

PROPERTIES OF ACTION POTENTIALS FROM INSECT MOTOR NERVE FIBRES

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INTRODUCTION

Extracellular recording from small nerve branches provides a method for recording from several nerve fibres simultaneously. This is extremely desirable in studying the organization of a neuronal system. Furthermore, extracellular records are insensitive to small movements, are stable over long periods, and can easily be repeated with subsequent experimental animals. They also involve relatively little dissection, and hence reduce the risk of injury to nerve fibres. We have derived various theoretical predictions for records taken from small nerve trunks using different electrode configurations (see Pearson, 1969). The present paper illustrates some of these theoretical results and their application to experimental work.

The preparations we have used are a levator motor nerve from the metathoracic segment of the cockroach and the tergal nerve to the third abdominal segment of the locust. The cockroach nerve is not ideal for this particular study, but is of interest in our work on the functional organization of motor units (Pearson & Bergman, 1969; Pearson & Iles, 1970) and on the patterns of neural activity during behavioural sequences. Use of a 'typical', rather than a specially selected nerve, illustrates the advantages and disadvantages of the various recording methods. The locust nerve, on the other hand, was selected because of its long length. The following properties of action potentials from different nerve fibres were measured in these preparations: monophasic and triphasic amplitudes, conduction velocity and duration. Triphasic or monophasic amplitudes were used to predict fibre diameter, as checked from histological sections. Conduction velocity was less useful since there were systematic deviations from the expected square root relation between velocity and diameter. The systematic effects of fibre diameter on the properties of nerve action potentials should be of general physiological interest.

METHODS

Cockroach preparation. The metathoracic nerve 6B ramus 4 (6Br₄ in the notation of Pipa & Cook, 1959) of *Periplaneta americana* contains motor fibres which innervate the main and posterior coxal levator muscles (181 and 182 in the notation of Carbonell, 1947). This nerve trunk is easily seen after pinning back the coxa to expose its dorsal surface and removing the tergal remotor muscles (174, 175 and 176) from the dorsal coxal rim (see Pearson & Bergman, 1969). Further dissection of the part of the coxal rim connected to the posterior coxal levator muscles reveals the branch point of

6Br₄; one branch going to the main levator muscles and the other to the posterior levator muscles. Neglecting the fine sensory branch 6Br₃ (which was cut from the main nerve), the unbranched length of the nerve 6B from ramus 2 to the branch point of 6Br₄ is about 2 mm.

Locust preparation. The tergal nerve to the third abdominal segment of *Locusta migratoria* has a long unbranched length (6 mm) and contains about twenty motor nerve fibres. The origin of this nerve is the third abdominal ganglion, which, together with the first two abdominal ganglia, is fused to the metathoracic ganglion. The tergal nerve was exposed by removing the ventral cuticle from the thorax and first three abdominal segments. The smaller fibres within this nerve are spontaneously active and the larger fibres are readily activated by light touch to the ventral abdominal segments.

Electrodes and recording equipment. All electrodes consisted of 75 μ silver wires. For recording a hook-shaped electrode was manipulated under the nerve and the nerve was carefully lifted until between 1 and 1.5 mm was clear of the haemolymph. This section was coated with petroleum jelly (Vaseline) to prevent drying. A second electrode was then placed in the haemolymph close to this section of nerve and the two electrodes were connected to a preamplifier. This gave a stable triphasic record. Extreme care was needed when lifting the nerve clear of the haemolymph to prevent damage to the individual fibres. A syringe was usually used to suck the haemolymph away from the nerve rather than attempting to draw the nerve out of the haemolymph. Signs of damage in a fibre were inflexions in the first positive or negative peaks and/or the absence of a third phase.

Several methods of monophasic recording were tried. The most satisfactory was to lift the nerve out of saline, coat it as described above, and then cut the nerve just distal to the point of recording. Immediately after cutting the nerve, the potential was diphasic, but within a few minutes a good and fairly stable monophasic potential was recorded.

To study the full time course of the action potentials, a second, more proximally situated electrode pair was used. Records from the proximal electrode pair could be pulse height analysed (Stein, 1968) so that a given fibre could be selected to trigger the oscilloscope sweep. The second pair of electrodes was also needed to measure conduction velocity. Because of the short length of the cockroach nerve, smaller lengths of nerve were lifted clear of the haemolymph when two sets of electrodes were used. The preamplifiers, a Tektronix Type 122 and an Isleworth Type A101, were connected to a Tektronix 502A oscilloscope, and the traces were photographed.

Source resistance. The source resistance of the action potentials was measured by placing a variable resistor across the input to the amplifier. The resistance value which reduced the amplitude to about half gave an approximate measure of the source resistance. This was less than 200 k Ω for monophasic recording and less than 100 k Ω for triphasic recording. These values are sufficiently low compared to the input resistance of the preamplifiers (10 M Ω) that no correction was needed. The total input capacitance (amplifier plus leads) to the Tektronix preamp (which was used for time course measurements) was about 110 pF, so the time constant for the input to the recording equipment was about 22 μ sec. for monophasic records and less for triphasic records. Thus, the frequency limitation was close to the high frequency cut

on the preamp (40 kc/s), and the shape of the action potentials could be accurately recorded.

Measurement of duration. Action potential duration may be measured in a variety of ways. For the small fibres with monophasic potentials less than 200 μ V, the slope of the rising and falling phases could not be accurately determined, so the method of Paintal (1966) was inappropriate. The positions in time when the action potential amplitude reached half its maximum value were readily determined, even in the presence of noise, and we have used the interval between the half-maximum amplitude points on the rising and falling phases as a measure of action potential duration.

Measurement of conduction velocity. Pearson (1969) showed that the positions of the first crossover point and the negative peak of the triphasic potential relative to the peak of the membrane potential are fairly insensitive to the length of restriction. Furthermore, the negative peak corresponds to within 0.04 msec. of the membrane action potential peak. The conduction time for the cockroach nerve fibres was measured as the time between the first crossover points at two separate pairs of electrodes with triphasic records, as the crossover points could be determined more accurately than the negative peaks. For the locust nerve, conduction times were measured between the negative peak of the triphasic record and the peak of the monophasic potential at a second electrode pair. The distance between electrodes was measured with a calibrated microscope eyepiece to an accuracy within 5% and was used for calculating conduction velocity.

Temperature. All experiments for measurement of conduction velocity or action potential duration were carried out at $20^{\circ} \pm 2^{\circ}$ C. In those experiments in which the temperature was not 20 $^{\circ}$ C, conduction velocity was corrected using a Q_{10} of 1.7 (Chapman & Pankhurst, 1967). The amplitude of the monophasic action potential is much less temperature sensitive so no correction was made.

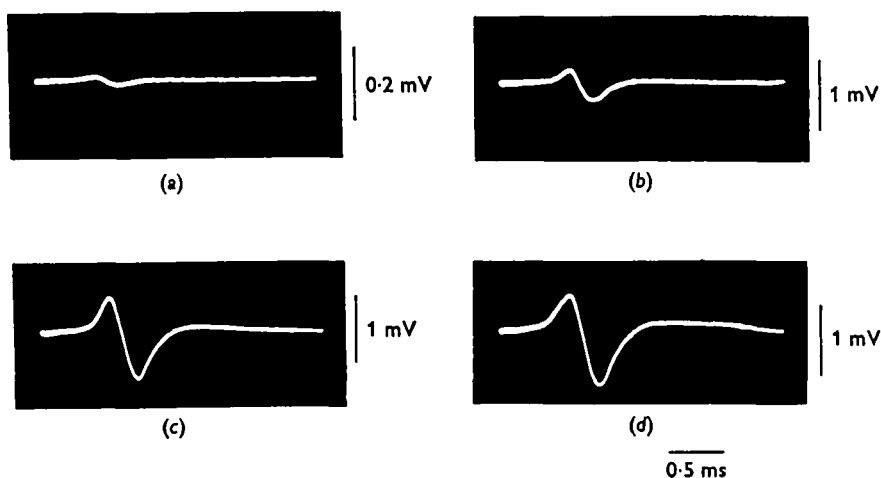
Histology. Only the cockroach nerve was examined histologically. Fixative (3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2-7.4) was poured on the nerve *in situ*. After fixation, the nerve was removed, dehydrated in ethanol and embedded in Araldite. One micron sections were cut starting from the peripheral end of the nerve until after the point where a sensory branch (nerve 6Br3) separated from the rest of the nerve. Sections were then stained with methylene blue and examined under a light microscope. The sensory branch can be seen on the right side of Pl. 1. At the level of this section, it had nearly separated from the larger motor nerve 6Br4. This was close to the point where electrical recording was normally made after removing the sensory branch. The various fibres were identified using criteria given in the text.

Thin sections were cut and routinely stained with an aqueous solution of uranyl acetate and then in lead citrate before examination in the electron microscope. Fibre diameters were calculated from measurements of cross-sectional areas in electron micrographs as described in the text. These were considerably more accurate, particularly for small fibres which were difficult to measure from the light micrographs. Agreement between light micrographs and electron micrographs of various magnifications was generally better than 20%.

RESULTS

Cockroach nerve

In an intact cockroach there are three or four different-sized fibres in nerve 6Br4 of the metathoracic segment which are continuously spontaneously active. In addition, a number of larger fibres tend to fire together in large bursts of activity. Summation and interaction of these fibres makes it difficult to distinguish single fibres accurately. If the abdominal and thoracic connectives are cut out to neurally isolate the segment, generally only six motor fibres are active even with strong mechanical stimulation. The amplitude of these six fibres recorded triphasically from an intact nerve varies over a wide range. Also the discharge pattern of each fibre is different (Pearson & Bergman, 1969) so each fibre can be readily distinguished from animal to animal. This ability to recognize corresponding fibres in a number of experimental animals is a great advantage. Results can then be averaged from a number of experiments to determine more accurately the properties of each motor nerve fibre. We have numbered these fibres from 1 to 6 in order of increasing amplitude and shall refer to them by number in the rest of the paper.

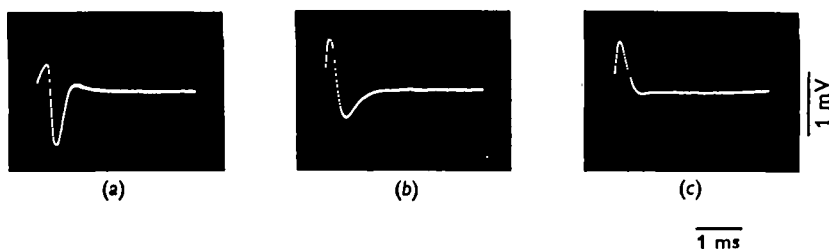


Text-fig. 1. Spontaneously occurring nerve action potentials recorded triphasically from an intact cockroach nerve in the animal's haemolymph (a) and as increasing lengths are lifted out of the haemolymph (b-d). Beyond a certain length the amplitudes of the first two phases do not increase though the waveform continues to spread out in time.

Amplitude. In a freshly dissected animal, the nerve lies in a thin layer of haemolymph. The potentials that can be recorded are small (Text-fig. 1a) but as one lifts the nerve free of the haemolymph, they rapidly increase in size and spread out in time (Text-fig. 1b and c). Beyond a certain length out of solution the amplitude does not increase further (Text-fig. 1d) although the time course continues to lengthen and the third phase continues to increase. The saturated values of the first two peaks are in the ratio 1:2 as predicted (Pearson, 1969) and indeed the shapes of the action potentials show good qualitative agreement with computed values.

If a long length of nerve is pulled out so that the maximum amplitudes are approached,

and one is careful not to injure the nerve, the amplitude of each fibre is quite reproducible (the standard deviation from animal to animal is about 15% of the mean). After coating the nerve with petroleum jelly (Vaseline) to prevent drying, stable records can be obtained for hours without the need for any saline. Cutting the nerve distal to the electrode changes the record from triphasic (Text-fig. 2*a*) to diphasic (Text-fig. 2*b*). The positive peak of the diphasic record is equal in size to the negative peak of the triphasic record and thus twice the first positive peak of the triphasic record. If the nerve is cut close to the electrode, the amplitude of the positive peak remains constant while the negative peak decreases in size and lengthens out. Soon, a good monophasic potential remains (Text-fig. 2*c*) which is an extracellular replica of the membrane action potential. Monophasic amplitudes were slightly more reproducible than triphasic amplitudes (the standard deviation from animal to animal was only about 10–15% of the mean amplitude), though they are not as stable as triphasic records. After 10 or 15 min the monophasic action potentials lengthen and then gradually decline in size. This process takes longer the greater the length of nerve, and is presumably due to the nerve 'running down' as it loses potassium and other substances from the cut nerve end.



Text-fig. 2. Comparison of waveforms obtained (a) from an intact cockroach nerve (triphasic record), (b) immediately after cutting the nerve distal to the recording electrode (diphasic record), and (c) a few minutes later (monophasic record).

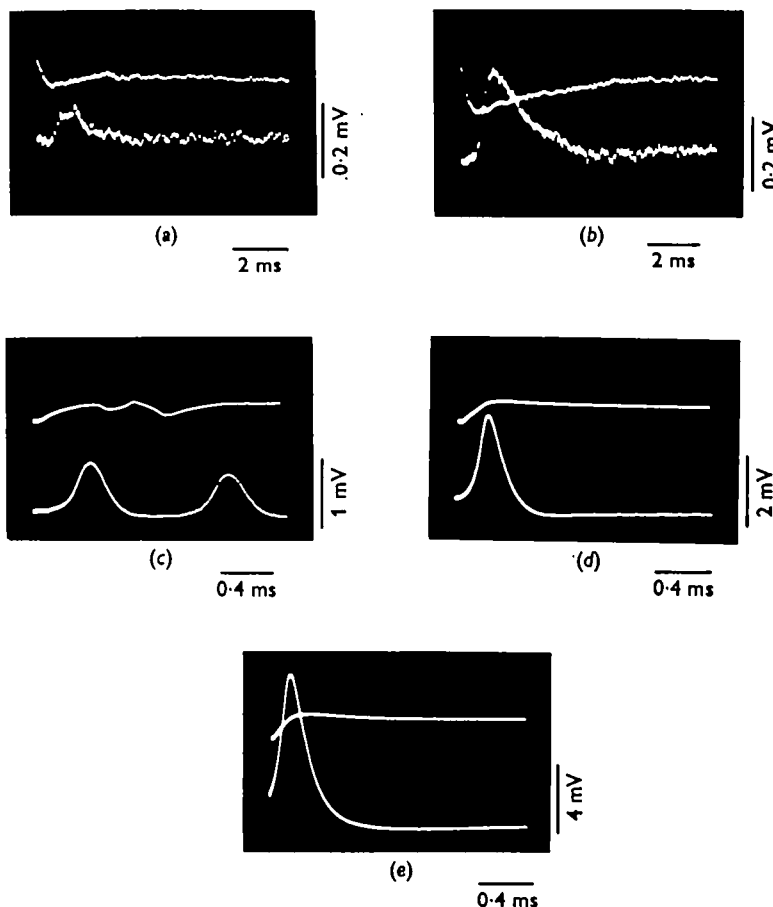
Text-fig. 3 shows monophasic records of the six efferent fibres which discharge with segment neurally isolated. A second pair of electrodes was placed proximally to trigger the oscilloscope so that the whole time course of the action potential could be seen.

For recording fibres 3–6 in Text-fig. 3, only a short length of nerve was pulled out at the proximal electrode to minimize interaction between the two pairs of electrodes over this short length of nerve. This means that the triphasic potentials were small and one cannot easily trigger off fibres 1 and 2. In another experiment, a longer length was pulled out for triphasic recording (and a shorter length for monophasic recording) to give records for fibres 1 and 2. The temperature in the first experiment was 3 °C higher than in the second.

The amplitudes of fibres 3 and 4 were sometimes indistinguishable. When they were different in size, fibres 3 and 4 could be identified by their spontaneous discharge patterns and response to mechanical stimulation. Also, fibre 3 is a branch of the common inhibitor (Pearson & Bergman, 1969) and can be readily distinguished if one records simultaneously from another of its branches.

Text-fig. 4 shows the relation between monophasic amplitude and the amplitude of both the first positive peak and the negative peak of the triphasic waveform. The extent of each symbol gives the standard deviation of the measurements from between

twelve and twenty-one experiments. Double logarithmic co-ordinates are used because of the wide range of amplitudes. The straight lines are the expected relationships when a sufficient length of nerve has been pulled out so that the triphasic records reach their maximum value. The upper data symbols give the amplitudes of the



Text-fig. 3. Monophasic action potentials recorded in the cockroach nerve from (a) fibre 1, (b) fibre 2, (c) fibres 4 and 3, (d) fibre 5, (e) fibre 6. The short time interval between the spontaneous occurrences of fibre 4 and 3 in (c) indicates that they are separate fibres although their amplitudes are only slightly different. Note the range of voltage and time scales used for the monophasic records. The upper traces are the triphasic records used to trigger the oscilloscope sweep.

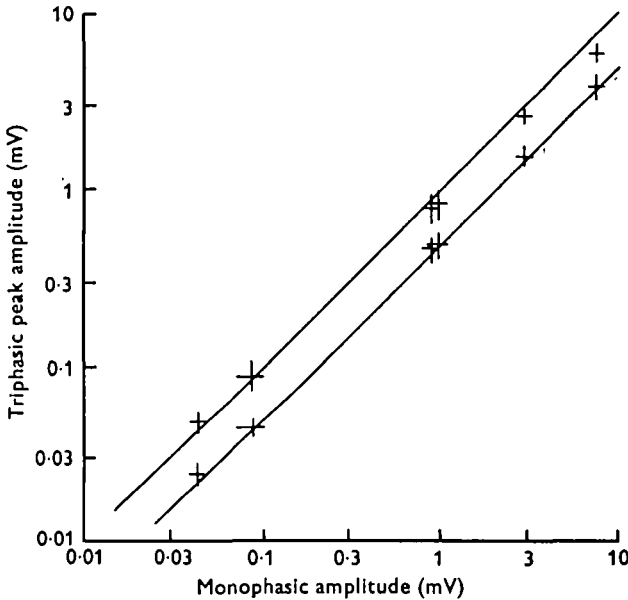
negative peaks in triphasic records from the six fibres. The upper straight line is the simple prediction that the amplitude of the negative peak of the triphasic record should equal the amplitude with monophasic recording. The lower data points give the amplitude of the first positive peaks in the triphasic records, and they should be equal to half the monophasic amplitudes (lower straight line).

The agreement between the data and the prediction is very good, particularly for the first positive peak. There is a small deviation in the negative peaks of the triphasic records for the largest fibres. They are slightly smaller than predicted, presumably because the length of nerve for recording was not quite long enough for the negative

peaks to reach their maximum values. Theoretically, it was found (Pearson, 1969) that the positive peak reached its maximum value somewhat earlier than the negative peak as the restricted length of nerve was increased relative to the conduction velocity of the fibre.

Predicted diameter. We have shown that the monophasic amplitudes in mV can be converted to fibre diameter in microns using the equation:

$$a = b \sqrt{(V_1/V_m)},$$



Text-fig. 4. The amplitude of the first positive peak (lower data symbols) and negative peak (upper data symbols) from triphasic recording plotted as a function of monophasic amplitude of the six active fibres in the cockroach nerve. The vertical and horizontal extent of the symbols gives the standard deviation of the values measured in a number of preparations. The solid lines are theoretical predictions as explained in the text. Double logarithmic co-ordinates.

where a is the fibre radius, b the trunk radius, V_1 the peak amplitude of the monophasic potential, and V_m the peak amplitude of the membrane action potential (see Pearson, 1969). From histological section and direct microscopic observation, $2b$ was about 70μ . The peak amplitude of intracellular action potentials, V_m must be assumed, and we shall use an amplitude of 100 mV which is about what Pichon & Boistel (1967) measured intracellularly from cockroach giant fibres. Then, if V_1 is the amplitude of the monophasic action potential in mV and d is the fibre diameter in μ , we have:

$$\begin{aligned} d &= 70 \sqrt{(V_1/100)} \\ &= 7 \sqrt{V_1}. \end{aligned} \quad (1)$$

The saturated peak-to-peak value expected from the triphasic records is $3/2$ times the monophasic amplitude, so diameter can also be calculated from the peak-to-peak triphasic amplitude which we denote by V_3 , where

$$d = 5.7 \sqrt{V_3}. \quad (2)$$

Predicted diameters using equations (1) and (2) are given in Table 1.

Histological measurement of diameter. Both light and electron micrographs from five fresh cockroach preparations were examined in detail, sometimes at more than one point along the nerve. Although one cannot equate with absolute certainty, the fibres observed electrically with those seen in fixed sections, a number of criteria were available to reliably identify the fibres in cross sections such as Pl. 1.

(1) Experiments using both reflex and electrical stimulation of intact animals indicate the presence of a number of fibres larger than the largest one (fibre 6) which shows activity with the segment neurally isolated. At least four such fibres have been recorded in single experiments so that most of the largest fibres do *not* correspond to any observed electrically.

(2) In no experiment was any motor fibre found which had an amplitude intermediate in size between the six fibres which show activity in the neurally isolated segment. One and only one sensory fibre which was intermediate in size between motor fibres 2 and 3 was regularly seen. If we call this fibre S, there should be a sequence of fibres of steadily increasing size in the order 1, 2, S, 3, 4, 5, 6.

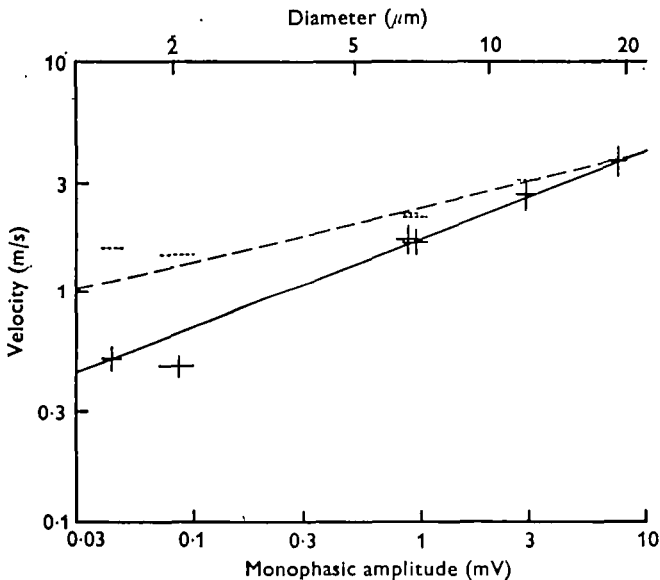
(3) The amplitudes of electrical records from fibres 3 and 4 are very similar in size, and so their diameters are presumably of similar size. The only two medium-sized fibres with comparable diameters are those at about $5\ \mu$ in Pl. 1. Having picked out these two fibres, it is straightforward to identify successively larger or smaller fibres and number them as indicated on Pl. 1.

Table 1

Axon	Monophasic amplitude (mV)	Predicted diameter (μ)	Triphasic p/p amplitude (mV)	Predicted diameter (μ)	Measured diameter (μ)
1	0.043	1.5	0.073	1.5	1.6
2	0.085	2.0	0.13	2.1	1.9
3	0.88	6.6	1.2	6.2	5.1
4	0.96	6.9	1.3	6.5	5.7
5	2.9	11.9	4.1	11.5	10.4
6	7.4	19.0	9.7	17.7	16.1

This labelling was confirmed from low power electron micrographs and higher power electron micrographs were taken of the fibres of interest. The cross-sectional areas were measured with a planimeter and average diameter was then calculated by dividing the cross-sectional area by $\frac{1}{4}\pi$ and taking the square root. The average measured diameters from five cockroach nerves are shown in Table 1, and the predicted diameters from spike amplitude measurements agree to within about 20% of these measured diameters. This is reasonably good agreement considering the inaccuracies inherent in both methods. However, the measured diameters are mainly smaller than predicted. Shrinkage during fixation could account for part of this difference since the average nerve diameter measured from the total cross-sectional area of the fixed nerves was $64\ \mu$, whereas the diameter measured from direct observation of the living nerve was $70\ \mu$. There could also have been systematic differences between the two groups of animals used or systematic errors resulting from the choice of constants in equations (1) and (2). Nonetheless, our results indicate that a good estimate of fibre diameter can be obtained simply by measuring the heights of extracellularly recorded action potentials from fibres in small nerves, and directly observing the diameter of the nerve *in situ*.

Conduction velocity. Conduction velocities of fibres 1 to 6 were measured in twelve experiments. Velocity appears to be slightly more reproducible (the standard deviations from animal to animal were only somewhat over 10% of the mean) than either monophasic or triphasic amplitude. However, the range of velocities is only a factor of 7, whereas amplitudes vary over more than a 100-fold range for the same fibres. If we consider the variation relative to the total range, it is clear that either monophasic amplitude or triphasic amplitude gives a more reproducible estimate of diameter. Text-fig. 5 shows a plot of conduction velocity against monophasic amplitudes on log-log co-ordinates, together with a good fitting straight line for all but fibre 2. The extent of each solid symbol gives the standard deviation of the values measured from animal to animal.



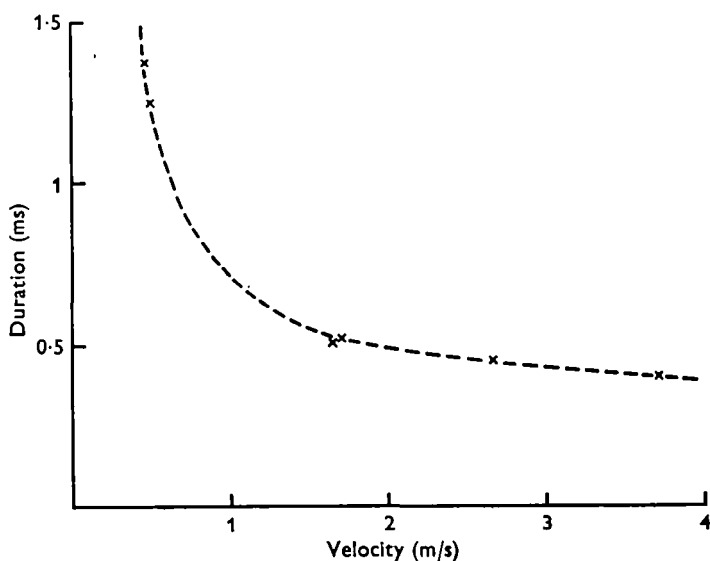
Text-fig. 5. Conduction velocity as a function of monophasic amplitude for the six active fibres in the cockroach nerve. Fibre diameter can be calculated from monophasic amplitude using equation (1) and diameter values are shown above. The extent of the solid data symbols represent the standard deviation of the measurements from animal to animal. The solid line drawn through the points has a higher than expected slope on double logarithmic co-ordinates (the dashed line gives the expected slope). Further explanations in text.

If the membrane properties and action potential waveform were independent of diameter, conduction velocity should increase as the square root of fibre diameter. Since the monophasic amplitude increases as the *square* of fibre diameter (as checked earlier against the histological sections) the expected slope of the velocity/monophasic amplitude relationship is 0.25. (A power function plots as a straight line on log-log paper and the slope gives the exponent.) The measured value of 0.39 is much greater and suggests that conduction velocity varies as the 0.78 power of fibre diameter. An even higher value would result if a line nearer to fibre 2 was drawn. Fibre 2 clearly deviates from the straight line relation and although its monophasic amplitude is double that of fibre 1, its conduction velocity is smaller. The reproducibility of the values from animal to animal and close agreement of monophasic and triphasic potentials suggest that selective partial damage of this fibre was not responsible.

If the conduction velocities determined for fibres 1 to 6 are used to estimate diameter (assuming velocity is proportional to the square root of diameter and taking as reference that the computed conduction velocity at 20 °C in a squid axon of 476 μ diameter is 19 m/s (Stein, unpublished calculations) then, apart from fibre 6, the estimated diameters are less than those measured from Pl. 1, as shown in Table 2. The largest errors occur for the smaller fibres with fibres 1 and 2 differing more than a factor of four from prediction. Thus the diameters, particularly of smaller fibres, are more accurately estimated from spike amplitude rather than from conduction velocity measurements.

Table 2

Axon	Velocity (m/s)	Estimated diameter from velocity (μ)	Measured diameter (μ)
1	0.51	0.34	1.6
2	0.47	0.29	1.9
3	1.71	3.9	5.1
4	1.66	3.6	5.7
5	2.66	9.3	10.4
6	3.71	18.2	16.1



Text-fig. 6. Action potential duration measured at half the peak amplitude as a function of velocity for fibres of the cockroach nerve.

Duration. The relationship between conduction velocity and monophasic action potential duration is plotted in Text-fig. 6. The shape of this curve agrees well with that found by Paintal (1966, 1967) for small mammalian fibres and suggests that there is a systematic relation between action potential duration and conduction velocity. Indeed, fibre 2, which appears to have an anomalously low conduction velocity also has a particularly long duration (see Text-fig. 3) and a smooth curve can be drawn through all the data points. Thus, there are factors related to fibre size that markedly affect both action potential duration and conduction velocity.

In fact, the deviations from predicted conduction velocities are roughly comparable

to the increase in durations. This can be shown by multiplying the conduction velocity of fibres 1 to 5 by an amount equal to the ratio of the duration of each fibre's action potential relative to that of fibre 6. These new values are represented as horizontal interrupted lines in Text-fig. 5 with the same lateral extent as previously. They lie close to the expected relation between conduction velocity and amplitude (an interrupted line with slope 0.25 has been drawn through the value for fibre 6).

Structural features. Electron micrographs were examined for systematic differences between large and small fibres which might account for their different electrical properties. One striking difference is seen in Pl. 2 and 3. Plate 2 shows two very large fibres while Pl. 3 shows fibres 1, 3, 4 and 6. The largest fibres have a prominent concentric arrangement containing half a dozen layers of glial processes. Between these layers is found a dense material thought to be an acid muco-polysaccharide (Smith & Treherne, 1963) which is stained very well by uranyl acetate and gives the nerves a characteristic appearance. This arrangement of glial processes and dense extracellular material is less prominent around the medium-sized fibres (2 to 3 layers) and virtually absent from the smallest fibres.

The intricate arrangement of glial cells also raises a question concerning the interpretation of recordings taken with electrodes external to the nerve, for these cells may alter the extracellular current pathways. The electron micrograph indicates that the peripheral organization is very similar to that described by Maddrell & Treherne (1967) in the neural connectives and ganglia of *Periplaneta*. Underlying the connective tissue sheath is a region of perineurium with numerous dense lines toward its inner border. At higher magnification (Pl. 4) these are seen to be composed of regularly spaced transverse bridges across the space between adjacent glial cell membranes. Similar transverse bridges, known as septate junctions have been described in the salivary gland of *Chironomus* and *Drosophila* (Bullivant & Lowenstein, 1968; Malhotra, 1969) where they have been shown to provide low resistance connexions between cells. If a similar function were present here, the effect of the glia on the electrical records was probably small. Also the outer sheath probably had little effect for Treherne (1961) found that even with the much thicker sheath surrounding central ganglia in the cockroach, removal of the sheath does not greatly affect the passive efflux of sodium ions from the central nervous system.

In conclusion, the properties of nerve action potentials vary systematically with fibre size, and there are important systematic differences in structure, although, as will be discussed later, the two cannot yet be correlated with certainty. These results are based on rather few fibres, so a second insect nerve was investigated.

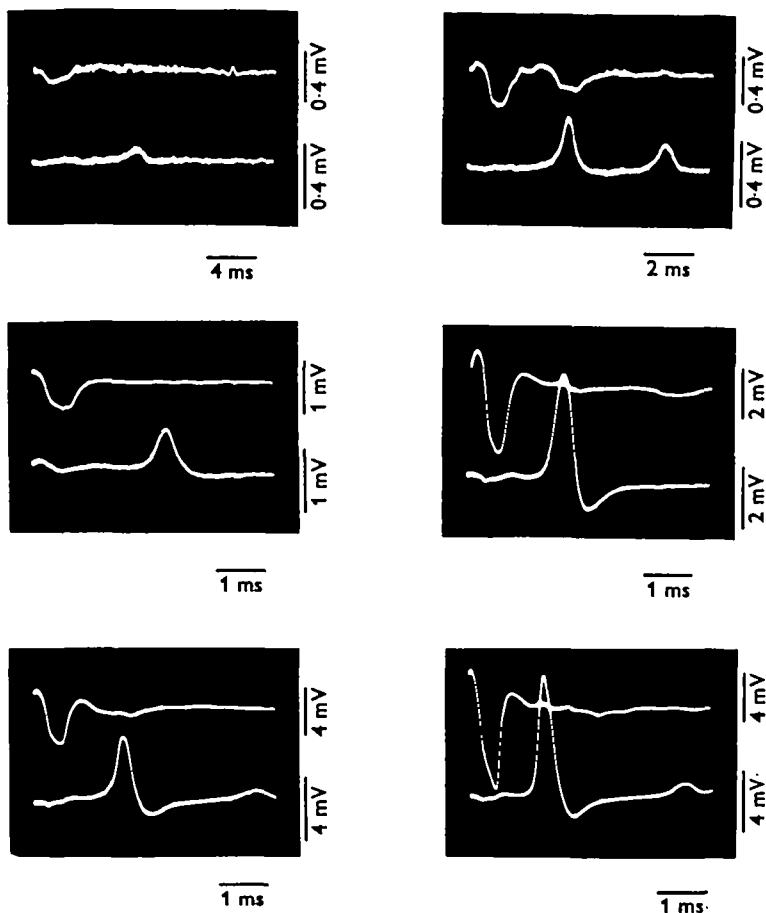
Locust nerve

The tergal nerve to the third abdominal segment of the locust contains a number of spontaneously active fibres. Further fibres can be activated by graded mechanical stimuli to the abdomen or the cercus. With this number corresponding fibres are not easily identified from animal to animal. However, this preparation has the advantage over the cockroach preparation that the nerve trunk is long enough to allow monophasic amplitude, duration and conduction velocity to be measured simultaneously for any particular fibre. Text-fig. 7 shows records from a number of different fibres in one experiment.

The diameter of the locust nerve measured by direct microscopic observation was about $55\ \mu$. Thus, assuming that the membrane action potential amplitude was $100\ \text{mV}$, the diameters of the nerve fibres are given by

$$d = 5.5 \sqrt{V_1}, \quad (3)$$

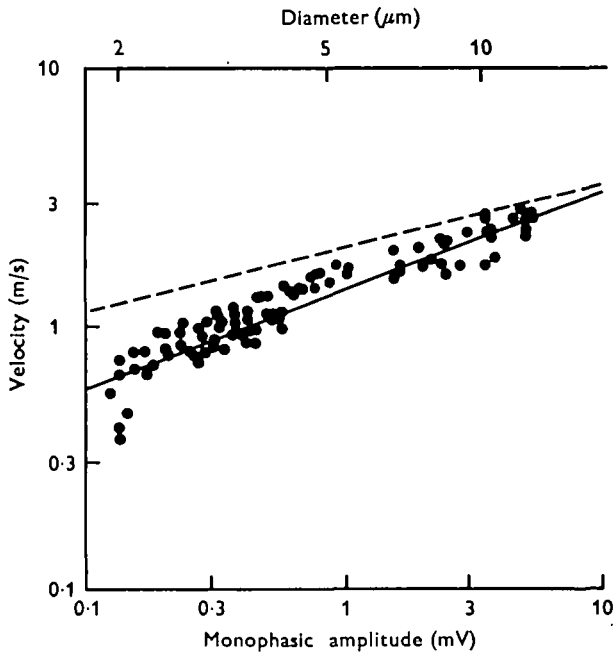
where V_1 is again the extracellularly recorded monophasic potential. Using equation (3), monophasic amplitudes can be converted to fibre diameter. Text-fig. 8 shows conduction velocity plotted both as a function of amplitude (bottom scale) and computed diameter (top scale). The solid line through the points is the relationship between



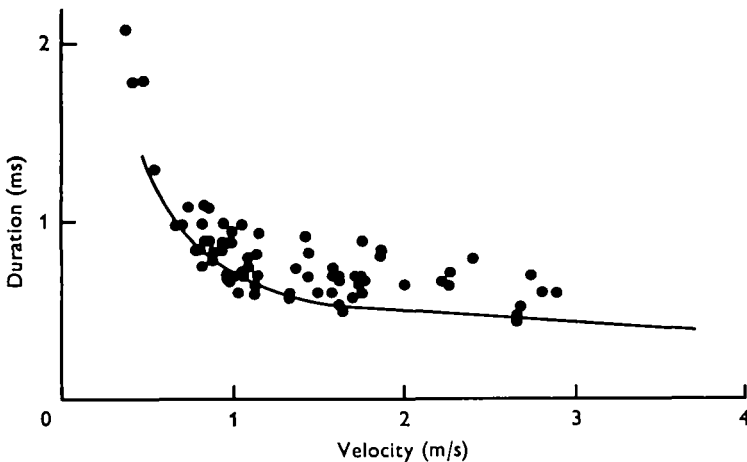
Text-fig. 7. Action potentials recorded from different fibres in a locust nerve. The upper trace shows triphasic records from a proximal electrode pair while the bottom trace gives monophasic records from a more distal electrode pair. Conduction time was measured between the negative peak of the triphasic record and the peak of the monophasic record. Note the different time and voltage scales.

fibre diameter and conduction velocity obtained from the cockroach nerve, and the agreement is quite good. The dashed line is the relationship which would be expected if velocity varied as the square root of diameter, taking for reference the conduction velocity ($19\ \text{m/s}$ at 20°C) computed for a nerve impulse propagating along a squid

axon of $476\ \mu$. The nerve of marine animals such as a squid has a very different composition from an insect nerve, and the proximity of the computed curve may be fortuitous. Nonetheless, it appears that, as fibre size increases, the velocities approach the expected velocities, but the smaller the fibre, the greater the deviation. There is



Text-fig. 8. Conduction velocity as a function of monophasic amplitude in a locust nerve. Fibre diameters were computed from equation (3) and the solid line gives the experimental results for fibres of equal size in the cockroach. The dashed line is the expected relationship from computations based on giant fibres of the squid assuming that membrane properties are independent of fibre size.



Text-fig. 9. Action potential duration measured at half the peak amplitude in the locust nerve as a function of conduction velocity. The solid line again represents the corresponding data from the cockroach nerve.

a suggestion in the locust data that the points do not fall on a single straight line (power function), but form a smooth curve. Fibres in the locust nerve for which the action potential velocity was less than 0.5 m/s (corresponding to fibre 2 in the cockroach nerve trunk) deviate most markedly from any sort of power function relationship between diameter and conduction velocity. Disregarding the fibres for which $v < 0.5$ m/s the slope of the line of best fit through the points in Text-fig. 8 is 0.35. This indicates that velocity increases as the 0.7 power of diameter for these fibres which is again considerably higher than the expected square root relationship.

Duration. Text-fig. 9 shows the action potential duration of fibres measured in five experiments as a function of conduction velocity. The solid line is again the corresponding relationship for the cockroach nerve. The duration of the action potentials from the locust nerves are slightly longer than those in the cockroach nerve, but show the same basic shape. As with fibre 2 of the cockroach nerve, the duration of the action potentials with conduction velocities less than 0.5 m/s are very long and fit on a smooth curve through the data points.

DISCUSSION

1. *Monophasic and triphasic recording*

Electrical records are commonly made using a cut end of a nerve lifted into some insulating medium (monophasic recording), or a short length of intact nerve lifted on a single electrode into an insulating medium (triphasic recording). The second electrode in both cases is in the solution bathing the preparation. One aim of this work was to determine the form of the potentials recorded in these situations, and how the amplitude of these potentials depended on the diameter of the fibres and the length of restriction.

It has been shown that the monophasic amplitude varies as the square of the fibre diameter for fibres 1 to 6 in the cockroach nerve. This relationship was predicted theoretically (Pearson, 1969). The variation in form of the triphasic potential was qualitatively similar to that predicted and for long lengths of restriction, the maximum amplitude of the first positive peak approaches half the monophasic amplitude, and the amplitude of the negative peak approaches the monophasic amplitude. Thus, for long lengths of restriction, the peak-to-peak amplitude of the triphasic potential also varies as the square of fibre diameter.

The only information that can be derived from monophasic recording which cannot be accurately determined from triphasic recording is the form of the membrane action potential. The triphasic method has two obvious advantages. First, it allows stable recordings to be made from an uninjured nerve for many hours. Secondly, since the nerve is intact, triphasic records can be made from afferent and efferent axons simultaneously during reflex acts. Therefore, unless information is required about the time course of the membrane action potentials, triphasic recording is generally preferable. There is, however, one practical advantage of monophasic recording. If the nerve trunks are very short, or space very limited, it may not be possible to restrict a sufficiently long length of nerve to obtain good triphasic potentials. Often in these cases, if the nerve is cut distally, a sufficient length can be obtained to get stable monophasic records.

2. Axon diameters

The determination of fibre diameter from the action potential conduction velocity in unmyelinated nerve fibres contained in small nerve trunks is unsuitable for a number of theoretical and practical reasons. A procedure for estimating the diameter of unmyelinated fibres from measurements of the amplitude of their extracellular potentials is therefore desirable. A method for doing this from monophasic amplitude measurements was suggested by Pearson (1969) (equation (1) of this paper), and has been used in this experimental investigation with reasonable success (for long lengths of restriction triphasic amplitude is proportional to monophasic amplitude; therefore a similar formula can be used to estimate axon diameter from triphasic records, e.g. equation (2)).

The estimated diameters from amplitude measurements for fibres 1 to 6 in the cockroach nerve were in reasonably good agreement with histological measure, although histologically measured diameters tended to be somewhat smaller than predicted. For the smaller fibres, these estimates were considerably more accurate than those made from conduction velocity, the latter underestimating the fibre diameters by more than a factor of 4. We concluded from the observations made on the cockroach levator nerve that, for short nerves containing small unmyelinated fibres, the simplest and most accurate method for estimating fibre diameter is from the amplitude of the extracellular potentials, rather than from conduction velocity.

One further point of interest is the diameters of fibres 1 and 2 in the cockroach nerve. It has sometimes been assumed (Guthrie, 1962) that peripheral cockroach nerve fibres larger than $5\ \mu$ are motor and those smaller than $5\ \mu$ are sensory. The above observation on the diameters of fibres 1 and 2 indicates that some motor fibres are much smaller than this value, and Chapman & Pankhurst (1967) have shown that some sensory fibres have diameters double this value.

3. Conduction velocity and duration

Another aim of this work was to use extracellular recording techniques to study some of the properties of action potentials in small unmyelinated fibres. The two properties of particular interest were action potential conduction velocity and action potential duration.

At present there is little direct experimental information on the conduction velocity-diameter relationship for small unmyelinated nerve fibres. Pumphrey & Young (1938) showed for large unmyelinated axons of the squid ($> 30\ \mu$) that conduction speed varies as the 0.61 power of diameter which is significantly larger than the expected value of 0.5. More recently, Burrows *et al.* (1965) studied this relationship in octopus, squid and sepioid, extending the range of diameters and found the best fitting power function over the range $2\text{--}480\ \mu$ had an exponent of 0.57, although there was no precise determination of fibre diameter in the $2\text{--}22\ \mu$ range which was approximately the range of interest here. The results presented above for the cockroach fibres (neglecting axon 2) showed that on a log-log plot of velocity against diameter the slope was 0.78, and was even larger if fibre 2 was included. The slope of the best fitting straight line for the locust tergal nerve is 0.70. However, the results for the locust suggest that there may be no single power function relating velocity and diameter, but that the slope of a log-log

plot increases continuously for the smaller axons. Thus, there appears to be considerable evidence that conduction velocity varies more rapidly than the square root of diameter in unmyelinated fibres, and that the deviations are more extreme the smaller the fibre.

In both the cockroach nerve and the locust tergal nerve, it was found that with decreasing fibre diameter, the duration of the action potential increased by an amount approximately proportional to the deviation of the conduction velocity from the expected square root relationship with diameter.

The explanation of these results is uncertain. Stein (unpublished calculations) showed that a decrease in the specific conductance of a unit membrane area to all ions increased action potential duration and conduction time by roughly equal amounts, in agreement with the experimental results. Changes in membrane capacity affect conduction velocity much more than duration, while changing the rate constants in the Hodgkin-Huxley equations affects conduction velocity much less than duration. Therefore, changes in membrane capacitance or rate constants *alone* could not account for the experimental results. Maddrell & Treherne (1967) suggest that the function of the concentric arrangement of glial processes and extracellular spaces containing an acid mucopolysaccharide is to regulate the ionic environment around the nerve cell. This would permit the larger cells which contain these structures to function despite large changes in ionic concentrations elsewhere in the extracellular space, but it would also permit these cells to function under normal conditions with the higher conductances per unit membrane area which our results suggest they possess.

However, this is not the only possible explanation. The concentric arrangement of glial processes suggests that the effective resistance of the larger nerve cells might be increased and the effective membrane capacitance decreased. This combined effect on resistance and capacitance might also account for the roughly proportional increase in the duration and deviation in the conduction velocity of nerve impulses. This explanation is difficult to reconcile with the results of Yamasaki & Narahashi (1959) who calculated an extremely high value of membrane capacity for giant fibres in the cockroach. However, their results depend critically on assumptions about axoplasmic resistivity and fibre diameter, neither of which was measured directly.

Paintal (1967) found similar changes in the duration of action potentials in mammalian axons of slowest conduction velocity, and he has evidence for structural changes with size (personal communication, 1968) which suggests that the effects of axon size on the properties of action potentials may be quite general. There are now also a number of studies indicating that other neuronal properties change systematically with size (Bullock, 1953; Henneman, Somjen & Carpenter, 1965; Kernell, 1965; Kennedy, 1967). This evidence must eventually be fitted into a general theory of the relation between neuronal size and function.

SUMMARY

1. The properties of nerve action potentials in small insect motor nerves were studied using extracellular recording electrodes.
2. A length of nerve was lifted out of solution and recordings were made with

respect to the solution either from an intact nerve (triphasic recording) or from near a cut end of the nerve (monophasic recording).

3. In a cockroach nerve, the number of spontaneously active fibres was small enough that corresponding nerve fibres could be identified in each preparation by their action potential amplitude and their pattern of activity. Under controlled conditions, the absolute amplitudes of either monophasic or triphasic records were reproducible and could be used to calculate fibre diameter. The calculations were confirmed from histological sections of the nerve.

4. Conduction velocity varied approximately as the 0.78 power of fibre diameter in a cockroach nerve and as 0.7 power of fibre diameter in a locust nerve. These values are considerably larger than the square root relation predicted if membrane properties are independent of fibre diameter.

5. Membrane properties probably vary with fibre diameter since the action potential duration increases dramatically for fibres below $5\ \mu$ in diameter.

6. For the cockroach nerve systematic structural differences between fibres of different sizes are also seen with the electron microscope and the relation of these to the functional differences is considered.

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EXPLANATIONS OF PLATES

PLATE 1

Cross-section of cockroach nerve 6Br₄ examined with a light microscope. The numbering of motor fibres 1-6 and of sensory fibre (s) is included on the plate. The sensory branch 6Br₃ is seen on the right (SN). Further explanation in text.

PLATE 2

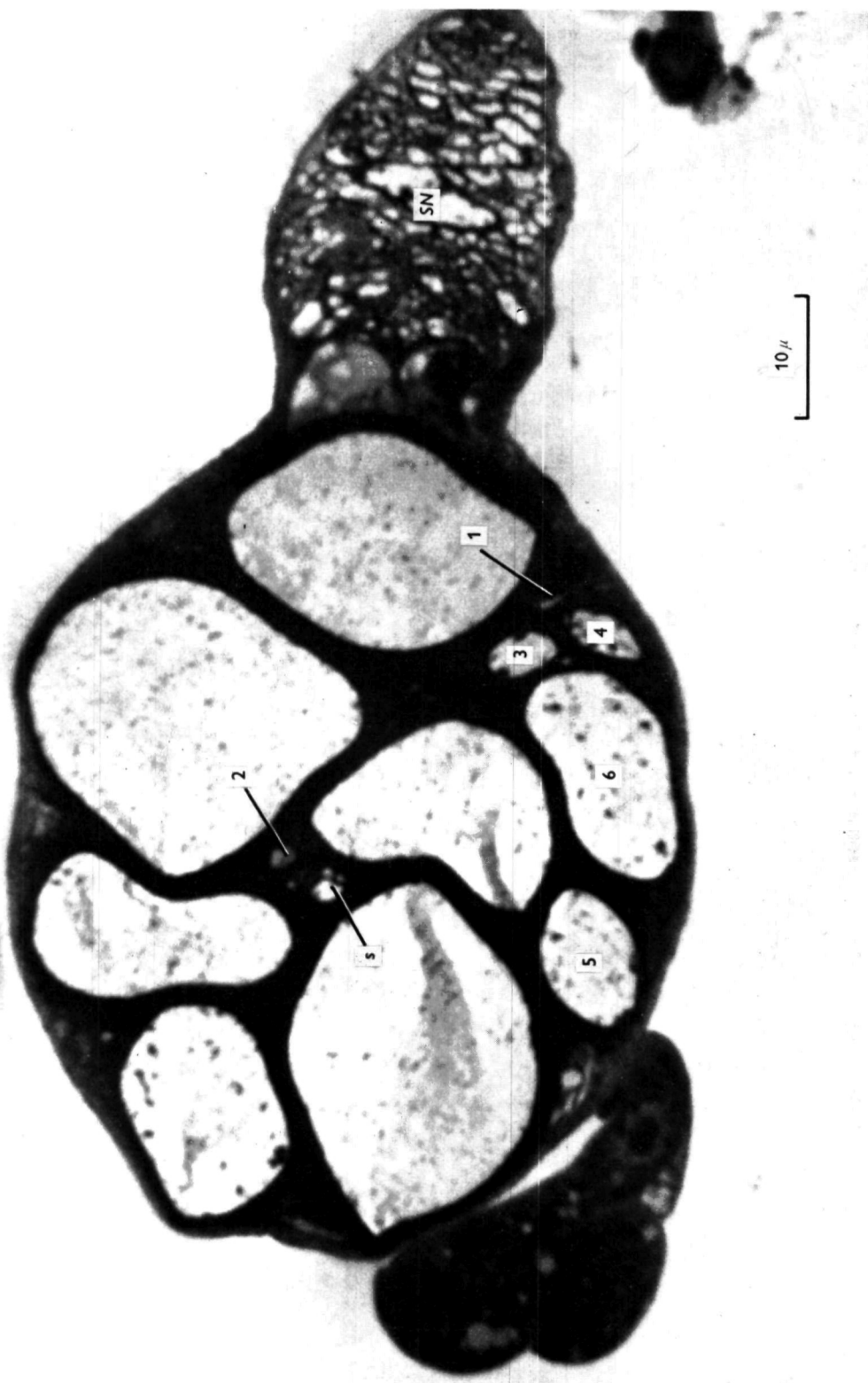
Electron micrograph showing the concentric arrangement of glial processes and dense extracellular material around two large motor fibres. Note also the thin dense lines (arrow) near the periphery of the nerve trunk. At higher magnification (Pl. 4) these are seen to be septate junctions.

PLATE 3

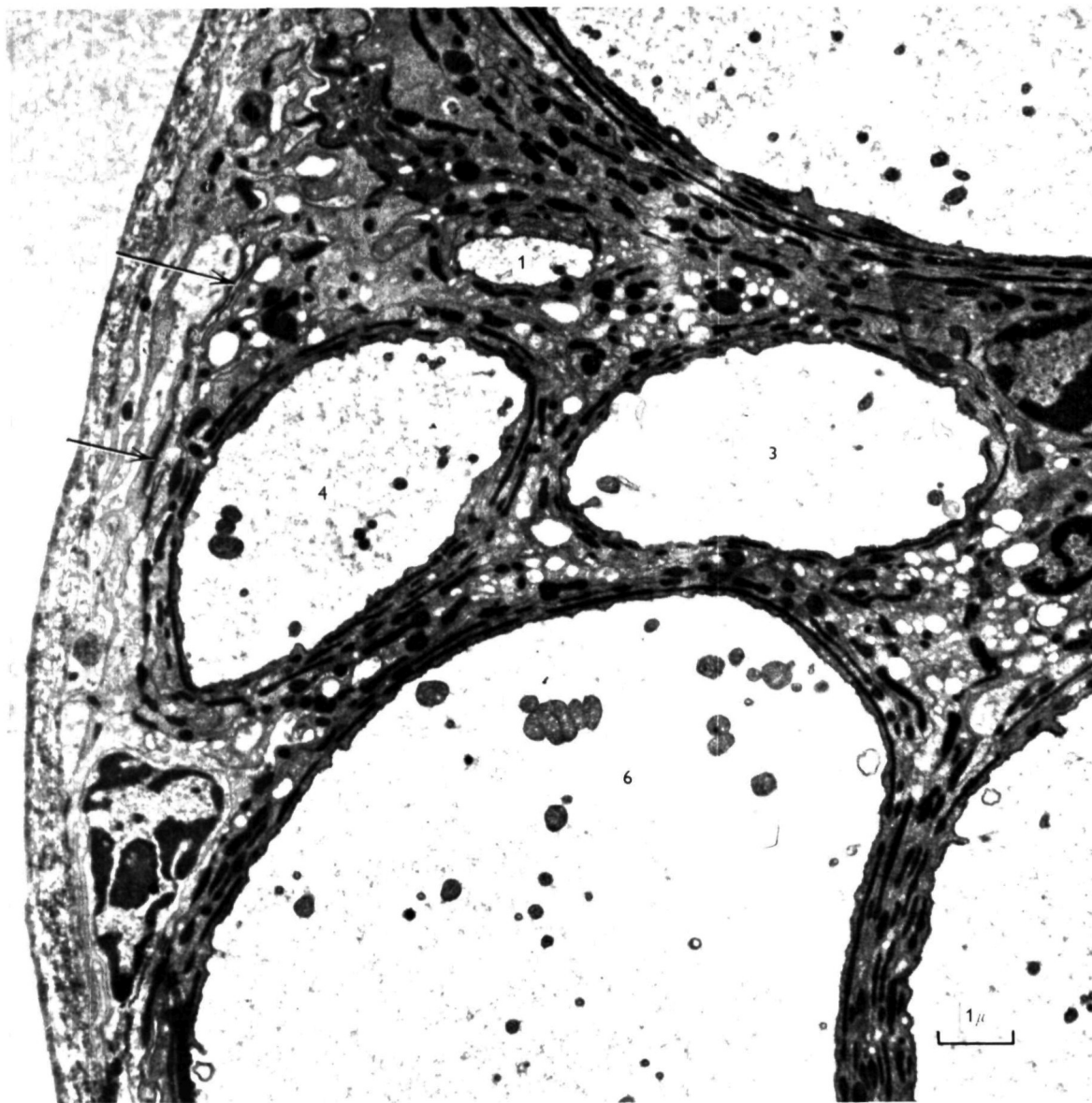
The concentric arrangement of glial processes and extracellular material is less prominent around the medium-sized axons (3 and 4) than around the larger fibres and virtually absent from the smallest fibre (1). Note also the thin dense lines (arrows) which show septate junctions at higher magnifications.

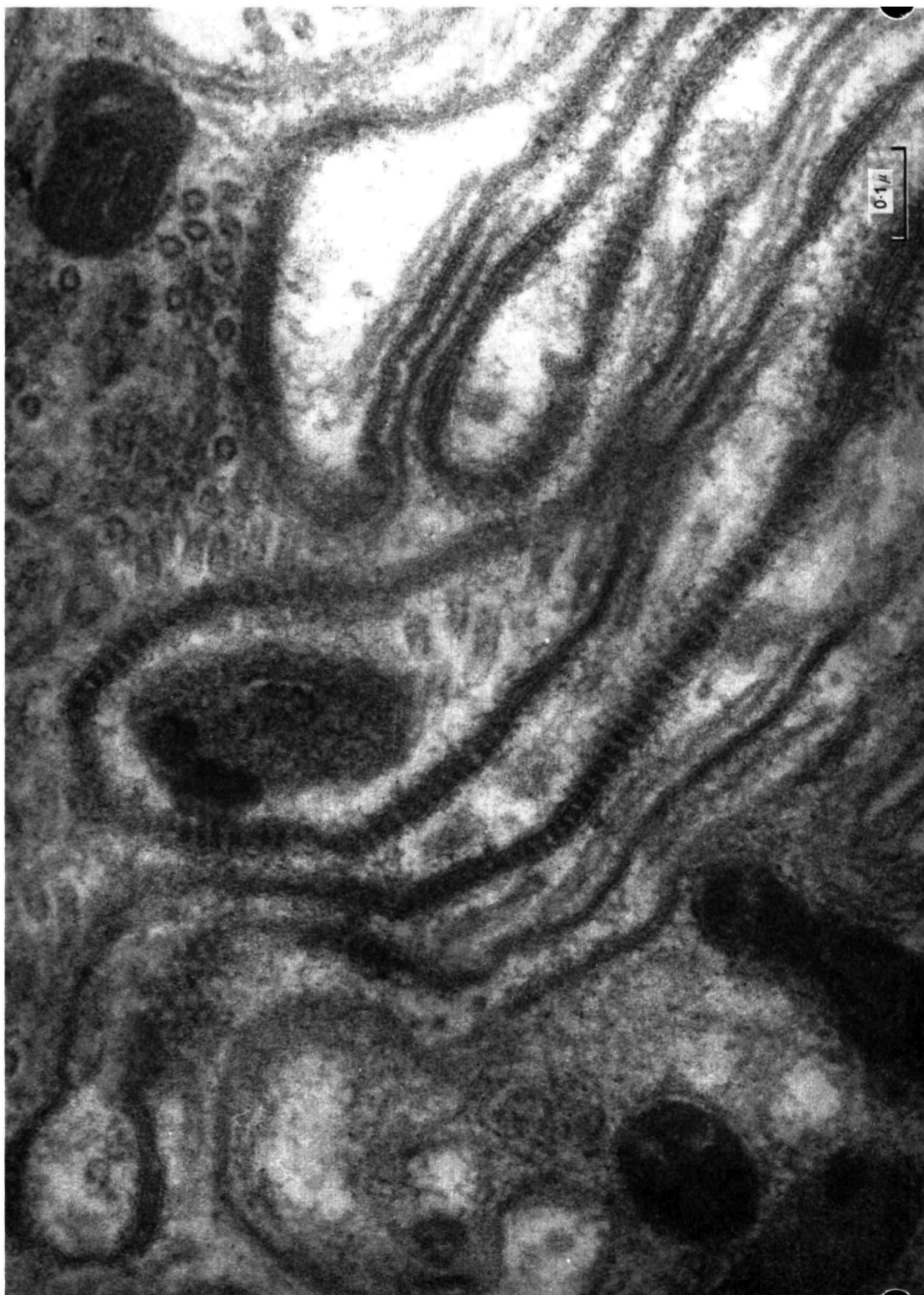
PLATE 4

Cell membranes of adjacent glial processes are joined by regularly occurring transverse bridges or 'septate junctions' which may provide low resistance pathways for ionic movements.









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