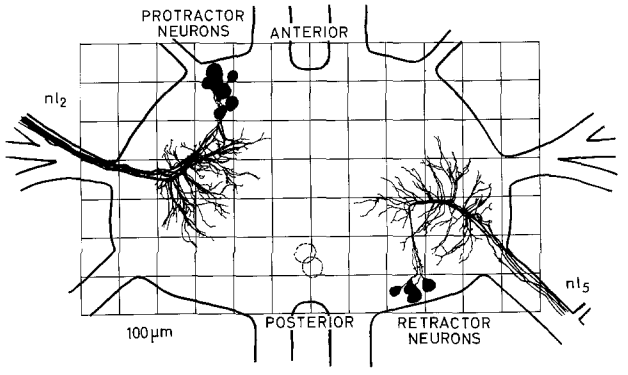

Fig. 9. Cobalt back fills from the cut end of nl_2 and nl_5 close to the nerve recording site. The nerve nl_5 joins the nervus cruris 300 µm from the ganglion. The two dotted circles show the position of pale cell fillings which correspond to the position of the locust common inhibitor (somata are in a ventral position)

electron microscope sections in the retractor unguis nerve of the stick insect.

Figure 8 shows the provisional innervation pattern for the muscles Ra and Rb determined by stimulating the nerve and recording the intracellular activity. Axon 5 is designated as fast (F), axons 3 and 4 produce semifast (sF) responses. Axon 2 is considered to be a slow (S) axon and axon 1 is assumed to be a common inhibitor. No junction potentials were found in any fibers of Ra and Rb that did not correspond to action potentials recorded in the anterior branch of nl_5 passing over muscle Ra.

Cobalt Staining

Figure 9 shows a camera lucida drawing of the motor neurons obtained by back filling the peripheral nerves nl_5 and nl_2 with cobalt chloride. The 100 µm grid is based upon the posterior margin of the nervus cruris as a reference. The paths of the axons and the dendritic field shape and cell body position for the retractor neurons correspond closely to a tergocoxal motor neuron reported by Burrows (1975). Either 3, 4, 5 or 7 neurons were filled in nl_5 depending upon the level at which the nerve was cut. For nl_2 either 5 or 8 neurones were filled.

The cell bodies range in diameter from 15–40 µm and in all high density fills a large pale cell body is found close to the ventral midline. No axon has been traced to this cell but it was always present in high density fills and was always displaced towards the filled side. It was not present when cobalt did not reach the ganglion and two cells appeared when the nerve branches on both sides were filled. The position of these cells is shown by the dotted outlines in Fig. 9. They occupy a position similar to that reported for the common inhibitor in locust (Burrows 1973).

In 2 out of 11 fills of nl_5 from a cut distal to the branch point 5 cells were found. As this group is not expected to include the common inhibitor cell this would suggest the possibility of 5 rather than 4 excitatory axons. So far all motor records can be analysed on the basis of a total of only 5 action potentials and it is possible that in these two instances a 6th axon may have been filled by leakage of cobalt into the cut lateral branch innervating muscles Rb and Rc and the body wall muscles.

Intraleg Reflexes

The retractor reflex at four different frequencies is shown in Fig. 10. At frequencies in the range 0.01–0.05 Hz only axons 1 and 2 were active. These axons were modulated by leg movements as slow as 1 degree/s. As the frequency increased axons 3 and 4 produced action potentials and at 10 Hz axon 5 produced one potential during the 1st cycle of the stimulus with a nerve spike amplitude smaller than that of the semifast units in this record. The reflex for all axons showed a maximum response at the onset of the stimulus which fell to a steady level after 1–2

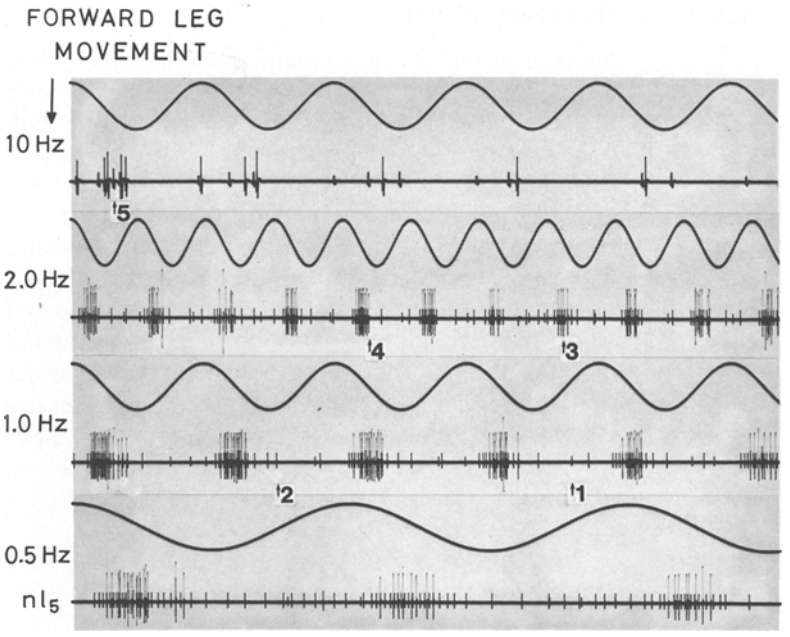

Fig. 10. Motor reflex recorded from nl_5 at 4 different frequencies of movement in one animal. All records show the beginning of a stimulus period. Individual axons are identified by number (1, 2, 3, 4, 5); see text

cycles, in axon 4 the number of potentials per cycle decreased steadily over the first 10–20 cycles. No unit showed an improved response with time after the onset of the oscillatory stimulus.

Figure 11 shows the phase histograms for the five axons. The phase maximum for axons 2, 3, 4 and 5 shows that these axons were most active during the maximum angular rate of change in forward movements of the leg. Axon 1 was most active at the end of the rearward movement of the leg. As the frequency of the stimulus was increased above 10 Hz the histograms show a shift of the peak to the right indicating that reflex responses are appearing later in the stimulus cycle. The displacement of the response can be seen to be about 10 ms for unit 3, 20 ms for unit 2 and 40 ms for unit 1. This may represent differences in either axon conduction velocity or central processing of the sensory input and may be sensitive to the rate of change of the stimulus as the frequency is increased.

The frequency distribution of the maximum activity of each axon differs and shows that each appears to play a different role in the control of the muscle (see Fig. 12). Axons 2, 3, 4, and 5 were most active in the frequency range from 2–10 Hz compared to the normal walking range of 1–3 Hz. Axon 5 had a peak around 5 Hz and axon 4 showed phasic behaviour responding strongly for only 10–20 cycles at the beginning of a period of stimulation. Axons 1, 2 and 3 were active over the whole frequency range, and were maintained as long as the stimulus was applied. All the larger axon potentials 3, 4 and 5 showed a pronounced peak of activity at the phase of maximum velocity of movement and little or no activity in other parts of the cycle except at the higher frequencies of 10 Hz and above where conduction and processing delays became significant. Axon 2 was atypical in this respect. At 5 Hz it showed a pronounced maxi-

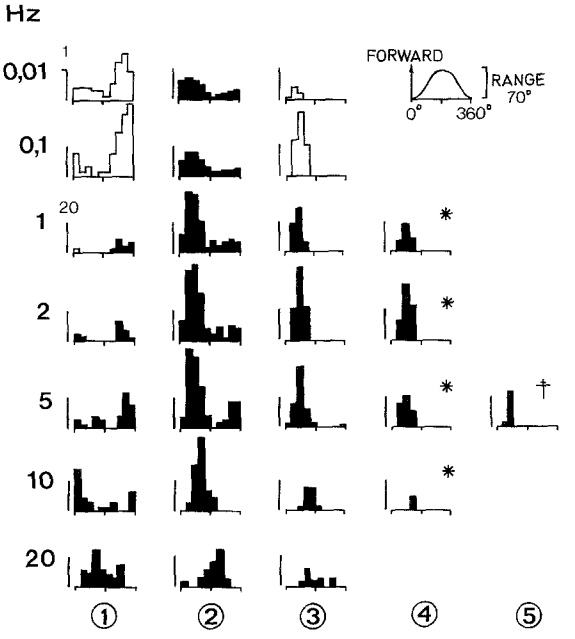

Fig. 11. Action potentials per 36° of phase angle for each identified axon. Open histograms have a scale of 1 impulse/bin and are averaged over the first 2–3 cycles. The closed histograms have a scale of 20 impulses/bin and are averaged over 10 cycles following the 5th cycle. The *histograms are averaged over the 1st 10 cycles of the stimulus. The † histogram is averaged over 40 cycles following the first 10 cycles. The axons are identified as a common inhibitor (1), slow (2), semi-fast (3, 4) and fast (5)

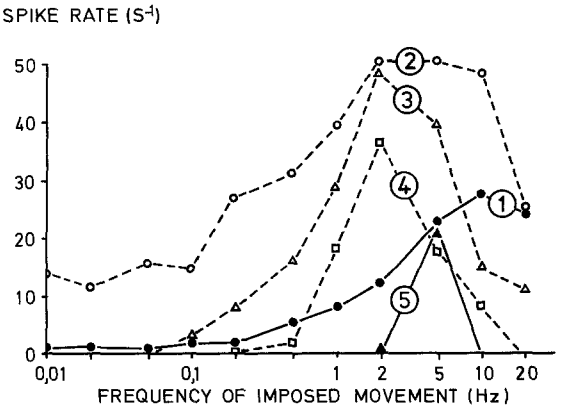

Fig. 12. Firing rate (spikes/s) at the peak of activity for the 5 axons of Fig. 11 as a function of stimulus frequency

mum in activity at the phase of maximum velocity during forward movement showing the normal resistance reflex. However, after the leg reached the forward position where the activity of axon 2 was a minimum there was a slight increase in the frequency as the leg moved to the rear. This was not a strong peak and was present in only two of the four animals examined and possibly resulted from a slight difference between the axis of leg rotation and the servomotor axis in these two instances.

Axon 1 had a maximum activity towards the end of the retraction stroke where the leg changed direction from rearward to forward movement at frequencies in the range 0.01–2 Hz. Above this frequency the peak began to show a delay shift and at both 5 Hz and 10 Hz a smaller peak occurred 180° out of phase with the main peak. The secondary peak occurred with all 4 animals and was a maximum just before the change of direction from forward to rearward movement at 5 Hz. Thus peaks in the activity of this unit were associated with the turning points of the sinusoid stimulus rather than coinciding with the maximum velocity of movement.

Interleg Reflexes

The possibility of reflexes between legs was examined for the meso- and metathoracic ganglia. Figure 4d shows the only example of an interleg reflex found in this study. This modulation of the spontaneous activity of an excitatory axon 2 in the left metathoracic nerve nl_5 was related in timing to the movement of the leg R3 from 0.1 Hz up to 2 Hz. The response disappeared after a brief burst of spontaneous leg movement. No retractor reflexes were found between the other meso- and metathoracic legs and no reflexes for the protractor nerve were found for any leg pair.

Discussion

No neurohemoral organ potentials of the type reported by Orchard and Finlayson (1976) have been found in electrophysiological recordings from nl_5 although they may be present in nl_2. The low firing rates of such units should not interfere with analysis but may give rise to occasional unidentified potentials in recording. Traces of the fatty sheath in the peripheral nerves sometimes caused poor recording immediately after hooking the nerve but the records improved within a few minutes probably due to slight movement of the hook along the nerve caused by muscle contractions during leg movement. The axon potentials recorded were similar in size to those found in other insects and using hook electrodes the firing pattern of up to 5 axons could be reliably distinguished even during the strongest movement reflexes.

Techniques such as cobalt filling, methylene blue and other forms of histological treatment were effective without modification in the stick insect. Furthermore the comparative anatomy at the muscle level and even at the neuronal level shows some similarity to that of the locust and it appears likely that preliminary identification of stained neurones may be possible by direct comparison with those already identified in locust.

Muscle Properties

Four of the axons of nl_5 produced depolarizing junction potentials in retractor muscle fibers which ranged in size from 100–500 μV for axon 2, 5–20 mV for axons 3, 4, and 30–50 mV for axon 5. There was a smooth graduation in properties for these axons with 3 and 4 showing weak twitches and 5 a much stronger twitch response. All required repetitive firing to achieve a strong contraction of the muscle fibers.

The resting levels in the fibers ranged from 15–55 mV and the junction potentials have a rising and falling phase similar to those found in the stick insect by Wood (1958) and Godden (1972). The cobalt filling of the excitatory axons as a group showed a close similarity in the general shape and branching pattern of the individual neurons which were all associated with one functional group of retractor muscles. There were minor differences in the final path to each cell body and not all of the neurons send dendrites along all the 1st order branches of their companions but this may have been caused by deficiencies in filling. Typically there were at least 3 branches in parallel for 4 excitatory axons. Comparison with a series of filled neurons from the locust in Fig. 9.2 of Burrows (1975) leads to immediate recognition of a 1st posterior tergo-coxal motoneuron as having all the features shown by the retractor neurons in Fig. 9. In the

protractor nerve filling, the branching pattern takes two forms suggesting that there may be 3 excitatory axons for the protractor, which would agree with the finding of 3 larger action potentials in nl_2 during the reflex and another 2 axons which perhaps innervate the body wall musculature. The presence of ventilatory motor spikes and sensory inflow from the body wall makes quantitative recording from this nerve difficult. Axon 1 is common to both nl_5 and nl_2 and Bässler and Storrer (1980) have shown that this same neuron is also an inhibitor of the extensor tibiae muscle and stimulation of this axon produced a reduction of the tension generated by the slow extensor motor neuron.

Axon 2 in nerve nl_5 is often spontaneously active and produced small excitatory junction potentials in the retractor muscle close to the tendon. The reflex activity of this axon suggests that it is an excitatory axon which is activated when the leg is moved forward. The small size of the excitatory junction potentials, absence of muscle twitches and graded contraction of some muscle fibers on the rare occasions when it could be preferentially stimulated indicate that this is a slow axon.

Axons 3 and 4 produce twitches in the muscle fibers under selective stimulation and excitatory junction potentials which are larger than those produced by axon 2 but usually smaller than those for axon 5. Axons 3 and 4 are referred to as semi-fast axons compared with the large excitatory junction potentials and much stronger twitch contractions produced by single action potentials in axon 5, which is termed fast.

In axon 1 the situation is not clear because it has not been possible to show a direct influence of stimulation of axon 1 on muscle tension due to difficulty in stimulating axon 2 reliably. However, there is strong support for the view that this axon is a common inhibitor, for the following reasons:

1. The unit is active 1:1 with a small unit frequently observed in nl_2 and nl_3, and this unit can be activated in nl_5 by stimulation applied to nl_2 or nl_3.

2. The same unit also fires 1:1 with the inhibitor of the extensor tibiae muscle (Bässler and Storrer 1980).

3. The axon is active during rearward movement of the leg, when the retractor muscle is relaxing.

4. The cobalt fillings stain cell bodies close to the ventral midline in a similar ventral position to the common inhibitor cell bodies of the locust which also innervate the extensor tibiae muscle in this insect.

5. Hyperpolarising potentials can be detected in a number of fibers close to the tendon. These are small both in amplitude and duration compared to those found in the extensor tibiae suggesting that either they have only a weak functional role or act via some mechanism other than changes in the membrane potential.

Intraleg Reflexes

The reflex examined here is stable and reproducible from insect to insect and is a further example of the proprioceptive reflexes first described by Pringle (1940), and most recently by Wong and Pearson (1976) in the cockroach, by Wendler (1972) and Bässler (1972) for the stick insect, and Runion and Usherwood (1968) and others in the locust. These are to be distinguished from the reflex studies of Wilson (1965) and Delcomyn (1971) on cockroach where interjoint reflexes are examined by either a general movement of the whole leg as in the Wilson experiment or a comparison of extensor femur activity with controlled movement of the tibia-femur joint as in the Delcomyn study. In the stick insect, only the forces or extracellular muscle activity were recorded and no information is available on the manner in which specific axons are excited. In particular, the activity of inhibitors was not examined during reflex movements.

The reflex observed in this work is highly reproducible and is present at very low speeds of leg movement as seen from Fig. 12. This suggests that the receptor involved has a strong tonic component. The phasic response has a dead time of about 10 ms for axon 3 which can be derived from the delay at 10 Hz in Fig. 11. This is between the values of 8 ms reported by Wendler (1972) for the depressor-femur muscles and 15 ms for the response to movement of the chordotonal organ in the tibial-extensor reflex (Storrer, personal communication).

The earlier report of very short latencies in the work of Wilson on leg reflexes in the cockroach is almost certainly unreliable as it was not based upon a simple reflex and the long delays in generating an adequate response suggest that a particular mode of oscillation is being excited in the cockroach ganglion. Perhaps this system contains anticipatory elements which produce a very short latency. The results of Delcomyn (1971) repeat this earlier work and indicate a similar time course for the leg reflex to that described here. Although the strength of the response was more variable.

The reflex response time for axon 3 is probably an overestimate of the real delay due to the low rate of change in the sine stimulus. A step function provides a more accurate measure of dead time, and Igelmund and Wendler (personal communication) have recently used this technique to measure a delay of 8 ms for an equivalent axon in this muscle.

The single hair reflexes described by Wong and Pearson (1976) with their very short response times of 2–3 ms represent a highly specialized monosynaptic system, probably used to protect the joint against damage caused by exceeding its designed range of movement. The excitatory reflex examined here shows a longer response time and is unlikely to be monosynaptic as it must be capable of gain changes and sign reversal under different behavioural conditions.

A reflex response that has not been examined in the earlier literature is that of the common inhibitor. It is of particular interest as several different proposals for the function of these axons have been put forward (Pearson 1973). In several insect muscles it has been established that hyperpolarizing junction potentials reduce the tension developed during slow contractions. This has recently been demonstrated for this particular axon in the extensor tibiae muscle (Bässler and Storrer 1980). However, the common inhibitor may innervate the antagonistic protractor and retractor coxae muscles in this instance, and its function is uncertain. It is also found that inhibitors tend to be active just before leg flexion in seeking and struggling movements (Pearson and Iles 1970) and just before protraction in walking movements (Runion and Usherwood 1968). Similar behavior is found here during imposed movement of the leg. The maximum response of the inhibitor occurs just before the leg changes direction from rearward to forward movement. This corresponds approximately to the posterior extreme position of the middle leg in free walking animals (Cruse 1976). Thus the reflex response is similar to the free walking locust result and suggests that this axon may improve the rates of relaxation in antagonist muscles when the direction of movement is changed. In addition, at frequencies of movement above 2 Hz a small peak appears just before the transition from forward to rearward movement at the anterior extreme position of the leg. Thus the common inhibitor is activated at both points in the cycle where the direction of movement is about to be reversed and the response is strongest for the transition from rearward to forward movement. This is the basic requirement for a walking step where the highest rate of relaxation would be required just before the fast protraction movement. For details of the activity of axon 1 during walking in the stick insect, see Graham and Wendler (1981).

Interleg Reflexes

The reflexes described so far function within one leg, and the isolation experiment shows that they depend upon the other ganglia of the nerve cord only so far as gain is concerned. Reflexes between legs have been mentioned by Wilson (1965) but are described as being unstable. A similar weak and unstable reflex was observed in the present work. This occurred once between R3 and L3 and tended to synchronize the movement of both legs by modulating the continuous activity of axon 2. This response appears to be similar to the sympathetic movements of the legs reported by Wendler (1964) in which forward or backward movement of a leg on one side caused similar movements on the contralateral side. No reflex interactions were found between the adjacent ipsilateral legs.

The failure to show strong static reflexes between the legs does not necessarily mean that the receptor system responsible for the intra-leg reflex is not involved in walking or other active movements of the legs. Indeed, the system has a very wide range of response and can follow movement reliably up to 5 Hz and possibly much higher and yet it is also capable of detecting movements of less than 0.1°/s. Thus it could provide both phasic and tonic information for the leg during either active or passive movements. Furthermore, the system appears to be protected within the body and is close to the ganglion. It would seem that this receptor is ideally situated to provide peripheral information that may be important in coordinating and controlling the active movements of the legs.

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