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NEUROMUSCULAR TRANSMISSION IN *PERIPATUS*

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SUMMARY

The electrical and mechanical responses of body, leg and jaw muscle of *Peripatus* to electrical excitation of their motor-nerves were examined. A small twitch was obtained from each muscle, whose strength increased stepwise with increasing stimulus strength. In jaw and body muscles as many as ten increments in height were obtained with increasing stimulus strength. Only a single twitch height was obtained from the claw retractor muscle. Tetanus:twitch ratio under supraminal stimulation was less than 2:1 for jaw muscles, about 50:1 for the claw retractor and about 6:1 for muscles moving the legs. The jaw-muscle twitch duration was 0·6 s, that for the leg muscles 1·2 s and for body muscles about 3·0 s. Large miniature junctional potentials were frequently recorded regardless of electrode location. Responses to neural stimulation consisted of small junctional potentials whose height was progressively increased with increasing stimulus strength, generally with three steps. With repetitive stimulation, facilitation of the second and third junctional potentials occurred, plus summation. A few fibres gave spikes to a single shock: most gave a few spikes sporadically, during repetitive stimulation only. No abrupt tension increments occurred in whole muscles when individual fibres spiked. We saw no evidence for peripheral inhibitory axons. The excitation of *Peripatus* muscle is by local graded junctional potentials at distributed nerve-on-muscle fibre synapses, together with action potentials. The latter are initiated only by larger junctional potentials compounded of multiple smaller ones summated and/or facilitated. The details of neuromuscular physiology are not compatible with the phylogenetic status commonly proposed for *Peripatus*.

INTRODUCTION

The Onychophora, comprising two families, the Peripatidae and the Peripatopsidae, are distributed throughout the world in tropical or semi-tropical rain forests and nearby moist regions. Anatomists have long been uncertain as to how to classify them. One opinion, that has been perpetrated by some writers of Zoology textbooks (e.g. Buchsbaum, 1976) regards them as living fossils (see also Burton, 1954; Delamare-Debouteville & Botosaneanu, 1970). They are considered to be relatives of an

ancestral form intermediate, and hence a 'missing link', between annelids and arthropods. The opinions of taxonomists has ranged from regarding them as allies of myriapods and insects in a taxon of phylum rank, the Uniramia (Manton, 1977), through lumping them together with annelids and arthropods as a single phylum, the Articulata (Beklemishev, 1969), to denying any connexion whatever with arthropods (Sharov, 1966). The negative opinion is shared by cuticle specialist Neville (1975) and cytologists Locke and Huie (1977) who compared the Golgi complexes of *Epiperipatus* with ones of various arthropods and found them distinctly different.

Physiologists have paid little attention to the Onychophora. It is known only that their dorsal longitudinal muscles contract in response to acetylcholine and that the contraction is potentiated by eserine (Ewer & Van den Berg, 1954; Florey & Florey, 1965). Florey & Florey (1965) found insensitivity to l. glutamate or gamma amino butyrate, unlike arthropod skeletal muscles. These pharmacological properties ally the Onychophora with the Annelida rather than the Arthropoda. The general features of neuromuscular physiology of both these major phyla are now well known, so we deemed it desirable to carry out comparative studies on *Peripatus* neuromuscular systems. The research quoted above did not include either neural stimulation or intracellular recording. These we have done for several muscles of the species found in the Puerto Rican rain forest, *Peripatus dominicae juanensis*.

P. dominicae is characterized by having 31 pairs of legs. The specimens we worked with ranged in length, determined at maximum extension during walking, from 3 cm for juveniles to 10 cm for mature adult females. A 10 cm specimen shortens to about 5·0 cm when at rest in damp vegetation and contracts further, to a minimum of about 3·5 cm, when disturbed. A lively *Peripatus* tends to have its body partially extended, though not to the extent seen during fast walking. It is ready to contract and spit the long strands of sticky protein material with which it traps prey. When disturbed it shortens markedly and rapidly, lifting and withdrawing the anterior part of the body. Such behaviour is of the kind one might expect to be mediated by giant fibres, such as Horridge (in Bullock & Horridge, 1965) indicated might be present in *Peripatus*. Schürmann & Sandeman (1976) have described giant axons in the ventral nerve cords of *Peripatoides* and Hoyle & Williams (1979) comparable ones in *P. dominicae*. The details of the anatomy of the innervation of *Peripatus* muscles have been reported by Hoyle & Williams (1979). Salient unique features are illustrated in Fig. 1. Branches termed muscle arms, that do not contain contractile material, arise from the muscle fibres and travel towards the nearest motor nerve branch. Each of these makes synaptic contacts with a few (generally at least three)

Fig. 1. Electron micrograph of a portion of a longitudinal muscle fibre illustrating the dual type of innervation of *Peripatus* muscle fibres; muscle arm on nerve, and nerve on muscle. The innervation is also multiterminal, with several junctions of each type associated with single muscle fibres, and each synaptic region is polyaxonal. Abbreviations: MA, muscle arm, which projects from a muscle fibre and passes towards a motor nerve; H, head section of a muscle arm, spreading out to contact motor axons in motor nerve; N, motor nerve, with two muscle arm head sections making multiple muscle on nerve synapses; NM, motor nerve on muscle fibre contact, with side-by-side synaptic contacts by several motor axons; E, end-plate type specialization at nerve on muscle fibre junction; Tr, tracheole; WT, wide invaginated tubules; Z, z material to which thin myofilaments are attached; C, collagen. We are greatly indebted to Melissa Williams for this micrograph.

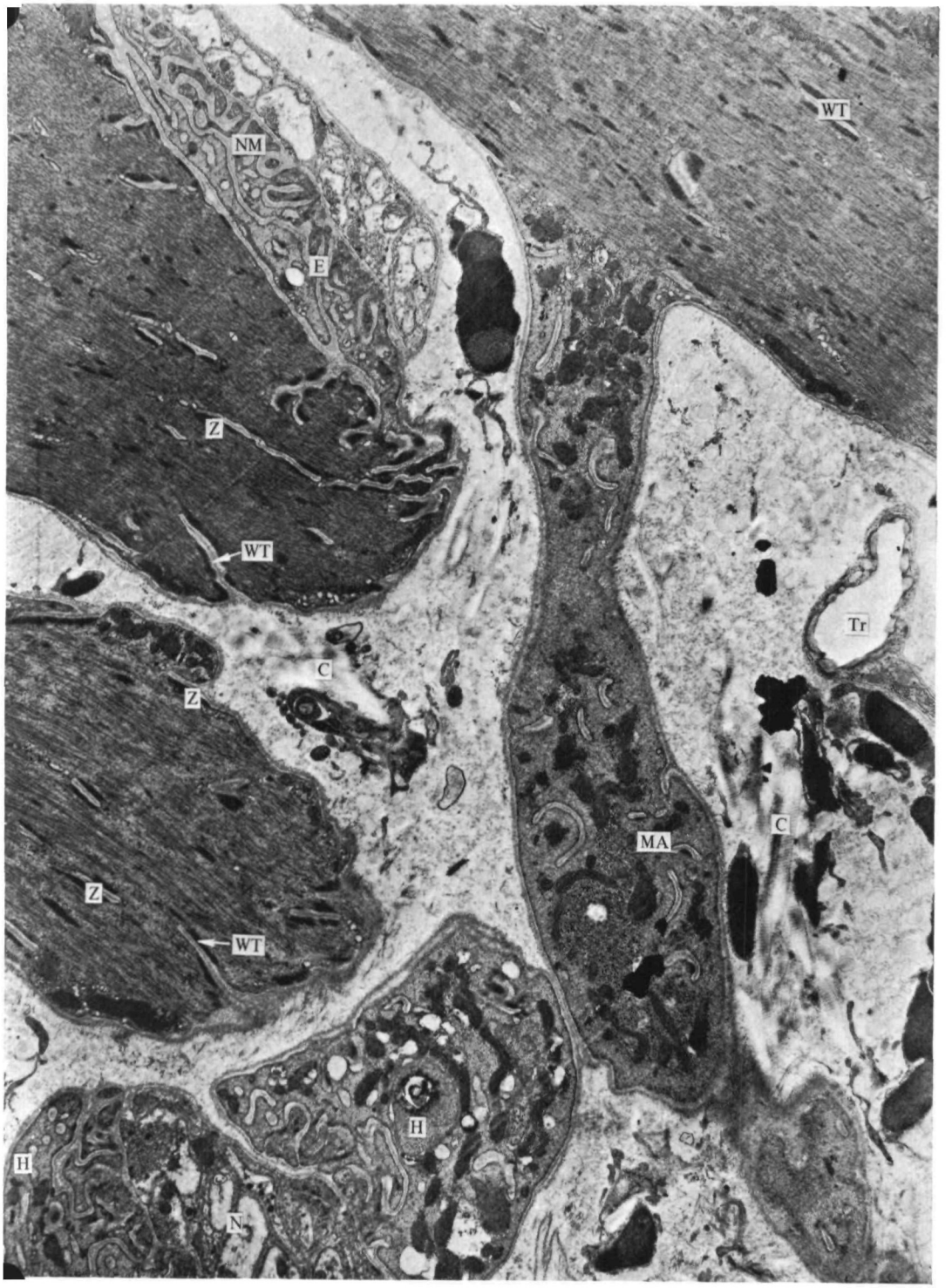

Fig. 1. For legend see facing page.

axons in the nerve. In addition, motor nerves send branches to the muscle fibres that synapse either directly with the muscle fibre or with a small projection arising from it. These nerve-on-muscle (NM) fibre contacts contain as many as eight motor axons, generally side by side, each of which makes a synaptic contact with the muscle fibre.

Peripatus muscle fibres are 1–40 μm in diameter and up to several mm long. Jaw and claw muscles terminate on arthropod-like apodemes at one end and are connected by collagen fibres to other muscles at the other. The basic ultrastructure of the muscle fibres, which is similar in different muscles, is that of a striated muscle but with the Z material scattered, not aligned into bands. Each fibre is invaginated not only by a small number of tubules of conventional T-tubule size, but also by a larger number of wide tubules with a lumen of about 0·13 μm. There are two sets of wide tubules, one running radially, the other longitudinally. The two systems do not contact each other. A large 40 μm diameter fibre has about 150 longitudinal wide tubules evenly distributed through the sarcoplasm. Radial wide tubules extend inwards for various distances, some to the centre of the fibre. Wide tubules also run inside the muscle arms.

These unusual features of innervation and muscle ultrastructure would render the *Peripatus* neuromuscular apparatus interesting regardless of the relevance of the research to helping settle the phylogenetic questions. Also, whatever its evolutionary status, its legs can serve as models for those from which arthropod legs evolved. The transition to jointed legs with a hard exoskeleton was the single most significant evolutionary achievement made by arthropods and it is not easy to imagine how it came about. A study of the onychophoran leg and its control may afford useful clues to interpreting arthropodan evolution.

MATERIALS AND METHODS

Peripatus dominicae juanensis Clark, 1913, were collected above 2000 ft in the rain forests of Puerto Rico. They were kept in a moist terrarium in an air-conditioned room at about 22 °C and fed upon termite larvae. All the experiments were carried out at this temperature, which falls within the range of that of their natural habitat (up to 25 °C during the day and well below 20 °C at night-time). At the time we started this research in 1975 there was no published data on *Peripatus* haemolymph. We tested insect saline (Hoyle, 1953) and finding it satisfactory continued to use it unchanged except for a reduction in magnesium content to one half. Campiglia (1976) published an analysis of *P. acacioi* haemolymph and Robson, Lockwood & Ralph (1976) of *P. moseleyi*. The latter proposed a saline based on their analysis. This is compared with the saline we used and also Campiglia's analysis in Table 1.

The animal was first held in the hand and rapidly cut longitudinally in the dorsal midline for all preparations except the dorsal longitudinal muscles and circular muscles. For the latter the cut was made in the ventral midline. All traces of gut and salivary glands were then removed, the body wall opened and gently stretched and pinned to a layer of Silgard in the bottom of a small dish. Further preparation depended on the particular muscle to be studied. Mechanical records were made

Table 1. *Ionic compositions of* Peripatus *saline/haemolymph, in m-equivalents/litre*

m-equiv	(*a*) Robson (1976)	(*b*) Campiglia (1976)	(*c*) Hoyle & del Castillo (this paper)
Na	107·6	93·5	140·0
K	5·0	3·4	10·0
Ca	3·3	6·8	4·0
Mg	0·7	1·0	4·0 or 2·0
HCO_3	0·6	6·0	4·0
H_2PO_4	0·1	7·7	6·0
Cl	117·0		158·0

(*a*) Saline suggested by Robson, Lockwood & Ralph (1976) based upon analysis of haemolymph of *P. moseleyi*.
(*b*) Analysis of haemolymph of *P. acacioi* by Campiglia (1976).
(*c*) Saline used (present work) for *P. dominicae*.

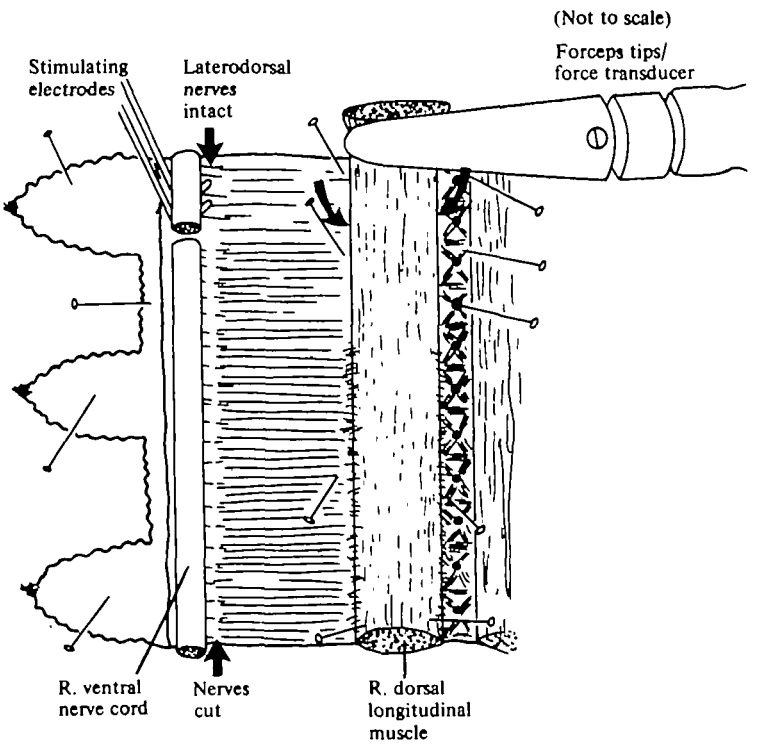

Fig. 2. Drawing to illustrate basic means of making neuromuscular preparations of *Peripatus*.

with the aid of an RCA 5734 mechanelectronic transducer by way of honed-down No. 5 forceps tips attached directly to the anode (Hoyle & Smyth, 1963). The device was micromanipulated into position and the tips opened to grip a skeletal hard part, a piece of body wall or one end of a muscle bundle.

The jaw muscles and retractors of the tiny claws at the tip of each foot are the only discrete muscles available in *Peripatus*, each having a movable chitinous hard part at one end. The remainder are all intimately associated with other muscle

tissue, to which their fibres are attached or with which they intermingle. Our general technique was to dissect part of the specimen containing a muscle, or part of a muscle, of interest, pin it out quickly on Silgard with the muscle uppermost, moistening it with saline. A forceps-tips/RCA 5734 force transducer was then micromanipulated into position and clamped directly onto an exposed end of a muscle (longitudinal or circular body) or skeletal hard part (jaw or claw). The clamped end was free from surrounding connective tissue and the other end firmly pinned down.

Next, the nearest major nerve trunk or nerve cord was located and freed by dissection until fine hook electrodes could be micromanipulated under it. The piece of nerve or cord was then cut away. A drawing illustrating the method is presented in Fig. 2.

Intracellular electrodes filled with 3 M-KCl and having resistances of 15–30 MΩ were used. Finely tapered platinum wires, insulated except at the tip, were used to stimulate a nerve branch near the muscle. The experiments were carried out at 27–28 °C. Preparations were made of the following muscles: dorsal longitudinal (DL), ventral longitudinal (VL), left and right ventro-lateral longitudinal (LVL, RVL), inner circular (IC), protractor of the jaw (JPr), retractor of the jaw (JRe), lateral jaw (JLa), remotor of the foot (Re), anterior depressor of the foot (ant D), retractor of the claw (RCl). Details of the anatomy of these muscles as well as of their innervation and ultrastructure are given in Hoyle & Williams (1979).

RESULTS

Mechanical activity

The characteristics of the mechanical responses obtained from different muscles of *Peripatus* were of sufficiently wide range to show that the muscles can be regarded as being functionally specialized. Differences in innervation, neuromuscular junctional transmission and intrinsic properties of the muscle fibres, all contribute though no major differences were detected in ultrastructure (Hoyle & Williams, 1979). But the fibres lack longitudinal organization so it was not possible to measure the *Peripatus* equivalent of sarcomere length, variation in which is a major concomitant of differences in contractile characteristics in diverse arthropod muscles (Hoyle, 1967; Elder, 1975; Atwood, 1977).

For descriptive purposes the muscles will be divided into major anatomical groupings, namely those of the body, leg and jaw, which have relatively slow, intermediate and fast speeds, respectively (though by arthropod standards even the fastest *Peripatus* muscle is to be regarded as slow). Speeds of relaxation differed more than did speeds of contraction in the onychophoran.

Muscles of the body

The longitudinal muscles of the body are opposed by weak outer, and strong inner, circular muscles that act antagonistically to regulate body stiffness and length. *Peripatus* often rears its front end off the ground, even during fast walking. This requires a maintained tonic contraction, not a quick one, and is a function of the dorsal longitudinal muscles (DL's). For these purposes the body muscles need to be able to maintain prolonged tonic contraction over a wide range of magnitudes.

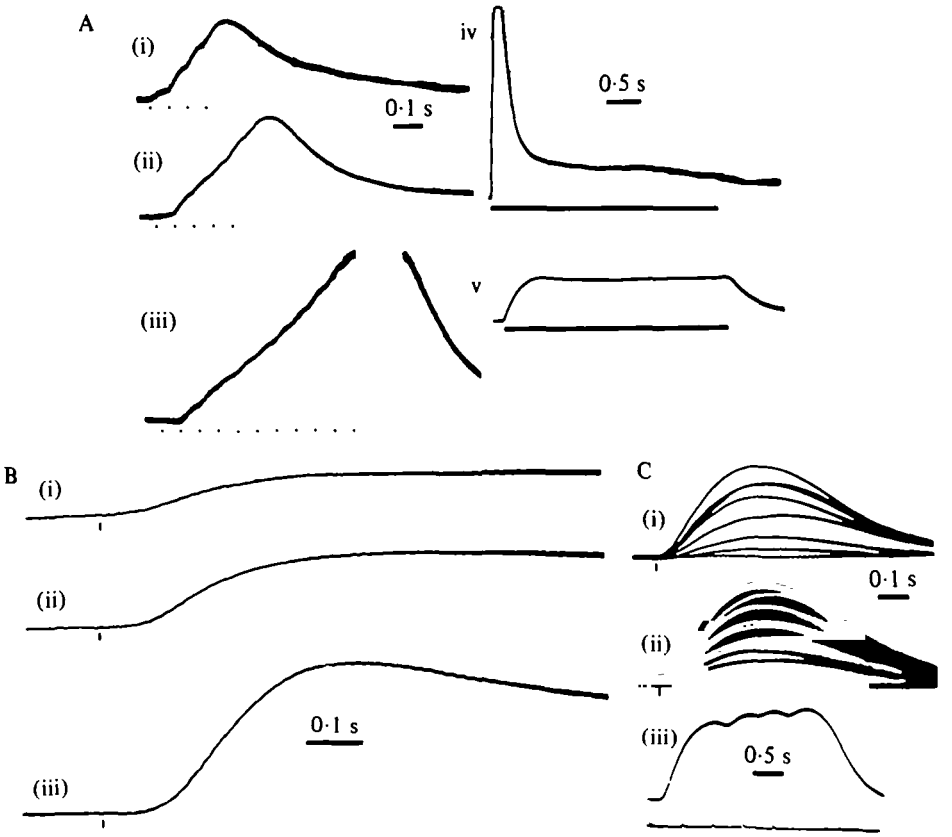

Fig. 3. Neurally evoked contractions of basic types of *Peripatus* muscle. Isometric force recordings.

(A) Ventral longitudinal muscle (intermediate speed). (i–iii) Tension staircase with continued stimulation. All same strength, at 15 Hz, repeated at 1 mm intervals; the initial contraction was progressively weaker, and facilitation of the second response greater. (iv) Supramaximal stimulus strength and a relatively high frequency (30 Hz) led to a fast phasic contraction which rapidly decays. (v) Same stimulus frequency as (iv) but at reduced strength; the fatiguing phasic response is missing, and replaced by a maintained tonic contraction.

(B) Inner circular muscle (the slowest). (i–iii) Responses to increasing intensity of single stimuli applied to nearby nerve cord.

(C) Jaw retractor muscle (the fastest). (i, ii) Responses to single stimuli applied to the nerve, which emanates directly from the brain. (iii) responses to repetitive stimulation at supramaximal strength.

In addition, quick defensive withdrawal, mediated exclusively by the dorsal, ventral and ventrolateral longitudinal muscle, requires that these muscles be able also to contract rapidly. To study DL's the preparation shown in Fig. 2 was used. A piece of body wall including about three of the feet of one side and both DL's was cut out after opening ventrally and pinned out on the dish. A small piece of nerve cord lying over one foot was severed from the remainder of the cord. Nerve branches to the nearby foot were severed but those travelling dorsally were left intact. The stimulating electrodes were placed under the piece of cord. All nerve trunks leaving the longer length of cord were severed. About half the DL nearest to the cord was freed from the body wall and the free end seized by a micromanipulated forceps

tips force transducer. The end attached to the body wall was firmly pinned to the Silgard.

A single shock of threshold strength applied to the nerve cord evoked a twitch with a rise-time to peak of 0·17–0·22 s and decay to baseline of 0·8–1·1 s. Increased stimulus strength evoked a stronger, but slower, twitch, rising to peak in 0·3 s and returning to baseline in 1·2–1·6 s. The twitch responses commonly, but not invariably, showed facilitation with repetition, particularly of the second in train. Maximally, this was about 3 × the amplitude of the first. Weaker stimuli evoked slower contractions, at frequencies above about 10 Hz, that were strongly frequency dependent.

There was no contraction in the DL muscle of the other half of the body when its partner was stimulated maximally via the nerve cord innervating it in preparations in which the commissural branches had not been severed. It is concluded from this that the left and right DL's are innervated only from the cord in their own half.

The ventral midline longitudinal muscle (VL) is a thin, flattened strip that runs on the inside of the inner circular muscle. A weak twitch was obtained from it, with a relatively fast rise-time of 0·06 s, and a slow decay to baseline in 0·8 s. The twitch height was always facilitated between the first and second responses in a train (Fig. 3A) and sometimes also between the second and third responses. Summation was extensive, with a tetanus:twitch ratio of about 30:1. When stimulated tetanically at 30 Hz or greater, at supramaximal strength, the tension rose rapidly to a peak in only 0·15 s but declined rapidly to a low plateau. At the same frequency, but with reduced stimulus strength, the rapidly rising phase was absent and a stronger steady tension was developed.

Both phasic and tonic contractions were obtained from VL, the former declining rapidly during continued stimulation. The two contractions were elicited together in response to strong stimuli at above about 20 Hz. Then the mechanical response consisted of a rapidly rising phase that reached a peak in about 500 ms followed after a brief plateau, by a decline to one-fifth peak amplitude after 2–3 s (Fig. 3A, iv). The lower level of tension was maintained with much less decrement and represents only the residual tonic component. By reducing the stimulus strength critically it was possible to obtain only the tonic response (Figure 3A, v).

The latency of the phasic mechanical response decreased with increasing stimulus strength. In this and in other *Peripatus* muscles such variable latency could be attributed to a marked dependency of excitation on stimulus strength. The phasic response was attributable to the initiation of spikes by the first few larger summed junctional potentials, with ensuing greater contraction activation. Thus, the muscle is capable of rapid phasic contraction if all the motor nerve fibres innervating it are excited at the same time at a sufficiently high frequency.

The inner circular muscle was studied by making thin strips partially isolated from the body wall. The nerve supply from a small piece of nearby nerve cord, about 0·5 mm long, was stimulated electrically. Only slow contractions and relaxations were obtained, even with maximal stimulation of the local nerve cord or branches at a high frequency. The minimal response required about 20 Hz, but a twitch occurred with sufficient stimulus strength. The contraction reached peak tension in 0·4–0·6 s and relaxed to baseline in about 2·7 s. The amplitude of the twitch was enhanced by increasing stimulus strength applied to the nerve over a wide range

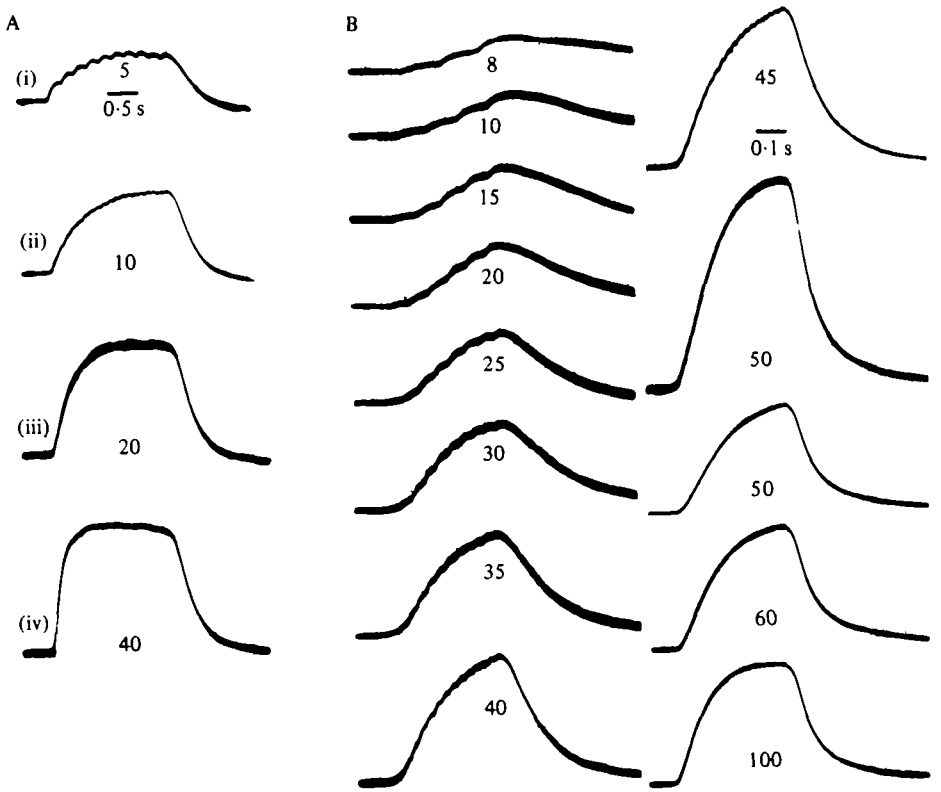

Fig. 4. Mechanical response to supramaximal nerve stimulation of leg and foot muscle at frequencies (Hz) stated. (A) Anterior depressor of the leg; development of tetanus. (B) Retractor of the claw; the gain was reduced to half for the second response at 50 Hz.

without any change in the time-course (Fig. 3B). Increased frequency of stimulation at constant stimulus strength increased total tension and duration of contraction without affecting the rate of rise. These results showed that the circular muscle comprises a homogeneous population of intrinsically slow muscle fibres.

Muscles of the jaw

Each of the jaw muscles tested, the retractor, the lateral and the protractor, gave similar responses to electrical stimulation of the relevant nerves, which each come directly from the brain. The minimum response was a small twitch, rising to a peak in 0·2–0·3 s and decaying to baseline in about 0·4 s (Fig. 3C, i, ii), so that twitch time is briefer than that of other *Peripatus* muscles. This is attributable to a higher rate of relaxation rather than greater speed of shortening. With increasing stimulus strength the twitch height grew progressively, with 6–10 steps (Fig. 3C, i, ii). Upon repetitive stimulation at low stimulus strength there was some facilitation and considerable summation. But at maximal stimulus strength there was but a small increment with repetition (Fig. 3C, iii) and a tetanus:twitch ratio of only about 1·5:1.

Muscles of the leg and foot

There are five locomotory muscles that operate each leg in stepping: promotor, remotor, levator, anterior depressor and posterior depressor (Hoyle & Williams, 1979). We experimented with each of these muscles and they gave similar results. Their twitch times were intermediate between those of the slow body muscles and the jaw muscles. We obtained somewhat better preparations of the remotor, which is the most powerful, and of the anterior depressor, than of their antagonists.

These muscles were prepared by cutting open a leg in the midline dorsally after firmly pinning out the body wall. Tension registration was achieved by seizing the claw by the forceps tips transducer. The relevant muscle was then isolated by cutting away all the other leg muscles. A small piece of nerve cord, with nerve branches to the muscle, was cut away from the rest of the body and placed on stimulating electrodes.

The twitch magnitude increased in 3–5 steps, with increasing strength of stimulation. The rise time to peak of a maximal twitch was as brief as 1·5 s, but the decay to baseline took 0·6–1·0 s. Tetanus:twitch ratios were about 9:1 (Fig. 4A). The mechanical response to the first stimulus was markedly larger than those to subsequent ones.

There are no major muscles operating the foot of *Peripatus*, but there is a conspicuous, long, thin muscle in the leg cavity that runs directly into the twin-tipped claw, which forms the apex of the foot.

The retractor of the claw is attached to the anterior base of the leg and contains about 30 parallel muscle fibres that taper gradually before attaching to the claw apodeme. They are innervated by a branch from the nerve cord, which passes close to the base of the leg. It was relatively easy to make a preparation of this muscle, which gave a weak twitch to a single shock. This rose to a peak in about 0·15 s and decayed to baseline in about 1 s. With repetitive stimulation at low frequencies the second and third increments in force were larger than the first (Fig. 4B). Peak force continued to increase by summation with increasing frequency up to about 50 Hz, and rate of rise of force continued to increase up to about 120 Hz.

Electrical activity

The range of types of electrical events observed, though varied, was basically similar no matter which muscle we were recording from. The entry of an electrode into a fibre was often followed immediately by local spiking, indicating membrane damage by the microelectrode. There was frequent electrode tip breakage at the moment of penetration. We attribute the two forms of damage to a meshwork of collagen fibres intimately associated with all *Peripatus* muscle fibres (Hoyle & Williams, 1979). At penetration, resting potentials were 22–62 mV, but they quickly fell to stable values of 19–48 mV, most being about 30 mV. In 45 stable penetrations in which we paid close attention to the potential, we obtained a mean of $31{\cdot}0 \pm 10{\cdot}7$ (S.E.). The smaller fibres, which range down to only a few microns, were always damaged by the electrode.

Many *Peripatus* muscles have fibres of 30–40 μm diameter, especially the 'giant' inner circular fibres as well as some fibres of dorsal longitudinal and jaw muscles.

The larger fibres almost always had resting potentials of 40 mV or more, and they always gave large spikes at penetration. Fibres with low resting potentials gave either small, probably graded, spikes or none. Owing to the difficulty of obtaining good results from the smaller fibres, we cannot give a precise overall evaluation of *Peripatus* muscle fibres, but we consider that electrical excitability is relatively high and that spikes are common. The typical result on penetration is shown in Fig. 5. Such spikes had maximum amplitudes of about 55 mV, and occurred at a maximum frequency of 10 Hz. The discharge resulting from penetration quickly declined in frequency and stopped. We did not detect a tension increment during the spiking and concluded that only the penetrated fibre was affected. All spikes had relatively long durations, with slow rise and decay times totalling about 70 ms. Both reflect a long time constant, with high resistance and capacitance, associated with the large number of wide invaginated tubules (Hoyle & Williams, 1979).

Junctional potentials

Even following cutting off the nerve supply, and after isolation of a muscle, there remained a continuous barrage of junctional potentials, in a range of sizes and shapes, with maxima reaching 5 mV. These we interpreted as unusually large miniature junctional potentials (m.j.p.s.) (del Castillo & Hoyle, 1979) but they might be due to impulses arising in the cut terminals since they overlapped in size with junctional potentials evoked by electric excitation of motor nerves and by reflex activation. At threshold, in all the *Peripatus* muscles studied, neural excitation evoked only a small (1–5 mV) junctional potential (j.p.). Stronger stimuli evoked somewhat larger (5–15 mV) j.p.s., by recruitment of motor axons innervating the same or nearby regions. The j.p.s. progressively recruited were of similar basic size to the first one initiated, with which they summated (Fig. 6*B*). There was no hint of a division into large and small categories. The larger, summed, junctional potentials initiated graded spike responses with undershooting after-potentials. These will be described below.

The synaptic currents associated with the junctional potentials were unusually obvious. A recording of junctional potential currents from the jaw retractor muscle is shown in Fig. 6A. It is interesting in that it shows progressive facilitation with repetition.

Regardless of recording electrode site, two basically different types of junctional potential were seen: ones with a sharply rising phase and sharp peak, and ones with a slowly-rising phase and rounded peak (Figs. 7, 8). The rounded ones were generally smaller than the others, but there was overlap. Neuromuscular synapses are of two kinds in *Peripatus*: conventional nerve on muscle (NM), and muscle on nerve (MN) junctions of a unique kind. The latter occur at distances up to a few hundred μm from the muscle fibres, which send arms towards a motor nerve. Each arm forms a broad expansion, or head section, that abuts the motor nerve and makes synaptic contacts with three or more motor axons inside the nerve (Fig. 1).

Rounded synaptic potentials must be due to synapses distant from the recording site. They are therefore either due to the MN synapses or to NM synapses located some distance from the recording site. We have not been able to devise any critical

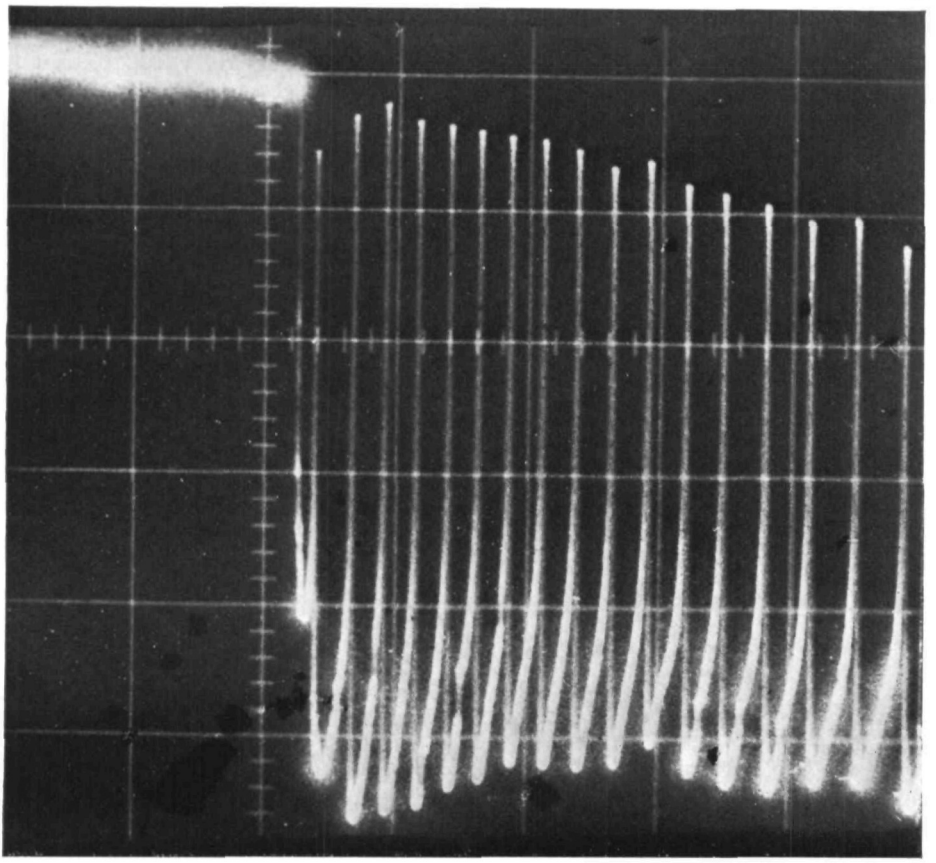

Fig. 5. Repetitive spikes associated with injury during penetration of a dorsal longitudinal muscle fibre during penetration of the electrode. Scale: vertical, 10 mV/division; horizontal. 0·5 s/division.

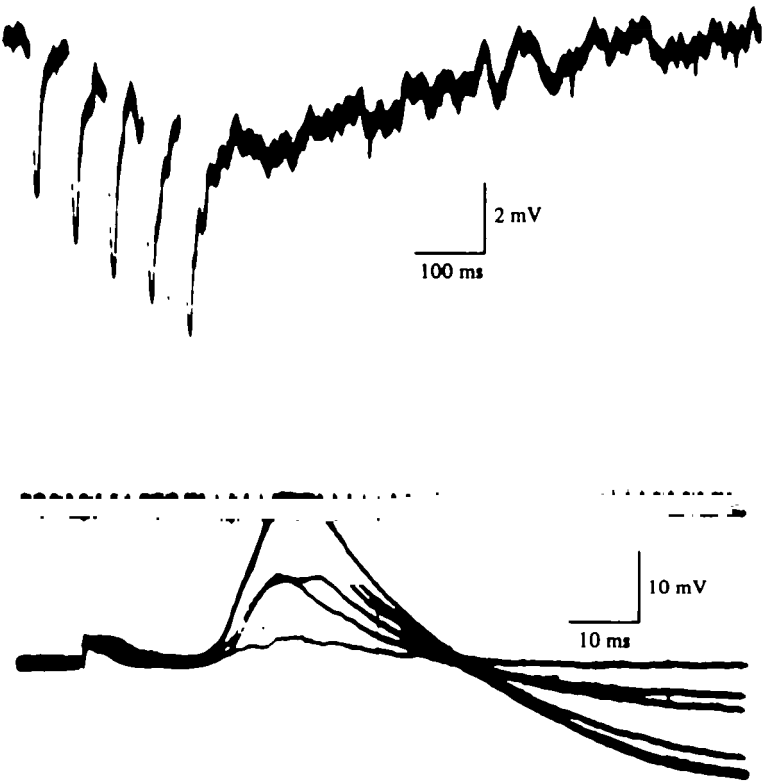

Fig. 6. Neuromuscular transmission to jaw retractor. (A) Extracellularly recorded synaptic current, showing facilitation. (B) Intracellularly recorded synaptic potentials during progressive increase in stimulus strength applied to the nerve. Three distinct steps in junctional potential size occurred, the larger ones evoking graded spike responses.

experiments that might enable us to decide between the alternatives, but it is reasonable to suppose, tentatively, that the smaller, rounded potentials are due to MN junctions and the sharp ones to NM junctions. Activation of contraction is achieved by quite small junctional potentials when the motor nerves are stimulated minimally. In arthropods this has been proven to be possible because there are many nerve terminals of a motor axon distributed along the surface of each muscle fibre (Hoyle, 1967) and because the excitation–contraction coupling thresholds of some muscle fibres is very close to the resting potential (Atwood, Hoyle & Smith, 1965). The local depolarizations cause graded local contractions. A similar system must be operating in *Peripatus* for the NM synapses, but can MN synapses similarly evoke contractions? We may also ask: are the same axons that are involved in MN contacts also involved in NM junctions? Unfortunately, we cannot yet answer these questions. A MN junction is clearly going to make a much smaller contribution than a NM junction to contraction, if their synaptic events are of similar magnitude. What, then, can be the value of the MN synapses?

When spikes appeared in the records they often could not be seen to be arising out of the sharp-peaked junctional potentials. Instead, they appeared to be propagating into the region from distant sources. These may well be the MN junctions. Since

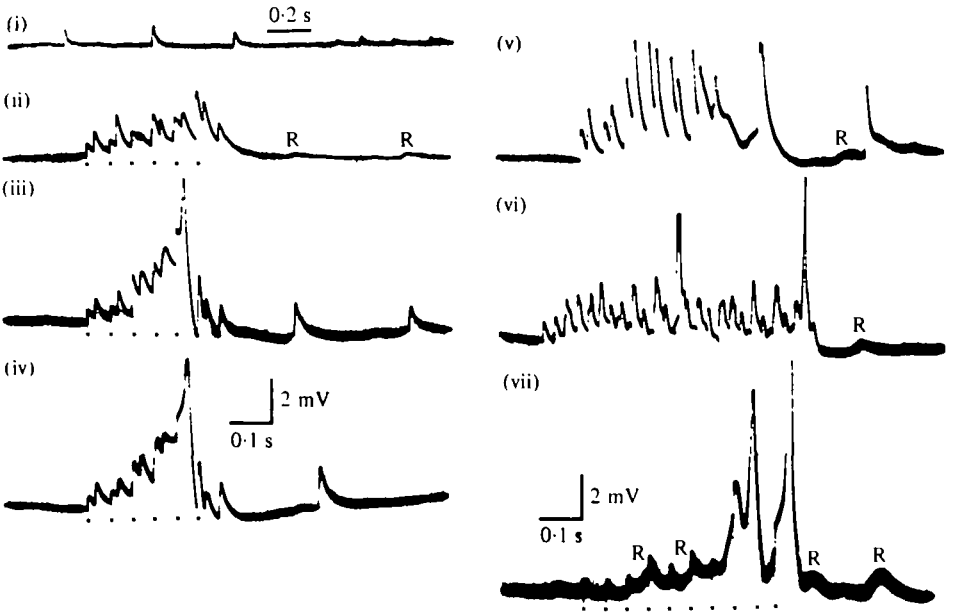

Fig. 7. Junctional potentials and spikes in giant inner circular muscle fibres. (i) Spontaneously occurring j.p.s. (ii–vii) Summating and facilitating j.p.s. evoked by brief trains of stimuli applied to the nerve cord. Most of the j.p.s. originated near the electrode and have sharp peaks. Others, arising at distant sites, are rounded (R).

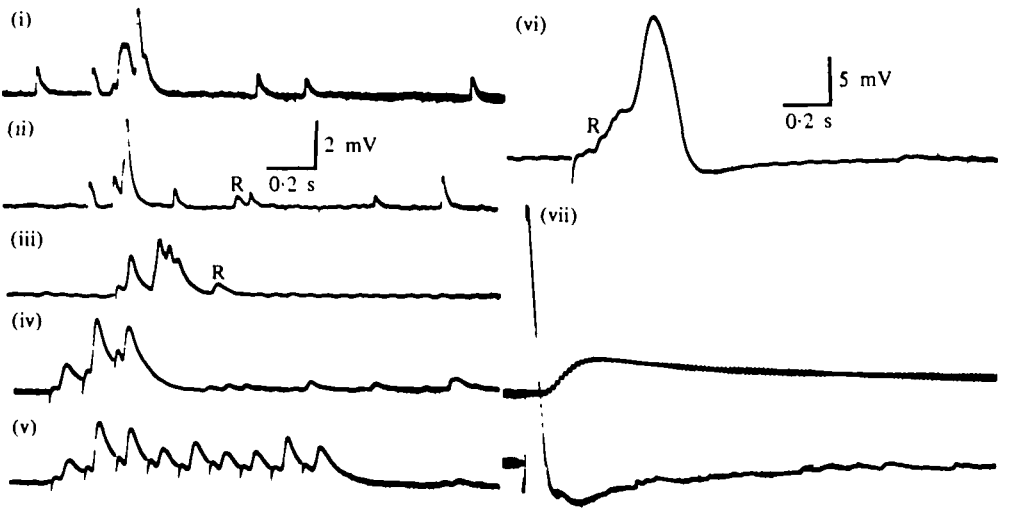

Fig. 8. Junctional potentials in retractor of the leg. (i, ii) Spontaneously occurring j.p.s. (iii–vi) Summating and facilitating j.p.s. evoked by brief trains of stimuli applied to the nerve cord. (vii) Spike evoked by single maximal shock. Twitch response shown above.

spikes cause larger contractions than junctional potentials they will be utilized by the animal when maximum possible speed and force are called for. A dual junctional system could be used to increase the probability of spiking in an emergency. Spikes are presumably propagated along the muscle arms and muscle fibres and activate the

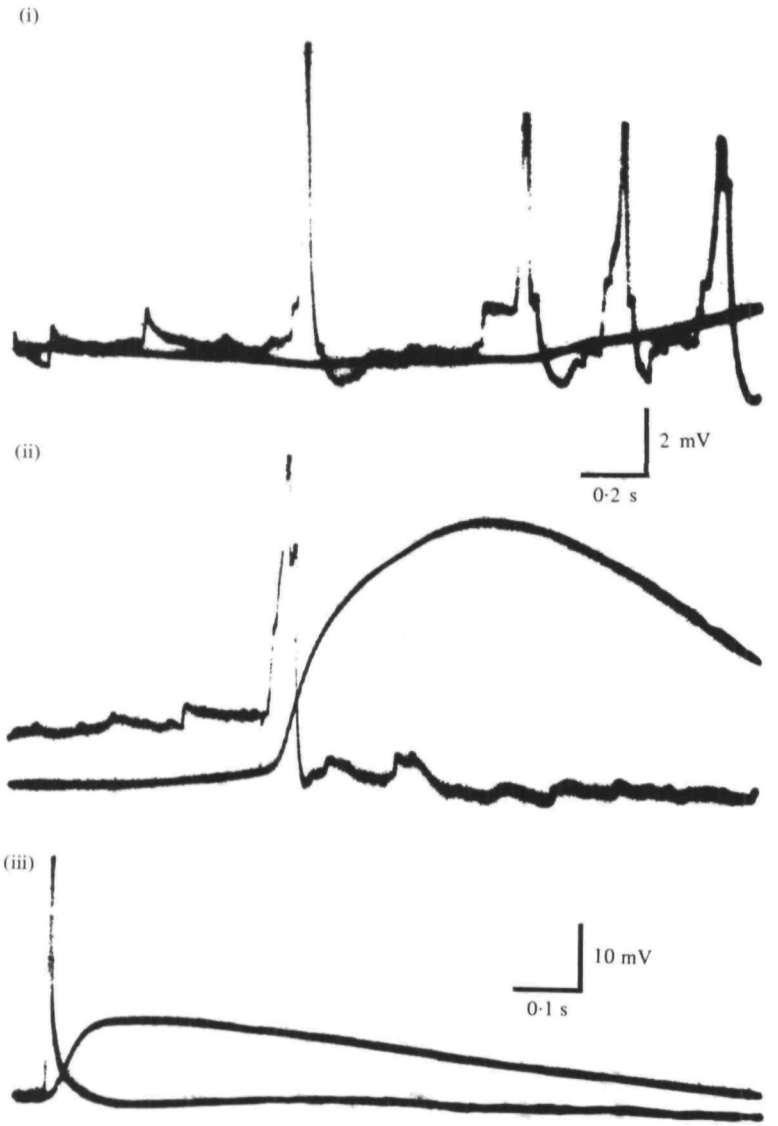

Fig. 9. Spikes in DL muscle. (i) During reflex activation. (ii, iii) In response to single strong shock applied to a small piece of nerve cord nearby. Lower traces are isometric force records.

contractile machinery maximally. An attractive possibility would be if there is a dual excitatory system, NM junctions being for slow activation via junctional potential and MN being for fast activation via spikes.

Action potentials

In some fibres of all muscles except the retractor of the claw we saw an occasional spike action potential. Spikes arose in response to strong reflex initiation, to maximal single electric shocks applied to a nerve, and to bursts of strong shocks at a frequency

of above 10 Hz (Fig. 9). In the latter, junctional potentials were summated and facilitated. Spiking was erratic, as it sometimes is also at crustacean neuromuscular junctions. Examples of the sporadic nature of spiking during burst stimulation are illustrated in Fig. 7, which were recorded from muscle fibres during electrical stimulation of the nerve branch close to the cord. The sizes of the spikes recorded were often quite small, the full range being 6–62 mV. Spikes larger than about 20 mV were recorded only at penetration and for a short time afterwards, so the small size was largely due to damage caused by the electrode. The presence of a regenerative, or spike, response was indicated by a rapid return phase and undershoot. These small spikes nevertheless served as a valid indicator of events at more distant, less deteriorated, sites, that presumably accurately reflect normal events. The spikes were variable in size; most are probably graded events that propagate with a decrement.

Apparent lack of peripheral inhibition

We at no time saw any hyperpolarizing junctional potentials in *Peripatus* muscle fibres during reflex, spontaneous or electrically excited activity. Nor was there any reduction in height of tension as we raised the stimulus strength during tetanus. We conclude that *Peripatus* may lack peripheral inhibition.

DISCUSSION

We examined electrical aspects of neuromuscular transmission, as well as tension development, for a majority of *Peripatus* muscles: dorsal, ventral and ventro-lateral longitudinal, inner circular, jaw operators, leg movers and retractor of the claw. These muscles are all relatively slow, the quickest being jaw muscles with a twitch time of about 0·6 s. The slowest was circular body-wall muscle, with a twitch time of about 3 s. Although serving different basic functions, all of these muscles are called upon to produce a wide range of forces and speeds. All share in tonic functions except, possibly, the jaw movers. The intrinsically slow speeds of the muscles necessarily limit *Peripatus* actions. The jaws cannot make complete movement cycles in less than 0·6 s, so they cannot tear food at a frequency greater than about 1·5 Hz, which has been noted as the cutting frequency (Kaestner, 1968). The muscles operating the legs require about a full second for a complete cycle, limiting efficient stepping to 2 Hz, which was the rate we observed in movies of *Peripatus dominicae* during fast walking. To activate a leg muscle for walking at top speed would require nearly simultaneous excitation of impulses in most of the several excitatory axons innervating it, at a frequency of 50 Hz or more, for 0·2 s.

With the possible exception of the retractor of the claw of the foot, all the muscles receive many motor axons. The jaw movers each receive at least eight, as judged by the twitch heights obtainable by graded strength stimulation, even though they are small and discrete, with only about 40 muscle fibres each. There are several hundred muscle fibres in the dorsal longitudinal and lateral longitudinal muscles. Each cluster of about 30 muscle fibres receives a motor nerve branch, and in these branches at least 12 motor axons were discerned (Hoyle & Williams, 1979). We did not find a corresponding number of discrete steps in physiological preparations, either because some are sensory neurons, or because some motor axons have similar

thresholds. However, a wide range of tensions could be obtained, depending on stimulus parameters, from all parts of the body.

Jaw muscles provided a contrast to the body and leg muscles, with both a quicker relaxation and a more intensive activation by a maximal single shock applied to the nerve. The tetanus:twitch (force) ratio of less than 2:1 under maximal stimulation, sets them apart from other *Peripatus* muscles, where the ratio is at least 6:1 (leg remotor) and as high as 50:1 (claw retractor). Whilst these differences in part represent the sizes of summed junctional potentials and evoked spikes, they must also reflect differences in the extent to which the contractile material is activated by similar depolarization. The slower muscles with the higher tetanus:twitch ratios are, like vertebrate smooth muscle, are only weakly activated, even by a spike. There is a system of dual innervation of *Peripatus* muscles, by a combination of muscle on nerve and nerve on muscle synapses that is unique in the animal kingdom (Hoyle & Williams, 1979). We were not able to discern any clear functional division associated with this division. However, we would like to propose a possible one. Junctional potentials are bound to be attenuated as they pass down muscle arms, reducing their effectiveness in causing contraction. This is not the case for regenerative spikes, such as are initiated in nematodes which have exclusively muscle on nerve synapses (de Bell, del Castillo & Sanchez, 1963; del Castillo, de Mello & Morales, 1967). We obtained spikes occasionally, in response to nerve stimulation, in all muscles except the retractor of the claw. Most of these lacked an inflexion in rising phase that would indicate initiation from a nearby junction; rather they were propagated in from a more distant one.

We suggest that muscle on nerve junctions of *Peripatus* represent sites for the initiation of spikes. They may even represent a parallel activation system whose purpose is to lead to greater activation when maximum speed is called for. *Peripatus* muscle fibres range widely in diameters but no evidence was found either anatomically (Hoyle & Williams, 1979) or physiologically in the present work, for a division into populations of different types of muscle fibres. It is now realized that subpopulations of muscle fibres having different ultrastructures and physiological properties are common in both crustacean (Atwood *et al.* 1965; Atwood, 1977) and insect (Elder, 1975; Cochrane, Elder & Usherwood, 1972; Hoyle, 1978) muscle. However, the ultrastructures of *Peripatus* muscles and neuromuscular synapses are both markedly different from those of either annelids or arthropods. Furthermore, the muscle arms making muscle on nerve contacts, although somewhat akin to those of nematodes (Rosenbluth, 1965; Ware *et al.* 1975), have no counterpart in either annelids or arthropods.

Annelid muscles that have been studied include longitudinal body-wall musculature of an earthworm (Chang, 1969; Hidaka, Ito & Kuriyama, 1969; Drewes & Pax, 1974), a polychaete (Wilson, 1960) and a leech (Stuart, 1970). All are innervated by a slow axon that produces facilitating junctional potentials and a slow, frequency-dependent contraction. They also receive a fast axon that evokes a larger synaptic response, giving rise to a twitch that rapidly declines with repetition, and some have peripheral inhibitory axons. Whilst some arthropod muscles receive two or three slow axons, plus two or three fast ones and one or two intermediates, many receive only one of each.

The mode of control of contraction in *Peripatus* muscle is by a combination of the number of motor neurones active and their frequency of discharge. This state of affairs is seen in flexor muscles of arthropods (Phillips, 1978), which receive up to eight motor axons. However, in *Peripatus*, unlike arthropod flexors, no single excitatory neurone exerts a strong activation of contraction. Summation of small junctional potentials initiated by different axons is an essential part of the control of contraction. In summary, it is evident that annelids, onychophorans and arthropods all rely on distributed, multiple synaptic activation of single muscle fibres, with both summation and synaptic facilitation playing major roles in the control of contraction. However, it is not possible to discern an evolutionary sequence, annelids being primitive, arthropods more advanced and *Peripatus* an intermediate. *Peripatus* has unique features that clearly separate it from either annelids or arthropods.

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